# CONSENSUS ON HYPERTHERMIA FOR THE 1990s

## Clinical Practice in Cancer Treatment

# ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY



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# CONSENSUS ON HYPERTHERMIA FOR THE 1990s

## Clinical Practice in Cancer Treatment

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PREFACE

Hyperthermia as a safe and effective cancer treatment modality is rapidly evolving propelled by widespread research and clinical efforts worldwide. Presentations on Hyperthermia experience are now commonplace at Oncology meetings, as are congresses dedicated entirely to the intertwined interactions between basic sciences and patient treatment that together are forming the structure of a new medical specialty. Such was the XII International Symposium on Clinical Hyperthermia held in Rome, Italy, April 27 - 29, 1989.

Papers presented therein constitute the backbone of this book. Biology research has provided data describing mechanisms of action for the cancer cell killing and physiological effects of Hyperthermia. Physics research has led to the development of equipment enabling treatment of many areas of the human body, as well as explained the limitations that still constrain our ability to treat, especially in the areas of deep seated tumor heating and non-invasive thermometry. The main question that will decide the future of this modality is that of its clinical use. To put it succinctly, what do we do with this potentially useful tool in an everyday clinical oncological practice...? This is the main question addressed in this book as "Consensus on Hyperthermia for the 1990s." The book includes 28 presented papers and 25 invited chapters from some of the leading experts in the field. Their basic mechanisms of action were physics principles, treatment quality assurance and especially, clinical indications. It was designed to provide a basis for the practicing oncologist for the understanding of the scientific merit of the modality, as well as its integration with practice of Radiation Therapy as well as Medical and Surgical Oncology. If some of this is achieved, it was worth it.

Special appreciation is extended to Dr. Betty J. Ciuchta for the many hours invested to organize and compose this book. Her personal sacrifice is a tremendous gain to our society and its membership.

Haim I. Bicher M.D.
Editor for the
International Clinical
Hyperthermia Society

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# CLINICAL USE OF REGIONAL HYPERTHERMIA

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For cancer control rates to significantly improve, increase in complete local control of the primary tumor is an important factor(1) . That distant metastasis can originate from uncontrolled local tumor cells has been repeatedly demonstrated, as in the results of adjuvant trials in breast cancer: Post operative radiotherapy reduced the frequency of distant metastasis equally as well as adjuvant chemotherapy. Radiation therapy proved to be actually better in post menopausal patients, including those given tamoxifen, which improved disease free survival in the chemotherapy group but not in the radiotherapy group (2).

Attempts to improve local control by giving higher irradiation dosage are frustrated by normal tissue damage, although achieving improved complete response (CR) rates as reported in the RTOG lung cancer studies,* CR with 75 Gy were 38.6%, 60 Gy 24.7%,and 50 Gy 23.1% (3). Attempts to gain therapeutic advantage by distinguishing tumor from normal tissue, primarily by altering or utilizing relative hypoxia of tumor, are ongoing (4).

Hyperthermia combined with definitive irradiation has achieved a more profound therapeutic advantage, both as to initial response and persistance of response than any other attempts at modification of standard radiotherapy. Reported long term results (5)in patients with multiple neck node metastases from squamous cell carcinoma of head and neck given 40 - 70 Gy with or without hyperthermia showed increased CR rates from 42% to 79%, a thermal enhancement ratio (TER) of 1.88. Persistance of CR at 2 years was 73 % with hyperthermia vs. 33% without. Another series also reporting results (6) in 31 patients with similar neck node

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Abbreviations used: CR=Complete Response, PR=Partial Response, NR=No response, SD=Stable Disease

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Edited by H. I. Bicher *et al.*
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recurrence of head and neck cancer or chest wall recurrence of breast cancer indicated that tumor regression of heated lesions was more rapid than with radiotherapy alone, with at least partial response at completion of treatment in 97% of patients given hyperthermia versus 58% of controls. Recurrence rate after two year follow up was calculated to be 0.03 per lesion at risk per 6 month interval in heated areas vs. 0.2 in control areas receiving radiotherapy only. In neither study (5,6) did the addition of heat cause any increase in early or late radiation effects in normal tissue.

Other publications comparing results of hyperthermia combined with radiotherapy with the same dose of radiotherapy alone have also shown a TER of about 2. In series reported from 18 institutions throughout the world using various schedules and techniques of both hyperthermia and irradiation for over 2000 tumors of various types and sites, TER ranges from 1.16 to more than 6, with a mean of 1.88 (5 - 27, 97) (Table 1). Survival after treatment of locally recurrent tumors has been shown to correlate with sustained CR. Two to 3 years after radiotherapy alone CR was 35%, and survival 32% while after combined treatment Cr was 72%, and survival 67%(6,16).

TABLE 1. Complete Response To Irradiation (RT) Alone Versus RT And Hyperthermia (HT)

| Author | | Evaluable Patients | RT Alone | RT And HT |
|---|---|---|---|---|
| Arcangeli | (5) | 163 | 38% | 74% |
| Scott | (6) | 62 | 39% | 87% |
| U | (7) | 14 | 14% | 86% |
| Kim | (8,9) | 238 | 39% | 72% |
| Overgaard | (10) | 101 | 39% | 62% |
| Corry | 11) | 33 | 0 | 62% |
| Hiraoka | (12) | 33 | 25% | 71% |
| Li | (13) | 124 | 29% | 54% |
| Hornback | (14) | 79 | 46% | 72% |
| Shidnia | (15) | 185 | 33% | 64% |
| Perez | (16) | 154 | 41% | 69% |
| Van Der Zee | (17) | 71 | 5% | 27% |
| Steeves | (18) | 90 | 31% | 65% |
| Dunlop | (19) | 86 | 50% | 60% |
| Goldobenko | (20) | 65 | 86% | 100% |
| Muratkhodzhaev | (21) | 313 | 25% | 63% |
| Lindholm | (22) | 85 | 25% | 46% |
| Valdagni | (29) | 78 | 36% | 73% |
| Emami | (24) | 116 | 24% | 59% |
| Marmor | (25) | 15 | 7% | 47% |
| Gonzalez | (26,27) | 46 | 33% | 50% |
| Sugimachi | (97) | 129 | 52% | 80% |

In other clinical studies published since 1977, the value of hyperthermia as an adjunct to radiotherapy in the treatment of superficial tumors has been affirmed (28 - 41). Hyperthermia for this purpose has been considered standard treatment since 1984. Hyperthermia has a direct cytotoxic and microcirculation effect, as well as enhancing the effect of irradiation, but results with hyperthermia alone are relatively poor with response rates of less than 60% primarily PR (11,19,39-41).

Most authors have reported no increase in acute or chronic normal tissue reaction in the radiation field with combined treatment as compared to the in same dose of radiotherapy given without hyperthermia, with the exception of thermal burns variously reported to occur in 5- 24% of treated patients. A few have found increased skin changes with combined treatment related to giving hyperthermia within a few minutes of high dose per fraction radiotherapy (43-45) or to heating normal tissue to more than 45 degrees Celsius (46).

Table 2 analyses results summarized in Table 1 to show results for the most commonly treated superficial tumors: chest wall recurrence of adenocarcinoma, neck node metastasis of squamous cell carcinoma and melanoma.

Table 2. Complete Response By Tumor Type And Site

| Type - Site | References | Evaluable Tumors | RT alone | RT and HT |
|---|---|---|---|---|
| Chest Wall, Adeno | 6,13,16,19,22, | 227 | 33-67% (42%) | 70-94% (79%) |
| Neck Nodes, Sq. Cell | 6,20,23,42 | 200 | 22-86% (53%) | 9-100% (87%) |
| Melanoma | 5,9,15,24,26,43 | 575 | 17-57% 37%) | 59-90% (68%) |

Factors affecting response have been analyzed by several groups. Tumor histology does not appear to be an important factor; therefore hyperthermia is particularly indicated for treatment of radioresistant tumors such as melanoma and sarcoma. Likewise local control does not depend on tumor site, so long as adequate heating is technically feasible (35,47). Tumor size has been shown to be a significant factor undoubtedly related to limited penetration of single applicator microwave equipment (3cm at 915 MHz, 4 cm at 300 MHz) and less homogeneous heating of larger tumors. CR rates for larger tumors (over 4 cm diameter) have been found to be significantly inferior (9,12,26,42,46,47). However, for patients given full dose radiation tumor size was not significant (23). Since tumor size is also a negative prognostic factor in radiotherapy, adjunct hyperthermia appears paradoxically to be relatively more important for control of larger tumors.

Tumor response is better when radiation is increased from 20 - 30 Gy to 32 -45Gy (35,47,48,49) but not significantly further improved with full dose irradiation in the 50 - 75 Gy range as commonly given previously unirradiated tumors (35). Not surprisingly, response correlates with ability to heat the tumor. Attempts to document this relationship and in particular to establish a thermal dose based on time-temperature isoeffect have had only moderate success. Temperature measurement is still primitive, employing thermometry probes inserted in tissue which adequately track only a small portion of tumor and tumor bed. In clinical practice it is generally agreed that minimum measured tumor temperature should remain at 42 degrees Celsius for at least 30 minutes while keeping normal skin below 45 degrees Celsius, which is the temperature at which pain and thermal burns occur. Considering multiple reports, minimum tumor temperature seems to be the best predictor of response (47,49,50).

In contrast, there is no agreement as to the optimal number of hyperthermia sessions, with abundant contradictory reports. Most clinics, including our own (28,29,35) have given hyperthermia twice a week, empirically based on the well established phenomenon of thermotolerance, defined as transient increase in resistance to a second heat application. In vitro studies show decay of thermotolerance over 30 - 72 hours (51), while in vivo data suggests resistance of both normal and cancer tissue to a second heat insult may persist even at 8 - 14 days (52). Thus even with weekly treatment only the first hyperthermia session should prove fully effective. A mitigating factor is that thermotolorance persists longer in normal than tumor tissue (52,53), related to partial inhibition of thermotolerance at low pH(55). Tumors have low pH, further reduced by Hyperthermia (55).

In clinical practice the influence of thermotolerance remains unclear. Results analyzed by number and frequency of hyperthermia treatments generally ignore other factors, such as radiation dose and fractionation. For superficial tumors relation of complete response rates to number of hyperthermia have variously been reported as inverse (56,57), equal (12,58), direct (33,59,60) or ambiguous (23,29,46,61)(Table 3).

For treatment of deep tumors, analysis of results from various institutions (Table 4), one of which did a retrospective comparison of 2 per week versus 5 per week treatment (61), show a clear advantage for a greater number of hyperthermia treatment sessions (14,35,61-67)(Table 5). Several authors have stated that thermotolerance does not appear to be a significant factor in cancer treatment, based on their clinical data (47, 60, 62,68).

Deep Hyperthermia has a significant effect in combination with both radiotherapy and chemotherapy. The CR rate for deep treatment is far less than for superficial hyperthermia, with some interesting exceptions such as

Table 3

CR Rates For Superficial Hyperthermia By Thermal Dose

| Author | | Site/ Type | # pts/ tumor | # Treatments wk | Total | CR % |
|---|---|---|---|---|---|---|
| Kim | (56) | Melanoma | 50 | 1 | 6 | 74 |
| | | | | 2 | 10 | 59 |
| Alexander | (57) | Multiple | 48 | 1 | 4 | 42 |
| | | | | 2 | 8 | 21 |
| Hiraoka | (12) | Multiple | 40 | 2 | 2-7 | 50 |
| | | | | 2 | 8-12 | 53 |
| Kapp | (58) | Multiple | 38 | 1 | 2 | 68 |
| | | | | 2 | 6 | 63 |
| Luk | (33) | Multiple | | 2 | 481-720min | 38 |
| | | | | 2 | 721+min | 75 |
| Arcangeli | (59) | Multiple | 23 | 1 | 5 | 64 |
| | | | | 2 | 10 | 78 |
| Leopold | (60) | Sarcoma | 17 | 1 | avg.4.4 | 38 |
| | | | | 2 | avg.7.3 | 100 |
| Bicher | (29) | Multiple | 121 | 2 | 8 | 65 |
| Bicher | (61) | Multiple | 154 | 2 | 10 | 41 |
| | | | | 5 | 25 | 55 |
| Valdagni | (23) | Neck | 17 | 2 | 2 | 85 |
| | | | | 2 | 6 | 80 |
| Valdagni | (46) | Neck | 27 | 2 | avg. 5.7 | 40 |
| | | | | 3 | avg. 5.7 | 71 |

Table 4

Hyperthermia For Deep Tumors

| Author | | Site | # pts | #tx | Response CR(%) | PR(%) |
|---|---|---|---|---|---|---|
| Howard | (63) | Pelvis | 20 | 1-7 | 1(5) | 5(25) |
| Hiraoka | (67) | Multiple | 40 | 4-13 | 6(15) | 19(47) |
| Petrovich | (64) | Multiple | 353 | 1-8 | 35(10) | 59(17) |
| Shimm | (65) | Multiple | 44 | 1-7 | 6(14) | 5(11) |
| Storm | (62) | Multiple | 960 | (Avg.12) | 85(9) | 268(28) |
| Baker | (66) | Multiple | 107 | 9-15 | 17(16) | 56(52) |
| Hornback | (14) | IIIB Cervix | 18 | 22-25 | 13(72) | |
| Bicher | (61) | Multiple | 29 | 10 | 5(17) | 9(31) |
| Bicher | (61) | Multiple | 92 | 25 | 19(21) | 48(52) |

Table 5

Literature Summary Results

Response vs. Number of Hyperthermia Treatments

In Deep Tumors

| #Tx | #Patients | CR+PR(%) | CR | PR |
|---|---|---|---|---|
| 1 - 8 | 417 | 27 % | 42 | 69 |
| 9 - 15 | 1136 | 41 % | 113 | 35 |
| 25 | 110 | 73 % | 32 | 48 |

definitive treatment of Stage IIIB cervix cancer (14) in which CR after irradiation alone was 48% versus 72% with combined treatment, or other deep lesions (lung, prostate, esophagus) (61). For superficial lesions (breast, head and neck) CR was also 71% (61). However, most series report a relatively poor CR rate for deep tumors probably related to the fact that most deep treatment has been for palliation in patients with bulky metastatic disease and perhaps also that heating to temperatures generally considered therapeutic has been less consistently achieved than in superficial tumors. At least 42 degrees Celsius has been obtained in 40 % (62) to 78%(63) of deep tumors in published reports that specify tumor temperature. Surprisingly response has not been shown to correlate well with minimum tumor temperature for deep tumors, CR + PR 34% at less than 42 degrees versus 38% at more than 42 degrees (64), and 69% under 43 degrees versus 53% over 43 degrees (67).

Regional deep treatment using magnetic induction (Magnetrode, Henry Medical Electronics, Los Angeles, CA.) (62) or Annular Phased Array (BSD Medical Corporation, Salt Lake City, UT) (64) has been associated with poor patient tolerance and compliance. Significant reaction (pain or systemic stress) occurs in 45% of patients treated with the currently most commonly used equipment (64). Local deep treatment using parallel opposed 300 MHz external applicators (POPAS, HBCI, Panorama City, CA) (61) has in contrast been quite well tolerated, with moderate perspiration but otherwise not different from superficial treatment.

Based on their gratifying clinical data, several authors have stated that hyperthermia treatment of deep tumors should no longer be considered investigational (61,62).

Investigation of the timing of hyperthermia and irradiation fraction indicates significant synergy at a separation of up to four hours or more(10). Although in vivo studies show maximal interaction with simultaneous treatment, two authors

have reported clinical studies comparing hyperthermia within 30 minutes of irradiation versus delay of 3-4 hours, with improved therapeutic gain using the latter regimen (43,45). While most groups have given hyperthermia following the radiation fraction , results using the reverse sequence have been similar (11,37,56).

The interaction of heat and chemotherapeutic agents has been extensively studied in vivo (69,70), since Hahn reported in 1975 that commonly used drugs show increased cell killing at increased temperatures (71). Such interaction is quite complex, however. Various mechanisms of action have been identified. Timing can be critical but differs with the agent; for instance Adriamycin and Actinomycin cytotoxicity is either inhibited or enhanced depending on when heat is applied.

The groups that compared chemotherapy alone with the same dose of the same drugs plus local or regional hyperthermia (72-75) all found significant increase in tumor response using combination treatment. No studies comparing hyperthermia alone with thermochemotherapy have been reported, and the benefit of adding chemotherapy to the hyperthermia regimen has not been established. The few reports including results in patients treated with hyperthermia combined with chemotherapy show fairly good tumor response rates but less than with hyperthermia and low dose irradiation and not clearly synergistic (60,62,76-84). Results in patients who had previously failed the same chemotherapy given without hyperthermia were equivalent to those who had not (62). Addition of immunostimulative agents to thermochemotherapy significantly increased survival (79).

Interstitial hyperthermia, employing the same implant techniques well established for endocurietherapy, has achieved higher and more uniform tumor temperatures as well as better response rates, particulary CR, than external (37,85) or intracavitary heating techniques (84). The limited published clinical experience using interstitial hyperthermia, all combined with endocurietherapy given to a total dose of 20 - 60 Gy, is summarized in Table 6 (37,85-92). All authors using interstitial hyperthermia combined with endocurietherapy agree that successful results depend on heating of the entire tumor to a minimum of 42 degrees Celsius with adequate implant geometry to include the complete tumor volume. Complications related to tumor necrosis occurred in 21% to 38 % of treatments, similar to endocurietherapy alone. Therapeutic advantage of combined treatment is suggested in comparison with historical controls (86), but no significant improvement over endocurietherapy alone has been claimed.

Preliminary results of hyperthermia for brain tumors are quite promising, safe, with palliation and surprising prolongation of survival. Three groups have used various techniques of interstitial hyperthermia alone in a total of 38 patients (93-95), while two have used external hyperthermia in 29 patients either alone (96) or with chemotherapy (97).

Analysis of hyperthermia treatment results for deep seated tumors from published data that specify site specific tumor response is shown in Tables 7,8 and 9. Tumor response rates range from 36% in the abdomen to 52% in the pelvis, but there is much wider variation among various institutions treating the same area, 18% to 71 % in the abdomen. One significant variable factor appears to be technique. In both chest and pelvis response rates have been better with intracavitary than external treatment (99, 74, 105 - 107), as also shown by Bicher (85), who found CR + PR 89% with interstitial, 87% with intracavitary, and 56% with external hyperthermia. In more recent reports, however, Bicher (61,98) has reported 78% Cr + PR in the pelvis and 74% in the chest using external treatment, most patients given daily hyperthermia for five weeks.

Table 6

Interstitial Hyperthermia Tumor Response

| Author | # treated | CR |
|---|---|---|
| Oleson (37) | 52 | 38% |
| Puthawala (86) | 43 | 86% |
| Cosset (87) | 23 | 83% |
| Emami (88) | 48 | 52% |
| Bicher (89) | 9 | 78% |
| Surmit (90) | 12 | 48% |
| Vora (91) | 16 | 68% |
| Lam (92) | 31 | 61% |

The vast majority of hyperthermia treatment has been for previous treatment failure or metastatic disease. The few reports of primary external treatment for locally advanced cancer are quite promising, with complete local control in the 70% - 80 % range for both superficial lesions, as previously mentioned, and for deep seated primaries of cervix (14) and lung (67,98). For pancreatic primaries complete control is rare, but significant tumor regression with prolonged survival has been achieved by hyperthermia combined with radiotherapy or chemotherapy (79,101).

Table 7

Hyperthermia For Intrathoracic Tumors

| Authors | Site | Technique | # | CR | PR | SD | CR+PR |
|---|---|---|---|---|---|---|---|
| Bicher (98) | All | External | 39 | 10 | 19 | 4 | 74 |
| Storm (62) | Lung | External | 147 | 9 | 38 | 57 | 32% |
| Baker (66) | All | External | 10 | 0 | 7 | NS | 70% |
| Sugimachi (99) | Esoph | Intracav | 25 | 6 | 14 | NS | 80% |
| Petrovich (100) | All | External | 19 | 2 | 4 | 7 | 32% |
| TOTAL | | | 240 | | | | 45% |

Table 8

Hyperthermia For Abdominal Tumors

| Authors | Site | # | CR | PR | SD | CR+PR |
|---|---|---|---|---|---|---|
| Bicher (101) | Pancreas | 14 | 1 | 9 | 2 | 71% |
| | Liver | 36 | 4 | 13 | 8 | 47% |
| Petrovich (100) | Liver | 28 | 1 | 12 | 8 | 46% |
| | Other | 62 | 2 | 30 | 8 | 64% |
| Storm (62) | Liver | 304 | 8 | 51 | 117 | 19% |
| | Other | 156 | 9 | 51 | 44 | 38% |
| Baker (66) | Liver | 14 | 0 | 9 | NS | 64% |
| | Other | 17 | 0 | 5 | NS | 29% |
| Sapozink (102) | All | 28 | 0 | 5 | NS | 18% |
| Moffat (78) | Liver | 215 | 0 | 106 | 18 | 49% |
| TOTAL | | 874 | | | | 36% |

Table 9

Hyperthermia For Pelvic Tumors

| Authors | Site | Technique | # | CR | PR | SD | CR+PR |
|---|---|---|---|---|---|---|---|
| Bicher (61) | All | External | 32 | 9 | 16 | 5 | 78% |
| Petrovich(100) | All | External | 55 | 4 | 33 | 13 | 67% |
| Storm (62) | All | External | 156 | 17 | 60 | 56 | 49% |
| Baker (66) | All | External | 28 | 1 | 19 | NS | 71% |
| Sapozink (103) | All | External | 39 | 5 | 14 | NS | 49% |
| Howard (63) | All | External | 20 | 1 | 5 | 6 | 30% |
| Steindorfer(104) | All | External | 15 | 2 | 6 | 4 | 53% |
| Shimm (65) | All | External | 32 | 5 | 3 | NS | 25% |
| Hornback (14) | Cervix | External | 18 | 13 | NS | NS | 72% |
| Kubota (105) | Bladder | External | 33 | 14 | 10 | NS | 73% |
| Szmigielski(106) | Prostate | Intracav | 15 | 3 | 5 | NS | 53% |
| Yerushalmi(107) | Prostate | Intracav | 32 | 14 | 13 | NS | 84% |
| Fujiwara (74) | Vagina | Intracav | 42 | 6 | 22 | 10 | 67% |
| TOTAL | | | 517 | | | | 52% |

Stable disease is an important tumor response category, not only because most of these patients have significant palliation and subjective improvement, but also because many tumors that fail to regress and are subsequently resected are found to contain no viable cancer cells (108).

The phenomenon of abscopal response with hyperthermia treatment has been specifically noted by two groups (106,109). Of 3 patients with bone metastasis who responded to intracavitary hyperthermia for locally progressive prostate cancer and followed at least one year, 2 showed complete disappearance of lesions documented by bone scan after 12 and 18 months(106). Several patients with melanoma locally recurrent in a limb as well as metastatic showed complete regression of all lesions following heated chemotherapy perfussion of the limb (109), with disease free survival at follow up for up to 15 years. Abscopal response presumably is related to stimulation of immune response, which has been demonstrated to occur after hyperthermia treatment (109).

The future of hyperthermia in cancer treatment appears most promising and exciting, but this has been true for years and

progress has been frustratingly slow. Most hyperthermia is still given at university centers. While there is a clear need for further well designed and well conducted prospective controlled clinical trials, hyperthermia needs to be more widely utilized in community oncology practice. Further research and development of hyperthermia equipment and thermometry is also needed; but current imperfect techniques of hyperthermia treatment provide significant clinical benefit.

Clinical data available now, as discussed herein, have established hyperthermia as safe and effective for tumors at any site, even including the brain. There should be no trepidation in using hyperthermia for treatment of any malignant tumor. Specific indications for hyperthermia are summarized in Table 10; but simply stated, hyperthermia is appropriate treatment for any patient with cancer unlikely to be adequately controlled by the other standard modalites - radiotherapy, surgery or chemotherapy.

Table 10

Indications For Local/Regional Hyperthermia Combined With Best Available Chemotherapy Or Irradiation

A. Local Failures Or Recurrence
   1. Breast, Chest Wall, Bone (Lung Liver)
   2. Head and Neck
   3. Skin (Advanced Basal Cell)
   4. Perineal

B. Regional Failure Or Recurrence
   1. Pelvis
   2. Neck
   3. Mediastinum

C. Metastasis
   1. Lung, Pleura
   2. Liver
   3. Bone (With Chemotherapy-Avoids Excessive Radiation To Marrow)

D. Advanced Stage Primaries (III Or IV)
   1. Head and Neck
   2. Esophagus
   3. Pelvis (Colon - Uterus - Cervix - Bladder - Prostate)
   4. Pancreas
   5. Stomach
   6. Breast (Including Inflammatory)
   7. Brain (Superficial Tumors)

E. Special Histology
   1. Melanoma
   2. Sarcoma
   3. Adenoidcystic Carcinoma

F. Patient Refusal Of Other Modalities

Only about 10% of our patients have previously untreated advanced primary disease (61), yet it is for these patients where hyperthermia can have the greatest impact on disease free survival. Future clinical trials will hopefully evaluate the potential benefits of adding hyperthermia to the treatment regimen of stage I and II malignancies. It is not unlikely that thermoradiotherapy will be shown to improve disease free survival for those patients as well as for those afflicted with more advanced disease.

Hyperthermia in the 1990's will undoubtedly become an accepted cancer treatment modality, widely used in community pratice, while research will continue at university and specialized centers. The main obstacle to accomplishing this objective is education, both of oncologists and medical specialists, as well as the patient population, which anxiously awaits the introduction of less toxic, scientifically developed effective cancer therapies. Hyperthermia has been all that, and will occupy its place in the next decade.

REFERENCES

1) Suit, HD; Westgate, SJ: Impact of improved local control on survival. Int. J. Radiat. Oncol. Biol. Phys. 12:453-458,1986.

2) Rutqvist, LE; Cedermark, B; Glas , U ;et al : Radiotherapy, chemotherapy, and tamoxifen as adjuncts to surgery in early breast cancer : A summary of three randomized trials. Int. J. Radiat. Oncol. Biol. Phys. 16:629-639,1989.

3) Emami, B; Perez, CA; Herskovich; A; et al : Phase I/II study of treatment of locally advanced (T3,T4) non-oat cell lung cancer with high dose radiotherapy (rapid fractionation) : Radiation Therapy Oncology Group study. Int. J. Radiat. Oncol. Biol. Phys. 15: 1021-1025,1988.

4) Chemical modifiers of cancer treatment, Paris, France, 21-25 March 1988. Int. J. Radiat. Oncol. Biol. Phys. 16: numbers 4 + 5. Whitmore, GF : Conference Summary 16: 887-889,1989.

5) Arcangeli,G; Benassi, M; Cividalli, A; et al: Radiotherapy and hyperthermia. Analysis of clinical results and identification of prognostic variables. Cancer 60: 950 - 956, 1987.

6) Scott, RS; Johnson, RJR; Story, KV;et al: Local hyperthermia in combination with definitive radiotherapy : Increased tumor clearance, reduced recurrence rate in extended Follow - up. Int. J. Radiat. Oncol. Biol. Phys. 10:2119-2123,1984.

7) U, R ; Noell, T ; Woodward, KT; et al : Microwave - induced local hyperthermia in combination with radiotherapy of human malignant tumors. Cancer 45: 636 - 646, 1980.

8) Kim, JH; Hahn, EW; Antich, PE: Radiofrequency hyperthermia for clinical cancer therapy. Natl. Cancer Inst. Monogr. 61:339-342,1982.

9) Kim, J H; Hahn, E W; Ahmed, SA; et al: Combination hyperthermia and radiation therapy for malignant melanoma. Cancer 50:478-482,1982.

10) Overgaard, J : The current and potential role of hyperthermia in radiotherapy. Int. J. Radiat. Oncol. Biol. Phys. 16: 535-549,1989.

11) Corry, PM; Barlogie,B; Tilchen, EJ;et al: Ultrasound induced hyperthermia for the treatment of human superficial tumors. Int. J. Radiat. Oncol. Biol. Phys. 8: 1225-1229,1982.

12) Hiraoka, M; Jo, S; Dodo, Y; et al : Clinical results of radiofrequency hyperthermia combined with radiation in the treatment of radioresistant cancers. Cancer 54: 2898 - 2904, 1984.

13) Li, RY; Wang, HP; Lin,SY; et al : Clinical evaluation of combined radiotherapy and thermotherapy on carcinoma of the breast. Clin. Oncol. 12: 73-76, 1985.

14) Hornback, NB; Shupe, RE; Shidnia, H; et al : Advanced stage III B cancer of the cervix treatment by hyperthermia and radiation. Gyn. Oncol. 23: 160-167,1986.

15) Shidnia, H; Hornback,NB; Shupe, RE ; et al : Correlation between hyperthermia and large dose per fraction in treatment of Malignant Melanoma ( Abstr.) Int. Clin. Hyperthermia Soc. Meeting, Lund Sweden, 1987.

16) Perez, CA; Kuske, RR; Emami, B; et al : Irradiation alone or combined with hyperthermia in the treatment of recurrent carcinoma of the breast in the chest wall. A nonrandomized comparison. Int. J. Hyperthermia 2: 179-187,1986.

17) van der Zee, J; van Rhoon, GC; Wike- Hooley,JL; et al : Clinically derived dose effect relationship for hyperthermia with low dose radiotherapy. Br. J. Radiol. 58:243-250,1985.

18) Steeves, RA; Severson, SB; Paliwal, BR; et al : Matched - pair analysis of response to local hyperthermia and megavoltage electron therapy for superficial tumors. Endocurietherapy/ Hyperthermia Oncol. 2:163-170,1986.

19) Dunlop, PRC; Hand, JW; Dickinson, RJ; et al : An assessment of local hyperthermia in clinical practice. Int. J. Hyperthermia 2:39-50,1986.

20) Goldobenko, GV; Durnov,LA ; Knysh,VI; et al : Experience in the use of thermoradiotherapy of malignant tumors. Med. Radiol. 32:36-37,1987.

21) Muratkhodzhaev, NK; Svetitsky,PV; Kochegarov,AA; et al: Hyperthermia in therapy of cancer patients. Med. Radiol. 32:30-36, 1987.

22) Lindholm,CE; Kjellen,E ; Nilsson, P ; et al: Microwave-induced hyperthermia and radiotherapy in human superficial tumors. Clinical results with a comparative study of combined treatment versus radiotherapy alone. Int. J. Hyperthermia. 3:393-411, 1987.

23) Valdagni, R; Amichetti, M; Pani, G : Radical radiation alone versus radical radiation plus microwave hyperthermia for N3 neck nodes : A prospective randomized clinical trial. Int.J. Radiat. Oncol. Biol. Phys. 15:13-24,1988.

24) Emami,B; Perez, CA ;Konefal,J ; et al: Thermoradiotherapy of malignant melanoma. Int. J. Hyperthermia 4:373-381, 1988.

25) Marmor, JB; Hahn , GM : Combined radiation and hyperthermia in superfical human Tumors. Cancer 46: 1986-1991, 1980.

26) Gonzalez Gonzalez, D ; van Dijk, JDP ; Blank, LECM ; et al : Combined treatment with radiation and hyperthermia in metastatic malignant melanoma. Radiother. Oncol. 6: 105-113,1986.

27) Gonzalez Gonzalez, D ; van Dijk, JDP ; Blank, LECM ; et al : Chest wall recurrence of breast cancer : Results of combined treatment with radiation and hyperthermia. Radiother. Oncol. 12:95-103:1988.

28) Bicher, HI ; Sandhu T ; Hetzel F ; Hyperthermia and radiation in combination : A clinical fractionation regime. Int. J. Radiat. Oncol. Biol. Phys. 6:867-870,1980.

29) Bicher, HI ; Hetzel F ; Sandhu, T : Results of phase I/II clinical trial of fractionated hyperthermia in combination with low dose ionizing radiation, Bicher HI, Bruley D (Eds.) : Hyperthermia . New York , Plenum Press 1982, pp 87-89

30) Hornback, NB; Shupe, RE; Homayon S; et al : Preliminary clinical results of combined 433 MHz microwave therapy and radiation therapy on patients with advanced cancer. Cancer 40 : 2854-2863, 1977

31) Hornback, NB; ,Shupe, RE; Shidnia, H; et al: Radiation and microwave therapy in the treatment of advanced cancer. Radiology 130 : 459-464, 1979.

32) Fazekas, JT ; Nerlinger, BS : Localized hyperthermia adjuvant to irradiation in superficial recurrent carcinomas : A preliminary report on 46 patients. Int. J. Radiat. Oncol. Biol. Phys. 7:1457-1463.1981

33) Luk, KH; Francis, ME; Perez, CA; et al : Combined radiation and hyperthermia : comparison of two treatment schedules based on data from a registry established by the Radiation Therapy Oncology Group (RTOG). Int. J. Radiat. Oncol. Biol. Phys. 10 : 801-809, 1984

34) Field, S ; Bleehen, NM : Hyperthermia in the treatment of Cancer. Cancer Treatment Reviews 6: 63-94, 1979.

35) Bicher, HI ; Wolfstein, RS ; Lewinsky, BS, et al : Microwave hyperthermia as an adjunct to radiation therapy : Summary experience of 256 multifraction treatment cases. Int. J. Radiat. Oncol. Biol. Phys. 12: 1667-1671, 1986.

36) Kim, JH ; Hahn, GM ; Benjamin, F : Treatment of superficial cancers by combination hyperthermia and radiation therapy. Clin. Bull. 9: 13-16, 1979.

37) Oleson, J; Manning, M ; Sim, D; et al : A review of the University of Arizona human clinical hyperthermia experience. Front. Radiat. Ther. Oncol. 18:136-143,1984.

38) Manning, M; Cetas,T; Miller, R; et al : Clinical hyperthermia : Results of a phase I clinical trial employing hyperthermia alone or in combination with external beam or interstitial radiotherapy. Cancer 49: 205-216, 1982.

39) Meyer, JL : The clinical efficacy of localized hyperthermia. Cancer Res. 44 : 4745s - 4751s, 1984.

40) Kim, JH; Hahn, EW : Clinical and biological studies of localized hyperthermia. Cancer Res. 39:2258-2261, 1979.

41) Marmor, JB; Pound, D; Hahn, GM : Clinical studies with ultrasound induced hyperthermia. Nat. Cancer Inst. Monogr. 61:333-337,1982.

42) Arcangeli, G ; Arcangeli, G ; Guerra, A ; et al : Tumor response to heat and radiation : Prognostic Variables in the treatment of neck node metastasis from head and neck cancer. Int. J. Hyperthermia . 1:207-217,1985.

43) Overgaard, J ; Overgaard, M ; Hyperthermia as an adjuvant to radiotherapy in the treatment of malignant melanoma. Int.J. Hyperthermia. 3: 483-501,1987.

44) Howard, GCW ; Sathiaseelan, V ; Freedman, L ; et al : Hyperthermia and radiation in the treatment of superficial malignancy :an analysis of treatment parameters, response and toxicity. Int. J. Hyperthermia 3: 1-8, 1987.

45) Arcangeli, G ; Nervi, C ; Cividalli, A ; et al : Problem of sequence and fractionation in the clinical application of combined heat and radiation. Cancer Res. 44 : 4857s - 4863s, 1984.

46) Valdagni, R ; Kapp, D.S ; Valdagni, C : N3 metastatic neck nodes managed by combined radiation therapy and hyperthermia : Clinical results and analysis of treatment parameters. Int. J. hyperthermia. 2: 189-200, 1986.

47) Oleson, JR ; Sim , DA ; Manning ,MR : Analysis of prognostic variables in hyperthermia treatment of 161 patients. Int J. Radiat. Oncol Biol. Phys. 10: 2231-2239, 1984.

48) Perez , CA ; Nussbaum, G ; Emami, B ; et al : Clinical results of irradiation combined with local Hyperthermia. Cancer 52 : 1597 - 1603, 1983.

49) van der Zee , J ; van Putten , WLJ ; van den Berg, AP ; et al: Retrospective analysis of the response of tumors in patients treated with a combination of radiotherapy and hyperthermia. Int. J. Hyperthermia. 2:337-349,1986.

50) Dewhirst, MW ; Sim, DA : The utility of thermal dose as a predictor of tumor and normal tissue responses to combined radiation and hyperthermia. Cancer Res. 44: 4772s-4780s, 1984.

51) Hahn, GM ; Hyperthermia and Cancer. New York, Plenum Press, 1982.

52) Urano, M ; Kahn, J : Differential kinetics of thermal resistance (thermotolerance) between murine normal and tumor tissues. Int. J. Radiat. Oncol. Biol. Phys. 12: 89-93, 1986.

53) Field, SB : Hyperthermia in the treatment of cancer. Phys. Med. Biol. 32:789-811, 1987.

54) Goldin, EM ; Leeper,DB : The effect of reduced pH on the induction of thermotolerance. Radiology 141:505-508, 1981.

55) Bicher, HI ; Hetzel, F : Effects of hyperthermia on normal and tumor microenvironment. Radiology 137: 523-530, 1980.

56) Kim, JH ; Hahn, EW ; Ahmed, SA ; et al : Clinical study of the sequence of combined hyperthermia and radiation therapy of malignant melanoma. In Overgaard, J (ED.):Hyperthermic Oncology 1984, Taylor and Francis, London-Philadelphia, 1984, pp 387-391.

57) Alexander, GA ; Moylan, DJ ; Waterman , FM ; et al : Randomized trial of 1 vs. 2 adjuvant hyperthermia treatments in patients with superficial metastasis(Abstr.) Presented at 35th Annual Meeting of the Radiation Research society, 2/21-26,1987.

58) Kapp, DS ; Bagshaw, MA ; Meyer, JL ; et al Optimization of hyperthermia and low dose irradiation in the treatment of superficial tumors : A prospective randomized trial of 2 versus 6 heat treatments (Abstr.) Presented at 33rd Annual Meeting of the Radiation Research Society, 5/5-9, 1985.

59) Arcangeli, G ; Nervi , C : The lack of clinical evidence of tumor thermotolerance after some schedules of combined heat (HT) and radiation (RT), In Overgaard, J (Ed.) : Hyperthermic Oncology 1984. Taylor and Francis, London and Philadelphia, 1984, pp 231-235.

60) Leopold , KA ; Harrelson , J ; Prosnitz , L ; et al : Preoperative hyperthermia for soft tissue sarcomas : Advantage of two versus one treatments per week. Int. J. Radiat. Oncol. Biol. Phys. 16:107-115, 1989.

61) Bicher, HI ; Wolfstein, RS : Local superficial and deep hyperthermia - Factors affecting tumor response and patient survival (Abstr). Presented at 37th Annual Meeting of the Radiation Research Society, seattle , Wash. 3/18-23/1989.

62) Storm, K ; Baker , HW ; Scanlon , EF ; et al : Magnetic induction hyperthermia. Results of a 5 year multi - institutional national cooperative trial in advanced cancer patients. Cancer 55 : 2677-2687,1985.

63) Howard, GCW; Sathiaseelan, V; King, GA; et al : Regional hyperthermia for extensive pelvic tumors using an annular phased array applicator: a feasibility study. British J. Radiol. 59:1195-1201,1986.

64) Petrovich, Z ; Langholz,B; Gibbs, FA ; et al : Regional hyperthermia for advanced tumors : A clinical study of 353 patients. Int. J. Radiat. oncol. Biol. Phys. 16:601-607,1989.

65) Shimm, DS; Cetas, TC; Oleson, JR ; et al : Regional hyperthermia for deep - seated maligancies using the BSD annular array. Int. J. Hyperthermia 4:159-170,1988.

66) Baker , HW ; Snedecor, PA ; Goss, JC; et al : Regional hyperthermia for cancer. Am. J. Surgery 143:586-590, 1982.

67) Hiraoka, M; Jo, S; Akuta, K ; et al : Radiofrequency capacitive hyperthermia for deep - seated tumors. II . Effects of thermoradiotherapy. Cancer 60 : 128-135, 1987.

68) Streffer, C; Van Beuningen, D; Marbach, J :Thermotolerance A barrier for the use of hyperthermia in tumor therapy/(abstr.) Int. J. Radiat. Oncol. Phys. 8:109,1982.

69) Engelhardt, R: Hyperthermia and drugs. Recent Results Cancer Res. 104:136-203, 1987.

70) Dahl, O: Hyperthermia and drugs. In Wathmong , JD and Ross, Wm(Eds): Hyperthermia . Blackie, Glasgow, 1986,pp121-153.

71) Hahn, GM; Braun, J: Har-kedar, I : Thermochemotherapy : Synergism between hyperthermia (42-43 degrees) and Adriamycin or bleomycin in mammalian cell inactivation. Proc. Nat. Acad. Sci. 72:937-940,1975.

72) Arcangeli, G; Cividalli, A; Mauro, F; et al : Enhanced effectiveness of Adriamycin and Bleomycin combined with local hyperthermia in neck node metastases from head and neck cancers. Tumori 65: 481-486,1979.

73) Kohno, I; Kaneshige, E; Fujiwara, K; et al : Thermochemotherapy for gynecological malignancies. In Overgaard, J (Ed) : Hyperthermic Oncology 1984. Taylor and francis. London and Philadelphia, 1984, pp 753-756.

74) Fujiwara, K; Kohno, I; Sekiba,K : Therapeutic effects of hyperthermia combined with chemotherapy on vulvar and vaginal carcinoma. Acta Med. Okayama 41:55-62, 1987.

75) Stehlin,JS; Giovenella,BC; de Ipolyi, PD ; et al : Results of eleven years of experience with heated perfusion for melanoma of the extremities. Cancer Research 39 : 2255-2257,1979.

76) Moffat, FL; Rotstein, LE ; Calhoun, K; et al : Palliation of advanced head and neck cancer with radiofrequency hyperthermia and cytotoxic chemotherapy. Can. J. Surg. 27:38-41, 1984.

77) Jacobs, SC ; Mc Clellan , SL; Maher, C; et al : Pre - cystectomy intra - arterial cis - diaminedichloroplatinum II with local bladder hyperthermia for bladder cancer. J. Urol. 131: 473-476, 1984.

78) Moffat, FL ; Gilas, T ; Calhoun, K; et al : Further experience with regional radiofrequency hyperthermia and cytotoxic chemotherapy for unresectable hepatic neoplasia. Cancer 55 : 1291-1295, 1985.

79) Falk, RE; Moffat, FL; Lawler, M ; et al : Combination therapy for resectable and unresectable adenocarcinoma of the pancreas. Cancer 57 : 685-688, 1986.

80) Cruciani, G ; Molinari, Al; Marangolo, M; et al : Applicability of local hyperthermia as adjuvant to systemic chemotherapy. Tumori 73 : 629-633,1987.

81) Dahl, O; Mella, A; et al : Clinical hyperthermia combined with drugs and radiation . A phase I/II study. Strahlentherapie und Onkolgie 163:446-448,1987.

82) Steindorfer, P; Jakse, R; Germann, G; et al : Hyperthermia as an adjuvant to radiation and/or chemotherapy in far advanced recurrences of the head and neck region. Strahlentherapie und Onkologie 163:449-452,1987.

83) Li, D-J; Hon, B-S : Preliminary report on the treatment of esophageal cancer by intraluminal microwave hyperthermia and chemotherapy. Cancer Treat. Rep. 71:1013-1019, 1987.

84) Pilepich MV; Jones, KG; Emami, CA; et al: Interaction of bleomycin and hyperthermia - results of a clinical pilot study. Int. J. Radiat. Oncol. Biol. Phys. 16:211-213, 1989.

85) Bicher, HI; Wolfstein, RS : Local hyperthermia for deep tumors - Experience with three techniques. J. Microwave Power 21: 21st International Microwave Power Symposium Summaries, 103-104, 1986.

86) Puthawala, AA; Syed, AMN; Sheikh, KMA; et al: Interstitial hyperthermia for recurrent malignancies. Endocurietherapy/ hyperthermia Oncology 1 : 125-131, 1985.

87) Cosset, JM; Dutreix, J; Haie, C; et al : Interstitial thermoradiotherapy : A technical and clinical study of 29 implantations perrformed at the Institut Gustave-Roussy. Int. J. Hyperthermia 1 : 3-13, 1985.

88) Emami, B ; Perez, CA ; Leybovich , L ; et al : Interstitial thermoradiotherapy in treatment of malignant tumors. Int. J. Hyperthermia. 3: 107-118, 1987.

89) Bicher, HI; Moore, DW; Wolfstein, RS : A method for interstitial thermoradiotherapy. In Overgaard, J(Ed): Hyperthermic Oncology 1984, Taylor and Francis , London and Philadelphia, pp 595-598 , 1984.

90) Surwit, EA; Manning, MR; Aristizabal, SA; et al : Interstitial thermoradiotherapy in recurrent gynecologic malignancies. Gyn. Oncol. 15 : 95-102,1983.

91) Vora, N; Forell, B; Joseph, C; Fearnot, N : Interstitial implant with interstitial hyperthermia. Cancer 50 : 2518-2523, 1982.

92) Lam, K ; Astrahan, M ; Langholz, B ; et al : Interstitial hyperthermia for recurrent or persistent tumors. Int. J. Hyperthermia 4: 259 - 266, 1988.

93) Marchosky, JA; Moran , C; Fearnot, N : Long term interstitial hyperthermia therapy for brain tumors using implanted catheters. (Abstr.)Int. Clin. Hyperthermia symposium, Indiana U., Indianapolis, 5/24-27,1988.

94) Winter, A; Laing, J; Paglione, R; et al : Microwave hyperthermia for brain tumors. Neurosurgery 17 : 387-399,1985.

95) Salcman,M; Samaras, GM : Interstitial microwave hyperthermia for brain tumors . Results of a phase I clinical trial. J. Neurooncology 1: 225-236, 1983.

96) Tanaka, R; Kim, CH; Yamada, N; et al : Radiofrequency hyperthermia for malignant brain tumors : Preliminary results of clinical trials. Neurosurgery 21:478-483,1987.

97) Silberman, AW; Rand, RW; Krag, DN ; et al : Effect of localized magnetic-induction hyperthermia on the brain. Cancer 57:1401-1404, 1986.

98) Bicher, HI; Wolfstein, RS : Treatment of intrathoracic lesions preliminary results(Abstr.). Presented at 37th annual meeting of the Rad. Res. Soc., Seattle Wash. 3/18 - 23, 1989.

99) Sugimachi, K; Inokuchi, K : Hyperthermochemoradiotherapy and esophageal carcinoma . Sem. in Surg. Oncol. 2:38-44,1986.

100) Petrovich, Z; Emami, B; Astrahan, M; et al: Regional hyperthermia with BSD-1000 annular phased array in the managment of recurrent deep seated malignant tumors. Strahlentherapie und Onkologie 163:430-433,1987.

101) Bicher, HI; Wolfstein, RS : Hyperthermia treatment for abdominal tumors(Abstr). Presented at 37th annual meeting of the Rad. Res. Soc., Seattle, Wash. 3/18 - 23, 1989.

102) Sapozink, MD ; Gibbs, FA; egger, MJ ; et al : Abdominal regional hyperthermia with annular phased aray. J. Clin. Oncol. 4 : 775-783, 1986.

103) Sapozink, MD ; Gibbs, FA ; Egger, MJ ; et al : Regional hyperthermia for clinically advanced deep-seated pelvic malignancy. Am. J. Clin. Oncol. 9: 162-169, 1986.

104) Steindorfer, P ; Germann, R ; Schneider, G ; et al : Our experience with an annular phased array hyperthermia system in the treatment of advanced recurrences of the pelvis. Strahlentherapie und Onkologie 163: 439-442, 1987.

105) Kubota, Y ; Shuin, T ; Miura, T ; et al : Treatment of bladder cancer with a combination of hyperthermia, radiation and bleomycin. Cancer 53: 199-202, 1984.

106) Szmigielski, S; Zielinski, H; Stawarz, B; et al : Local microwave hyperthermia in treatment of advanced prostatic adenocarcinoma. Urol. Res. 16:1-7,1988.

107) Yerushalmi, A; Shani, A ; Fishelovitz, Y; et al : Local microwave hyperthermia in the treatment of carcinoma of the prostate. Oncology 43: 299-305, 1986.

108) Storm, KF; Scanlon, EF; Baker, HW; et al : Tumor stabilization after hyperthermia : An important criterion of response to thermal therapy. J. Surg. Oncol. 34: 143-149,1987.

109) Stehlin, JS; Greef, PJ ; de Ipolyi, PD; et al : Heat as an adjuvant in the treatment of advanced melanoma: an immune stimulant. Houston Med. J.4: 61-82, 1988.

# THE BASIS FOR HYPERTHERMIA BECOMING THE FOURTH CANCER TREATMENT MODALITY IN THE 1990'S

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At and following the International Symposium of Hyperthermia, sponsored by the American College of Radiology at Baltimore in 1978, there was much optimism based upon biological and physical data that heat as the fourth modality for the treatment of cancer would evolve. In spite of firm foundations for the clinical use of heat and in spite of extensive engineering toward the application of space age technology to adequately heat cancer patients and with financing from several sources many questions remain unanswered. Many investigators previously guardedly optimistic have gradually become disappointed and somewhat pessimistic, and financing has tightened over the past decade. Much of the present clinical work, due to equipment limitations, is directed largely to more superficial cancers most available to surgery and radiation for definitive treatment. Many are asking "Wither hyperthermia in the 1990's?"

The discovery of fire eons ago increased the available world's food supplies at quantum levels thereby catalyzing the exponential growth of the world population and the available heat was utilized at least 2000 years ago (Rawajawa) in hopes of curing some of these by use of cautery. Hopefully sufficient advances can be realized as we enter the final decade of this century to fulfill this 2000 year dream. This book has been compiled by a number of noted scholars with expertise in the field of hyperthermia to form a consensus for the potential advances of hyperthermia into the 1990's. Much of the remainder of this chapter will be devoted to the summary of the fundamentals of hyperthermia as of the late 1980's so that the various authors need not repeat some of these basic facts upon which their work and predictions are based.

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increasing knowledge as to the specific defects of the immunocompetence found in cancer patients hopefully a rational application of hyperthermia to improve such patient's immunocompetence can be developed.

## TECHNIQUES OF DELIVERING HYPERTHERMIA

Many believe that cancer is basically a systemic disease and therefore any rational treatment plan must treat the whole body. It may be that Mother Nature's use of fever may be an effective systemic procedure. Due to the inherent toxicity of total body treatment (core temperature should not exceed 41.8oC for the usual treatment interval), other investigators have preferred more intense heating (at least 43.0oC) in hopes of sterilizing the local tumor area while stimulating a favorable immune response to effect some systemic benefit. Inherent in many heating systems is the fact that the borders cannot be sharply defined and therefore local heating actually becomes enlarged into a regional pattern which can be beneficial since many cancers spread early in continuity.

A number of systems, many complex and expensive, have been developed to heat patient's total body in hopes of producing a cure. Some of the early methods were somewhat primitive and consisted of simply emerging a patient's body into a heated tub of paraffin (later an investigator who experienced a leakage of paraffin onto two laboratory floors substituted water) with only the head emerged. Frequently the patient needed to inspire heated gases to obtain adequate temperatures. Earlier (1935) Warren, heating systemically by enclosing the body in a cabinet with carbon filament lamps and radiofrequency diathermy, was able to reach temperatures of 41oC to 43oC with evidence of tumor regression and relief of symptoms. Tested for systemic heating has been all types of environments which can be temperature controlled such as iron lungs, space suits, blankets, and more recently, infra-red chambers (discussed by Dr. Robins in a later chapter). Some have used space age technology to control the body core temperature within a tenth of a degree Celsius by extracorporal perfusion in which the patient's blood is heated and then returned to maintain a desired temperature (Dr. Koga discusses this technique in a later chapter). Extensive data 8,9,10 has been collected showing enhanced responses when heat is added to perfused cytostatics into extremities with malignancies. Some investigators, knowing that if sufficient wattage is wrapped about the patient, the body's mechanism for dissipating heat can be overcome and the patient can be maintained safely in a state of therapeutic hyperthermia with conventional hyperthermia equipment more frequently used for regional heating. An equal effect may be obtained if a somewhat lower temperatures are maintained for a longer period of time. Hence, a number of investigators have recommended that the core temperature be reduced to less toxic levels but maintained for hours.

Various forms of electronic equipment have been used in attempts to heat tumors at some depth. These include radiofrequency, microwave and ultrasonic modalities. Coupling

techniques must be employed so as to optimize delivery of their energies into the body. They all also suffer from the fact that absorption differs amongst various tissues, thus, the heating pattern at the depth of the tumor may be quite inhomogeneous. So as to adequately heat tumors at a depth at which tumors are frequently found (at or beyond eight centimeters) attempts are being made to utilize microwaves at lower frequencies (there is a greater penetrability at lower frequencies) with specially designed antennae with multiple fields so as to focus heat at depth with well defined borders. As these portals of entry become greater in size and as heat diffuses laterally it is better to define such heating patterns as regional and not local.

Unfortunately, all anticancer agents demonstrate toxicity against the surrounding normal cells as well as the target malignant cells. So as to maximize these toxic effects to the malignancy efforts have been made to confine the heat to the volume of the tumor similar to what has been done for decades in the use of radioactive sources (brachytherapy). This may be done in quite a similar manner by the insertion of an antenna or electrodes instead of radioactive sources and heating these with proper instrumentation with either microwave or radiofrequency electromagnetic radiations. Another interesting technique is the entrapment or injections of magnetic compounds into the area of the tumor which can be heated by external sources. By choosing the proper compounds which lose their magnetic properties abruptly at a given temperature (Curie Point) a method for adequate controlling temperatures may be developed.11,12

There are advantages and disadvantages to each of the techniques and methods discussed above. Unfortunately, none are optimum, all have inherent deficiencies because Mother Nature has been cruel with reference to the physics of depositing energy at any depth into the body. Furthermore, in order to monitor these patients quality assurance must include temperature measurements, not merely at one point but at multiple points because of the evidence that responses may depend upon the minimum temperature obtained (Oleson).13 Efforts have been successful in the development of instrumentation permitting use of small catheters which measure multiple temperatures along their axis. However, these all require minor surgical procedure for their insertion which is time consuming and not particularly comfortable to the patient. This is particularly true when the patient is reminded that this is merely for monitoring purposes and not a treatment procedure. In spite of exhaustive attempts to develop a non-invasive thermometry system none have proven to be practical or sufficiently accurate. If the temperature gradient of a heating unit could be sufficiently tight, i.e., a rapid fall off of temperature outside the defined beams of the modality being used then possibly sufficient cytotoxic temperatures could be delivered within the tumor volume with relatively nontoxic levels short distances from the tumor into normal tissue such that thermometry would not be as necessary as it is with today's limited technology. Such technique could incorporate also the deposition of energy sufficiently

rapid, that the normal conduction of heat would not result in over-heating of neighboring normal tissues.

TUMOR VASCULARITY

To be discussed later are the biological differences in the micro environment of the tumor cells from that of normal cells, i.e., increased acidity and nutritional deprivation, etc., which render them more sensitive to temperature increments which may be due in part to the differences in vascularity with tumors as compared to the surrounding normal tissues. Westermark was an early investigator to conclude that the thermoregulation within tumors was deficient based upon his heating of rodent tumors with radiofrequency units to 45oC-50oC which produced marked regression of the tumors without damage to the surrounding tissues. Subsequently, numerous investigators using a diversity of techniques and compounds, i.e., dyes, radioisotopes, etc., have shown blood flow and perfusion within tumor tissues to be significantly less (2-15%) of that in the surrounding normal tissues, therefore, tissue efflux heat less well and act as heat sinks.14,15

Such differences may be explained by the neovascularity of tumors which differ markedly from that of normal tissues and in many cases permit the differential diagnosis of normal tissues from malignant tissues on the basis of angiographic studies. Although upon such studies tumors appear quite vascular and at surgery bleed profusely, the architecture is such that metabolic compounds and heat are poorly cleared from such areas. Tumor growth usually lags behind that of the proliferating neovascularity which forms under the influence of tumor angiogenesis factor. Adjacent venules of the host become hyperemic and dilated. As small buds develop into small branches forming collectively a tumor capillary network. Tumors accelerate their growth only when such networks are sufficiently mature to permit blood flow. The vessels in such neovascularity angle sharply with much twisting and bending with irregular areas of constrictions and dilatations. These drain into numerous tortuous sinusoids and patchy areas of dilatation leading to further stasis. Frequently such capillaries are longer, wider and with greater distances between capillaries than that of the normal tissues. Such structural differences are important since oxygen can diffuse only approximately 150 micron from a capillary; as a result anaerobic metabolic pathways become more prominent in tumor cells at greater distances from capillaries, and also explains the observation of Thomlinson and Gray16 that for the human bronchogenic carcinoma cores of tumor whose diameters exceeded 200 micron contained central areas of necrosis. Clinically macroscopic necrosis is observed and 99% of the cells are hypoxic in tumors over two centimeters in diameter. At the normal usual venous pressure of oxygen of 40 mm of mercury, the diffusion length of oxygen is less than 100 micron. With increasing tumor growth the length and degree of dilatation of such capillaries increases disproportionately resulting in further stasis of blood at times the extravascular pressure may exceed the artericlar pressures. As a result17,18 with increasing tumor weight the blood flow gradually decreases.

Such vessels do not respond to adrenalin, histamine or acetylcholine Natadze,19 while unfortunately attempts to use such physiological differences for clinical advantage have not been successful.

Most tumors may show an increase in blood supply at temperature elevation to 40oC-41oC but at higher temperatures blood flow tends to decrease and with further increases there is collapse of the micro circulation enhancing the temperature differentials of tumors vs normal tissues.

There are indications that these poorly formed immature vascular beds are more sensitive to heat than are the normal vascularity. (Sugaar and LeVeen20) found in human lung tumors following hyperthermia with radiofrequency hyperthermia for one day a reduced flood flow in the capillaries of the tumor and dilatation of the efferent vessels. These changes progressed to showing degenerative changes with marked fibrinoid necrosis within their walls and many were functionally and anatomically destroyed. Such necrotic vessels were found to be infiltrated with small lymphocytes suggesting that in part the effects of hyperthermia is due to the actions of T-lymphocytes. With more intense heating (45oC or greater for 15-30 minutes with radiofrequency) Storm, et al,21 demonstrated more severe damage of coagulation necrosis and vascular thrombosis two weeks following five courses of such hyperthermia.

The erratic and often chaotic vascular patterns of tumor results not only in poor diffusion of nutrients but also of heat exchange. The small but proven temperature increments relative to the surrounding normal structures combined with the increased thermosensitivity due to nutritional deprivation makes hyperthermia an enticing and viable treatment modality. Its synergistic enhancement of the response rates of tumor cells to both radiation and chemotherapy are well documented. Whether or not any therapeutic gain results from such adjuvant combinations will depend upon whether or not the associated increased blood flow within the surrounding normal tissue undergoing hyperthermia gives sufficient increased oxygenation resulting in increased radiation damage, or if due to the increased blood flow and vascular permeability the concentration of drugs become greater in normal tissues. In either case the addition of heat may have a negative impact.

## RATIONALE FOR HYPERTHERMIA TREATMENT OF CANCER PATIENTS

Lambert22 may have been the first to observe a differential cytotoxicity to heat between normal cells and tumor cells. If heat is to be an effective anticancerous treatment modality, like all other agents, it must exhibit acceptable toxicity to normal tissues while sufficiently toxic to tumors to cause their regression. Westermark5 demonstrated this differential in 1927 when heating rats with diathermy and found that the normal tissues were not damaged when adjacent tumors were heated for 180 minutes at 44oC or 90 minutes at 45C producing tumor necrosis. Subsequently many

workers23-32 using tissue culture or animal models have been able to confirm this. Interestingly, Crile33 found that tumors implanted in mice excised immediately after heating grew in recipient animals upon transplantation but those so transplanted four hours after heating did not grow- suggesting an antitumor immune effect. Later Cavaliere demonstrated the same phenomena clinically in his osteosarcoma patients. Those whose amputation was delayed for one month had less metastases. Crile also later demonstrated several caveats of hyperthermia in that he noted when tumors were preheated they were less sensitive to further heating (thermotolerance) and the tumors could be made more sensitive by clamping the blood supply to the tumor. In Overgaard's work as well as others a relationship was noted between temperature and time of exposure. They also reported autolytic disintegration of the heat treated tumor cells with subsequent proliferation of connected tissue and scarring, changes not demonstrated in surrounding normal tissues. Giovanella's work demonstrated cell lines acquiring malignant potential either in vitro or vivo are accompanied by increased sensitivity to heat. LeVeen noted malignant cells heated in vitro survival depended not only upon temperature obtained but also the time exposed to such temperature. At temperatures between 42oC and 47oC exposure times sufficient to kill sarcoma cells (inability to migrate from fragments of tissue within a plasma clot) were found to be non-toxic under the same conditions to mesenchymal non-tumorous cells. Stehlin, et al,34 demonstrated that human normal melanocytes from the uvea of the eye were less thermosensitive than human melanoma cells. His group then demonstrated similar findings in a wide variety of human neoplastic cells and their non-neoplastic counterparts. These differentials in thermosensitivity are enhanced when such tumors are growing in the host rather than in tissue culture.35-37

Many38-40 have studied the micro environment of tumors searching for the various factors which may account for the enhanced thermosensitivity. Due to the resultant nutritional deprivation from poor blood flow the thermal sensitivity of these cells is further enhanced and in the hypoxic and anaerobic environment cells have aerobic glycolysis metabolic pathways with the formation of lactic acid and increased acidity in these tissues. This lower pH is considered by many to be an important factor in increased sensitivity. This is based on the fact that in vitro40-43 thermosensitivity has increased when pH is lowered. The pH is further reduced by accumulation of acidic catabolites. Von Ardenne and associates44-45 in the early 1970's had attempted to use this phenomena by producing hyperglycemia which stimulates glycolysis with further production of lactic acid. It is felt that hyperglycemia leads to a chain of events resulting in cancer cell death through stimulating glycolysis, increased lactic acid, decreased intercellular pH, lysosomal damage which under the influence of hyperthermia releases hydrolytic enzymes resulting in cell death. This hypothesis has been tested for a number of years on patients. (Some results are discussed later by Dr. Osinski).

Recently the biological effects of heat have been classified into cytotoxic and radiosensitizing. The thermosensitivity of the former is enhanced by nutritional deprivation such that these cells may be more thermolabile than the normal adjacent environment even if there is no temperature gradient. The cytotoxic effect also varies markedly amongst human cells and tissues although significant differences may not be noted between the malignant cells and their normal counterparts. Those cells in the S-phase of the cell cycle are more sensitive to heat. Although the cytotoxic effect may result in minimal decrease in tumor size much of this is due to the death of the S cells which are most resistant to radiation, and similar differences are also true for hypoxic cells which are more resistant to radiation; therefore, making a combination of radiation and heat most attractive. Cytotoxic effects of hyperthermia are independent of any temporal relationship with radiation and it may be dependent upon the rate of heating with temperature and time of exposure, cell line, and the preheating history of the cells. In contrast the latter type, radiosensitization, is dependent upon the temporal relationship of combined irradiation. Evidence that heat may impair the ability of the cell to repair sublethal radiation damage also makes the combination attractive. Heat alone produces immediate responses which are short-lived and because many of the above factors are also operative when heat is combined with chemotherapy tri-modality studies using radiation and/or chemotherapy, plus heat have been recommended.46-48

While the preceding section explains the fact that temperature gradients exist between tumors and their surrounding structures and that under their poor microenvironment they may exhibit increased thermosensitivity controversy exists as to the possible biochemical site of the primary interaction. Much data supports the hypothesis that damage to the cellular membrane results in increased permeability with the loss of normal osmo-regulatory function. It is known that the lipid composition of membranes relates to thermosensitivity and that cholesterol is an effective regulator of membrane permeability. Compounds such as polyamines49 and local anesthetic agents which alter the permeability of membranes also influence cellular thermosensitivity. The intratumoral injection of rat tumors with procaine50 has potentiated the effects of heat and by in vitro studies it has been demonstrated polyamines will inhibit the development of thermoresistance within cell lines and assist in their overcoming thermal tolerance. Overgaard51,52 has suggested an intracellular site with increased lysosomal enzyme activity with electron microscopy demonstrating an increased number of lysosomes within 24 hours of heating mammary carcinomas. He suggests that increased acidity produces further release of hydrolytic enzymes leading to cell death. Yet the DNA extracted from bacteria following heating is unable to repair the breakage of its normal structure suggesting that the primary site may be more central in the DNA. Precise localization of the primary injury which leads to cell death will assist during the next decade in the development of hyperthermia as a variable competitor for

cancer management particularly if agents can be identified which will selectively alter cellular membranes or intracellular structures to further increase the thermosensitivity differential between the tumor cells and normal cells.

## HEAT SHOCK PROTEINS

The human body has proven itself ingeniously adaptive at developing protective measures against attacks by many potentially harmful agents. Unfortunately, heat is not an exception. Subsequent to Crile's observation in 1968 that preheated tumors became less sensitive to further heating, many others have noted thermotolerance which is thought to be due to the body's release of heat shock proteins. Many[53-59] studying a diversity of biological systems for equally diversified purposes have found the ability of these biological systems to develop various degrees of resistance with increased temperatures. Development of such thermotolerance should be differentiated from the inherited ability to survive high temperatures, i.e., heat resistance. While much of this work is in tissue culture or animal models, Hahn[60] has shown with the use of ultra-sound in patients with more superficial tumors following a single heat treatment subsequent temperatures required to produce similar responses may be as much as 2oC greater. Interestingly, Gerner, et al,[61] has shown that polyamines have the ability to overcome such thermotolerance.

Numerous workers have demonstrated that the body in response to the challenge of heat releases specific proteins, "heat-shock proteins", for protection.[62-66] These proteins have been identified as having molecular weights between 68,000 and 70,000 whose functions are unclear. The development of such heat shock proteins may be inhibited by the use of chemotherapy.[67-70] (This phenomena is discussed in more detail subsequently by Dr. Geovanella).

Due to the fractionated pattern of radiation therapy, hyperthermia, when added, must be integrated with a fractionated schedule. There is considerable variation in the kinetics and the development of thermotolerance between different tissues and attempts to predict this tolerance between a given tumor or normal tissue is difficult. Apparently the degree of subsequent thermotolerance will vary directly with the maximum of the initial heat treatment.[71] To overcome this phenomena, most people recommend large time intervals between heat sessions at least 48 hours and many, once a week.[72]

## COMBINED MODALITY TREATMENT - HYPERTHERMIA AND RADIATION

There are three quite rational reasons as to why the

combination of these two agents would be beneficial:
1) Both agents are cell cycle specific - those in the S-phase are more sensitive to heat and most resistant to radiation.
2) Hypoxic cells, which comprise the major population of many tumors, are most sensitive to heat while quite resistant to radiation.

3) Heat has a radiosensitizing effect possibly due to its inhibition of the cell's ability to repair sublethal radiation trauma.

Observations by many investigators at many Centers throughout the world in past years showed an enhanced response of palliation when they combined heat with x-rays. Comparisons of the numerous clinical results reported about the world indicate that local and regional hyperthermia augments the effectiveness of tumor response over that of radiation therapy alone even though the studies vary markedly as to the type of hyperthermia, fractionation of radiation, sequence in timing of the agents, and types of cancers study. Selawry, et al,73 has estimated that tumor temperatures obtained in much of the early work was between 38-42oC and then for short intervals of time of 10-20 minutes which is inadequate by today's standards. Furthermore, randomized trials have not been performed because of the difficulty of thermometry and quality assurance. Due to the fact that protocols usually call for minimum temperatures of 43oC only more superficial cancers can be treated due to the limitations of present heating units. Unfortunately, one of the few randomized trials, RTOG Study, concluded there is not a significant difference with the addition of heat to radiation. Numerous critiques of this study have been done. Analysis of those lesions under three centimeters in diameter revealed significant benefit by the addition of heat. Again, in spite of the enormous amount of works comparing the combined use of heat and radiation, much controversy persists as to whether or not radiation should precede or follow the delivery of hyperthermia, the doses of both agents, and the frequency of thermotherapy. The latter is important because of the release of heat shock proteins and the opinion by many that development of thermotolerance which may last for several days to as many ten days.

To determine the effectiveness of such combination therapy, comparisons have been made of that dose of radiation alone necessary to produce the same response as a dose of radiation combined with heat. (TER). Overgaard using his own work and extensive literature review has calculated such TER'S for a number of cancers, many in the range of 1.5 and that of melanoma approximately 2.0. This enhancement gain without corresponding increased toxic effect upon the surrounding normal structures one should anticipate as we go into the 1990's, particularly for certain specified melanomas, heat will be mandatory to the adjuvant management of melanoma. (Dr. Shidnia discusses this in more detail in a subsequent chapter).

## CONCLUSIONS

In the opening paragraphs of this chapter, justification was given for some increasing pessimism and lack of funding over the past decade concerning the potential use of hyperthermia. In spite of the sound basis for this pessimism, there are early indications that during the next decade, opinion may again swing from its nadir to levels of optimism. Work presented later in this book would warrant such optimism.

There are increasing numbers of centers investigating heated perfusion of extremity malignancies with cytotoxics, showing significant benefits to an escalating number of patients. While this technique has limited applicability, it is proving that when heat can be delivered at adequate temperatures under controlled conditions, response rates can be improved. Although not as convincing, there is increasing evidence that some of the current units are heating sufficiently to improve results.

There is some promise that technology will develop sophistocated heating units which will heat sufficiently rapidly with well-defined beams that target volumes can be heated to effective temperatures without toxic temperatures to neighboring tissues, somewhat similar to the Gamma Knife or Radiation Surgery.

Of more potential is the possibility that the parallel studies of the biological effects of heat upon the immune system and the exponential increase of our knowledge concerning specifics of the complex immuno-compentence system will result in the marriage of these two modalities which together will be a very effective fourth modality in the 1990's. Current clinical trials may prove the impressions of our former colleagues that multiple bacterial toxins are effective, economical, and relatively non-"toxic" to normal tissues.

With the increasing convincing evidence to be gained by combining hyperthermia with chemotherapy, we can anticipate greater interest in this combined modality during the next decade, hopefully with meaningful statistics at the end of the decade. Also, for the same reasons, well-designed protocols for tri-modality therapies will become more widely employed.

In view of the proven potential benefit of heat and its relative lack of toxic effects, technology must be found for its clinical application. Possibly we can paraphrase Hippocrates, "If cancer cannot be cured by heat in the next decade, then it cannot be cured."

## REFERENCES

1. Overgaard, J., The Current and Potential Role of Hyperthermia in Radiotherapy. Int. J. Radiation Oncology Biol. Phys., Vol. 16, pp. 535-549.

2. Busch, W. 1866): Uber den Einfluss. Welchen heftigere erysipeln aug organisierte Neubildungen ausuben.

3. Coley, E.B., The Treatment of Malignant Tumors by Repeated Inoculations of Erysipelas: with a Report of Ten Original Cases. American Journal of Medical Science, 105: 487, 1893.

4. Strauss, A., et al: Immunological Resistance to Carcinoma Produced by Electrocoagulation. Surg. Gynecol. Obstet. 121: 989-996, 1965.

5. Westermark, H., 1927: The Effect of Heat upon Rat Tumors. Scandinavial Archives of Physiology, 52, 257-322.

6. Muller, C.: Eina Neue Behandlungs methode bosartiger Geschwelste. Munchen. Med. Wchnschr; 1910, 57, 1490-1493.

7. Warren, S.L.: Preliminary Study of Effects of Artificial Fever upon Hopeless Tumor Cases. Am. J. Roentgenol. Rad. Therapy, 1935, 33, 76-87.

8. Cavaliere, R., Moricca, G., Caputo, A.: Regional Hyperthermia by Perfusion. In: Proceedings of the First International Symposium on Cancer Therapy Hyperthermia and Radiation, eds. M.J. Wizenberg, Robinson, J.E., Chicago: American College of Radiology, 1976, pp. 251-265.

9. Stehlin, J.S., et al: Hyperthermic Perfusion of Extremities for Melanoma and Soft Tissue Sarcoma. Recent Results Cancer Res., 59:171-185, 1977.

10. Ghussen, F., Kruger, I., Groth, W., Stutzer, H.: The Role of Regional Hyperthermic Cytostatic Perfusion in the Treatment of Extremity of Melanoma. Cancer, Vol. 61, pp. 654-659, 1988.

11. Au, K.S., Cetas, T.C., Shimm, D.S., Sinno, J.S., Haider, S.A., Buechler, D.N., Lutz, W. R., Cassady, J.R.: Interstitial Ferromagnetic Hyperthermia and Brachytherapy: Preliminary Report of a Phase I Clinical Trial. Endocurietherapy/Hyperthermia Oncology 1989; 5:127-136.

12. Neyzari, Ali, Cheung, A. Y.: A Review of Brachyhyperthermia Approaches for The Treatment of Cancer. Endocurietherapy/Hyperthermia Oncology 1985; 1:257-264.

13. Oleson, J.R.; Sim, D.A.; Manning, M.R.: Analysis of Prognostic Variables in Hyperthermia Treatment of 161 Patients. Int. J. Radiat. Oncol. Biol. Phys. 10:2231-2239, 1984.

14. Goldacre, R.J., and Sylven, B.: A Rapid Method of Studying Tumour Blood Supply Using Systemic Dyes. Br. J. Cancer 16:306, 1962.

15. Jain, R.K.: Temperature Distributions in Normal and Neoplastic Tissues During Normothermia and Hyperthermia. In Thermal Characteristics of Tumors: Applications in Detections and Treatments Eds. R.K. Jain and P.M. Gullino. Ann. NY Acad. Sci. 335:48-66, 1980.

16. Thomlinson, R.H., and Gray, L.H.: The Histological Structure of Some Human Lung Cancers and the Possible Implications for Radiotherapy. Br. J. Cancer 9:539-549, 1955.

17. Song, C.W. et al. Effect of Hyperthermia on Vascular Function in Normal and Neoplastic Tissues In Vivo. Ann. N.Y. Acad. Sci. 335:35-47, 1980.

18. Jain, R.K.; Weissbrod, J.; and Wei, j.: Mass Transfer in Tumors: Characterization and Applications in Chemotherapy. Adv. Cancer Res. 33:251-311, 1980.

19. Natadze, T.G.: Regulation of Blood Circulation in Malignant Tumors. Vopr. Onkol. 5:14-23, 1959.

20. Sugaar, S., LeVeen, H.H.: A Histophatologic Study on the Effects of Radiofrequency Thermo Therapy on Malignant Tumors of the Lung. Cancer 43:767, 1979.

21. Storm, F.K. et al. Human Hyperthermic Therapy: Relationship Between Tumor Type and Capacity to Induce Hyperthermia by Radio Frequency. Am. J. Surg. 138:170-174, 1979b.

22. Lambert, R.A.: Demonstration of the Greater Susceptibility to Heat of Sarcoma Cells. JAMA 59:2147-2148, 1912.

23. Stevenson, H.N.: The Effect of Heat Upon Tumor Tissues. J. Cancer Res. 4:54, 1919.

24. Rohdenburg, G.L.: Fluctuations in the Growth of Malignant Tumors in Man, with Special Reference to Spontaneous Recession. J. Cancer Res. 3:193-225, 1918.

25. Crile, G., Jr.: Selective Destruction of Cancers after Exposure to Heat. Ann. Surg. 156:404-407, 1962.

26. Cavaliere, R. et al. Selective Heat Sensitivity of Cancer Cells - Biochemical and Clinical Studies. Cancer 20:1351-1381, 1967.

27. Muckle, D.S., and Dickson, J.A. The Selective Inhibitory Effect of Hyperthermia on the Metabolism and Growth of Malignant Cells. Br. J. Cancer 15:771-778, 1971.

28. Dickson, J.A.: The sensitivity of Human Cancer to Hyperthermia. In Proceedings of Conference on Clinical Prospects for Hypoxic Cell Sensitizers and Hyperthermia, Eds. W.L. Caldwell and R.E. Durand. Madison, Wisc.: University of Wisconsin Press, 1978, pp. 174-193.

29. Overgaard, K., and Overgaard, J.: Investigations on the Possibility of a Thermic Tumor Therapy-Shortwave Treatment of a Transplanted Isologous Mouse Mammary Carcinoma. Eur. J. Cancer 8:65-78, 1972.

30. Giovanella, B.C. et al.: Selective Lethal Effect of Supranormal Temperatures on Mouse Sarcoma Cells. Cancer Res. 33: 2568-2578, 1973.

31. Giovanella, B.C.; Lohman, W.A.; and Heidelberger, C.: Effects of Elevated Temperatures and Drugs on the Viability of L-1210 Leukemia Cells. Cancer Res. 30: 1623-1631, 1970.

32. Marmor, J.B.; Hahn, N.; and Hahn, G.M.: Tumor Cure and Cell Survival after Localized Radio Frequency Heating. Cancer Res. 37:879-883, 1977.

33. Crile, G., Jr.: The Effects of Heat and Radiation on Cancers Implanted into the Feet of Mice. Cancer Res. 23:372-380, 1963.

34. Stehlin, J.S. et al.: Results of Hyperthermic Perfusion for Melanoma of the Extremities. Surg. Gynecol. Obstet. 140:339-348, 1975.

35. Kim, S.H.; Kim, J.H.; and Hahn, E.W.: Enhanced Killing of Hypoxic Tumor Cells by Hyperthermia. Br. J. Radiol. 48:872-874, 1975.

36. LeVeen, H.H. et al.: Tumor Eradication by Radio Frequency Therapy. JAMA 235:2198-2200, 1976.

37. Storm, F.K. et al.: Normal Tissue and Solid Tumor Effects of Hyperthermia in Animal Models and Clinical Trials. Cancer Res. 39:2245-2251, 1979a.

38. Gerweck, L.E.: Influence of Microenvironmental Conditions on Sensitivity to Hyperthermia or Radiation for Cancer Therapy. In Proceedings of Conference on Clinical Prospects for Hypoxic Cell Sensitizers and Hyperthermia, Eds. W.L. Caldwell and R.E. Durand. Madison, Wisc: University of Wisconsin, 1978, pp. 113-126.

39. Dickson, J.A., and Calderwood, S.K.: Temperature Range and Selective Sensitivity of Tumors to Hyperthermia: A Critical Review. In Thermal Characteristics of Tumors: Applications in Detection and Treatment, Eds. R.K. Jain and P.M. Gullino. Ann. NY Acad. Sci. 335:180-207, 1980.

40. Overgaard, J., and Nielsen, O.S.: The Role of Tissue Environmental Factors on the Kinetics and Morphology of Tumor Cells Exposed to Hyperthermia. In Thermal Characteristics of Tumors: Applications in Detection and Treatment, Eds. R.K. Jain and P.M. Gullino. Ann. NY Acad. Sci 335:254-280,1980.

41. Freeman, M.L.; Dewey, W.C.; and Hopwood, L.E.: Effect of pH on Hyperthermic Cell Survival. J. Natl. Cancer Inst. 58:1837-1839, 1977.

42. Suit, H.D., and Gerweck, L.E.: Potential for Hyperthermia and Radiation Therapy. Cancer Res. 39:2290-2298, 1979.

43. Urano, M. et al.: Response of a Spontaneous Murine Tumor to Hyperthermia: Factors which Modify the Thermal Response In Vivo. Radiat. Res. 83:321-322, 1980.

44. Von Ardenne, M.: Selective Multiphase Cancer Therapy:

Conceptual Aspects and Experimental Basis. Adv. Pharmacol. 10:339-380, 1972.

45. Von Ardenne, M., and Kruger, W.: The use of Hyperthermia Within the Frame of Cancer Multistep Therapy. In Thermal Characteristics of Tumors: Applications in Detection and Treatment, Eds. R.K. Jain and P.M. Gullino. Ann. NY Acad. Sci. 335:356-361, 1980.

46. Herman, T.S., et al: Rationale for Use of Local Hyperthermia with Radiation Therapy and Selected Anticancer Drugs in Locally Advanced Human Malignancies. Int. J. Hyperthermia, 1988, Vol. 4, No. 2, 143-158.

47. Sugimachi, K.; Matsuda, H.; Ohno, S.; Fukuda, A.; Matsuoka, H.; Mori, M.; Kuwano, H.: Long-Term Effects of Hyperthermia Combined with Chemotherapy and Irradiation for the Treatment of Patients with Carcinoma of the Esophagus. Surg. Gynecol. Obstet., 1988, Oct. 167(4), pp. 319-323.

48. Matsufuji, H.; Kuwano, H.; Kai, H.; Matsuda, H.; Sugimachi, K.: Preoperative Hyperthermia Combined with Radiotherapy and Chemotherapy for Patients with Incompletely Resected Carcinoma of the Esophagus. Cancer, 1988, Sep 1, 62(5) pp. 889-894.

49. Gerner, E.W. et al.: Factors Regulating Membrane Permeability after Thermal Resistance. In Thermal Characteristics of Tumors: Applications in Detection and Treatment, Eds. R.K. Jain and P.M. Gullino. Ann. NY Acad. Sci. 335:254-280, 1980.

50. Yatvin, M.B.: The Influence of Membrane Lipid Composition and Procaine on Hyperthermic Death of Cells. Int. J. Radiat. Biol. 32:513-521, 1977.

51. Overgaard, J.: Ultrastructure of a Murine Mammary Carcinoma Exposed to Hyperthermia In Vivo. Cancer Res. 36:983-995, 1976a.

52. Overgaard, K., and Overgaard, J.: Investigations on the Possibility of a Thermic Tumor Therapy. I. Short-wave Treatment of a Transplanted Isologous Mouse Mammary Carcinoma. Eur. J. Cancer 8:65-78, 1972.

53. Gerner, E.W., and Schneider, M.J.: Induced Thermal Resistance in HeLa Cells. Nature 256:500-502, 1975.

54. Gerweck, L.E.: Modification of Cell Lethality at Elevated Temperatures: the pH effect. Radiat. Res. 70:224-235, 1977.

55. Dewey, W.C.; Sapareto, S.A.; and Betten, D.A.: Hyperthermic Radiosensitization of Synchronous Chinese Hamster cells: Relation Between Lethality and Chromosomal Aberrations. Radiat. Res. 76:48-59, 1978.

56. Henle, K.J.; Bitner, A.F.; and Dethlefsen, L.A.: Induction of Thermotolerance by Multiple Heat Fractions in Chinese Hamster Ovary Cells. Cancer Res. 39:2772-2778, 1979.

57. Bede Z, Diven, Z., Xiaoxiong, W. (Abstr)(1980) Clinical Effects of Radiation Combined with Radiofrequency Diathermy in Bladder Cancer. Third International Symposium of Cancer Therapy by Hyperthermia. Drugs and Radiation. Fort Collins, Colorado, June 22-26.

58. Busch, W. (1866): Uber den EinfluB, Welchen Heftigere Erysipeln auf Organisierte Neubildungen Ausuben. Verhandl Naturh Preuss Rhein Westphal 23:28-30.

59. Edrich J., Liep, H.D. (1986): Microwave Thermography for Control of Microwave Induced Hyperthermia: Potentials, Limitations and Preliminary Results. In: Streffer, C., Herbst, M., Schwabe, H. (Eds) Lokale Hyperthermie. Deutscher Arzteverlag, Cologne, pp 121-125.

60. Hahn, G.M. Radiofrequency, Microwaves, and Ultrasound in the Treatment of Cancer: Some Heat Transfer Problems. Paper Read at the Conference on Thermal Characteristics of Tumors: Applications in Detection and Treatment, March 1979, New York, N.Y.

61. Gerner, E.W. et al. Factors Regulating Membrane Permeability Alter Thermal Resistance. Ann. N.Y. Acad. Sci. 335:215-233, 1980a.

62. Leeper, D.B., Karamuz, J.E., Henle, K.J. (1977). Hyperthermia Induced Alterations in Macromolecular Synthesis. Radiat. Res. 70:610.

63. Leeper, D.B., Karamuz, J.E., Henle, K.J. (1977). Effect of Inhibition of Macromolecular Synthesis on the Induction of Thermotolerance. Proc Am Assoc Cancer Res 18:139.

64. Li, G.C., Petersen, N.S., Mitchell, K.H. (1982). Induced Thermal Tolerance and Heat Shock Protein Synthesis in Chinese Hamster Ovary Cells. Br J. Cancer (SupplV) 45:132-136.

65. Subjeck, J.R., Shyy, T., Shen J. Johnson RJ (1983) Association Between the Mammalian 110,000-Dalton Heat-Shock Protein and Nucleoli. J. Cell Biol 97:1389-1395.

66. Tsukeda H., Maekawa H., Izumi, S., Nitta, K. (1981). Effect of Heat Shock on Protein Synthesis by Normal and Malignant Human Lunt Cells in Tissue Culture. Cancer Res 41:5188-5192.

67. Kamura, T., Nielsen, O.S., Overgaard, J., and Andersen, A.H. (1982): Development of Thermotolerance During Fractionated Hyperthermia in a Solid Tumor In Vivo. Cancer Res 41:1744-1748.

68. Nielsen, O.S., Overgaard, J. (1982): Importance of Preheating Temperature and Time for the Induction of Thermotolerance in a Solid Tumour In Vivo. Br J Cancer 46:894-903.

69. Nielsen, O.S. (1984): fractionated Hyperthermia and Thermotolerance. Danish Medical Bulletin 31:376-390.

70. Overgaard, J., Nielsen, O.S. (1983): The Importance of Thermotolerance for the Clinical Treatment with Hyperthermia. Radiotherapy and Oncology 1:167-178.

71. Nielsen, O.S., Overgaard, J., Kamura, T. (1983): Influence of Thermotolerance on the Interaction Between Hyperthermia and Radiation in a Solid Tumour In Vivo. Br. J. Radiol, 56, 267-273.

72. Leopold, K.A., Harrelson, J., Prosnitz, L., Samulski, T.V., Dewhirst, M.W., Oleson, J.R.: Preoperative Hyperthermia and Radiation for Soft Tissue Sarcomas: Advantage of Two vs. One Hyperthermia Treatments Per Week. Int. J. Radiation Oncology Biol. Phys. Vol. 16, pp. 107-115.

73. Selawry, O.S., Carlson, J.C., Moore, G.E.: Tumor Response to Ionizing Rays and Elevated Temperatures. Am. J. Roentgenol, Rad. Therapy and Nuclear Med., 1958, 80, 833-839.

# LOCAL TUMOR HYPERTHERMIA IN THE 1990s

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There is compelling evidence that heat alone is tumoricidal and enhances efficacy of radiation therapy and cytotoxicity of many drugs. However, it is almost certain that during the next decade, heat will be used primarily as an adjuvant to surgery, radiation and chemotherapy rather than alone as the first line of treatment. It is also known that the threshold of thermal damage for normal tissues is not significantly different from that for most tumors, and there is increasing evidence that in normal tissues as in tumors the damage threshold is lower for heat plus drugs and/or radiation than for heat, drugs or radiation alone. For example, in biopsies obtained from the normal liver tissue overlying a tumor in a patient with localized, metastatic liver carcinoma, it was found that whereas heat at 42°C for 20 min., before initiation of chemotherapy with 5-Fluorouracil, caused only dilatation and engorgement of capillaries, when the liver was heated in presence of the drug extensive hemorrhages in the parenchyma were observed. Similar toxicity was observed in other tissues such as the kidney. However, it is now generally recognized that to achieve tumor control it is the proliferative and infiltrative tumor margin which must be heated to therapeutic temperature. Therefore to keep normal tissue toxicity at an acceptable level, tumor bed tissues and any critical normal tissues must remain at a lower temperature. Thus, it is important that heating devices must heat most of the tumor, specially its edge, and least of the normal tissue.

## DEVICE EVALUATION STUDIES

With these criteria for optimal heating in mind a comparative clinical evaluation of some of currently used heating devices was undertaken at the Harvard-MIT Hyperthermia Center, Massachusetts Institute of Technology, Cambridge, Mass., U.S.A. Dr. Joseph F. Simeone, Interventional Radiologist from the Massachusetts General Hospital, Boston, Mass., placed thermometric probes in the treatment volume under CT or Real Time Ultrasound guidance as described below, as well as obtained biopsies. Dr. Phillip Stork, Pathologist from Brigham & Women's Hospital, Boston, Mass., examined the biopsy and autopsy materials. Approximately 60% of the 133 patients entered into this study were direct referrals, the rest were referrals from Departments of Radiation Medicine and Medical Oncology of the Massachusetts General Hospital; the Dana Farber Cancer Institute and the Joint Center for Radiation Therapy; and Boston University Medical Center. Of the 133 patients studied only 88 patients in whom 2 or more heating devices

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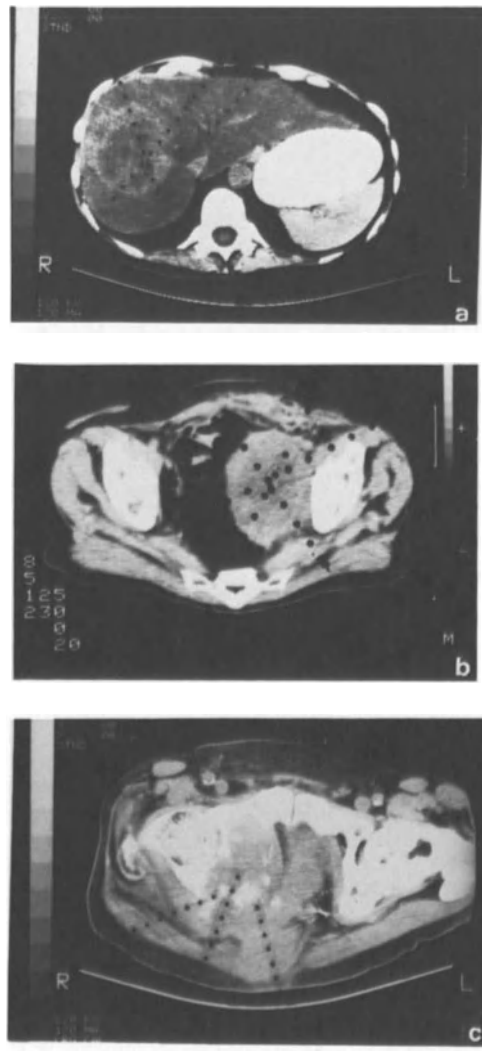


Fig. 1 Examples of thermometry in deep tumors. a.: Liver b.: Urinary Bladder c.: Pelvic Chondrosarcoma

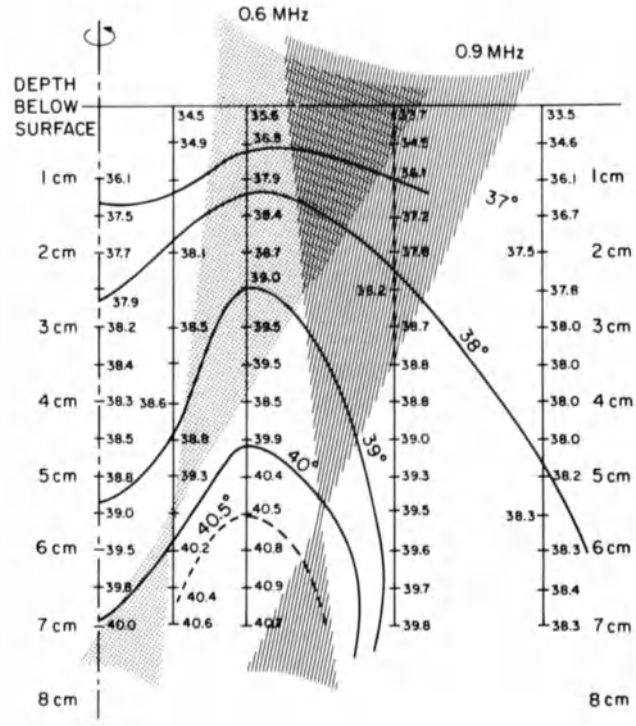


Fig. 2 Half-plot of symmetric temperature distribution in the liver of a patient with diffuse carcinoma due to two concurrent SIMFU beams (0.6 MHz and 0.9 MHz). The insonation beams are shown as simple triangles, omitting the half power focal zone width and length for clarity. Circular scan patterns of 2.0 cm and 6.0 cm diameter, at focal plane 5.5 cm below skin surface. Isotherms at 1° C intervals interpolated from 5 sets of thermometric data at different relative locations of pattern with respect to vertical catheter. Isotherm of 40.5° C is shown as a broken line; highest temperature measured was 40.9° C at depth of 6.5 cm, 2.0 cm from pattern centerline. Note that lower than optimal frequencies resulted in high temperature rise below focal plane.

were compared are included in the analyses in this paper. In the initial screening studies, as extensive thermometry as permissible was conducted. Generally temperatures were measured at 12 to 75 or more points during each session. Examples of the points at which temperatures were measured along the tracks of insertion of thermometric probes are shown in Fig. 1a,b, and c. In the initial device screening studies the temperature at any of the points monitored was not allowed to exceed a subtherapeutic temperature of 41.5°C in order to ensure safety to normal tissues and to preclude any heat induced changes in between trials with different devices. Most comparisons were made on the same day with the same thermometry using non-perturbing probes specific to the heating modality. Isotherms were plotted from steady state temperatures stored in the computer. Fig. 2 shows the heating beams superimposed on the isotherms in a case of a cholangiocarcinoma of the liver. Temperatures were measured at

65 to 70 points. Other examples are presented elsewhere (1). In previous animal studies *in vivo*, where more extensive thermometry at 600 to 800 locations was performed it was found that all the tissue within an isotherm line was heated uniformly to that temperature (2).

Table 1 presents the data on 309 device evaluations performed in 88 patients. Three to four devices were evaluated in each patient. Note that many of the superficial tumors were overlying bone. Details on the SIMFU and its operation are published in references 3-11. In this study, generally, 2 or 3 insonation heads, operating at different ultrasonic frequencies were used for heating deep and/or large tumors. The method of use of other devices was similar to that detailed in reference 12.

The detailed data from these studies are being published elsewhere (Lele, Goddard & Blanter, in press). The performance of different devices for effective and safe heating as defined by ability to heat tumor, specially its edges, to 41.5° C, without higher temperatures outside tumor volume, is summarized in Tables 2 to 5.

In shallow tumors the performance of the devices depended on the volume of tumor (Table 2). All of the devices tested could heat small volumes satisfactorily. The performance of the EM Horns was considerably lower for tumors of a moderate volume. The low score was due to inability to localize heating to tumor, that is, inability to spare normal tissues. With SIMFU the failures were due to pricking sensation on skin. No complaints of pain attributable to bone-heating were noted. None of the devices could heat large tumors effectively and safely. Magnatherm and SIMFU were not used as they could not cover large areas.

The limitations and toxicity encountered in heating shallow tumors by these devices are shown in Table 3. It should be pointed out that no narcotics were administered to the patient for these studies unless they were receiving them regularly for control of pain from their disease.

Table 1. Device Evaluations

| | 99 Shallow <3 cm | 210 Deep >3 cm |
|---|---|---|
| | EM Horns, Magnatherm SIMFU | EM Horns, AA Magnetrode, SIMFU |
| Head & Neck | 8 | 57 |
| Thorax | 59 | 43 |
| Abdomen | 30 | 54 |
| Pelvis | 0 | 45 |
| Extremities | 2 | 11 |

Thermotron was not available for evaluation.

EM Horn = Electromagnetic Radiation Antenna; Magnatherm = EM Pancake coil; SIMFU = Scanned Intensity Modulated Focused Ultrasound; AA = RF Annular Array; Magnetrode = Single Turn Coil

Dose-limiting pain and systemic stress, during the heating trials were the major factor which led to lack of success in heating the tumor to 41.5° C, mostly in tumors 50 to 500 ml in volume. When the tumor was "successfully" heated, the excessive normal tissue temperature represented the "biological cost" or risk. A temperature exceeding 41.5° C in deep tissue was defined as "excessive" in this study.

Results on heating performance, limitations and toxicity in deep tumors are summarized in Tables 4 and 5. Only SIMFU could effectively and safely heat tumors up to 500 ml in volume and up to a depth of 14 cm; tumors larger than 500 ml were heated in consecutive sessions. EM Horns and Magnetrode were effective in fewer instances. The maximum depth of heating with these was about 7 cm. The low performance of AA is attributed to systemic stress and excessive normal tissue temperature as presented in Table 5.

## THE FUTURE OF SIMFU

The MIT SIMFU thus does well for both deep and superficial tumors and is being adopted by a number of institutions and manufacturers. However, the current equipment is complex and mechanically scanned, requires highly trained operators for therapy planning, and safe and effective use of the SIMFU. Long set up time and tedious data acquisition and display reduce the patient throughput rate. The equipment in the future is expected to be electronically scanned, and will have semi-real time, 3-D temperature

Table 2. Effective & Safe Heating Performance
To 41.5° C

| | % of Shallow Tumors | | |
|---|---|---|---|
| | ml. <50 | 50 - 500 | >500 |
| EM Horns | 45 | 24 | 0 |
| Magnatherm | 71 | 66 | |
| SIMFU | 81 | 79 | |

Table 3. Limitations & Toxicity (%)
Note: Multiple Toxicities in Same Patient

| | Shallow Tumors | | |
|---|---|---|---|
| | E.M. Horns | Magnatherm | SIMFU |
| Dose-limiting | | | |
| Pain (Skin, Bone) | 33 | 21 | 10 |
| Systemic Stress | 9 | 0 | 0 |
| Excessive Normal Tissue Temp. | | | |
| Superficial (Burns) | 20 | 9 | 0.7 |
| Deep | 17 | 3 | 2 |
| No Toxicity or Limitation | 53 | 76 | 86 |

and heat dose display. "Expert Systems" capable of learning and decision making will reduce operator training requirements. Simplified direct coupling will reduce set up time. SIMFU yields remarkably uniform temperature distributions right to the edge of the treatment volume, if the conditions of its use are optimized utilizing the flexibility and versatility of the present MIT research system. Reverification of heating performance will be essential when the system is re-engineered and simplified for routine clinical use.

## THE FUNDAMENTAL PROBLEM IN LOCAL HYPERTHERMIA

The current thermometry involves multi-point temperature measurement throughout the treatment area, at each session. It is a highly invasive, high risk, time consuming and costly procedure. Sophisticated imaging equipment and services of an interventional radiologist are required. The need for its repetition at each session is unacceptable to most patients. Whether, in the foreseeable future, noninvasive thermometry with required sensitivity and spatial resolution will be practicable or will remain a dream is, at the best uncertain. Therefore, great emphasis is laid on thermal modeling.

Table 4. Effective & Safe Heating Performance

| | % of Deep Tumors | | | |
|---|---|---|---|---|
| | ml. | <50 | 50-500 | >500 |
| EM Horns | | 8 | 13 | 18 |
| AA | | 0 | 1 | 1 |
| Magnetrode | | 0 | 13 | 22 |
| SIMFU | | 63 | 75 | 73 |

Table 5. Limitations & Toxicity (%)
Note: Multiple Toxicities in Same Patient

| | Deep Tumors | | | |
|---|---|---|---|---|
| | EM Horns >4 cm. Depth | AA Depth | Magnetrode >6 cm. Depth | SIMFU to 15 cm. Depth |
| Dose-limiting | | | | |
| Pain (Skin, Bone) | 41 | 70 | 23 | 10 |
| Systemic Stress | 12 | 95 | 40 | 0 |
| Excessive Normal Tissue Temp. | | | | |
| Superficial (Burns) | 22 | 25 | 5 | 0.7 |
| Deep | 21 | 86 | 30 | 2 |
| No Toxicity or Limitation | 45 | 5 | 45 | 86 |

However, the use of thermal modeling for prediction of tissue temperatures during hyperthermia is totally dependent on quantification of the local tissue blood perfusion. But blood perfusion varies from tissue to tissue, place to place and differently at different temperatures and therefore needs to be measured at numerous points, throughout the treatment at each session. The procedure will be as invasive as current direct temperature measurement, which it is hoped to replace.

## A POTENTIAL SOLUTION : RAPID HYPERTHERMIA

The most attractive solution to these problems is "Rapid" hyperthermia. The concept of Rapid Hyperthermia is that a heat dose biologically equivalent to that in current practice is delivered in a time span too short for significant heat transport. Fig. 3 shows the Iso-Dose Plot for heat induced histological damage to mammalian tissue *in vivo*. Note that a dose equivalent to 30 min. at 42.5° C can be delivered in a couple of seconds (time too short for significant heat transport) at about 57° C.

Fig. 4 shows the temperature recorded at depth in brain of an experimental animal *in vivo*, with the SIMFU in stationary mode. But note that the focal volume is small. In "stepping, mode" the SIMFU can produce Rapid Hyperthermia in the entire tumor under computer control by following the 3-D Tumor Outlines (Fig. 5a) or even ablate the tumor - non-invasively at higher heat-doses. It has been coupled to a "diagnostic system" (Fig. 5b) to keep track of any blood vessels, bone, etc. and to avoid them. Animal studies have established its safety, and protocols for a Phase I study are currently under development. The feasibility of Rapid Hyperthermia by using SIMFU in a scanning mode has recently been determined in a theoretical study (13).

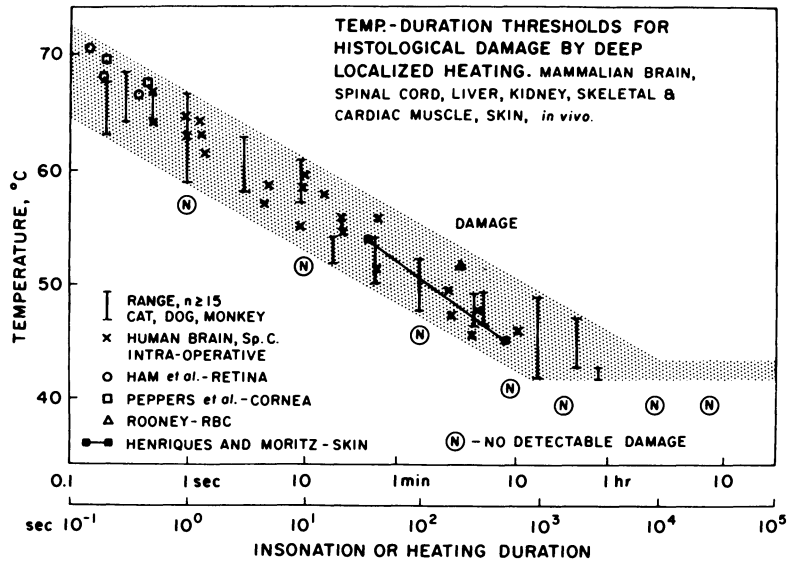


Fig. 3 Iso-dose plot for heat induced, histologically detectable injury to 'solid' mammalian tissues *in vivo*

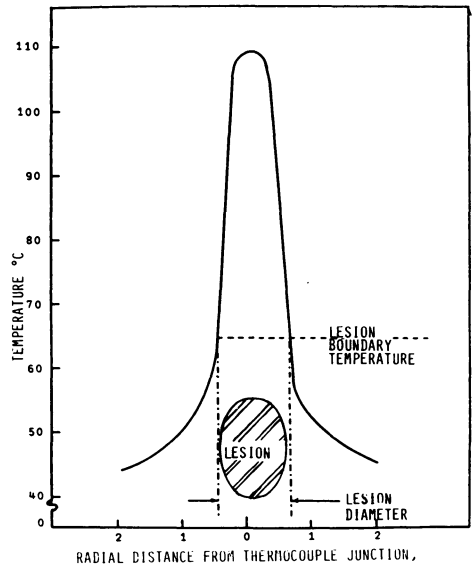


Fig. 4 Profile of instantaneous temperature elevation produced at depth in tissue within the focal region of a focused ultrasonic beam. All tissue enclosed with the volume labelled "lesion" will be subjected to a therapeutic hyperthermic dose.

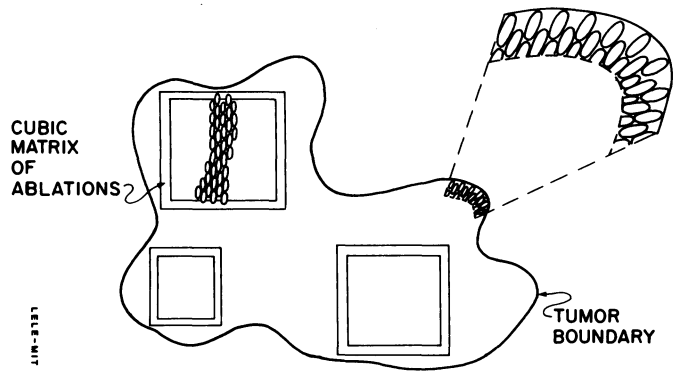


Fig. 5a Use of SIMFU in "stepping" mode for Rapid Hyperthermia of sequential volumes of tissue

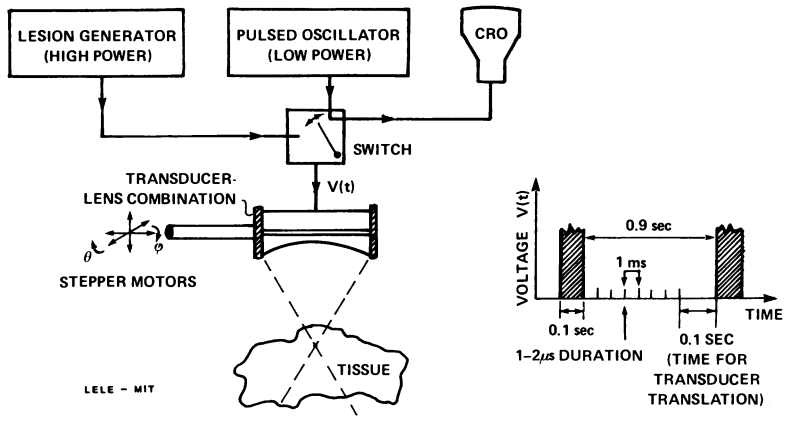


Fig. 5b Schematic diagram of the combined "Diagnostic-Therapeutic" SIMFU system

In summary, EM techniques will continue to be used for some time because of their ubiquity and simplicity. However, since they can produce only regional and not local hyperthermia (6), they will be gradually replaced by SIMFU-like approaches for production of localized hyperthermia, specially in deep tumors. Local tumor hyperthermia later in the 1990's is likely to be based on:

1. Rapid Hyperthermia
   - minimizing thermometry requirements

2. Simplified SIMFU based equipment and treatment procedure to increase throughput rate

3. Use of expert systems to reduce personnel requirements

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REFERENCES

1. Lele, P.P., "Advanced Ultrasonic Techniques for Local Tumor Hyperthermia", Radiologic Clinics of North America 27 (3): 559-575 (1989).
2. Lele, P.P. and Parker, K.J., "Temperature Distributions in Tissues during Local Hyperthermia by Stationary or Steered Beams of Unfocused or Focused Ultrasound", British J. Cancer 45 (Suppl. V): 108-121 (1982).
3. Lele, P.P., "Local Hyperthermia by Ultrasound" in "Physical Aspects of Hyperthermia", G.H. Nussbaum (Ed.), American Association of Physicists in Medicine, American Institute of Physics, Inc., New York, NY, pp. 393-440 (1982).

4. Lele, P.P., "Physical Aspects and Clinical Studies with Ultrasonic Hyperthermia" in "Hyperthermia in Cancer Therapy", Chapter 16, F.K. Storm (Ed.), G.K. Hall and Co., Publisher, pp. 333-367 (1983).
5. Lele, P.P. et al, "Scanned Focused Ultrasound System for Hyperthermia of Deep Tumors" in "Hyperthermic Oncology, 1984", Jens Overgaard (Ed.), Taylor & Francis, London, Publishers, Vol. 1: 625-628 (1984).
6. Lele, P.P., "Ultrasound: Is It the Modality of Choice for Controlled, Localized Heating of Deep Tumors?" in "Hyperthermia Oncology, 1984", Jens Overgaard (Ed.), Taylor & Francis, London, Publishers, Vol. 2: 129-154 (1985).
7. Lele, P.P., "Rationale, Technique and Clinical Results with Scanned, Focused Ultrasound (SIMFU) System", Proc. IEEE Eighth Annual Conf. of the Engineering in Medicine and Biology Society, pp. 1435-1440 (1986).
8. Lele, P.P. and Goddard, J., "Optimizing Insonation Parameters in Therapy Planning for Deep Heating by SIMFU", Proc. of the IEEE Ninth Annual Conf. of the Engineering in Medicine and Biology Society, CH2513-0: 1650-1651 (1987).
9. Lele, P.P., Goddard, J. and Blanter, M., "Clinical Results with Intensity Modulated, Scanned, Focused Ultrasound (SIMFU) System" in "Syllabus: A Categorical Course in Radiation Therapy: Hyperthermia", R.A. Steeves and B.R. Paliwal (Eds.), Radiological Soc. of North America, Oak Brook, ILL, pp. 159-170 (1987).
10. Lele, P.P., "Ultrasound Hyperthermia" in "Encyclopedia of Medical Devices and Instrumentation", J.G. Webster (Ed.), John Wiley and Sons, NY, 3: 1599-1612 (1988).
11. Lele, P.P., "The MIT Scanned, Intensity-Modulated Focused Ultrasound (SIMFU) System", in "Proc. of the 5th Int. Symp. on Hyperthermic Oncology", August 29 - September 3, 1988, Kyoto, Japan (In Press).
12. International J. Hyperthermia, Vol. 4: 1-132.
13. Davis, B.J. and Lele, P.P., "A Theoretical Study of Rapid Hyperthermia by Scanned Focused Ultrasound", American Society of Mechanical Engineers Winter Annual Meeting, San Francisco, 1989, (In Press).

# CONSENSUS OF HYPERTHERMIA FOR THE 1990s

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Even though it is difficult to determine the first time heat or a hot instrument was used for treatment of malignant tumors, by the year 2000 B.C., the use of cauterization for treatment of tumors was one of the methods of therapy. Hippocrates also was aware of the effect of fever in treatment of infectious disease, as well as malignant tumors, but it was not until the second half of the 1800s that we have documented evidence of resolution of sarcomas by hyperthermia. In 1866, Bush reported several papers in medical literature claiming the relationship on disappearance of malignant tumors. This, followed by Coley's innovative work in 1893, published in The American Journal of Medical Science, showed impressive results of fever on malignant tumors.

Due to lack of interest and the difficulty in achieving the high temperatures, this work got lost in piles of medical literature. Then, in 1971, Coley's work was published by Miller and Nicholson and showed clearly that there was an advantage in using fever in the treatment of malignant tumors, about two to three degrees above the baseline temperature. Since then, many centers have tried to use this modality as a radiation or chemotherapy sensitizer, or even an immune system stimulant, with some success.

Different methods of heat production, such as hot water, hot wax, radio frequencies, microwave, radiant heat systems, hot air, ultrasound, extra corporeal hyperthermia, and electrical currents, have been used. Each method has its own deficiency and advantage. The problem of the nonthermal effect of the microwave has not been solved yet, and due to lack of uniform methods of heat delivery, reproduction of treatment methods and comparisons of methods of treatment are rather difficult. Non-uniformity of heat distribution, efficiency of blood circulation taking heat away from the tumor, and poor depth of penetration of the heat makes any clinical study very difficult to obtain statistically sound conclusions.

In the past 10 to 15 years, there have been many new works done in the field of hyperthermia; many improvements in instrumentation of thermometry; as well as new problems of which we were not aware. There have been some clinical studies, all of which probably should be repeated. There is some new interest in the use of hyperthermia in benign conditions.

I believe in the 1990s, we have to look into many aspects of hyperthermia in the following categories:

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1. Instrumentation

   a. We need equipment that will give uniform heat to any desired depth, similar to ionizing radiation. This could be achieved by use of ultrasound with different frequencies, either from a fixed source or a moveable source.

   b. Radio frequency or microwave by use of multiple applicators or moveable applicators.

   c. Combining regional or whole body hyperthermia with local hyperthermia for more uniform heat to the area.

2. Thermometry

   a. We need a non-invasive way of documenting the temperature across the field and the depth of the field. This probably can be achieved by MRI or radio frequency, or tomo-graphic system. However, better instrumentation and uniform heat would make the problem of thermometry easier. Without documented temperature or energy absorbed in each area, no logical conclusion can be obtained.

   The problem of time/temperature unit should be solved by way of animal studies or laboratory studies to overcome this obstacle.

3. In laboratory fields, the value of heat shocked protein and thermal tolerance in clinical setups should be tested. Whether or not heat shocked protein can be used as a guide for treatment or not depends on our work in this field.

   The subject of the non-thermal affect of non-ionizing radiation should be addressed and this problem solved.

4. In the field of immunology, much work should be done to find out the effect of different temperatures on the immune system. This method should be utilized for future treatment.

5. Interstitial hyperthermia probably has seen the most progress. Today, we can say that with the help of computers, we can deliver heat to almost any point. However, utilization of this form of treatment, because of invasiveness, is rather limited.

6. In the clinic, we have to prove the number of treatments necessary to control the tumor per week and in total. The same is true for ionizing radiation. There are many papers contradicting each other, and this problem should be addressed. Conductive interstitial hyperthermia is very promising and exciting.

   Predictive assays will help us to determine the effect of treatment during the course of therapy. The use of radiation therapy and hyperthermia, chemotherapy and hyperthermia, radiation therapy, chemotherapy and hyperthermia should be started with a uniform protocol, documented tumor size, localization, temperature in different areas, and timed. With timed temperature to each point, we should be able to make some conclusions. The use of extracorporeal hyperthermia should be more uniform and practiced.

7. There is a new interest in the field of non-oncological use of hyperthermia, such as in benign hypertrophic prostate and skin lesions such as psoriasis, that should be carefully approached.

In summary, in the 1990s, I feel there are lots of exciting projects that probably each Center and Discipline should take part in. I see that we will use more and more hyperthermia in conjunction with radiation and chemotherapy.

# MICRONUCLEI ASSAY - A PREDICTIVE VARIABLE FOR TUMOR RESPONSE TO TREATMENT

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## ABSTRACT

Our preliminary data indicate that the formation of micronuclei (MN) in treated tumor cells is a predictive variable for tumor response to treatment. In a pilot study involving four patients who received both radiation therapy and hyperthermia, fine needle aspirate (FNA) samples were taken and analyzed before therapy, and after each 1000 centigray (cGy) up to 3000 cGy. The results indicate a correlation between increasing formation of micronuclei and decreasing tumor volume. All of the patients in this Study have had their tumors under control for at least one year.

Our preliminary data demonstrated that a high level of micronuclei in tumor cells correlates with favorable response of the tumor to treatment with radiation and heat. The assay is easy to perform and FNA biopsy could be done in the clinic with minimal discomfort to the patient.

## INTRODUCTION

Radiation therapy, as practiced today, often controls many malignant tumors. Those tumors, which remain refractory to standard treatment, might respond if adequate dosages of radiation were administered.

Perhaps one of the most significant challenges in the field of radiation therapy today pertains to the identification of variables which can be utilized to predict treatment response in tumors.[1] It would be extremely helpful if it were possible to predict tumor responsiveness, either before initiation, or during the course, of radiation therapy. One promising predictive variable is the frequency of micronuclei formation in treated tumor cells.

The technique of FNA may possibly develop into one of the most useful methods of collecting tumor cells for many types of assays or analyses. This technique allows the researcher to draw cell samples from practically any area of the tumor. Thus, a pool of several FNA biopsies from the same tumor would likely provide a better representation of the whole tumor than a single surgical biopsy. In addition, the FNA technique will also allow us to study cell survival *in vivo* as a function of time.[2] Establishment of MN formation as a variable for prediction of radiation response would be a major breakthrough in cancer treatment, especially when the assay can pro-

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vide results within a few hours, as compared to two weeks for the tumor stem cell cloning assay.

## MATERIALS AND METHOD

In a pilot study involving four patients who received both radiation and hyperthermia, FNA samples were taken for flow cytometric analysis before therapy was initiated and after each 1000 cGy, up to 3000 cGy. The techniques of FNA and MN assay have been described previously.[3,4]

### Patient No. 1

(E.L.) - A 67-year old female with diagnosis of malignant fibrous histiocytoma, previously treated to 6000 cGy. After six months, the tumor recurred and at that time measured 3 x 4 x 10 cm. Examination of FNA biopsy obtained prior to therapy confirmed the histological diagnosis. Radiation therapy was started with 200 cGy at 32 MeV electrons daily, five times a week, concomitantly with hyperthermia at 433 MHz for one hour twice a week. FNA was repeated 24 hours after each 1000 cGy.

### Patient No. 2

(J.P.) - A 73-year old male with proven diagnosis of recurrent squamous cell carcinoma of the head and neck. He had two separate areas of involvement: 4 x 4 x 4 cm and 3 x 3 x 3 cm. Examination of FNA prior to therapy confirmed the histological diagnosis, and the patient started with 200 cGy at 13 MeV electron beams daily, five times a week. FNA was repeated 24 hours after each 1000 cGy. The patient was also treated with hyperthermia twice a week for one hour at 915 MHz.

### Patient No. 3

(M.C.) - A 57-year old female with history of recurrent squamous cell carcinoma of head and neck. The tumors were multiple and measured 2 cm in diameter each. FNA prior to treatment confirmed the diagnosis and the MN count was high (80%). The patient started with 200 cGy at 10 MeV electron beams daily, five times a week. FNA was repeated 24 hours after each 1000 cGy. The patient also was treated with hyperthermia at 915 MHz twice a week for one hour.

### Patient No. 4

(D.M.) - A 58-year old male with diagnosis of malignant fibrous histiocytoma. The tumor measured 11 x 9 x 6 cm. FNA prior to treatment proved the diagnosis, and the patient started treatment at 19 MeV electron beams with 200 cGy daily, five times per week. FNA was repeated 24 hours after each 1000 cGy. The patient also was treated with hyperthermia at 453 MHz for one hour twice a week.

## RESULTS

The results are shown in Figures 1A and 1B. Patient No. 1 showed correlation of increasing numbers of micronuclei with decreasing tumor volume. Patient No. 2 showed an initial decrease in the number of micronuclei followed by an increase which correlated with a corresponding decrease in tumor volume. By the end of the therapy, no cells could be drawn from the tissue of this patient. On pathological examination, the tissue showed necrosis. In Patient No. 3, it was interesting to note that the number of micronuclei was highest (about 80%) a week prior to radiation therapy. The reason for this initial high frequency is not known. During the treatment, the number of micronuclei decreased with a corresponding decrease in the tumor volume.

By the end of the therapy, the tumor had completely disappeared. This suggests that an increase in micronuclei prior to radiation therapy may also predict a good response.[5] In Patient No. 4, the number of micronuclei rose during the treatment with a corresponding decrease in the tumor volume. By the end of therapy, no tumor was evident, and pathological examination of the tissue showed only necrosis. At one year follow-up, all patients are alive with no evidence of disease.

## DISCUSSION

The formation of micronuclei, as discussed by Midlander and Brock, appears to be the result of enhancement in radiation-induced DNA double-strand break formation (DSBs).[1,5-7] Accentric chromosome fragmentation seems to be respresentative of radiation damage to the chromosomes.[2,8,9]

Brock and Williams studied the survival of Chinese hamster cells after irradiation with the use of both the standard colony forming assay and the micronuclei assay. They observed that the survival curves for the treated cells obtained by both methods were comparable. In addition, the micronuclei assay could provide quick feedback on tumor response to radiation, which in turn, would help the physician determine if adjustments or changes should be made in the course of therapy.

We have shown that formation of high levels of MN in tumor cells following radiation and heat correlates with favorable response of the tumor to treatment. The measurement of MN in FNA samples will become a reliable way to predict patient's response to irradiation and hyperthermia.

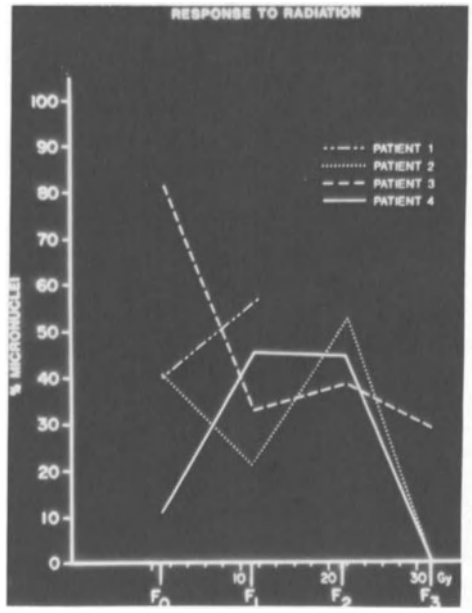


FIGURE 1A

$F_0$ = FNA prior to treatment

$F_1$ = FNA after 1000 cGy

$F_2$ = FNA after 2000 cGy

$F_3$ = FNA after 3000 cGy

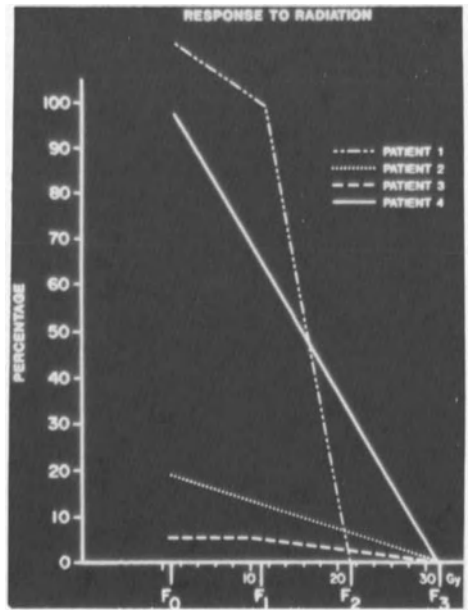


FIGURE 1B

$F_0$ = FNA prior to treatment

$F_1$ = FNA after 1000 cGy

$F_2$ = FNA after 2000 cGy

$F_3$ = FNA after 3000 cGy

REFERENCES


1. W.A. Brock, M. Williams, Kinetics of micronucleus expression in synchronized irradiated Chinese hamster ovary cells, Cell Tissue Kinet 18:247-254 (1985).
2. I. Diehl-Marshall, M. Bianchi, Induction of micronuclei by irradiation with neutrons produced from 600 MeV protons, Br J Radiol 54:530-532 (1981).
3. W.N. Crabtree, R-N. Shen, C. Stine, H. Shidnia, P. Young, M. Hartson, The efficacy of flow cytometric analysis of micronuclei to predict murine tumor cell response to x-ray irradiation and hyperthermia. Presented at the International Hyperthermic Society, Kyoto, Japan, Proceedings in press. (1988).
4. M. Nusse, J. Kramer, Flow cytometric analysis of micronuclei found in cells after irradiation, Cytometry 5:20-25 (1984).
5. W.A. Brock, M.H. Maor, L.J. Peters, Cellular radiosensitivity as a predictor of tumor radiocurability, Radiat Res 104:290-296 (1985).
6. W.A. Brock, M. Williams, M.L. Meistrich, D.J. Grdina, Micronucleus formation as a measure of surviving fraction in irradiated cell populations. In: J.J., Broerse, G.W. Barendsen, eds., Proceedings of the 7th International Congress of Radiation Research, Amsterdam: Martinus Nijhoff, p B4-03 (1983).
7. W.A. Brock, M. Williams, V.A. Bhadkamkar, G. Spitzer, F. Baker, Radiosensitivity testing of primary cultures derived from human tumors. In: K.H. Karcher, ed., Proceedings of the 3rd International Meeting on Progress in Radio-Oncology, Vienna, Austria (1985).
8. S.J. Grote, G.P. Joshi, S.H. Revell, C.A. Shaw, Observations of radiation-induced chromosome fragment loss in live mammalian cells in culture, and its effect on colony-forming ability. Int J Radiat Biol 39:395-408 (1981).
9. G.P. Joshi, W.J. Nelson, S.H. Revell, C.A. Shaw, X-ray-induced chromosome damage in live mammalian cells and improved measurements of its effects on their colony-forming ability, Int J Radiat Biol 41:161-181 (1982).

# INSTRUMENTATION FOR CLINICAL HYPERTHERMIA

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## INTRODUCTION

The number of institutes using hyperthermia as a clinical treatment for cancer has grown steadily during the last two decades. Most of the pioneering institutes which entered the hyperthermia field before 1980 started to perform hyperthermia treatment with instrumentation borrowed from rehabilitation departments and their equipment consisted often of a single microwave or radiofrequency generator with simple applicators. Today, these institutes use their own "in-house" developed heating systems with high demands on technical specifications. Frequently, they use applicator arrays to adapt the treatment area to size, depth and location of the tumor to be heated.

The institutes approaching the hyperthermia field face the problem of deciding whether they should develop their own hyperthermia system or buy a commercial system. In-house development of a hyperthermia system is however, labor intensive, and normally it takes a long time before a failure-proof and efficient hyperthermia system can be built. Purchasing a commercial hyperthermia system has the important advantage that the hyperthermia treatment can be started within a short time base; and that most of the technical pitfalls have already been dealt with. However the question as to which commercial hyperthermia system to buy remains to be solved and the answer to this question will vary strongly from institute to institute, and is dependent upon a number of conditions.

Generally, within a hyperthermia system, 4 separate units can be distinguished: the applicators, the generators, the thermometry system and the computerized temperature registration and power control (Fig.1). Each unit forms an indispensable part of the whole system and the required specifications of each unit will depend greatly upon the tumor site to be heated. Therefore, for each institute entering the field of hyperthermia the first and most

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important request comes from the clinician, who has to provide the physicist with a good and clear insight of the tumor locations to be heated. Only after this discussion has been closed satisfactorily does it become possible to specify the demands which need to be fulfilled by the hyperthermia system.

The objective of this chapter is to present a short overview of the heating techniques available at present, and to discuss some of the developments expected within the near future. This will be done by discussing the demands of the first three parts of the heating system and the specific

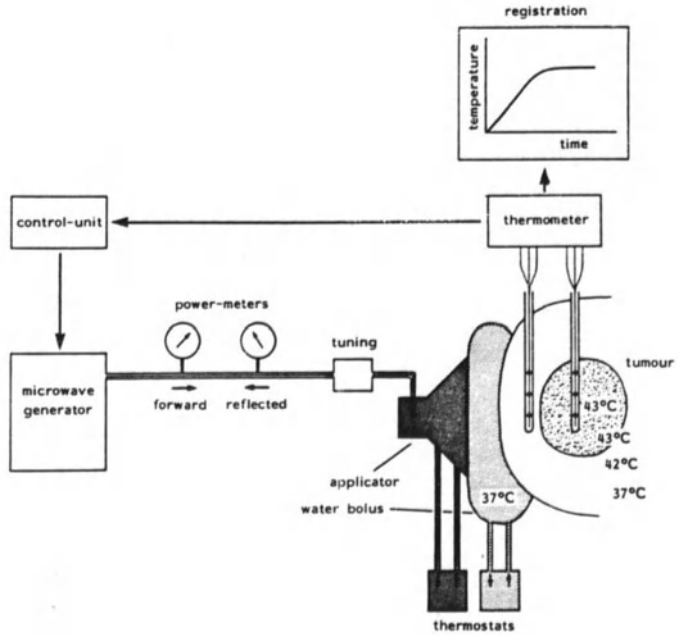


Figure 1. Schematic representation of the set-up of the instrumentation for clinical hyperthermia.

advantages and disadvantages of the various solutions. The advantages of computerized temperature registration and power control is obvious and needs no further explanation. We refrain from promoting any of the commercial hyperthermia system available or any special heating technique.

Finally, the composition of the staff of a hyperthermia department and the problem of quality assurance and its

relevance towards the clinical outcome of the hyperthermic treatment is addressed.

## TECHNICAL ASPECTS OF HYPERTHERMIC TREATMENT APPLICATORS

Roughly, the heating devices can be divided into two categories:
a) hyperthermia system for superficial tumors (depth < 4cm).
b) hyperthermia system for deep seated tumors (depth > 4cm).
With regard to interstitial hyperthermia systems we confine ourselves to referring to several other manuscripts within this book.

### a) Hyperthermic treatment of superficial tumors

Analysis of a number of clinical studies (Dunlop et al., 1986, Emami et al., 1988, Howard et al., 1987, Kapp et al., 1988, Lindholm et all., 1987, Perez et al., 1989, Scott et al., 1988, Shimm et al., 1988, Tsykiyama et al., 1987, Van der Zee et all., 1987, published during the last 3 years shows that approximately 50% (n=498) of the superficial tumors which are treated with hyperthermia are located in the chest wall, nearly 30% (n=290) in the head & neck region, 13% (n=132) in the extremities and the remaining 7% (n=68) in other areas of the body. Most of the reporting institutes used electromagnetic (EM) energy, transferred by waveguide or microstrip patch and spiral applicators (Dunlop et al., 1986, Kapp et al., 1988) to heat the tumors. The use of ultrasound applicators for clinical superficial hyperthermia is reported by approximately 30% of the institutes. This is illustrated in Figure 2. A more or less similar distribution exists for hyperthermia system produced by the various manufacturers as appears from Figure 2.

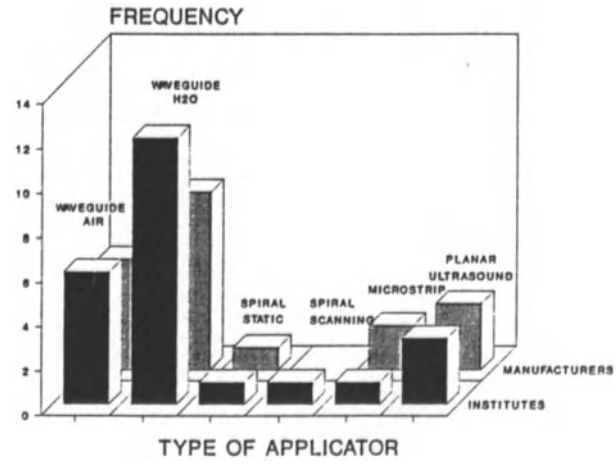


Figure 2. Distribution of various types of applicators for superficial hyperthermia used by the institutes and produced by the manufacturers.

Electromagnetic applicators

As is apparent from Figure 2 the majority of the institutes use waveguide or horn applicators rather than microstrip patch or spiral applicators to transform the EM energy into the tumor. An example of a horn applicator is given in Fig. 3. A small dipole is used to transmit the energy into the horn applicator. The length (D) of the dipole and its distance (L) to the reflecting backwall are critical to obtain proper operating of the applicator (Collin, 1960). The tuning stub enables the operator to tune the applicator for achieving a minimum of reflected power.

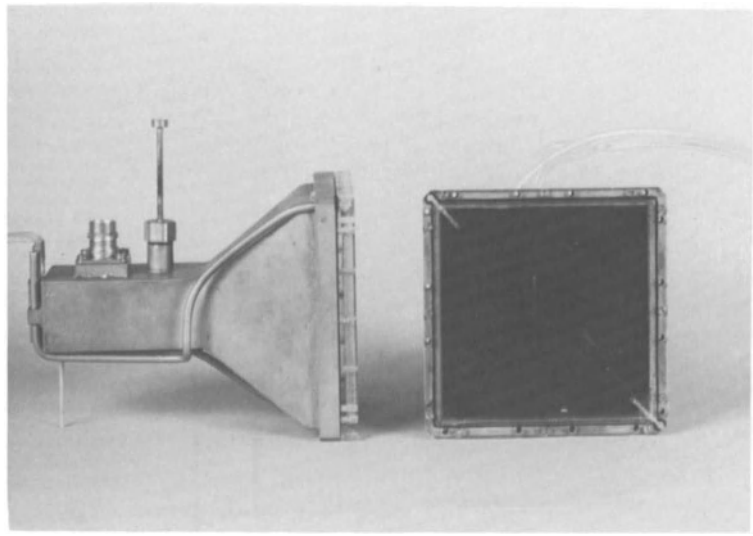

Figure 3. a) Photograph of set of waterfilled horn applicators operating at 433 MHz. The aperture size is 10x10 $cm^2$.

The frequencies used vary from 100 to 2450 MHz with a clear preference for the lower frequencies that are allowed for industrial, scientific and medical (I.S.M.) applications. These frequencies are 915 MHz for the U.S.A. and 434 MHz for Europe. The low weight and small size of microstrip applicators offer an important advantage over the bulky and rigid waveguide applicators which explains the recent growing interest into the clinical use of this type of applicator.

A common characteristic of waveguides as well as of microstrip applicators is that the penetration depth in homogeneous muscle tissue is highly dependent upon the aperture size of the applicator. As shown in a recent review by Hand (1987), the penetration depth for small aperture sizes is more or less constant (2cm) for frequencies between 100 and 1000 Mhz, regardless of the type of applicator (Table 1). Only for large apertures (size greater than 1.5-2 times the waveguide in muscle tissue) the penetration depth equals the penetration depth of the plane wave EM-field. In the latter case penetration depth can increase up to 6 cm at the lower end of the frequency range. However, in

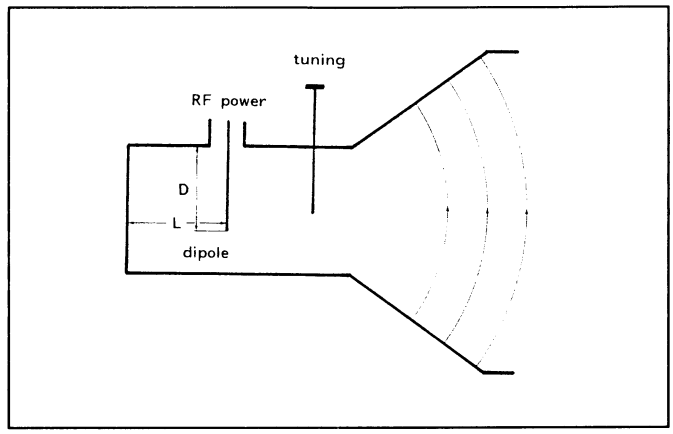


Figure 3 b) Schematic drawing of the applicator

perspective of the spatial control of the energy distribution the aperture sizes at these low frequencies will be impractical for clinical use. Therefore, the penetration depth of most non-invasive electromagnetic (EM) applicators is only sufficient for hyperthermia treatment as superficial located tumors at the chest wall and in the head and neck region. Furthermore, it means that the tumor extension must be not deeper than 4 cm , otherwise the tumor can not be heated adequately by using these applicators.

Other common characteristics of waveguides as well as of microstrip applicators are the small effective treatment area compared to the aperture size, and the need of a waterbolus in front of the aperture. This waterbolus is meant in the first place to reduce the high intensities which exist in the region of the nearby field.

Additional functions of the waterbolus are to:
- adapt the aperture of the applicator to the surface of the treatment field
- control skin temperature to improve the homogeneity of the temperature distribution and to prevent the occurrence of skin burns
- reduce the electromagnetic leakage in order to obtain safe levels of EM radiation in the treatment room.

Table 1

Penetration depth as function of frequency and aperture size

| Penetration Depth [cm] | Frequency [MHz] | | | | |
|---|---|---|---|---|---|
| Aperture size [ cm x cm ] | 100 | 200 | 400 | 700 | 900 |
| 4 x 4 | 1.7 | 1.7 | 1.7 | 1.9 | 2.0 |
| 8 x 8 | 2.8 | 2.8 | 2.8 | 3.1 | 3.3 |
| 10 x 10 | 3.2 | 3.2 | 3.3 | 3.4 | 3.3 |
| 15 x 15 | 4.7 | 4.2 | 3.6 | 3.1 | 3.1 |
| 20 x 20 | 5.5 | 4.5 | 3.6 | 3.2 | 3.1 |

In the Dr. Daniel den Hoed Cancer Center approximately 60% of the chest wall fields treated, can extend over an area of more than 200 $cm^2$. Such large treatment fields can only be heated adequately when multiple applicators are used simultaneously. The most important advantage of an array of coherent radiating applicators is that the effective treatment area approaches that of the total aperture size (Margin and Peterson, 1989). However, no substantial increase of penetration depth is obtained. In our experience individual control of the amplitude of each applicator is an absolute requirement for the clinical use of such applicator arrays. Whereas at present experimental and theoretical research on applicator arrays has been performed mainly with simple applicator geometries and equal amplitudes to each applicator, future research is needed under conditions like those encountered in the clinic. This accounts especially for the effective treatment set-ups with unequal amplitudes, non-coherent radiation and variable shape and composition of the surface anatomy.

Under clinical circumstances the use of the relatively bulky and rigid waveguide applicators becomes cumbersome, and they are difficult to adapt to the shape of the tissue to be treated. Of great interest for the future use of applicator arrays is the recent development of flexible microstrip applicators (Fessenden et al., 1988, Johnson et al., 1988). These applicators consist of a large number of small microstrip antennas, spiral or leaky stripline, which can render the set-up of the clinical heat treatment for large tumor areas more reproduceable and time-effective.

Ultrasound applicators

Important physical advantages of ultrasound (Hunt,1987) over electromagnetic heating techniques are the low absorption coefficient and the short wavelength in human tissue. These two characteristics of ultrasound enables the use of various applicator techniques to obtain a high deposition of energy at depth. However, the clinical use of ultrasound is hampered by two distinct disadvantages:
-Ultrasound energy has a high absorption in bone. This causes high temperature in bone, which is often treatment limiting.
-Large reflections occur at the interfaces between soft tissue and bone or air. This causes a severe complication, or even prevents the achievement of adequate heating of tissues beneath air cavities or bone structures.

The ultrasound system for superficial hyperthermia commonly use planar ultrasound transducers with diameters varying from 1cm to 10cm. A well designed planar ultrasound applicator will have a sufficient thick water column in front of the transducing element. Like with the electromagnetic applicator, the water column is used to match the acoustic impedance of the body to the tranducer and to eliminate the rapidly varying contribution of the nearby field. Secondly, the water bolus can be used to control the temperature of the superficial tissue layers.

Clinically, the planar ultrasound transducer offers the same possibilities as the microwave waveguide or horn applicators to adapt the treatment field to the size of the tumor. The important advantage of the increased penetration depth of ultrasound can not be exploited for tumors located immediately above bone, such as chest wall recurrences and, to a lesser degree, head and neck tumors.

Here, too, heating of large tissue areas can only be obtained by using more complex applicator configurations. Several ways to extend the treatment area are being presently investigated: mechanical scanning with one or two planar transducers, electronically scanning with a segmented tranducer, or the use of a multiple transducer array (Dickinson, 1984). By selective activation of each tranducer within the array, the field size can be shaped to the treatment field. The latter system is now commercially available from several corporations.

Table 2. Clinical performance of superficial hyperthermia system/applicator in terms of average tumor temperature.

| Tumor site | Reference | Hyperthermia system/applicator | | | | | |
|---|---|---|---|---|---|---|---|
| | | US | MI | Microwaves | | | |
| | | | | Waveguides | | Spiral | |
| | | | | air* | water* | static | scan. |
| Head & Neck | Shim et al., 1988 | 39.9 (6) | 39.1 (9) | 40.9 (22) | 41.5 (26) | | |
| | Kapp et al., 1988 | 42.2 (27) | | | 41.8 (15) | 42.6 (53) | 42.0 (40) |
| | Corry et al., 1988 | 42.9 (38) | 39.5 (3) | | | | |
| Chest wall | Shim et al., 1988 | 39.6 (2) | 39.6 (30) | 40.8 (6) | 41.6 (25) | | |
| | Kapp et al., 1988 | | | 40.9 (5) | 41.8 (60) | 43.1 (57) | 42.4 (280) |
| | Corry et al., 1988 | 42.9 (26) | 41.4 (43) | | | | |
| Extremities | Shim et al., 1988 | 39.7 (2) | 39.6 (7) | | 41.6 (7) | | |
| | Kapp et al., 1988 | 42.9 (6) | | | 40.5 (7) | 42.3 (43) | 42.2 (40) |
| | Corry et al., 1988 | 43.2 (25) | 41.8 (23) | | | | |

Body of table: treatment temperature achieved (oC)
(n) = number evaluations.
US = ultrasound: planar tranducers.
MI = magnetic induction systems:magnetrode, solenoidal and coaxial pair magnetic applicators.
Microwaves: frequency range 100 - 2450 MHz;
* = coupling medium between applicator and tissue surface is air or water.

Clinical performance

The clinical performance of various superficial hyperthermia applicators, expressed as the average tumor temperature achieved by three groups participating in the NCI Hyperthermia Equipment Evaluation Contractor's Group is given in Table 2. The limited amount of data prohibits definitive conclusions but allows making some general remarks. Generally, with electromagnetic waveguide and microstrip (patch or spiral) applicators, as well as ultrasound applicators, therapeutic temperature distributions could be obtained under clinical conditions. However, heating is always heterogeneous. The large variations in temperatures obtained by groups for the other devices at all three tumor sites seems to reflect the differences in skill of each group to handle the hyperthermia device in the best manner. For the extremities, heating with ultrasound or microwaves with static or scanning spiral applicators seems to result in better temperatures. As expected, ultrasonic heating of tumor lesions in the head and neck and chest wall area was often limited by bone pain at the underlying ribs or mandible. Furthermore, there is a tendency that waveguide with air as coupling medium between the applicator and skin, do not provide adequate heating either in the head and neck or the chest wall area. Similarly, all other devices seem to outperform magnetic induction at these 3 superficial tumor locations. A direct comparison of waveguide applicators to microstrip applicators was reported by Kapp et al., 1988. They found consistently better temperatures with static and scanning microstrip applicators.

b) Deep seated tumors

The most important feature of an ideal hyperthermia system for treatment of deep seated tumors would be its ability to deposit the majority of the power in the periphery of the tumor. Secondly the system should be simple and safe, causing no systemic stress and providing good access to the patient during treatment, preferably by not requiring contact with the body surface. Only focused scanned ultrasound (US) and coaxial pair magnetic (CPM) applicators have theoretically the ability to deposit the majority of the power in the periphery of the tumor. However, in clinical treatments with non-homogeneous loads, this ideal energy distribution will be difficult to obtain.

With ultrasound, reflection of waves at interfaces between soft tissue and air or bone and high energy absorption in bone results in 70% of the pelvis treatments in limiting normal tissue heating and pain (Table 3). At present extensive studies are performed by various groups to investigating the use of scanning in single or multiple trajectories with focused transducers. The dynamic scanning algorithms are expected to improve the control of the energy distribution with associated reduction of pain. The scanning US technique is especially promising for relatively small

tumor lesions, where it may result in a highly selective heating of the tumor. However, when large tumor volumes need to be heated it may be too difficult to increase the speed of scanning to a level at which the temperature can be kept at a therapeutic level throughout the whole tumor. Also, due to the large scanning area and the relative small focal

Table 3. Evaluation of various devices for deep heating.

DEVICE EVALUATIONS

| AUTHOR | TREATMENT INTOLERANCE #DE | PAIN | DISC | NT | BLAD | NONE | TEMP. %SESSIONS %T > 42 |
|---|---|---|---|---|---|---|---|
| APA | | | | | | | |
| Shimm et al.'88 | | | | | | | + 20 |
| Kapp et al. '88 | 108 | 86 | 10 | | | 12 | 19* |
| Sapozink et al. AA2Q | 6 | 2 | 5 | | 0 | | 85 |
| '88 AA3Q | 1 | 1 | 1 | | 0 | | 100 |
| AA4Q | 62 | 58 | 27 | | 11 | | 78 |
| Pilepich et al. '88 | 30 | 24 | 6 | | | | 68 |
| Howard et al. '86 | 63 | | | | unfoc | | 78 |
| Issels et al. '88 | 25 | 11 | | | perf | | 68 |
| Total | 78% | | 24% | | 16% | 11% | |
| Power limiting | 32% | | 33% | 8% | | 13% | |
| MI | | | | | | | |
| Corry et al. '88 | | 13 | 10 | 9 | | | 40 |
| Sapozink et al. '88 | 14 | 13 | | | | | 27 |
| Total | 85% | | | | | | |
| Power limiting | 74% | | 22% | 7% | | 7% | |
| US | | | | | | | |
| Corry et al. '88 | 4 | 4 | 2 | | | | 23 |
| Kapp et al. '88 | 33 | 20 | | | | 10 | 15 |
| Roemer & Hynyen '89 | 28 | 13 | | | | | 53** |
| Total | | 57% | | | | 30% | |
| Power limiting | | -----65%----- | | | | 9% | |
| COND | | | | | | | |
| Abe et al. '86 | | | | | | | 65 |
| Hiraoka et al. '86 | | | | | | | 60 |

*deep heating
**%T> 42.5
APA: BSD1000 annular phased array.
MI: *coaxial pair magnetic applicator; **Magnetrode applicator.
US: planar ultrasound transducers.
Cond: capacitor plate applicators: Thermotron.

volume, substantial heating of the tissue in front of the tumor may occur. Finally, the presence of bone and air in the vicinity of the tumor will remain, causing problems. This problem will remain, though to a lesser extent, with the use of focused US.

The coaxial pair magnetic (CPM) system performs well when applied to deep seated tumors in the thorax but, unfortunately, heating of tumors in the pelvic area is poor due to local pain (Corry et al., 1988). The inability to control circulating currents around large pelvic bones limits the use of this technique to eccentrally located tumors.

Several other non-invasive electromagnetic (EM) devices have been developed using either quasi-static or radiative methods to transfer the energy to the patient. All of these are unable to deposit the power selectively in the tumor.

Radiofrequency capacitive systems, with the E-field perpendicular to the body axis, have a high power deposition in front of the electrodes and this causes overheating or the subcutaneous fat tissue. The preferential heating of the fat layer may be adequately counteracted (Abe et al.,1986, Hiraoka et al,. 1987, Kato et al., 1985) with efficient precooling, provided that the fat layer does not exceed a thickness of 2 cm. This apparent feature is caused by the fact that in Western countries Japanese patients generally have less subcutaneous fat than European or American patients. Therefore the limitations of this method should be kept well in mind.

Inductive concentric coil devices have zero power deposition at the center of the patient and therefore clinical use should be restricted to eccentric tumors.

Generally the radiative devices are expected to more effective in obtaining deep heating, especially for patients with more extensive layers of subcutaneous fat. These devices generate a circumferential E-field distribution around the patient, characterized by electrical field lines parallel with the body axis and, as a consequence of interference, high power deposition at the center of the body with minimal heating of the subcutaneous fat tissue. The circumferential E-field produces an internal maximum.

The ability of frequencies above 50Mhz to steer the SAR maximum and adapt the energy distribution to obtain an optimal temperature distribution has been demonstrated clinically and theoretically by several groups (Strohbehn et al., 1986, Sathiaseelan, 1986, Sapozink et al., 1986). Especially, for the treatment of eccentrically located tumors the optimization techniques are of great value. For centrally located pelvic tumors, high SAR gradients exists with the tumor with maximum SAR induced in the flank and hip region (Strohbehn et al., 1986); and here less improvement might be expected from SAR steering. In this situation smaller SAR gradients and better ratios of normal tissue to tumor SAR might be expected at lower frequencies.

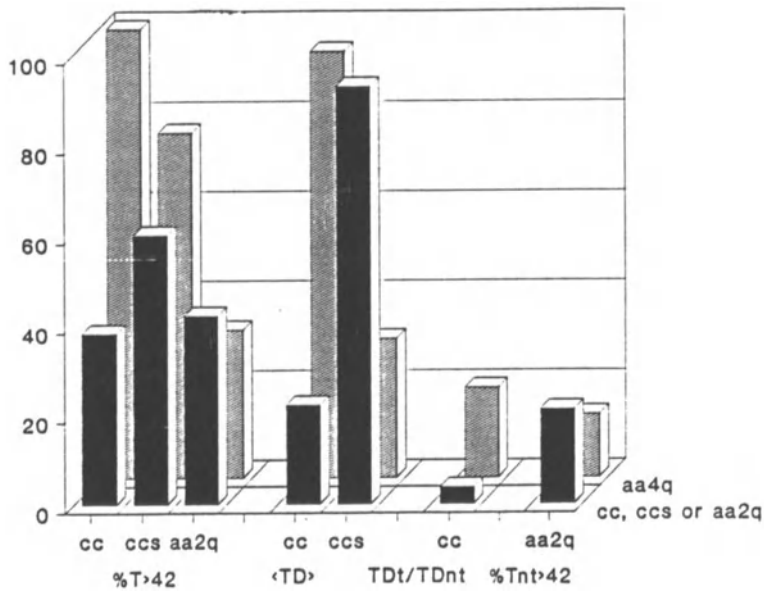


Figure 4. Clinical comparison between three techniques for deep heating using different thermal dose parameters.%T> 42: percentage of treatments with a tumor temperature above 42 degrees C; <TD>: mean equivalent thermal dose in tumor; TDt/TDnt: ratio of thermal dose for tumor and normal tissue; %Tnt> 42: percentage of treatments with normal tissue temperature above 42 degrees C. Concentric coil (cc) versus Annular phased array 4 quadrants (aa4q); Capacitor plate system (ccs) versus Annular phased array 4 quadrants (aa4q); and Annular phased 2 quadrants (aa2q) versus Annular phased array 4 quadrants (aa4q). After data by Sapozink (1986). When the radius of the tissue cylinder is equal to the focal width, for elliptical cross-section as at the lower abdomen this leads to a choice of frequency between 20 and 100 Mhz. Selective tumor heating can only be obtained when tumor blood flow is low compared to that of the surrounding normal tissue. Of the radiative devices the Annular Phased Array (AA) of the BSD company is widly used for clinical treatments. Direct clinical comparison of various deep heating systems (Fig.4) has shown that the AA is superior to the concentric coil (Sapozink et al., 1986), and has an equal heating efficiency to the Thermotron R.F. capacitive system when used for patients with a fat thickness less than 1.5cm (Egawa et al., 1988). However, in approx. 90% of the clinical treatments local pain, general discomfort and rise of normal tissue temperature is power limiting with this device (Table 2).

Several promising electromagnetic deep heating systems are presently under investigation. The coaxial TEM applicator (De Leeuw et al., 1987) is unique concerning its solution for the waterbolus. With this applicator design the patient floats within the waterbolus within the applicator. Due to the lack of waterpressure on the skin of the patient a better treatment tolerance is expected. The coaxial applicator can be used over a broad frequency range and has the possibility to adapt the axial extent of the energy distribution. With the Ring Capacitor Plate (RCP) system (Franconi et al., 1987, Van Rhoon et al., 1988) a circumferential E-field distribution can be created at low frequencies (Hiraoka et al., 1987). Like the TEM applicator the RCP applicator has the capability to adapt the axial extent of the energy distribution. An important advantage of the RCP applicator is its small size which is comparable to that of the Sigma-60 applicator.

However, the major instrumentional improvement has come from the introduction of the BDS2000 Sigma-60 applicator system. The enormous reduction in size and highly efficient waterbolus device of the Sigma-60 applicator in comparison with the APA-system is an important improvement to the comfort of the patient. Whether the ability of this system to control the location of the SAR maximum by steering of the amplitude and phase of the four antenna pairs will result in improved temperature distribution is presently the subject of various clinical studies.

Generators

As there are only a few demands to be met by the radiofrequency or microwave output signal, each high power generator is suitable to use for hyperthermic treatments. No essential difference exists between generators used with electromagnetic applictors or with ultrasound applicators. The most important demand on the generator is that it must be able to deliver high output power and withstand high amounts of reflected power (20%) for a long duration of time ($>$ 8 hours). Furthermore there are a number of requirements such as continuous power control, watchdog for reflected power, which are met easily met by the common generator designs. Of course each generator has to have a set of indicators to monitor forward and reflected power, calibrated to read the actual power delivered at the applicator. In a recent quality assurance investigation (Shrivastava et al., 1988) it was found that the power loss in the transmission lines is approximately 5%.

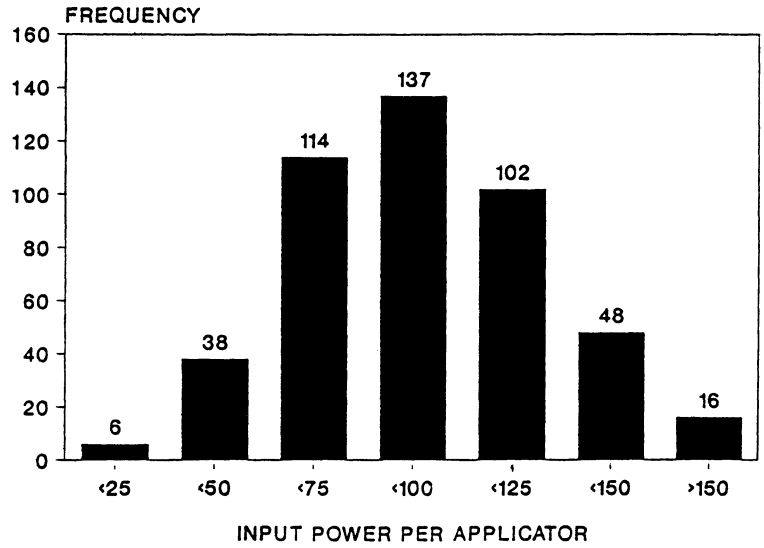


Figure 5. Frequency distribution of the maximum microwave power per applicator needed during clinical treatment.

Depending upon the application, the frequency range and maximum power output of the generator, is chosen. For ultrasound, the appropriate frequency range will be from 100 kHz to 10 MHz with a maximum power output of 200 W. With electromagnetic hyperthermia systems the frequency varies over a much larger range. With superficial heating systems single frequency generators are dominating; in the U.S.A. 915 MHz and in Europe 433 MHz. In our department we found that for 90% of the applications a maximum microwave power output of 150 W per applicator is sufficient to heat all tumor locations (Fig. 5). Occasionally, more than 150 W per applicator is needed. When tumors are located in the head and neck region, more than 150 W per applicator is occasionally needed. For deep heating systems multiple broad band, range 1-200 Mhz, generators with a maximum power output of 500 W each are recommended. The interstitial hyperthermia systems can be divided in two groups. The system with a quasi-static behavior operate at a frequency range of 500 kHz to 30 MHz and the radiative systems operate from 300 MHz upto 2450 MHz. The power output per applicator is relativly low and generally 10 to 25 W will be sufficient. For optimal flexibility of power control every applicator should be fed by its own generator. This holds for all hyperthermia systems, ultrsound or electromagnetic, deep or superficial. A minimum of 2 and 8 generators is needed for superficial and interstitial hyperthermia system respectively.

## THERMOMETRY

Although good progress has been made with the development of non-invasive thermometry systems, the temperature measurement under clinical circumstances is still fully dependent upon invasive thermometry probes. In recent years good improvements have been obtained with temperature sensors that do not disturb the E.M. fields. Currently, several reliable thermometry system are available from several companies.

The high resistive thermistor probes developed by Bowman have the best performance on calibration accurcy, drift, response time, etc., but have as a severe disadvantage, their large diameter (1.1 mm) and limited probe length. The optical thermometry systems have been continously improved and good progress is made with regard to short term drift, accuracy, flexibility and probe diameter ($\leq$ 0.8 mm for containing 4 sensor points). A common disadvantage of the non-perturbing thermometry system is that they are expensive. A major advantage is that the sensor operates independent with respect to the probe direction in relation to the electromagnetic field. This always presents a problem with the commonly used thermocouples. Additionally, methods have been developed to

obtain accurate temperature reading with conventional thermometry systems using the cheaper thermocouple sensors. With these latter systems, careful placement of the probes is necessary to reduce temperature artifacts caused by probe heating, perturbation of the electromagnetic field or interference on the electronic readout system.

For ultrasound, completely upperturbing probes still are not available. However, by proper choice of the catheter and probe material the errors in the temperature reading can be substantially reduced and accurate thermometry is possible. An extensive review on thermometry systems is presented in the chapter on thermometry within this book.

Essential for the quality of the temperature measurement is a regular testing and verification of the specifications of the thermometry system. Therefore within each hyperthermia department a complete set-up to perform an accurate temperature calibration procedure should be available. A common calibration set-up consists of a high quality mercury in glass thermometer (20-50 degrees C, 0.05 division, calibration standard traceable) and a well stirred waterbath with a temperature stability of + 0.05 degrees C. Better standard ($\pm$0.005 degrees C) can be obtained when a Gallium melting point cell is used. The check of the thermometry system should be performed minimally once each day when the system is in use; preferably before and after each patient treatment session. Although the above procedure provides a reliable manner to measure the temperature it will not provide the important information on the quality of the measured temperature distribution. Parameters with impact on the quality of measurement are, the number of probes, their spacing and their location. Temperatures measured at the periphery of the tumor specifically add to better quality of measurement.

The need of a high quality measurement of temperature distribution is obvious: it forms the input for all "thermal dose"-response studies. Furthermore, the result of the hyperthermic treatment appears to be related to the minimum temperature (Oleson et al., 1984, Arcangeli et al., 1985, Van der Zee et al., 1986) and therefore it is essential to obtain good information on the temperature distribution. Therefore, clinical hyperthermic treatment should be performed with two thermometry catheters inserted within the tumor tissue. Such catheters consist of thin plastic tubes, placed in the (tumor) tissue before treatment (Van der Zee et al., 1987). Within each catheter the temperature must be measured at multiple locations by multisensor probes or by "thermal mapping". The position of each catheter should be such that at least one temperature measuring point is located in the tissue each beneath applicator used. At the same time a high quality measurement of the temperature distribution enables quantification of the quality of the heat treatment. A simple and valuable performance parameter may be represented by the percentage of probes which

indicate an equivalent temperature above a certain index temperature, e.g. ETV (Sim et al., 1984). Information on temperature distribution can further be used to perform multi-institutional device evaluations. This may lead to the choice of optional heating techniques for specific tumor localizations.

## HYPERTHERMIA STAFF

A good hyperthermia technique is only part of what we need to perform hyperthermia well. To provide the minimal requirements for high quality treatments, the staff of a hyperthermia department should consist of an interactive group of various disciplines: a physician, a physicist and a hyperthermia technician. A summary of the qualifications needed by the hyperthermia staff was recently published: (Luk, 1988)

- the physician who prescribes and supervises hyperthermia should be a licensed clinician with special training in oncology and the basic biological and physical principles of hyperthermia, as well as adequate experience in clinical applications of hyperthermia. A minimum of six months of additional training in hyperthermia should be required to achieve the needed knowledge and clinical proficiency.

- the physicist, who is responsible for all the physical and quality aspects of the hyperthermia equipment, must be trained at the level of a radiation physicist. Additional training in thermometry, electromagnetic and thermal treatment modeling, and the physics of ultrasound, radiofrequency and microwaves is required, as well as a thorough knowledge of the biological and physiological responses of tumor and normal tissues to heat. Furthermore, an excellent knowledge of the operation, maintenance, and quality assurance procedures of hyperthermia treatment is required.

- the hyperthermic technician who administers the hyperthermia treatment should be trained in basic health and physical sciences such as normally given to a radiation therapy technician. Supplementary, special training in the operation, maintenance, and quality assurance procedures of hyperthermia equipment and clinical application of the hyperthermia treatment is required.

## QUALITY ASSURANCE

With respect to clinical treatment each institute active within the field of hyperthermia should realize that good hyperthermia equipment does not guarantee a good hyperthermia treatment. A highly motivated and experienced staff, together with a strict confirmation of the treatment quality assurance and assessment (QA) guidelines is an absolute requirement to obtain reliable and comparable clinical results of the different patients. A recent

publication showed the impact of such QA problems on the therapeutic outcome of the RTOG 81-04 study (Perez et al., 1989). For example. a significant correlation between local control and tumor coverage by at least the 25% iso-SAR contour (66% vs 17%) a significantly increased complete response (CR) rate for the patients treated in recent series (71%) compared with an earlier series (23%). In both series the same radiotherapy schedule was used and the greatly improved results reflects the progressive heating techniques. In conclusion: constant alertness and concerted effort of the entire clinical hyperthermia staff is an essential prerequisite for a continuous increase of the quality of even the "best" hyperthermic treatment to assure an optimal clinical outcome for the patient.

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REFERENCES

1) Abe M., Hiraoka M., Takahashi M., Egawa S., Matsuda C., Onoyama Y., Morita, K., Kakehi M. and Sugahara T., 1986, Multi-institutional studies on hyperthermia using an 8 MHz radiofrequency capacitive heating device (Thermotron RF-8) in combination with radiation for cancer therapy, Cancer, 58:1589.

2) Arcangeli, G., Arcangeli, G., Guerra, A., Lovisolo, G., Cividalli, A., Marino, G., and Mauro, F., 1985, Tumor response to heat and radiation: prognostic variables in the treatment of neck node metastases from head and neck cancer, Int. J. of Hyperthermia, 1:207.

3) Corry, P.M., Jabboury, K., Kong, J.S., Armour, E.P., McCraw, F.J. and LeDuc, T., 1988, Evaluation of equipment for hyperthermia treatment of cancer, Int. J. Hyperthermia, 4:53.

4) De Leeuw, A.A.C., and Lagendijk, J.J.W., 1987, Design of a clinical deep body hyperthermia system based on the 'coaxial TEM' applicator, Int. J. Hyperthermia, 3:413.

5) Dickinson, J.R., 1984, A non-rigid mosaic applicator for local ultrasound hyperthermia, in: Hypertheric Oncology I, Ed. J. Overgaard, Taylor and Francis, London, 671.

6) Dunlop, P.R.C., Hand, J.W., Dickinson, R.J. and Field S.B., 1986, Assessment of local hyperthermia in clinical practice, Int. J. Hyperthermia, 2:39.

7) Egawa, S., Tsukiyama, I., Akine, Yl, Kajiura, Y., Ogino, T. and Yamshita, K., 1988. Hyperthermic therapy of deep seated tumors: Comparison of the heating efficiencies of an APA and a capacitively coupled RF system, Int. J. Radiat. Oncol. Biol. Phys., 14:521.

8) Emami B., Perez, C.A., Konefal, J., Pilepick, M.V., Leybovich, L., Straube, W., Von Gerichten, D., Hederman, M.A., 1988, Thermoradiotherapy of malignant melanoma, Int. J. Hyperthermia, 4:373.

9) Fessenden, P., Lee, E.R., Kapp, D.S., Traczy-Hornoch, P., Prionas, S.D. and Sullivan, D.M., 1988, Improved microwave (MW) applicators for surface hyperthermia, 5th International Symposium on Hyperthermic Oncology (ISHO), Kyoto 1988, p31-a-10.

10) Franconi, C., 1987, Hyperthermia heating technology and devices, in: Physics and Technology of Hyperthermia, S.B. Field and C. Franconi, eds. Martinus Nijhoff Publishers, Dordrecht/Boston/Lancaster, 80.

11) Hand, J.W., 1987, Electromagnetic applicators for non-invasive local hyperthermia, in: Physics and technology of hyperthermia, S. B. Field and C. FRanconi, eds. Martinus Nijhoff Publishers, Dordecht/Boston/Lancaster, 189.

12) Hiraoka, M., Jo S., Akuta K., Nishimura Y., Takahashi M. and Abe M., 1987, Radiofrequency capacitive hyperthermia for deep-seated tumors. II. Effects of thermoradiotherapy, Cancer, 60:128.

13) Howard, G.C.W., Sathiaseelan, V., Freedman, L. and Bleehen, N.M., 1987, Hyperthermia and radiation in the treatment of superficial malignancy: an analysis of treatment parameters, response and toxicity, Int. J. Hyperthermia, 3:1.

14) Hunt, J. W., 1987, Principles of ultrasound used for hyperthermia, in: Physics and technology of hyperthermia, S.B.Field and C. Franconi, eds. Martinus Nijhoff Publishers, Dordrecht/Boston/Lancaster, 354.

15) Ishida, T., and Kato, H., 1980, Muscle equivalent agar hantom for 13.56 MHz RF-induced hyperthermia, Shimane J. Med. Sc., 4:134.

16) Issels, R.D., Wadepohl, M., Tiling, K., Mueller, M., Sauer, H. and Willmanns, W., 1988, Regional Hyperthermia combined with systemic chemotherapy in advanced abdominal and pelvic tumors: First results of a Pilot Study employing an annular phased array applicator, Recent Results in Cancer Research, 107, 236.

17) Johnson, R.H., Preece, A.W. and Murfin, J.L., 1988, Flexible electromagnetic hyperthermia applicators, Abstracts of the 5th International Symposium on Hyperthermic Oncology (ISHO), Kyoto, p44-a-6, 317.

18) Kapp, D.S.,Fesswnden, P., Samulski, T.V., Bagshaw, M.A., Cox, R.S., L, E.R., Lohrbach, A.W., Meyer, J.L. and Prionas, S.D., 1988, Stanford University institutional report. Phase I evaluation of equipment for hyperthermia treatment of cancer, Int. J. Hyperthermia, 4:75.

19) Kato, H., Hiraoka, M., Nakajima, T., Ishida, T., 1985, Deep-heating characteristics of an RF capacitive heating device, Int. J. Hyperthermia, 1:15.

20) Lindholm, C.E., Kjellan, JE., Nilsson, P. and Hertzman, S., 1987, Microwave induced hyperthermia radiotherapy in human superficial tumors: clinical results with a comparative study of combined treatment versus radiotherapy alone, Int. J. Hyperthermia 3:393.

21) Luk, K.H., 1988, Training and certification issues in hyperthermia. In: Biological, Physical and Clinical Aspects of Hyperthermia, Medical Physics Monograph 16, Ed. B.R. Paliwal, F.W. Hetzel, M.W. Dewhirst, American Institute of Physics, Inc., New York, 476.

22) Magin, R.L., Peterson, A.F., 1989, Noninvasive microwave arrays for local hyperthermia: A review, Int. J. Hyperthermia, 429.

23) Myerson, R.J., Perez, C.A., Straube, W., Kushe, R.R., Leybovich, L. Von Gerichten, JD., 1989, Tumor control in long-term survivors following superficial hyperthermia, North America Hyperthermia Group (NAHG), Seattle, 1989, Ck-3.

24) Nishimura Y., Hiraoka, M., J S., Akuta K., Nagata Y., Takahashi, M. and Abe M., 1986, Radiofrequency capacitive hyperthermia combined with radiation for lower abdomional and pelvic deep-seated tumors, In: Hyperthermic Oncology Japan, Proc. 3th JSHO, Ed. Y Onoyama, Mag Bros Inc.: 371.

25) Oleson, J.R., Sim, D.A., Manning, M.R., 1984, Analysis of prognostic variables in hyperthermia treatment of 161 patients, Int. J. Radiat. Oncol. Biol. Phys., 10:2231.

26) Perez, C.A., Gillespie, B., Pajak, T., Hornback, N.B., Emami, B. and Rubin, P., 1989, Quality assurance problems in clinical hyperthermia and their impact on theraeutic outcome: A report by the radiation therapy oncology group, Int. J. Radiat. Oncol. Biol. Phys., 16:551.

27) Pilepich, M.V., Myerson, R.J., Emami, B.N., Perez, C., Leybovich, L., and Von Gerichten, D., 1987, Regional hyperthermia: a feasibility analysis, Int. J. Hyperthermia, 3:347.

28) Rhoon, G.C., van, Visser, A.G., Berg, P.M., van den and Reinhold, H.S., 1988, Evaluation of ring capacitor plates for regional deep heating, Int. J. Hyperthermia, 4:33.

29) Roemer, R.B., 1989, Personnel communication.

30) Sapozink, M.D., Gibbs, F.A., Thomson, J.W., Eltingham, J.R., and Stewart, J.R., 1986, A comparison of deep regional hyperthermia from an annular array and a concentric coil in the same patients. Int. J. Radiat. Oncol. Biol. Phys., 11:179.

31) Sapozink, M.D., Gibbs, F.A., Gibbs, P. and Stewart, J.R., 1988, Phase I evaluation of hyperthermia equipment-University of Utah Institutional Report, Int. J. Hyperthermia, 4:117.

32) Sathiaseelan, V., 1986, High frequency techniques: deep heating with an annular phased array, IEEE, Eighth Annual Conference of the Engineering in Medicine and Biology Society, 1474.

33) Scott, R., Gillespie, JB., Perez, C.A., Hornback, N.B., Johnson, R., Emami, B., Bauer, M. and Pakuris, E., 1988, Hyperthermia in combination with definitive radiation therapy: Results of a phase I/II RTOG study, Int. J. Radiat. Oncol. Biol. Phys., 15:711.

34) Shimm, D.S., Cetas, T.C., Oleson, J.R., Cassady, J.R., and Sim, D.A., 1988, Clinical evaluation of hyperthermia equipment: The University of Arizona Institutional REport for the NCI Hyperthermia Equipment Evaluation Contract, Int. J. Hyperthermia, 4:39.

35) Shrivastava, P.N., Saylor, T.K., Matloubieh, A.Y., Paliwal, B.R., 1988, Hyperthermia quality assurance results, Int. J. Hyperthermia, 4:25.

36) Sim, D.A., Dewhirst, M.W., Oleson, J.R., and Grochowski, K.J., 1984, Proc 4th International Symposium on Hyperthermic Oncology (ISHO), Aarhus, Denmark, 1984, Estimating the therapeutic advantage of adequate heat ( ), 359.

37) Strohbehn, J.W., Paulsen, K.D. and Lynch, D.R., 1986, Physical Techniques in Clinical Hyperthermia, Research Study Press, 383.

38) Tsykiyama, I., Yamashita, K., Kajiura, Y., Akine, Y., Egawa, S. and Yawagawa, S., 1987, REsults of a non-controlled trial of hyperthermia combined with radiation for superficial tumors, Int. J. Hyperthermia, 3:503.

39) Van der Zee, J., Van Rhoon, G.C., Broekmeijer-Reurink, M.P. and Reinhold, H.S., 1987, The use of implanted closed-tip catheters for the introduction of thermometry probes during local hyperthermia treatment series, Int. J. Hyperthermia, 3:337.

40) Van der Zee, J., Van Putten, W.L.J., Van den Berg, A.P., Van Rhoon, G.C., Wike-Hooley, J.L., Broekmeyer-Reurink, M.P., and Reinhold, H.S., 1986, Retrospective analysis of the response of tumors in patiens treated with a combination of radiotherapy and hyperthermia. Int. J. Hyperthermia, 2:337.

**THE USE OF HYPERTHERMIA IN CANCER TREATMENT**

# An Analysis of Clinical Investigations on Deep Seated Malignant Tumor Control with a Radiofrequency Capacitive Hyperthermia System (THERMOTRON RF-8).


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## INTRODUCTION

In response to the challenge of treating deep-seated tumors without undue damage to surrounding normal tissues, a radiofrequency (RF) capacitive heating system known as the Thermotron RF-8 (Yamamoto Vinyter Co., Osaka), with its many innovative engineering remedies, was developed, and it has enabled multi-institutional controlled clinical hyperthermia trials targeted to major organ site neoplasms, i.e. lung cancer, pancreatic cancer, gastric cancer, hepatocellular cancer, rectal cancer, urinary bladder cancer, etc.

This paper is based on recent clinical data collected by a multi-institutional investigation group in Japan. These data were made available to the Hyperthermia Research Coordination Committee by the investigators as a part of a collaborative research endeavor for further communication. We herein present the clinical results which were collected following the adjuvant therapeutic application of 8 MHz RF capacitive hyperthermia with radiotherapy or with chemotherapy for deep-seated tumors.

## MATERIALS AND METHODS

THE HEATING DEVICE: Some limitations and possible solutions to conventional RF capacitive heating devices are listed in Table 1.

Briefly, the RF generator has an 8 MHz self-excited oscillation circuit with a maximum power output of 1.5 KW. RF energy is transmitted from a generator via two coaxial cables. The RF is applied through a pair of electrodes placed on opposite sides of the body, and power is distributed locally or regionally through the interaction of electrical fields produced between the parallel-opposed electrodes. The inner ring of the gantry with the electrodes can be rotated 180 degrees to facilitate the heating of various target sites in the body. Electrode sizes vary


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Table 1

Possible Solutions to Some of the Limitations of Conventional RF Capacitive Heating Devices

| LIMITATION | POSSIBLE SOLUTION |
|---|---|
| 1. Difficult to heat tumors deep in the body. | Large electrodes and a special matching system. RF - 13.56 MHz to 8 MHz |
| 2. Electrode edge effects. | An overlay-bolus system with contact medium and Rogowski's flat-plate electrodes. |
| 3. Excessive heating of the subcutaneous fatty layer. | A cooling system for the skin and subcutaneous tissues. |
| 4. Electrical mismatching due to movement and temperature changes in the human body. | A self-exciting oscillator. |
| 5. Asymmetry and poor reproducibility of heating patterns. | A movable gantry with fixed electrodes. |

from 6 to 30 cm in diameter. A predictable thermal-field cross section corresponding to approximately 70% of the electrode surface is possible. Deep-seated tumor heating depends largely upon the ratio of the diameter of the electrodes to the thickness of the load between the two opposed electrodes. A suitable combination of electrodes is selected according to the size and location of the tumor. The surface of the electrode is covered with flexible water pads and/or an overlay bolus system. A temperature controlled saline solution, which, electrically, is nearly the equivalent of muscle, is perfused into the water pad or overlay bolus system so that excessive heating of the skin and subcutaneous fatty tissue can be avoided. Also, this bolus pad, when used with sufficient contact medium such as electrode coupling cream or gel, makes it possible to position the electrodes to the body surface smoothly, and enables the RF energy to be applied relatively homogeneously to uneven contours on the body. The bolus water temperature can be adjusted to avoid overheating of surrounding normal tissue while heating the target tumor at a therapeutic level.

The Thermotron RF-8 has four built-in thermocouple channels for temperature measurement. These sensors are teflon-coated probes of copper/constantan micro-thermocouples (Type IT-18, Sensortek, New Jersey). The thermometry system with the micro-thermocouples connected to an automatic temperature and power feedback controller provides an accuracy of +/- 0.2°C. The high RF wave filter is inserted into the thermometry system which makes it possible to measure the temperature during RF thermotherapy. Indwelling catheter sheaths (18-19 gauge) are placed into varying depths of the tumor and adjacent normal tissues after local anesthesia with Lidocaine, and thermocouples are inserted inside the catheters. The temperature in the tumor and surrounding normal tissues is monitored continuously during heating, and temperatures are shown on a digital and computer-generated graphic monitoring display system which includes a hard copy print-out mode. The skin surface temperature in the heating field is also monitored. Full details of this system have been described by others (1, 9, 16).

Independently reported thermal profile data by several institutional groups using the Thermotron RF-8 capacitive hyperthermia system have shown the efficiency of deep-target heating (1- 5, 7-10, 12-14, 16-17).

HEATING: Hyperthermia was usually applied 1-2 times a week immediately

after radiotherapy. For those receiving thermochemotherapy protocols, the drug was administered before or during the heating session. The aim of hyperthermia was to heat the tumor to above 42.0°C with each session lasting 40 to 60 minutes. Tables 8-15 show the number of heating sessions given in each organ site tumor with radiotherapy or chemotherapy.

RADIOTHERAPY: Radiotherapy was given to patients using 6-10 MeV x-rays in two opposing anterior-posterior fields. In some cases, a three portal or rotational technique was used. The calibration, collimation, and beam blocking techniques were those routinely utilized in radiation therapy.

CHEMOTHERAPY: Chemotherapy was administered by i.v. dripping injection or by per os or suppository methods before or during heating. In those patients with hepatocellular carcinoma involving chemoembolization, degradable starch microspheres (DSM), Pharmacia AB, Uppsala, Sweden, were used.

PATIENTS: Those cases with histologically-confirmed recurrent or locally advanced malignant tumors in specific organ sites were eligible for participation in this investigation (see tables 2 through 7).

EVALUATION: Since the majority of patients who entered into this investigation had advanced diseases, the evaluation of treatment response was assessed primarily on local tumor response. Tumor response criteria were based upon the following categories: Complete Response (CR) for complete disappearance of the tumor in the treated field; Partial Response (PR) for a more than 50% regression of tumor volume; Minor Regression (MR) for a 25-50% regression of tumor volume; No Change (NC) for less than 50% reduction in tumor volume; and Progression of Disease (PD) for increase of of tumor volume. Techniques for objective assessment included plain field x-ray, CT, MRI, ultrasound images, and endoscopic examinations. A performance status scoring system was also utilized: 0=asymptomatic; 1=symptomatic, ambulatory; 2=less than 50% bed-ridden; 3=more than 50% bed-ridden; 4=100% bed-ridden.

## RESULTS

Clinical results of this investigation were collected from January, 1985 to December, 1988 by seven participating institutions in Japan. There were a total of 177 evaluable cases using a common protocol for each visceral organ site tumor. 54% (96) of these cases received radiation therapy plus hyperthermia, of which 5 cases were pre-operative lung cancers and 9 cases were pre-operative rectal cancers. A total of 81 cases (46%) were in the chemotherapy plus hyperthermia group. Thermoradiotherapy results on lung cancer, pancreatic cancer, rectal cancer, and urinary bladder cancer are presented in tables 8, 9, 13 and 14 respectively. Thermochemotherapy results on pancreatic cancer, gastric cancer, hepatocellular cancer, and urinary bladder cancer are presented in tables 10, 11, 12a, 12b and 15 respectively.

LUNG CANCER: The measurement of the thermal profiles of 21 of these lung tumors indicated that over 86% (18/21) achieved therapeutic levels of hyperthermia, while 3 out of 21 cases attained temperatures in the range of only 40-41°C. Patients in the radical treatment group were treated with a radiation dose of 40-70 Gy in fraction sizes of 4 Gy, and patients in the preoperative group were treated with a total of 30 Gy in fraction sizes of 2.5 Gy. Table 8 indicates that the thermoradiotherapy response showed an 80% favorable response rate which consisted of 3 CR plus 13 PR in 20 tumors, and that there were 3 MR cases along with 1 NC. An analysis of the favorable response rate according to histological types includes a

TABLE 2

LUNG CANCER PATIENT CHARACTERISTICS

| No. of Cases | Stage | Histology | Tumor Vol. cm3 (mean) | Performance Status (mean) |
|---|---|---|---|---|
| 13 | IIIA | Squamos cell ca:8 | 33-1047 (232) | 1-4 (2.4) |
| 4 | IV | Adenocarcinoma :7 | | |
| 3 | Recur. | Large cell anaplastic ca :4 | | |
| Preoperative Treatment | | | | |
| 5 | IIIA | Squamous cell ca:5 | 120-448 (160) | 1-3 (1.8) |
| 25 | | 25 | | |

TABLE 3

PANCREATIC CANCER PATIENT CHARACTERISTICS

| No. of Cases | Stage | Histology | Tumor Vol. cm3 (mean) | Performance Status (mean) |
|---|---|---|---|---|
| 6 | III | Adenocarcinoma:23 | 6.3-140* (73) | 1-4 (3.0) |
| 28 | IV | Unknown primary :11 | | |
| 34 | | 34 | | |

*maximum area

TABLE 4

GASTRIC CANCER PATIENT CHARACTERISTICS

| No. of Cases | Stage | Histology | Performance Status (mean) |
|---|---|---|---|
| 4 | II | Adenocarcinoma: 32 | 1-4 (3.1) |
| 29 | IV | Unknown primary : 1 | |
| 33 | | 33 | |

TABLE 5

HEPATOCELLULAR CARCINOMA PATIENT CHARACTERISTICS

| No. of Cases | Stage | Histology | Tumor Volume $cm^3$ (mean) | Performance Status (mean) |
|---|---|---|---|---|
| 3 | II | Hepatocellular carcinoma: 18 | 2.5-15.0* (7.7) | 0-3 (0.5) |
| 15 | III | | | |
| 18 | | 18 | | |

*maximum diameter

TABLE 6

RECTAL CANCER PATIENT CHARACTERISTICS

| No. of Cases | Stage | Histology | Tumor Volume $cm^3$ (mean) | Performance Status (mean) |
|---|---|---|---|---|
| 1 | Duke A | Adenocarcinoma: 9 | | 2-3 (3.0) |
| 7 | Duke B-1 | | | |
| 1 | Duke B-2 | | | |
| 34 | Recur. | Adenocarcinoma: 34 | 48-1053 (748) | 2-4 (2.9) |
| 43 | | 43 | | |

TABLE 7

URINARY BLADDER CANCER PATIENT CHARACTERISTICS

| No. of cases | Stage | Histology | | Tumor Vol. $cm^3$ (mean) | Performance Status (mean) |
|---|---|---|---|---|---|
| 5 | I | Transitional cell carcinoma | G1: 1 | 0.6-88.0 (32.7) | 0-4 (2.0) |
| 6 | II | | G2: 8 | | |
| 8 | III | | G3: 14 | | |
| 3 | IV | Squamous cell carcinoma | : 1 | | |
| 2 | Recur. | | | | |
| 24 | | | 24 | | |

total of 6 cases (1 CR plus 5 PR) out of 8 squamous cell carcinoma (sq.c.c.), resulting in a response rate of slightly over 75%, and a total of 6 cases (1 CR plus 5 PR) out of 7 adenocarcinoma, producing an 85% response rate. There were also 3 cases of large cell anaplastic carcinoma, and the response was 1 CR and 2 PR. The remaining 5 cases with sq.c.c. were pre-operative protocol cases which resulted in 2 PR, 1 MR, and 2 NC. A follow-up duration of 11-12 months was observed in 4 out of 5 cases. With regard to the subjective complaints of these patients, a marked reduction or the disappearance of hemophytis, cough, pain, etc. was observed in 21 out of 25 cases (84%). A similar improvement rate of the performance status (PS) was obtained with a minimum survival range of 2 months to a maximum 41 months to date. Concerning side effects, 7 patients experienced pain during hyperthermia session.

PANCREATIC CANCER: The measurement of the thermal profiles of tumors located at this site showed that hyperthermic temperature levels ranging from 40.5°C to 43.6°C were achieved in a total of 34 cases. A group of 12 patients were in the thermoradiotherapy group (Table 9), and another 22 patients were in the thermochemotherapy group (Table 10). For the thermoradiotherapy group, radiotherapy was given with a total of 50-60 Gy in fraction sizes of 1.8-2.0 Gy administered over 5-6 weeks. Thermochemotherapy consisted of MMC and 5-Fu derivatives. Mitomycin $C_{4mg}$ were given twice a week. Uracil $ftoraful_{300-600mg}$, ftoraful $suppository_{750-1000mg}$, and $5\text{-}fluorouracil_{250mg}$ were given daily. Thermoradiotherapy results in Table 10 showed a 33% favorable response rate which consisted of 1 CR and 3 PR in 12 pancreatic tumors, as well as 2 MR cases and 6 NC. An analysis of thermochemotherapy results in Table 10 showed a 36% favorable response rate which consisted of 3 CR and 5 PR in 22 pancreatic tumors, in addition to 2 MR cases and 8 NC. There were 4 cases in the PD category. A reduction of pain was noted in over 80% (28 out of 34) of the patients who had received heat in combination with radiation or chemotherapy. An improvement of PS was noted in slightly over 40% (14 out of 34) of the patients.

GASTRIC CANCER: The temperature was measured by inserting thermocouple sensors via an alimentary tube. The therapeutic temperature was aimed at 42°C but the actual achieved temperature varied from 40°C to better than 43°C. However, no corresponding clinical results could be observed in tumors at this organ site. Chemotherapy of gastric cancers involved the same procedure as with pancreatic cancers. $MMC_{4mg}$ were given twice a week. $5\text{-}fluorouracil_{250mg}$, 5-Fu derivatives with uracil $ftoraful_{300-600mg}$, and ftoraful $suppository_{750-1000mg}$ were administered daily. Table 11 indicates that the thermochemotherapy response showed a 39% favorable response rate which was comprised of 3 CR plus 10 PR in 33 cases of gastric cancer, and 3 MR cases along with 13 NC. The remaining 4 cases were in the PD category. An analysis of the histopathological types shows that a close to 43% response rate was achieved with 2 CR and 4 PR out of 14 tubular adenocarcinoma. Out of 11 cases of poorly differentiated adenocarcinoma, a 27% response rate was achieved with 1 CR and 2 PR. Transient side effects included occasional cases of reduced appetite, abdominal pain, discomfort of the abdomen, ascites, G.I. tract bleeding, passage disturbance, nausea, vomiting, weight loss, etc. Gradually, most of these patients achieved a marked improvement in their quality of life. The only observed side effect attributable to hyperthermia was fatty tissue induration in only one case.

HEPATOCELLULAR CANCER: The measurement of the thermal profiles of tumors located in liver showed that hyperthermic temperature levels in the range of 42°C or above were achieved. It was noticed that DSM embolization helped boost liver tumor temperatures 1-2°C higher than the attempt with hyperthermia alone. Chemotherapy involved the use of $adriamycin_{30-40mg}$

TABLE 8

LUNG CANCER THERMORADIOTHERAPY RESPONSE

| No. of Cases | | Radiation Dose | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|---|
| 20 | Radical treatment | 40-70Gy (4Gy/frac) | 4-14* | 3 | 13 | 3 | 1 | 0 | 16 (80) |
| 5 | Preoperat. | 30Gy@ | 4-14* | 0 | 2 | 1 | 2 | 0 | 2 (40) |

@2.5Gy/frac. *Heat was given 1-2x/week.
Relief of Symptom: 80%
Performance Status Improvement: 80%
Modified from: M. Kakehi, et al., 89

TABLE 9

PANCREATIC CANCER THERMORADIOTHERAPY RESPONSE

| No. of cases | Radiation Dose | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 12 | 50-60Gy (1.8-2.0Gy/frac) | 8-10* | 1 | 3 | 2 | 6 | 0 | 4(33) |

*Heat was given 2x/week.
Relief of Sumptom: 90%
Performance Status Improvement: 33%
Modified from: M. Kakehi, et al., 89

TABLE 10

PANCREATIC THERMOCHEMOTHERAPY RESPONSE

| No. of cases | Drugs Doses | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 22 | $MMC_{4mg}$ +5-Fu derivatives ($UFT_{300-600mg}$, FT $suppo_{750-1000mg}$, $5\text{-}Fu_{250mg}$) | 10* | 3 | 5 | 2 | 8 | 4 | 8(36) |

*Heat was given 2x/week.
Relief of Symptom: 77%
Performance Status Improvement: 45%
Modified from: M. Kakehi, et al., 89

TABLE 11

GASTRIC CANCER THERMOCHEMOTHERAPY RESPONSE

| No. of Cases | Drugs Doses | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 33 | $MMC_{4mg}$ +5-Fu derivatives ($UFT_{300-600mg}$, FT-$suppo_{750-100mg}$, $5\text{-}Fu_{250mg}$) | 6-40 (2x/week) | 3 | 10 | 3 | 13 | 4 | 13(39) |

Relief of Symptom: 67%
Performance Status Improvement: 45%
Modified from: M. Kakehi, et al., 89

TABLE 12A

HEPATOCELLULAR CARCINOMA THERMOCHEMOTHERAPY RESPONSE

| No. of Cases | Drugs Doses | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 18 | $ADM_{30-40mg}$ using DSM chemoembolization | 4-16* | 1 | 9 | 3 | 5 | 0 | 10(56) |

*Heat was given 2x/week.
Relief of Symptom: 89%
Performance Status Improvement: NC
Modified from: M. Kakehi, et al., 89

TABLE 12B

RESPONSE RATE COMPARISON AFTER CHEMOEMBOLIZATION OF HEPATOCELLULAR CARCINOMA WITH AND WITHOUT HYPERTHERMIA

| No. of Cases | Type of Treatement | Tumor Size | CR | PR | NC | PD | RESPONSE RATE % | CR+PR |
|---|---|---|---|---|---|---|---|---|
| 26 | without hyperthermia | $\leq$ 7cm | 2 | 9 | 4 | 0 | 73%(11/15) | 42%(11/26) |
| | | > 7cm | 0 | 0 | 11 | 0 | 0%( 0/11) | |
| 18 | with hyperthermia | $\leq$ 7cm | 0 | 5 | 2 | 0 | 71%(5/7) | 56%(10/18) |
| | | > 7cm | 1 | 4 | 6 | 0 | 46%(5/11) | |

Modified from: M.Kakehi, et al., 89

administered in three courses over four weeks. Table 12a indicates that chemotherapy combined with DSM embolization resulted in a 55% favorable response rate which was comprised of 1 CR plus 9 PR in 18 cases of hepatocellular carcinoma, and 3 MR cases along with 5 NC. A reduction of symptomatic complaints was observed in 89% (16 out of 18) of the patients. Side effects such as fatty tissue induration with pain were observed in 2 out of 18 patients. Table 12b compares response rates while taking in account tumor sizes, chemoembolization and whether or not hyperthermia was used. It is apparent that the addition of hyperthermic treatment resulted in a better tumor response rate for those tumors larger than 7cm in size. The one year follow-up survival rate after chemoembolization combined with hyperthermia was 83% while the rate for patients who received the same treatment without hyperthermia was 58%. Figure 1 represents survival curves constructed by the Kaplan & Meier method.

RECTAL CANCER: Therapeutic temperatures in the range of 41°C to better than 44.5°C were in fact achieved in this study. Table 13 indicates a 47% favorable response rate which consisted of 2 CR and 14 PR in 34 recurrent rectal tumors, with 1 MR and 14 NC. Patients in this group were treated with 1.8-2.0 Gy daily five times per week for a total of 50-60 Gy over 5-6 weeks. 1 out of 3 patients experienced pain during the hyperthermia treatment which disappeared at the end of treatment. Thirteen patients have had a follow-up period of more than 3 months after the combined treatment. A survival duration of a minimum of 3 months to a maximum of 24 months was noted. In addition, there were 9 preoperative cases with thermoradiotherapy. Each patient in this subgroup was treated with a total of 40 Gy over 4 weeks with the same daily radiation dose fractionation routine as in recurrent tumor cases. All 9 cases have achieved PR. An improvement of subjective symptoms such as pain, bleeding and disturbance of defecation was noted. There have been no observable side effects.

URINARY BLADDER CANCER: Temperature measurement in 20 patients showed that all achieved therapeutic temperatures of 42.0°C or above. Table 14 indicates a 69% favorable response rate which consisted of 2 CR and 9 PR in 16 transitional cell tumors, with 1 MR, 3 NC and 1 PD. Patients in this group were treated with a total radiation dose of 40 Gy in over 5 weeks with a fraction size of 4 Gy. Chemotherapy consisted of adriamycin $_{40mg}$, 5-fluorouracil$_{,500mg}$ pnos, cisplatin$_{50mg}$, and M-VAC (methotrexate$_{40mg}$, vincristin$_{4mg}$, adriamycin$_{40mg}$, and cyclophosphamide$_{70mg}$). Table 15 indicates the results following thermochemotherapy. A 75% favorable response rate was obtained which consisted of 2 CR and 4 PR in a total of 8 cases, with 1 each in the MR and NC categories. An analysis of the tumor stage and grade levels on all urinary bladder tumor cases in this study showed the following: 82% achieved a favorable response rate which consisted of 3 CR and 6 PR in 11 patients with T1 and T2. Of 13 patients with T3 and T4, a 61% favorable response rate occurred which consisted of 1 CR and 7 PR. With regard to histopathological types, there were only 3 PR out of 5 cases with a G1 level of malignancy, and an 84% favorable response rate in 19 patients with a G2 level, with 4 CR and 12 PR. A 50% one-year survival rate (12 out of 24 patients) following hyperthermia combined with radiation or chemotherapy has been observed. 12 cases experienced side effects such as fatty tissue induration, burns, pain and pollakisuria. 33%, or 8 patients, have shown an improvement of PS, and one case has shown a deterioration of PS.

CASE ILLUSTRATION: As shown by selected patients in Figures 2 through 6, the adjuvant use of hyperthermia with radiation therapy or chemotherapy can offer remarkable responses with select patients.

TABLE 13

RECTAL CANCER THERMORADIOTHERAPY RESPONSE

| No. of Cases | | Radiation Dose | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|---|
| 34 | Recurrent | 50-60Gy* | 8-10** | 2 | 14 | 1 | 17 | 0 | 16(47) |
| 9 | Preoperative | 40Gy* | 10 | 0 | 9 | 0 | 0 | 0 | 9(100) |

*1.8-2.Gy/frac. **Heat was given 2x/week.
Performance Status Improvement: 89% Relief of Symptom: -
Modified from: M. Kakehi, et al., 89

TABLE 14

URINARY BLADDER CANCER THERMORADIOTHERAPY RESPONSE

| No. of Cases | Radiation Dose | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 16 | 40Gy (4Gy/frac) | 10** | 2 | 9 | 1 | 3 | 1 | 11(69) |

**Heat was given 2x/week.
Relief of Symptom: Asymptomatic
Performance Status Improvement: NC
Modified from: M. Kakehi, et al., 89

TABLE 15

URINARY BLADDER CANCER THERMOCHEMOTHERAPY RESPONSE

| No. of Cases | Drugs Doses | Heat | CR | PR | MR | NC | PD | CR+PR (%) |
|---|---|---|---|---|---|---|---|---|
| 8 | $ADM_{40mg}$ plus $5\text{-}Fu_{150mg\ pnoa}$, $CDDP_{50mg}$, $M_{40mg}$-$VAC_{4mg}$ (each combination) | 10** | 2 | 4 | 1 | 1 | 0 | 6(75) |

**Heat was given 2x/week.
Relief of symptom: Asymptomatic
Performance Status Improvement: NC
Modified from: M. Kakehi, et al., 89

CASE NO. 1: A 40 year-old male with a hepatocellular carcinoma of 10.5 x 8.5 cm measured by CT received 16 courses of hyperthermia and 8 courses of adriamycin 250 mg chemoembolization using DSM. A reduction in tumor size was confirmed in angiograph. A relatively low density was observed in CT (see Figure 2). The patient is still alive with a survival period of over 16 months since the combined treatment.

CASE NO. 2: A 74 year-old female with urinary bladder cancer (T3N2MO, TCC Grade 3) was given 4 hyperthermia courses after chemotherapy with MVAC, i.e., methotrexate 40 mg, vincristin 4 mg, adriamycin 40 mg, and cyclophosphamide 70 mg. The patient has survived for over 30 months after the initial treatment (see Figures 3 & 4) and continues to do well.

CASE NO. 3: A 76 year-old male with a recurrent rectal tumor size of 12 x 8 x 10 cm in CT measurement received 6 courses of hyperthermia with a total radiation dose of 66 Gy. The patient achieved complete response (CR) status, and has now been alive for 11 months following the initial treatment (see Figures 5 & 6).

DISCUSSION

Therapeutic temperatures in the range of 41°C to better than 44°C were achieved in the majority of deep-seated tumors investigated. This study has demonstrated that the Thermotron RF-8 is a clinically safe and effective hyperthermia system for the heating of tumors. Other than tolerable pain and/or the excessive heating of fatty tissue with very obese patients who could not be cooled adequately, the heating system could be used without undue fear of adverse side effects.

Patient characteristics in this investigation consisted mostly of advanced disease status, and the nature of this study precluded conducting a concurrent study of a controlled group with radiation only or chemotherapy only. The exception to this was the chemoembolization study of hepatocellular carcinoma with and without hyperthermia. Response rates obtained by conventional treatments for the above types of patients made the justification of a randomized concurrent control group difficult.

There is ample clinical evidence showing that the adjuvant use of hyperthermia can significantly increase tumor responses to radiation for superficially located tumor (review papers 11, 15). Available reports on the clinical safety and effectiveness of an 8 MHz RF capacitive heating device known as the Thermotron have also reconfirmed such favorable tumor responses to hyperthermia combined with radiation.

Our analysis of clinical data clearly confirms previous reports (1-5, 7-10, 12-14, 16-18) indicating the safety and effectiveness of 8 MHz RF capacitive hyperthermia in treating different types of refractory tumors in visceral organ sites. A significant improvement in performance status and/or a marked reduction in subjective complaints associated with advance diseases after combined treatment is of considerable interest.

The use of hyperthermia both locally and regionally produces no additional adverse systemic effects in conjunction with radiotherapy or chemotherapy. The minor side effects noted in local target heating fields were mostly limited to transient types which soon resolved themselves. Treatments were tolerated well by the majority of patients, all of whom had advanced diseases. Additional details on this clinical investigation are expected to be reported by the investigating group in the near future. As with the development of radiotherapy, further advances in hyperthermia technology and its techniques will surely produce increasingly superior treatment results.

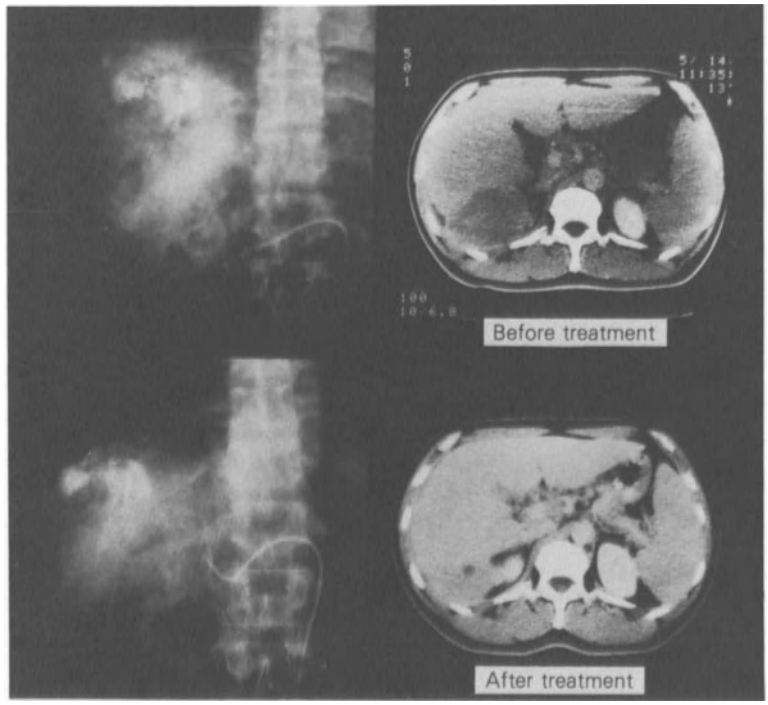


Figure 1. Patient survival curves on hepatocellular carcinoma following chemoembolization using DSM with and without hyperthermia (M. Kakehi, et al., 89).

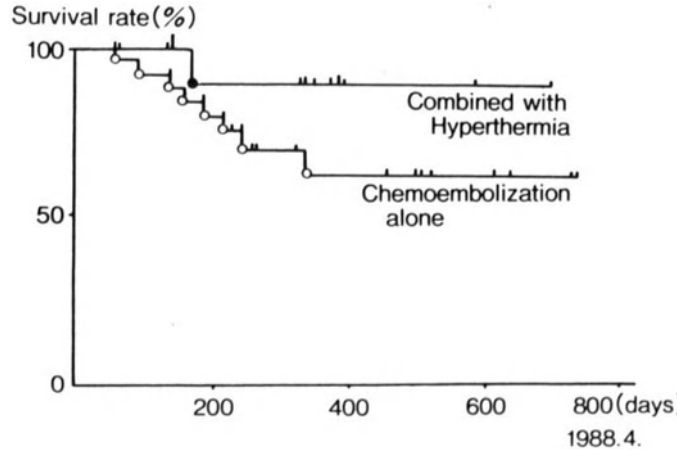


Figure 2. Angiographs and CT scans of Case No. 1 showing the hepatocellular carcinoma response following chemo-embolization using DSM and hyperthermia (M. Kakehi, et al., 89).

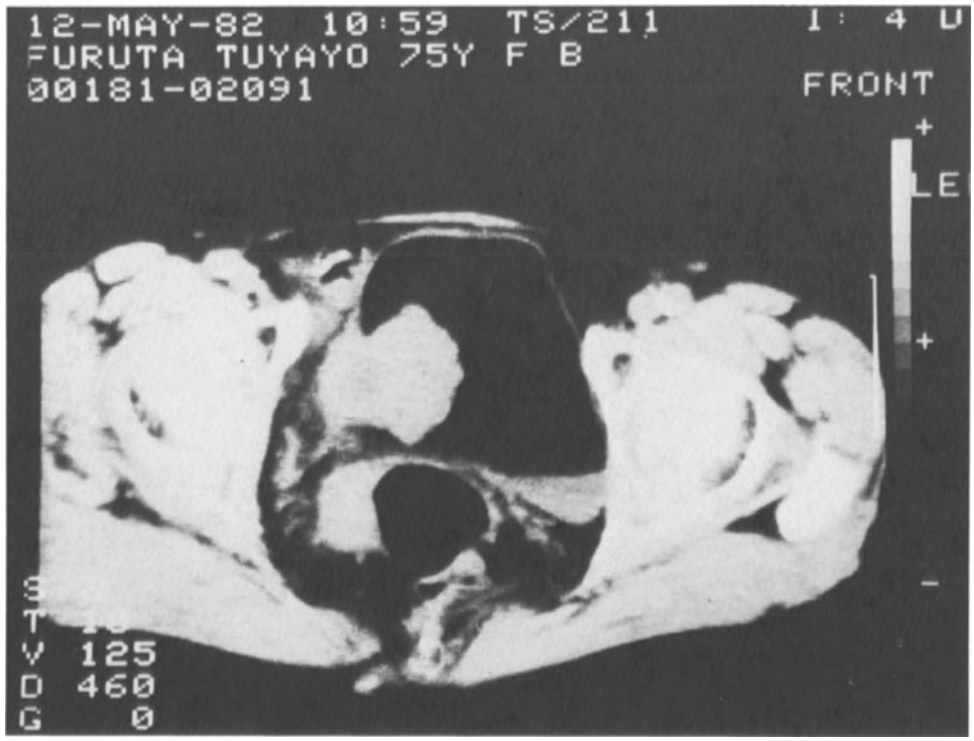


Figure 3. CT scan of Case No. 2 showing urinary bladder cancer before therchemotherapy (M. Kakehi, et al., 89).

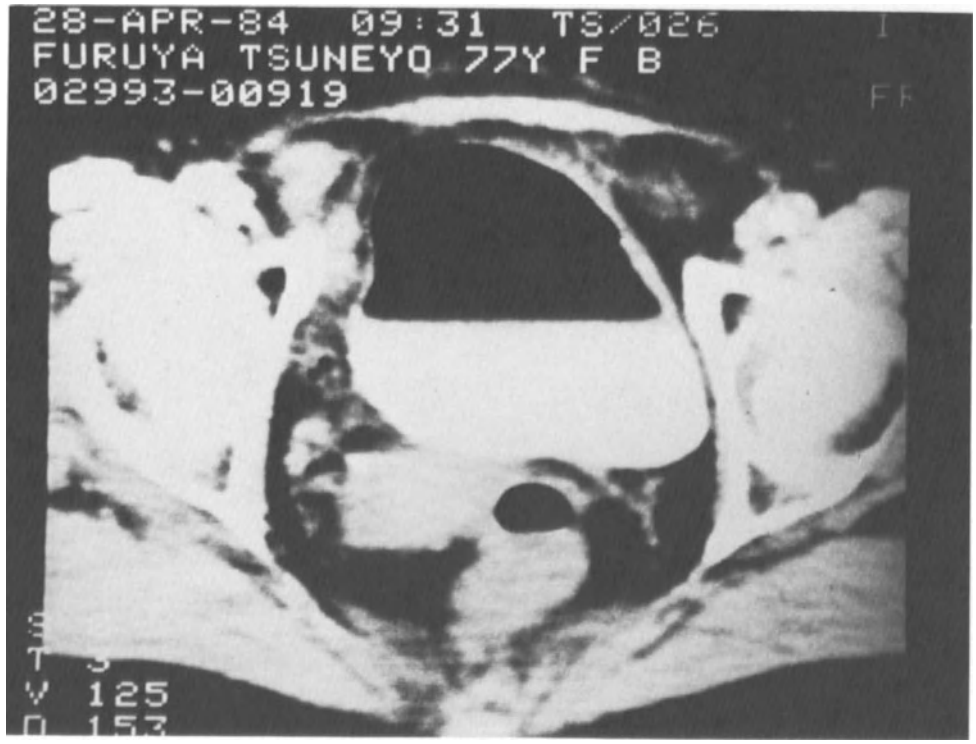


Figure 4. Case No. 2 after thermochemotherapy (M. Kakehi, et al., 89).

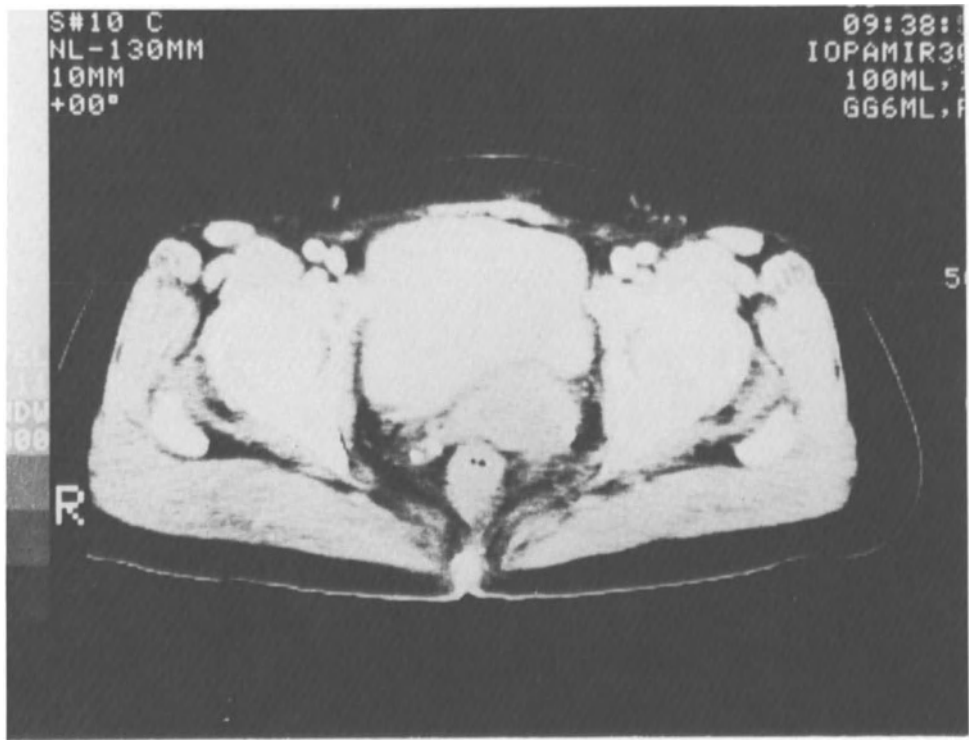


Figure 5. CT scan of Case No. 3 showing a recurrent rectal carcinoma, before thermoradiotherapy (M. Kakehi, et al., 89).

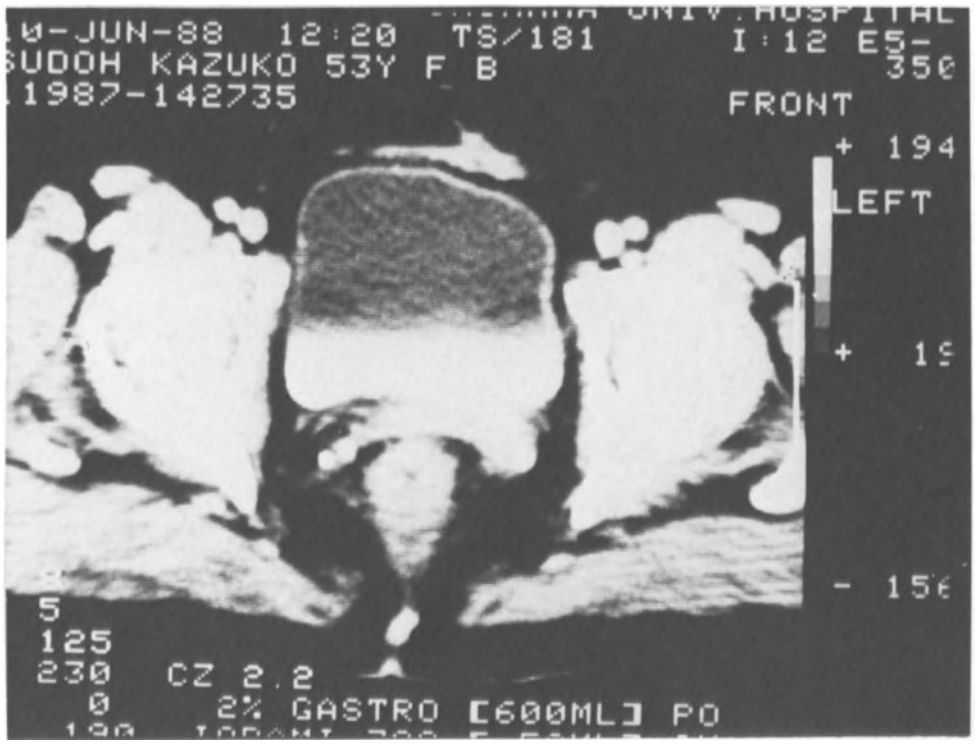


Figure No. 6. CT scan of Case No. 3 after thermoradiotherapy (M. Kakehi, et al., 89).

Currently, there are several other joint clinical hyperthermia research investigations being conducted by multi-institutional clinical hyperthermia research groups in Japan. Unfortunately, many of these reports in Japan are not available in English, which hinders the effective exchange of data to colleagues outside of Japan.

## CONCLUSION

In conclusion, controlled RF capacitive hyperthermia as applied according to protocol on those anatomic regions investigated to date reconfirms the safety of its clinical use as well as its potential usefulness in controlling malignant tumors considered to be refractory to conventional treatments alone. The relative flexibility and ease of using this hyperthermia system opens up a wider applicability for hyperthermia in cancer treatment. Therefore, the role of hyperthermia can be expected to gain greater significance in the management of tumor control in the 1990's.

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## REFERENCES

1. Abe M, Hiraoka M, Takahashi M, Egawa S, Matsuda T, Onoyama Y, Morita K, Kakehi M and Sugahara T: Multi-institutional studies on hyperthermia using an 8-MHZ radiofrequency capacitive heating device (Thermotron RF-8) in combination with radiation for cancer therapy. Cancer 58: 1589-1595, 1986.

2. Hiraoka M, Jo S, Akuta K, Nishimura Y, Takahashi M and Abe M: Radiofrequency capacitive hyperthermia for deep-seated tumors. I. Studies on thermometry. Cancer 60: 121-127, 1987.

3. Hiraoka M, Jo S, Akuta K, Nishimura Y, Takahashi M and Abe M: Radiofrequency capacitive hyperthermia for deep-seated tumors. II. Effects of thermoradiotherapy. Cancer 60: 128-135, 1987.

4. Hiraoka M, Jo S, Dodo Y, Ono K, Takahashi M, Nishida H and Abe M: Clinical Results of radiofrequency hyperthermia combined with radiation in the treatment of radioresistant cancers. Cancer 54: 2898-2904, 1984.

5. Hiraoka M, Jo S, Takahashi M and Abe M: Thermometry results of RF capacitive heating for human deep-seated tumors. In: Overgaard J, ed. Proceedings of the 4th International Symposium on Hyperthermic Oncology. London: Talor & Francis, 1984, pp. 803-806.

6. Kakehi M, Ueda K, Mukojima T, Hiraoka M, Seto O, Akanuma A and Nakatsugawa S: Multi-institutional clinical studies on hyperthermia combined with radiotherapy or chemotherapy in advanced cancers. Int J Hyperthermic Oncol: In Press.

7. Kato H, Hiraoka M, Nakajima T and Ishida T: Deep-heating characteristics of an RF capacitive heating device. Int J Hyperthermia i: 15-28, 1985.

8. Kodama K, Doi O, Tatsuta M, Kuriyama K and Tateishi R: Development of postoperative intrathoracic chemothermotherapy for lung cancer with objective of improving local cure. Cancer: In Press.

9. Lee CKK, Song CW, Rhee JG and Levitt SH: Clinical experience with Thermotron RF-8 capacitive heating for bulky tumors: University of Minnesota Experience. Radiologic Clinic of North America 27: 543-558, 1989.

10. Matsuda T, Sugiyama A and Nakada Y: Studies of radiofrequency hyperthermia (First report). Fundamental studies of Thermotron hyperthermia. J Japn Soc Cancer Ther 18: 1904-1914, 1983.

11. Meyer JL, Kapp DS, Fessenden P and Hahn GH: Hyperthermic Oncology: Current biology, physics and clinical results. Pharmac Ther 42: 251-288, 1989.

12. Nakajima K and Hisazumi H: An experimental study of temperature distribution in prostatic cancer at 8 MHz RF heating using agar phantom. Japn J Hyperthermic Oncol 1: 131-137, 1985.

13. Nakajima K, Hisazumi H, Misaki T, Naito K, Uchibayashi T, Koshida K, Ueki O, Kobashi K, Yamamoto H, Fuse H, Takashima M, Nitta M, Asari T and Saito Y: A combined treatment of 8MHz radiofrequency hyperthermia and THP-adriamycin or irradiation for bladder carcinoma. Japn J Hyperthermic Oncol 2: 383-388, 1986.

14. Onoyama Y: Studies of the combined use of hypoxic cell sensitizers and hyperthermia with radiation in human cancer therapy. IAEA-TECDOC-492, Vienna, 1989.

15. Overgaard J: The current and potential role of hyperthermia in radiotherapy. Int J Radiat Oncol Biol Phys 16: 535-549, 1989.

16. Song CW, Rhee JG, Lee CKK and Levitt SH: Capacitive heating of phantom and human tumors with an 8 MHz radiofrequency applicator (Thermotron RF-8). Int J Radiat Oncol Biol Phys 12: 365-372, 1986.

17. Yanagawa S, Sone Y, Doi H and Yamamoto G: A new procedure for the prevention of surface overheating in deep hyperthermia using RF capacitive heating equipment. Japn J Hyperthermic Oncol 1: 187-191.

18. Yoshikawa T, Oyamada H, Ichikawa H, Naito Y, Ueda S, Tainaka K, Itani K, Seto O, Sugino S and Kondo M: Antitumor effect and indication of chemoembolization using degradable starch microspheres and regional hyperthermia in patients with hepatocellular carcinoma. J Japn Soc Cancer Ther 24: 786-792, 1989.

# HEAT SHOCK PROTEINS

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The finding that tumor cells are more thermosensitive than their normal counterparts (1-4) prompted research on the effect of heat on normal and neoplastic cells. In 1970, the phenomenon of thermotolerance was described for the first time (5). Cells of L1210 leukemia after being exposed to sublethal hyperthermia (52% of BDF1 mice survivors after inoculation of $1x10^4$ L1210 cells treated for 2 hours at 42°C in Fischer medium versus 0% survivors after inoculation of same number of cells treated first at 40°C for 4 hours and afterward at 42°C for 2 hours). This result appears paradoxical. Cells that had received a more prolonged heat treatment (4 hours at 40°C + 2 hours at 42°C) survived better than cells less treated (2 hours at 42°C only). According to the nomogram used, the difference between 50% and 0% mice survival observed corresponded to a one-log difference in cell killing. It appeared that pre-treatment with heat rendered the cell capable to withstand further exposure to the same injury better than cells not pre-treated.

Many studies followed (6-8) and the phenomenon was named thermotolerance to distinguish it from thermoresistance. This distinction is important because thermotolerance is transient and not heritable. It is, in other words, a somatic phenomenon and not a mutation which induces a permanent heritable status which should be called thermoresistance - as heritable resistance to chemicals is called chemoresistance. It must be said that, although thermotolerance has been found to be a rather widespread phenomenon, present in different degrees in almost all the organisms investigated, thermoresistance is a much rarer phenomenon.

Reports of thermoresistance on tumor cells were, until now, very few and, in our laboratory, the most strenuous efforts to obtain thermoresistant tumor cell lines has consistently failed. Thermotolerance, on the contrary, is easily induced in every system tested and maintained for variable lengths of time, never surviving cell division (9). For some time, the molecular mechanism of thermotolerance remained obscure until the discovery of the production of a new class of proteins in Drosophila after heat shock (10). It was soon found afterward that the production of heat shock proteins was a general phenomenon (11-12) through all the various cell species from bacteria to eukaryotic ones, and their synthesis was correlated with the induction of thermotolerance (12-14). It was quickly found also that these proteins or HSP are present in more than one class.

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In mammalian cells, heat shock (i.e., 20-30 minutes at 43°C or a longer period of time at lower temperatures down to a minimum of at least 2-3°C above the physiological temperature of the animal) induces synthesis of four major classes of HSP designated 27, 70, 89 or 90 and 107. These designations are based on the respective molecular weight expressed in kilodaltons. A close temporal relationship has been found between thermotolerance and HSP. HSP synthesis (70, 89 and 107) is detectable immediately after heat shock and returns to normal levels as soon as the thermotolerance peaks and levels. Also, HSP levels remain high through the period of thermotolerance and drop when thermotolerance falls, disappearing with the return of thermosensitivity. Agents that induce thermotolerance were found to stimulate synthesis of HSP (15,16).

Direct proof of the causal relationship between HSP and thermotolerance has been achieved recently (17) when it was found that microinjection of monoclonal antibodies to HSP70 into fibroblasts rendered these much more sensitive to heat treatment. Control cells microinjected with other antibodies withstood the same heat treatment with no ill effects. This was confirmed shortly afterward (18) when it was observed that suppressing expression of the HSP70 gene rendered the cells so modified they were more thermosensitive. HSP are now thought to act by stabilizing cell proteins against denaturation, promoting the renaturation of partially denatured proteins and assisting other proteins in moving from one part of the cell to another (19-21), unfolding and chaperoning them (hence, the naming recently of such classes of proteins unfoldases and chaperonins).

Interest in these proteins is increasing at a tremendous rate because they appear to be involved not only in protecting cells from the effect of heat but in many other cell functions. HSP are common to almost all cells and are very similar, even in very different species (antibodies against an HSP of a bird will react readily with an HSP of the same class obtained from a shrimp). This suggests that their function(s) are very basic if they are found practically in all the animal kingdom, including bacteria and yeast.

HSP are also synthesized in response to other stimuli which are injurious to the cell as contact with heavy metals and oxygen deprivation and the interpretation of their role in such contigencies suggests again protection against protein denaturation as the most likely mechanism of action. Unstressed cells contain large amounts of HSP and it is thought that these constitutional levels of HSP are the guarantors of the normal level of cell resistance to heat and other stresses. This interpretation has been reinforced by the finding that cells not expressing normal levels of HSP are very thermosensitive (18). It will be very interesting to study the levels of HSP in bacteria thermophila which can live at temperatures of 60-70°C. It has always been very puzzling to every biologist how the proteins of such organisms manage to remain in the native state carrying on the enzymatic reactions necessary for the survival and reproduction of such bacteria. Another interesting and more practical problem is the role of the HSP in the higher thermosensitivity of neoplastic cells when compared to their normal counterparts (1-4). This phenomenon is quite widespread, being present in rodent and human cells alike, and it appears as soon as the cell is transformed (2-3). Two explanations are possible. The first is the production in the neoplastic cells of smaller amounts of HSP than in normal cells when heat shock is applied. The second is a mutation in the neoplastic HSP gene(s) which renders the protein product less effective in protecting the neoplastic cells, although the amount of HSP present is the same as in the normal cell.

Recently, very provocative results have been obtained which demonstrate binding of HSP with oncogene products (22-23). In this case, it is possible that the oncogene activated by mutation produces a protein with increased

affinity for HSP, depleting the transformed cell of it and increasing hence its thermosensitivity. Alternatively, it can be postulated that it is the level of the oncogene product which is raised in the transformed cells without any qualitative difference. The published data so far do not allow for choosing one explanation over the other (23).

It is well known that oncogenes can transform cells by both mechanisms, i.e., activation by both mutation or by product excess consequent to gene amplification or to gene overexpression. It is, however, very probable that the original observation of increased thermosensitivity of the transformed cells will soon find its molecular explanation.

There is at the present moment an enormous amount of work being done on HSPs and the literature on the subject has already reached gargantual dimensions. These studies can result in important advances for hyperthermic treatment in the near future if it is confirmed that blocking HSP activities with monoclonal antibodies or other agents results in increased thermosensitivity of the treated cells. However, this very attractive possibility still requires considerable amounts of experimental works and many hurdles to be successfully negotiated before entering the realm of practicality.

At the present, the most important practical consequence of the HSP discovery for the hyperthermia practitioner is the caveat against too closely spaced hyperthermia treatments.

REFERENCES


1. R. Cavaliere, E. C. Ciocatto, B. C. Giovanella, C. Heidelberger, R. O. Johnson, M. Margottini, B. Mondovi, G. Moricca, and A. Rossi-Fanelli. Selective Heat Sensitivity of Cancer Cells - Biochemical and Clinical Studies, Cancer 20:1351 (1967).
2. T. T. Chen and C. Heidelberger. Quantitative Studies on the Malignant Transformation of Mouse Prostate Cells by Carcinogenic Hydrocarbons In Vitro, Int. J. Cancer 4:166 (1969).
3. B. C. Giovanella, A. C. Morgan, J. S. Stehlin, and L. J. Williams. Selective Lethal Effect of Supranormal Temperatures on Mouse Sarcoma Cells, Cancer Res. 33:2568 (1973).
4. B. C. Giovanella, J. S. Stehlin, and A. C. Morgan. Selective Lethal Effect of Supranormal Temperatures on Human Neoplastic Cells, Cancer Res. 36:3944 (1976).
5. B. C. Giovanella, W. A. Lohman, and C. Heidelberger. Effects of Elevated Temperatures and Drugs on the Viability of L1210 Leukemia Cells, Cancer Res. 30:1623 (1970).
6. E. W. Cerner and M. J. Schneider. Induced Thermal Resistance in HeLa Cells. Nature 256:500 (1975).
7. E. W. Cerner, et al. A Transient Thermotolerant Survival Response Produced by Single Thermal Doses in HeLa Cells, Cancer Res. 36:1035 (1976).
8. E. W. Cerner. "Thermotolerance in Hyperthermia in Cancer Therapy." F. K. Storm, ed., G. K. Hall, Boston, Mass. (1983), pp. 141-162.
9. K. J. Henle and D. B. Leeper. Interaction of Hyperthermia and Radiation in CHO Cells: Recovery Kinetics, Radiation Res. 66:505 (1976).
10. A Tissieres, H. K. Mitchell, and U. M. Tracy. Protein Synthesis in Salivary Glands of Drosophila Melanogaster: Relation to Chromosome Puffs, J. Mol. Biol. 84:389 (1974).
11. L. McAlister and D. B. Finkelstien. Heat Shock Proteins and Thermal Resistance in Yeast, Biochem. Biophys. Res. Comm. 98:819 (1980).
12. G. C. Li and Z. Werb. Correlation Between Synthesis of Heat Shock Proteins and Development of Thermotolerance in Chinese Hamster Fibroblasts, PNAS 79:3219 (1982).

13. J. R. Subjectk, J. J. Sciandra, D. F. Chao, and R. J. Johnson. Heat Shock Proteins and Biological Response to Hyperthermia, Br. J. Cancer 45:127 (1982).
14. J. Landry, D. Berner, P. Chretian, L. M. Nicole, R. M. Tanguay, and N. Marceau. Synthesis and Degradation of Heat Shock Proteins During Development and Decay of Thermotolerance, Cancer Res. 42:2457 (1982).
15. G. C. Li and G. M. Hahn. Ethanol-Induced Tolerance to Heat and Adriamycin, Nature 274:699 (1978).
16. G. C. Li. Induction of Thermotolerance and Enhanced Heat Shock Protein Synthesis in Chinese Hamster Fibroblasts by Sodium Arsenite and by Ethanol, J. Cell Physiol. 115:115 (1983).
17. K. T. Riabowol, L. A. Mizzen, and W. J. Welch. Heat Shock is Lethal to Fibroblasts Microinjected with Antibodies Against HSP70, Science 242:433 (1988).
18. R. N. Johnson and B. L. Kucey. Competitive Inhibition of HSP70 Gene Expression Causes Thermosensitivity, Science 242:1551 (1988).
19. M. J. Lewis and H. R. B. Pelham. Involvement of ATP in the Nuclear and Nucleolar Functions of the 70KD Heat Shock Protein, EMBO J. 4:3137 (1985).
20. R. J. Deshaies, B. D. Koch, Werner, M. Washburne, E. A. Craig, and R. Schekman. A Subfamily of Stress Proteins Facilitates Translocation of Secretory and Mitochondria Precursor Polypeptides, Nature 332:880 (1988).
21. W. J. Chirico, M. G. Waters, and G. Blobel. 70K Heat Shock Related Proteins Stimulate Protein Translocation into Microsomes, Nature 332:802 (1988).
22. O. Pinhasi-Kimni, D. Michalovitz, A. Ben-Zeev, and M. Oren. Specific Interaction Between the P53 Cellular Tumor Antigen and Major Heat Shock Proteins, Nature 320:182 (1986).
23. H. W. Sturzbecher, P. Chumakov, W. J. Welch, and J. R. Jenkins. Mutant P53 Proteins Bind HSP 72/73 Heat Shock Related Proteins in SV40 Transformed Monkey Cells, Oncogene J. 1:201 (1987).

# BIOCHEMICAL AND ULTRASTRUCTURAL CHANGES IN THE HYPERTHERMIC TREATMENT OF TUMOR CELLS: AN OUTLINE

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## INTRODUCTION

Among the therapeutic strategies that have recently been proposed and are currently in use in the cancer treatment, hyperthermia is one of the most commonly employed.

The idea that heat, at temperatures above the normal physiological value, could be used in cancer therapy goes back to over a century ago from occasional observations of spontaneous tumor regressions in concurrence with high fever. From a historical point of view, it is necessary to recall that the disappearence of a histologically diagnosed sarcoma of the face was described after attacks of erysipelas with concomitant high fever (1). Similarly, the complete regression of multiple recurrent melanomas was noticed in a patient affected by erysipelas, after several days of fever over 40°C (2). In 1893, Coley suggested to artificially induce fever, by inoculating bacterial toxins, for the treatment of tumors; the regression of sarcomas in patients receiving deliberate infections of erysipelas was reported (3). Attemps were soon made to take advantage of these findings for the treatment of cancer by using locally or systemically applied heat in various forms (4-6). In 1967, long-term regression of malignant tumors in humans by hyperthermic limb perfusion was reported (7).

Considerable literature has now been accumulated on the effect of heat on tumor cells and it has been asserted that neoplastic cells are more easily damaged by supranormal temperatures than normal cells either *in vitro* or *in vivo* and that thermosensitivity is a general property of neoplastic cells, acquired with the malignant transformation (8). According to some authors, on the other hand, a correlation between heat sensitivity and malignant character cannot be assessed unambiguously (9). However, the extent of the heat sensitivity of normal and neoplastic cells is variable and depends on several parameters that generally differ for the *in vitro* and *in vivo* conditions. This fact

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makes hazardous to directly transfer information obtained from in vitro experiments to the in vivo treatment of tumors.

In in vitro conditions, tumor cells show reduced survival when exposed to temperature values between 40 and 43°C: in this range, the effect of heat increases with the temperature increase. The selectivity of tumor cells' sensitivity to heat, as compared to normal ones, appears to be reduced at higher temperature values, so that it becames impractical to employ temperatures too much higher than those considered normal for the tissue or cell culture or living organism being studied. In fact, excessive heat damages normal cells so fast that it is unfavourable to use such extreme conditions both in the in vitro experiments and the in vivo therapy. Furthermore, it is worthwhile mentioning that the sensitivity of cells to heat also depends on the time of exposure; in fact, after a long exposure the cytotoxic effect of heat is no longer proportional to the duration of the heat treatment (9). Thermosensitivity of tumor cells varies also in dependence of the phase of their mitotic cycle. Cells in S phase are more sensitive to hyperthermia (10). The pH value of the culture medium can also affect the survival of tumor cells exposed to hyperthermic conditions. In fact, cells maintained under acidic conditions exhibit enhanced sensitivity to the heat damage (11).

Moreover, the different cellular responses to heat have been shown to depend also on the modalities of exposure to high temperature. In fact, heat may induce thermotolerance in the tumor cells, i.e. temporary resistance to subsequent reheating. The kinetics of thermotolerance depend on the extent of the initial treatment. The greater the effectiveness of the initial treatment, the greater the maximum effect of thermotolerance (12-15). On the other hand, when cells are heated at approximately 43°C or above, and then reheated at 42°C or below, then the treatment at low temperature is more effective than if given alone, a phenomenon known as step-down sensitisation. The step-down effect rises immediately after the first heating and decays rapidly in relation to the time course of induction and decay of the thermotolerance (16).

Elevated temperature affects tumors both in vitro and in vivo. In the latter case, however, other factors are also active, among which immunological reactions and tumor vascularization appear to be significant.

Immunological phenomena are probably involved to explain the therapeutic effect of hyperthermia, but only in a narrow range of temperature (17). It was demonstrated that Ehrlich ascite cells increased their immunogenicity with consequent inhibition of tumor growth in mice inoculated with viable cancer cells when heated at 42.5°C for 3 h. If the incubation was extended to longer times (6 h), the increase of immunogenicity was absent (17). The above mentioned relationship between heat and immunogenicity can explain the different result obtained when experiments are performed under in vivo or in vitro conditions. In fact, as previously found, biochemical damage is only one of the

parameters to be taken into account for explaining the heat sensitivity of tumors. It is a matter of fact that often the in vivo treatment gives better results than the in vitro experiments. A further evidence of the correlation between heat and immunity is given by the decrease in the growth rate of both the primary tumor and metastases observed when only the primary tumor was heated, which strongly supports the hypothesis of an heat-inducible enhancement of host's immunological response (18).

Tumors up to growth stage of $10^6$ cells are mostly avascular, and nutrients and oxygen are supplied by diffusion which leads to conditions of acidosis. If on one hand this situation suggests favorable conditions for the therapeutic effect of hyperthermia, on the other hand the non homogeneous blood perfusion in the tumor can rarely result in an uniform distribution of temperature when heat is applied (19). In this context, great importance is turned to the resolution of the technical problems related to the production and application of heat in in vivo conditions (20).

After exposure of mammalian tumor cells to high temperature, a great number of different morphological and physiological alterations may be observed, although it is not yet clear whether the changes observed are involved in the process of cell killing or they may on the opposite have a protective effect. Ultrastructural studies have demonstated variations in the morphology of almost all subcellular structures, while functional studies have evidenced changes in intracellular salt concentration and in metabolic pathways such as protein and nucleic acid synhteses, DNA replication and respiration . Glycolysis is probably not depressed at least during the first time of exposure at high temperature (21). As a consequence, it is difficult to ascribe to a specific cellular component the responsibility for the cellular sensitivity to high temperature; it appears instead plausible that concomitant phenomena triggerd by heat and related each other may be involved.

However, a key role in the cellular response to heat might be ascribed to proteins. In fact, the activation energy for thermal denaturation of several proteins lie in the same range of temperatures as that employed in the hyperthermic treatment which in turn corresponds to that for cessation of cell growth and cell viability (9). A direct damage to the nucleic acids, and particularly DNA, of tumor cells caused by heat doesn't appear to be probable; in fact the melting point of DNA is generally, at physiological pH, over 50 °C (22). Furthermore, the direct involvement of lipids seems less probable since no lipid phase transition has been found in the range of the hyperthermic temperatures critical for cell survival (23).

## HYPERTHERMIA AND THE PROTEINS INVOLVED IN THE NUCLEAR FUNCTION

When tumor cells are exposed to hyperthermia, changes in the nuclear structure and function have been observed. An irreversible inhibition of the incorporation of $^3H$ thymidine

into DNA of about 95% has been described in Novikoff hepatoma after 2 h of incubation at 42°C or 30 min at 43 °C, as well as inhibition of the incorporation of $^{3}H$ uridine in RNA (24). Furthermore, exposure of HeLa cells to hyperthermia between 43 and 48°C has been shown to result either in the inhibition of replicon initiation and DNA replicative elongation or in reduced elongation rate of initiated replicons (25). As a consequence, in replicating DNA, single-strand nicks can occur which may represent the molecular lesions responsible for the chromosome aberrations produced to some extent in all cells by heat (26,27) . On the other hand, in the S phase a log-linear correlation has been reported between the extent of chromosome lesions and cell inactivation (26). Other than DNA replicative processes, also DNA repair synthesis was observed to be affected by heat. In fact, after exposure to 42.5 °C of Chinese hamster V79 cells, significant inhibition of DNA repair synthesis following exposure of cells to UV irradiation, which causes dimerization of adjacent pyrimidine residues, has been reported (28). Furthermore, heat has been reported to inhibit the repair of X-ray-induced DNA damage (29,30). A protein mechanism involved in the inhibition of DNA replication and/or repair observed during hyperthermic exposure may be represented by heat denaturation of replicative enzymes. DNA polymerase B has been shown to be damaged by heat treatment (31) but, according to some authors, the loss of polymerase activity after heating is not correlated with the cell death . In fact, when acute or chronic thermotolerance has been induced in CHO cells by single or combined heat exposure, the thermal sensitivity of polymerase B was lowered while step-down heating of the cells doesn't alter the sensitivity of polymerase B to the second treatment (32). Also polymerase A, enzyme involved in the repair of X-ray induced DNA damage, is partially inactivated by heat, but doesn't appear to play a major role in the observed repair inhibtition. In fact, when HeLa cells are used to investigate possible mechanism of inhibitory action of hyperthermia on DNA repair, differential inhibitory kinetics of heat and aphidicolin, an inibitor of polymerase A, are observed on DNA repair and no full additivity of the respective effects has been reported for various combinations of these agents (33).

On the other hand, a study performed on CHO cells showed that hyperthermic treatment at 45.5 °C induces non-specific increase, proportional to the heat treatment duration, of high molecular weight non-histone proteins isolated with DNA (34). Furthermore, when HeLa cells are heated at 45 °C, the nuclear matrix protein mass has been observed to increase linearly increasing the exposure time to heat, and an exponential relationiship is observed between heat-induced cytotoxicity and nuclear matrix protein mass increase (35). Alterations in the nuclear matrix protein mass has been also shown to be correlated in a log-linear manner with the inhibition of DNA single-strand-break repair (36). On the other hand, the nuclear matrix has been proposed to form scaffold on which DNA is organized into structural and funtional domains (37). Hyperthermic alterations of this structure might lead to inhibition of nuclear function (semiconservative replication

of DNA, DNA damage repair and DNA transcription into RNA and / or RNA processing) by physically obstructing the replicative enzymes or by altering the state of DNA supercoiling. Indeed, replicon initiation is associated with the nuclear matrix and requires the maintenance of DNA supercoiling or the ability to change it (38). Thus, inhibition of replicon initiation may result from the absorption of proteins at the sites of replicon initiation and this process may be inhibited until some or all of them are removed. The involved proteins may be the above described non-histonic ones, or the result of a redistribution of nuclear and cytoplasmatic proteins onto the cell nucleoskeleton from other cell compartments.

## HYPERTHERMIA AND THE HEAT SHOCK PROTEINS

Besides changes in DNA synthesis and repair, hyperthermic treatment has been shown to determine complex modifications also in RNA and protein syntheses. When Novikoff hepatoma cells were exposed at 43 °C for 2 h, inhibition of incorporation of both $^{3}H$ uridine in RNA and $^{14}C$ amino acids in proteins of about 88% and 95%, respectively, was reported (24). Furthermore, the rate of total protein synthesis has been shown to decrease rapidly upon shifting the incubation temperature of HeLa cells from 37 to 42 °C, but the synthesis of proteins recovered during continued incubation at 42°C or when the cells were transferred back to 37°C (39).

In numerous works, the recovery of protein synthesis has been reported to be due to the synthesis of a specific set of proteins called heat shock proteins, hsps (40,41). However, the induction of hsps is a general phenomenon obseved in almost all organisms and cultured cell lines after exposure to sublethal temperature as well as to other stimuli which are injurious to the cells, such as recovery from hypoxia , contact with heavy metal, ethanol, etc. (42).

In eukaryotic cells the heat shock proteins, identified by SDS-polyacrilamide gel electrophoresis, may be classified in four major groups designed 16-28, 69-70, 80-90, 110, based on their respective molecular weights expressed in kilodaltons. Hsps of the same size are so similar each other in the diffent cell types and organisms that antibodies against a particular heat shock protein from one species usually cross-react with similar proteins from phylogenetically distant species. In particular, the hsps with molecular weight around 70 and 90 Kd seem to be the most highly conserved in nature. However, some variability among the species exists as regards the electrophoretical mobility of hsps, and for each class of hsps several isoforms may be recognized (43).

Each specific temperature value reached during the heating process induces the expression of a specific hsp pattern. In <u>in vitro</u> conditions, the pattern of hsps induced in tumor cells has been reported to change in dependence of the different cell lines, culture conditions, modality of hyperthermic treatment. Although the hsps are generally expressed also in normal cell and/ or in normothermic conditions, qualitative and quantitative differences may be

observed in tumor cells. HeLa cells heated at 42°C for 2 h or 45°C for 10 min show an increase in protein synthesis of 70, 73, 78, 85, 92, 105 Kd, expressed at a reduced level under normal growth conditions. However, an additional protein with molecular wheigh of 90 Kd has been found to be produced only at 42°C, but not at 37°C nor at 45°C. Although the kinetics of the induction of this protein is the same as one of the major heat shock proteins, the 70 Kd one, the 90 kd protein seems to undergo a regulatory mechanism different from the other heat shock proteins. In fact, although a variety of agents such as ethanol, arsenite, $CuSO_4$ , puromicyn, canavanine etc. induce the other heat shock proteins, the 42°C specific protein is not induced by such treatments (44). Furthermore, differential heat shock response has been reported for primary human cell cultures and established cell lines. After hyperthermic treatment, an increase of hsp 70 synthesis of about 20-25 fold has been observed in established cell lines SV80 and Ht-1080, respectively, while the increase varied from 7-14 fold in primary cell cultures, fibroblasts F2300 and Meningioma T2300. In addition to hsp 70, a new protein called T 70 and characterized by similar size , appeared in 42°C cell line while it was completely absent in primary cell culture (45).

When the hsp induced synthesis was studied in cultured lung cells, the synthesis of 70 Kd protein was shown to be markedly stimulated in normal, malignant and SV40-trasformed cells, while 90 and 100 Kd proteins were demonstrated to be stimulated only in malignant and SV40 transformed cells. Morever, different heat exposure conditions are reported to be necessary for induction, i.e, 43°C for 1 h for normal cells and 41°C for 1 hour for malignant cells (46).

On the other hand, some works indicate that there are few differences between normal and tumor cells. In fact, a study on the heat shock response of a syngenic rat mammary adenocarcinoma cell clone and a rat lung endothelial cell clone reports few differences in heat shock protein synthesis after exposure at 42°C for 1 h. In fact, the hsp groups having greater molecular weight have been shown to be comparable in the clones studied. However, some apparent differences in synthesis rate of hsp between the two cell types have been observed and they might have a key role in the phenotypic differences between these cells (47).

Despite the induction of hsps by heat is a well characterized phenomenon, although not very specific, the biological role of these proteins is unclear. Taking into account that synthesis of hsp is observed in response to different enviromental insults (42), the involvment of hsps in protecting cell from damage in adverse situations may be assumed. In the *in vivo* hyperthermic treatment of rats it has been reported a marked decrease in photoreceptor degeneration when their retinas were exposed to bright light as compared to the animals kept in normothermic conditions. Such protection has been reported to be concomitant with retinal synthesis of three types of heat shock proteins (48). A protective role of hsps also seems supported by a good correlation established between the induction of the elevated synthesis of the hsps and the development of thermotolerance (49), in addition to the isolation and

characterization of heat-resistant variants of mammalian cells that express elevated level of hsps (50). Moreover, a brief incubation at 45°C has been reported to be lethal to fibroblasts after microinjection of antibodies against hsp 70, indicanding that functional hsp 70 is required for survival of these cells during and after thermal stress (51). Furthermore, suppressing the expression of hsp 70 gene has been shown to make these modified cells more thermosensitive (52).

The correlation between induction of hsps and development of tolerance to subsequent thermal shock contrasts with the above mentioned correlation between cytotoxicity and heat-induced increase in nuclear matrix-associated proteins (35). However, a possible concatenation can exist. In fact, acute heat shock might result in a redistribution of critical cell proteins and their absorpition onto the cell nucleoskeleton (35). The subsequent functional sequestration of these proteins results in cytotoxicity. The elevated synthesis and replacement of a similar set of proteins which occur during milder heat stress produces a thermotolerant state. This speculation is supported by the observation that a specific set of polypeptides with molecular mass similar to the HeLa hsps have been observed to become tightly associated with the cell's nucleoskeleton during acute thermal shock (53). On the other hand, a role of hsps in stabilization of the structure of proteins involved in metabolic processes critical for cell survival has been suggested (54).

Hsps might act by stabilizing cell proteins against denaturation, promoting the renaturation of partially denatured proteins or assisting other proteins in moving from one part of the cell to another. Hsp 90, together with an uncharacterized phosphoprotein, have been reported to interact with a membrane protein , pp 60, in a complex soon after its synthesis (55). After the dissociation of this complex, pp 60 is activated and inserted into the plasma membrane in a concerted manner (56).

However, a role of hsp in cell growth and differentiation has also been suggested. In mouse embrional carcinoma cells, differentiation promoted by retinoic acid or the turn to blastocyst stage has been shown to be accompanied by the synthesis of hsp 68 and hsp 115, proteins that were not induced by heat treatment (57).

## HYPERTHERMIA AND MEMBRANE PROTEINS

After heat treatment in the range of 41-45 °C, wide alterations are observed in the cellular membranes, which serve central roles in maintaining the biochemical activity of the cells. It is unclear, however, if membranes represent the primary target of heat damage or rather the damage is a consequence of events triggered in different cellular sites.

The morphological and physiological alterations in the plasma membrane are well characterized and include changes in cellular surface morphology, surface charge, attachment of cells to substrate, binding of growth factors, permeability (58).

The formation on the plasma membrane of projections, blebs, ruffles, nodules or invaginations is usually observed in the heat damaged cells (59,60). An electron microscopy study, carried out on Chinese hamster V79 fibroblasts has shown, after heat treatment at 42-45°C for variable times, the heat-dose-dependent occurence of remarkable interruptions in the plasma membrane as well as in the nuclear membrane (61).

After exposure at 42°C for 15 min the electrophoretic mobility of mastocytoma cells has been shown to decrease, recovering the normal value after incubation at 37°C for 24 h . However, when prolonged hyperthermic exposure (60 min to 42°C ) is used, no recovery occurres (62). The decrease of the surface charge density, after hyperthermic exposure (43 °C), has been established also for two leukemic cell lines (63). Moreover, in L fibroblasts the alteration in charge distribution seems to affect the attachment of the cell to the substrate (64). Changes in membrane potential have been observed: hyperthermic exposure (38-42°C) for 1-2 h has been reported to bring about 6-15 mV depolarization of the plasma membrane of normal hamster lymphocytes and a 2-6 mV hyperpolarization of tumor cells (65).

High temperature may also affect the membrane permeability so that normal transmembrane gradients of ions and metabolites are disturbed. Heat causes an immediate and very pronounced( but reversible) increase in the rate of passive diffusion of low molecular weight substances through the membranes (66). Treatment at 40-42 °C irreversibly modifies the potassium-dependent trancellular migration of glutamate of Novikoff hepatoma cells (67). An increase of $^{3}H$ uridine permeability in RK-13 cells after incubation at 42°C for 1-3 h, as well as the release of radiolabeled proteins from 3T3 cells are observed (68,69). In suspensions of mastocytoma P815 cells, the content of potassium and chloride ions , measured by radioisotopic technique, has been shown to decrease when temperature is raised from 37 to 43 °C with a concomitant increase in the proton concentration: the decrease of the ions concentration, particularly potassium, and the decrease in cellular pH have been proposed to affect the cell survival (70).

When the measure of the intracellular concentration of free chloride and potassium ions is performed in murine neuroblastoma cells, reduced intracellular $K^{+}$ and $Cl^{-}$ concentrations are observed only in a small number of cells that had a distinctly mottled morphology and included the trypan blue, while no significant changes has been detected in the cells that exclude the dye, even when clonogenic survival is less than 1 %. The results obtained seem to indicate that the perturbation of the ion content is not the mechanism by which hyperthermia initiates reproductive cell death , but a consequence of it (71).

Moreover, the function of some cellular membrane proteins has been reported to change after exposure to heat. Functional membrane protein damaged by heat may be represented by the membrane receptors. A lowered affinity for insulin and epidermal growth factor has been shown, although the different receptors appear to be differently

affected by heat; in fact, the binding of epidermal growth factor to CHO cell has been shown to be less sensitive to heat than insulin binding (72,73). Other important proteins reported to be affected by heat are particular membrane ATPases . The ability of the Ca-ATPase of sarcoplasmic reticulum to transport calcium has been shown to be reduced at 40-45°C (74), and a redistribution of cellular calcium may occur resulting in important cellular events (75). In fact, calcium ion is biologically very active, acting either directly on as a second messanger in most biochemical processes (76). Hyperthermia at 43°C has been reported to cause also modification of membrane Na-K ATPase, leading, at least in part, to the decrease of $^{86}Rb$ transport in rabbit kidney RK-13 cells (68).

The molecular mechanism by which hyperthermia can affect the membrane function is yet unclear, taking into account that it is difficult to distinguish between direct effect due to the proteins, to the lipids or effect dependent to lipid-protein or protein-protein interactions.

In numerous works a key role of the membrane's proteic components has been proposed. The hyperthermic conditions that affect the cell survival have been reported to determine changes in the protein conformation rather than in the lipid structure. In fact, mithocondria and whole cell homogenates of different cultured Chinese hamster cell lines, studied using either an electron spin resonance label or a fluorescent probe, have not shown the existance of lipid transitions at a temperature (40-41.5°C) which affects the cell survival, whilst the irreversible transition in protein structure or arrangement has been observed (77).

Moreover, in a quantitative freeze-fracture structural study performed on the plasma membrane of Chinese hamster V 79 fibroblasts after exposure of the cells at 43°C for 1 h, an increase in density and size of intramembranous particles, elements assumed to represent the membrane proteins, has been shown. A rearrangment and /or unfolding of membrane proteins, a greater rate of membrane protein synthesis as well as the apposition on the membrane of newly synthesized proteins might be responable for the observed phenomenon (78).

On the other hand, changes in the synthesis of lipids are reported to occur in V79 Chinese hamster cells. Subtoxic heat treatment seems to determine an increase of cholesterol and a decrease in the phospholipids during the 24 h period after the heat treatment (79). This variation in lipid composition might represent the adaptative response to heat. In fact, hyperthermic treatment has been reported to increase the fluidity of membranes (80). In general, increased concentration of cholesterol and saturated fatty acid in lipids rigidify membranes, while increased levels of unsaturated fatty acids in lipids increase membrane fluidity. Phospholipid head groups have also significant effects on acyl chain motion (23). However, the lipid fluidity *per se* is most probably of little importance for the cell, but it becomes important for the stabilization of the protein conformations. In fact, the lipid composition of cellular membranes has been reported to affect the heat

sensitivity of mammalian cells in a manner dependent on the proteins. A correlation between the lipid (both cholesterol and phospholipids)/protein weight ratio and the increased resistence of mammalian cultured cells to elevated temperature has been suggested, taking into account the fact that the cell lines which are more resistant to hyperthermia-induced cell killing contained both more cholesterol and more phospholipid relatively to protein in particulate fraction (81).

The importance of lipidic component is, however, substantiated by the observation that changes induced in fatty acid composition can affect the cellular response to heat. Changes in the fatty acid composition can be obtained in cultured cell lines by adding a specific fatty acid to the culture medium. The type of change induced in fatty acid pattern of a cell and the effect on its thermosensitivity are, however, reported to be dependent on the fatty acid used as a consequence of the different degree of fluidity induced (82).

The possibility to enhance cell thermosensitivity by altering lipid composition may have a therapeutic imortance. Modulation of tumor membrane lipid composition can be performed even *in vivo* by varying the diet. The sensitivity of mice solid tumors to local hyperthermic treatment has been reported to increase by feeding the animals a diet enriched in linoleic acid (83). In this context an enhancement of hyperthermic effect has been observed after liposomal vesicle treatment of a cultured cell line (84). In fact, when cultured M14 melanoma cells were treated at 42.5 °C for 3 h after a preincubation with empty multilamellar liposomes contained 1-$\alpha$- dipalmitoylphosphatidylcholine, the plating efficiency was found to be decreased significantly. Research on the mechanisms by which empty liposomes can affect *per se* the response of cells to hyperthermic conditions are in progress in our laboratory.

## HYPERTHERMIA AND THE PROTEINS INVOLVED IN THE OXYGEN FREE RADICAL PRODUCTION

Among the proteic functions inside the tumor cells that can be involved in the cellular response to heat shock, particular emphasis should be turned to the enzymes involved in the production and scavanging of the oxygen free radicals. In fact, the increase of the steady state concentrations of the superoxide radical $O_2^-$, hydrogen peroxide $H_2O_2$ and the more reactive hydroxyl radical $OH^{\bullet}$ (that is formed by $H_2O_2$ and $O_2^-$ in Haber-Weiss rection catalyzed by transition metal ions), could be responsable for the cellular damage and death consequent to hyperthermic treatment. These products of the partial reduction of molecular oxygen are known to interact with various cell components leading to inactivation of enzymes, degradation of DNA and polysaccharides and induction of lipid peroxidation (85).

Hyperthermic treatment may be supposed to determine the conditions for increasing the oxygen radical flux affecting, by means of a complex cascade of events, the rate of radical production at the mitochondrial and microsomal redox chains level as well as at the level of oxidases and oxygenases.

Generally, free $O_2^-$ and $H_2O_2$ can be detected in mitochondria when electrons bypass the terminal acceptors of the redox chains and directly reduce $O_2$ via a single-electron step. At the stage where single-electron transfer metals (Fe,Cu) and semiquinones (flavin, Coenzyme A) are involved, single-electron transfer to oxygen may occur (85,86). Heat shock could promote the mithochondrial production of oxygen radicals probably by disrupting the organization of the electron transport assemblies of the membrane. In this context, modifications in the structural and functional characteristics of the mithochondria have been reported by different authors. On the other hand, mithocondrial modifications can be induced also as a consequence of heat damage to the cytoskeletal components (87), but also in this case an involvement of oxy radical in disruption of the cytoskeleton has been suggested (88).

Ultrastructural studies have shown that heat can induce mitochondrial morphological changes, similar to the modifications produced by unclouplers of oxidative phosphorilation (89). In Chinese hamster V 79 fibroblasts after 1 h of treatment at 42°C, the mitochondrial cristae appear dilated and the mitochondria swollen; the intracristal spaces appear enlarged after 1 h of treatment at 43°C and very irregular and dilated cristae are exhibited after exposure at 45°C for 1 h (61). Furthermore, in Chinese hamster spheroids incubated at 42°C, an initial but short increase in oxygen consumption, followed by a precipitous decline, has been shown to occur (90). A selective and irreversible damage to the respiration was observed in human and experimental tumors. In Novikoff hepatoma cells, for example, after 1.5 h incubation at 42 °C, about 75 % inhibition of oxygen uptake was observed (21), whereas in minimal deviation hepatoma cells a longer incubation time is necessary for observing the inhibitory effect (7). It should be pointed out that in regenering liver cells, used as normal control tissue, only less than 20% inhibition was observed, in the same experimental conditions. Absence of selective heat sensitivity of respiration has been observed in tumor acellular preparations (21). On the other hand, probably due to the different experimental conditions employed, in the isolated mitochondria of Ehrlich ascite tumor cells the rate of oxygen consumption and the redox state of the respiratory carriers has been reported to be affected by heat, the cythochrome segment being indicated as the most heat-sensitive part of the respiratory chain (91). The heat in a range of 41-45°C has been reported to uncouple oxidative phosphorilation of mouse liver mithocondria and, in this case, the succinate-citochome c site seems to be more sensitive to high temperature than citochrome c-oxygen site (92). Moreover, when evaluating the cyanide-resistant respiration as an upper-limit measure of intracellular production of $O_2^-$, it has been reported that it rapidly increases in rat lung slices under heat shock and remains elevated for 1h of exposure of the tissue at 41°C (93).

The rate of production of $O_2^-$ and $H_2O_2$ can also be affected by heat at the level of the oxidases. In this regard, a special emphasis should be given to the oxidative degradation of hypoxanthine to xanthine, and of xanthine to

urate by xanthine oxidase. In fact, as a consequence of insufficent ATP production due to decreased respiration, an increase of cytosolic $Ca^{2+}$ may occur, which in turn activates a protease capable of converting xanthine dehydrogenase to the oxidase. Concomitantly, depletion of cell ATP results in an elevated concentration of AMP, which is then metabolized to adenosine, inosine, hypoxanthine and xanthine (94).

Other possible sources of $O_2^-$ could be represented by cyclooxygenase and lipooxygenase, since hyperthermia has been reported to increase phosphatidylinositol I turnover and to release arachidonic acid, the substrate for cyclooxygenase and lipooxygenase (95).

An accelerated rate of production of the oxygen free radicals could also result as consequence of the increased rate of enzymatic activity of amine oxidases on polyamines, which are organic polycations present at high concentrations into the cells and, at particularly high levels in cancer cells. These molecules are known to enhance the cytotoxic effect of heat when added to the medium of cell cultures (96). Although a model has been proposed for polyamine modulation of hyperthermic cytotoxicity according to which they may interact with targets on the outer membrane surface , evidences have been accumulated suggesting that the oxidation products of polyamines produced by amine oxidases (aldehydes and hydrogen peroxide) may be responsable for their toxic effect on tumor cells (97). In fact, the addition of diamine oxidase (DAO) to Ehrlich ascite cells strongly enhances the cytotoxic effect of heat (98). Immobilized DAO injected intraperitoneally into Ehrlich ascite tumor bearing mice, inhibited tumor growth (99).

Although the production of free radicals is dependent on $O_2$ availability, a role of oxy radicals in the killing effect of heat is possible even under hypoxic conditions. In fact, the uncoupling of oxidative phosphorilation can occur in mitochondria even at low oxygen tension (100) and, on the other hand, Km values for oxygen around 24-240 uM are known for xanthine oxidase, while the oxygen tension, even under hypoxic condition, ranges from 1-100 uM (101).

Against oxidative processes mediated by oxygen derived free radicals, cells have evolved some systems of protection. In particular, antioxidant enzymes such as superoxide dismutases (SOD) contained in both mitochondria and cytosol which catalyze the dismutation of $O_2^-$ to $O_2$ and $H_2O_2$ , catalase in the peroxisomes (CAT) which metabolizes $H_2O_2$ to $O_2$ and $H_2O$ , and glutathione peroxidase (GSH-Px) that is present in the cytosol and which, by oxidizing glutathione (GSH), metabolizes $H_2O_2$ or organic hydroperoxide. The catalytic function of the these three enzymes in the cells is concerted so that catalase and gluthatione peroxidase match the $H_2O_2$ production derived from $O_2^-$ dismutation (102).

In the tumor cells, high steady state concentration of heat-induced oxygen free radicals might be sustained by the characteristic pattern of the antioxidant enzymes . Low activity of either the cytoplasmatic Cu-Zn SOD or the mitochondrial form containing Mn with respect to their

normal counterparts have been found in tumor cells (103). Similarly, also catalase and gluthatione peroxidase activities were found to be low (104,105).

On the other hand, also the metabolic ratio between the formation of $H_2O_2$ by SOD and its removal by GSH-Px has been claimed to be important in determining the resistence to intracellular oxidative stress. In fact, Ehrlich ascite cells that have a GSH-Px/SOD ratio much lower than normal controls have been demonstarted to be extremely sensitive to intracellular and extracellular $O_2^-$ and $H_2O_2$ flux (106,107).

In cloned normal mouse embryo cells and their Simian virus 40-transformed derivatives, a correlation between low levels of antioxidant enzymes and thermosensitivity has been demonstarted (108). The transformed cells that have been shown to possess undetectable Mn SOD and markedy low level of Cu-Zn SOD, CAT and GSH-Px activities are selectively killed by exposure to lethal hyperthermia, whereas the normal cells, having significantly higher enzyme activities, have been demonstrated to be resistant to hyperthermic injury. On the other hand, in both cell types the hyperthermic effect appear enhanced after a pretreatment with diethyldithiocarbamate (DDC), an inhibitor of Cu-Zn SOD. Chinese hamster cells have also been reported to be sensitized to heat by treatment with DDC at doses that don't affect cell viability in normothermic condition (109).

Furthermore, it has been reported that GSH-depleted-cells are more sensitive to heat shock (110,111). This finding might support the involvment of oxygen radicals in the cytotoxic effect of hyperthermia, taking into account that GSH plays an integral role in the maintenance of the cellular redox state and detoxification (112).

Fluxes of superoxide or other oxygen free radicals, induced by treatment of the cells with redox active compounds are known to increase the intracellular SOD level. These SOD-enriched cells seem to be protected against subsequent exposure to the free radical-producing agents (113, 114). An induction of SOD after hyperthermic treatment has been reported, suggesting a correlation with the development of thermotolerance. In CHO and ovarian carcinoma cells, a rise in Cu-Zn SOD activity was reported after heating to induce thermotolerance (115). An increase of the synthesis of Cu-Zn SOD has been observed also in rat lung either *in vitro* or *in vivo* (93). The induction of the antioxidant enzymes has been reported to require different times with respect to the induction of heat shock proteins. On the other hand, while the induction of heat shock proteins involves *de novo* trascription and selective translation of the respective mRNA species (41), the increased synthesis of Cu-Zn SOD by lung appears to be mediated by posttrascriptional events (93). Sublethal hyperthermia has been reported to cause an induction of CAT, GSH-Px Cu-Zn SOD also in mouse embryo cells but several hours seem to be necessary for the induction (108).

CONCLUSION

The results herewith reported indicate that tumor cells

are selectively damaged when heated under appropriate conditions of time and temperature.

"Selectively" means that the heat-induced damage provoked in normal control cells has a much lower extent or is even practically absent, under the same experimental conditions with respect to the cancer cells.

A specific biochemical target of the hyperthermic damage has not been so far identified, occurring most probably a simultaneous concurrence of different causes.

Anyway, concerning the in vivo applications of hyperthermia as a therapeutic technique for cancer treatment, it should be recalled that the effect of eventual biochemical lesions provoked by heat on the impairment of the cell survival should be considered in addition to other secondary physiological modification involving the cytotoxic effect of heat. Such mechanisms include, among the others, enhancement of immune response and factors involving the vascularization of tumor tissue.

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REFERENCES

1. W. Bush, Uber den Einfluss welchen Heftigere Erysipeln Zuweilen auf Organisierte Neubildungen Ausuben, Verhandl Naturh. Preuss. Rhein. Westphal. 23: 28 (1866).
2. P. Burns, Die Heilwirkung des Erysipels auf Geschwulste, Beitr. Klin. Chir. 3: 443 (1887).
3. W. B. Coley, The treatment of malignant tumors by repeated inoculations of erysipelas-With a report of ten original cases, Am. J. Med. Sci. 105: 487 (1893).
4. F. Westermark, Uber die Behandlung des Ulcerirended Cervixcarcinoms, Mittel Konstanter Warme, Zbl. Gynak. 1335 (1898).
5. J. F. Percy, Heat in the treatment of carcinomas of the uterus, Surg. Gynec. Obstet. 22: 77 (1916).
6. R. Kirsch and D. Schmidt, Erste Experimentelle und Klinische Erfahrungen mit der Ganzkorper-Extrem-Hyperthermie, in: Aktuelle Probleme aus dem Gebiet der Cancerologie, W. Doerr, F. Linder, and G.Wagner, eds. Heidelberg,Springer-Verlag pp. 53 (1966).
7. R. Cavaliere, E. C. Ciocatto, B. C. Giovannella, C. Heidelberger, R. O. Johnson, M. Fagottini, B. Mondovi', G. Moricca and A. Rossi-Fanelli, Selective heat sensitivity of cancer cells. Biochemical and clinical studies, Cancer 20: 1351 (1967).
8. B. C. Giovanella, Actions of hyperthermia on tumor cells cultured in vitro, in: Selective heat

sensitivity of cancer cells, A. Rossi Fanelli, R. Cavaliere, B. Mondovi', G. Moricca eds. Springer-Verlag, Berlin, Heidelberg, New York pp. 36 (1977).
9. S. B. Field, Biological aspects of hyperthermia, in Physics and thecnology of hyperthermia, S. B. Field and C. Franconi eds. NATO ASI Series E: Applied Sciences, 127, pp. 634 (1987).
10. R. J. Palzer and C. Heidelberger, Influence of drugs and synchrony on the hyperthermic killing of HeLa cells, Cancer Res. 33: 422 (1973).
11. B. Mondovi', Temperature range and selective sensitivity to hyperthermia, in: Thermal characteristics of tumors: application in detection and treatment, R. K. Jain and P.M. Gullino eds, Ann. New York Acad. Sci. New York pp. 202 and 231 (1980).
12. K. J. Henle and L. A. Dethlefsen, Heat fractionation and thermotolerance: a rewiew, Cancer Res. 38: 1843 (1978).
13. G. M. Hahn, Hyperthermia and cancer, Plenum Press, New York and London (1982).
14. S. B. Field and R. L. Anderson, Thermotolerance: a review of observations and possible mechanisms, Natl. Cancer Inst. Monogr. 61: 193 (1982).
15. S. B. Field, Clinical implication of thermotolerance in: Hyperthermic Oncology 1984, Vol 2, J. Overgaard ed., London, pp. 235 (1985).
16. J. Jung, A generalised concept for cell killing by heat, Radiat. Res. 106: 56 (1986).
17. B. Mondovi', A. Scioscia Santoro, R. Strom, R. Faiola and A. Rossi Fanelli, Increased immunogenicity of Ehrlich ascites cells after heat treatment, Cancer 30: 885 (1972).
18. R. Cavaliere, B. Mondovi', G. Moricca, G. Monticelli, P.G. Natali, F. S. Santori, F. Di Filippo, A. Varanese, L. Aloe and A. Rossi Fanelli, Regional perfution hyperthermia, in : Hyperthermia in cancer therapy, F.K. Storm ed. G.K. Hall Medical Publ. Boston, Massachusetts, pp. 369 (1983).
19. J. Otte, Hyperthermia in cancer therapy, Eur. J. Pediatr. 147: 560 (1988).
20. J. W. Strohbehn, Hyperthermia and cancer therapy: a review of biochemical engineering contributions and challenges, IEEE Trans. Biomed. Eng. 31: 779 (1984).
21. B. Mondovi', A. Finazzi Agro', G. Rotilio, R. Strom, G. Moricca and A. Rossi Fanelli, The biochemical mechanism of selective heat sensitivity of cancer cells: I. Studies on cellular respiration, Europ. J. Cancer 5: 129 (1969).
22. S. Lewin and D. S. Pepper, Variation of the melting temperature of calf-thymus DNA with pH and type of buffer, Arch. Biochem. Biophys. 109: 192 (1965).
23. J. R. Lepock, Involvement of membranes in cellular responses to hyperthermia, Radiat. Res. 92: 433 (1982).
24. B. Mondovi', A. Finazzi Agro', G. Rotilio, R. Strom, G. Moricca and A. Rossi Fanelli, The biochemical

mechanism of selective heat sensitivity of cancer cells: II. Studies on nucleuc acids and protein synthesis, Europ. J. Cancer 5: 137 (1969).
25. R. L. Warters and O. L. Stone, The effects of hyperthermia on DNA replication in HeLa cells, Radiat. Res. 93: 71 (1983).
26. W. C. Dewey, A. Westra, H. H. Miller, and H. Nagasawa, Heat induced lethality and chromosomal damage in synchronized Chinese hamster cells treated with 5-bromodeoxyuridine, Int. J. Radiat. Biol. 20: 505 (1971).
27. G. K. Livingston and L. A. Dethlefsen, effects of hyperthermia and X irradiation on sister chromatid exchange(SCE) frequency in Chinese hamster ovary (CHO) cells, Radiat. Res. 77: 513 (1979).
28. A. Bozzi, G. Mariutti, B. Mondovi' and R. Strom, Effect of temperature on DNA repair synthesis in V79 mammalian cells exposed to ultraviolet light, Bull. Mol. Biol. Med. 12: 59 (1987).
29. G. C. Li, R. G. Evans and G. M. Hahn, Modification and inhibition of repair of potentially lethal X-ray damage by hyperthermia, Radiat. Res. 67: 491 (1976).
30. G. P. Raaphorst, E. I. Azzam and M. Feeley, Potentially lethal radiation damage repair and its inhibition by hyperthermia in normal hamster cells, mouse cells, and transformed mouse cells, Radiat. Res. 113: 171 (1988).
31. D. K. Dube, G. Seal and L. A. Loeb, Differential heat sensitivity of mammalian DNA polymerases, Biochem. Biophys. Res. Commun. 76: 483 (1977).
32. E. Dikomey, W. Becker and K. Wielckens, Reduction of DNA-polymerase B activity of CHO cells by single and combined heat treatment, Int. J. Radiat. Biol. 52: 775 (1987).
33. H. H. Kampinga and A. W. T. Konings, Inhibition of repair of X-ray-induced DNA damage by heat: the role of hyperthermic inhibition of DNA polymerase A activity, Radiat. Res. 112: 86 (1987).
34. S. P. Tomasovic, G. N. Turner and W. C. Dewey, Effect of hyperthermia on nonhistone proteins isolated with DNA, Radiat. Res. 73: 535 (1978).
35. R. L. Warters, L. M; Brizgys, R. Sharma and J. L. Roti Roti, Heat shock (45 C ) results in an increase of nuclear matrix protein mass in HeLa cells, Int. J . Radiat. Biol. 50: 253 (1986).
36. R. L. Warters, L. M. Brizgys and B. W. Lyons, Alterations in nuclear matrix protein mass correlate with heat-induced inhibition of DNA single-strand-break repair, Int. J. Radiat. Biol. 52: 299 (1987).
37. R. Hancock, Topological organization of interphase DNA: the nuclear matrix and other skeletal structures, Biol. Cell 46: 102 (1982).
38. M. R. Mattern and R. B. Painter, Dependence of mammalian DNA replication on DNA supercoiling. I. Effects of ethidium bromide on DNA synthesis in permeable Chinese hamster ovary cells, Biochim. Biophys. Acta 563: 293 (1979).

39. E. D. Hickey and L. A. Weber, Modulation of heat-shock polypeptide synthesis in HeLa cells during hyperthermia and recovery, Biochemistry 21: 1513 (1982).
40. M. J. Schlesinger, M. Ashburner and A. Tissieres eds. , Heat shock- from bacteria to man, Cold Spring harbor Laboratory, Cold Spring Harbor, New York (1982).
41. R. H. Burdon, Heat shock and heat shock proteins, Biochem. J. 240: 313 (1986).
42. K. W. Lanks, Modulators of the eukaryotic heat shock response, Exp. Cell Res. 165: 1 (1986).
43. S. N. Alahiotis, Heat shock proteins. A new view on the temperature compensation, Comp. Biochem. Physiol. 75B: 379 (1983).
44. T. Hatayama, K. Honda, and M.Yukioka, Hela cells synthetize a specific heat shock protein upon exposure to heat shock at 42 C but not at 45 C, Biochem. Biophys. Res. Commun. 3: 957 (1986).
45. W. W. Richter and O.G. Issinger, Differential heat shock responce of primary human cell cultures and established cell lines, Biochem. Biophys. Res. Commun., 141: 46 (1986).
46. H. Tsukeda, H. Maekawa, S. Izumi and K. Nitta, Effect of heat shock on protein synthesis by normal and malignant human lung cells in tissue culture, Cancer Res. 41: 5188 (1981).
47. S. P. Tomasovic, L.S. Ramagli, R. A. Simonette, M. J. Wilson, and L.V. Rodriguez, Heat-stress protein of rat lung endothelial and mammary adenocarcinoma cells, Radiat. Res. 110: 45 (1987).
48. M. F. Barbe, M.Tytell, D . J. Gower, and W.J. Welch, Hyperthermia protects against light damage in rat retina, Science 241: 1817 (1988).
49. G. C. Li and Z. Werb, Correlation between synthesis of heat shock proteins and the development of thermotholerance in Chinese hamster fibroblast, Proc. Natl. Acad. Sci. Usa 79 : 3219 (1982).
50. A. Laszlo and G. C. Li, Heat-resistant variants of Chinese hamster fibroblasts altered in expression of heat shock proteins, Proc. Natl. Acad. Sci. Usa 82: 8029 (1985).
51. K. T. Riabowl, L. A. Mizzen and W.J. Welch, Heat shock is lethal to fibroblasts microinjected with antibodies against hsp 70, Science 242: 433 (1988).
52. R. N. Johnston, and B. L. Kucey, Competitive inhibition of hsp 70 gene expression causes thermosensitivity, Science 242: 1551 (1988).
53. W. Welch and J. R. Feramisco, Purification of the major mammalian heat shock proteins, J. Biol. Chem. 257: 14949 (1982).
54. M. J. Schlesinger, Heat shock proteins: the seach for functions, J. Cell Biol. 103: 321 (1986).
55. A. Courtneidge and J.M. Bishop, Transit of pp60 to the plasma membrane, Proc. Natl. Acad. Sci. Usa, 79: 7117 (1982).
56. R. J. Deshaies, B. D. Knoch and R. Schekman, The role of stress proteins in membrane biogenesis, TIBS 13: 384 (1988).

57. M. Morange, A. Diu, O. Bensaude and C. Babinet, Altered expression of heat shock proteins in embrional carcinoma and mouse early embrionic cells, Mol. Cell. Biol. 4: 730 (1984).
58. W. Leyko and G. Bartosz, Membrane effects of ionozing radiation and hyperthermia, Int . J. Radiat. Biol. 49: 743 (1986).
59. P. S. Lin, P.S. Lui and S. Tsai, Heat induced ultrastructural injuries in limphoid cells, Exp. Mol. Pathol. 29: 281 (1978).
60. H. Bass, J. L. Moore and W. T. Coarkley, Lethality in mammalian cells due to hyperthermia under oxic and hypoxic conditions, Int. J. Radiat. Biol. 49: 743 (1978).
61. G. Arancia, P. Crateri Trovalusci, G. Mariutti and B. Mondovi', Ultrastructural changes induced by hyperthermia in Chinese hamster V 79 fibroblasts, Int. J. Hyperthermia 5: 341 (1985).
62. C. Sato, J. Nakayama, K. Kojma, Y. Nishimoto, W. and Nakamura, Effect of hyperthermia on cell surface charge and cell survival in mastocytoma cells, Cancer Res. 41: 4107 ( 1981).
63. M. Kapiszewska, K. Hyrc and K. Cieszka, Effect of hyperthermia on cell surface charge and cell survival in two cell lines L5178Y, Hyperthermic Oncology 1984, Vol I, Summary paper, edited by Overgaard (London, Philadelphia: Taylor and Francis) pp. 41-44 (1984).
64. R. L. Anderson, S. Leeman, R. Parker, M.J. Hedges, P. W. Vaughan, and S. B. Field, Attachment of fibroblasts following hyperthermia and ultrasound, Int. Radiat. Biol. 46: 399 (1984).
65. R. B. Mikkelsen and B. Koch , Thermosensitivity of the membrane potential of normal and simiam virus 40-transformed hamster lymphocites, Cancer RES. 41: 209 (1981).
66. R. Strom, A. Scioscia Santoro, C. Crifo', A. Bozzi, B. Mondovi' and A. Rossi Fanelli, The biochemical mechanism of selective heat sensitivity of cancer cells. IV. Inhibition of RNA synthesis, Europ. J. cancer 9: 103 (1973).
67. R. Strom, P. Caiafa, B. Mondovi' and A. Rossi-Fanelli, Effect of temperature on potassium-dependent stimulation of trans cellular migration in normal and neoplastic cells, FEBS Lett. 3: 343 (1969).
68. S. Szmigielski and M. Janiak, Membrane injury in cells exposed in vitro at 43 C hyperthermia, Cancer Therapy by Hyperthermia and Radiation, edited by C. Streffer, D. von Beuningen, F. Dietzel, E. Rottinger, J. E. Robinson, Scherer, S. Seeber, and K. R. Trott (Baltimore Munich: Urban and Schwarzenberg) pp. 169-171 (1978).
69. E. N. Alexandrova, G. S. Kalendo and N. G. Serebryakov, Effect of hyperthermia on early radiation reactions of HeLa cells at the stationary stage of growth, Radiobiologiya 24: 468 (1984).
70. P. N. Yi, Cellular ion content changes during and after hyperthermia, Bioch. Biophys. Res. Commun. 91: 177 (1979).

71. M. J. Borrelli, W. G. Carlini, B. R. Ransom and W. C. Dewey , Ion-sensitive microelectrode measurements of free intracellular chloride and potassium concentrations in hyperthermia-treated neuroblastoma cells, J. Cell. Physiol. 129: 175 (1986).
72. S. H. Calderwood and G. M. Hahn, Thermal sensitivity of insulin-receptor binding, Biochim. Biophys. Acta, 756: 1 (1983).
73. B. E. Magun and C. W. Fennie, Effect of hyperthermia on binding, internalization, and degradation of epidermal growth factor, Rad. Res. 86: 133 (1981).
74. K. H. Cheng, S. W. Hui and J. R. Lepock, Protection of membrane ATPase from thermak inactivation by cholesterol, Cancer Res. 47: 1255 (1987).
75. A. Malhotra, L. P. M. Heynen and J. R. Lepock, Role of extracellular calcium in the hyperthermic killing of CHL V79 , Radiat. Res. 112: 478 (1987).
76. J. Landry, P. Crete, S. Lamarche and P. Chretien, Activation of Ca-dependent processes during heat shock: role in cell thermoresistence, Radiat. Res. 113: 426 (1988).
77. J. R. Lepock, K. H. Cheng, H. Al-Qysi and J. Kruuv, Thermotropic lipid and protein transitions in Chinese hamster lung cell membranes: relationiship to hyperthermic cell killing, Can. J. Biochem. Cell Biol. 61: 421 (1983).
78. G. Arancia, W. Malorni, G. Mariutti and P. Trovalusci, Effect of hyperthermia on the plasma membrane structure of Chinese hamster V79 fibroblasts: a quantitative freeze-fracture study, Radiat. Res. 106: 47 (1986).
79. M. H. Ringdahl, B. Anderstam and C. Vaca, Heat-induced changes in the incorporation of H acetate in membrane lipids, Int. J. Radiat. Biol. 52: 315 (1987).
80. C. R. Hackenbrock, M. Hochli and R. M. Chau, Calorimetric and freeze fracture analisis of lipid phase transitions and lateral traslational motion of intramembrane particles in mithocondriaa membranes, Biochim. Biophys. Acta 455: 466 (1976).
81. A. E. Cress, P. S. Culver, T. E. Moon and E. W. Gerner, Correlation between amounts of cellular membrane components and sensitivity to hyperthermia in a variety of mammalian cell lines in culture, Cancer Res. 4: 1716 (1982).
82. M. M . Guffy, J. A. Roserberg, I. Simon and C. P. Burns, Effect of cellular fatty acid alteration on hyperthermic sensitivity in cultured L1210 murine leukemia cells, Cancer Res. 42: 3625 (1982).
83. M. B. Yatvin, N. M. Abuirmeileh, J. W. Vorpahl and C. E. Elson, Biological optimization of hyperthermia: modification of tumor membrane lipids, Eur. J. Cancer Clin. Oncol. 19: 657 (1985).
84. L. Marcocci, N. Laudonio, G. Zupi, E. Poggi, C. Greco, A. Bozzi, I. Mavelli, G. Rotilio and B.

Mondovi', Liposomes and heat sensitivity of tumor cells, J. Exp. Clin. Res. 7: 1 (1988).
85. J. DiGiuseppi and I. Fridovich, The toxicology of molecular oxygen, CRC Critical. Rev. Toxicol. 12: 315 (1984).
86. T. Ramasarma, Generation of H O in biomembranes , Biochem. Biophys. Acta 694: 69 (1982).
87. W. J. Welch and J. P. Suhan, Morphological study of the mammalian stress response: characterization of changes in cythoplasmic organelles, cytoskeleton, and nucleoli, and the apparence of intranuclear actin filaments in rat fibroblasts after heat-shock treatment, J. Cell Biol. 101: 1198 (1985).
88. M. Younes and C. P. Siegers, Interrelation between lipid peroxidation and hepatotoxic events, Biochem. Pharmacol. 33: 2001 (1984).
89. P. Buffa, V. Guarriera-Bobyleva, V. Muscatello, I. Pasquali Ronchetti, Conformational changes in the mithocondria associated with uncoupling of oxidative phosphrylation in vivo and in vitro, Nature 226: 272 (1970).
90. R. E. Durand, Potentiation of radiation lethality by hyperthermia in a tumor model: effect of sequence, degree and duration of heating, Int. J. Radiat. Oncol. Biol. Phys. 4: 401 (1978).
91. A. Floridi, A. Nista, M. G. Paggi, L. Pellegrini, A. Bagnato, M. Fanciulli and A. Caputo, Effect of hyperthermia on electron transport in Ehrlich ascites tumor mithicondria,Exp. Mol. Pathol. 46: 279 (1987).
92. E. N. Christiansen and E. Kvamme, Effect of thermal treatment on mithocondria of brain, liver, ascites cells, Acta Physiol. Scand 76: 472 (1969).
93. M. A. Hass and D. Massaro, Regulation of the synthesis of superoxide dismutases in rat lungs during oxidant and hyperthermic stresses, J. Biol. Chem. 263: 776 (1988).
94. J. M. McCord, Oxygen derived free radicals in postischemic tissue injury, New Engl. J. Med. 312: 159 (1985).
95. M. A. Steven and S. K. Calderwood G. M. Hanh, Rapid increases in inositol triphosphate and intracellular Ca after heat shock, Biochem. Biophys. Res. Commun. 137: 826 (1986).
96. E. Ben-Hur and E. Riklis, Enhancement of thermal killing by polyamines , Rad. Res. 78: 321 (1979).
97. B. Mondovi' and P. Riccio, Biological basis of thermosensitivity of tumor cells, Proceeding of II international conference on Applications of Physics to Medicine and Biology Z. Bajzer, P. Baxa, C. Francioni, pp. 297 (1984).
98. B. Mondovi', P. Guerrieri, M. T. Costa and S. Sabatini, Amine oxidases inhibitors and biogenic amines metabolism, Adv. Polyamine Res. 3: 75 (1981).
99. B. Mondovi', P. Gerosa and R. Cavaliere, Studies on the effect of polyamines and their products on Ehrlich ascites tumors, Agents and Actions, 12: 450 (1982).

100. R. S. Kramer and R. D. Pearlstein, Reversible uncoupling of oxidative phosphorilation at low oxygen tension, Proc. Natl. Acad. Sci. USA 80: 5807 (1983).
101. D. P. Jones, Hypoxia and drug metabolism, Biochem. Pharm. 30: 1019 (1981).
102. I. Mavelli and G. Rotilio, Enzymatic protection against intracellular oxidative processes, in: Advances on oxygen radicals and radioprotection. A. Breccia, C. L. Greenstock, M. Tamba , eds. Lo Scarabeo, pp. 65 (1983).
103. L. W. Oberley and G. R. Buettner, Role of superoxide dismutase in cancer, Cancer Res. 39: 1141 (1979).
104. A. Bozzi, I. Mavelli, A. Finazzi Agro', R. Strom, A.M. Wolf, B. Mondovi' and G. Rotilio, Enzyme defense against oxygen derivatives. II. Erythrocytes and tumor cells, Molec. Cell. Biochem. 10: 11 (1976).
105. I. Mavelli, G. Rotilio, M. R. Ciriolo, G. Melino and O. Sapora, Antioxygenic enzymes as tumor markers: a critical reassesment of the respective roles of superoxide dismutase and gluthatione peroxidase, in: Human marker, Cimino, Birkmayer, Klavins, Timental, Salvatore eds. pp. 883 (1987).
106. A. Bozzi, I. Mavelli, B. Mondovi', R. Strom and G. Rotilio, Differential sensitivity of tumor cells to externally generated hydrogen peroxide. Role of glutathione and related enzymes, Cancer Biochem. Biophys. 3: 135 (1979).
107. A. Bozzi, I. Mavelli, B. Mondovi', R. Strom and G. Rotilio, Differential cytotoxicity of daunomycin in tumor cells is related to glutathione-dependent hydrogen peroxide metabolism, Biochem. J. 194: 369 (1981).
108. R. A. Omar, S. Yano and Y. Kikkawa, Antioxidant enzymes and survival of normal and Simiam Virus 40-transformed mouse embryo cells after hyperthermia, Cancer Res. 47: 3473-3476 (1987).
109. P. L. Lin, L. Kwock and C.E. Butterflield, Diethyldithiocarbamate enhancement of radiation and hyperthermic effcts on Chinese hamster cells in vitro, Rad. Res. 77: 501 (1979).
110. J. B. Mitchell, A. Russo, T. J. Kinsella and E. Glatstein, Glutathione elevation during thermotolerance induction and thermosensitization by gluthatione depletion, Cancer Res. 43: 987 (1983).
111. M. L. Freeman, A. W. Malcolm and M. J. Meredith, Decreased intracellular glutathione concentration and increased hyperthermic cytotoxicity in an acid enviromental, Cancer Res. 45: 504 (1985).
112. B. Chance, H. Sies and S. Boveris, Hydroperoxide metabolism in mammalian organs, Physiol. Rev. 59: 527 (1979).
113. J. D. Crapo and J. M. McCord, Oxygen-induced changes in pulmonary superoxide dismutase assayed by antibody titration, Am. J. Physiol. 231: 1196 (1976).
114. S. E. Dryer, R. L. Dryer and A. P. Author, Enhancement of mitochondrial cyanide-resistent superoxide dismutase in the liver of rats treated

with 2,4-dinitrophenol, J. Biol. Chem. 255: 1054 (1980).

115. D. P. Loven, D. L. Leeper and L. W. Oberley, Superoxide dismutase levels in Chinese hamster ovary cells and ovarian carcinoma cells after hyperthermia or exposure to cycloheximide, Cancer Res. 45: 3029 (1985).

# INTERSTITIAL HYPERTHERMIA (IHT): TECHNICAL PROBLEMS AND METHODOLOGY

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## INTRODUCTION

Utilization of interstitial applicators in clinical hyperthermia offers useful advantages such as more homogeneous and greater heating of the target volume and also smaller energy absorption in surrounding tissues. For thermal effectiveness a particular precision of the instruments must be used in the characterization of antennas. The intent of this work is to present three methods for characterization of interstitial applicators and planning methodology for clinical use.

## MATERIALS AND METHODS

In our institute, the available system is the SAPIC sv03/A made by Aeritalia, Gruppo Sistemi Avionici ed Equipagggiamenti, Casell Torinese. This system allows electromagnetic generation, power delivery in patients, measurement and registration of temperature data. It also includes a program which simulates temperature distribution into heated volumes: according to the simulation results, it is possible to optimize all treatment parameters. For interstitial hyperthermia, the system works in local mode: parameter control and regulation are completely committed to operator experience; the computer and all the peripheral unities are in fact totally unfitted. The system itself is able to prevent every wrong command on the operator's behalf and to manage situation that can damage treatment security: for this purpose there is a hardware logic included in the system.

The interstitial antennas are dipole ones, and have a length of 125 mm and a diameter of 2mm. The junction is posed at 3.5 cm from the distal extremity of the antenna: the characterization made shows that heating reaches the maximum value exactly at that point. The antennas are covered by teflon; the range of interest is microwave range and the working frequency is 915 MHz. They are controlled by a 8 channel power drive that is able to distribute the total output power of 1- 8 parts according to the number of applicators.

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To control the power distribution of antennas we have used optical fibers and an Infrared Thermograph. The first ones are GaAs optical fibers that allow temperature measurements with an accuracy of 0.1 C. They have stability, precision and durability and show an excellent bio-compatibility. They give eight temperature measurements through four single sensors (0.3 mm in diameter) and a multiple sensor which allows four measurements using only an optical fiber. They have a disadvantage: their information is limited to few points. Because of that we didn't limit our characterization to this kind of measurement but also used the thermograph which offers good accuracy and satisfactory information about the temperature reached after heating. The available system is the Thermal Video System, TVS-300 Series; it is a piece of equipment used to measure the temperatures at various points of the volume of interest under non-contact, and display in pseudo color, it's temperature distribution on a TV monitor. TVS primarily consist of an infrared camera head, an image processor and a monitor. The camera head will receive infrared rays emitted from the surface of an object and convert them to electrical signals. The processor will convert electrical signals from the camera head to digital signals, record their signals on a frame memory and then process these signals to display thermal image in real time in color or monochrome in 16 levels of shading on a color monitor screen. It can accomplish displays of various modes of thermal image by it's built-in microprocessor such as displays of selected shade level, still image displays, temperature indication of a desired point of thermal image and time and message displays.

The simulation program, which is used to compare experimental, to calculated antennas characterization, is included in the SAPIC sv03/A system. It was developed by a collaboration between Aeritalia and Electriconic and Automatic Department of Engineering University of Ancona. It calculates the irradiated field in phantom; this means SAR (Specification Absorption Rate) determination for a muscle simulated phantom. Data related to Sar was compared to data obtained from experiments (with optical fibers and Thermograph). When the simulation program is used to optimize interstitial treatment, it calculates the temperature distribution from surrounding heating exchange, the power delivered by the applicator (by SAR), the power taken away by the blood flow, and the power delivered by metabolism: These latter parameters are deduced by a TC or ecographic image.

Interstitial Hyperthermia is carried out in combination with Brachytherapy with 192Ir. The protocol treatment provides two interstitial Hyperthermia sessions of 45 minutes at 42-45 degrees C with an intermediate Brachytherapy session (30Gy in 30 hours).

## CHARACTERIZATION

The characterization was performed in a muscle simulation phantom: they were parallel pipes 12.5 cm thick

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## CHARACTERIZATION

The characterization was performed in a muscle simulation phantom: they were parallel pipes 12.5 cm thick in which there were inserted the antennas through teflon guides. The thermal distribution was studied in a plane only because of the heating symmetry. The optical fibers were positioned in parallel lines with the antennas, the thermograph camera in front of the phantom surface.

Fig. 1 shows the thermal behavior of the optical fibers.
Fig. 2 the isotherms obtained through the thermograph.
Fig. 3 shows the S.A.R. images calculated by the simulation program of a four antenna array.

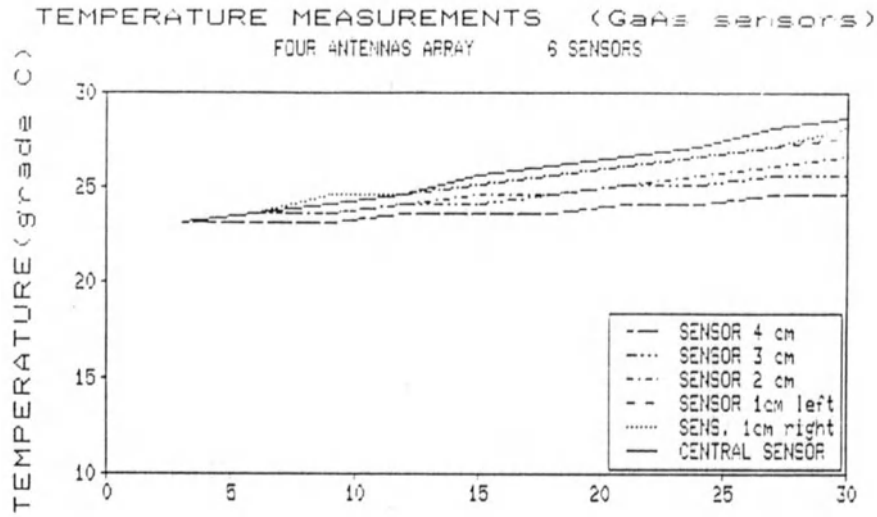


Fig. 1. Temperature behavior obtained by optical fibers

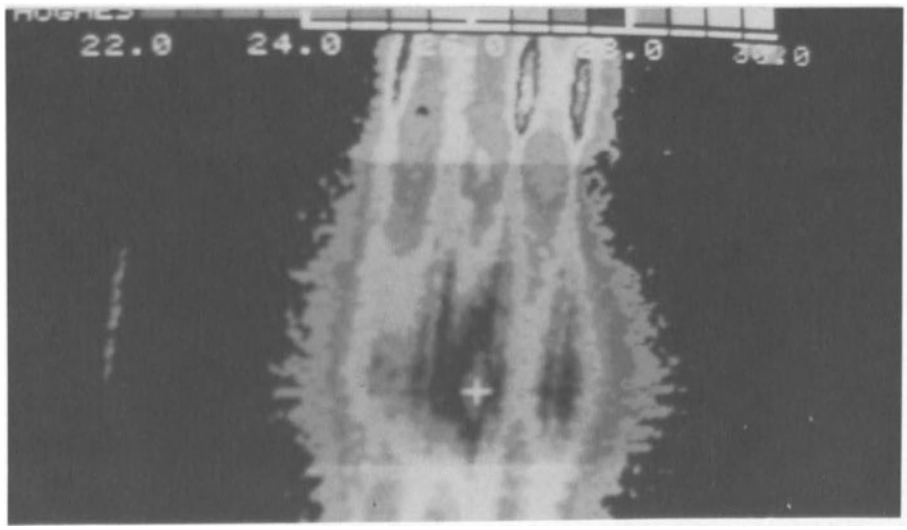


Fig. 2. Isotherms obtained by Infrared Thermograph

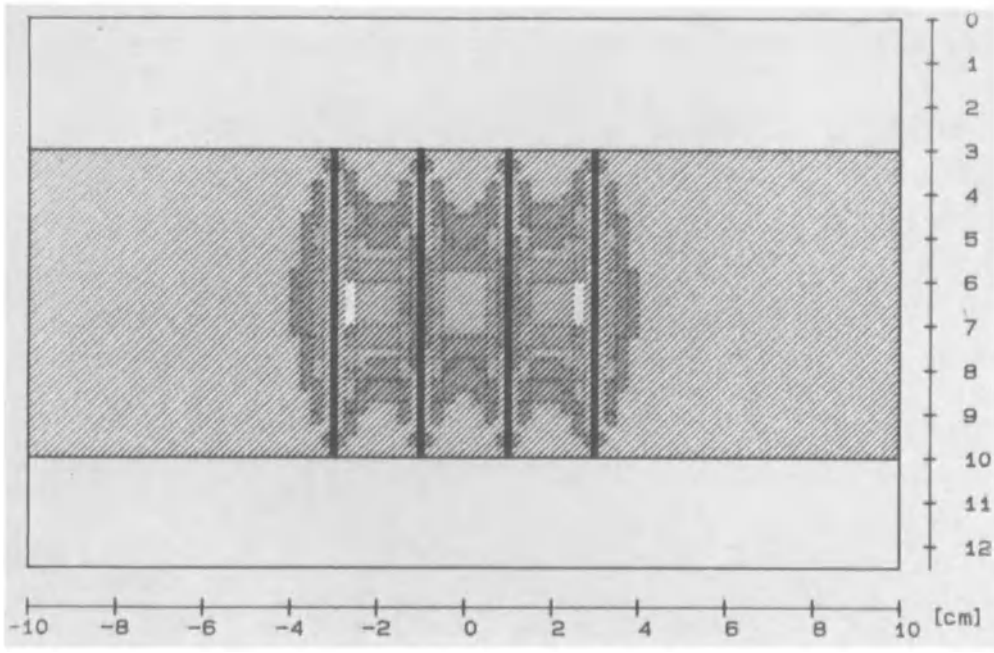


Fig. 3. SAR distribution calculated by simulation program

CLINICAL CASE

A patient affected by a recurrent soft tissue sarcoma of the forearm was treated with a combination of interstitial hyperthermia with 192Ir. A treatment planning was studied according to the lesion geometry and size: the disposition and the number of the radioactive sources was appropriately selected. By ecography images an anatomical map of the lesion was designed; then the antennas were placed in order to have the best homogeneity of heating and the SAR was calculated. On this ground the hyperthermic treatment was simulated. Fig. 4 and Fig. 5 show the modality of the two treatments, and Fig. 6 the characteristic of the implantation.

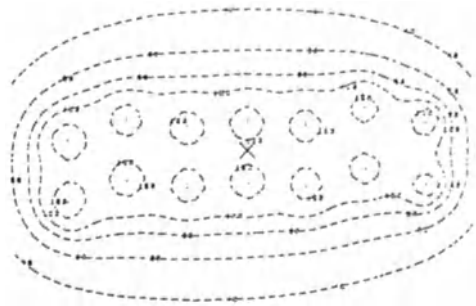

Fig. 4. Planning for Brachytherapy treatment.

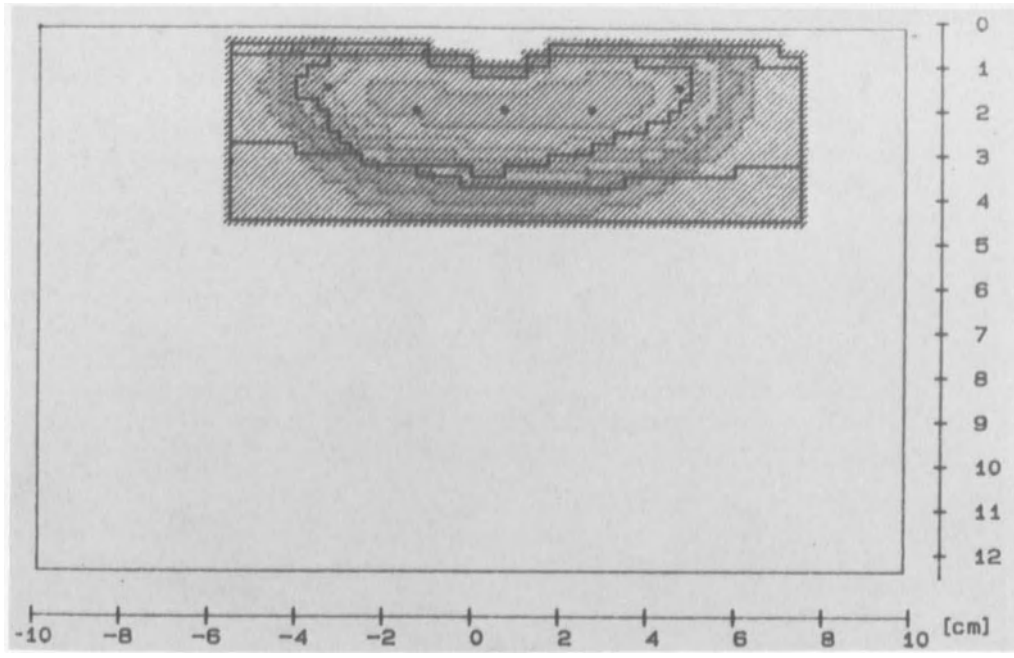


Fig. 5. Temperature distribution obtained by simulation.

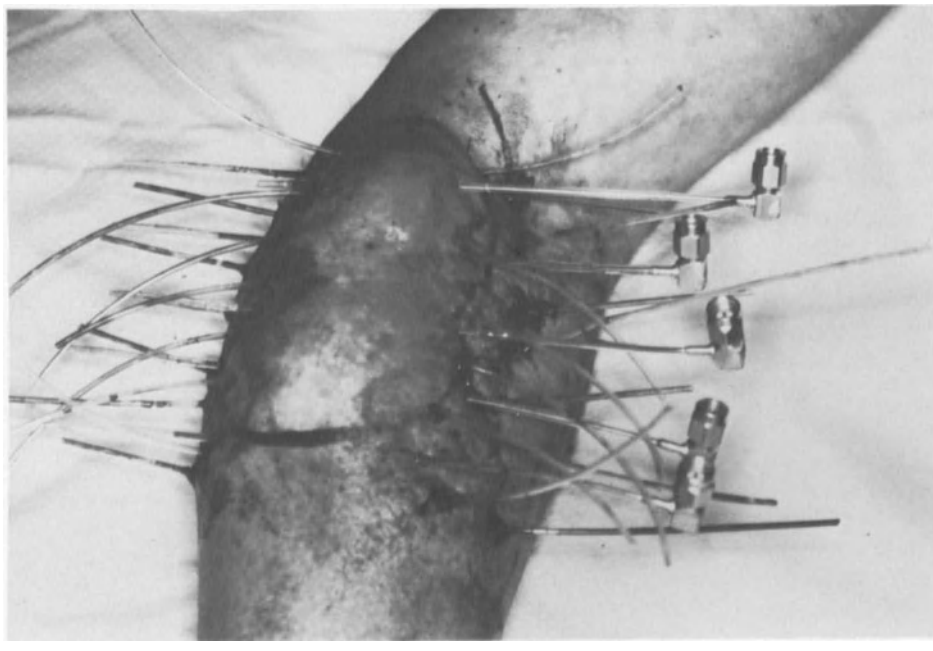

Fig. 6. Implant representation.

CONCLUSIONS

One week after the treatment, there was a blister on the skin in correspondence of a cranial antenna.

Four months after the treatment, the patient was operated on with a conservative procedure and now he is living without disease or complications.

REFERENCES

1) Bicher HI, Moore DW, Wolfstein RW: A method for interstitial thermoradiotherapy. Proceed of 4th Inter Symp on Hyperthermic Oncology, Aahrus, 595-598, 2-6/6/1984.

2) Cosset JM, Dutries J et al: Les tecnicque interstitielles de l'hyperthermia. Bull Cancer 68,3: 268-272, 1981.

3) Cosset JM, Dutreix J et al: Interstitial thermoradiotherapy a technical and clinical study of 29 implantations performed at the Institute gustave Roussy. International Journal of Hyperthermia vol 1, 3-13, 1985.

4) Emami B, Marks J, Perez C et al: Treatment of human tumors with interstitial irradiation and hyperthermia. Proceed of 4th Inter Symp on Hyperthermic Oncology. Aahrus, 583-586, 2-6/6/1984.

5) Joseph CD, Astrahan M, Lipsett J et al: Interstitial hyperthermia and interstitial iridium 192 implantation: a technique and preliminary results. Int J Radiat Oncol Biol Phys, vol 7: 827-833, 1981.

6) Ogno G, Audone B, Bolla L, Ragona R, Orecchia R, Gabriele P: A computer program for predicting temperature distributions in clinical Hyperthermia. Abstract from ESHO Meeting, Cardiff. Int Journ of Hyperth, vol 3, 6, 1987.

7) Ongo G, Bolla L, Audone , Gabriele P, Ragona R, Anglesio S: An automatic multifrequence computerized system for Hyperthermia (the SAPIC sv03/A by Aeritalia). Abstracts from the ESHO Meeting, CArdiff. Int Journ of Hyperth, vol 3, 6, 1987.

8) Selmo L, Ogno G, Ozzello F, Munoz F, Tseroni V: Applicatori intersiziali multipli: termometria sperimentale. Atti VIII Con Naz della Sex Aut Onc Rad (SARO), pag 727-730. St Vincent (AO), 23-26/9/1987.

# CONDUCTIVE, INTERSTITIAL HYPERTHERMIA:

# A NEW MODALITY FOR TREATMENT OF INTRACRANIAL TUMORS[1]

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Malignant brain tumors comprise a devastating class of diseases with an overall dismal prognosis. The incidence of primary malignant gliomas, the most common category of primary intracranial tumors, is 12,000 to 15,000 patients per year in the United States.[1,2] Metastatic brain tumors are reported in an additional 100,000 patients per year.[3,4] Whereas the survival of patients with metastatic intracranial tumors is often determined by widespread systemic disease, in primary intracranial malignancies local recurrence represents the major source of failure. Despite aggressive cytoreductive surgery, radiation therapy, and chemotherapy, the clinical outcome is generally grim. Salcman (1980) in a review of 1561 glioblastoma cases treated with maximal resection, with or without the addition of radiation or radiation plus chemotherapy, found a two-year survival of 10%.[5] Walker (1978) in a study of 222 patients with anaplastic gliomas found 1% survival at 24 months with surgery and radiation therapy, and 5% survival at 24 months with surgery, BCNU and radiation.[6] Multiple combinations of modalities and different approaches have been explored quite extensively by many investigators. Even studies resorting to extreme approaches using high doses of radiation and chemotherapy have yielded only limited benefits to a minority of the population at risk.[4,5,7-12] As expected, these cases have been accompanied by significant side effects and complications.[13,14] Regrettably, anticipated refinements in standard therapies are not expected to appreciably improve the prognosis for patients with malignant gliomas.[9,15,16]

Interstitial delivery of therapy to brain tumors has a substantial appeal, because local application of radiation, chemotherapy, or heat therapy may allow doses otherwise intolerable to the whole brain to be delivered directly to the tumor. Intratumoral implantation of radiation sources and chemotherapy carriers is being explored actively at several centers.[17-19] This review focuses on interstitial delivery of local heat therapy by conductive techniques--i.e., conductive, interstitial hyperthermia--in the treatment of intracranial tumors.

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## INTRODUCTION

The concept of hyperthermia or heat as a therapeutic modality for malignancies is not new. The history of hyperthermia over the past 5,000 years has been amply discussed in recent reviews.[20-22] What is new is the ability to produce controlled localized elevation of temperature inside and around tumors, fostered by advances in electronic and computer technology during the last two decades. Interstitial delivery carries the advantage of direct deposition of energy in the form of heat into the tumor tissue, allowing treatment of deep seated lesions with minimal insult to surrounding normal tissues.

The first modern clinical report of interstitial hyperthermia as a treatment modality for brain tumors was published by Sutton in 1971.[23] He reported the short-term application of an invasive, resistive heating system, placed into an area presumably bearing tumor; chemotherapy was administered at the same time. Because of the nonexistence of computerized tomography, and the limited number of cases, it is difficult to know what benefits were accrued. In 1981, Salcman and Samaras reported the use of single-microwave-antenna systems operating at 2450 mHz in six patients who were treated sequentially with brachytherapy and chemotherapy.[22,24] In 1985, Winter *et al.* presented a report of 12 patients with malignant gliomas of different grades, treated with multiple microwave antennae systems also operating at 2450 mHz.[25] Subsequently Roberts *et al.* in 1986 reported the results of treatment in six patients with malignant gliomas sequentially treated with interstitial hyperthermia and Iridium-192 brachytherapy.[17,18] Roberts's microwave antenna array operated at 915 mHz. All of the above investigators have limited their treatments to 60 minutes or less at each session (with one or two sessions administered to most patients) in combination with brachytherapy and/or chemotherapy, therefore blurring the specific effect of hyperthermia.

The major significance of these early studies was that they showed the remarkable ability of the brain to withstand implantation of multiple catheters. Despite the inconclusive long-term results, technical feasibility and reasonable patient tolerance of interstitial catheter implantation were well demonstrated. Side effects were limited and reasonable in the context of the underlying disease.[17,18,22-25]

### Interstitial Hyperthermia

Previous experience by the authors with interstitial brachytherapy led to the development of the concept of volumetric interstitial hyperthermia therapy.[14,26,27] By volumetric interstitial therapy, we mean the stereotactic placement of multiple catheters or therapeutic modalities in a symmetric pattern, in sufficient quantity to completely span the imaged tumor volume. With volumetric interstitial hyperthermia, the heating pattern can be contoured to tumor geometry. In this sense, volumetric interstitial hyperthermia allows the elevation of temperature in a defined volume of tissue above a cytocidal level. This approach allows effective treatment of the entire tumor mass including the actively growing neoplastic cells located peripheral to the contrast enhanced tumor margin.

Among interstitial hyperthermia techniques previously available, microwave and radiofrequency based approaches can produce physical side effects in normal tissues, especially unexpected hot spots in hard-to-predict locations. The fundamental physical properties of these radiant energy sources make it very difficult to rigorously control power deposition in a precisely configured pattern that fits the irregular tumor geometry. With conductive hyperthermia techniques (ferromagnetic thermoseeds, hot water perfusion, or electrically heated catheters), however, the maximum temperatures occur at known points: the conductive heating sources themselves. The temperature distributions in surrounding tissue are, as will soon be discussed, predictable and in principle controllable to a high degree of

accuracy.[28] Furthermore, no energy is deposited directly in the tissue. Instead, energy is applied to each heat source, which in turn warms tissue in close proximity by simple thermal conduction and blood convection.

After exploring the various alternatives available for interstitial hyperthermia generation, we selected a conductive system using electrically heated catheters because of its ease of operation, its clearly focused and predictable thermal effects, and its ease of temperature regulation. The use of heated interstitial catheters obviates the possible physical side effects that microwave, radiofrequency or ultrasound can produce in normal tissues; implantation of catheters into the tumor in a uniform pattern and selecting the heating element length to correspond to the dimensions of the tumor results in a singular match of treatment to target. Heat delivery to nearby normal tissues is thus limited, decreasing the incidence of side effects. Furthermore, when hyperthermia is provided by electrically heated catheters, the therapy is inherently suited to computer control, with the potential ability to continuously compensate for changing blood flow by varying heat delivery. Because of the ease of operation of such a system, prolonged hyperthermia (i.e., 72 hours) can be administered without much additional effort.

**The Process of Conductive Interstitial Hyperthermia**

The following temperature parameters are important in understanding the process of conductive hyperthermia using electrically heated catheters: internal catheter temperature, catheter surface temperature, and minimum tumor tissue temperature. Because of necessities of catheter construction, there is a temperature gradient through the sheath of the catheter such that the surface temperature is lower than the internal temperature by a predictable amount. The catheter surface temperature is thus the maximum tissue temperature. Because of heat transfer created by thermal conduction and blood perfusion in the tumor, radial gradients of temperature extending from the outer surface of the catheter are characteristic. When an array of heated catheters is implanted, the thermal profiles from adjacent catheters merge to produce relatively broad, flat thermal valleys in the regions of the interstices of the array.[28] Figure 1 shows thermal profiles for a three-catheter section within a larger array of catheters. Phantom studies and computer simulations have indicated an effective intercatheter distance to be 15 mm.[28]

The temperature distributions created in perfused tissue have been intensively studied by computer simulations relating power, blood flow, and minimum tissue temperature.[28] The temperature distributions, similar to that shown in Figure 1, are typified by a regular series of thermal peaks and elevated valleys. The peaks represent the catheter surface temperature, and the elevation of the valley floor between peaks is the minimum tissue temperature achieved between catheters. The base plane represents arterial blood temperature. Since the main route for heat loss from the interior valleys is blood perfusion, with only minimal conduction to adjacent tissues, three dominant variables can be identified: (1) heater spacing, (2) blood flow, and (3) catheter surface temperature. By correlating these three factors in computer simulation studies, the necessary internal catheter temperature needed to obtain a desired minimum therapeutic temperature can be calculated. Once minimum therapeutic temperature is achieved throughout the entire volume of tumor, the thermal dose can be controlled.

Conductive, interstitial hyperthermia was studied in patients with malignant brain tumors. Recurrent or progressive intracranial tumors, identified by enhancement on a CT scan, underwent implantation of the interstitial catheters and subsequent hyperthermia treatment as later described. Further computer modeling of complete tissue temperature distributions, with varying heating catheter temperatures based upon data from 11 patients, revealed temperature distributions similar to that shown in Figure 2. In this analysis, the

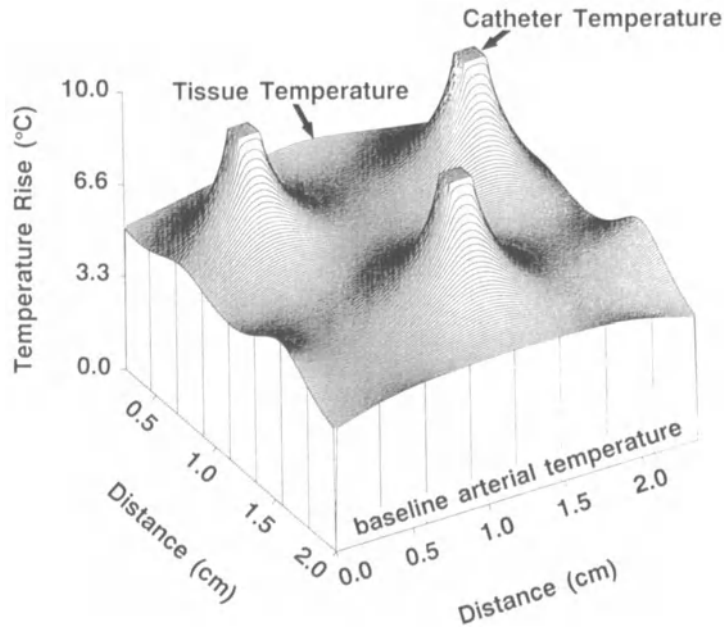


Figure 1. Computer modeling, based on the bioheat equation, of temperature profiles from heated catheters within a tumor.[29]

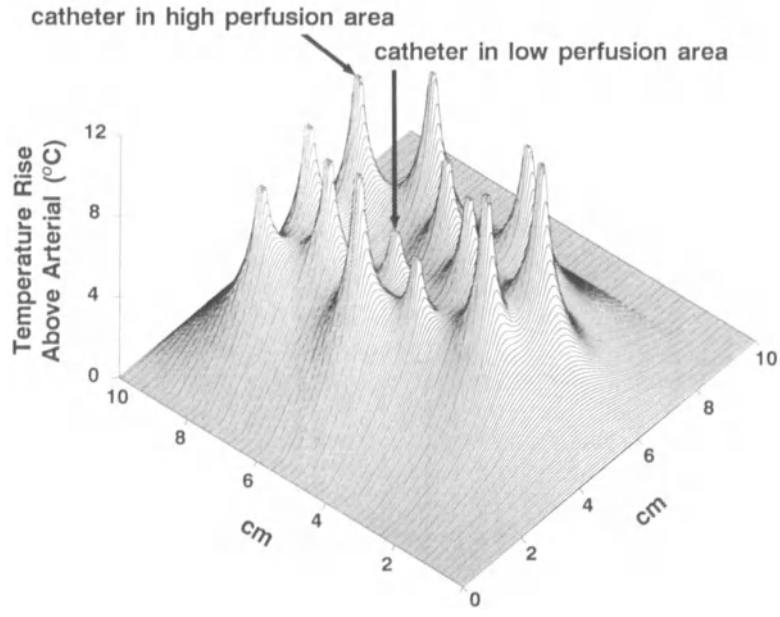


Figure 2. Temperature profiles calculated for a typical treatment (zero corresponds to arterial temperature).[29] Thirteen catheters were implanted to encompass the tumor volume. Power to each catheter was adjusted under computer control to compensate for varying blood perfusion as described in reference 28.

actual catheter geometry (reconstructed from CT records) and catheter power (recorded by the computer control system during treatment) were taken as inputs to a heat transfer simulation program based upon the bioheat equation,[29] resulting in a calculation of the complete temperature profile in a plane through the tumor. Temperatures from the simulation were compared to temperatures measured at the corresponding points in the tumor by independent thermometry during treatment. The thermal peaks and valleys that developed during treatment could be estimated with an accuracy of $\pm 0.6°C$.[29]

## THE HYPERTHERMIA SYSTEM

The conductive interstitial hyperthermia system (Volumetric Hyperthermia Treatment System, Model VH 8500, Cook Inc., Bloomington, Indiana) used in these investigations is comprised of implantable catheters (Figure 3) which are connected to computer-controlled hyperthermia generators (Figure 4). The system is capable of controlling up to 16 hyperthermia catheters and monitoring 16 channels of independent thermometry. Treatment data are automatically recorded by the system for subsequent analysis.

The hyperthermia catheters used for treatment of brain tumors are thin (2.2 mm outside diameter) and uniformly cylindrical (Figure 3). The tip of each catheter is elliptically tapered to allow easy penetration of tissue, with the rest of the catheter following the same trajectory, minimizing distortion of tissue and injury to blood vessels. The intratumoral portion of the catheter is semirigid and contains a heating element of variable length (2 to 8 cm) and a centrally placed thermistor. The heating element is embedded in a thermally conducting, electrically insulating plastic. The proximal portion of the catheter contains the electrical contacts which permit detachable coupling with cables to computer-controlled power sources in the hyperthermia generators.

In addition to heat-emitting catheters, independent thermometry catheters can also be implanted to confirm the level of heat delivery to the tissue. The independent thermometry catheters are 1.2 mm in diameter and have either one temperature sensing point or four separate temperature sensing points, spaced 1 cm apart along the implanted segment.

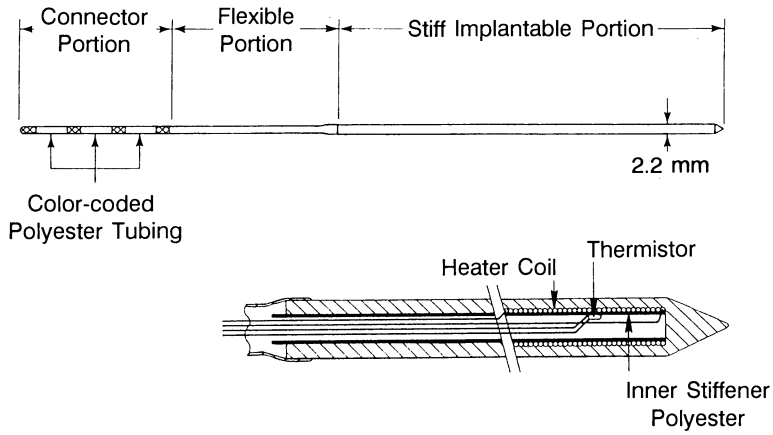


Figure 3. Hyperthermia catheters are comprised of a stiff, implantable portion with variable heating segment length, a flexible portion, and a connector portion allowing connection, via detachable cables, to the hyperthermia generators. Each catheter has a thermistor inside the heater coil.


Figure 4. The conductive, interstitial hyperthermia system.

The hyperthermia generators (Figure 4) control the electrical energy delivered to the heating element in each hyperthermia catheter. The generators are provided with a built-in test, safety check, and automatic calibration. The generators regularly monitor the actual heater voltage, heater current, reference voltage, and heater and tissue temperatures, and communicate this information to the computer control system.

The computer control system regulates each step of the treatment process. The control software utilizes programmable prescription parameters including treatment temperature, start time, duration, configuration, and number of treatments. Temperature data generated throughout treatment are continuously recorded. A real-time color graphic display demonstrating the temperature data from individual hyperthermia catheters and independent thermometry catheters is also provided. Individual catheter temperatures are measured with a resolution of 0.1°C.

## INTERSTITIAL CATHETER IMPLANTATION

Interactive CT scanning is used for the percutaneous implantation of hyperthermia and thermometry catheters. The entire procedure is performed in the CT suite, which also serves as an operating room. This time-saving approach allows CT localization, treatment planning, and stereotactic surgical implantation of up to 16 hyperthermia catheters in approximately 2 hours. The interstitial catheters are fundamentally far less invasive than the method of Tanaka *et al.*,[30] in which large, 9 cm diameter radiofrequency applicators are applied directly to an exposed cerebral hemisphere of a patient to achieve local hyperthermia.

For the treatment of brain tumors, hyperthermia catheters are positioned in staggered, parallel arrays so that they are separated by 15 mm. The patient is positioned on the CT table with the tumor uppermost, and the head secured in a stereotactic stabilization frame. After the scalp is prepared, a template, attached to the stabilization frame, is positioned over the tumor to guide catheter implantation. A white strip along the edge of the template is used to register the CT scanner at zero using the CT alignment laser. To plan

catheter implantation, distance from the template surface to the far side of the tumor and distance across the tumor are measured from the CT scan; this determines the appropriate implantation depth and heating segment length (2 to 8 cm), respectively, for a given catheter. Measurements are repeated for each aperture of the template intersecting the tumor on serial CT images spaced at 7.5 mm. Twist drill holes (2.5 mm) are then drilled through the desired apertures, and hyperthermia catheters having the appropriate heater lengths are implanted through the template to the measured depths. For independent thermometry catheters, 1.5 mm holes are drilled through smaller apertures in the template.

After implantation, CT scans are taken to confirm hemostasis and catheter placement (Figure 5). If needed, any adjustments in depth or placement are made and confirmed by rescanning. A cranial dressing of layered foam and silicone elastomer is used to secure the catheters, and the patient is transferred to the intensive care unit. Catheters are connected to the system with detachable cables and the treatment prescription is entered into the computer. The patients are typically awake or sleeping normally during treatment, and are allowed minimal activity at the bedside during hyperthermia delivery; between sessions of hyperthermia delivery, patients can be disconnected from the generator and are free to ambulate as allowed by their conditions and hospital procedure. Conductive hyperthermia treatment is administered, according to the protocol now to be described.

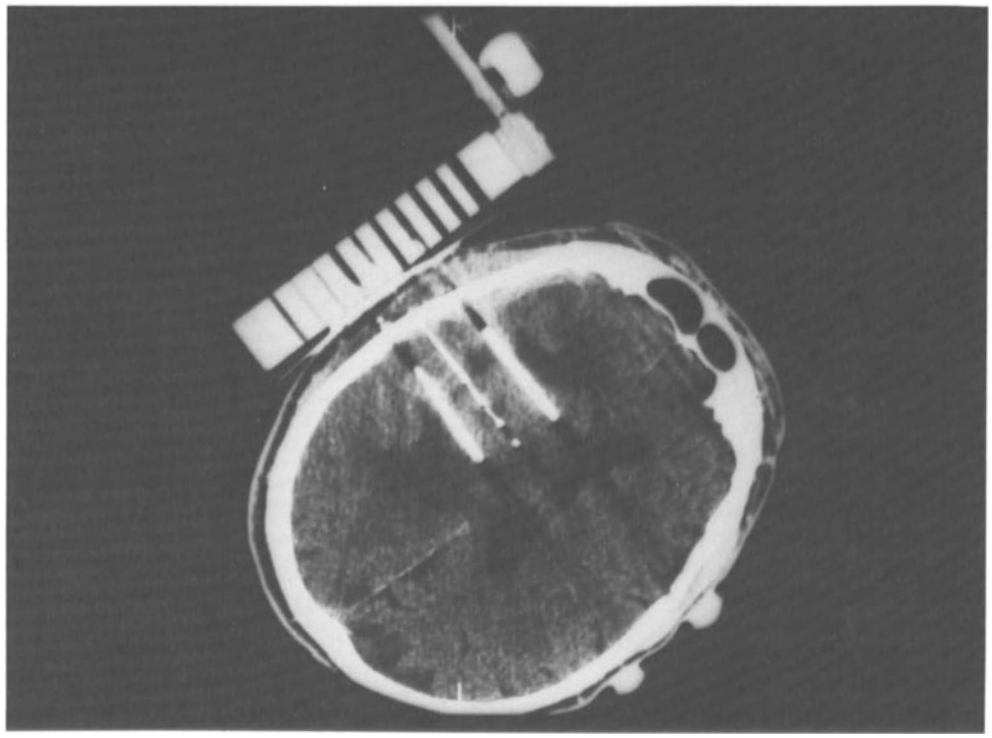

Figure 5. CT scan showing a cross section of the head, with catheters implanted in the tumor through the CT-mounted template. Two hyperthermia catheters and one four-sensor independent thermometry catheter are visible in the image.

## ONGOING CLINICAL INVESTIGATIONS

### Development of the Protocol

Because clinical data on long-term interstitial hyperthermia were not available, the clinical investigation proceeded stepwise. In the initial five patients, one-hour fractions of hyperthermia were delivered using an array of three hyperthermia catheters with heating element lengths of 2.5 cm, followed by one hour of post-treatment temperature monitoring. The early treatments were administered every 4 hours for 7 consecutive days, accumulating a total of 42 hours of hyperthermia using maximal heating catheter temperatures of approximately 44.5°C. This thermal dose was found to be well tolerated by the patients, and moderately effective, demonstrated on CT scans as necrosis in the area of catheter placement. In retrospect, it is clear that many tissue regions were not adequately heated in this initial protocol.

Expansion and technological improvement of the system accommodated an increased number of catheters, allowing treatment of a larger tumor volume, and longer treatment fractionation in the next five patients. Up to 16 heating catheters, with heating element lengths from 2 to 6 cm, could be controlled simultaneously. In all subsequent patients, catheters with heating element lengths from 2 to 8 cm were used. In addition to heating a larger volume of tumor, the duration of treatment was increased from one hour every four hours to two hours every four hours, and then to three hours every four hours. Three-hour fractions delivered every four hours and post-treatment temperature monitoring of five minutes was found to be well tolerated. Approximately one hour of normothermia between hyperthermia treatment fractions allowed for patient care. Total treatment duration was then 72 hours of hyperthermia delivered over approximately 96 hours.

Patients were maintained on appropriate medication regimes of steroids, anti-convulsants, and antibiotics. In addition, Dextran 40 was administered (600 ml/day) for prophylaxis against venous thrombus formation and thromboembolism. Procainamide was administered in a low dose (100-200 mg/hr) to potentiate the effects of hyperthermia by affecting the tumor cell membrane, altering the permeability and fluidity.[31] Throughout treatment, patients were monitored for vital signs, core body temperature, serum electrolytes, and neurologic status. If neurologic condition deteriorated during treatment, CT scans were obtained to assess for intracranial hemorrhage or mass effect. Hyperthermia was discontinued prior to completion if clinical condition indicated. At the termination of treatment, the catheters were explanted. Treatments were repeated at four to six week intervals, until three cycles were completed or evidence of viable tumor disappeared.

For the first 112 treatment cycles in this series, catheters were controlled by maintaining each catheter at the prescribed heating element temperature, typically about 50°C. This provided average catheter surface temperatures (maximum tissue temperatures) of about 45°C and average minimum tumor temperatures of about 42°C. For later treatments, power delivered to individual catheters was adjusted based on continuous estimates of mass heat transfer in the region of each catheter[28] to obtain a minimum tumor temperature of 41 to 43°C, despite local blood flow variation.

### Clinical Results

Experience with this technique in 50 patients was analyzed. This represents implantation of 1471 catheters and total hyperthermia delivery of 7153 hours. The number of patient admissions for catheter implantation totaled 124 with an average of 2.5 per patient. The average number of catheters implanted for each treatment cycle was 11.8, and an average of 58 hours of hyperthermia were delivered during each treatment cycle. The

patients' ages ranged between 16 and 69 with an average of 46.5 years. A total of 34 were males and 16 were females. All tumors were recurrent after biopsy and/or cytoreductive surgery with radiation therapy, except for one patient with metastatic melanoma who had received only chemotherapy. Nine patients received chemotherapy in addition to radiation therapy. Tumors treated included 9 high grade astrocytomas, 33 glioblastoma multiforme, and 8 metastatic tumors. Results of all versions of the evolving hyperthermia protocol, including those now believed to be less effective, are included in the present analysis.

Survival time from diagnosis to death ranged from 16 to 539 weeks (median 67 weeks) for all patients treated with conventional therapy followed by conductive, interstitial hyperthermia. Survival time from first hyperthermia treatment was up to 123 weeks (median 22 weeks); hyperthermia treatments began 9 to 52 weeks (median 40 weeks) after diagnosis. Survival times are still increasing for this group of patients because of continued patient survival. Two year survival (from diagnosis) in patients with recurrent malignant glioma (glioblastoma multiforme or astrocytoma with anaplastic features) was 33%. None of the patients with metastatic tumors achieved two year survival, due to their systemic disease.

In 21 patients, post-treatment tumor volume responses were determined by comparing contrast-enhanced CT images from pretreatment scans with those obtained during follow-up after hyperthermia therapy. Using a modified response criterion suggested by Storm *et al.*,[32] responses were identified as tumor regression (38% of cases) with a more than 25% decrease in observable tumor volume, stabilization (48% of cases) with a 25% decrease to 25% increase in tumor volume, or progression (14% of cases) with a greater than 25% increase in tumor volume.

The interstitial hyperthermia therapy was well tolerated by most patients. During treatment, the patients were unable to discern periods of intracranial temperature elevation. However, patients had intracranial complications (seizures and brain edema) and cardiovascular complications (deep vein thrombosis and pulmonary embolism) typical of brain tumor patients. For the 133 treatments, generalized seizures occurred in 8 patients, 5 during treatment and 3 following treatment; 12 patients also had focal seizures during treatment. No focal seizures were reported following treatment. Debulking craniotomies were performed in 5 patients after hyperthermia treatment to control mass effect. Delayed craniotomies for chronic edema or progressive brain necrosis have not been necessary. Five ventriculostomies were performed, three for intracranial pressure monitoring and two for management of increased intracranial pressure. Deep vein thrombosis and/or pulmonary embolism was noted post-implant in 9 patients. Vena cava filters were placed in 10 patients either before, during, or after the therapy. One patient died of a myocardial infarction; he had severe coronary artery disease undiagnosed prior to implant. Complications related to catheter placement included intracranial hemorrhage and infection. Out of 133 treatments, hemorrhage was minimal and resolved in 15, remote to catheter implantation in 2, and major in 4. Scalp infections followed 3 of the 133 implants and meningitis followed 1. Two patients had elevated body temperature, but in the majority of the patients heating had little effect on core body temperature.

## DISCUSSION AND FUTURE DIRECTIONS

The production of localized temperature elevations inside and around brain tumors by conductive interstitial techniques has now become feasible, as has been demonstrated in our laboratory and clinic. Several characteristics of hyperthermia contribute to its attractiveness as a therapeutic tool. Because fever is a physiologic event,[33-38] one would expect that hyperthermia, or artificial fever, would have no documented cumulative toxicity when administered within reasonable parameters. Prolonged doses or multiple treatments are

thus limited only by technological constraints. The effects of hyperthermia are related to temperature and duration of application; both of these parameters can be controlled, allowing for a wide spectrum of doses that can be tailored to the precise requirement of various neoplastic tissues.

The effects of hyperthermia are enhanced by hypoxic, hypovascular, hypometabolic and hyperacidic environments.[20,39-47] These are environments in which radiation and chemotherapy are usually less effective,[35,37,39,42,43,45,46,48-57] supporting arguments regarding the potential for synergistic effect between these treatment modalities. In addition, larger tumors are poorly vascularized, with vessels deficient in autoregulatory mechanisms.[40,42,47,58-61] These vessels may be relatively ineffective in heat removal by blood perfusion. Therefore relatively high temperatures at the core of the tumor may result.[61,62] It has also been argued that tumor microvasculature is more heat sensitive than normal vasculature, which would yield disruption of microcirculation at thermal doses not injurious to normal vessels. In addition, clotting or destruction of neovasculature occurs at temperatures below those which clot or destroy normal vasculature.[40,41,63,64] By focusing hyperthermia on the mass of tumor, neoplastic cells that are not cytotoxically injured may still succumb from destruction of their vascular supply. Mitotic cells and cells in the synthetic phase appear to be extremely sensitive to hyperthermia doses.[43,44,46,65-72] In addition, metabolically quiescent cells appear to be sensitive to doses of hyperthermia.

The protocol for conductive, interstitial hyperthermia was designed with thermal tolerance in mind. The treatments were divided into four-hour fractions (three hours hyperthermia and one hour normothermia) to allow initiation of subsequent treatment before the predicted time at which thermal tolerance should appear. Available evidence indicates that thermal tolerance usually develops between five and six hours after a thermal challenge. It was felt that a long-term heat treatment could overwhelm the mechanisms that lead to thermal tolerance before they had an opportunity to develop.

Initially, treatment extended for seven days. One factor which influenced this decision was the surgical risk associated with implantation of catheters in the brain, which demands that maximal benefits be obtained. The seven day period was used as a maximum since clinical experience with other percutaneous implanted catheters, such as ventriculostomies, has shown that after seven days, the rate of infection increases rapidly. Enhanced CT scans of the brain obtained at 48 to 96 hours after initiation of treatment were compared with enhanced CT scans obtained at the end of treatment. At approximately 72 hours of treatment, maximum benefits were obtained: no additional CT-depicted injury to the tumor accrued after this time. Consequently, the duration of a treatment cycle was limited to 72 hours. This 72 hour cycle duration will need to be further examined as data accumulate.

The patients' tolerance to long-term volumetric interstitial hyperthermia has proved quite reasonable. Most patients were awake, able to converse and assist in self-care during prolonged hyperthermia treatments. Discomfort was felt to be related to scalp penetration for percutaneous insertion and was relieved with mild analgesics such as acetaminophen. Increasing neurologic dysfunction present previous to treatment continued to worsen during treatment. This was an expected event, considering the advanced stage of the recurrent disease, the location of many of the tumors, the infiltrating nature of gliomas, and the addition of a volumetric implant. However, after removal of the catheters there was usually progressive clinical improvement over the ensuing weeks or months to the point that many patients returned to the pre-implantation neurologic status or better. This remarkable improvement in function weeks and months after treatment was not seen in the

experience gained with brachytherapy, and allowed aggressive management of tumors in areas that otherwise would have been considered unapproachable.

The prolonged survival in many of these patients after recurrence of their tumors, which had been previously treated with the most aggressive modalities of treatment, argues in favor of hyperthermia as a modality of treatment. Further investigation with newly diagnosed tumors in patients who are not in rapid decline from recurrent tumors and with conductive, interstitial hyperthermia in combination with other treatment modalities is required to more fully determine the true efficacy this approach.

The shortcomings of conductive, interstitial hyperthermia are gradually being elucidated and often eliminated with experience. Initially, the number of catheters implanted was inadequate. However, as tolerance to initial hyperthermia doses was documented, higher thermal doses were delivered. Improvement of the hyperthermia system allowed for greater dose application. Initially, a uniform thermal application was employed. Although heterogeneity of blood flow, which would produce variable tumor tissue temperatures, was expected, the enormous disparity of blood flow between regions of a single tumor, found subsequently in our research, was unexpected. Therefore, in order to compensate for the unpredictable and variable blood flow, a method for regularly estimating the thermal effects of blood flow had to be developed.[29] Further utilization of catheter temperature and power data allowed for prediction of the minimum tumor tissue temperature in real time, allowing for appropriate adjustment of catheter power to avoid cold spots. Individual control of each heater in an array of catheters compensates for variable flow through different regions of the tumor, leading to a well-defined, uniform thermal dose for each component of the tumor.

For future *in vitro* and *in vivo* investigation to be meaningful, clear delineation of the hyperthermia treatment is required. The following parameters appear to be important: volume of tumor, minimal temperature, maximal temperature, minimal thermal dose (minimal temperature applied for a determined duration of time), maximal thermal dose (maximal temperature applied for a determined amount of time), percentage of tumor volume exposed to different thermal doses, surviving fraction, volume of surviving fraction, minimal thermal dose applied to surviving fraction, and maximal thermal dose applied to surviving fraction. A method for correlating treatment temperature and duration to fraction of volume of tumor exposed to a minimal thermal dose is necessary; the correlation of these factors with outcome parameters would thus facilitate description and comparison of various hyperthermia treatments and serve as guidelines for reproducible clinical dosimetry.

Conductive interstitial hyperthermia is thus a rapidly evolving technology that shows considerable promise as adjunctive, and perhaps even primary therapy for intracranial neoplasms.

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## REFERENCES


1. Kornblith PL, Walker MD, Cassady JR. *Neurologic Oncology.* Philadelphia: JB Lippincott, 1987.
2. Salcman M. The morbidity and mortality of brain tumors. *Neurologic Clinics* 1985; 3:229-257.
3. Silverberg E, Lubera JA. Cancer Statistics, 1986. *Cancer* 1986; 36:9-25.
4. Obbens EAMT, Geun LG. Treatment options for recurrent brain metastasis. *Cancer Bulletin* 1986; 38:45-48.
5. Salcman M. Survival in glioblastoma: historical perspective. *Neurosurgery* 1980; 7:435-439.
6. Walker MD, Alexander E, Hunt WE, MacCarty CS, Mahaley MS, Mealey J, Norrell HA, Owens G, Ransohoff J, Wilson CB, Gehan EA, Strike TA. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic glioma. *J Neurosurgery* 1978; 49:333-343.
7. Douglas BJ, Worth AJ. Superfractionation in glioblastoma multiformae--results of a phase II study. *Int J Radiat Oncol Biol Phys* 1982; 8:1787-1794.
8. Garfield J. Recent status and future role of surgery for malignant supratentorial gliomas. *Neurosurgery Review* 1986; 9:23-25.
9. Kornblith PL, Walker M. Chemotherapy for malignant gliomas. *J Neurosurgery* 1988; 68:1-17.
10. Ransohoff J, Kelly P, Laws E. The role of intracranial surgery for the treatment of malignant gliomas. *Seminars in Oncology* 1986; 13:27-37.
11. Shapiro WR, Shapiro JE. Principles of brain tumor chemotherapy. *Seminars in Oncology* 1986; 13:56-69.
12. Gutin PH, Phillips TL, Wara WM, et al. Brachytherapy of recurrent malignant brain tumor with removable high activity iodine-125 sources. *J Neurosurgery* 1984; 60:61-68.
13. Sheline GE, Wara WM, Smith V. Therapeutic irradiation and brain injury. *Int J Radiat Oncol Biol Phys* 1980; 6:1215-1228.
14. Simpson J, Marchosky JA, Moran CJ et al. Volumetric intertitial irradiation of glioblastoma multiforme. *Endocurietherapy/Hyperthermia Oncology* 1987; 3:161-170.
15. Nelson DF, Urtasum RC, Saunders WM, Gutin PH, Sheline GE. Recent and current investigations of radiation therapy of malignant gliomas. *Seminars in Oncology* 1986; 12:46-55.
16. Kelly PJ, Eamest IVF, Kall BA, Goerss SJ, Schilthauer B. Surgical options for patients with deep seated brain tumors: computer assisted stereotactic biopsies. *Mayo Clinic Proc.* 60:223-229, 1985.
17. Roberts DW, Coughlin CT, Wong TZ, Fratkin JD, Douple EB, Strohbehn JW. Interstitial hyperthermia and iridium brachytherapy in treatment of malignant glioma. *J Neurosurg* 1986; 64:581-587.
18. Conzlilin CT, Douple EB, Strohbehn JW, Eaton WL, Trembly ES, Wong TZ. Interstitial hyperthermia in combination with brachytherapy. *Radiology* 1983; 148:285-288.
19. Borok TL, Winter A, Lang J, Pazlione R, Sterzer F, Similar I, Plafker J. Microwave hyperthermia radiosensitized iridium-192 for recurrent brain malignancy. *Medical Dosimetry* 1988; 13:29-36.
20. Hahn GM. *Hyperthermia and Cancer.* New York: Plenum Press, 1982.
21. Hornback NB. *Hyperthermia and Cancer: Human Clinical Trial Experience.* Vol. 1, Boca Raton: CRC Press, 1984.
22. Salcman M, Samaras G. Hyperthermia for brain tumors: biophysical rationale. *Neurosurgery* 1981; 9:327-335.

23. Sutton CH. Tumor hyperthermia in the treatment of malignant gliomas of the brain. *Trans Am Neurol Assoc* 1971; 96:195-197.
24. Salcman M, Samaras GL. Interstitial microwave hyperthermia for brain tumors. *J Neuro-oncology* 1983; 1:225-235.
25. Winter A, Laing J, Paglione R, Stenzer F. Microwave hyperthermia for brain tumors. *Neurosurgery* 1985; 17:387-399.
26. Marchosky JA, Moran CJ, Fearnot NE, et al. Volumetric interstitial hyperthermia: Phase I clinical study. *Abstracts of Papers for the 8th Annual Meeting of the North American Hyperthermia Group* (Philadelphia, PA), April 16-21, 1988, p. 46.
27. Moran CJ, Marchosky JA, Fearnot NE, et al. A stereotactic system for volumetric implantation in the brain. *Abstracts of Papers for the 8th Annual Meeting of the North American Hyperthermia Group* (Philadelphia, PA), April 16-21, 1988, p. 47.
28. Babbs CF, Fearnot NE, Marchosky CJ, Jones JT, Plantenga TD. Theoretical basis for controlling minimal tumor temperature during interstitial conductive heat therapy. *IEEE Trans Biomed Eng* 1989 (in press).
29. DeFord JA, Babbs CF, Patel UH, Fearnot NE, Marchosky JA, Moran CJ. Accuracy and precision of computer simulated tissue temperatures in individual human intracranial tumors treated with interstitial hyperthermia (submitted to *Int J Hyperthermia*, 1989).
30. Tanaka R, Choong HK, Nobuhisa Y, Yoshiaki S. Radiofrequency hyperthermia for malignant brain tumors: preliminary results of clinical trials. *Neurosurgery* 1987; 21:478-483.
31. Yatvin MB. The influence of membrane lipid composition and procaine on hyperthermic death of cells. *Int J Radiat Biol* 1977; 32:513-521.
32. Storm FK, Scanlon EF, Baker HW, Roe D, Motton DL. Tumor stabilization after hyperthermia: an important criterion of response to thermal therapy. *J Surg Oncol* 1987; 34:143-149.
33. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas with a report of original cases. *Am J Med Sci* 1893; 105:487-511.
34. Larkin JM, Edwards WS, Smith DE, Clark PJ. Systemic thermotherapy: description of a method and physiologic tolerance in clinical subjects. *Cancer* 1977; 40:3155-3159.
35. Muckle DS, Dickson JA. The selective inhibitory effect of hyperthermia on the metabolism and growth of malignant cells. *Br J Cancer* 1971; 25:771.
36. Nauts HC, Swift WE, Coley BE. The treatment of malignant tumors by bacterial toxins as developed by the late William B. Coley, MD, reviewed in the light of modern research. *Cancer Research* 1946; 6:205-216.
37. Overgaard J (ed). *Hyperthermia Oncology*. London: Taylor and Francis, 1984.
38. Warren SI. Preliminary study of the effect of artificial fever upon hopeless tumor cases. *Am J Roent* 1935; 33:75-87.
39. Song CW, Rhee JG, Levitt SH. Effect of hyperthermia on hypoxic cell fraction in tumor. *Int J Radiat Oncol Biol Phys* 1983; 8:851-856.
40. Song CW, Loshima A, et al. Implication of blood flow in hyperthermia treatment of tumors. *IEEE Trans Biomed Eng* 1984; 31:9-16.
41. Song CW. Role of vascular physiology in hyperthermia. In Steeves RA, Paliwal BR, Eds. *Syllabus--A Categorical Course in Radiation Therapy and Hyperthermia.* Oak Brook, IL: The Radiologic Society of North America, 1987.
42. Song CW, Kang MS, et al. The effect of hyperthermia on vascular function, pH, and cell survival. *Radiology* 1980; 137:795-803.
43. Dickson JA, Ellis HA. Temperature range and selective sensitivity of tumors to hyperthermia: a critical review. *Ann NY Acad Sci* 1980; 335:180-204.

44. Dickson JA, Calderwood SK. Thermosensitivity of neoplastic tissues in vivo. In: Storm, FK (ed). *Hyperthermia in Cancer Therapy*. Boston: G.K. Hall Medical Publishers 1983; 63-140.
45. Dewey WC, Freeman ML, et al. Cell biology of hyperthermia and radiation. In Meyn RE, Withers HR (eds) *Radiation Biology in Cancer Research*. New York: Raven Press, 1980, 589-621.
46. Cavaliere B, Ciocatto EC, Giovanella BC, Heidelberg C, Johnson R, Margottini M, Mondovi B, Moricca G, Rossi-Fanelli A. Selective heat sensitivity of cancer cells--biochemical and clinical studies. *Cancer* 1967; 20:1351-1381.
47. Emami B, Song CW. Physiological mechanisms in hyperthermia: a review. *Int J Radiat Oncol Biol Phys* 1984; 10:289-295.
48. Dewey WC, Thrall DE, Gillette EL. Hyperthermia and radiation--a selective thermal effect on chronically hypoxic tumor cells in vivo. *Int J Radiat Oncol Biol Phys* 1977; 2:99-105.
49. Dewey WC. Interaction of heat with radiation and chemotherapy. *Cancer Research* 1984; 44S:4714S-4720S.
50. Dewhirst MW, Sinox DA. Estimation of therapeutic gain in clinical trials involving hyperthermia and radiotherapy. *Int J Hyperthermia* 1986; 2:165-178.
51. Gerwech LE, Richards B, Jennings M. The influence of variable oxygen concentrations on the response of cells to heat and/or x-irradiation. *Radiation Research* 1981; 85:315-320.
52. Gerwech LE, Richards B. Influence of pH in the thermal sensitivity of cultured human glioblastoma cells. *Cancer Research* 1981; 41:845-849.
53. Hetzel FW. Introduction to the biology of hyperthermia. In: Steeves RA, Paliwal BR (eds). *Syllabus: A Categorical Course in Radiation Therapy: Hyperthermia*. Oak Brook, IL: Radiological Society of North America 1987; 7-15.
54. Nielsen DS, Overgaard JL. Effect of extracellular pH on thermotolerance and recovery of hyperthermia damage in vitro. *Cancer Research* 1979; 39:2772-2778.
55. Overgaard K, Overgaard J. Investigations on the possibility of a thermic tumor therapy. *Eur J Cancer* 1972; 8:65-78.
56. Overgaard J. Effect of hyperthermia on malignant cells in vivo: a review and a hypothesis. *Cancer* 1977; 39:2637-2646.
57. Roti Roti JL. Heat induced cell death and radiosensitization: molecular mechanisms. *National Cancer Institute Monograph* 1982; 61:3-10.
58. Dudar TE, Jain RK. Differential responses of normal and tumor microcirculation to hyperthermia. *Cancer Research* 1984; 44:605-612.
59. Mantyla MJ. Regional blood flow in human tumors. *Cancer Research* 1979; 39:2304-2306.
60. Chan RC, Babbs CF, Vetter RJ, Lamar CH. Abnormal response of tumor vasculature to vasoactive drugs. *J National Cancer Institute* 1984; 72:145-150.
61. Voorhees WD, Babbs CF. Hydralazine-enhanced heating of transmissible venereal tumor implants in dogs. *Eur J Cancer Clin Oncol* 1982; 18:1027-1034.
62. Babbs CF, DeWitt DP, Voorhees WD, McCaw JS, Chan RC. Theoretical feasibility of vasodilator enhanced local tumor heating. *Eur J Cancer Clin Oncol* 1982; 18:1137-1146.
63. Milligan AJ, Conran PB et al. Prediction of blood flow from thermal clearance during regional hyperthermia. *Int J Radiat Oncol Biol Phys* 1983; 197-210.
64. Strohbehn JW, Trembly BS, Douple EB. blood flow effects on the temperature distribution from an invasive microwave antenna array used in cancer therapy. *IEEE Trans Biomed Eng* 1982; 29:649-661.
65. Bhuyan BK, Day KJ, et al. Sensitivity of different cell lines and of different phases in the cell cycle to hyperthermia. *Cancer Research* 1984; 37:3780-3784.
66. Christiansen EN, Kuamme E. Effects of thermal treatment in mitochondria of brain, liver, and ascites cells. *ACTA Physiol Scand* 1969; 76:472-484.

67. Gillette EL. Clinical use of thermal enhancement and therapeutic gain for hyperthermia combined with radiation or drugs. *Cancer Research* 1984; 44S:4836S-4841S.
68. Harris JJ, Jones WC, Solley GW. The effect of temperature on tumor growth. *Proc Am Assoc Cancer Research* 1962; 3:326.
69. Perez CA, Kuske RR, Ememi B, Fineberg B. Irradiation alone or combined with hyperthermia in the treatment of recurrent carcinoma of the breast in the chest wall. *Int J Hyperthermia* 1986; 2:179-187.
70. Warters RL, Roti Roti JL. Hyperthermia and the cell nucleus. *Radiat Research* 1982; 92:458-462.
71. Warters RL, Stone OL. The effects of hyperthermia on DNA replication in HeLa Cells. *Radiat Research* 1983; 93:71-84.
72. Weinstein JN, Magin RI, Yatvin MB, Zaharko DS. Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. *Science* 1979; 204:188-191.

# HYPERTHERMIA FOR THE TREATMENT OF BRAIN TUMORS

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Brain cancer therapy often produces disappointing results even though there have been great strides in the treatment of cancer in general. Of the primary brain tumors, glioblastoma multiforme is by far the most common (32) and unfortunately, the most rapidly fatal (30, 31). Following surgical resection, without any other therapy, median survival of patients with glioblastoma multiforme is approximately 20 weeks. The addition of postoperative radiation extends median survival to approximately 39 weeks, and when combining surgery, radiation therapy and chemotherapy the median survival can be extended to approximately 55 to 60 weeks (31, 32, 33, 52). When compared to the most common solid malignancies of other parts of the body, glioblastoma multiforme has the most rapidly fatal course (30, 31). Many malignant gliomas are surgically accessible and 'gross total removal' is not uncommonly obtained, however, since tumor cells spread along fiber tracts and can be found far from the center of the tumor, malignant cells are usually left behind and recurrences are the norm (5). Glioblastoma multiforme has a biologically heterogeneous population of cells and in order for therapy to be completely effective and achieve total cell kill it must be directed at each of the various cell types (34, 35). Radiation therapy and chemotherapy in addition to surgery are unable to obtain total cell kill though they seem to slow the growth of these aggressive tumors. In 1989, in the United States, it is estimated that there will be approximately 15,000 new cases of brain malignancies with approximately 11,000 deaths (2).

Metastatic lesions to the brain also produce a great deal of morbidity and mortality, with mean survival of untreated intracerebral metastasis being approximately 4 weeks (55). Median survival of treated patients is extended to just under 4 months (55). Nearly 20 per cent of all patients suffering with systemic cancer will have one or more intracranial metastasis which occasionally is the cause of the ini-

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tial presenting symptoms (32, 55). Approximately ten percent of newly diagnosed patients with lung cancer will harbor an intracranial metastasis (3). Metastases tend to be better localized within the brain than primary gliomas, and solitary lesions lend themselves to a more thorough surgical removal. Recurrences however, are common and prophylactic whole brain radiation is usually administered postoperatively (10).

Many investigators are working toward prolonging survival, improving the quality of survival and ultimately curing patients suffering with intracranial cancer. Hyperthermia has the potential of aiding us in achieving these goals. It can be applied to those cancer cells left behind following surgery, can kill those cells that tend to be radioresistant, and can potentiate the effects of both radiotherapy and chemotherapy (6, 7, 11, 12, 28, 38, 41). Hyperthermia, unlike radiation, has no known cumulative toxicity and therefore can be safely reapplied to recurrent lesions (16).

## Brain Hyperthermia Techniques

There are numerous ways of achieving elevated temperatures within the brain (Table 1). Whole body hyperthermia is able to heat the brain along with the rest of the body. It is limited to a temperature of 42 ºC due to hepatocyte sensitivity* and requires expertise in its administration in order to protect the patient from its particular potential complications (36,40).

Perfusion techniques require the surgical isolation and cannulation of the appropriate artery and vein. It is an excellent technique in limb malignancies, such as melanoma (46), but to our knowledge has not been used on patients clinically.

Radiofrequency capacitive heating is able to heat relatively large areas of deep tissues. This technique requires the use of large metallic

Table 1. *Methods of achieving brain hyperthermia*

| |
|---|
| Whole Body Hyperthermia |
| Isolated perfusion |
| Radiofrequency Capacitive Heating |
| Interstitial Radiofrequency Hyperthermia |
| Ferromagnetic Thermoseed Induction |
| Ultrasound Hyperthermia |
| Microwave Hyperthermia |
| Magnetic Loop Induction |

* Some authors believe that neurons are damaged by temperatures greater than 42 ºC. However, studies by other investigators and work we have performed indicate that neurons can tolerate temperatures in the range of 42.5 to 43 ºC for periods of at least 60 minutes.

paddle-shaped transmitters which are placed adjacent to the skin over the area to be treated. There is significant surface heating and methods for cooling the plates are required. Radiofrequency also does not penetrate bone, so large areas of the skull must be removed prior to the administration of hyperthermia (50).

Interstitial radiofrequency hyperthermia utilizes a gold-plated brass intracranial electrode coupled with a flexible headband-like aluminum extracranial electrode. Using an 8 MHz oscillating radiofrequency generator the intracranial electrode is able to generate intratumoral temperatures of 44 ºC while maintaining surrounding brain temperatures below 42 ºC (19).

Ferromagnetic seeds can be surgically implanted into the tumor and later heated with the use of external radiofrequency or magnetic energy (18, 23). This technique focuses the heat around the seeds as the seeds are the objects being heated by the external energy source.

Ultrasound uses high frequency sound waves transmitted via implantable antennae and imparts kinetic energy to the target tissue which is rapidly and spontaneously converted into heat (4, 22). Energy is focused to a small target area around the tip of the ultrasound antenna. Ultrasound antennae can be inserted into catheters which may already be in place for interstitial seed brachytherapy, thus obviating the need for a special surgical procedure.

Microwave energy ranges from 300 MHz to 3000 MHz (13, 29) and the three frequencies most commonly used are 434 MHz, 915 MHz, and 2450 MHz. We work with 434 MHz which has a relatively long wavelength and can heat large areas of tissue. It also can penetrate the skull (13) and therefore offers the advantage of not requiring implantable devices. In the United States, a Faraday cage must be employed when using 434 MHz because this frequency interferes with short wave radio transmission (17). Both 915 MHz and 2450 MHz microwave energy sources focus their energy to smaller target areas, and like ultrasound, use implantable antennae which can be inserted into previously placed catheters for brachytherapy (26).

Magnetic loop induction, like 434 MHz microwave, can heat the brain through the intact skull without the need for invasive devices (43, 44). This technique uses a self-resonant, circular applicator which has impedance-matching circuitry that operates at 13.56 MHz (49). The applicator creates concentric circular flux lines of an electric field which can generate deep intratumoral heating while sparing surface structures (49).

## Optimal Heating

Optimal dosing, timing, and heating techniques are still under investigation. If one wished to focus energy to a small volume of tissue, then interstitial radiofrequency hyperthermia, inductive heating of ferromagnetic seeds, ultrasound, and 915 MHz and 2450 MHz microwave are the best methods. To heat larger areas of the brain,

radiofrequency capacitive heating, 434 MHz microwave and magnetic loop induction are the best methods. Whole body hyperthermia is best suited to treat the patient with multiple metastatic lesions though it is also used when only brain disease is present (40).

The region of recurrence is generally accepted to be a 2 cm rim around the tumor (15) and therapy should be directed at the tumor and this area. We agree with this but also believe that glioblastoma multiforme is a whole brain disease and as such treatment of brain tissue away from the lesion is also important. Dr. P. C. Burger has stated:

> "It has been our experience that recurrent lesions that have run their course and killed the patient, freely infiltrate the cerebrum, and often neoplastic cells can be found in the opposite cerebral hemisphere, cerebellum and brain stem." (5)

Whole brain hyperthermia has the potential advantage of treating distant tumor cells that would otherwise have been left unheated by local therapies. The possibility of neuronal injury when heating the whole brain must be considered. Many authors refer to the inability of normal neurons to tolerate temperatures greater than 42 ºC (1, 4, 21, 22, 45, 51). We have found that the normal canine brain can tolerate at least one hour of heating at temperatures in excess of 42.5 ºC with no evidence of neuronal injury (25). In our study, the normal, living, canine brain was heated through the intact cranium to greater than 42.5 ºC for one hour or more with transcranial 434 MHz microwave via two cradle antennae. The animals were sacrificed 3 or 7 days following the administration of hyperthermia and underwent an extensive histopathological analysis. No abnormalities, other than those that could be directly ascribed to the temperature monitoring probe tracts, were found (25). Other authors have also found that normal brain tissue can tolerate 42.5 ºC to 43 ºC (14, 20, 37, 42).

The use of heat as a therapeutic modality is complicated by difficulty achieving even, predictable temperature elevations because different tissues respond to energy sources differently, and blood flow varies from tissue to tissue, from time to time, and as a function of temperature (47). In general however, malignancies within the brain act as heat sinks (40). Because of this, regardless of the technique used, it is possible to obtain intratumoral temperatures much above that of the surrounding brain, thereby lessening the risk of brain injury and increasing the chance for successful tumoricidal effects. Work is being done with synthetic liposomes that can be designed to release their contents at a predetermined temperature (53). It is possible that some day these will carry therapeutic agents through the bloodstream and deposit them in heated tumors thereby decreasing systemic exposure.

It is well known that hyperthermia potentiates the effects of radiotherapy (6, 28, 38) and many chemotherapeutic agents (7, 11, 12, 41). This potentiation is more pronounced when hyperthermia is applied simultaneously or within a short time of the other therapeutic modality (7, 9, 11, 12, 24, 28, 38) . The cell kill rate for the most ef-

fective known chemotherapeutic agent in the treatment of gliomas, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (39), increases nearly linearly with increasing temperature above 39 ºC (7, 48). Many investigators are using the various methods of administering hyperthermia and combining them with radiotherapy and or chemotherapy in order to determine the optimum dosing schedule (26, 29, 41, 44, 54).

Brain hyperthermia has been associated with an initial rise in intracranial pressure followed by a return toward baseline as temperature increases (43). We do not know the occurrence of any deleterious effects of brain hyperthermia related to elevated intracranial pressure.

## Our Clinical Work

In the treatment of brain malignancy we use 434 MHz microwave applied transcranially via two cradle antennae. Treatment periods last one hour each. For patients receiving radiotherapy (which is given in a fractionated fashion, 5 days a week, over several weeks) we administer hyperthermia three days a week (Monday, Wednesday, and Friday) immediately following radiation. For those patients receiving chemotherapy, we administer hyperthermia during or immediately following chemotherapy and again the following day.

We have thus far treated 24 patients with intracranial malignant disease; eight of primary glial origin and 16 of metastatic origin. Twelve of these patients showed improvement of symptoms with stabilization or decrease if the size of their lesions. The patients that showed improvement received on average 7 to 21 treatments. The patients that did not do well received on average of 1 to 5 treatments. This latter group, in general was suffering from very advanced disease and was not able to complete therapy. No adverse effects related to the hyperthermia therapies were noted in any of the patients we treated.

Figure 1 shows computerized tomography scans of a patient with metastatic renal cell cancer who was treated with hyperthermia in addition to radiation therapy. The top row of pictures are the patient's admission films and the bottom row are his follow-up films 6 months later. At the time of presentation this patient was a 65 year old white gentleman 5 1/2 years status post left nephrectomy for renal cell carcinoma. On the evening of admission he developed sudden onset of headache, nausea, vomiting, and unsteady gait. Physicial exam was significant for a patient with severe headache, nausea, vomiting, and generalized weakness. Neurological examination found only a right partial homonomous hemianopsia. Computerized tomography scan showed a large enhancing lesion in the left medial occipital lobe with hemorrhage within the tumor and ventricular system and two smaller lesions, one in each frontal lobe. The patient was treated with radiation therapy and hyperthermia and showed dramatic improvement in

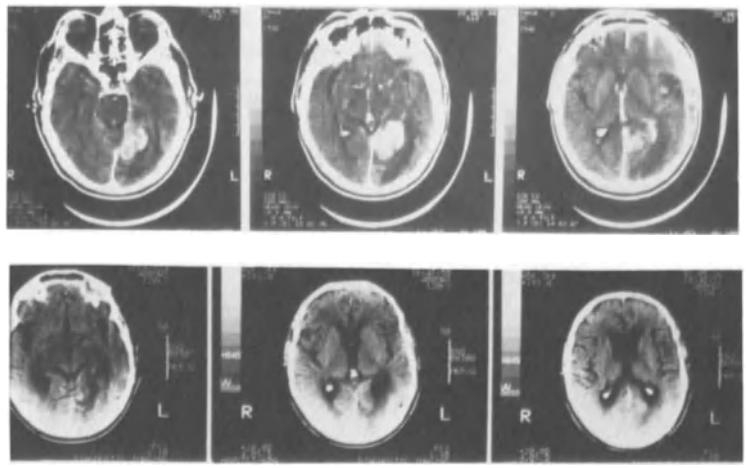

Figure 1. Effect of hyperthermia combined with radiation therapy. The computerized tomography scan in the top row was performed prior to therapy and the scan in the bottom row was done six months after therapy. Although the angle of the gantry is different in these two examinations, the area of interest in the occipital lobe is well seen in both. Note that the lesion in the medial left occipital lobe is nearly gone in the follow-up study. The patient also had lesions in each frontal lobe (not shown) which were not visualized on the follow-up examination.

his symptoms as well as near complete resolution of his lesions on follow-up computerized tomography scans.

## Summary

Brain malignancy, either primary or metastatic, in general is associated with a very poor outcome in spite of the best therapy modern medicine has to offer. The multimodality approach appears to offer the best chance of achieving our goal of improved survival (quality and quantity) and ultimately cure. Hyperthermia, though its application to brain cancer remains experimental, has proven itself in its ability to improve cancer therapy results. There are many methods available to apply hyperthermia to the brain and its application, thus far, has been quite safe. Continued research in this exciting field is warranted.

## REFERENCES

1 Allen, I. V. Cerebral changes in experimental heatstroke. Ir. J. Med. Sc. 6:517-527; 1964.

2 American Cancer Society, Cancer Facts and Figures - 1989; monograph No. 5008-LE; 1989.

3 American Cancer Society, Facts on Cancer of the Brain; monograph No. 2648-LE; 1988.

4 Britt, R. H.; Lyons, B. E.; Pounds, D. W.; Prionas, S. D. Feasibility of ultrasound hyperthermia in the treatment of malignant brain tumors. Med. Instrum. 17(2):172-177;1983.

5 Burger, P. C.: Malignant astrocytic neoplasms: Classification, pathologic anatomy, and response to treatment. Seminars in Oncology 13:18-26, 1988.
6 Crile, G. Heat as an adjunct to the treatment of cancer. Cleve. Clin. Q. 28:75-89; 1961.
7 Da Silva, V. F.; Raaphorst, G. P.; Goyal R.; Feeley, M. Drug cytotoxicity at elevated temperature. In vitro study on the U-87MG glioma cell line. J. Neurosurg. 67:885-8; 1987.
8 Dewey, W. C.; Holahn, E. V. Hyperthermia - Basic biology. Prog. Exp. Tumor Res. 28:198-219; 1984.
9 Dunlop, P. R. C.; Howard, G. C. W. Has hyperthermia a place in cancer treatment? Clin. Radiol. 40:76-82; 1989.
10 Gelber, R. D.; Larson, M.; Borgelt, B. B.; Kramer, S..: Equivalence of Radiation schedules for the palliative treatment of brain metastases in patients with favorable prognosis. Cancer 48:1749-1753, 1981.
11 Hahn, G. M. Potential for therapy of drugs and hyperthermia. Cancer Res. 39:2264-2268; 1979.
12 Hahn, G. M.; Li, G. C. Interactions of hyperthermia and durgs. Treatments and probes. NCI. Monogr. 61:317-323; 1982.
13 Hand, J. W.; ter Haar, G. Heating techniques in hyperthermia. 1. Introduction and assessment of techniques. Br. J. Radiol. 54:443-466; 1981.
14 Harris, A. B.; Erickson, L.; Kendig, J. H.; Mingrino, S.; Goldring, S. Observations on selective brain heating in dogs. J. Neurosurg. 19:514-521; 1962.
15 Hochberg, F. H.; Pruitt, A.: Assumptions in the radiotherapy of glioblastoma. Neurology 30:907-911; 1980.
16 Hornback, N. B.: Historical aspects of hyperthermia in cancer therapy. In: Steeves, R. A. ed. *Hyperthermia.* The Radiologic Clinics of North America 27:481-488, 1989.
17 Hornback, N. B.; Shupe, R.; Homayoon, S.; Joe, B. T.; Sayoc, E.; George, R.; Marshall, C. Radiation and Microwave therapy in the treatment of advanced cancer. Radiology 130:459-464; 1979.
18 Kobayashi, T.; Kida, Y.; Tanaka, T.; et. al.: Magnetic induction hyperthermia for brain tumor using ferromagnetic implant with low Curie temperature. J. Neurooncol. 4:175-181, 1986.
19 Koga, H.; Mori, K.: Interstitial radiofrequency hyperthermia. Clinical application to brain tumor. abstr. *XII International Symposium on Clinical Hyperthermia.* Rome, April 27-29, 1989.
20 Lin, J. C.; Guy, A. W.; Kraft, G. H. Microwave selective brain heating. J. Microwave Power 8:275-281; 1973.
21 Lyons, B. E.; Britt, R. H.; Strohbehn, J. W. Localized hyperthermia in the treatment of malignant brain tumors using an interstitial microwave antenna array. IEEE Trans. Biomed. Eng. BME-31(1):53-62; 1984.
22 Lyons, B. E.; Obana, W. G.; Borcich, J. K.; Kleinman, R.; Singh, D.; Britt, R. H. Chronic histological effects of ultrasonic hyperthermia on normal feline brain tissue. Radiat. Res. 106:234-251; 1986.
23 Moidel, R. A.; Wolfron, S. K.; Selker, R. G.; Weiner, S. B.: Materials for selective tissue heating in a radiofrequency electromagnetic field for the combined chemothermal treatment of brain tumors. J. Biomed. Mater. Res. 10:327-334, 1076.
24 Overgaard, J.: Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue *in vivo.* Int. J. Radiat. Oncol. Biol. Phys. 6:1507-1517, 1980.
25 Ricca, G. F.; Drerup, M. L.; Dohan, F. C. Jr.; Page, R. C.; Clark, D. C.; Robertson, J. T.: Transcranial 434 MHz microwave hyperthermia in a canine model. Submitted for publication.
26 Roberts, D. W.; Coughlin, C. T.; Wong, T. Z.; Fratkin, J. T.; Douple, E. B.; Strohbehn, J. W. Interstitial hyperthermia and iridium brachytherapy in treatment of malignant glioma. A phase I clinical trial. J Neurosurg 64:581-587; 1986.
27 Roberts, D. W.; Strohbehn, J. W.; Coughlin, C. T.; et. al.: Iridium-192

brachytherapy in combination with interstitial microwave-induced hyperthermia for malignant glioma. Appl. Neurophysiol. 50:287-291; 1987.
28 Rohdenburg, G. L.; Prime, F. The effect of combined radiation and heat on neoplasms. Arch. of Surg. 2:116-129; 1921.
29 Salcman, M.; Feasibility of microwave hyperthermia for brain tumor therapy. Prog. exp. Tumor Res. 28:220-231; 1984.
30 Salcman, M.; Glioblastoma multirorme. Am. J. Med. Sci., 279:84-94, 1980.
31 Salcman, M.; Survival in glioblastoma: Historical perspective. Neurosurgery, 7:435-439, 1980.
32 Salcman, M. The morbidity and mortality of brain tumors. A perspective on recent advances in therapy. Neurol. Clin. 3(2):229-257; 1985.
33 Salcman, M.; Kaplan, R. S.; Ducker, T. B., et. al.: Effect of age and reoperation on survival in the combined modality treatment of malignant astrocytoma. Neurosurgery, 10:454-463, 1982.
34 Salcman, M.; Kaplan, R. S.; Samaras, G. M.; Ducker, T. B.; Broadwell, R. D. Aggressive multimodality therapy based on a multicompartmental model of glioblastoma. Surgery 92(2):250-259; 1982
35 Salcman, M.; Samaras, G. M. Hyperthermia for brain tumors: Biophysical rationale. Nuerosurgery 9:327-335; 1981.
36 Salcman, M.; Samaras, G. M.; Mena, H.; Monteiro, P.; Garcia, J. Whole body hyperthermia: potential hazards in its application to glioblastoma. In: Paoletti, P., Walker, M. D., Butti, G., Kenerich, R., eds. *Multidisciplinary Aspects of Brain Tumor Therapy.* Amsterdam: Elsevier; 1979:351-356.
37 Samaras, G. M.; Salcman, M.; Cheung, A. Y,; Abdo, H. S.; Schepp, R. S. Microwave-induced hyperthermia: an experimental adjunct for brain tumor therapy. Natl. Cancer Inst. Monogr. 61:477-482; 1982.
38 Sapareto, S. A.; Rapphorst, G.; Dewey, W. C. Cell killing and sequencing of hyperthermia and radiation. Int. J. Radiat. Oncol. Biol. Phys. 5:343-347; 1979.
39 Schold, Jr., S. C.; Cairncross, J. G.; Bullard, D. E. Chemotherapy of primary Brain Tumors. In: Wilkins, R. H.; Rengachary, S. S., eds. *Neurosurgery.* New York, NY: McGraw-Hill Book Co; 1985:1143-1153.
40 Selker, R. G.; Hyperthermia in the treatment of intracranial turmos. In: Wilkins, R. H.; Rengachary, S. S., eds. *Neurosurgery.* New York, NY: McGraw-Hill Book Co; 1985:1159-1163.
41 Silberman, A. W.; Morgan, D. F.; Storm, F. K.; Rand, R. W.; Benz M.; Drury, B.; Morton, D. L. Combination hyperthermia and chemotherapy (BCNU) for brain malignancy. Animal experience and two case reports. J. Neurooncol. 2:19-28; 1984.
42 Silberman, A. W.; Morgan, D. F.; Storm, F. K.; Rand, R. W.; Bubbers, J. E.; Brown, W. J.; Morton, D. L. Localized magnetic-loop induction hyperthremia of the rabbit brain. J. Sur. Oncol. 20:174-178; 1982.
43 Silberman, A. W.; Rand, R. W.; Krag, D. N.; Storm, F. K.; Benz, M.; Drury, B.; Morton, D. L. Effect of localized magenitic-induction hyperthermia on the brain. Temperature versus intracranial pressure Cancer 57:1401-1404; 1986.
44 Silberman, A. W.; Rand, R. W.; Storm, F. K.; Drury, B.; Benz, M.; Morton, D. L. Phase 1 trial of thermochemotherapy for brain malignancy. Cancer 56:48-56; 1985.
45 Sneed, P. K.; Matsumoto, K,; Stauffer, P. R.; Fike, J. R.; Smith, V.; Gutin, P. H. Interstitial microwave hyperthermia in a canine brain model. Int. J. Radiat. Oncol. Biol. Phys. 12:1887-1897; 1986.
46 Stehlin, J. S.; Giovanella, B. C.; de Impolyi, P. D.; et. al.: Results of hyperthermia perfusion for melenoma of the extremity. Surg. Gynecol. Obstet. 140:338-340, 1975.
47 Stewart, J. R.; Gibbs, F. A. Hyperthermia in the treatment of cancer. Perspectives on its promise and its problems. Cancer 54:2823-2830; 1984.
48 Storm, K. F.: Clinical hyperthermia and chermotherapy. In: Steeves, R. A., ed. *Hyperthermia.* The Radiologic Clinics of North America 27:481-488, 1989.
49 Storm, K. F.; Harrison, W. H.; Elliott, R. S.; Morton, D. L.: Physical aspects of localized heating by magnetic-loop induction. In: Storm, K. F., ed.

*Hyperthermia In Cancer Therapy.* Boston, Massachusetts: G. K. Hall Medical Publishers; 1983:305-313.

50 Tanaka, R.; Kim, C. H.; Yamada, N.; Saito, Y.: Radiofrequency hyperthermia for malignant brain tumors: Preliminary results of clinical trials. Neurosurgery 21:478-483, 1987.

51 Thrall, D. E.; Page, R. L.; Dewhirst, M. W.; Meyer, R. E.; Hoopes, P. J.; Kornegay, J. N.: Temperature measurements in normal and tumor tissue of dogs undergoing whole body hyperthermia. Cancer Research 46:6229-6235; 1986.

52 Walker, M. D.; Green, S. B.; Byar, D. P.; Alexander, E, Jr.; Batzdorf, U.; Brooks, W. H.; Hunt, W. E.; MacCarty, C. S.; Mahaley, M. S. Jr.; Mealey, J. Jr.; Owens, G.; Ransohoff, J.; Robertson, J. T.; Shapiro, W. R.; Smith, K. R. Jr.; Wilson, C. B.; Strike, T. A.: Randomized comparison of radiotherapy and nitorsoureas for the treatment of malignant glioma after surgery. N. Engl. J. Med. 303:1322-1329, 1980.

53 Weinstein, J. N.; Magin, R. L.; Yatvin, M. B.; Zaharko, D. S. Liposomes and local hyperthermia: Selective delivery of methotrexate to heated tumors. Science 204:188-191; 1979.

54 Winter, A.; Laing, J.; Paglione, R.; Sterzer, F. Microwave hyperthermia for brain tumors. Neurosurgery 17:387-399; 1985.

55 Zimm, S.; Wampler, G. L.; Stablein, D.; Hazra, T.; Young, H. F.: Intracerebral metastases in solid-tumor patients: Natural history and results of treatment. Cancer 48:384-394, 1981.

# HYPERTHERMIA AND THE LIVER

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## LIVER NEOPLASM AND ITS TREATMENT

The liver is the organ most commonly involved in spread of gastrointestinal cancer. Of the synchrounous liver metastases found at laparotomy, 20-25 % are due to colorectal cancer. Approximately 50 % of colorectal tumors will sooner or later give rise to liver metastases. The outcome for patients with primary and secondary liver cancer is dismal, with mean survival of only a few months for primary and six to eight months for secondary cancer. Liver resection may induce a 2-year survival of approximately 30 % in patients with restricted tumor spread. These facts justify an aggressive surgical treatment in selected cases. In patients with scattered metastases in the liver dearterialization and/or chemotherapy has been used but the results are on the whole rather disappointing with a tumor response of 20-30 %. Neither dearterialization nor chemotherapy have induced any significant prolongation of survival.

In the search for other means of controlling liver tumor growth hyperthermia has been introduced as a complementary mode of treatment (Crile, 1961; Parks et al, 1978; Storm et al 1979). Hyperthermia offers a theoretically interesting prospect arising from its suggested selective effect on tumor tissue, leaving normal tissue intact.

## METHODS FOR INDUCTION OF LIVER HYPERTHERMIA

In humans survival has been reported after rectal temperatures as high as 44.4°C. The upper limit for induced whole body hyperthermia is below 43°C. whole body hyperthermia can be induced by immersion in hot fluid, perfusion with extracorporal heat exchange or by exposition to radiant heat or microwaves.

Local and regional hyperthermia of the liver can be induced by focused ultrasound, perfusion or by electromagnetic heating. During local heating a significant temperature gradient can be obtained between the liver and the rectum (Fig 1, Hugander et al, 1984).

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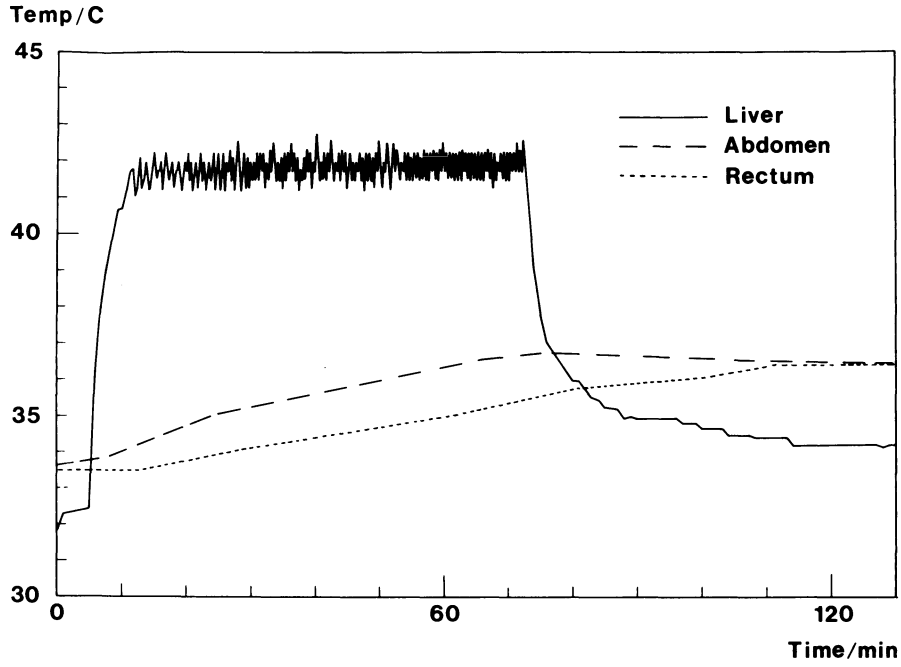


Fig 1. Temperature profile during local liver hyperthermia.

## HYPERTHERMIC EFFECTS ON THE LIVER

Before a patient with liver cancer is treated with hyperthermia some important factors should be considered; extent of tumor growth, tumor type, concomitant treatment with hepatotoxic drugs, earlier hepatic injury are factors that could have a negative impact on the postoperative liver function.

The toxic and therapeutic effect of hyperthermia on liver tissue can be increased by alteration of liver blood flow, for example by hepatic artery ligation, injection of degradable microspheres or by temporary occlusion of the portal blood supply. Alterations in the blood supply may effect the maximum temperature as well as the metabolism in normal tissue and tumor tissue.

Under normothermic conditions the temperature of the liver is approximately 0.7-1$^{o}$C higher than the intraabdominal temperature. This difference is caused by the high metabolism of the liver cells, as the liver is the most important internal heat source for maintaining the body temperature. During whole body hyperthermia the liver temperature remains close to the intraabdominal temperature whereas after concluded heating the difference increases, indicating a less efficient heat transfer from the liver.

In the perfused liver increases of lactate, pyrovate, glucose, urea, potassium, ALP, GOT and LDH indicate a liver injury at temperatures above 42$^{o}$C. Other criterias for liver injury are decreased oxygen consumption and increased ammonia production (Skibba et al, 1986).

In animal experiments during constant conditions concerning blood flow, oxygenation and pH, isolated perfusion of the rat liver in vitro induced pronounced centrilobular vacuolization at 41°C. A severe dissociation of hepatocytes occured at 42°C (Bowers et al, 1981). In vivo perfusion of dog livers at 43°C microscopic analyses revealed a diffuse hepatocyte vaculopathy progressing to ballooning degeneration (Boddie et al, 1979). When combining 42°C whole body hyperthermia with hepatic artery occlusion and transient portal ven occlusion centrilobular necrosis occur in the liver parenchyma. These changes do not occur during hyperthermia without circulatory manipulation (Holmin et al, 1987).

Clinical reports concerning hyperthermic effects on the liver including parenchymal cell necrosis, cholestasis and liver necrosis. A considerable incidence of liver toxicity and insifficiency occur after whole body hyperthermia as well as after liver perfusion (Robins et al, 1989, Hafström, 1989). No definite correlation excists between the temperature level and extent of liver injury.

## HYPERTHERMIC EFFECTS ON LIVER TUMORS

Theoretically regional hyperthermic treatment of the liver should be of value providing that the blood flow in the tumors is below that of the surrounding parenchyma, thus resulting in a temperature difference between tumor and normal parenchyma. In this way a selective heating of tumor tissue makes hyperthermia suitable for the treatment of scattered metastases (Fig 1.).

No controlled studies have been performed concerning the effect of hyperthermia on liver tumors. Most reports describe the effect of combination with chemotherapy or radiotherapy. A combination of whole body hyperthermia and chemotherapy caused decreased tumor size, regression of pain and regression of jaundice in patients with hepatic metastases of colonic cancer, cholangiocarcinoma, undifferentiated carcinoma and adenocarcinoma of the gall bladder (Pettigrew et al, 1974). In patients with melanoma metastases of the liver, regional hyperthermia combined with DTIC induced tumor regression and pain relief in more than 50 % of patients treated (Storm et al, 1982). Similar effects on liver metastases of colorectal cancer are reported after deep microwave hyperthermia combined with radiotherapy or chemotherapy (Petrovich et al, 1988).

## EVALUATION OF RESPONSE

To evaluate the response to hyperthermia treatment a combination of diagnostic tools have to be used. If tumor size measurement by CT or ultrasound is used the tumoricidal effect of hyperthermia treatment may be disguised by "tumor swelling" caused by necrosis. Registration of tumor size should therefore be combined by biopsies to reveal the extent of tumor necrosis. In human liver tumors vascular necrosis and trombosis after hyperthermia treatment may prevent absorption of the destroyed tumor, resulting in fibrous replacement with little change in tumor size.

## THE FUTURE FOR LIVER HYPERTHERMIA

Most patients exposed to liver hyperthermia are in an advanced stage of cancer disease with pain and discomfort caused by bulky tumor masses. A good palliaton including pain relief and reduction in tumor size may motivate the use of hyperthermia, althought no increase in survival yet has been proven. Additive or synergistic effects of the combination of chemotherapy and hyperthermia may be used in decreasing the toxicity of chemotherapeutic agents.

To further develop liver hyperthermia into a useful and safe technique for treatment of primary and secondary liver cancer less toxic and less expensive methods for induction of liver hyperthermia have to be developed. Interesting studies are presently performed in different Japanese Centres, using the effect of vasoactive drugs, microspheres or occlusors to improve the tumoricidal effect on liver tumors.

Research resources should be concentrated on the demonstration of beneficial effects of hyperthermia and thermochemotherapy in animal experiments as well as in controlled clinical trials with the long term purpose of adjuvant treatment after surgery of advanced colorectal cancer, pancreatic cancer, primary hepatoma and other neoplasms where current therapy fail to improve survival and quality of life.

## REFERENCES


Boddie AW, Booker L, Mullins JD, Buckley CJ, McBride CM., 1979, Hepatic hyperthermia by total isolation and regional perfusion in vivo. J Surg Res., 26:447-457.

Bowers W, Hubbard R, Wagner D, Chrisholm P, Murphy M, Leav I, Hamlet M, Maher J., 1981, Integrity on perfused rat liver at different heat loads. Lab Invest., 44:99-104.

Crile G Jr, 1961, Heat as an adjunct to the treatment of cancer: experimental studies. Cleveland Clin Quart., 28:75.

Hafström LO, Unpublished data.

Holmin T, Hugander A, Hägerstrand J, Ingvar C, Stridbeck H, 1987, The effect of hyperthermic ischemia on normal liver parenchyma. Eur Surg Res., 19, suppl 1, 103-4.

Hugander A, Hafström LO, Jönsson PE, Bolmsjö M, Persson B, Stenram U, 1984, Local hyperthermia in treatment of experimental liver tumors. Cancer, 53:1686-91.

Parks LC, Minaberry D, Nely B et al, 1978, Extracorporealinduced systemic hyperthermia: effects on man and cancer. Surg Forum, 29:148-150.

Petrovich Z, Langholz B, Astrahan M, Emami B, 1988, Deep microwave hyperthermia for metastatic tumors of the liver. Recent Results Cancer Res., 107:244-8.

Pettigrew RT, Galt JM, Ludgate CM, Smith AN, 1974, Clinical effects of whole body hyperthermia in advanced malignancy. Brit Med J., 4:679-682.

Robins HI, Hugander A, Cohen JD, 1989, Whole body hyperthermia in the treatment of neoplastic disease. Radiologic Clinics of North America, Vol 27:3, 603-10.

Skibba JL, Quebbeman EJ, Kalbfleisch JH, Sonsalla JC, Petroff RJ Jr, 1986, Alterations in biochemical functions during hyperthermic isolation-perfusion of the human liver. J Surg Res., 41 (4):432-8.

Storm FK, Harrisson WH, Elliott RS, Morton DL, 1979, Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials. Cancer Res., 39:2245-2251.
Storm FK, Kaiser LR, Goodnight JE, Harrison WH, Elliott RS, Gomes AS, Morton DL, 1982, Thermochemotherapy for melanoma metastases in the liver. Cancer, 49:1243-1248.

# QUALITY CONTROL OF A HYPERTHERMIA SYSTEM

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## SUMMARY

Characterization of the applicators, of the thermometers and of the cooling (bolus) system of hyperthermic apparatus must be made before starting hyperthermic treatment and also starting with a frequency depending on the technical features of equipments that make up the whole system. This data gives the radiotherapist some useful parameters for the selection of applicators and general knowledge of the accuracy of the system.

## INTRODUCTION

The acceptance of our automatic computerized system for oncology hyperthermic therapy (SAPIC SV03/A, Aeritalia, Italy) installed at National Cancer Institute of Milan forced us not only to calibrate the thermometers and the bolus system but also to study the distribution of the absorbed energy following irradiation.

## MATERIALS AND METHODS

### Characterization of applicators

Characterization in high water content tissue of the hyperthermic applicators following E.S.H.O. protocol (1987) is made irradiating, for a few seconds with high power, a plane homogeneous phantom of dielectric characteristic like muscle (Cetas 1985). The water bolus is used for having a good transmission of electromagnetic waves. Measurements were made in two phantoms whose compositions are listed in Table 1 (Chou et al. 1984, Nilsson 1984). Measured dielectric constant and electric conductivity (see definition in NCRP report N.67, 1981) determined with accuracy of about 3%, are given for phantoms MW1 in Table 2.

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Table 1. Recipe of materials of two phantoms simulating high water content tissue in microwave region:

| Phantom material | % Composition MW1 | MW2 |
|---|---|---|
| NaCl | 0.99 | 1.2 |
| $H_2O$ | 75.15 | 58.8 |
| Polyethlene powder | 15.44 | - |
| Tx - 150 (super-stuff) | 8.42 | 17.5 |
| Sugar | - | 22.5 |

Table 2. Measured dielectric $\varepsilon_r$ constant and electric conductivity $\sigma$ of simulating phantoms. Last row data, taken from NCRP Report N.67 are given for comparison.

| Phantom or tissue | Microwave frequency MHz | $\varepsilon_r$ | $\sigma$ S/m |
|---|---|---|---|
| Mw1 phantom (20°C) | 434 | 47.5 | 1.46 |
| " " | 915 | 46.9 | 1.58 |
| MW2 phantom (20°C) | 434 | 53.9 | 0.80 |
| " " | 915 | 50.6 | 1.15 |
| Muscle (37°C) | 434 | 53 | 1.18 |
| " | 915 | 51 | 1.28 |

Specific absorption rate (SAR) patterns in muscle equivalent phantoms for surface and interstitial applicators were measured in a clinical set-up with an accuracy of about 10%. SAR is given by the expression:

$$SAR \quad \frac{4,186 \cdot c \cdot \Delta T}{t} \quad \frac{W}{kG} \qquad (1)$$

where:

c = specific heat, cal/ °C.g (c= 0.87 cal / °C.g for MW2 phantom);

T = temperature increase in phantom. °C;
t = microwave high power pulse duration, s.

SAR can be normalized to the net power used to heat the phantom for comparison between different applicators.

Temperature distribution has been detected by a tele thermal imaging system (mod.3300, Hughes, USA) with a temperature difference sensitivity of 0.1°C interfaced to a personal computer able to aquire and analyze images giving isothermal distribution of the temperature increase due to absorbed energy in phantom. We set up an image processing card (mod. PIP-640, Matrox, Canada), an additional high resolution color monitor (mod. HF-1400,Mitsubishi, Japan) and a dot color printer (mod.PJ-1080A, Canon Japan) for hard copy of images with 8 colors.

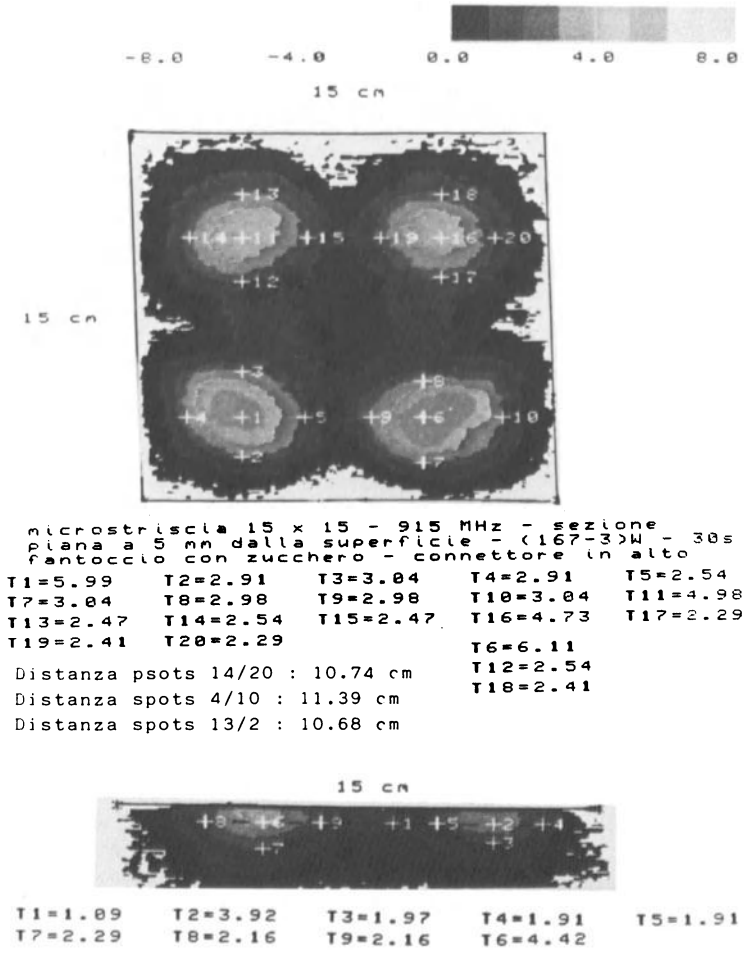


Figure 1. Temperature rise distribution in muscle equivalent phantom following contact irradiation with the 4 lobes microstrip at 915 MHz and power of 164 W for 30 s. The figure at the top gives the increase of temperature in a plane at a depth of 5 mm from the surface of the phantom while the figure at the bottom gives the increase of temperature in a parallel plane to the beam axis intersecting 2 lobes. Different intervals of temperature are visualized with different colors. The temperature rise in each each pixel of the image selected by a cross can be displayed as well as the distance between two points thus giving the possibility of measurement of the effective field size and of the penetration depth.

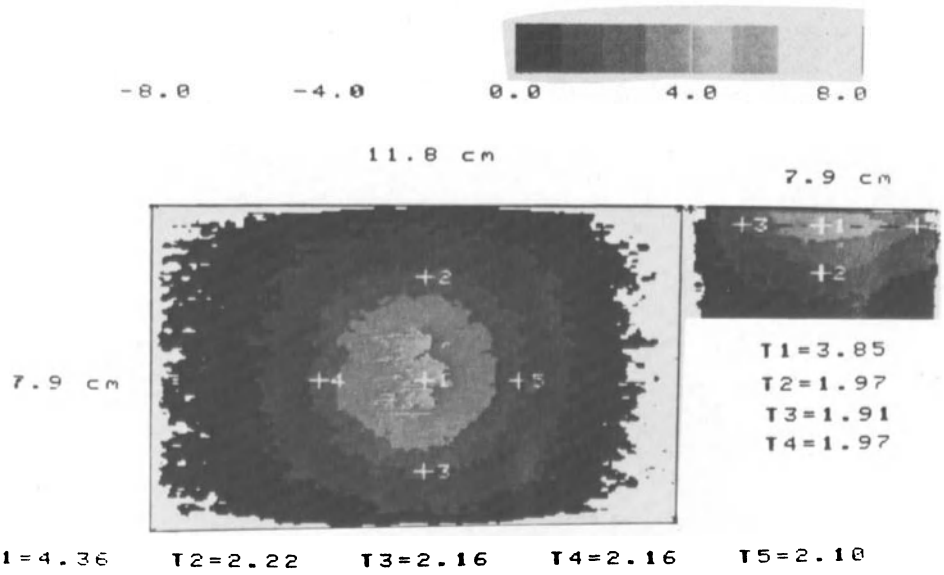


Figure 2. Temperature rise distribution in muscle equivalent phantom following superficial irradiation with a loaded waveguide external applicator working at 434 MHz emitting a power of 50 W for 30 s. The figure at left gives the increase of temperature in a plane at a depth of 5 mm from the surface of the phantom and the figure at the right gives the increase of temperature in a plane containing the beam axis and parallel to the short side of the applicator.

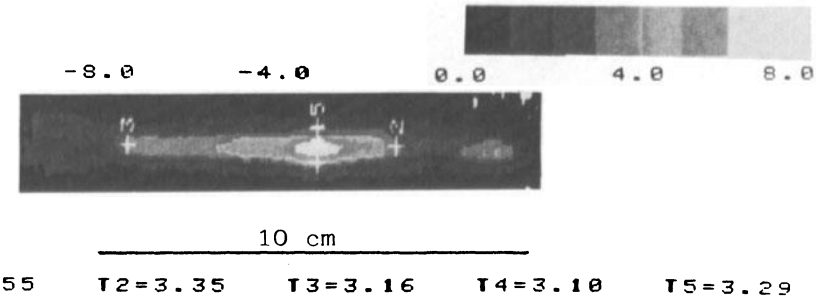


Figure 3. Temperature rise distribution for interstitial applicator at 915 MHz - 12 - 20 s in muscle equivalent phantom. The interstitial antenna is inserted in phantom 10 cm.

## Control of calibration of thermometric probes

Calibration of the thermometric probes is made by the system each day before starting applications. Weekly they are tested in a water thermostatic bath using a calibrated certified mercury thermometer.

## RESULTS

Effective field sizes and penetration depths for surface applicators listed in Table 3 were determined from SAR distribution (Figures 1 and 2 ). To characterize interstitial antenna the maximum diameter of field taken at isoSAR 50% around the applicator is reported (Figure 3 and Table 4).

Table 3. Results of contact applicator's characterization. The dimensions of radiating aperture and, between parenthesis, the external geometrical dimensions are reported in the first column.

| Contact applicators | Frequency MHz | Penetration depth,cm | Effective field size, cmxcm |
|---|---|---|---|
| waveguide 5x9(7.9x11.8) | 434 | 2.2 | 5.0 x 5.0 |
| waveguide 13x13 (15x15) | 915 | 2.0 | 11.0 x 12.0 |
| microstrip5.8x8.6(7.5x12) | 434 | 1.7 | 3.9 x 4.3 |
| " " " " " | 915 | 1.6 | 3.5 x 4.4 |
| microstrip5.2x5.4(6.7x7.5) | 434 | 1.8 | 3.1 x 5.7 |
| " " " " " | 915 | 1.5 | 3.7 x 5.9 |
| 4lobes microstrip10.8x12.6 (15x15) | 434 | 2.1 | 11.7 x 11.2 |
| " " " " " | 915 | 1.8 | 11.7 x 10.8 |

Table 4. Characterization of interstitial applicators. Interstitial antennas are 10.8 cm long, the interstitial applicators (bottom row) are 1.2 cm apart simulating radioactive implant geometry.

| Interstitial applicators Number | Freq. MHz | Length of insertion in phantom, cm | Field diameter cm |
|---|---|---|---|
| 1 | 915 | 7 | 0.8 |
| 1 | 915 | 10 | 0.6 |
| 2 | 915 | 7 | 2.2 |

In the range 30-47°C the value of the temperature measured by each of the 12 thermometric probes is at maximum 0.5 °C different from the value given by a temperature standard. In order to have the real temperature within the bolus during treatment the correction factor to be applied to the displayed bolus temperature has been determined.

REFERENCES

1) Cetas, T.C., 1982, The philosophy and use of tissue-equivalent electromagnetic phantoms,"Physical aspects of hyperthermia" G.H.Nussbaum, ed., American Institute of Physics, NY, 441-461.

2) Chou, C.K., Chen, G.W., Guy, A.W., and Luk, K.H., 1984, Formulas for preparing phantom muscle tissue at various radiofrequencies. Bioelectromagnetics 5, 435-441.

3) European Society for Hyperthermic Oncology, Technical Commitee, 1987, Quality assurance guidelines for ESHO protocols.3 draft.

4) National Council on Radiation Protection and Measurements, 1981, "Radiofrequency electromagnetic fields". NCRP Report N. 67, Washington, D.C., 54-91.

5) Nilsson P., 1984, Physics and technique of microwave-induced hyperthermia in the treatment of malignant tumors. Doctoral dissertation, Lund university, 11-36.

# USE OF LOCAL HYPERTHERMIA FOR THE TREATMENT OF BENIGN PROSTATIC HYPERPLASIA

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## INTRODUCTION

Local hyperthermia (LH) combined with radiation therapy (RT) has been used for the last 3 decades for the treatment of a variety of human tumors (1-6). To-date, most of the reports in oncologic hyperthermia (HT) deal with results obtained from treatments encompassing variations in tumors, tumor site, protocol of treatment and HT equipment. The normally advanced stage of the disease on admittance to LH permits only short follow-up periods and no long term response information is available.

Clinical treatment of benign tumors by HT alone has not been reported in the literature prior to 1985 (7). In 1980 we started the treatment of benign prostatic hyperplasia (BPH) with LH alone (7). In this chapter, I review our 8 years of experience and I summarize our group's results (7-9), regarding initial response, response duration, acute and long term side effects from HT.

## INTRACAVITARY HYPERTHERMIA

The HT system used for the treatment of prostatic tumors and the method of application have been described in detail elsewhere (7-9). Briefly, LH at 42.5-43°C is delivered using a transrectal applicator operating at 2.45 Ghz. The applicator is equipped with an appropriate cooling system to avoid overheating of the rectal mucosa and rectal wall. The applicator is positioned in the rectum and maximal heat energy is aimed towards the prostatic mass. This method permits heating of the tissue comprising the prostatic tumor with minimal heating of non invaded tissues. Thus, a deep seated tumor can be heated without the need for large electro-magnetic fields required if treated by external antennae.

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Table 1. Response of BPH patients with an indwelling catheter to local hyperthermia

| AFTER TREATMENT | | AFTER 6 - 70 MONTHS | |
|---|---|---|---|
| Free of catheter satisfactory voiding | Failed | Free of catheter satisfactory voiding | Failed |
| 57% | 43% | 47% | 53% |

Thirty seven patients entered the study, three non evaluable. Catheter carried prior to LH: range 2-12 months, mean 6.7 months. Age: range 59-86 years, mean 73 years. Number of sessions: range 6-19, mean 13. Follow-up: 6-70 months, mean 49 months.

## PATIENTS AND TREATMENT PROTOCOL

Since 1980, all the patients received LH twice weekly in 12-16 sessions, each lasting 1 hour. Treatments were given without additional medication, on an outpatient basis, with no sedation or anaesthesia.

One hundred and five patients, most of them poor surgical risk patients with BPH entered the study. Thirty seven carried an indwelling catheter from one to twelve months before admission to treatment. The second group consisted of 68 patients with severe prostatic symptoms. Five patients were lost to follow-up and 6 stopped treatment. For evaluation, the patients were categorized into the following study groups: catheter carrying and patients without a catheter who underwent one course of treatment, patients who were relieved of their symptoms, deteriorated to their pretreatment obstructions and underwent a second course of treatment.

## RESULTS

The results of treatment as detailed in tables 1-3 were derived from a scoring system designed for evaluating urological symptoms prior to and after treatment (7). In the catheter carrying group, at the end of the treatment course, 57% of the treated patients were relieved of their catheter and resumed satisfactory voiding. Forty seven percent of the treated patients were free of their catheter until 70 months after treatment. The post treatment results were maintained in 82% of the catheter relieved patients during a follow-up period of 70 months (Table 1).

Table 2. Response of BPH patients without a catheter to local hyperthermia

| AFTER TREATMENT | | |
|---|---|---|
| Marked improvement | Improvement | Failed |
| % | % | % |
| 38 | 45 | 17 |

TOTAL IMPROVEMENT 83 %

| AFTER 6-80 MONTHS | | |
|---|---|---|
| Same or better than after treatment | Less than after treatment but satisfactory | Regressed to pretreatment |
| % | % | % |
| 43 | 26 | 31 |

TOTAL IMPROVEMENT 72 %

Sixty eight patients entered the study, five non evaluable. Age: range 50-85 years, mean 71 years. Number of sessions: range 6-19, mean 13. Follow-up, 6-80 months, mean 53 months.

Eighty three percent of patients initially with severe prostatic symptoms were improved after LH. Relief from prostatic symptoms was maintained in 72% of the patients of this group, until 80 months after treatment (Table 2). It should be noted that there were cases in which no improvement was noted after LH, but urological symptomatology improved a few weeks later.

Most BPH patients are elderly people, suffering from a variety of diseases, a part of which denies them of prostatectomy. During the follow-up period of about 8 years, ten patients died of these diseases. In this group, 69% were free of their urological problems and resumed normal voiding until their death, namely LH freed them of their urological obstructions during their whole life time from treatment to their death.

Eight patients underwent two courses of treatment. These patients benefited from the first course, but later, symptoms recurred to pretreatment symptomatology. As can be seen from table 3, the period to recurrence of obstructions was not related to the patient's age, nor to the number of sessions in the first course of treatment or to the prostate's size. None of the treated patients experienced thermal damage or burns. No reactions of the rectal mucosa such as erythema, edema, or ulceration were observed and treatment was generally well tolerated.

Table 3. Response to LH of BPH patients who underwent two treatment courses

| ----1ST COURSE----- | | | | | ----2ND COURSE----- | | | |
|---|---|---|---|---|---|---|---|---|
| Catheter | Age | No. sessions | Status | Period months | No. sessions | Status | Period months | Alive Dead |
| - | 71 | 15 | Imp | 7 | 12 | Imp++ | 36 | D |
| - | 65 | 11 | Imp++ | 9 | 9 | Imp++ | 32 | A |
| + | 73 | 15 | Free | 3 | 13 | Free | 25 | A |
| - | 75 | 7 | Imp++ | 24 | 9 | Imp++ | 13 | A |
| - | 74 | 11 | Imp | 29 | 7 | Imp | 17 | A |
| - | 75 | 16 | Imp | 7 | 10 | Imp | 15 | D |
| - | 62 | 8 | Imp | 7 | 9 | Imp++ | 8 | A |
| - | 72 | 9 | ImP | 8 | 9 | Imp | 6 | A |

Patients underwent a first course of treatment which resulted in improvement (IMP), marked improvement (IMP++), which lasted as specified in Period months. After this period, urological obstructions recurred and the patients underwent a second course of treatment.

## DISCUSSION

Most of the clinical experience with HT is still with superficial and subcutaneous tumors. Treatment of deep seated tumors with HT involves difficulties in technology which is not yet advanced to assure heat delivery to tumors situated deep in the body, without heating of noninvaded, unmonitored sites in the applicator to tumor pathway and the lack of non invasive thermal dosimetry at depth. To apply HT to a deep seated tumor with existing technology, a tumor in the vicinity of a natural cavity was selected. This allows close proximity of the applicator to the tumor, so that low microwave power will be sufficient to reach hyperthermic temperatures in the targeted volume and concomitantly minimize normal tissue heating in the applicator to tumor pathway.

I have selected the prostate as the first site for intracavitary HT because it is the site of two diseases in the male, prostatic carcinoma (6,8,9) and BPH and because of the easy administration of treatment through the rectal cavity. Prior to its clinical application, the technique was tested in animals. We investigated the thermal tolerance of normal tissues in the vicinity of the treated prostate. The histolopathological evidence obtained in this study indicated that our LH technology can be safely applied for the clinical treatment of prostatic tumors (10).

BPH is a common disease of the aging male, resulting in varying degrees of urinary obstructions and catheterization. At the present time, there is no effective medical management for BPH and the only effective treatment is surgery (11). However, because of the advanced age of most patients with BPH, surgery

is contraindicated in a significant number of cases. Hence, the importance of a non surgical method for the treatment of BPH is self evident.

This study is unique with respect to treatment with LH alone of a benign tumor, in a patient population homogeneous with respect to tumor site, treated with the same machine and same LH protocol and 8 years of follow-up which enables analysis of short and long term side effects of LH. The long lasting relief from catheterization and improvement in symptoms in the patients without a catheter, demonstrate the method's efficacy.

During our 8 years of application and follow-up, there were no acute or long term thermal damages, attesting to the safety of the HT technology employed. Moreover, the absence of acute and long term toxicity permitted the application of a second course of treatment in patients who regressed to their pretreatment obstructions. The recurrence after the first course is a natural etiology of a slow growing benign tumor that was reduced in size slightly below the critical value permitting a temporary relief of obstructive symptoms.

We have been repeatedly asked about a relationship between the efficiency of treatment versus prostate size. From our 8 years of experience, no conclusion can be drawn regarding this subject. We have noticed cases in which the treatment was efficient in patients with large prostates, while failure occurred in patients with smaller prostates. The explanation of this phenomena might be that BPH develops many years before manifestation of obstructive symptoms. The enlarged prostate causes urinary obstructions after reaching a critical size, resulting in urinary obstructions. If a treatment succeeds in reducing the prostate size below this critical value, the obstruction will disappear, leading to satisfactory voiding. Hence, the absolute size of the prostate after treatment is not the measure of failure or success of treatment, but the reduction in size to a value permitting satisfactory voiding.

In conclusion, the results achieved and 8 years of follow-up indicate that LH is an effective modality for the treatment of BPH.

## PROSPECTS OF HYPERTHERMIA TOWARDS THE 1990'S

The future of HT as a clinical treatment modality depends on its use in a wide spectrum of diseases. At present, clinical HT is almost completely limited to cancer therapy, as an adjuvant to radio- or chemo- therapy. Additional past proven uses of HT and its potential as a therapeutic modality for other diseases are ignored. Three objective difficulties limit the use of oncologic HT and prevent its acceptance as a standard clinical treatment: the physical difficulties of depositing energy in deeply situated tumors, inability to control regional blood flow and the lack of non-invasive thermometry for deep seated tumors HT. These limitations have seriously hampered the acceptance of HT for the treatment of primary tumors and oncological HT is the last choice treatment and most patients are referred to it after failure of conventional methods.

Hyperthermia will never pass the stage of a clinical trial and will continue to be the treatment of last choice, unless its

use will be extended to a wide spectrum of diseases. This will enable the medical community to gain experience in its use towards its acceptance as a treatment modality for various diseases, including cancer. In this section I want to make justice to the forgotten role played by elevated temperatures in medicine and emphasize its potential use in the treatment of non malignant diseases.

Elevation of local, regional or systemic temperature of an organism results in cellular changes which may lead to the cell's death, affects the tissues causing vasodilation, antalgic effect, anti-inflammatory effect, local immunity modifications and systemic. The use of HT in medicine resides on the basic fact that temperatures above the normal core temperature (fever) play a role in the host's defence mechanism (12-20). Fever is produced by pyrogenic substances in the blood that are released when the body is encountered with foreign antigens, regardless of whether the invader is a virus, bacteria, or other foreign molecules, or when inflammation due to other causes takes place. This temperature dependence of the immune response (21-22) could therefore contribute to the selective value of fever, and its role in the host's defence mechanism. In fever, the thermal regulatory mechanisms function normally but maintain an elevated body temperature, i.e., the body's responses are directed to reaching a new higher thermal regulatory set point.

Fever has been considered beneficial until the late 1800's. Prior to the advent of antibiotic drug therapy, diseases such as orchitis, asthma, multiple sclerosis and infectious diseases such as syphyllis and gonococcal infections, including endocarditis and pneumococcal meningitis were treated by hyperthermia(23-28). The sub sequent development of antipyretic drugs and their wide use led to categorizing fever as a harmful by product of disease because most antipyretic drugs such a the salicy lates, are not only antipyretics but also analgesics. A drug which is purely antipyretic might have no effect on the pain which accompanies most infections: however, the dual function of aspirin, may lead to the conclusion that pain relief is the direct result of lowering fever.

In accord with this reasoning, I have extended the use of HT and applied it in 3 diseases, in addition to malignant tumors. I have applied heat alone for the treatment of benign prostatic hyperplasia, to viral rhinitis, and to allergic rhinitis. The clinical application of LH to benign prostatic hyperplasia was initiated by our group in 1980. The first results of two years experience were reported in 1982. The updated results with a follow-up of 8 years were detailed in the first part of this chapter.

I shall describe the methodology which led me to apply HT to two other disease. The analysis of this methodology, based on the relationships between the effects of heat on the cause of disease, may point out the approach to extend the use of HT as a clinical tool to additional diseases.

## LOCAL HYPERTHERMIA FOR THE TREATMENT OF VIRAL AND ALLERGIC RHINITIS

Viral development is temperature sensitive. Different temperatures inhibit viral proliferation according to the

strain's thermal tolerance and the host's normal temperature at which the virus is maintained. Temperatures above the normal host's temperature may block viral development and lead to the virus' death.

After infection by a virus and before the action of antibodies, leukocytes accumulate around infected cells, lactic acid accumulates and the pH drops to levels deleterious to viral development, in concert with the activity of cellular metabolism resulting in the increase in temperature of the inflammatory zone. Subsequently, a pyrogenic substance is released by leukocytes, acting on the central nervous system and fever is produced at temperatures deleterious to many viral strains which may inhibit viral replication. In addition, elevated temperatures increase lysosomal lesions and lysosomal enzymes which destroy viral RNA are liberated, leading to a block in viral development. It is well established that heat has a direct adverse effect upon viability of viruses (29 -30), regardless of the stage of infection and maintaining the host at higher temperatures increase the host's resistance to infection, as with gastroenteritis virus (31). Hence, fever is a major factor in recovery from primary virus infections (32 33).

The function and morphology of the upper respiratory passages allow close contact of antigenic material with the local immune apparatus, stimulation of the immunoresponsive system, resulting in an allergic reaction manifesting itself as allergic rhinitis. The biochemical mediators of allergic reactions of the immediate hypersensitivity type involving immunoglobulin E (IgE) antibodies are fairly well known (34-35). Antibodies of this class (reagins) participate in immediate-type hypersensitivity reactions (36). The thermal lability of IgE is a long recognized fact (37), demonstrated by the loss of IgE ability to bind to basophils and mast cells membranes upon heating the molecule[38]. The histamine - releasing mechanism in anaphylaxis was found to be highly temperature dependent and heating tissues in the temperature range of 42.5°C to 43.5°C produced persistent inactivation of the anaphylactic mechanism (39).

Because of the cooling produced by air flow, the temperature of the nasal turbinates is lower than the core temperature, varying between 31°C-35°C (40). Hence, when activated in response to contact with a virus or an antigen in the nasal passages, the febrile response is unable to elevate the temperature in the nose to those achieved during fever in the inner parts of the body. Hence, local HT to the nasal passages should assist the body to elevate the temperature of this anatomical site. The aforementioned known biological facts on the effects of elevated temperatures on the cause of disease, form the rationale for the application of LH to the nose in patients suffering from viral and allergic rhinitis.

In both diseases, LH was applied at 43°C to the upper respiratory passages, without supplementary drugs. In patients afflicted with viral rhinitis, LH suppressed the symptoms of the disease in 78% of the treated patients (41).

Using the same HT technology and method of treatment, we applied local HT to the nasal passages to patients suffering from perennial and seasonal allergic rhinitis. Seventy five

percent and 68% of the patients were free of symptoms 1 week and 1 month respectively, after treatment (42). In another study, local HT partially or fully inactivated the anaphylactic reaction in 76% of patients, as found by challenge with the appropriate allergen; 10 days after treatment (43). It should be emphasized that these results were achieved with LH, without supplementary drugs.

The method of analysis of the relationships between hyperthermia and the cause of disease leading to the selection of a disease for treatment with hyperthermia and the results obtained, emphasize the potential of HT in the treatment of additional diseases. The experience gained from treatment by HT of nonmalignant diseases, permitting long follow-up and analysis of results will pave the way towards the acceptance of HT as a method in medicine, including cancer therapy.

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REFERENCES

1. G. Arcangeli, A. Cividalli, G. Creton, G. Lovisolo, F. Mauro, C. Nervi, Tumor control and therapeutic gain with different schedules of combined radiotherapy and local hyperthermiain human cancer. Int. J. Radiat. Oncol. Biol. Phys. 9: 1125 (1983).
2. P. Corry, B. Barlogie, W. Spanos, E. Armour, H. Barkley, M. Gonzales, Approaches to clinical applications of nonionizing and ionizing radiations. In: Radiation biology in cancer research, R.E. Meyn, and H.R. Withersed.ed., p 637. Raven Press, New York.(1980).
3. J. Overgaard, Fractionated radiation and hyperthermia: Experimental and clinical studies. Cancer 48:1116 (1981).
4. R.S. Scott, R.J.R. Johnson, H. Kowal, R.M. Krishnam-setty, K. Story, L. Clay, Hyperthermia in combination with radio-therapy. A review of 5 years experience in the treatment of superficial tumors. Int. J. Radiat. Oncol. Biol. Phys. 9: 1327 (1983).
5. U.R. Noell, K.T. Woodward, R.I. Worde, B.T. Fishburn, L.S. Miller, Microwave-induced local hyperthermia in combination with radiotherapy of human malignant tumors. Cancer 45: 638 (1980).
6. A. Yerushalmi, A. Shani, Y. Fishelovitz, J. Arielly, D. Singer, E. Levy, R. Katsnelson, E. Rakowsky, J.A. Stein, Local microwave hyperthermia in the treatment of carcinoma of the prostate. Oncology 43: 209(1986).
7. A. Yerushalmi, Y. Fishelovitz, D. Singer, I. Reiner, J. Arielly, I. Abramovici, R. Katsnelson, E. Levy, A. Shani, Localized deep microwave hyperthermia (LDMW) in the treatment of poor operative risk patients with benign prostatic hyperplasia. J. Urol. 133: 873-876 (1985).
8. A. Yerushalmi, Hyperthermia treatment of prostate tumors In: Hyperthermia in cancer treatment, L.J. Anghileri, and J. Robert, ed., vol. 3, p 89. CRC Press, Inc. Boca Raton, Florida (1986).
9. A. Yerushalmi, Localized, non-invasive deep microwave hyperthermia for the treatment of prostatic tumors: the

first 5 years. In: Recent Results in Cancer Research, R.D. Issels and W. Wilmanns ed., vol. 107, p 141. Springer-Verlag, Berlin - Heidelberg (1988).
10. A. Yerushalmi, Z. Shpirer, I. Hod, F. Gotteffeld, D.D. Bass, Normal tissue response to localized deep microwave hyperthermia in the rabbit's prostate. Int. J. Radiat. Oncol. Biol. Phys. 9: 77-82 (1983).
11. P.C. Walsh, Benign prostatic hyperplasia. In: Campbell's Urology, J.H., Harrison, R.F., Gittes, A.D.,Perlmutter, T.A., Starney and P.C., Walsh, WB Saunders, eds., p 949 Philadelphia,(1979).
12. A. Kirn, F. Keller, How viruses may overcome non-specific defences in the host. Phil. Trans. R. Soc. Lond. B 303: 115-122 (1983).
13. J.R.Schmidt, A.F. Rasmussen, The influence of environ-mental temperature on the course of experimental herpes simplex infection. J. Infect. Dis. 107:356 (1960).
14. S. Furuchi, Y. Shimizu, Efect of ambient temperature on multiplication of attenuated transmissible gastro-enteritis virus in the bodies of newborn piglets. Infect. Immun. 13:990 (1976).
15. J.F. Bell, G.J. Moore, Effects of high ambient tempera-ture at various stages of rabies virus infection in mice. Infect. Immun. 10:510 (1974).
16. A.R. Rich, C.M. McKee, The mechanism of a hitherto un-explained form of native immunity to type III pneumo-coccus. Bull. Johns Hopkins Hosp. 59:171 (1936).
17. H.A. Bernheim, .T. Bodel, P.W. Paskenase, E. Atkins, Effects of fever on host defense mechanisms after infection in the lizard Disposaurus dorsalis. Br. J. Pathol. 59:76 (1978).
18. L.K. Vaughn, M.J. Kluger, Fever and survival in bacte-rially infected rabbits. Fed. Proc. 36:511 (1977).
19. L.E. Carmichael, F.D.Barnes, Effect of temperature on growth of canine herpesvirus in canine kidney cell and macrophage cultures. J. Infect. Dis. 20:664 (1969).
20. D.L. Walker, W.D. Boring, Factors influencing host-virus interactions. III. Further studies on the alteration of coxsackie virus infection in adult mice by environmental temperature. J. Immunol. 80:39 (1958).
21. E. Atkins, J.D. Feldman, L. Francis, E. Hursh, Studies on the mechanism of fever accompanying delayed hyper-sensitivity. The role of the sensitized lymphocyte J. Exp. Med. 135:1113 (1972).
22. H.D. Jampel, G.W. Duff, R.K Gershon, E. Atkins, S.K., Durum, Fever and immunoregulation III. Hyperthermia augments the primary in vitro humoral immune response. J. Exp. Med. 157:1229 (1983).
23. W.M. Simpson, H.W. Kendell, Experimental treatment of early syphilis with artificial fever combined with chemo-therapy. Am. J. Syph. Gonorrhea Vener. Dis. 21:526 (1937).
24. I.L. Bennett, A. Nicastri, Fever as a mechanism of resistance. Bacteriol. Rev. 24:16 (1960).
25. P.S. Hench, Clinical notes on the results of fever therapy in different diseases. Proc. Staff Meet. Mayo Clin. 10:662 (1935).
26. M.F. Shaffer, J.F. Enders, J. Wilson, The effect of artificial fever and specific antiserum on the organism present in cases of type III pneumococcus meningitis. J. Clin.Invest. 17:133 (1938).

27. K.J. Dorrington, B.R. Smith, Conformational changes accompanying the dissociation and association of immunoglobulin G subunits. Biochim. Biophys. Acta 263:70 (1970).
28. M.J. Kluger, D.H. Ringler, M.R. Anver, Fever and survival. Science 188: 166-168 (1975).
29. R. Depoux, Infection des cellules de glande sosmaxillair de chien cultivées in vitro par un virus rabique fixé. Can J. Microbiol. 10:527 1964.
30. R.E. Kissling, D. R. Reese, Anti-rabies vaccine of tissue culture origin. J. Immunol. 91:362 (1963).
31. S. Furuuchi, Y. Shimizu, Effect of ambient temperatures on multiplication of attenuated transmissible gastro-enteritis virus in the bodies of newborn piglets. Infect. Immun. 13:990, 1976.
32. S. Baron, Mechanism of recovery from viral infection. Advan. Virus Res. 10:39, (1963).
33. A. Lwoff, Factors influencing the evolution of viral diseases at the cellular level and in the organism. Bacteriol. Rev. 23:109, (1959).
34. K.F. Austen, S.I. Wasserman, E.J. Gotetz, In: Molecular Basis of the Acute Allergic Reaction. K. Johansson, B. Strandberg, ed.,p 293, Plenum Press, New York, (1976).
35. H.H. Bennich, S.G.O. Johansson, Structure and function of human immunoglobulin E. Advan. Immunol. 13:1 (1971).
36. S.G.O. Johansson, H.H. Bennich, T. Berg, The clinical significance of IgE. Prog. Clin. Immunol. 1:35 (1972).
37. A.F. Coca, E.F. Grove, Studies in hypersensitiveness. A study of the atopic reagin. J. Immunol. 10:445 (1925).
38. K, Ishizaka, T. Ishizaka, A.E.O. Menzel, Physiochemical properties of reaginic antibody. VI. Effect of heat on gamma E, gamma G and gamma A antibodies in the sera of ragweed sensitive patients. J. Immunol. 99:610 (1967).
39. J.L. Mongar, H.O. Schild, Effect of temperature on the anaphylactic reaction. J. Physiol. 135:320 (1957).
40. J.E. Walker, R.E. Wells, E.W. Merrill, Heat and water exchange in the respiratory tract. Am. J. Med. 30:259 (1961).
41. A. Yerushalmi, A. Lwoff, Traîtement du coryza infectieux et des rhinites persistantes allergiques par la thermothérapie: C.R. Acad. Sc. Paris, D. 291:957 (1980).
42. A, Yerushalmi S, Karman, A. Lwoff, Treatment of perennial allergic rhinitis by local hyperthermia. Proc. Natl. Acad. Sci. USA. 79:4766 (1982).
43. M. Schata, W. Jorde, Local hyperthermia in the treatment of allergic rhinitis. Atemwegs und Lungenkrankheiten Dustrieverlag. In press.

# EXTRACORPOREALLY INDUCED TOTAL-BODY HYPERTHERMIA FOR DISSEMINATED CANCER

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## INTRODUCTION

Although the mechanisms of the anticancer effects of hyperthermia have remained obscure, it is now generally accepted that the heating temperature must be at least 41.0°C or greater to achieve cancer regression. Clinically applied thermochemotherapy can be divided into local and total-body hyperthermia (TBHT). Local hyperthermia has been primarily used in the treatment of superficial and localized malignant tumors. Because advanced cancer infiltrates neighboring organs and is often metastatic to other distant organs, TBHT appears to be a reasonable and a promising approach. Although various methods for TBHT have been reported, we have been performed TBHT using an extracorporeal circuit modified from the original of Parks et al.[2] In this chapter, we describe the clinical effect of TBHT combined with anticancer chemotherapy and some problems in this area.

## METHOD FOR TOTAL-BODY HYPERTHERMIA

Various approaches[2-9] for TBHT as shown in Table 1 have been reported, e.g. the heating gas inhalation method accompanied by the molten paraffin method, the hyperthermic chamber method, the heat-blanket method, the water-perfusion suit method, and the radiant heat device method. Irrespective of the heating method used, it is indispensable to control the body temperature and to monitor bodily reactions during treatment. However, these methods seem to be poorly suited for rapid induction of high temperatures and specific precise temperature control.

Parks et al.[2] reported that experimentally, a uniquely modified heat exchanger incorporated in an extracorporeal circuit was capable of safely inducing systemic hyperthermia for significant periods. Based on this experimental study, they treated patients with bronchogenic carcinoma[2]. Although this method is somewhat cumbersome, it facilitates the rapid induction of hyperthermia and provides for accurate temperature control. Therefore, in our cinical introduction, TBHT was induced using a femoral arteriovenous shunt and an extracorporeal circuit incorporating a heat exchanger according to our modification of the method of parks et al. (Fig. 1).

Our method is as follows: the inguinal region was incised and a 15-cm loop of a 6 mm vascular graft (Goretex, U.S.A.) was anastomosed end-to-side to the common femoral artery and vein as an arteriovenous shunt. The shunt

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Table 1. Methods of Total-Body Hyperthermia

| Reporter | Method |
|---|---|
| Warren, S.L. (1935)[3] | Carbon filament lump |
| Suryanarayan, C.R. (1966)[4] | Water bath |
| Pettigrew, R.T. (1974)[5] | Heating gas inhalation<br>Molten paraffin |
| Larkin, J.M. (1977)[6] | Water blanket |
| Euler-Rolle, J. (1978)[7] | Siemens hyperthermic chamber |
| Bull, J.M. (1979)[8] | Water perfusion suit |
| Parks, L.C. (1979)[2] | Extracorporeal circuit incorporated a heat exchanger |
| Robins, H.I. (1983)[9] | Radiant heat device |

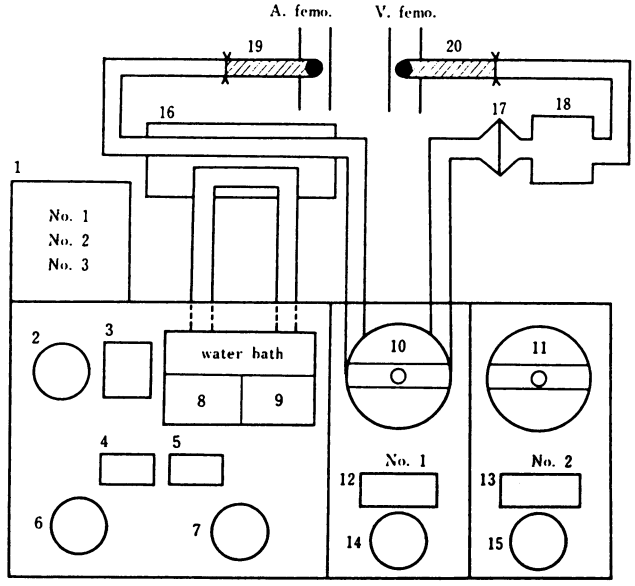


Fig. 1. Extracorporeal circuit for total-body hyperthermia. (1) Digital thermal display; (2) Water bath pressure; (3) Rectal temperature; (4) Maximum point of rectal temperature during maintenance (42°C) (5) Minimum point of rectal temperature during maintenance (41.5°C); (6) Water bath pressure; (7) A-V shunt volume controller; (8) Water bath temperature; (9) Water bath temperature controller; (10,11) Roller pump; (12,13) Digital tachometer; (14,15) Rotation controller; (16) heat exchanger; (17,18) Blood filter; (19,20) A-V graft.

volume was 1,350 to 1,900 ml/min (average 1740 ml/min). The shunt was placed under the skin and the shunt wound was allowed to heal for several days. With the patients under general endotracheal anesthesia, TBHT was induced. A 3 to 5 incision was made and the graft was brought through the opening. Heparin (100 units/Kg) was administered and the graft was clamped and divided. Both ends of the graft were connected to the extracorporeal circuit primed with 800 ml lactated Ringer's solution. Heparin (100 units/Kg) was infused intravenously every hour during TBHT. The arterial limb of the circuit was led through a roller pump set to maintain the flow at 1,000 to 1,500 ml/min. The patient's temperature was continuously monitored from the esophagus and rectum with thermister probes. The heating time from normal body temperature to a final rectal temperature of 41.5 to 42.0°C was about 30 to 50 min. The final rectal temperature was maintained for 3 to 5 hr and then the patient's temperature was decreased by lowering the water-bath temperature to normal body temperature.

Upon completion of the hyperthermic treatment the extracorporeal circuit was separated from the graft. The ends of the graft were anastomosed end-to-end and the skin incision was closed. We attempted to induce TBHT at least four times at intervals of 7 to 14 days. However, some of the patients received this treatment only once because of a general poor condition or complication.

## CLINICAL EFFECTS

### Physiologic Effects

In this treatment, not only concerous tissue but also normal organs are heated to a non-physiological level for a long time, raising concerns that damage to the normal organs may ensue, although it appears that the damaging effects of hyperthermia are relatively grater on malignant than normal cells [11]. A typical time course of the temperature is shown in Fig. 2.

Cardiovascular Function. The cardiovascular changes during hyperthermia were monitored continuously by electrocardiography, peripheral arterial and central venous pressures and checked periodically by blood gas determinations and Swan-Ganz thermodilution catheters inserted percutaneously[12] (11 patients, 23 treatments).

The heart rate and cardiac output were always increased during hyperthermia. The heart rate was 73±13/min and cardiac output was 5.3±0.7 l/min before the shunt was connected to the extracorporeal circuit; when the rectal temperature reached 41.5°C, these values increased to 118±15/min ($p < 0.001$) and 10.3±2.5 l/min ($p < 0.001$), respectively. Neither value returned to the pretreatment level within 1 hr after termination of hyperthermia. Peripheral arterial and central venous pressures were little affected during hyperthermia. The systolic pulmonary pressure were elevated ($p < 0.01$) only when the rectal temperature reached 41.5°C; it declined gradually thereafter. The diastolic pulmonary and pulmonary wedge pressures were not affected. $PaO_2$ was little affected. While the base excess and pH declined gradually, no progressive metabolic acidosis occurred. The increased cardiac output associated both with placement of A-V shunt and hyperthermia was well tolerated.

These findings suggest that the increase of tissue oxygen consumption and the dilation of peripheral blood vessels induced by hyperthermia were compensated for by an increase in both the heart rate and the cardiac output. The observed cardiovascular changes were not markedly different from those reported for the water perfusion suit method[13] and the heat-blanket method.

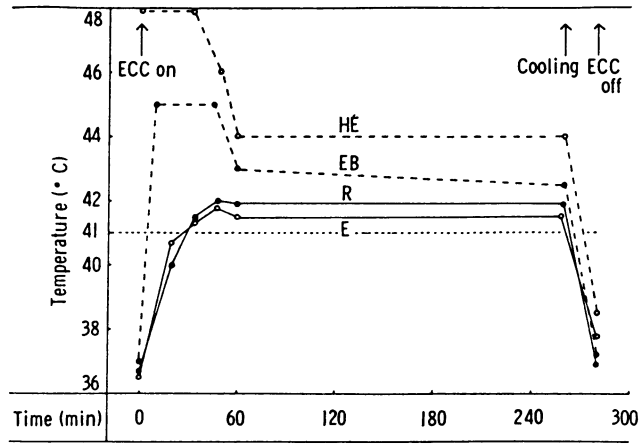


Fig. 2. Typical time course and temperature changes during hyperthermic treatment. ECC, Extracorporeal circuit; HE, Heat exchanger; EB, Extracorporeal blood; R, Rectum; E, Esophagus

Biochemical Examination of Serum. In our series, slight and transient liver dysfunction in SGOT, SGPT, and serum bilirubin was observed; however, the abnormal values in parameters of liver and renal function tended to return to the pretreatment levels about 1 week after TBHT. Some investigators[6,8] described elevations in serum bilirubin, alkaline phosphatase, SGOT, and SGPT when patients were heated greater than 41.8°C suggesting that the liver is susceptible to heat.

Hematologic Examination. There was no instance of hemolysis as determined by measurement of free serum hemoglobin. Thrombocytopenia was observed most frequently after TBHT. Total leukocyte count was usually elevated after TBHT and this raise was in the polymorphonuclear cell fraction, while the proportion of lymphocytes actually decreased. These changes in peripheral counts returned to normal levels 1 week after TBHT in most patients.

Immunological Effects. Prolonged exposure of the total body of cancer patients to high temperature may bring about a disturbance in their immune response, possible leading to cancer proliferation or metastasis. We investigated the effect of in vitro hyperthermia on murine and human lymphocytes from the viewpoint of cell-mediated immunity[14], i.e., PHA-induced blastogenesis and cytotoxic activity (Winn assay) of murine lymphocytes were reduced at 39°C and especially at 42°C hyperthermia. In human lymphocytes, rosette formation inhibition depended on heating time and temperature. PHA-induced lymphocyte blastogenesis was markedly inhibited at 42°C. These results indicate that host immunocompetence is adversely affected by TBHT. In our TBHT-treated patients, we observed transient decreases in lymphocyte blastogenesis, the C3c component, lymphocyte rosetting, and IgG, but these parameters recovered to pretreatment levels within 1 week after TBHT[15]. These clinical results were different from the results of in vitro hyperthermia. However, not only the effects of TBHT, but also the effects of extracorporeal circuit, anesthesia, and combined use of anticancer drugs must be taken into consideration. DeHoratius et al[16] subjected patients with malignant diseases to TBHT and noted no remarkable changes in T-cells and a decrease in the C3c component. Gee et al.[17] reported that in advanced cancer patients, TBHT produced a reduction of cell-mediated immunity;

however, total recovery occurred within a few days. Our and other workers results indicate that TBHT therapy leads to a transient decrease in the host immune response. Therefore, we believe that there is no necessity for giving special consideration to a long-standing reduction of cell-mediated immunity in TBHT therapy.

## Therapeutic Effects And Complication

The results of collected patients treated with extracorporeally induced TBHT are described[18]. A total of 444 TBHT treatments were administered to 168 patients with far-advanced miscellaneous cancers in seven Japanese hospitals between 1980 and 1985. Of these patients, 148 (88.1%) had had unsuccessful chemotherapy and/or radiation therapy. Fifty-five of these patients (33.3%) had primary tumors and 112 (66.7%) had secondary metastatic or recurrent tumors after surgical resection of the primary tumor. The majority of patients was in generally poor condition and 55 patients (32.7%) had been receiving continuous intravenous hyperalimentation because of poor nutritional status at the time of initiation of TBHT treatment.

Tumor response was evaluated as follows: complete response (CR); complete regression of all recognizable disease for at least 4 weeks; partial response (PR); 50% to 99% decrease in the sum of the two longest dimensions of all lesions for at least 4 weeks; minor response (MR); 25% to 49% regression in these parameters for at least 4 weeks; no change (NC); less than 25% decrease or increase in these parameters; and progressive disease (PD); greater than 25% increase in the parameters or the appearance of new lesions. The mean number of treatments was 2.64 (range, 1-8) and the mean maintenance time of temperaure (41.5-42°C; rectal temperature) was 11.4 hr (range, 2-44 hr) per patient.

Therapeutic Effects. We could not evaluate any antitumor effects in 36 of 168 patients (21.4%). Three of 36 patients had no measurable disease and 33 had died before the evaluation was made. Twenty-four of 33 patients died of complications that resulted from TBHT. Another cause of death was rapid deterioration in general condition (cachexia) after the first treatment; nine patients died before an evaluation could be made, without any evidence of obvious complication. The incidence of these deaths from TBHT treatment increased with reduced pretreatment performance status.

As shown in Table 2, the antitumor effects of TBHT on malignancy were evaluated in 132 patients. Overall, regression of malignancy was found in 39 of 132 patients (29.5%). There were 2 (1.5%) CRs, 30 (22.7%) PRs, and 7 (5.3%) MRs. The most favorable result was obtained in patients with lung cancer. Although this resposne rate is far from satisfactory, if we consider that almost all patients in this series had been unsuccessfully treated by other methods and were in the terminal stage of their disease, this result is encouraging as a multimodal cancer treatment. Table 3 shows the anticancer effects of TBHT analyzed with respect to the site where the major malignant lesions exist, irrespective of whether these are primary or secondary lesions. A positive response to TBHT was observed in tumors situated in the lung, liver, lymph nodes, and soft tissue, but there was a poor response by tumors in the peritoneum (peritoneal dissemination), bone, stomach, pancreas, and large bowel. However, there were no differences in the responses of tumors categorized by their histologic patterns. It is very interesting to note that, in this series, favorable antitumor effects were obtained exclusively in patients whose dominant tumors were in the lung, liver, limph nodes, and soft tissue. Although we are unable to explain the reason for this phenomenon, this result may be important and useful, hereafter, for evaluation of patients as possible candidates for treatment by TBHT.

Patients undergoing TBHT were simultaneously receiving a variety of chemotherapeutic drugs. There was no clear relationship between an objec-

Table 2. Antitumor Effects of Total-Body Hyperthermia on Malignancy

| Malignancies | No. of evaluable patients | Antitumor Effects* (%) | | | | |
|---|---|---|---|---|---|---|
| | | CR | PR | MR | NC | PD |
| Gastric cancer | 30 | | 5 (16.7) | | 15 (50.0) | 10 (33.3) |
| Large bowel cancer | 29 | | 5 (17.2) | 1 ( 3.4) | 13 (44.8) | 10 (34.5) |
| Lung cancer | 22 | 1 ( 4.5) | 7 (31.9) | 2 ( 9.1) | 7 (31.8) | 5 (22.7) |
| Breast cancer | 10 | | 2 (20.0) | 2 (20.0) | 6 (60.0) | |
| Pancreatic cancer | 6 | | | | 5 (83.3) | 1 (16.7) |
| Renal cancer | 4 | | 1 (25.0) | | 1 (25.0) | 2 (50.0) |
| Malignant melanoma | 4 | | 1 (25.0) | | 2 (50.0) | 1 (25.0) |
| Liver cancer | 3 | | 2 (66.7) | | 1 (33.3) | |
| Uterine cancer | 4 | | | 1 (25.) | 3 (75.) | |
| Malignant lymphoma | 2 | 1 (50.0) | 1 (50.0) | | | |
| Pharyngeal cancer | 2 | | | | 1 (50.0) | 1 (50.0) |
| Testicular cancer | 2 | | 2(100.0) | | | |
| Esophageal cancer | 1 | | 1(100.0) | | | |
| Bladder cancer | 1 | | | 1(100.0) | | |
| Ovarian cancer | 2 | | | | 1 (50.0) | 1 (50.0) |
| Thyroid cancer | 1 | | 1(100.0) | | | |
| Choledochal cancer | 2 | | | | 1 (50.0) | 1 (50.0) |
| Osteosarcoma | 2 | | 1 (50.0) | | 1 (50.0) | |
| Others | 5 | | 1 (20.0) | | 1 (20.0) | 3 (60.0) |
| Total | 132 | 2 ( 1.5) | 30 (22.7) | 7 ( 5.3) | 58 (43.9) | 35 (26.5) |
| | | | 39 (29.5) | | | |

*CR: complete response; PR: Partial response; MR: minor response; NC: no change; PD: progressive disease

Table 3. Antitumor Effects Analyzed by Site of Malignancy

| Cancer site | No. of evaluable patients | Antitumor effects (%) | | | |
|---|---|---|---|---|---|
| | | CR | PR | MR | NC,PD |
| Lung | 41 | 1 (2.4) | 12 (29.3) | 1 ( 2.4) | 27 (65.9) |
| Liver | 21 | | 8 (38.1) | 1 ( 4.8) | 12 (57.1) |
| Lymph nodes | 15 | 1 (6.7) | 3 (20.0) | 2 (13.3) | 9 (60.0) |
| Soft tissue | 14 | | 4 (28.6) | 3 (21.4) | 7 (50.0) |
| Peritoneum | 14 | | 1 ( 7.1) | | 13 (92.9) |
| Bone | 9 | | 1 (11.1) | | 8 (88.9) |
| Stomach | 8 | | 1 (12.5) | | 7 (87.5) |
| Pancreas | 6 | | | | 6(100.0) |
| Large bowel | 4 | | | | 4(100.0) |

tive tumor response and the nature of the simultaneous chemotherapy as shown in Table 4. A slight favorable response was observed when cisdiamminedichloroplatinum (II) (CDDP; 30-60 mg/$m^2$ per treatment) was administered alone or as the main drug in multiple combination chemotherapy than adriamycin and/or mitomycin C were employed. We were enable to elucidate the effect of the timing of the administration of the drugs on the antitumor response. When TBHT was performed without combined chemotherapy, no PR was obtained, and MR was found in only two of six patients.

Table 4. Combined Anticancer Chemotherapy and Anticancer Effects

| Drugs | No. of evaluable patients | Antitumor effects (%) | | | |
|---|---|---|---|---|---|
| | | CR | PR | MR | NC,PD |
| CDDP* | 63 | 1 (1.6) | 17 (27.0) | 4 ( 6.3) | 41 (65.1) |
| Adriamycin | 32 | 1 (3.1) | 6 (18.8) | | 25 (78.1) |
| Mitomycin C | 23 | | 5 (21.7) | 1 ( 4.3) | 17 (73.9) |
| ACNU** | 4 | | | | 4(100.0) |
| Carbazilquinone | 2 | | 1 (50.0) | | 1 (50.0) |
| Bleomycin | 2 | | 1 (50.0) | | 1 (50.0) |
| None | 6 | | | 2 (33.3) | 4 (66.7) |

*CDDP: cis-diamminedichloroplatinum (II); **ACNU: nimustin hydrochloride

There was a relationship between the response of the tumors and the pretreatment performance status of the patients. An objective response was found in only 3 of 29 evauable patients (10.3%) whose pretreatment performance status (Karnofsky's criteria[19]) had been less than 30%. On the other hand, an objective response was obtained in 36 of 103 evaluable patients (35.0%) whose performance was 31% or more. The number of TBHT treatments also was closely related to the patients' pretreatment performance status. Ninety patients received one or two TBHT treatments and 78 patients received TBHT more than three times. An objective response was observed in 13 of 55 evaluable patients (23.6%) who received one or two treatments and 26 of 77 evaluable patients (33.8%) who received more than three treatments.

An analysis of the tumor response with respect to the presence or absence of previous treatment before TBHT showed that 148 patients had received chemotherapy, chemotherapy combined with radiotherapy or radiotherapy alone. In these patients an objective response was obtained in 35 of 115 evaluable patients (27.8%). Whereas, in 20 patients who had received no previous treatment, an objective response was observed in 7 of 17 evaluable patients (41.2%). One year survival rates for patients with tumor regression (CR, PR, MR), NC, and with PD were 36.4%, 17.3%, and 0%, respectively.

Complications. The incidence of complications and the mortality increased in proportion to the reduction of the performance status of patients before TBHT as shown in Table 5. Six patients died of lung complication (lung edema in four and pneumonia in two) and six died of hepatic insufficiency. All six patients who died of hepatic insufficiency had manifested obstructive jaundice before TBHT. Five patients died of bleeding from cancer (gastric cancer in two cases; lung cancer, breast cancer, and liver cancer in one case each) and three patients died of massive bleeding from other site (intratracheal, intra-abdominal, and superior mesenteric aneurysmal bleeding in one case each). Two patients died of renal insufficiency and femoral wound infection (sepsis). None of the patients with manifested neurotoxicity died. Overall, thirty-three of 168 patients (19.6%) died of complications or of cachexia without complications after relative short period of TBHT. Furthermore, 20 of 33 patients who died had poor performance status of less than 30%, by Karnofesky's classification, before TBHT. These results suggest that we should reconsider the bases for selection of patients for treatment with TBHT and that we should pay greater attention to the possible development of fatal complications.

Table 5. Complications due to Total-Body Hyperthermia

| Complication | Pre-treatment performance status: Karnofsky scale (No. of treated patients) | | | | Total No. of patients |
|---|---|---|---|---|---|
| | 100-71% (30) | 70-50% (39) | 50-31% (50) | 30-20% (49) | |
| Pulmonary complication | 2(1) | 1(1) | 3(1) | 5(3) | 11(6)* |
| Hepatic insufficiency | | | 3(2) | 5(4) | 8(6) |
| Hemorrhage in cancer site | | | | | |
| Gastric cancer | | | 2(1) | 1(1) | 3(2) |
| Liver cancer | | | 1(1) | | 1(1) |
| Lung cancer | | | | 1(1) | 1(1) |
| Breast cancer | | 1(1) | | | 1(1) |
| Hemorrhage in another site | | | | | |
| Trachea | 1(0) | | 1(1) | | 2(1) |
| Intraabdominal | | | | 1(0) | 1(1) |
| Reptured aneurysma | | | 1(1) | | 1(1) |
| Gastro-intestinal | | 2(0) | 2(0) | 1(0) | 5(0) |
| Cardiovascular shock | | 1(1) | 1(1) | 1(0) | 3(2) |
| Renal insufficiency | 1(0) | | 1(0) | 2(1) | 4(1) |
| Local infection (Sepsis) | 1(1) | | 2(0) | | 3(1) |
| Neurotoxicity | | | | | |
| Foot drop | 3(0) | 2(0) | 2(0) | 3(0) | 10(0) |
| Hallucinations | | 2(0) | 3(0) | 1(0) | 6(0) |
| Ataxia | 2(0) | 3(0) | | 1(0) | 7(0) |

*No. of deaths in parentheses.

## PROBLEMS IN TOTAL-BODY HYPERTHERMIA

Treatment by extracorporeally induced TBHT involves several unfavorable aspects, such as high cost; high incidence of fatal complication as well nonfatal neurologic or muscle[2,20] toxicity; relative low 1-year survival rates, even in patients with tumor regression; a transient decrease in host-immunocompetence[15]; and an increased possibility of enhanced distant metastases, as observed experiment with an animal model[21]. However, we can anticipate some useful antitumor effects from TBHT in hopeless patients with terminal cancer, who have received unsuccessful prior treatment, when TBHT is the multimodal cancer therapy of last resort. Actually Engelhardt[22] recently reported the updated result of TBHT induced by the hyperthermic chamber. In a randomized study for patients with small cell lung cancer; one receiving chemotherapy alone, and the other receiving the same chemotherapy plus TBHT, responses (CR+PR) were 8/22 in the normothermic treatment arm and 15/22 in the hyperthermic treatment arm. Further, mean duration of response was significantly longer in hyperthermic group and there were only minimal differences in toxicity. Any way, further efforts are seemed to be necessary to increase the safety of our TBHT and to minimize complications, as well as to augment its antitumor effects.

### Patient Selection

TBHT should not be performed on patients in poor condition and with obstructive jaundice. Full attention should be paid for an A-V shunt flow volume because an excessive flow rate causes the development of lung edema even in patients in good general condition. TBHT should be performed more

than three times for patients whose dominant tumors are in the lung, liver, lymph nodes, and soft tissue, irrespective of whether the tumors are primary or secondary.

## Thermochemosensitivity of Human Tumor Cells

To augment the antitumor effects of TBHT, the use of antitumor drugs during hyperthermia seems to be indispensable. Which chemotherapeutic agent is most valid? Experimentally, both in vitro and in vivo, an augmented antitumor effect has been observed when hyperthermia has been combined with a number of chemotherapeutic agents[23-25]. Ideally, in the future, the selection of patients for treatment of TBHT combined with anticancer drugs should be decided by the results of thermosensitivity and thermochemosensitivity assay, performed on the cancer cells of each patient. Such assays for sensitivity to hyperthermia have been reported already using xenografts of human tumors in nude mice[26] and by a human tumor stem cell assay[27].

Tables 6 and 7 show our results of thermochemosensitivity assay against colorectal cancers by the human tumor clonogenic assay[28]. The rate of higher sensitivity (70% and over in ratio to colony inhibition) of cancer cells to cis-diamminedichloroplatinum, 5-fluorouracil and mitomycin C was 13.8%, 24.1% and 48.3%, respectively. The rate of higher sensitivity to hyperthermia alone was 29.6% and 55.6% at 42°C and 43°C, respectively. The sensitivity was augmented when hyperthermia was combined with administration of anticancer agents. Even in tumor cells that were not sensitive to anticancer drugs, sensitivity was enhanced when combined with hyperthermia. These results indicate that human tumor clonogenic assay may be useful as a predictive test for the application of thermochemotherapy and for individualization of chemotherapy. Further efforts are necessary to introduce assays for sensitivity into the clinical applications of TBHT.

Table 6. Drug Sensitivity of Human Tumor Cells

| | | Inhibition ratio (%) in colony formation* | |
|---|---|---|---|
| | Anticancer drugs** | Sensitivity rate of 50-60% | Sensitivity rate of 70% and over |
| CDDP | (0.2ug/ml) | 8/29(27.6) | 4/29(13.8) |
| 5-Fu | (1.0ug/ml) | 12/29(41.4) | 7/29(24.1) |
| MMC | (0.3ug/ml) | 20/29(69.0) | 14/29(48.3) |

*(1-colonies in treated group/colonies in nontreated group)x100.
**( ): 1/10 of the maximum concentration in blood.
Abbreviations: CDDP, cis-diamminedichloroplatinum; 5-Fu, 5-fluorouracil; MMC, mitomycin C

## Timing Between Hyperthermia And Chemotherapy

The selective enhancement of drug delivery to tumors is an important factor in the effectiveness of thermochemotherapy in not only local hyperthermia but also TBHT as well as standard normothermal chemotherapy. It has been suggested that hyperthermia exerts a great influence on blood flow in both normal tissues and tumors. With respect to the changes of blood flow that accompany local hyperthermia, it is generally accepted that the relative increase in the rate of blood flow is large in normal tissue but rather small in tumors during hyperthermia at 42-43°C[29,30]. But, there is little information about it during TBHT. We studied the effect of TBHT on blood flow in tissues during TBHT by hydrogen clearance method[31]. Fig. 3 shows

Table 7. Sensitivity of Human Tumor Cells to Drugs plus Hyperthermia

| Heating (1 hr) | Anti-Cancer drugs* | Inhibition ratio (%) in colony formation: Sensitivity rate of 50-69% | Sensitivity rate of 70% and over |
|---|---|---|---|
| 42°C | — | 15/27(55.6) | 8/27(29.6) |
| | CDDP | 17/26(65.4) | 8/26(30.8) |
| | 5-FU | 17/26(65.4) | 13/26(50.0) |
| | MMC | 22/26(84.6) | 17/26(65.4) |
| 43°C | — | 21/27(77.8) | 15/27(55.6) |
| | CDDP | 23/26(88.5) | 19/26(73.1) |
| | 5-FU | 23/26(88.5) | 20/26(76.9) |
| | MMC | 25/26(96.1) | 24/26(92.3) |

*Concentrations and abbreviations are the same as in Table 1.

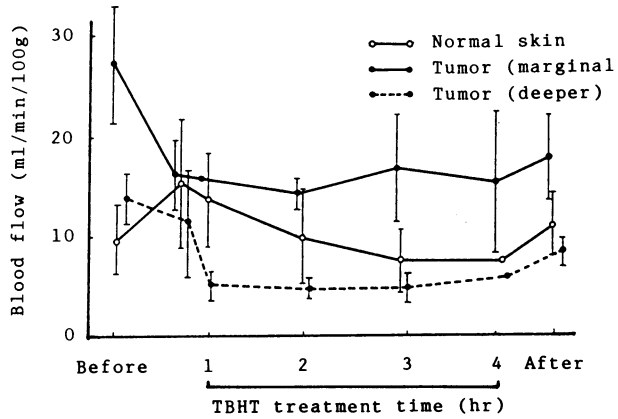


Fig. 3. Changes of Tissue Blood Flow during TBHT

the changes of blood flow (3 treatments) in tumor, and normal skin that overlays the tumor in patient with local recurrence after surgery from colon cancer (a 46-year-old man). The decreased tendency in tumor blood flow in both marginal (at a depth of 1.5cm from the skin) and core (at a depth of 4.5cm) sites. This result may indicate, under TBHT, that anticancer drugs should be administered during the initial phase of TBHT.

Further study is necessary to clarify an optimal timing between TBHT and chemotherapy.

REFERENCES

1. K. Overgaard and J. Overgaad, Investigation on the possibility of a thermic tumor therapy, Eur J Cancer. 8:65 (1972).
2. L.C. Parks, M. Minaberry, D.P. Smith, and W.A. Neely, Treatment of far-advanced bronchogenic carcinoma by extracorporeally induced systemic

hyperthermia, Thorac Cardiovasc Surg. 78:883, (1979).
3. S.L. Warren, Preliminary study of the effect of artificial fever upon hopeless tumor cases, Am J Roentgenol 33:75, (1935).
4. C.R. Suryanarayan, Certain interesting observations during and after hydrohyperthermic chemotherapy in advanced malignancies (preliminary survey), Indian J Cancer. 3:176 (1966).
5. R.T. Pettigrew, J.M. Galt, C.M. Ludgate, and A.N. Smith, Clinical effects of whole-body hyperthermia in advanced malignancy, Br Med J. 21:679, (1974).
6. J.M. Larkin, W.S. Edvards, D.E. Smith, and P.J. Clark, Systemic thermotherapy: description of a method and physiologic tolerance in clinical subjects, Cancer. 40:3155, (1977).
7. J. Euler-Rolle, A. Driesching, E. Vormittag, C. Tschakakoff, and P. Polteraner, Prevention of cardiac complications during whole-body hyperthermia by beta-receptor blockage, in: "Cancer Therapy by Hyperthermia and Radiation," C. Streffer, ed., Urban & Schwarzenberg, Baltimore (1978).
8. J.M.C. Bull, D. Lees, W. Schuette, J. Whang-Peng, R. Smith, G. Bynum, R. Atkinson, J.S. Gottdiener, H.R. Gralnick, T.H. Shawker, and V.T. DeVita, Whole-body hyperthermia: phase-1 trial of a potential adjuvant chemotherapy, Ann Intern Med. 90:137, (1979).
9. H.I. Robins, J. Grossman, T.E. Davis, J.P. AuBuchon, and W. Dennis, Preclinical trial of a radiant heat device for whole-body hyperthermia using a procine model, Cancer Res. 43:2018, (1983).
10. S. Koga and M. Maeta, Total-body hyperthermia and chemotherapy, in: "Hyperthermia in Cancer Treatment," Vol.III, L.J. Anghileri & J. Robert, eds., CRC Press, Boca Raton (1986).
11. B.C. Giovanella, A.C. Morgan, J.S. Stehlin, and L.J. Williams, Selective lethal effect of supranormal temperatures on mouse sarcoma cells, Cancer Res. 33:2568, (1973).
12. M.Maeta, S. Koga, N. Shimizu, H. Kanayama, R. Hamazoe, T. Karino, T. Yamane, and M. Oda, Effect of extracorporeally induced total-body hyperthermia for cancer on cardiovascular function, Jpn Heart J. 25: 993, (1984).
13. D.E. Lees, Y.D. Kim, M.B. Bull, J.M. Bull, J. Whang-Peng, W. Schuette, R.Smith, and T.E. MacNamara, Anesthetic management of whole-body hyperthermia for the treatment of cancer, Anesthesiology. 52:418, (1980).
14. A. Izumi, S. Koga, and M. Maeta, Effects of in vitro hyperthermia on murine and human lymphocytes, Cancer. 51:2061, (1983).
15. S. Koga, A. Izumi, M. Maeta, N. Shimizu, Y. Osaki, and H. Kanayama, The effects of total-body hyperthermia combined with anticancer drugs on immunity in advanced cancer patients, Cancer. 52:1173, (1983).
16. R.L. DeHoratius, J.M. Hosea, E.D. Van Epps, W.P. Reed, W.S. Edwards, and R.C. Williams, Immunologic function in human before and after hyperthermia and chemotherapy for disseminated malignancy, J Natl Cancer Inst. 58:905, (1977).
17. A.P. Gee, A.D. Williams, R.T. Rettigrew, and A.N. Smith, The effects of whole-body hyperthermia therapy on the general immunocompetence of the advanced cancer patient, in: "Cancer Therapy by Hyperthermia and Radiation," C. Streffer, ed., Urban & Schwarzenberg, Baltimore (1978).
18. M.Maeta, S. Koga, J. Wada, M. Yokoyama, N. Kato, H. Kawahara, T. Sakai, M. Hino, T. Ono, and K. Yuasa, Clinical evaluation of total-body hyperthermia combined with anticancer chemotherapy for far-advanced miscellaneous cancer in Japan, Cancer. 59:1101, (1987).
19. D.A. Karnofsky, Meaningful clinical classification of the therapeutic response to anticancer drugs. Clin Pharmcol Ther. 2:709, (1961).
20. S.Koga, M. Maerta, N. Shimizu, Y. Osaki, R. Hamazoe, M. Oda, T. Karino, and T. Yamane, Clinical effects of total-body hyperthermia combined with anticancer chemotherapy for far-advanced gastrointestinal cancer, Cancer. 55:1641, (1985).

21. M. Oda, S. Koga, and M. Maeta, Effects of total-body hyperthermia on metastasis from experimental mouse tumors. Cancer Res. 45:1532, (1985).
22. R. Engelhardt, Whole-body hyperthermia, in: "Abstract of the 5th International Symposium on Hyperthermic Oncology", Kyoto, Japan (1988).
23. J.B. Marmor, D. Kozak, and G.M. Hahn, Effects of systemically administered bleomycin or adriamycin with local hyperthermia on primary tumor and lung metastasis, Cancer Treat Rep. 63:1279, (1979).
24. S. Mizuno, M. Amagi, and A. Ishida, Synergistic cell killing by antitumor agents and hyperthermia in cultured cells, Gann. 71:471, (1980).
25. B. Barlogie, P.M. Corry, and B. Drewindo, In vitro thermochemotherapy of human colon cancer cells with cis-dichloro-diammineplatinum (II) and mitomycin C, Cancer Res. 40:1165, (1981).
26. M.H. Shiu, A.S. Cahan, J. Fogh, and J.G. Fortner, Sensitivity of xenografts of human pancreatic adenocarcinoma in nude mice to heat and heat combined with chemotherapy, Cancer Res. 43:4014, (1983).
27. B.D. Mann, F.K. Storm, D.L. Morton, C.A. Bertelsen, E.L. Korn, L.R. Kaiser, and D.H. Kern, Predictability of response to clinical thermochemotherapy by the clonogenic assay, Cancer. 52:1389, (1983).
28. A. Murakami, S. Koga, and M. Maeta, Thermochemosensitivity: augmentation by hyperthermia of cytotoxicity of anticancer drugs against human colorectal cancers, measured by the human tumor clonogenic assay, Oncology. 45:236, (1988).
29. H.I. Bicher, F.W. Hetzel, T.S. Sandhu, S. Frinak, P. Veupel, M.D. O'Hara, and T. O'Brien, Effects of hyperthermia on normal and tumor microenvironments. Radiology. 137:523, (1980).
30. C.W. Song, J.G. Rhee, and S.H. Levitt, Blood flow in normal tissues and tumors during hyperthermia, JNCI. 64:119, (1980).
31. K. Aukland, B.F. Bower, and R.W. Berliner, Measurement of local blood flow with hydrogen gas, Circulation Res. 4:164, (1964).

# RADIANT HEAT SYSTEMIC HYPERTHERMIA CLINICAL TRIALS

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## ABSTRACT

Results of a Phase I study led our group to the conclusion that a radiant heat system provides a safe and effective method of inducing 41.8°C whole body hyperthermia (WBH) which is suitable for combination therapy. The review to follow summarizes our progress in combining WBH with other cancer-treatment modalities, including ionizing irradiation, chemotherapy and immunotherapy.

## INTRODUCTION AND BACKGROUND

As most cancers which are refractory to conventional therapy are systemic in nature, we have focused our attention on whole body hyperthermia (WBH). Although research relating to WBH has continued for over 15 years, many clinicians have avoided systemic hyperthermia as it has been labor-intensive, has required complex equipment, and has been associated with appreciable toxicity (27). Our belief that WBH could contribute to the control of systemic disease led to an analysis of the various systems available for WBH (5, 18), including a comprehensive review of the published WBH clinical experience as well as physiological thermoregulatory principles. Based on this assessment, we predicted that a simple system based on the control of radiant heat exchange to supplement metabolic heat production might provide several advantages, including: a) decreased morbidity, b) elimination of general anesthesia and endotracheal intubation, c) improved patient comfort, and d) favorable cost-benefit considerations. In order to test the feasibility and validity of our projections regarding the use of radiant heat as a basis for a WBH technology, we embarked on aggressive preclinical and clinical research programs (11-28).

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Results of a Phase I study led our group to the conclusion that a radiant heat system (RHS) provides a safe and effective method of inducing WBH which is suitable for combined modality therapy (15). This conclusion has been substantiated in a series of second-generation studies consisting of approximately 600 WBH treatments (14, 15, 23-27).

The biophysical basis for the use of the WBH-RHS is well reviewed in a series of papers (5, 15, 18, 27). The WBH-radiant heat device consists of a cylinder surrounded by specialized heating cables (Enthermics Medical Systems, Inc.). The essence of the system relates to exposing the patient to a low-density radiant heat (while preventing evaporatiave heat losses). As radiant energy exchange is highly efficient, i.e., a function of the fourth power of the absolute temperature of surfaces involved (i.e., the patient and the wall of the apparatus), this methodology is relatively efficient in terms of heating times. When patients achieve target temperature, their hyperthermic state is almost entirely maintained by metabolic heat production.

Patients are placed within the cylinder with their heads exposed and the treatment chamber is humidified to prevent evaporative heat losses. The physiological basis for the operation of the system relates to two principles: 1) radiant heat exchange is the major component of thermal regulation in man when evaporative heat losses are minimized; 2) as core temperature increases there is a nonlinear increase in metabolic rate.

Patients undergoing treatment receive intravenous (i.v.) fluids to compensate for fluid losses. The drugs lidocaine and thiopental are used as the primary sedative during WBH as they display synergistic sedative effects when used in combination (15). Lidocaine also provides cardiac arrhythmia prophylaxis; thiopental provides seizure prophylaxis (15). We have also shown that these drugs may have antineoplastic activity in the setting of hyperthermia (13, 16).

For patient comfort, the drugs droperidol, levorphanol and i.v. diazepam are also utilized during therapy (15). The goal of this sedation is to have a calm patient who can sleep intermittently, yet respond lucidly to verbal stimulation, retain laryngeal reflexes, and continue spontaneous respirations at a rate greater than 10 breaths per minute.

The various physiological changes observed during WBH with this system have been reviewed in detail elsewhere (14, 15, 19, 21, 22, 27) and beyond the scope of this review.

In brief, patients undergoing radiant heat WBH with the system described here always have increases in both heart rate and cardiac output. The degree of cardiac stress is minimal in preselected patients, as reflected by normal pressures seen during swan ganz catheterization; stroke volume has been shown to be relatively constant during treatment. Hepatic, renal, hematologic, endocrine, and central nervous system toxicity has not been significant to date.

## MULTIMODALITY CLINICAL TRIALS

### WBH and Ionizing Irradiation

We have recently tested the use of WBH with ionizing irradiation in two clinical pilot studies (20, 25, 26). One program integrated 41.8°C WBH into a typical radiotherapy regimen for unresectable nonsmall cell lung carcinoma

confined to the thorax. The 6 patients in this pilot study (26) received WBH (41.8°C x 75 minutes) 4 times during the first 2 weeks of a 6-week radiotherapy course. Hyperthermia was initiated within 15 minutes after irradiation. For these 2 weeks, the spinal cord was shielded to avoid the theoretically increased risk of transverse myelitis with WBH-irradiation. In so doing, part of the mediastinum and contralateral hilar region were partially shielded. For the last 4 weeks patients received only conventional radiotherapy reaching a net dose of 60 Gy.

Interestingly, toxicity did not appear to be increased by adding WBH. It was formally estimated that the net incidence of significant morbidity was at most 37% (95% confidence interval), which is not unusual for irradiation alone. Specifically, radiation pneumonitis occurred to the same extent as would be expected with 60 Gy irradiation alone; 1 patient developed moist desquamation. None of the patients developed transverse myelitis.

Gross disease responded in 5 of the patients, but 2 of these recurred with pericardial effusions. Although pericardial effusions are common in this setting, it is conceivable that mediastinal shielding could have been a contributing factor. Because of this possibility, the treatment program for future studies was modified to "sandwich" each hyperthermia treatment between two irradiation fractions. This change is expected to increase thermal radiosensitization during the first 2 weeks to compensate for the partial shielding effect to neoplastic cells.

A second study (25) at the University of Wisconsin combined WBH with total body irradiation (TBI) in the treatment of 8 patients with low-grade B-cell neoplasms (7 nodular lymphomas, 1 chronic lymphocytic leukemia). Usually TBI is complicated by treatment-limiting thrombocytopenia and leukopenia which occur during the course of irradiation. However, preclinical studies using the AKR murine leukemia model suggested the possibility of a therapeutic gain by giving TBI with WBH (11, 31). Treatment therefore consisted of TBI at 12.5 cGy twice a week up to a net dose of 1.5 to 2.0 Gy; each TBI treatment was followed immediately by WBH (41.8°C x 75 min).

In these 8 patients, there were 3 complete responses, 4 partial responses, and 1 objective response (48% tumor reduction) with a median time to treatment failure of 9.4 months (90% confidence interval). In addition to achieving frequent responses, myelosuppression was not treatment-limiting. Indeed, the results obtained were suggestive that WBH might be protective against radiation-induced thrombocytopenia. This result is in sharp contrast to previous TBI studies and to patients treated concomitantly at our center using TBI and lonidamine, a putative radiosensitizer.

Admittedly, patients were not randomized to the TBI-WBH or TBI-lonidamine studies. However, it is still interesting that TBI-lonidamine produced only 1 complete response, 4 partial responses (out of 10 patients versus 7 of 8 patients in the WBH study), and a median time to treatment failure of just 2.4 months (versus 9.4 months in the WBH study). Because of these differences, it was felt that a formal randomized comparison of TBI-WBH versus TBI alone is warranted.

WBH and Lonidamine

Lonidamine is also of interest as a cytotoxic chemotherapeutic agent in its own right and as a nonmyelosuppressive drug which has supra-additive tumoricidal interactions with hyperthermia. Because of these properties, we have also combined lonidamine with WBH in a Phase I clinical trial (24). This investigation utilized a hyperthermia "escalation" schema reaching two 41.8°C WBH treatments (75 minutes) each week. Three groups of patients

received lonidamine at 60, 180 and 360 mg/m$^2$ per day (Groups A, B and C respectively).

As would be expected with lonidamine, 16 of the 24 patients reported myalgias with 2 patients requiring 20 to 30% dose reductions. One patient required a 25% dose reduction for central nervous system toxicity reflected as diminished concentration, depression, and photophobia.

Posthyperthermia (93 treatments at 41.0°C and 105 treatments at 41.8°C) 5 patients developed fatigue and lethargy and 5 patients reported headaches. The same number of patients reported nausea and some episodes of vomiting, which ended within 12 hours after treatment. This emetogenic effect seemed related to thiopental-induced gastric stasis and responded to metoclopramide. Encouragingly, the combined WBH-lonidamine regimen did not produce myelosuppression or any other significant toxicity.

In this study, 20 patients were evaluable for response. There were 2 partial responses in Group B (nodular lymphoma and appendiceal adenocarcinoma); there was measurable clinical improvement in 1 patient with an adenocarcinoma of the urachus. In Group C, we observed 1 complete remission (lung cancer), 2 improvements (lung cancer and melanoma) and 3 disease stabilizations greater than 100 days (adenocarcinoma).

WBH and Interferon

We have also combined WBH with human lymphoblastoid alpha interferon based on preclinical studies showing significant antiproliferative and cytotoxic interactions between the two agents (11, 28). In a Phase I study (28), 17 patients were treated with WBH alone (40.5°C for 75 minutes), with interferon alone (1, 3 or 10 x $10^6$ units/m$^2$ i.m.), and then with both WBH and interferon (interferon 1 hour before WBH). This design permitted comparisons of the toxicities of WBH or interferon alone and in combination.

At the highest treatment level, patients eventually received interferon at 1 x $10^7$ units/m$^2$ i.m. for 6 consecutive days with WBH on days 4 and 6. The treatment schedule permitted the development of tachyphylaxis to interferon-related fever and patient temperatures 24 hours after WBH were not significantly different for single agent as opposed to combination therapy. Furthermore, repeated toxicity and pharmacologic evaluations did not show significant differences between single agent and combination therapy. There also was no evidence of cumulative toxicity in 6 patients who received maintenance combination therapy for up to 1 year. A trend toward increased natural killer cell activity and antibody-dependent cellular cytotoxicity with the combination of WBH and interferon was observed.

Collectively, these findings demonstrate that twice weekly WBH treatments can be safely integrated into relatively high-dose interferon regimens. In addition, while this was a Phase I study (in heavily pretreated patients), it should be noted that there were partial responses in a 37-year-old woman with nodular lymphoma and in a 38-year-old male with metastatic melanoma. Additionally, 3 patients with metastatic disease (1 melanoma and 2 leiomyosarcomas) have experienced exceedingly long disease stabilizations (i.e., 4.2, 3.1 and 2.3 years, respectively) and continue to show no evidence of disease progression.

Carboplatin and WBH--A New Clinical Trial

The combination of hyperthermia and the chemotherapeutic agent, cisplatin, has generated considerable interest as an anticancer therapy as hyperthermia has been found to enhance the tumoricidal effect of cisplatin

*in vitro*, *in vivo*, and clinically using regional hyperthermia (i.e., limb perfusion) (1, 8, 29).

Unfortunately, cisplatin in combination with hyperthermia currently has limited clinical utility. For many cancer patients, local or regional hyperthermia may have only palliative benefits because of the spector of systemic metastases (11). In addition, the combination of cisplatin and WBH has been associated with unacceptable clinical toxicity including severe renal injury not seen with other drugs using the same hyperthermia systems (6, 10, 34). This increased nephrotoxicity may result from increased renal cisplatin uptake as suggested from canine experiments (10). In addition, despite vigorous antiemetic prophylaxis, cisplatin frequently causes severe or intractable nausea and emesis (34). Because of the risk of aspiration pneumonitis, these side effects are undesirable with any WBH system which does not require endotracheal intubation (15).

Carboplatin is an appealing alternative to cisplatin in the setting of WBH. Carboplatin and cisplatin appear to have the same active intermediate and have similar spectra of antitumor activity (2, 7, 9, 30). Carboplatin is also much less emetogenic than cisplatin (2, 7, 30) and carboplatin does not cause nephrotoxicity (2, 7) even at the very high doses used in the setting of autologous bone marrow transplantation. In addition, we have recently demonstrated supra-additive cytotoxic interactions between hyperthermia and carboplatin at systemic hyperthermia temperatures (3). The thermal enhancement ratio for carboplatin is substantial (3.32 at 41.8°C) (3) and is nearly identical to the thermal enhancement ratio for cisplatin when the two drugs are simultaneously compared in the same cell line (4). We have further found the combination is well tolerated in a murine model at dose levels consistent with clinical practice, with results that also show therapeutic enhancement (32, 33). The presumptive biophysical basis for this therapeutic enhancement, i.e., selective killing of neoplastic cells in comparison to normal hematopoietic tissue, is discussed elsewhere (12). Thus, based on the aforementioned preclinical data, a Phase I clinical trial of the combination of carboplatin and WBH has been initiated at the University of Wisconsin Clinical Cancer Center.

## WBH As Part of a Preparative Regimen for Allogeneic Bone Marrow Transplantation

Another area of interest for our group is the use of WBH in the setting of bone marrow transplantation (11, 17) Preliminary results in this program have been promising (23). The rationale for the use of WBH in this clinical setting is reviewed in detail elsewhere (11, 17, 23). Preclinical data suggests that the addition of WBH to ablative radiotherapy should have cytoreductive advantages. Immunological benefits including decrease in host-mediated rejection of allogeneic donor marrow have similarly been predicted. Other potential immunological features relating to the use of WBH in the setting of allogeneic bone marrow transplantation include the effects of hyperthermia on tumor immunogenicity as well as the expression of graph versus host disease. Preliminary clinical data support the aforementioned speculation regarding the positive role of WBH in this clinical setting (23).

## SUMMARY

In summary, response data and the lack of toxicity observed in our ongoing clinical trials show that prospective randomized (Phase III) multi-institutional studies are feasible. It is the goal of this review to generate greater interest in the potential use of WBH as an adjunct in the treatment of neoplastic diseases.

REFERENCES


1. Barlogie, B., Corry, P.M. and Drewinko, B. *In vitro* thermochemotherapy of human colon cancer cells with cis-dichlorodiammineplatinum (II) and mitomycin C. Cancer Res. 40:1165-1168, 1980.
2. Calvert, A.H., Harland, S.J., Newell, D.R., Siddik, Z.H., Jones, A.C., McElwain, R.S., Wiltshaw, E., Smith, I.E., Baker, J.M., Peckham, M.J., and Harrap, K.R. Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. Cancer Chemother. Pharmacol. 9:140-147, 1982.
3. Cohen, J.D., and Robins, H.I. Hyperthermic enhancement of cis-diammine-1,1-cyclobutane dicarboxylate platinum (II) cytotoxicity in human leukemia cells *in vitro*. Cancer Res. 47:4335-4337, 1987.
4. Cohen, J.D., Robins, H.I., and Schmitt, C.L. Interactions of hyperthermia with carboplatin, cisplatin, and etoposide in human leukemia cells *in vitro*. Cancer Letters, in press, 1989.
5. Dennis, W.H., and Robins, H.I. The need for analysis of modern technologies for health care prior to development. (In) Applied Systems and Cybernetics, vol. 4, pp. 1744-1749, New York, Pergamon Press, 1981.
6. Gerad, H., Egorin, M.J., Whitacre, M., Van Echo, D.A., and Aisner, J. Renal failure and platinum pharmacokinetics in three patients treated with cis-diammine-dichloroplatinum (II) and whole body hyperthermia. Cancer Chemother. Pharmacol. 11:162-166, 1983.
7. Koeller, J.M., Trump, D.L., Tutsch, K.D., Earhart, R.H., Davis, T.E., and Tormey, D.C. Phase I clinical trial and pharmacokinetics of carboplatin (NSC 241240) by single monthly 30-minute infusion. Cancer 57:222-225, 1986.
8. Meyn, R.E., Corry, P.M., Fletcher, S.E., and Demetriades, M. Thermal enhancement of DNA damage in mammalian cells treated with cis-diammine-dichloroplatinum (II). Cancer Res. 40:1136-1139, 1980.
9. Mong, S., Huang, C.H., Prestayko, A.W., and Crooke, S.T. Effects of second-generation platinum analogs on isolated PM-2 DNA and their cytotoxicity *in vitro* and *in vivo*. Cancer Res. 40:3318-3322, 1980.
10. Riviere, J.E., Page, R.L., Dewhirst, M.W., Tyczkowska, K., and Thrall, D.E. Effect of hyperthermia on cisplatin pharmacokinetics in normal dogs. Int. J. Hyperthermia 2:351-358, 1986.
11. Robins, H.I. The role of whole body hyperthermia in the treatment of neoplastic disease, its current status and future prospects. Cancer Res. 44:4878s-4883, 1984.
12. Robins, H.I., Cohen, J.D., and Schmitt, C.L. The relationship of thermal enhancement (TE) to temperature for carboplatin (CBDCA). Rad. Res., NAGH, Seattle, WA, March 18-23, 1989.
13. Robins, H.I., Dennis, W.H., Martin, P.A., Sondel, P.M., Yatvin, M.B., and Steeves, R.A. Potentiation of differential hyperthermic sensitivity of AKR leukemia and normal bone marrow cells by lidocaine or thiopental. Cancer 54:2831-2835, 1984.
14. Robins, H.I., Dennis, W.H., Neville, A.J., Hugander, A., Shecterle, L.M., Martin, P.A., Gillis, W.K., Grossman, J., Steeves, R.A., and Davis, T.E. Whole body hyperthermia clinical trials. Proc. IEEE/Engineering Medicine and Biology Society 1:361-364, 1985.
15. Robins, H.I., Dennis, W.H., Neville, A.J., Shecterle, L.M., Martin, P.A., Grossman, J., Davis, T.E., Neville, S.R., Gillis, W.K., and Rusy, B.F. A nontoxic system for 41.8°C whole body hyperthermia: Results of a Phase I study using a radiant heat device. Cancer Res. 45:3937-3944, 1985.
16. Robins, H.I., Dennis, W.H., Slattery, J.S., Lange, T.A., and Yatvin, M.B. Systemic lidocaine enhancement of hyperthermia-induced

tumor regression in transplantable murine models. Cancer Res. 43:3187-3191, 1983.
17. Robins, H.I., Dennis, W.H., Steeves, R.A., and Sondel, P.M. A proposal for the addition of hyperthermia to treatment regimens for acute and chronic leukemia. J. Clin. Oncol. 2:1050-1056, 1984.
18. Robins, H.I., Grossman, J., Davis, T.E., Dennis, W.H., and AuBuchon, J.P. A preclinical trial of a radiant heat device for whole body hyperthermia using a porcine model. Cancer Res. 43:2018-2022, 1983.
19. Robins, H.I., Hugander, A., Besozzi, M., Schmitt, C.L., Martin, P.A., Zager, L.V., and Grossman, J. Beta-blockade during whole body hyperthermia: A toxicity study in the dog. J. Comp. Vet. Oncology, in press.
20. Robins, H.I., Hugander, A., Steeves, R., and Dennis, W.H. Radiotherapy and hyperthermia for lung cancer. Int. J. Radiat. Oncol. Biol. Phys. 12:147, 1986.
21. Robins, H.I., Kalin, N., Shelton, S.E., Martin, P.A., Shecterle, L.M., Barksdale, C.N., Neville, A.J., and Marshall, J. Rise in plasma beta-endorphin, ACTH, and cortisol in cancer patients undergoing whole body hyperthermia. Horm. Metab. Res. 19:441-443, 1987.
22. Robins, H.I., Kalin, N., Shelton, S.E., Shecterle, L., Marshal, J., and Barksdale, C.N. Neuroendocrine changes in patients undergoing whole body hyperthermia. Int. J. Hyperthermia 3:99-105, 1987.
23. Robins, H.I., Longo, W.L., Hugander, A., Bozdeck, M., Schwartz, B., Steeves, R.A., Flynn, B., Trigg, M., and Sondel, P.M. Whole body hyperthermia combined with total body irradiation and chemotherapy as a preparative regimen for allogeneic bone marrow transplantation. Cancer J. 1:180-183, 1986.
24. Robins, H.I., Longo, W.L., Lagoni, R.K., Neville, A.J., Riggs, C., Schmitt, C.L., Hugander, A., and Young, C. A Phase I trial of lonidamine with whole body hyperthermia in advanced cancer. Cancer Res. 48:6587-6592, 1988.
25. Robins, H.I., Longo, W.L., Schmitt, C.L., Lagoni, R.K., Neville, A.J., Giese, W., and Steeves, R.A. Whole body hyperthermia and total body irradiation in the treatment of B cell neoplasm. Proc. 5th Int. Symp. Hyperthermic Oncol., in press, 1988.
26. Robins, H.I., Longo, W.L., Steeves, R.A., Lagoni, R.K., Hugander, A., Neville, A.J., O'Keefe, S., Giese, W., and Schmitt, C.L. A pilot study of whole body hyperthermia and local irradiation for advanced nonsmall cell lung cancer confined to the thorax. Inter. J. Radiat. Oncol. Biol. Phys. 15:427-431, 1988.
27. Robins, H.I., and Neville, A.J. Biology and methodology of whole body hyperthermia. In: Hyperthermia in Cancer Treatment, vol. 3, L.V. Anghileri and J. Robert, eds., CRC Press, Boca Raton, FL, 1986, pp. 184-206.
28. Robins, H.I., Sielaff, K.M., Storer, B., Hawkins, M.J., and Borden, E.C. A Phase I trial of interferon-αLy with whole body hyperthermia in advanced cancer. Cancer Res. 49: 1989.
29. Roseman, J.M., Tench, D., and Bryant, L.R. The safe use of cisplatin in hyperthermic isolated limb perfusion systems. Cancer 56:742-744, 1985.
30. Rozenzweig, M., von Hoff, D.D., Slavik, M., and Muggia, F.M. Cis-diammine-dichloro (II), a new anticancer drug. Ann. Int. Med. 86:803-812, 1977.
31. Steeves, R., Robins, H.I., Miller, K., Shecterle, L., Kruk, M., and Dennis, W. Interaction of whole body hyperthermia and irradiation in the treatment of AKR mouse leukemia. Int. J. Radiat. Biol. 52:935-947, 1987.
32. Tapazoglou, E., Robins, H.I., Khatana, A., Schmitt, C.L., Cohen, J., Franken, K., Corry, P., and Sapareto, S.A. The enhancement of cytotoxicity of carboplatin (CBDCA) by whole body hyperthermia (WBH) in the murine AKR leukemia. AACR Abstract 29:1342, 1988.

33. Tapazoglou, E., Robins, H.I., Khatana, A., Schmitt, C.L., Cohen, J., Franken, K., Corry, P., and Sapareto, S.A. Whole body hyperthermia (WBH) potentiation of carboplatin (CBDCA) cytotoxicity in murine AKR leukemia. Rad. Res. Soc.--NAHG Abstract Cd-13, 1988.
34. Wondergem, J., Bulger, R.E., Strebel, F.R., Newman, R.A., Travis, E.L., Stephens, L.C., and Bull, J.M.C. Effect of cis-diamminedichloroplatinum (II) combined with whole body hyperthermia on renal injury. Cancer Res. 48:440-446, 1988.

# WHOLE BODY HYPERTHERMIA AND INTRAPERITONEAL CARBOPLATIN IN RESIDUAL OVARIAN CANCER

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## ABSTRACT

Intraperitoneal (IP) cisplatin-based chemotherapy provides prolonged disease-free survival in some women with residual peritoneal ovarian cancer following systemic chemotherapy. This review presents several considerations which support the combined use of IP carboplatin and whole body hyperthermia in this patient population. This novel treatment approach is predicated on projected advantages which relate to improved therapeutic index.

## INTRODUCTION AND BACKGROUND

Intraperitoneal (IP) drugs administered in a large volume of solvent (e.g., 2 liters) only gradually enter the systemic circulation (8, 12, 24, 25) although most IP drug ultimately reaches the general circulation to affect systemic metastases (e.g., more than 90% with cisplatin) (8, 12, 24). Due to slow drug egress from the peritoneal space and the relative rapidity of systemic drug elimination, the IP drug concentration and the IP area under the concentration-time curve (i.e., "AUC") can greatly exceed the systemic drug concentration and systemic AUC (8, 12, 24). As a result, due to local drug diffusion, tissue drug concentrations can significantly exceed systemic drug levels for at least several millimeters into a peritoneal tumor (e.g., 4 mm after 120 mg cisplatin/$m^2$ IP) (21). This phenomenon has been applied in the treatment of primary ovarian cancer which typically remains limited to the peritoneal cavity until relatively late in its disease course (8, 24).

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Cisplatin and carboplatin, which appear to have the same active intermediate, have similar activity for IP chemotherapy of ovarian cancer. These platinum analogues show very similar efficacy against ovarian cancer (27, 33) and they are the most active antineoplastic drugs in this disease (7, 26, 27, 33). Both drugs also exhibit a steep dose-response relationship (19, 27). Intraperitoneal cisplatin achieves approximately a 15 to 30-fold dose advantage (AUC peritoneum divided by AUC plasma) based on actual clinical measurements of IP and serum drug levels (12, 18, 24, 30). Dose advantages for IP carboplatin may be modestly higher based on data from 2 patients (29 and 50 fold) for whom such measurements exist (22). A higher dose advantage is expected due to carboplatin's faster plasma clearance (3, 6, 16, 22) and due to carboplatin's greater molecular weight which causes slower egress from the peritoneal space (24). Overall, the depth of carboplatin penetration into tumor should equal that of cisplatin (24). Both drugs are well tolerated at high IP doses such as 200-300 mg carboplatin/$m^2$ (22), up to 120 mg cisplatin/$m^2$ or up to 270 mg cisplatin/$m^2$ with systemic thiosulfate protection (12, 20, 21).

Regimens employing such cisplatin doses produce long-term, disease-free survival in up to 69% of ovarian cancer patients who have minimal residual intraabdominal tumor ($\leq$2 cm in diameter) following their initial cisplatin-based systemic chemotherapy (13). Unfortunately, residual peritoneal ovarian cancer proves resistant to IP cisplatin regimens in at least 30% of these "good risk" patients as well as in 100% of patients whose residual disease exceeds 2 cm in diameter (13). Such treatment failures could reflect both the intrinsic drug resistance of some tumors or inadequate drug penetration into larger tumor nodules (13, 24). In the proposal to follow, we will outline the potential role of whole body hyperthermia (WBH) as an adjunct to IP carboplatin to overcome such therapeutic obstacles.

## WBH AND IP CARBOPLATIN

An analysis of recent preclinical studies provides a foundation for the projected improvement in efficacy and therapeutic index when using WBH as an adjunct to IP carboplatin chemotherapy. Such an approach should produce substantial heat-carboplatin tumoricidal synergism, could enhance the depth of carboplatin penetration into peritoneal tumor, and could produce a therapeutic gain via several mechanisms.

### Advantages of Carboplatin

Carboplatin is preferable to cisplatin in the WBH setting. Combining cisplatin and WBH has resulted in prohibitive nephrotoxicity in both preclinical (34) and clinical (11) studies. Thermal enhancement ratios for heat-cisplatin cytotoxicity (1, 5, 10, 23) are very similar to estimates of the thermal enhancement ratio for cisplatin nephrotoxicity (34). As a result, WBH may produce little or no real increase in cisplatin's therapeutic index (5). In addition, cisplatin's emetogenic effects pose a risk for aspiration and subsequent pneumonitis when using a system for WBH which does not require endotracheal intubation (4, 29).

In contrast, carboplatin is much less emetogenic than cisplatin (3, 6, 15, 16) and carboplatin does not cause nephrotoxicity (3, 16) even at the very high doses used in the setting of autologous bone marrow transplantation (L. Einhorn, Indiana University, personal communication). In addition, we have recently demonstrated supra-additive cytotoxic interactions between hyperthermia and carboplatin at WBH temperatures (4). The thermal enhancement ratio for carboplatin killing is substantial and is essentially the same as the thermal enhancement ratio for cisplatin when the two drugs are simultaneously compared in the same cell line (5). For example, the thermal

enhancement ratio is 3.32 at 41.8°C in the JM human T cell leukemia (4); i.e., 10 μg carboplatin/ml at 41.8°C is equivalent to 33.2 μg/ml at 37.0°C even after correcting for direct thermal killing.

WBH and the Therapeutic Index of IP Carboplatin

WBH could increase the therapeutic index of IP carboplatin therapy due to certain factors related to heat-drug sequencing. In particular, we have found that the hyperthermia-carboplatin sequence profoundly affects the degree of synergism achieved (4). Simultaneous heat and carboplatin causes maximal synergism, heat before carboplatin causes much less synergism, and heat after carboplatin causes minimal or no synergism (4). Thus, WBH can be given during the first 1 or 2 hours of IP carboplatin instillation when IP concentrations are highest and little drug has reached the systemic circulation (12, 13, 22). Thereafter, patients can be promptly returned to normal body temperature [in 30 minutes or less (29)] to minimize systemic heat-drug synergism.

Myelosuppression, the dose-limiting toxicity of carboplatin (3, 6, 15, 16), should be the most important systemic cytotoxic effect when combining WBH and carboplatin. In regard to carboplatin's myelosuppression, it should be noted that radiant heat WBH is nonmyelosuppressive, produces minimal morbidity, and as a single agent can produce brief tumor responses (29).

Unique aspects of the heating of bone marrow during radiant heat WBH also have implications relating to myelosuppression. We (14) and others (31) have noted that bone marrow temperatures remain approximately 0.6°C lower than the core body temperature and other body sites during the plateau phase of WBH (i.e., when patients are maintained steadily at the target temperature). In addition, we have recently shown that the degree of hyperthermia-carboplatin synergism increases with increasing temperature (4). For instance, in the JM cell line, the thermal enhancement ratio for carboplatin killing is 1.89 at 40.5°C and increases to 3.32 at 41.8°C (4). Because of this relationship, bone marrow should experience less cytotoxic effect than would the remainder of the body during hyperthermia-carboplatin therapy.

WBH and Platinum Resistance

Combining hyperthermia and carboplatin might also address problems related to the platinum resistance of residual IP ovarian cancer cells. Hyperthermia and cisplatin together can overcome cisplatin resistance in rodent ovarian cancer cells *in vitro* (32). This effect is ideal for clinical IP chemotherapy which is given primarily (13) to eradicate persistent IP ovarian cancer which is resistant to the initial systemic cisplatin therapy.

The ability to overcome platinum resistance might be related to the accelerated formation of reactive cisplatin metabolites which occurs at elevated temperatures (28, 35). However, platinum resistance frequently correlates closely with decreased platinum uptake into tumor cells (1, 9, 17, 32) including in human ovarian carcinoma cells (1). Thus, overcoming resistance may especially reflect the increased platinum permeability of tumor cells that occurs at elevated temperatures (32).

These physicochemical effects have special implications for IP therapy. Greater cell permeability to platinum agents might improve the depth of drug penetration into tumor tissue. This would address the probable cause of IP treatment failure in many patients with disease greater than 2 cm in diameter (13). In addition, accelerated formation of active

platinum metabolites should increase the efficacy of IP carboplatin and also decrease the rate of entry of intact reactive platinum into the systemic circulation.

In addition to tissue permeability, the duration and concentration of drug exposure are important determinants of drug penetration (8, 12, 13, 22, 24, 25, 30). In this regard, studies of regional hyperthermia in dogs have noted that the transit of cisplatin out of the peritoneum is substantially slower in heated than in unheated animals ($t_{\frac{1}{2}}$ 133 minutes vs. $t_{\frac{1}{2}}$ 68 minutes, $p<0.001$) (35).

## SUMMARY

Taken collectively, the preceding considerations and observations suggest several mechanisms by which WBH and carboplatin could increase the efficacy and therapeutic index of IP platinum therapy. A necessary prelude to such an approach is a clinical Phase I trial combining WBH and systemic carboplatin. Such a study, supported by the Investigational Drug Branch of the National Cancer Institute (USA), is currently in progress at the University of Wisconsin Clinical Cancer Center.

## REFERENCES


1. Andrews, P.A., Velury, S., Mann, S.C., and Howell, S.B. cis-Diammine-dichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. Cancer Res. 48:68-73, 1988.
2. Barlogie, B., Corry, P.M., and Drewinko, B. *In vitro* thermochemotherapy of human colon cancer cells with cis-dichlorodiammine-platinum(II) and mitomycin C. Cancer Res. 40:1165-1168, 1980.
3. Calvert, A.H., Harland, S.J., Newell, D.R., Siddik, Z.H., Jones, A.C., McElwain, P.G., Raju, S., Wiltshaw, E., Smith, I.E., Baker, J.M., Peckham, M.J., and Harrap, K.R. Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. Cancer Chemother. Pharmacol. 9:140-147, 1982.
4. Cohen, J.D., and Robins, H.I. Hyperthermic enhancement of cis-diammine-1,1-cyclobutane dicarboxylate platinum(II) cytotoxicity in human leukemia cells *in vitro*. Cancer Res. 47:4335-4337, 1987.
5. Cohen, J.D., Robins, H.I., and Schmitt, C.L. Interactions of hyperthermia with carboplatin, cisplatin, and etoposide in human leukemia cells *in vitro*. Cancer Letters, in press.
6. Curt, G.A., Grygiel, J.J., Corden, B.J., Ozols, R.F., Weiss, R.B., Tell, D.T., Myers, C.E., and Collins, J.M. A Phase I and pharmacokinetic study of diamminecyclobutanedicarboxylatoplatinum (NSC 241240). Cancer Res. 43:4470-4473, 1983.
7. Decker, D.G., Fleming, T.R., Malkasian, G.D. *et al.* Cyclophosphamide plus cisplatinum in combination: Treatment program for stage III or IV ovarian carcinoma. Obstetrics and Gynecology 60:481-487, 1982.
8. Dedrick, R.L., Myers, C.E., Bungay, P.M., and DeVita, V.T. Jr. Pharmacokinetic rationale for peritoneal drug administration in the treatment of ovarian cancer. Cancer Treat. Rep. 62:1-11, 1978.
9. Eichholtz-Wirth, H., and Hietel, B. The relationship between cisplatin sensitivity and drug uptake into mammalian cells *in vitro*. Brit. J. Cancer 54:239-243, 1986.
10. Fisher, G., and Hahn, G.M. Enhancement of cis-platinum(II)diamminedichloride cytotoxicity by hyperthermia. National Cancer Institute Monographs 61:255-257, 1982.

11. Gerad, H., Egorin, M.J., Whitacre, M., Van Echo, D.A., and Aisner, J. Renal failure and platinum pharmacokinetics in three patients treated with cis-diamminedichloroplatinum(II) and whole body hyperthermia. Cancer Chemotherapy and Pharmacology 11:162-166, 1983.
12. Howell, S.B., Pfeifle, C.L., Wung, W.E., Olshen, R.A., Lucas, W.E., Yon, J.L., and Green, M. Intraperitoneal cisplatin with systemic thiosulfate protection. Ann. Int. Med. 97:845-851, 1982.
13. Howell, S.B., Zimm, S., Markman, M., Abramson, I.S., Cleary, S., Lucas, W.E., and Weiss, R.J. Long-term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. J. Clin. Oncol. 5:1607-1612, 1987.
14. Hugander, A., Robins, H.I., Martin, P., and Schmitt, C. Temperature distribution during radiant heat whole-body hyperthermia: experimental studies in the dog. Int. J. Hypertherm. 3:199-208, 1987.
15. Joss, R.A., Kaplan, S., Goldhirsch, A., Sessa, C., Brunner, K.W., and Cavalli, F. A Phase I trial of cis-diammine-1,1-cyclobutane dicarboxylate platinum II (Carboplatin, CBDCA, JM-8) with a single dose every five-week schedule. Invest. New Drugs 2:297-304, 1984.
16. Koeller, J.M., Trump, D.L., Tutsch, K.D., Earhart, R.H., Davis, T.E., Tormey, D.C. Phase I clinical trial and pharmacokinetics of carboplatin (NSC 241240) by single monthly 30-minute infusion. Cancer 57:222-225, 1986.
17. Kraker, A.J., and Moore, C.W. Accumulation of cis-diamminedichloroplatinum(II) and platinum analogues by platinum-resistant murine leukemia cells *in vitro*. Cancer Res. 48:9-13, 1988.
18. Lambert, H.E., and Berry, R.J. High dose cisplatin compared with high dose cyclophosphamide in the management of advanced epithelial ovarian cancer (FIGO stages III and IV): Report from the North Thames Cooperative Group. Brit. Med. J. 290:889-893, 1985.
19. Levin, L., and Hryniuk, W.M. Dose intensity analysis of chemotherapy regimens in ovarian carcinoma. J. Clin. Oncol. 5:756-767, 1987.
20. Markman, M., Cleary, S., and Howells, S.B. Nephrotoxicity of high-dose intracavitary cisplatin with intravenous thiosulfate protection. Eur. J. Clin. Oncol. 21:1015-1018, 1985.
21. McVie, J.G., Dikhoff, T., Van der Heide, J., *et al.* Tissue concentration of platinum after intraperitoneal cisplatin administration in patients. Abstract, Proc. Amer. Assoc. Cancer Res. 26:162, 1985.
22. McVie, J.G., ten Bokkel Huinink, W.W., Dubbelman, R., Franklin, H., van der Vijgh, W., and Klein, I. Phase I study and pharmacokinetics of intraperitoneal carboplatin. Cancer Treat. Rev. 12:35-41, 1985.
23. Meyn, R.E., Corry, P.M., Fletcher, S.E., and Demetriades, M. Thermal enhancement of DNA damage in mammalian cells treated with cis-diamminedichloroplatinum (II). Cancer Res. 40:1136-1139, 1980.
24. Myers, C.E. The use of intraperitoneal chemotherapy in the treatment of ovarian cancer. Sem. Oncol. 11:275-284, 1984.
25. Myers, C.E., and Collins, J.M. Pharmacology of intraperitoneal chemotherapy. Cancer Invest. 1:395-407, 1983.
26. Omura, G., Blessing, J.A., Ehrlich, C.E., *et al.* A randomized trial of cyclophosphamide and doxorubicin with or without cisplatin in advanced ovarian carcinoma. Cancer 57:1725-1730, 1986.
27. Ozols, R.F., Behrens, B.C., Ostchega, Y., and Young, R.C. High dose cisplatin and high dose carboplatin in refractory ovarian cancer. Cancer Treat. Rep. 12:59-65, 1985.
28. Riviere, J.E., Page, R.L., Dewhirst, M.W., Tyczkowska, K., and Thrall, D.E. Effect of hyperthermia on cisplatin pharmacokinetics in normal dogs. Int. J. Hypertherm. 2:351-358, 1986.
29. Robins, H.I., Dennis, W.H., Neville, A.J., Shecterle, L.M., Martin, P.A., Grossman, J., Davis, T.E., Neville, S.R., Gillis, W.K., and Rusy, B.F. A nontoxic system for 41.8°C whole body hyperthermia: Results of a Phase I study using a radiant heat device. Cancer Res. 45:3937-3944, 1985.

30. ten Bokkel Huinink, W.W., Dubbelman, R., Aartsen, E., Franklin, H., and McVie, J.G. Experimental and clinical results with intraperitoneal cisplatin. Sem. Oncol. 12:43-46, 1985.
31. Thrall, D.E., Page, R.L., Dewhirst, M.W., Meyer, R.E., Hoopes, P.J., and Kornegay, J.N. Temperature measurements in normal and tumor tissue of dogs undergoing whole body hyperthermia. Cancer Res. 46:6229-6235, 1986.
32. Wallner, K.E., DeGregorio, M.W., and Li, G.C. Hyperthermic potentiation of cis-diamminedichloroplatinum(II) cytotoxicity in Chinese hamster ovary cells resistant to the drug. Cancer Res. 46:6242-6245, 1986.
33. Wiltshaw, E., Evans, B., and Harland, S. Phase III randomised trial of cisplatin versus JM8 (carboplatin) in 112 ovarian cancer patients, stages III and IV. Abstract, Proc. Amer. Soc. Clin. Oncol. 4:121, 1985.
34. Wondergem, J., Bulger, R.E., Strebel, F.R., Newman, R.A., Travis, E.L., Stephens, L.C., and Bull, J.M.C. Effect of cis-diamminedichloroplatinum(II) combined with whole body hyperthermia on renal injury. Cancer Res. 48:440-446, 1988.
35. Zakris, E.L., Dewhirst, M.W., Riviere, J.E., Hoopes, P.J., Page, R.L., and Oleson, J.R. Pharmacokinetics and toxicity of intraperitoneal cisplatin combined with regional hyperthermia. J. Clin. Oncol. 5:1613-1620, 1987.

# OVERVIEW OF WHOLE BODY HYPERTHERMIA EXPERIENCE AT AMERICAN INTERNATIONAL HOSPITAL

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Whole body hyperthermia (WBH) with general anaesthesia,utilizing the heated water blanket technique of Barlogie et al[1] and of Larkin[2], have been utilized in 427 patients at American International Hospital (AIH) from 1978 thru 1987. After an early developmental phase, we now treat to achieve a core body temperatures of 42.2°C for two hours with a rapid cool-down phase. Most patients received WBH once each month with chemotherapy beginning 18 hours after completion of WBH.This was done in an attempt to utilize WBH in its optimum conditions, and to utilize a wide range of chemotherapy drugs, given in combinations and doses felt optimum for patient care in the absence of WBH. These studies were primarily intended to confirm the safety of both forms of therapy given sequentially.

Our 1985 protocol was designed to evaluate the ability of WBH to induce remissions in patients who have received two monthly cycles of combination chemotherapy without apparently achieving an early response. Patients only then become eligible for WBH. The ability of WBH to induce remission in this refractory group was then studied. Survival statistics from this group cannot thus be compared to studies which report all treated patients, because our WBH patients were selected for this poor prognostic factor.

Eligibility criteria include: histologically proven malignancies, with life expectancy > 12 weeks, Zubrod performance status <=3, age > 15 years, serum creatinine < 2 mg/dl, pulmonary vital capacity > 75% of predicted, and an absence of brain metastases. Patients with prior radiotherapy to the spine are accepted, but we prefer to exclude those with radiotherapy to the spine within 90 days. Cardiac screening is important, for patients with significant cardiac disease are unlikely to tolerate the stresses of WBH. Electrolyte abnormalities should be corrected and the electrocardiogram should not evidence a significant arrhythmia. Consultation with cardiologists, and special studies, may be needed to help determine eligibility for WBH.

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Table 1. Actuarial Per Cent Surviving and Median Survival (in months) in 427 Patients Receiving WBH and Chemotherapy from 10/78-12/87

| PRIMARY SITE | N | LOST | 1 YR | 3 YR | 5 YR | MEDIAN SURVIVAL (mos) |
|---|---|---|---|---|---|---|
| Lymph Nodes | 26 | 2 | 69% | 53% | 32% | 43.6 |
| Kidney | 11 | 2 | 51% | 20% | 20% | 12.3 |
| Breast | 144 | 9 | 56% | 24% | 16% | 11.2 |
| Bone | 23 | 2 | 60% | 14% | 9% | 11.1 |
| Colon | 42 | 3 | 39% | 3% | 0% | 7.4 |
| Ovary | 17 | 3 | 30% | 22% | 11% | 7.0 |
| Melanoma | 22 | 3 | 36% | 21% | 14% | 6.0 |
| Myeloma/CLL | 10 | 0 | 40% | 20% | 20% | 6.0 |
| Lung | 22 | 5 | 47% | 12% | 0% | 5.5 |
| Stomach | 18 | 0 | 22% | 0% | 0% | 4.0 |
| ALL SITES | 427 | 37 | 46% | 18% | 11% | 8.1 |

OVERALL SURVIVAL

From 10/78 through 12/87, 1011 WBH therapies have been adminstered to 427 patients under various protocols approved by the Institutional Review Board for Research and Ethics. The survival of the composite groups is presented in Table 1, which indicates the actuarial survival and median survival of our patients, reported as of 12/31/88. Here, N is the number of patients, and the number lost to followup is indicated. Individual tumor groups will be discussed separately below.

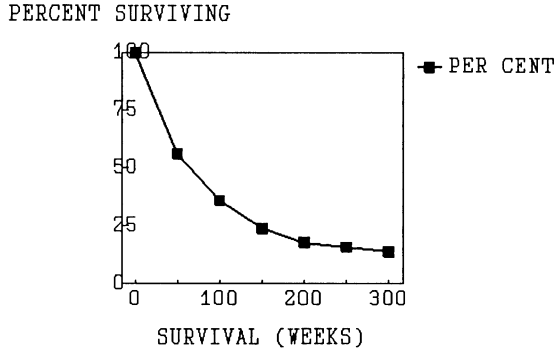


Chart 1. Survival of 144 patients with refractory metastatic breast cancer who received WBH.

TWO CASE HISTORIES

Patient E.D., 37 years old, had metastatic breast cancer which relapsed after adjuvant CMF chemotherapy and had a partial remission with combination chemotherapy with doxorubicin, cyclophosphamide, and 5FU. She received chemotherapy with vinblastine and mitomycin C, but had progressive, widespread bone metastases. She then presented to AIH with large palpable metastases extending from the skull and ribs. She received WBH followed by constant infusion 5FU, and had

shrinking of palpable metastases within 48 hours of WBH, and was dramaically pain free within 5 days when the initial course of 5FU completed. She continued with 3 monthly cycles of WBH-chemo, and then continued with 5FU only, but relapsed after 6 months.

Patient S.W., a 27yo male with initially a Stage IIB Hodgkins Disease, relapsed after several combination chemotherapy regimens. He began chemotherapy in 11/86 with BCNU, doxorubicin, vinblastine, and bleomycin. Only stable disease was evident by 3/87 when WBH was added to the same regimen. He then achieved a partial remission within one week, and received no further therapy until 8/87 when disease relapse occurred, which again responded to the same combination of WBH and chemotherapy. Due to the patients' unreliability, therapy has been sporatic and persistent Hodgkin's has been noted, but he continues with minor symptoms at this time.

BREAST CANCER

We have completed a more comprehensive review of our evaluable patients with breast cancer who began therapy at AIH from 1982 - 1986, including 42 patients who received WBH and chemotherapy and 44 who received only chemotherapy[3]. This is reported in detail elsewhere in this volume[4]. Proportional-hazards statistical analysis identified that survival depended upon Zubrod performance status, tissue hormone receptor (Estrogen and Progesterone), number of prior chemotherapy regimens that previously were failed, and infiltration of tumor into visceral sites. These factors were independant of the effects of WBH. Clinical indicators of response included complete and partial remissions in accordance with the World Health Organization guidelines[5] and an additional category of minor response is added, signifying patients with stable disease by WHO guidelines but with significant pain relief of at least two levels. In this group including extensively pretreated patients, 38 patients responded either completely, partially, or minimally. A logistic regression model of response[6,7] identified an increased chance of response with a positive estrogen receptor assay ($p=0.0031$) or with the use of Adriamycin ($p=0.031$). A decreased chance of response was noted in patients with prior chemotherapy for metastatic disease ($p=0.012$) or in patients with visceral metastases ($p=0.02$).

Our breast cancer studies identified several interesting aspects:

a) The overall groups of 28 evaluable patients who were matched for prognostic factors, had median time to tumor progression (TTP) and median survival that were similar to matched control patients, in spite of the fact that WBH patients were selected on the basis of not having an early response to chemotherapy.

b) Upon relapse from a prior chemotherapy regimen, the survival advantage associated with positive estrogen and progesterone receptors was lost and receptor positive patients then had a median duration of response of only 76 days.

c) In 32 patients who previously failed chemotherapy regimens, there seems to be a doubling of median TTP in WBH as compared to chemotherapy-only patients, however small

sample sizes limit confidence in a statistical evaluation of this observation.

d) The median TTP of our patients with liver metastases were 164 and 103 days in WBH and control patients. These results could not be correlated with the doxorubicin dose intensity calculated in mg/$M^2$/wk. This apparent survival advantage favoring WBH was not evident in the overall groups because of dilution of the effects by good risk patients with positive hormone receptor tests or with previously untreated

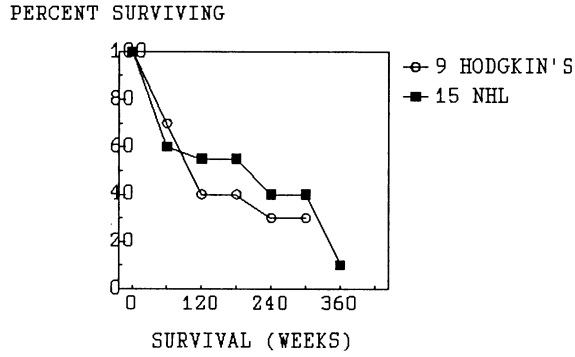


Chart 2. Survival of 9 patients with refractory Hodgkin's Disease and 15 patients with refractory Non-Hodgkin's Lymphoma.

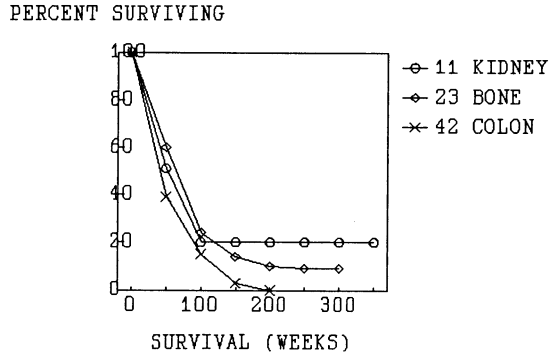


Chart 3. Survival of patients with refractory disease who received WBH and chemotherapy, including 11 with Hypernephroma, 22 with Sarcomas, and 38 with Colon Carcinoma

patients whose salvage therapy was effective. The design of the studies to include only patients without an excellent response with initial chemotherapy may well underlie these conclusions. Current studies for breast cancer patients include randomized prospective trials to clarify the role of WBH, including trials for patients with, and without prior doxorubicin therapy.

OTHER TUMORS

Significant responses may be achieved with patients with lymphoma or myeloma treated with or without concomitant chemotherapy. Significant responses have been evident in patients with sarcomas, especially uterine leiomyosarcoma, hepatoma, and bowel adenocarcinomas. Detailed studies are in progress in these tumor types.

TOXICITY

Details of subjective toxicity, categorized according to WHO guidelines[5] for 216 WBH treatments for 101 patients given from 11/16/82 to 2/8/89 are listed in Table 4.
The usual transient laboratory changes of dilutional anemia, mild thrombopenia, hyponatremia and hypophosphatemia were common. The clinically expected subjective toxicities thus include edema, weakness, and fatigue, which may well persist for several days. Confusion and central neurological signs are occasionally seen following WBH, but usually resolve within 4 days, although two episodes took several weeks to resolve. One patient with refractory lymphoma,recent doxorubicin and spinal radiation developed paraplegia immediately following WBH. No deaths associated with WBH were noted since 1982 when current eligibility criteria were adopted.

Table 4. Toxicity Experienced during 216 WBH Treatments. Each number indicates the percent of therapies with > Gr 2 WHO toxicity. N = the number with this data available.

| Toxicity | N | Interval Prior to WBH | < 24 hrs After WBH | 1-4d After WBH |
|---|---|---|---|---|
| Zubrod level | 215 | 12% | 86% | 20% |
| Analgesia | 215 | 30% | 43% | 33% |
| Pulmonary Function | 216 | 0% | 1% | 2% |
| Diarrhea | 215 | 1% | 3% | 2% |
| Nausea/Vomiting | 215 | 0% | 0% | 0% |
| Stomatitis | 215 | 0% | 0% | 0% |
| Cardiac Arrhythmia | 215 | 0% | 1% | 0% |
| Cardiac Function | 215 | 0% | 0% | 0% |
| Skin Burns | 215 | 0% | 0% | 0% |
| Fatigue | 215 | 2% | 79% | 18% |
| Neuropathy | 215 | 0% | 1% | 1% |
| Weakness | 216 | 1% | 69% | 38% |
| Confusion/Stroke | 215 | 0% | 4% | 1% |
| Edema | 216 | 1% | 69% | 38% |
| Hemorrhage | 216 | 3% | 0% | 1% |
| Fever | 215 | 1% | 1% | 1% |
| Infection | 215 | 1% | 1% | 1% |

CONCLUSION

With studies designed to utilize WBH in patients eligible only once extreme clinical risks were present, we have demonstrated that WBH is clinically manageable in patients with extensive, metastatic disease. WBH has been shown to facilitate remissions in selected patients who were refractory to chemotherapy. This was true even though early studies did not demonstrate prolonged remissions with WBH alone. It does

remain disappointing, however, to continue to observe median remission durations undr one uear in most subgroups of these heavily pretreated patients. These studies doe not provide any useful clues regarding the mechanism for the occasional effectiveness of WBH. Since survival appeared to be prolonged in breast cancer patients whose chemotherapy regimen was not changed, reversal of chemotherapy resistance may have occurred in several patients. Prolonged remissions have been evident particularly in patients with liver metastases, suggesting that effectiveness may be organ specific. Prolonged remissions have not correlated with thermal dose intensity or with dose intensity of doxorubicin in the breast cancer patients, suggesting that other mechanisms of activity may be involved. Nevertheless, clinical remissions may be achieved in patients whose disease is refractory to multiple chemotherapy regimens, and transient benefit and pain control can provide individual patients new hopes. Our studies continue, and include additional studies of immune function during and after WBH, and well as prospective randomized trials to further delineate the clinical role of WBH.

REFERENCES


1. B. Barlogie, P. M. Corry, E. Yip, et al, Total Body Hyperthermia with and without Chemotherapy for Advanced Human Neoplasms, Cancer Res. 39:1481 (1979).

2. J. M. Larkin, W. S. Edwards, D. E. Smith, and P. J. Clark. ystemic Thermotherapy: Description of a Method and Physiologic Tolerance in Clinical Subjects, Cancer 40:3155 (1977).

3. R. D. Levin, R. Sanchez, Y. D. Kim, A Mellijor, M. A. Doyle, W. Simonich, and R. M. Williams. Studies of Whole Body Hyperthermia (WBH) with Chemotherapy (CH) in Metastatic Breast Cancer, Submitted.

4. R. D. Levin, R. Sanchez, Y. D. Kim, A. Mellijor, M. A. Doyle, W. Simonich, and R. M. Williams. Whole Body Hyperthermia Experience in Breast Cancer at American International Hospital. This volume.

5. A. B. Miller, B. Hoogstraten, M. Staquet, et al. Reporting results of Cancer treatment, Cancer 47:207 (1981).

6. M. Friedman, The use of Ranks to Avoid the Assumption of Normality Implicit in the Analysis of Variance, J. Amer. Statis. Assn. 32:675 (1937).

7. D. R. Cox, Regression Models and Life Tables, J. Roy. Statist. Soc. 34B:187 (1972).

# HYPERTHERMIA AND CHEMOTHERAPY

William P. Galen

Changes produced in the cytotoxic effects of chemotherapeutic agents on cancer cells by hyperthermia have been demonstrated in vitro and in vivo. Increasing cell toxicity has been shown as the temperature is raised from 40 to 45 degrees centigrade. The mechanisms responsible for these temperature effects on cell killing by anticancer drugs are not entirely understood. In a recent review by Herman (1), possible explanations for this net increase in DNA damage seen after exposure to anticancer drugs and hyperthermia include the following:

1. Hyperthermia may increase the drug uptake by cancer cells.
2. Hyperthermia may alter the intracellular distribution of the drug.
3. Hyperthermia may alter metabolism of the drug.
4. Heat may increase the drug reaction rate with DNA or inhibit DNA repair.
5. Any combination of the above events.

Not all chemotherapeutic drugs become more cytotoxic at elevated temperatures. Cell killing by AMSA and ARA C is actually inhibited by hyperthermia. The vinca alkaloids and the antimetabolites, 5-FU and methotrexate, are only additively cytotoxic with hyperthermia. Complex interactions between the anti-tumor antibiotics Adriamycin and Actinomycin D have been described in which either increased killing or protection can take place, depending on the timing of the drugs and the heat. In general those anticancer drugs whose cytotoxicities are increased at higher temperatures show greater supra-additive lethality as the temperature is progressively increased from 40 degrees centigrade to 45 degrees centigrade. Conversely, the cytotoxicity of Adriamycin, Bleomycin, Cisplatin and BCNU is inhibited when the tissue is cooled and exposure to the drug occurs at temperatures below 37 degrees centigrade. Adriamycin cytotoxicity appears to be particularly affected by lower temperatures. This may explain the protective action of scalp cooling on Adriamycin- induced alopecia.

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Data from Donaldson et al, 1978; Kim et al, 1978; Hahn 1979; Marmor 1979; b Teicher et al,1981 a; Adams, et al, 1982; Herman 1983 b; Goldfeder and Newport 1984).*In hypoxic cells.

The rate of heating of tissues prior to exposure to chemotherapeutic drugs may alter the cytotoxicity. It has been shown with BCNU, Cisplatin, Bleomycin and Adriamycin that a one hour exposure to these drugs at 42.4 degrees centigrade causes significantly less cell killing after a three hour transit from 37 degrees centigrade to 42.4 degrees centigrade than does immediate exposure to the drug

Table I. Cytotoxic interaction between anticancer drugs and hyperthermia.

| Supra-additive | Additive | Less than additive |
|---|---|---|
| Adriamycin | Vincristine | AMSA |
| Actinomycin D | Vindesine | Ara-C |
| Bleomycin | 5-Fluorouracil | |
| BCNU | Methotrexate | |
| Cisplatin | | If heat precedes drug: |
| Cyclophosphamide | | |
| Melphalan | | Adriamycin |
| Mitoxantrone | | Actinomycin D |
| Mitomycin C | | |
| thio-TEPA | | |
| Misonidazole* | | |
| 5-thio-Dglucose* | | |

at 42.4 degrees centigrade. Thus, the more rapid heating possible with local or regional hyperthermia may have the advantage of an increased cytotoxic interaction between the anicancer drug and hyperthermia than those techniques used to induce whole body hyperthermia which require heating over two or three hours to reach that temperature.

Effectiveness of chemotherapy depends upon the anticancer agents reaching the tumor cells in adequate concentrations to be cytotoxic. Changes in the intratumor circulation may profoundly affect the distribution of the drugs. These changes may be produced by rapid tumor growth with inadequate vascularization, prior surgery or radiation treatment. Methods used to enhance the drug concentration have included the following:

1. Arterial infusion chemotherapy provides a very high drug concentration on the initial pass through. This is used in hepatic artery, hypogastric artery and external carotid artery infusion programs.
2. Isolation perfusion techniques used for extremity melanomas and sarcomas provide a more sustained high concentration of drug exposure to the cancer cells, while decreasing or preventing systemic toxicity.
3. Chemoembolotherapy can provide the high drug concentration of arterial infusion therapy with the prolonged tumor dwell time produced by embolizing the arterial supply so the drug will not be rapidly washed out of the tissue. This is accomplished by injecting slurries of chemotherapy drugs mixed with contrast agent and either Gelfoam, microspheres or collagen particles into the supplying artery.
4. Intracavitary chemotherapy provides a multifold drug concentration advantage for treating the surface two or three cell layers involved by tumor.

Combining the above techniques with hyperthermia should enhance the tumor cell kill and produce better and more durable responses than seen with conventionally administered chemotherapy.

Clinical trials of hyperthermia and chemotherapy have confirmed the benefits of the combined treatment in patients with advanced disease who had failed after repeated standard treatments with surgery, radiation, therapy and chemotherapy. Generally, the chemotherapy drugs were used in conventional dosages and protocols with only the addition of hyperthermia.

Whole body hyperthermia (WBH) has been extensively studied by Joan Bull (2) who reported responses in 10 of 19 (53%) of heavily pretreated advanced sarcoma patients following WBH and BCNU chemotherapy. Sanchez (3), Levin (4), et al, reported responses in patients with advanced breast, cervix, biliary, sarcoma, and lymphoma given WBH and chemotherapy. A subsequent series of patients with advanced breast cancer (6) showed increased disease free and overall survival in hormone receptor negative patients given WBH and Adriamycin based chemotherapy vs. controls treated with the same chemotherapy alone. However, when the intensive care and cost in dollars and personnel presently required for safe WBH is coupled with the nondurable nature of most responses so far seen, WBH will probably remain the province of those centers that are currently studying its use.

Regional hyperthermia (RH) provided by a variety of techniques has been used in conjuntion with chemotherapy drugs. The problems of adequately heating tumors deep below the surface, while protecting surrounding and superficial tissues have limited the applications. Techniques using ultrasound, radiofrequency, and microwave energy sources as well as hot water baths and hyperthermic arterial perfusions have all been reported to augment the effects of anticancer

drug therapy and produce responses in patients who had previously failed standard treatments. There has been no increase in systemic or local toxicity to the drugs when regional hyperthermia was used. In 1985, Creech (5) first reported on the arterial perfusion technique for treating recurrent malignant melanoma of the extremity. Stehlin (6) reported approximately doubling that response rate by heating the perfusate to 40 degrees centigrade. Probably the best study to demonstrate the value of hyperthermic perfusion in the treatment of extremity melanoma is Ghussen's (7) study which randomized patients to identical surgical treatment with and without the addition of hyperthermic Melphalan perfusion. The randomization of patients was stopped when analysis after 550 days showed 21 local recurrences in the surgery only group vs. 4 recurrences in the perfusion group. A five year followup reported 26 recurrences and 8 deaths in 54 patients in the control group vs. 6 recurrences and 1 death in 53 patients in the perfusion group.

Klein (8) has treated 28 patients with advanced melanoma of the extremities with hyperthermic perfusion of Cis-Platinum, suggesting its use as an alternative to Melphalan. Pommier (9) reported treating 22 patients with extremity melanomas and 35 with soft tissue sarcomas with hyperthermic Cis-Platinum perfusions. Early data in both studies showed excellent local control. Survival data is not yet available to compare the results with those of Melphalan.

Storm (10) summarized 194 patients with advanced and recurrent visceral cancers treated with standard dose chemotherapy and hyperthermia (13.5 mHz-Magnetrode). Fifty five (28%) of the patients showed regression of their disease and 83 (43%) had stable disease. Two hundred and eleven patients who had previously failed chemotherapy, when retreated with the drugs and hyperthermia, showed regression in 40 (19%) and stabilization in 64 (30%).

Pilepich (11) reported the results of Bleomycin and hyperthermia therapy in 12 patients (8 squamous cell carcinoma of the head and neck, 4 adenocarcinoma of the breast) with persistant or recurrent disease following conventional treatment including full dose radiotherapy. Four patients went into complete remission and 6 showed a partial response. This study took advantage of the marked temperature dependence of Bleomycin described by Roizin-Towle (12) and for the first time showed an effect from treatment of advanced breast cancer with Bleomycin.

Galen (13) reported the results of treating 10 patients with advanced pelvic colorectal metastases with aortic infusion chemotherapy using Cis-Platinum and FU combined with pelvic hyperthermia using the magnetrode (13.5 mHzRF). All patients had extensive prior therapy with surgery, radiation and chemotherapy. Tumor temperatures over 41 degrees centigrade were recorded in 7 of the patients. Eight of the 10 patients responded subjectively and objectively. Median survival in the responders was 13.5 months vs. 4

months in the nonresponders. Four of the 8 deaths in the responders were due to cancer outside the treated region.

Moffat (14) et al, described a mixed series of 178 hepatic malignancies treated with RF hyperthermia at 13.5 mHz and systemic or hepatic artery infusion chemotherapy. In 37 patients with previously untreated hepatic metastases of colorectal cancer, 29 (78.4%) had partial regression and 3 (8.1%) showed stable disease with a median survival of 36+ weeks.

Herman (15) reported progress on a current study of combined chemotherapy with weekly systemic Cis-Platinum, radiation therapy, and regional hyperthermia to 43 degrees centigrade in patients with advanced head and neck cancer and recurrent breast cancer. Seven of 14 patients reached CR and the balance a PR.

Khandekar (16) has described the results of treating 43 patients with unresectable pancreatic cancer with chemotherapy (CCNU and FU), radiation therapy (4,000 rads in 20 fractions split course) and hyperthermia (13.56 mHz-Magnetrode). Of 36 patients in which tumor temperature measurements were made in this difficult area, 24 (67%) had temperatures over 41 degrees centigrade and 12 of these exceeded 42.5 degrees centigrade. There were two complete responses with survival at 44 and 53+ months. There were 18 patients with PR with a median survival of 14.3 months.

Sugimachi (17) treated 297 patients with squamous cell carcinoma of the thoracic esophagus. One hundred and eighty three had a subtotal esophagectomy following preoperative chemotherapy of Bleomycin 5 mg. IV twice weekly for 3 weeks, radiation therapy to 30 gray, and in 62 of these patients hyperthermia with a radiofrequency system using an endotract electrode in the esophagus twice weekly for 3 weeks. The 114 patients who did not undergo resection received the same chemotherapy, 46.9 gray radiation therapy, and in 31 patients hyperthermia twice weekly for 6 weeks. Of those patients undergoing esophagectomy, 43.2% of the hyperthermia-chemotherapy-radiation therapy (HCR) group were alive at 5 years compared to only 14.7% of the chemotherapy-radiation therapy (CR) group.

Koga (18) and Fujimoto (19) have reported on the use of continuous hyperthermic peritoneal perfusion with Mitomycin-C (CHPP-M) in patients with peritoneal cancer. Koga randomized 60 patients with macroscopic serosal invasion but no macroscopic peritoneal metastases, who had undergone curative surgery for gastric cancer into 2 groups. Thirty two received CHPP-M and 28 did not. The CHPP-M was performed for 50-60 minutes while the patient was still in the operating room under general anesthesia. At 30 months 83% of the CHPP-M patients were alive vs. 67.3% of the control. Fujimoto treated 6 patients with peritoneal recurrence after radical surgery for gastrointestinal cancer with the same treatment program combined with surgical resection of the recurrent tumors and intestinal bypass anastomosis when

indicated. Malignant ascites, present in 5 of the 6 patients, cleared and did not reappear. One patient with gastric cancer died after 5 months with hepatic metastases. Five of the 6 remaining patients show no evidence of disease at 8-17 months following treatment.

I have listed many of the studies demonstrating the clinical value of adding hyperthermia to chemotherapy in patients with advanced cancer. The treatments devised for these patients with otherwise untreatable disease will hopefully lead to improvements in survival and perhaps cure of many less advanced malignancies.

## PREDICTIONS AND HOPES FOR THE 1990'S

### Chemotherapy drugs

I believe the 1990's will see further exloitation of this steep temperature response seen with Cis-Platinum and Bleomycin. Carboplatin has similar anticancer effects as Cis-Platinum. In vitro studies have demonstrated synergistic effect with heat. The decreased nephrotoxicity, nausea and vomiting, when Carboplatin is used, will simplify treatment and decrease the hospitalization necessary for the hydration and antimetics used with Cis-Platinum.

### Chemotherapy administration

The use of central access and venous port systems has simplified the administration of systemic chemotherapy. Repeated arterial infusion access can be provided with polyurethane catheters connected to subcutaneous ports. These can be inserted through a left axillary artery approach to a site in the aorta distal to the renal artery origin for pelvic infusion therapy. Similarly, catheters placed in the common hepatic artery can be connected to a subcutaneous port.

### Intraperitoneal hyperthermic perfusion

Intraperitoneal chemotherapy has already shown value in the treatment of recurrent ovarian cancer with small volume residual disease. The trial of hyperthermic perfusion in this disease is the natural next step to increasing the durability of the response. Similarly, Duke's B and C colon carcinoma treatment with adjuvant intraperitoneal hyperthermic perfusion is a trial that must be done.

4. Combinations of chemotherapy, radiation therapy, and hyperthermia should be evaluated in bulky nonresectable malignancies that may become resectable after treatment. Trimodality therapy is just beginning.

### Intraluminal hyperthermia

Dr. Sugimachi has already pointed the way in esophageal cancer. The 1990's will see the development of

other intraluminal electrodes such as an intraurethral catheter for prostate cancer, biliary catheters for cancers of the common bile duct and head of the pancreas, and bronchial catheters for endobronchial treatment. Controlled heating of local malignancies is the goal of hyperthermia. Hyperthermia enhances the anticancer effectiveness of our chemotherapy drugs. We need to learn how to best use them together. The 1990's will see the movement of hyperthermia therapy into the regular clinical practice of oncology.

REFERENCES


1) Herman TS, Teicher BA, Jochelson M, Clark J, Svensson G, and Coleman CN, 1988: Rationale for use of local hyperthermia with radiation therapy and selected anticancer drugs in locally advanced human malignancies. Int. J. Hyperthermia, 4, 143-158.

2) Bull JMC, Mansfield B, Jabboury K, Cronau L, and Allen S: An update of whole body hyperthermia (WBH) + BCNU in advanced sarcoma. Proc. ASCO; 5:144, 1986.

3) Sanchez R, Mellijor A, Kim Y, and Levin R: Whole body hyperthermia with chemotherapy in advanced refractory malignancies. Proc. ASCO; 6:176. 1987.

4) Levin RD, Sanchez R, Kim YD, Mellijor A, Doyle MA, Simonich W, and Williams RM: Whole body hyperthermia (WBH) in metastatic breast cancer. Proc. ASCO; 7:100, 1988.

5) Creech O, Krementz ET, Ryan RF: Chemotherapy of cancer: Regional perfusion utilizing an extracorporeal circuit. Ann Surg. 1985; 148:616-632.

6) Stehlin JS: Hyperthermic perfusion with chemotherapy for cancer of the extremities. SG&O, 1969; 129:305-308.

7) Ghussen F, Kruger I, Groth W, and Stutzer H: The role of regional hyperthermic cytostatic perfusion in the treatment of extremity melanoma. Cancer 61:654-659, 1988.

8) Klein ES, Brenner HJ, Schneebaum S, and Ben-Ari GY: Regional hyperthermic perfusion for malignant melanoma of the limb using Cisplatin. Proc. ASCO; 7:951, 1988.

9) Pommier RF, Moseley HS, Cohen J, Huang CS, Townsend RA, and Fletcher WS: Pharmacokinetics, toxicity, and short-term results of Cisplatin hyperthermic isolated limb perfusion for soft-tissue sarcoma and melanoma of the extremities. Am. J. Surg. 155:668-671, 1988.

10) Storm FK: Efficacy of radiotherapy and chemotherapy in regional hyperthermia of visceral cancer. Abst. Intern. Clin. Hyperthermia Symposium, 1988.

11) Pilepich MV, Jones KG, Emami BN, Perez CA, Fields JN, and Myerson RJ: Interaction of Bleomycin and hyperthermia-- results of a clinical pilot study. Int. J. Rad. Onc. Biol. Phys., 16: 211-213, 1989.

12) Roizin-Towle L, Hall EJ, and Capuano L: Third International Symposium: Cancer therapy by hyperthermia, drugs and radiation: NCI Monograph 61, 1982:149.

13) Galen WP, Baker H, Gallucci J, and Horowitz I: Abstract, Am. Soc. of Clin. Hyperthermic Oncology-- fourth annual meeting 1987.

14) Moffat FL, Gilas T, Calhoun K, Falk M, Dalfen R, Rotstein LE, Makowka L, Howard V, Laing D, Benturi D, Langer J, Falk RE: Further experience with regional radiofrequency hyperthermia and cytotoxic chemotherapy for unresectable hepatic neoplasia. Cancer 1985; 55:12

15) Herman TS, Teichner BA, Jochelson MS, Clark JR, Svensson GK Jansen JL and Coleman CN: Systemic Cisplatin (CDDP), local hyperthermia and radiation for treatment of locally advanced human malignancies. Abst. 8th Ann. Meeting, North American Hyperthermia Group, P 21, 1987.

16) Khandekar J, Scanlon E, Garces R, Berk R, Locker G, and Imperato J: A phase II study on combined therapy with radiofrequency (RF), hyperthermia (HT) with Magnetrode (M), radiation(RT), and chemotherapy (CT) with 5-Fluorouracil (5-FU) and nitrosoureas (NU) in pancreatic cancer (PC). Proc. ASCO; 8:405, 1989.

17) Sugimachi K, Matsuda H, Ohno S, Fukuda A, Matsuoka H, Mori M, and Kuwano H: Long-term effects of hyperthermia combined with chemotherapy and irradiation for the treatment of patients with carcinoma of the esophagus. SG&O, 1988; 167:319-323.

18) Koga S, Hamazoe R, Maeta M, Shimizu N, Murakami A, and Wakatsuki T: Prophylactic therapy for peritoneal recurrence of gastric cancer by continuous hyperthermic peritoneal perfusion with Mitomycin-C. Cancer 1988; 61:232-237.

19) Fujimoto S, Shrestha RD, Kokuben M, Kobayashi K, Kiuchi S, Takahashi M, Konno C, Ohta M, Koike S, Kitsukawa Y, Mizutani M and Okui K: Clinical trial with surgery and intraperitoneal hyperthermic perfusion for peritoneal recurrence of gastrointestinal cancer. Cancer 1989; 64:154- 160.

# TREATING ADVANCED MELANOMA WITH HYPERTHERMIA IN A PRIVATE SURGICAL ONCOLOGY PRACTICE

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From time to time oncologists are faced with difficult cancer problems for which standard methods of treatment hold little promise of success. Whereas we have long advocated the use of multiple therapeutic agencies with some success, the advent of clinically usable hyperthermia has added to our ability to manage even more complex problems, especially those which have failed to respond to standard measures.

This presentation is in support of this concept and the real place which hyperthermia has earned in cancer therapy. Specifically, four advanced localized metastatic melanomas (two in the head and neck region, one in the groin region, one in the chest wall) were treated either with heat alone (one patient) or heat in combination with radiation therapy and chemotherapy (three patients). The hyperthermia was supplied by capacitive hyperthermia at 13.56 MHz in one patient while microwave hyperthermia (300 MHz CL 5000) was used in three patients. The documented responses will be demonstrated for both presentation and general comment.

## CASE 1

C.W. is a 74 year old man who presented with locally advanced fungating ulcerating metastatic melanoma on the left side of his head and neck in February 1988. The primary melanoma was widely excised from the occiput in October 1985. By April 1986 a left posterior neck nodal metastasis developed, treated by bilateral posterior neck dissection followed by 3000 rads of radiation therapy. By May 1987 a left radical neck dissection was needed to remove recurrent metastatic disease. Systemic chemotherapy using Cisplatinum, Velban, and DTIC was subsequently administered followed by Actinomycin D, tumor necrosis factor and most recently recombinant alpha-2 interferon. By late 1987 it was clear that all the above measures had failed to control the disease which was now progressing while the patient was deteriorating.

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Upon presentation to me the metastatic mass measured 12 x 10 cm, projected 5 cm above the level of the skin, and was fixed to the left posterior mandible, parotid, mastoid and upper neck regions. The central mass was friable and ulcerated.

Treatment consisted of a combination of arterial chemotherapy, radiation therapy and localized hyperthermia. Chemotherapy and hyperthermia were administered simultaneously while radiation therapy followed immediately. Hyperthermia was administered in daily 90 minute fractions using the CL 5000 (Bicher) single applicator 300 MHz microwave hyperthermia unit maintaining an average of 42° C.

Intra-arterial chemotherapy was administered through a surgically placed catheter in the left external carotid artery via the superior thyroid artery. Its position and the extent of possible chemotherapy distribution was confirmed by injecting fluorescein dye under an ultraviolet lamp. This demonstrated a remarkable intense localized distribution of the dye throughout all the melanoma. Catheters were positioned through the tumor mass into which thermocouples could be inserted for temperature monitoring during hyperthermic treatments. Chemotherapy consisted of arterial Cisplatinum 100 $mg/m^2$ over 1 hour followed by DTIC 200 $mg/m^2$ over 2 hours on day 1 while the DTIC was given on days 2 through 5.

Radiation therapy was fractionated into daily doses over a 3 week period for a total of 3900 rads.

The cancerous mass was slow to respond but after 2 months a 50 percent reduction in the size was noted with healing of the ulcerated area. A repeat short course of arterial chemotherapy with Cisplatinum and DTIC with hyperthermia was administered. No further radiation was given. After approximately 5 months all evidence of disease had disappeared and, to date, 18 months after starting treatment, he remains free of disease and in robust health.

CASE 2

M.C., a 68 year old woman, presented in September 1988 with a large ulcerated recurrent melanoma involving the right groin region, measuring 12 cm in diameter. Prior repeated surgical attempts at removal failed to control the disease. Immunotherapy in the form of inoculations with irradiated melanoma cells likewise proved fruitless. After being informed that nothing more could be done for her at a major cancer treatment center, she sought assistance at our clinic. No prior radiation therapy or chemotherapy had been given.

Multiple small pulmonary nodules represented the only evidence of metastatic disease upon further evaluation. Treatment consisted of combined intra-arterial chemotherapy, radiation therapy and microwave hyperthermia.

Chemotherapy was given via a percutaneous arterial catheter which was introduced into the right external iliac artery. Its position was confirmed by injecting fluorescein dye under an ultraviolet light demonstrating excellent staining of the

entire tumor bearing and ulcerated areas. This is a good indication of the potential distribution of the chemotherapy administered directly through the arterial line. Chemotherapy consisted of Cisplatinum 100 mg/$m^2$ over 1 hour followed by DTIC 200 mg/$m^2$ over 2 hours on day 1. On days 2 through 5 only DTIC 200 mg/$m^2$ was introduced arterially. All chemotherapy was administered simultaneously with hyperthermia.

Radiotherapy was administered for a total of 4500 rads tumor dose with Cobalt 60 fractionated over a period of 3.5 weeks, each treatment immediately following the hyperthermia sessions.

Hyperthermia was provided by the 300 MHz CL 5000 apparatus (Bicher) for 17 daily treatments each lasting 90 minutes. Thermocouples were placed in several previously positioned catheters in the tumor mass, a temperature of approximately 42$^{o}$ C being maintained throughout each session.

Within 3 months of completing the treatments the entire mass had resolved completely except for a residual 2.5 cm flat ulcerated area which failed to heal. A biopsy of this area after 6 months revealed residual melanoma which was retreated with hyperthermia and radiation therapy (2000 rads in 8 fractions with the 9 MEV electron beam). The small ulcer has remained stable since. The pulmonary nodules are unchanged after 12 months while a left iliac bone metastasis was recently successfully treated with radiation therapy and hyperthermia.

CASE 3

J.T. is a 41 year old man who presented in August 1988 with a large 10 cm mass of metastatic melanoma on the left upper chest wall behind the pectoral muscles, extending into the left axilla with swelling and pain in the left arm. This had occurred despite several prior surgical attempts at removal. A metastatic survey revealed no cancer elsewhere. No prior chemotherapy or radiation therapy was administered. Surgical removal at this stage was considered impossible because of axillary vessel and possible brachial plexus involvement.

Treatment consisted of combining systemic chemotherapy with localized radiation therapy and hyperthermia.

Chemotherapy consisted of a 5 day course of intravenous DTIC 200 mg/$m^2$ per day over 2 hours with a single dose of intravenous Cisplatinum 100 mg/$m^2$ given on the first day over 1 hour. Each treatment was given while the local hyperthermia treatment was in progress.

Radiation therapy to the tumor followed immediately after each daily hyperthermia session for a total of 3000 rads with Cobalt 60 teletherapy over 10 days. An additional 1500 rads was given 3 weeks later with hyperthermia only for each of 5 days.

Each of 15 daily localized hyperthermia treatments was provided by two 300 MHz parallel opposed applicators (CL 5000 - Bicher) one positioned anteriorly, the other posteriorly opposite the tumor with the patient in a sitting position.

Average temperatures of 42° C were recorded by thermocouples in percutaneously placed catheters in the tumor. Other than superficial blisters on the skin no complication was noted.

Within 3 months the tumor mass had markedly reduced in size and by 6 months had completely disappeared resulting in full use of the left arm with complete disappearance of the swelling. All these responses were documented by CT scans.

Unfortunately subsequent metastatic disease developed in the left lung, successfully treated with capacitive hyperthermia, radiation therapy and systemic chemotherapy. The response, however, was short-lived as he developed subsequent brain metastasis followed by progressive bilateral pulmonary disease which ended his life. The treated mass in the chest wall remained under control.

CASE 4

E.D., a 50 year old man, presented with a large mass occupying most of the left side of the head and neck, measuring 15 cm in diameter. A prior biopsy was strongly suggestive of metastatic melanoma although no primary lesion was ever detected. Paralysis of the left vocal cord with right lateral flexion of the neck produced difficulty with speech and respiration. No prior surgery or radiation therapy was applied except for chemotherapy and 2 recent total body hyperthermia treatments which produced no benefit. Surgical removal was deemed technically impossible.

Treatment consisted entirely of localized hyperthermia. This was administered by a 13.56 MHz capacitive device through copper plates coupled to the skin overlying the tumor both anteriorly and posteriorly. Four 3 hours treatments over a period of 10 days resulted in no change in the size of the tumor but fluctuation became apparent. A needle introduced into the mass produced several hundred ccs of dark liquid followed rapidly by reexpansion of the mass as bleeding took place into the mass. After an additional 2 hyperthermic treatments, surgical removal was recommended when it became clear that the size of the mass could not be reduced. An extended left radical neck dissection was performed and to our surprise the mass was more easily removed than anticipated due to a thick, rind-like capsule having developed as a result of extensive necrosis in response to the hyperthermia treatments.

As best it could be evaluated histologically, all tumor was necrotic.

The patient is alive and well 10 years later.

CONCLUSION

A question frequently raised when such cases are presented concerns the role of hyperthermia in procuring such gratifying responses. The fourth case is, of course, exempt because hyperthermia was the only treatment prior to surgical removal to produce total necrosis of the tumor. Any suspicion that

hyperthermia had any part to play in the other three cases must also be equally leveled at what part chemotherapy or radiation therapy had in the response. In the absence of controlled prospective trials one has to make educated inferences based not only on the clinical evidence but also on the proven effectiveness of elevated temperatures on cancer cells in the laboratory and its known enhancement of both chemotherapeutic agents and radiation therapy. This will leave little doubt that, all things considered, hyperthermia was the common denominator to effect the dramatic responses witnessed in the cases presented. This only serves to encourage the continued application of hyperthermia in treating those tough cases we face all too often.

# THE ROLE OF HYPERTHERMIC PERFUSION IN THE TREATMENT OF TUMORS OF THE EXTREMITIES

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## INTRODUCTION

Approximately 60% of all soft tissue and osteogenic sarcomas and 50% of all melanomas arise in the extremities. Treatment of these tumors has traditionally been limited to local surgical procedures, often resulting in a non-functioning limb or amputation, particularly in the case of sarcoma and in a local recurrence rate primarily related to the biological tumor aggressiveness. A high incidence of micrometastatic nodules has been found in most high-grade sarcomas and in high-risk melanomas in the form of "skip" or "in transit" metastases. This suggests that these tumors cannot be managed as locally confined neoplasms, being a regional disease "ab initio" and requiring loco-regional treatment.

With this aim in mind, the technique of hyperthermic perfusion (H.P.) for the treatment of patients affected with tumors of the extremities was adopted in 1964 at the Regina Elena National Cancer Institute.

The first clinical results obtained with H.P. in treating limb tumors were satisfactory in terms of tumor control. However, a high incidence of complications was observed due to the high temperatures (42.5°-43°C) employed to reach a tumoricidal effect. Following this first experience, the technique was modified to include antineoplastic drugs into the perfusional circuit. The synergism obtained with the combined treatment permitted reducing the temperatures as well as the duration of the perfusion without decreasing the effectiveness of the treatment. Hyperthermic antiblastic perfusion (H.A.P.) is therefore routinely employed today.

Our experience over the past 20 years with different multimodality treatment protocols which all include perfusion as the first step, followed by surgery, radiotherapy or regional chemotherapy, combined in different manners, is reported. The impact of each approach on patient survival is also discussed.

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## MATERIALS AND METHODS

### Technique

The technique of H.A.P. has been previously described (1). Only few details are therefore reported.

The axillary and iliac vessels are cannulated for the upper and lower limbs respectively. The iliac approach is preferred for two main reasons:

1) during the isolation of the iliac vessels, the intraoperatory examination of the obturator and iliac nodes permits making a more accurate staging in the event of a melanoma;
2) the femoral approach may be employed for a second perfusion in the case of a recurrence.

The cannulae are connected to an extracorporeal circuit, equipped with an oxygenator, a pump and a heat exchanger. As soon as the tumor and muscle temperature reaches the preestablished level of approximately 41.5°C, the antineoplastic drugs are directly injected into the arterial circuit line. Melphalan, at a dosage of 10 mg/liter of limb volume, is employed for melanoma, while a combination of Melphalan and Actinomycin D (0.8 mg/Kg and 0.015 mg/Kg) is used for soft tissue and osteogenic sarcomas. The antineoplastic drug Cisplatin has been recently adopted for these tumors at a dosage of 3.2 mg/Kg of body weight which, in a dose-escalation study, was shown to be the maximum tolerable dose for a temperature of 41.5°C (2).

### Clinical Material

To December 1988, a total of 416 patients affected with tumors of the extremities has been treated with H.A.P. The staging system of the M.D. Anderson Hospital and Tumor Institute was adopted for melanoma. Table I presents the stratification of the melanoma patients according to stage.

TABLE I

STRATIFICATION OF MELANOMA PATIENTS ACCORDING TO DISEASE STAGE

| Stage | No. of Patients |
|---|---|
| I | 80 |
| II | 8 |
| III A | 47 |
| III B | 28 |
| III AB | 28 |
| IV N | 28 |
| IV V | 29 |
| Total | 248 |

IV N = Extraregional node involvement
IV V = Visceral metastases excluded from the evaluation

Stage I melanoma patients are considered eligible for H.A.P. only if the tumor thickness is $\geq$ 1.5 mm. Prophylactic lymphadenectomy is never performed and regional nodes are removed only if histologically involved (Stages IIIB-IIIAB). Extraregional node involvement was present in 28 patients, during the isolation of the iliac vessels. These patients were classified at Stage IV N and were included in the evaluation. Twenty nine patients with visceral metastases, classified at Stage IV V and treated with H.A.P. only for palliation, were therefore excluded from the evaluation. After the H.A.P., metastatic nodules which had not completely disappeared were surgically removed at the time of their maximum reduction generally 20 days after the perfusion.

Two different protocols have been adopted for the treatment of osteogenic sarcomas. In the first series of patients, amputation was routinely performed four weeks after the H.A.P. because the real effectiveness of H.A.P. was not completely known. At that time, demolitive surgery was considered the treatment of choice.

After the satisfactory results achieved in terms of both tumor necrosis and patient survival, the treatment protocol was modified. If the tumor is resectable, the patient is treated with H.A.P., an "en bloc" resection and bone reconstruction with metallic endoprosthesis or bone autografts. In the case of unresectable tumors, H.A.P. is always carried out. If the tumor becomes resectable, conservative surgery is performed; otherwise the patient is amputated.

Eligibility criteria for an "en bloc" resection includes the following:

a) more than 10 years of age, when statural growth has, for the most part, been completed;
b) the possibility of performing a wide excision, including the tissue surrounding the tumor;
c) no metastases present

To December 1988, a total of 79 osteogenic sarcoma patients have been treated. Thirty three were submitted to amputation and 41 were treated with conservative surgery. Of the 38 patients to be treated with amputation, only 30 may be evaluated for 5 refused amputation after the perfusion and were not included in the protocol. Three of the 33 patients were lost to follow-up at 2, 3 and 4 years respectively and could therefore not be evaluated. Regarding the group of patients treated with H.A.P. and conservative surgery, 2 out of 41 may not be evaluated because of inaccurate preoperative staging. Only 39 were therefore included in the protocol.

The American Joint Committee staging system was adopted for soft tissue sarcomas. The stratification of the patients according to the stage of the disease and the treatment protocol is presented in Table II.

Seven out of 89 patients presented visceral metastases, other than local relapse. They were therefore treated with only palliative intents and were not included in the evaluation. Two patients died during the postoperative period. The remaining 80 received further therapy according to different protocols, as follows:

a) H.A.P. + surgery delayed amputation or excision, depending on the size, site and shrinkage of the tumor mass;
b) H.A.P. + continuous infusion of intra-arterial Adriamycin (ADR) or radiotherapy to improve regional disease control and increase the possibility of conservative surgery, while evaluating the impact on long-term cure.

It should be emphasized that 51 of the 80 patients (63.7%) were classified at Stage III or IV A.

A continuous infusion of ADR was generally initiated within 10 days after the H.A.P. Three cycles, 10 mg/day for 10 days each, were administered at 10-day intervals, followed by a

TABLE II

DISTRIBUTION OF PATIENTS TREATED WITH H.A.P. ACCORDING TO STAGE AND TREATMENT PROTOCOL

| Treatment | Stage | | | | |
|---|---|---|---|---|---|
| | I | II | III | IVA | Total |
| Excision | 2 | 9 | 4 | 7 | 22 |
| Amputation | 3 | 5 | 3 | 14 | 25 |
| ADR Intra-arterial Infusion + Excision | 2 | 3 | 7 | 7 | 19 |
| Radiotherapy + Excision | - | 5 | 4 | 5 | 14 |
| Total | 7 | 22 | 18 | 33 | 80 |

Stage III-IVA: 51/80
ADR: Adriamycin

delayed wide excision. Radiotherapy was carried out by external beam source within 4 weeks after the H.A.P. All the patients received a dosage varying from 45 to 60 Gy, depending on the local tolerance. A delayed excision was performed 3 to 4 weeks later.

## COMPLICATIONS

Major complications, namely a high incidence of postoperative deaths, non-functional limbs and arterial ruptures or thrombosis requiring amputation occurred during the first pilot experience employing H.P. alone (3).

In the patients treated with H.A.P., the heat exchanger temperature was lowered because of the synergism existing between hyperthermia and antineoplastic drugs. In this manner, complications were kept at a minimum.

The postoperative death rate was only 3%. As regards morbidity, most of the complications were vascular. Two arterial ruptures requiring dacron prostheses occurred in patients treated for melanoma. In one patient with osteogenic sarcoma, an arterial embolism occurred at the tibio-peroneal trunk which was unsuccessfully treated with embolectomy, later requiring amputation of the thigh.

Of the patients treated for sarcoma, one was amputated because of the development of postoperative gangrene of the limb. Another patient treated with H.A.P. + ADR infusion + excision developed arteritis with progressive arterial insufficiency of the limb requiring amputation 14 months after the perfusion. A patient treated with H.A.P. + radiotherapy + excision presented postactinic necrosis of the femur with pathologic fracture, resistant to any form of orthopedic management. This patient as well was submitted to amputation 30 months after the perfusion.

Chronic insufficiency and moderate but irreversible neuromyopathy occurred in 2 patients treated with high-dose Cisplatin (5.0 mg/Kg of body weight) during the H.A.P. Since both the systemic and local toxicity appeared to be strictly Cisplatin dose-related, a safe therapeutic dose of 3.2 mg/Kg of body weight was established and has been employed since then (2).

Other minor local complications of the perfused limb occurred which were mainly in the range of Wieberdink's grade II to III classification (4).

## RESULTS

### Melanomas

The incidence of loco-regional relapse in the Stage I melanoma patients was 21.2%. Two patients (2.5%) developed in transit metastases, regional node relapses developed in 14 patients (17.5%) and 1 patient (1.2%) had both skin and node metastases. Eight patients presented distant metastases with no loco-regional relapse.

The 5 and 10-year disease-free survival rates observed were 62.3% and 56.9% respectively, while the 5 and 10-year overall survival rates were 80.8% and 77.2% respectively. These figures may be considered quite satisfactory considering the high risk population treated: 43 (53.9%) out of 80 patients had a tumor thickness $\geq$ 3 mm and the level was $\geq$ IV in 66 (82.5%) of these patients.

For recurrent limb melanoma, H.A.P. is today considered the treatment of choice. Table III compares the 5-year overall survival rates obtained with this technique to those obtained with surgery:

TABLE III

FIVE-YEAR OVERALL SURVIVAL RATES OF STAGE II, III A, III B AND III AB MELANOMA PATIENTS TREATED WITH H.A.P. OR SURGERY

| Author | II | III A | III B | III AB |
|---|---|---|---|---|
| H.A.P. | | | | |
| Cavaliere | 87.5 | 56.2 | 39.5 | 27.1 |
| Koops | 86 | 67 | 46 | 40 |
| Stehlin | 77 | 81 | 54 | 45 |
| Krementz | 64 | 38 | 59 | 34 |
| Total | 78.6 | 60.5 | 47.6 | 36.5 |
| Surgery | - | - | - | - |
| W.H.O. Karakoussis | 36 | 27-30 | 24-40 | 0-16 |

It is readily apparent that at every disease stage, H.A.P. has provided better results than surgery. Although the present report is a retrospective evaluation, the differences between the two treatments in terms of survival rates are so great that no doubts should exist regarding the superiority of H.A.P. as opposed to surgery.

Osteogenic Sarcomas

The results obtained with H.A.P. for treating osteogenic limb sarcoma are presented in the following Table:

TABLE IV

RESULTS OBTAINED IN OSTEOGENIC LIMB SARCOMA PATIENTS TREATED WITH H.A.P.

| Treatment protocol | % of necrosis | 5-year L.R. control (%) | 5-year overall survival (%) |
|---|---|---|---|
| H.A.P.* + Amputation | 80-100 | 100 | 63.3 |
| H.A.P.** + Resection | 80-100 | 97.5 | 64.7 |

* median follow-up: 90 months
** median follow-up: 50 months
L.R. : loco-regional

In the first group of patients treated with H.A.P. and amputation, histology showed a high degree of necrosis of the neoplastic tissue (80-100%). Regional recurrences were never observed and the 5-year overall survival rate was 63.3%.

In the second group of 39 patients treated with H.A.P. and conservative surgery, the percentage of limb necrosis was in the same range as in the patients treated with demolitive surgery. A 5-year actuarial loco-regional control rate of 97.5% was obtained and a local recurrence, clearly due to a technical error in the surgical resection, was observed in only 1 patient. The principles of the "en bloc" resection had not been respected as the section margin was too close to the tumor. The 5-year overall survival rate obtained was 64.7% which almost overlaps the survival rate of patients treated with demolitive surgery.

These results seem to indicate that amputation is not mandatory for osteogenic sarcoma patients.

Soft Tissue Sarcomas

The results obtained with the different treatment protocols adopted were evaluated with regard to three end points: (i) the percentage of conservative surgery; (ii) loco-regional control, and (iii) survival.

In the group of patients treated with H.A.P. and surgery, a delayed excision after the tumor mass shrinkage was possible in only 27 out of 47 patients (57.4%). In the remaining 20 patients (42.6%) who were mostly Stage IV A patients, amputation was required. The therapeutic effectiveness did not appear to be satisfactory as far as the long-term results are concerned, because of the high incidence of local recurrences (24.9%) and distant metastases. The overall 5-year survival rate was 46.8% (Table V).

TABLE V

FIVE-YEAR LOCO-REGIONAL CONTROL AND SURVIVAL RATES IN SOFT TISSUE SARCOMA PATIENTS

| Protocol | 5-year loco-regional control | 5-year survival | |
|---|---|---|---|
| | | D.F. | O.V. |
| H.A.P. | 75.1 | 32.1 | 46.8 |
| H.A.P. + ADR Infusion | 84.2 | 49.6 | 74.5 |
| H.A.P. + Radiotherapy | 100 | 70.1 | 70.1 |

ADR : Adriamycin
D.F.: Disease-free
O.V.: Overall

To improve loco-regional disease control and increase the rate of limb salvage treatments, the protocol was modified: 19 patients received an ADR intrarterial infusion after the H.A.P. Both the local control and survival rates improved. Conservative surgery could be performed in 17 out of 18 patients (94.4%). One patient was excluded because of amputation for progressive arteritis 14 months after the H.A.P. Local recurrences occurred in only 14.8% of the cases and the 5-year disease-free and overall survival rates were 49.6% and 74.5% respectively.

In the last group of patients treated with H.A.P. + radiotherapy, conservative surgery could be performed in all the patients. An irreversible limb impairment due to postactinic femur necrosis was present in 1 patient, requiring amputation 30 months after the perfusion. No local recurrences have been observed thus far and the 5-year disease-free and overall survival rate is 70.1%.

## DISCUSSION

Substantial *in vitro*, *in vivo* and clinical evidence indicate that hyperthermia has a significant anticancer activity, particularly when combined with chemotherapy. This combination has been employed for treating aggressive melanomas, soft tissue sarcomas and osteogenic sarcomas of the extremities which, subclinically, often spread loco-regionally before systemic dissemination.

In theory, the technique of hyperthermic antiblastic perfusion seems to be tailored for the treatment of these extremity tumors for several reasons: 1) the perfusional treatment involves the entire tumor-bearing limb, with possible control of metastatic foci; 2) the technique permits achieving homogeneous elevated temperatures that can have a tumoricidal effect; 3) high concentrations of antineoplastic drugs (6-10 times greater than those given systemically) can be injected into the perfusional circuit with a higher tumor drug uptake; 4) an elevated temperature and drugs potentiate each other's activity; and 5) the tumor mass "shrinkage" after the H.A.P. permits carrying out conservative rather than demolitive surgery.

The role of H.A.P. for the treatment of high risk Stage I limb melanoma patients has still not been completely defined. Nevertheless, retrospective analyses and more recently, the prospective randomized study of Ghussen have demonstrated the superiority of H.A.P. vs. surgery (5). For recurrent limb melanoma, H.A.P. is considered the treatment of choice.

However, despite almost twenty years of experience and wide clinical implementation, the results obtained with H.A.P. are not homogeneous and clarifications must still be made regarding the prognostic factors able to influence the disease outcome.

With the aim of identifying tumor and/or treatment-related factors, a multiparametric analysis on 139 recurrent limb melanoma patients treated with H.A.P. was carried out to verify the influence of the prognostic factors on the following end-

points: tumor response, loco-regional control, disease-free and overall survival rates. The tumor response (according to W.H.O. criteria) was evaluated in 82 out of 139 patients: a complete response (C.R.) was obtained in 39% of the patients while a partial response (P.R.)($\geq$ 50%) was achieved in 39.7% of the patients. Clinical factors with a statistically significant correlation to the complete response were: the number of lesions ($p = 0.000003$), the node status ($p = 0.014$) and the tumor localization ($p = 0.03$).

With regard to the treatment factors, the highest number of C.R.s was observed in patients whose tumor temperature was 41.5°C and maintained for the entire duration of the treatment ($p = 0.02$). When the drug dose, temperature and the number of lesions were simultaneously evaluated for their influence on tumor response, only the last two categories statistically influenced the number of C.R.s. Nevertheless, a crosstabulation of drug dose ($\leq$ or $>$ standard dose) and minimum tumor temperature (min. T) ($<$ or $>$ 41.5°C) in relation to the C.R., showed that the exploitation of the tumor response may be achieved only when the two agents are properly employed. In fact, the percentage of success of the treatment performed with a min. T $<$ 41.5°C and a standard dose was 15.4%, rising to 20% in patients treated with a higher than standard dose and to 35.3% in those treated with a min. T $>$ 41.5°C. The C.R. rate was 59.4% employing the simultaneous association of a min. T $>$ 41.5°C and a $>$ standard drug dose, showing a significant improvement which was higher than that expected in the case of an additive relation and indicates the presence of a synergistic effect.

As far as loco-regional control is concerned, the multivariate analysis on the basic series of 139 patients shows that only the number of lesions has a significant predictive value ($p = 0.0003$). The 5-year loco-regional control rate was 86.2% in patients with a single lesion, as opposed to 52.3% in patients with multiple lesions. When the analysis was carried out on the subgroup evaluated for tumor response (82 patients), both the number of lesions ($p = 0.09$) and the tumor response ($p = 0.008$) were selected as significant predictive categories. When all the categories were evaluated on the basic series of 139 patients in relation to disease-free survival, only the number of lesions and the last disease-free interval were selected as statistically significant factors. In the subgroup of 82 patients, only the tumor response showed a statistically significant predictive value for disease-free survival. Regarding overall survival, the multivariate analysis showed that only the node status, the number of lesions and the number of previous relapses independently influence survival. In the subgroup of 82 patients, age, tumor response, sex and the number of previous relapses were selected as independent categories.

The analysis, carried out with the aim of identifying the treatment- and/or tumor-related factors able to influence the disease outcome, permits making the following conclusions:

1) in patients with the same tumor burden, a min. T $>$ 41.5°C gave a higher rate of C.R.;
2) the association of a min. T $>$ 41.5°C and standard drug doses results in an exploitation of the treatment efficacy (a 59.4% rate of C.R.);
3) the tumor response has shown to statistically influence the

loco-regional control (75.3% at 5 years for C.R. vs 41.5% for P.R. + S.D., p = 0.009), the disease-free survival (51.4% for C.R. vs 15% for P.R. + S.D.) and, together with other clinical factors, overall survival;

4) since the clinical parameters cannot be influenced, the appropriate treatment, in terms of both drug dose and level of hyperthermia, is mandatory for its impact on tumor response.

The results obtained with H.A.P. for the treatment of osteogenic sarcomas may be considered quite satisfactory. In all the cases, the histological findings showed a marked necrosis of the neoplastic tissue, whereas the normal tissue surrounding the tumor showed only minor damage. This regressed almost completely 1 month after the perfusion, as demonstrated in the amputated limb. Moreover, surgical removal is facilitated by the sclerosis of the tumor mass and its reduction after the H.A.P. and the perfusion directly includes the eventual "skip" metastasis as well, with a preoperative incidence ranging around 20-25% according to Enneking and Kagan (6).

These considerations prompted us to undertake conservative treatment after the perfusion. Treatment with H.A.P. has provided excellent results in terms of limb salvage. Nine patients treated with conservative surgery responded so well to H.A.P. that the tumor became resectable, enabling us to spare the limb. In addition, the technique permitted obtaining complete loco-regional control (excluding the only local recurrence caused by a technical error in the "en bloc" resection). The 5-year overall survival rate overlaps that obtained with demolitive surgery (64.7% vs 63.3% respectively), demonstrating that amputation is not mandatory in the treatment of osteogenic limb sarcoma (7).

In our experience, H.A.P. did not provide satisfactory loco-regional control for soft tissue sarcomas. In contrast to what occurred in osteogenic sarcomas, this could be explained by the characteristics of the patients included in our trial: many had been previously treated with surgery, radiotherapy and/or chemotherapy. The former two treatments can reduce the blood circulation and drug uptake, due to sclerosis while tumors previously treated with chemotherapy can become less responsive to further treatments. Moreover, 51 (63.7%) of the 80 patients in this series were at Stages III-IV A, with a high risk of relapse. The association of the ADR infusion or radiotherapy with H.A.P. before tumor removal gave a better loco-regional control and permitted carrying out a higher rate of conservative surgery without impairing limb functionality (8).

It could be hypothesized that the potential efficacy in tumor control may be further improved by employing more specific drugs for soft tisuue sarcomas, other than LPAM and DACT. These were scarsely effective when used as single agents for treating soft tissue sarcomas. A significant therapeutic gain could be derived from the use of ADR, which, other than possessing a synergism with heat, has a well documented tumor specific activity.

As far as survival is concerned, trials employing H.A.P. have obtained quite satisfactory results with rates ranging

between 65 and 75% at 5 years (9,10). In evaluating these results, two principle considerations should be recalled:

1) in most of the reported series, half of the patients presented recurrences and it is known that local relapse is followed by pulmonary metastasis in 30 to 60% of these patients; and
2) none of the patients treated with H.A.P. received systemic chemotherapy which, in some randomized clinical trials, has shown to increase both the disease-free and the overall survival rates.

In conclusion, hyperthermic perfusion as a first step of a multimodality approach, plays an important role in the treatment of tumors of the extremities.

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REFERENCES

1. R. Cavaliere, B. Mondovì, G. Moricca, and F. Di Filippo, 1983, Regional perfusion hyperthermia in: "Hyperthermia in Cancer Therapy," F.K. Storm, ed., G.H. Hall, Boston, pp. 369-399.
2. F. Di Filippo, A.M. Calabrò, and C. Citro, Isolated hyperthermic perfusion with Cis-diammine-dichloroplatinum (II) in the treatment of limb tumors. Phase I study: Toxicity and pharmacokinetics, Exp Clin Cancer Res 6: 257 (1987).
3. R. Cavaliere, E.C. Giocatto, and B. Giovanella, Selective heat sensitivity of cancer cells: Biochemical and clinical studies, Cancer 20: 1357 (1967).
4. J. Wieberdink, C. Benckhuysen, and R.P. Braat, Dosimetry in isolation perfusion of limb by assessment of perfused tissue volume and grading of toxic tissue resections, Eur J Cancer Clin Oncol 18: 905 (1982).
5. F. Ghussen, A prospective randomized study of regional extremity perfusion in patients with malignant melanoma, Ann Surg 200: 764 (1984).
6. F. Enneking, and A. Kagan, "Skip" metastases in osteosarcoma, Cancer 36: 2192 (1975).
7. R. Cavaliere, F. Di Filippo, F.S. Santori, The role of hyperthermic perfusion in the treatment of limb osteogenic sarcoma, Oncology 44: 1 (1987).
8. F. Di Filippo, A.M. Calabrò, and A. Cavallari, The role of hyperthermic perfusion as first step in the treatment of soft tissue sarcoma of the extremities, W J Surg 12: 332 (1988).
9. P.M. Lethi, Improved survival for soft tissue sarcoma of the extremities by regional hyperthermic perfusion, local excision and radiation therapy, Surg Gynecol Obstet 162: 149 (1986).
10. J.S. Stehlin, B.C. Giovanella, and A.E. Gutierrez, Fifteen years' experience with hyperthermic perfusion for treatment of soft tissue sarcoma and malignant melanoma of the

extremities, Front Radiat Ther Oncol 18: 177 (1984).
11. C.P. Karakoussis, D.F. Temple, and R. Moore, Prognostic parameters in recurrent malignant melanoma, Cancer 52: 575 (1982).
12. E.T. Krementz, Regional perfusion: Current sophistication, What next? Cancer 57: 416 (1986).
13. H.S. Koops, H. Martijn, and J. Oldhoff, Regional perfusion in the treatment of patients with locally metastasized malignant melanoma of the limbs, Eur J Cancer 17: 477 (1981).

# DEVELOPMENT OF AN ELECTRICAL IMPEDANCE TOMOGRAPHY SYSTEM FOR NONINVASIVE TEMPERATURE MONITORING OF HYPERTHERMIA TREATMENTS

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## INTRODUCTION

It has been shown that it is possible to obtain information about the distribution of impedance within an object by measuring the voltage or the current on electrodes spaced on the object coming from a current or a voltage source. (Brown and Barber 1982, Barber and Brown 1984)

The electrical impedance of tissue depends on its temperature. We have previously suggested its use for non-invasive temperature monitoring of hyperthermia treatments.(Persson et al. 1988) The aim of the present paper is to demonstrate the Lund electrical impedance tomography system and its potential use for non-invasive monitoring of temperature changes during hyperthermia treatment of cancer.

## ELECTRICAL IMPEDANCE TOMOGRAPY SYSTEM

### Technical description

We have built a system utilizing 16 electrodes, which are used for alternatively driving current and measure voltages. The buffers, which are attached to the object, measure the voltages and are connected to 16 instrument differential amplifiers. The PC-computer controls the equipment, collect data and make the reconstruction and presentation. (Blad et al. 1988)

A block diagram of the system is shown in Figure 1. The voltage differences from the buffers are amplified and detected in the detector unit. 16 DC-levels are converted to digital values in the A/D converter. The current generator supply the object with a known current.

### Image reconstruction algorithms

The present algorithm relies an enhanced back projection technique, using 15 curved fields and 16 projections. Figure 2 shows a picture of the equipment and the lay out of a screen showing different possibilities of the software. The picture of measurement shows different resistivity points.

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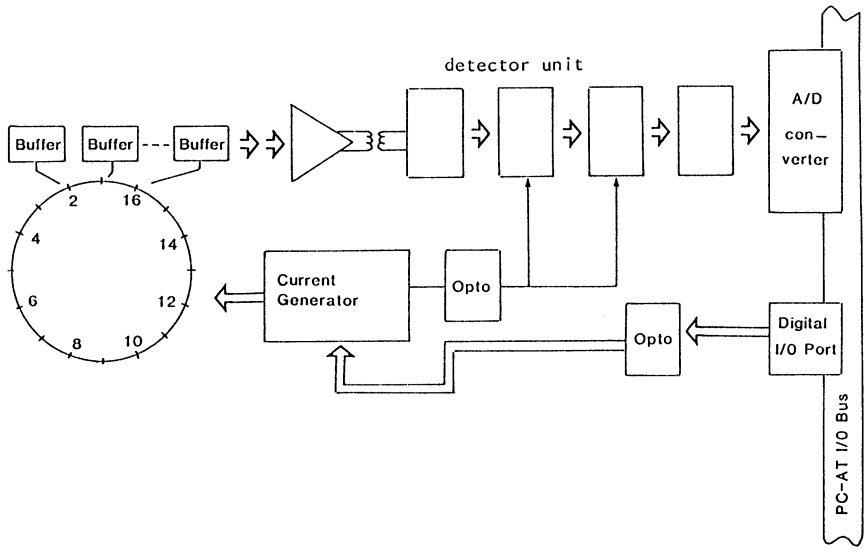


Fig. 1. A blockdiagram of the system. The voltage differences from the buffers are amplified and detected in the detector unit. 16 DC-levels are converted to digital values in the A/D converter. The current generator supply the object with a known current.

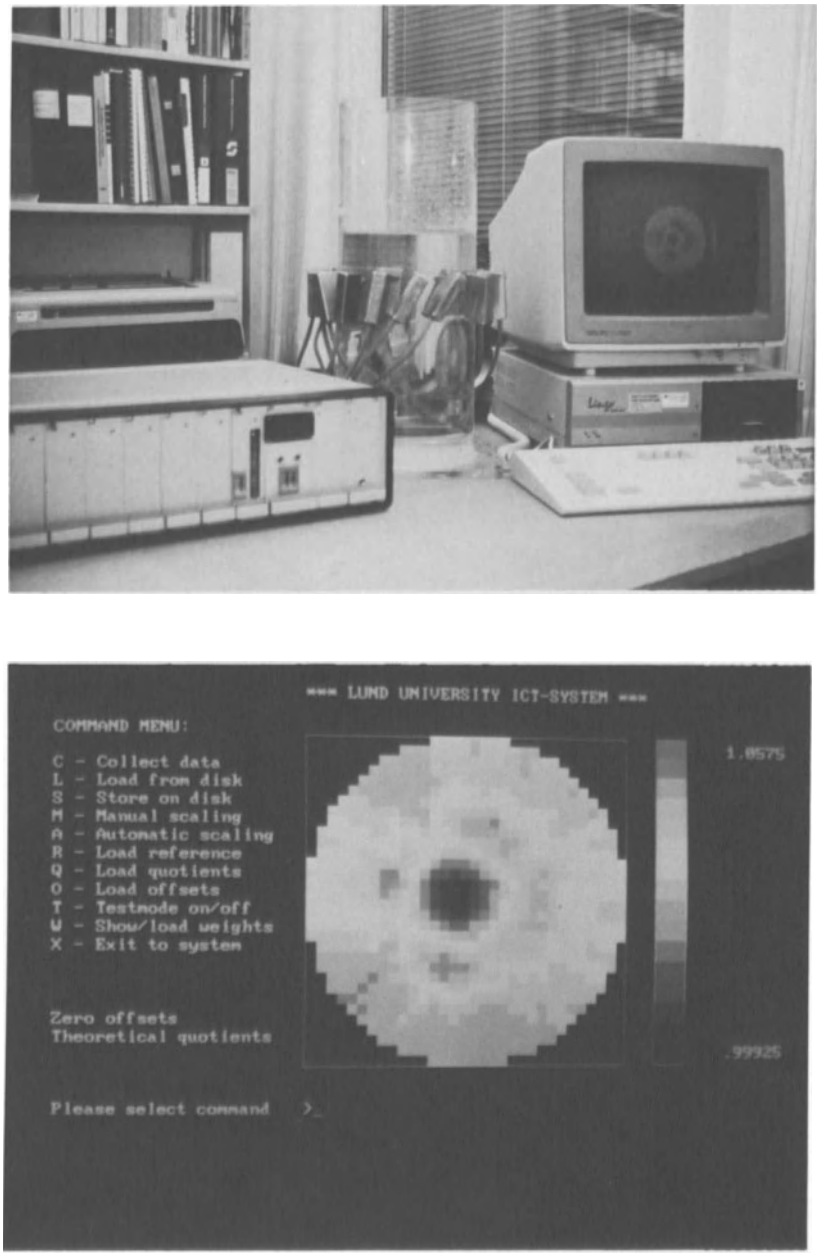


Fig. 2. Picture of the equipment and the lay out of a screen snowing different possibilities of the software. The picture of measurement shows different resistivity points.

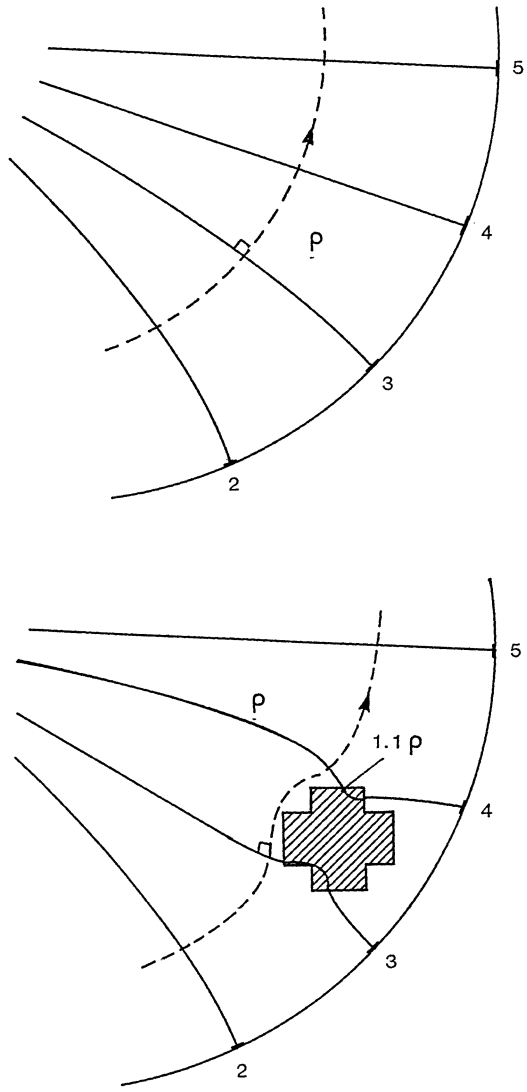


Fig. 3. Distortion of the equipotential lines by an area of 10% increase in resistivity.

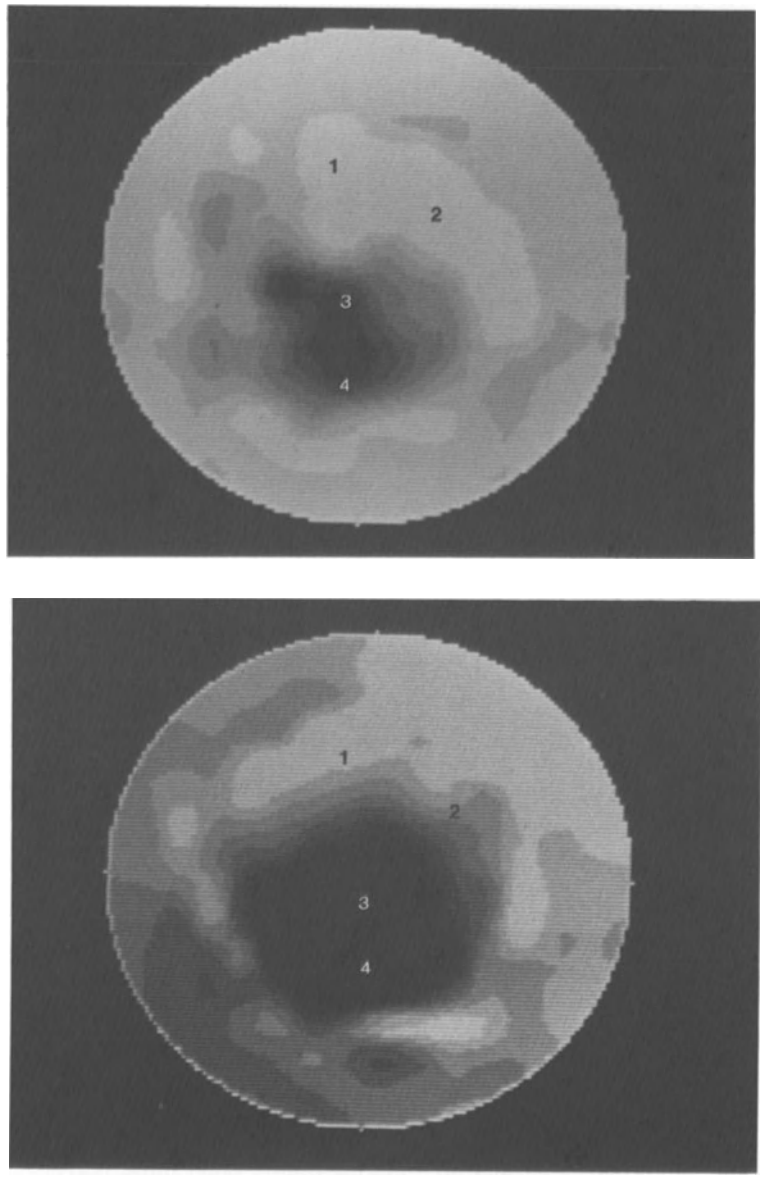


Fig. 4 The temperature change as a function of time for various region of interest in a phantom.

The back projection method which is used for image reconstruction makes it necessary to make rather coarse assumption about the equipotentials. Thus the equipotentials in the objects are supposed to be the same as for homogeneous object. To overcome this problem we also calculate the equipotentials. This is done by using the Finite Element method. Figure 3 shows how an area of 10% increase in resistivity distorts the equipotential lines.

Our aim is to implement an iterative method proposed by Yorkey (1986). The basic idea is to adapt an assumed resistivity distribution to the real one. This can be done by assuming a distribution calculating the potential differences on the boundary and comparing them with the measured voltages. This process is repeated until the voltages match. The voltages are calculated using the Finite Elements Method mentioned earlier. The resistivity distribution is determined by a modified Newton-Raphson recursive formula. The drawbacks of the method are that the calculation is very time consuming. Presently FEM-calculation takes hours to do, using PC-computers. One way of reducing the time would be to make the first assumption of the resistivity distribution using the fast back projection, then make the refinements using the iterative method based on FEM. (Blad et al. 1988)

## TEMPERATURE MONITORING

### Temperature sensitivity and resolution

The sensitivity and resolution properties of the EIT system have been tested by using a matrix of resistive electrically heated points embedded in a tissue equivalent phantom (ground beef). Temperature dependence of resistivity is about 2% per °C. It is possible to achieve an image of resistivity change caused by about 0.2 °C temperature sensitivity. The result of a single point source indicates that with the sensitivity of the present equipment it is possible to resolve 0.5 °C. With two point sources it was demonstrated that spots with one °C temperature difference could be separated at 4 cm distance.

### Dynamic temperature monitoring in a phantom

Four thin polythene tubes are placed in a tissue-equivalent phantom (ground beef). Through tubes 1 and 2 are flowing cold water with a temperature of 7 degrees and through tubes 3 and 4 are flowing hot water of 42 degrees. The temperature of the surrounding phantom-material is 22 degrees.

Measurements were performed every minute after start and the reconstructed images show the temperature changes from the starting conditions. In the first pictures all tubes are open, then the cold water was closed and only warm water was on, and at the end the cold water was closed and the warm water was opened again. The temperature change as a function of time for various region of interest can also be presented. The results from measurements at two different times are presented in Figure 4.

### Thermal self-regulating seeds

We used the same phantom for inductive heated thermal self-regulating ferromagnetic seeds. To overcome the problem of heating the wires and the electrodes placed on the object we have to use very thin isolated copper wires (Litz wire). No heating in electrod arrangement was observed. But a resistivity change in the phantom at the area with 5 self-regulating ferromagnetic seed was shown in the image.

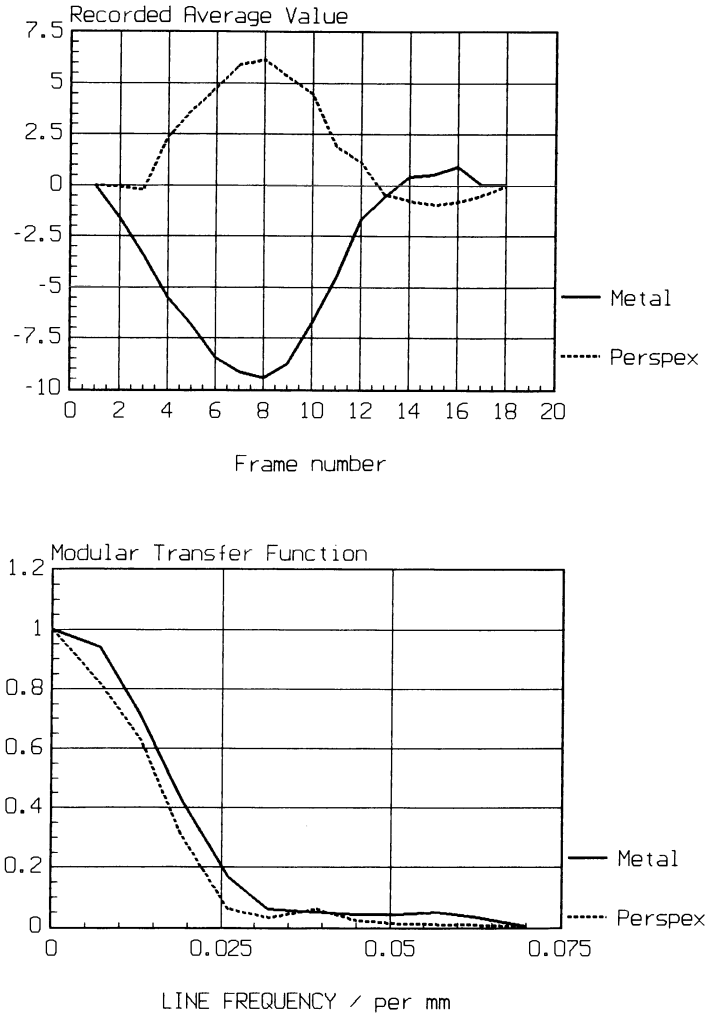


Fig. 5. Modulation transfer function offer possibility to get an estimation about the image quality of the system. A plate of high resistivity and another with low resistivity is put into a water phantom. The measured line spread functions and MTF(below) is presented in the diagram.

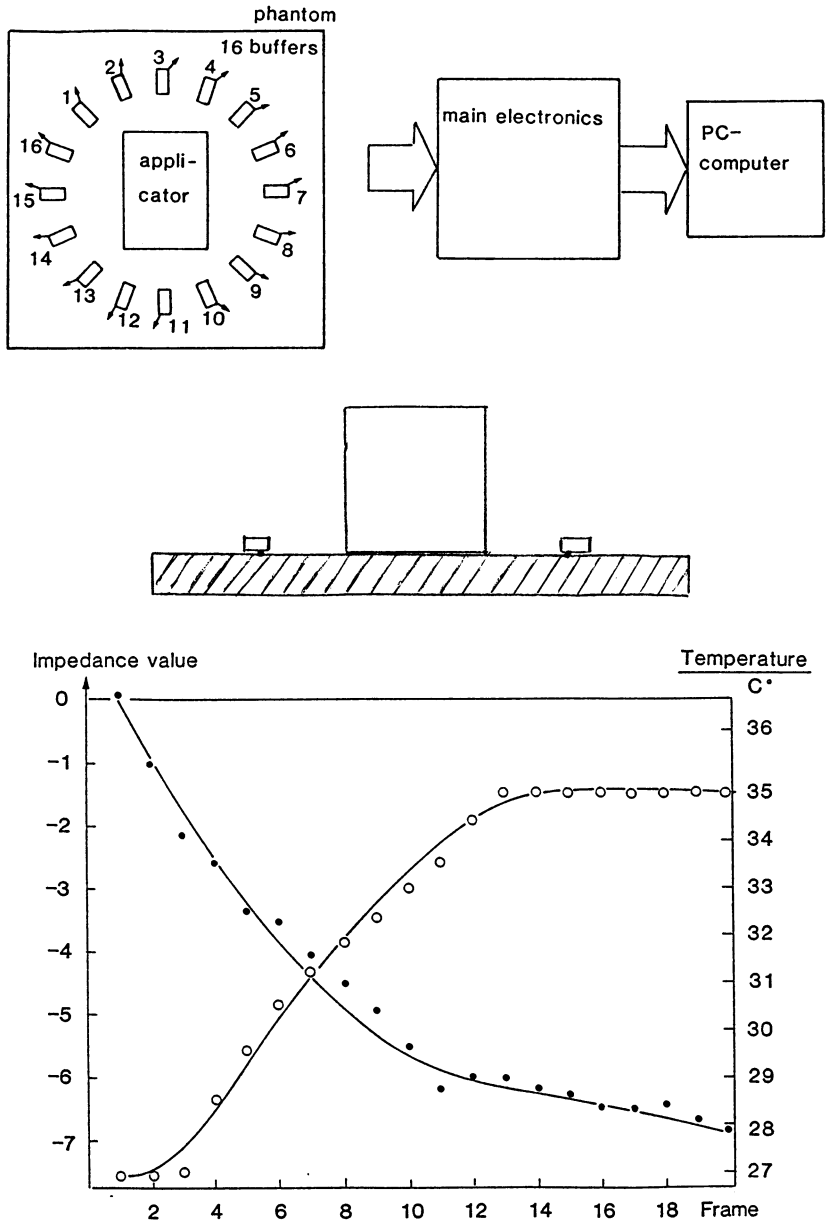


Fig. 6. An applicator were placed upon the phantom and the temperature in the centre was measured. The experimental arrangement and diagrams of measurements are shown in this figure.

Modulation transfer function

Modulation transfer function offer possibility to get an estimation about the image quality of the system. A plate of high resistivity and another with low resistivity is placed into a water phantom and imaged with ECT. The result of measured line spread functions and calculated MTF is presented in Figure 5.

Hyperthermia treatment

Next experiments describe temperature measurements together with hyperthermia treatment. No electrical interference between the systems was observed. The aim of the first part of the experiment was to heat up a tissue equivalent phantom consisting of so called super stuff. An applicator were placed upon the phantom and the temperature in the centre was measured. The experimental arrangement and diagrams of measurements are shown in Figure 6.

## CONCLUSIONS

We have demonstrated that the Electrical Impedance Tomography method is useful for non-invasive temperature monitoring and that it is sensitive enough to use in hyperthermia treatments. The sensitivity and resolution properties have been tested by using a matrix of point heat sources embedded in a tissue equivalent phantom (ground beef). The result of a single point source indicates that with the sensitivity of the present equipment it is possible to resolve ± 0.5 °C. With two point sources it was demonstrated that spots with one degree temperature difference could be separated at 4 cm distance. The result of a single point source indicates that with the sensitivity of the present equipment it is possible to resolve ± 0.5 °C. With two point sources it was demonstrated that spots with one degree temperature difference could be separated at 4 cm distance.

We have also studied the influence of flow in a phantom of ion-permeable collodium tubing in a phantom. The results of these measurements indicate that the EIT method is insensitive to flow in a phantom and thus probably to blood flow.

The method is very promising in non invasive monitoring of clinical hyperthermia treatments and for thermal mapping.

## REFERENCES


Barber, D.C., and Brown B.H., 1984, Applied Potential Tomography, J. Phys. E.: Sci. Instrum., 17, 723.

Brown, B.H., and Barber, D.C., 1982, "Proc. Hospital Physicists' Association Annual Conf.", Sheffield, Glasgow, Blackie.

Blad, B., Bertenstam, L., Göransson, G., Lindblad, U. and Persson B.R.R., 1988 Development of an electrical impedance tomography system. "Presented at the World Congress on Medical Physics and Biomedical Engineering", Aug. 6-11, 1988 San Antonio TX, USA.

Persson, B.R.R, Blad, B., Bertenstam, L. (1988) The use of electrical impedance tomography (EIT) for non-invasive temperature monitoring of hyperthermia treatments. "Poster presented at 5th International Society of Hyperthermic Oncology", August 28- Sept. 2, 1988, Kyoto, Japan.

Yorkey, T.J., Webster, J.G., and Tomkins, W.J., (1986) An optimal impedance tomographic reconstruction algorithm, "IEEE/ Eigth Annual Conference of the Engineering in Medicine and Biology Society", pp 339.

# THE ROLE OF PARAMEDICAL PERSONNEL IN THE FIELD OF CLINICAL HYPERTHERMIA; PAST, PRESENT AND FUTURE

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The use of radiofrequency or microwave induced hyperthermia for the treatment of cancer is still a very new, challenging field. Although much has been learned in the past few years about the use of this modality through clinical trial and error, formal training for physicians and paramedical personnel is very limited. This paper looks at the role of paramedical personnel in the clinic and offers suggestions of formal training regimens to prepare personnel to render safe, effective treatment under the direction of the attending physician.

The emergence of clinical hyperthermia facilities began in the United States in the late 1970's. At that time customized, institutionally constructed equipment was primarily used as no FDA approved, commercially manufactured equipment was available. Clinic personnel were usually drafted from existing hospital staff. These people came from a variety of backgrounds, but primarily consisted of either nursing or radio-therapy. No formal training was available; previous training in related medical fields was utilized, and under the direction of the attending physician, experience was gained through on the job training. By the early 1980's, the number of institutions using local hyperthermia continued to grow. Several commercial companies received FDA Investigational Device Exemptions and began to manufacture and market superficial hyperthermia equipment. Initially, training for operation of this equipment was very limited. As the need developed, manufacturers began offering week-long training programs, and user conferences to upgrade training to the most current levels. Numerous institutions, after gaining clinical experience, began offering workshops and training seminars; certificates of graduation were awarded upon completion of the course. National and international organizations were formed to provide hyperthermia personnel with a forum for training and discussion in the development of the field of hyperthermia. The International Clinical Hyperthermia Society was formed in 1980; the North America Hyperthermia Group was formed in 1981, and the American Society for Clinical Hyperthermic Oncology in 1984. Committees in existing oncology societies were also formed to investigate hyperthermia and the training of paramedical personnel; the American Society for Therapeutic Radiation Oncology, The American Association of Physicist in Medicine and the American Society of Radiologic Technologists formed such committees.

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In exploring the role of paramedicals in hyperthermia past and present, not much has changed. Upon adopting hyperthermia as a therapeutic modality, institutions usually utilized existing staff to assist in the hyperthermia clinic. The majority of programs were under the auspices of the Radiation Oncology Department, however some were located in either surgical or medical oncology. R.N.'s, R.T.'s, physicists, etc. were enlisted to learn hyperthermia equipment, procedures, and therapy. These paramedicals took on the enormous task of learning foreign equipment with very little instruction, learning the asis of hyperthermia and the application of treatment, learning, with the physician and the physicist, the implantation of catheters for thermometry and interstitial antennae. One had to become famililar enough with the equipment to troubleshoot problems and many times makect repair via telephone instructions from the manufacturer. Pursuing an understanding of the physical and biological aspects of hyperthermia was paramount along with a working understanding of such foreign concepts as E. fields, S.A.R.'s and time versus temperature curves. In addition to the technical and medical aspects of hyperthermia, paramedical personnel many times were responsible for establishing the administration of the hyperthermia clinic; policy and procedures, treatment records, patient files, charging procedures, quality assurance, data collection, etc. Many employees in new hyperthermia clinics experienced early "burn out" and left the field. In 1984 a report was published by Dr. Kenneth Luk, et. al., supported by a contract from the National Center for Devices and Radiological Health, Food and Drug Administration, Public Health Services. In this report,four hospitals using hyperthermia were selected; nine people working in hyperthermia were interviewed. These people came from a variety of backgrounds; nursing: Radiation Therapy: Medical Physics: and Medical Laboratory. This report was published over 5 years ago, so I won't outline it's results in detail. But I would like to point out a few key responses that are still applicable today. Regarding orientation to hyperthermia equipment, 44 percent of the respondents rated their orientation as inadequate, over half of the respondents related a high stress level associated with their job, and most respondents felt they were underpaid. In comments regarding stress, prejudice towards hyperthermia was cited, along with the responsibility of making decisions regarding treatment. One example given was making the decision to stop treatment as a result of patient intolerance. Also working in a small setting absent of co-workers was cited as a stress factor. Within the past year, the American Society of Radiologic Technologists developed a task force to investigate training and certification in hyperthermia. A number of questionnaires were sent out to various institutions. All the results are not in; however, Ms. Pam Scott, chairperson for the task force, agreed to share some of the early responses. Approximately 95 percent lof respondents are R.T.T.'s. Approximately 50 percent felt they did not receive adequate training. People expressed the need for clinical training. A majority of respondents work less than 20 hours a week in hyperthermia. What is being reinforced here from earlier statements is: paramedicals in hyperthermia come from a variety of backgrounds, adequate training is limited, and formal training is needed.

In 1985, the International Clinical Hyperthermia Society formed a committee to investigate the training and certification of hyperthermia personnel; I was appointed chairman of that committee. Since that time, I have pursued input from organizations, manufacturers, and individuals. Repeatedly I have been unable to get people to respond to requests for input, recommendations or suggestions. Ms. Scott relates similiar difficulties with their questionnaire. I do not feel this reflects apathy as much as the situation where those in hyperthermia do not have time to contribute. Most clinics are small and a majority of the duties are the responsibility of the paramendical staff. I would like to take this

opportunity to ask physicians and hospital administrators to encourage and support their hyperthermia staff to take an active role in hyperthermia society meetings, workshops, and manufacturer user conferences. Not only are they exposed to the most current events in hyperthermia, but they are also encouraged to share their experiences with their peers and contribute to the group information that can help everyone perform optimum hyperthermia treatments.

Hyperthermia has proven itself as an effective modality in the treatment of cancer, whether used alone or in combination with other modalities. It is a form of treatment with a bright future full of growth and expansion. The time has come for us in hyperthermia to move forward. It is time to strive for therapeutic and scientific excellence, to establish and to pursue formal training programs that will certify both physician and paramedical staff, and to achieve acceptance in the medical community as a safe, effective, scientifically based modality of treatment.

When originally asked to present this paper, it was supposed to be regarding nursing procedures in hyperthermia. Throughout this presentation, I have referred to paramedicals rather than nurses, and have demonstrated in various examples of backgrounds why. I do not feel it should be a requirement for working in hyperthermia to be a registered nurse, or a certified radiotechnologist. Even though backgrounds in nursing and radiotherapy would be extremely advantagous, it should not be a limiting factor. Many unlicensed people with a great deal to offer can function very effectively in hyperthermia upon completing training courses that will be discussed later. In the final phase of my presentation, I would like to discuss the role of paramedicals in hyperthermia in the future.

First, I would like to address the ongoing question of what is the appropriate job title for paramedical personnel. Although many institutions have job titles representing previous degrees, I believe a seperate and formal job title should be adopted. The most frequently used are technician, technologist, and clinical specialist. I have always disliked the title technician; it is an inaccurate description of the job, and is a title that is quite overused. For example, nursing aides at our institution are called patient care technicians, file clerks are called medical records technicians, and cafeteria employees are called food service technicians. It is pretty much the same situation with technologist. According to Webster's Seventh Collegiate Dictionary, a technologist is "involved in technical processes that increase productivity of machines and eliminates manual operations or operations done by older machines." Neither of these titles accurately describe the job. I have long been an advocate of the title hyperthermia therapist. As in many medical disciplines, (i.e. physical therapy, occupational therapy, respiratory therapy, speech therapy) one is trained and certified in their respective fields and is titled therapist. Back to Webster's, "a therapist is one specializing in therapy, especially a person trained in methods of treatment and rehabilitation other than the use of drugs or surgery". The physician, who is the clinical specialist, writes the order for treatment; the therapist carries out that order. The therapist is involved in discussing with the physician the type of treatment indicated, the type of applicators to be used, positioning of the patient, etc. The physician relies on the therapist because of their expertise in delivering safe, effective, and comfortable treatment. In many institutions, there is always a physician present in the hyperthermia treatment area. In equally as many institutions, the physician is not always present. The therapist is responsible, on a daily basis, to make many decisions regarding treatment within the paramenters dictated by the physician's order. The therapist may suggest to the physician medications

that the patient may require, based upon their observations of the patient. The therapist usually does the initial paperwork and consent forms for treatment. The therapist usually does the daily set up and application for treatment. The therapist is usually responsible for programming treatment parameters and maintaining the hyperthermia equipment during treatment. The therapist is usually responsible for monitoring the patient during treatment, doing vital signs when indicated, constantly insuring the patient maintains correct position, constant monitoring of thermometry, and being constantly available to respond to immediate patient needs. It is usually the therapist who coaxes the patient to tolerate the longest possible treatment at the highest possible power levels. It is the therapist who takes down the treatment set up and helps the patient prepare for the next scheduled treatment. It is the therapist who works with nursing staff in coordinating medications and chemotherapy. Quite often, the therapist is called upon by the patient for advice in nutrition, activity, medication, and even social and personal conflicts.

Secondly, training and certification of hyperthermia therapists needs to be addressed. In setting up any type of training program, several things need to be considered. I do not feel at this point and time, that it is reasonable to require someone to attend a year long training program at a college or junior college. However, I feel a variety of courses can be required; the applicant pursuing them as they can. Previously degreed people would probably already have most of the required credits. Also, there is the practice of "grandfathering certificates" to those who have previous experience in hyperthermia. I feel this is reasonable as long as those people meet certain requirements. These requirements include a certifying letter from the attending physician that this person is capable and qualified to perform hyperthermia, and meeting the basic college credit requirements.

There have been many suggestions as to what should be required in the training program. I feel these are all valid suggestions. They include: medical terminology, biology and oncologic biology, anatomy and physiology, clinical oncology, clinical patient care, basic physics of heat, basic pharmacology including chemotherapy, theory of electromagnetics, basic computer science, and a course in hyperthermia including the basis of hyperthermia, thermometry, equipment, and various treatment types. I also feel internships should be required upon completion of the required courses. There are now a variety of institutions around the U.S and Europe with quality experience in hyperthermia. A number of these institutions should be designated for internships in hyperthermia. In addition, all hyperthermia therapists should be required to join at least one existing hyperthermia society, and to attend at least one meeting a year to keep current on hyperthermia related matters.

Another question remains as what governing body should certify hyperthermia therapists. As mentioned earlier, there are a number of groups with an interest in the role of the hyperthermia therapist. I have serious doubts if these groups will ever come to an agreement on what should be required in the training and functions of the therapist. For this reason, I suggest the International Clinical Hyperthermia Society take the initiative and accept this responsibility. Guidelines, whether it be the afore mentioned, or additional ones, should be set up. Upon satisfactory completion of these requirements, the society should issue the applicant a certificate indicating the applicant has completed training requirements and meets the societies' criteria for performing safe and effective hyperthermia therapy. I feel it is irrelevant whether other hyperthermia groups recognize this certification. It will

demonstrate to the scientific community and medical governing boards that the society and the hospitals using hyperthermia are striving to offer the highest level of safe, effective, quality therapy.

What will the role of the hyperthermia therapist be in the 1990's? As I mentioned earlier, this field has a wide potential for growth. The certified hyperthermia therapist will play a greater role in the planning of treatment and assisting the physician in setting up treatment guidelines. The therapist will continue in the field of administration of the hyperthermia clinic, taking full responsibility in daily scheduling of patients, maintenance of patient files, ordering and maintaining stock supplies, processing patient charges, taking part in preventative maintenance of equipment and documentation thereof, organizing clinic budgets, and working with other ancillary departments involved with hyperthermia; namely nursing sevice, pharmacy, housekeeping, diet service, hospital administration and quality assurance. In the clinic setting, the hyperthermia therapist will be responsible for administering I.M. and P.O. medications in accordance with individual hospital policy. They will be qualified to monitor chemotherapies administered during treatment. Under the direction of the physician, they will be responsible for ordering routine x-rays and blood testing. They will play an increasing role in assisting the physician in the clinic and removing him from routine duties; i.e. prepping catheter sites, urinary catheter insertion, dressing changes, inserting butterfly intravenous catheters, and injecting local anesthetics. In many cases I predict the physician will be able to train the certified hyperthermia therapist for thermometry catheter insertion intc superficial tumors.

# PROCEDURES FOR IMPROVING THERAPEUTIC GAIN

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A number of studies (Bhuyan et al., 1977; Freeman et al., 1980; Kim et al., 1980; Haveman, Hahn, 1981) have determined that the best conditions to realize a maximum antitumor hyperthermia (HT) effect are the following: low pH, hypoxia, slow blood flow, deficiency of nutrients and energy and increase in cell numbers in the S-phase of a cell cycle. It was shown that tumor tissue pH is selectively decreased under induced hyperglycemia (IHG) (see the reviews by Wike-Hooley et al., 1984; Zavrid et al., 1987). According to this phenomenon von Ardenne (1966, 1972) has suggested the use of IHG for enhancement of thermosensitivity.

Haveman and Hahn (1981) have studied the potential effect of "glucose deficiency" on cells damaged by HT. They paid special attention to the deficiency of energy under this condition. They believe that availability of energy plays an important role not only for greater cell damage under HT but also for better tumor thermosensitivity in comparison with the normal tissue.

The above mentioned data is contradictory for use of IHG for enhancement of HT efficacy. Some authors refer to the paper of Dickson, Calderwood (1979), who did not obtain considerable enhancement of thermosensitivity of Yoshida sarcoma after intraperitoneal glucose injection when tumor pH dropped by 7.2 to 6.6. Those who support or who oppose the use of the combination of IHG and HT have not investigated whether the combined treatment is advisable.

After taking into account the high selectivity of decreasing pH in tumor and it's possible role in enhancement of HT efficacy, we performed a series of experiments in attempt to elucidate this disputable problem. The most important data is given below.

In the beginning we optimized the schedule of IHG. Previous studies used long-term infusion (up to 24 hr.) of glucose solution. Within 60-90 min. following the glucose infusion tumor pH decreased significantly and then remained stable. Further administration of glucose did not change the tumor level. Explanation of this phenomenon are the

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following: inhibition of glycolysis under low pH, overloading of substrate (glucose) and deficiency of cofactors and activators, decreasing of tumor blood flow rate that produces glucose supply in a diminished quantity.

With this in mind, some questions appeared: Is a long-term glucose infusion advisable? What is an optimal hyperglycemic schedule?

Long-term glucose infusion may result in a considerable increase in glycemia level, showing various disorders of the organism to reach homeostasis. To avoid this phenomena, it was suggested that a glucose solution with a varying infusion rate occur with the aim of keeping a glycemia level of 25-27 mmol/1. This level is optimal for a tumor acidification (Ardenne von, 1972). There are difficulties in maintaining the glycemia level for a long-term period. Even with an optimum solution there is no guarantee in obtaining a necessary tumor pH and no insurance against dramatic rises of glycemia level.

In our experiments bearing the Brown-Pearce and Guerin carcinoma utilizing normal rabbits and rats and animals, glycemic level's oscillations were significant: $\pm$ 25 mmol/1 (at the assigned one of 27.75 mmol1/1) Under the glucose infusion with variable rate. Use of balanced algorithm of the glycemia level control that was developed by V.M. Glushkov Institute for Cybernetics with our collaboration, provided a glycemia value of 25 $\pm$ 2.2 mmol/1 during 4-6 hours only. Our experiments were not always successful. A number of cases were without significant tumor acidification.

Determination of some glucose metabolism indices after the different IHG schedules in rats bearing the mammary gland carcinoma RMK-1 and Guerin carcinoma provided the following results: at the 180-240th min. of glucose infusion a glycemia increases to 34 $\pm$ 7 mmol/1, lactatemia + 6.5$\pm$1 mmol/1, hexokinase activity of tumor and liver cells decreases by a factor of 2-3, liver glycogen content increases by a factor of 1.5 while tumor content does not change, tumor lactate content increases by a factor of 2-3 while liver and muscle content was unchanged practically. Lactatemia level is appreciably increasing after the 180th min. of glucose infusion while the tumor lactate content does not increase. After 24 hours following the IHG schedule: 120 min. glucose infusion at a rate of 80 mg/kg per min,-tumor lactate content still enhanced (initial value is 31. 7 $\pm$ 1.4 mmol/g, at the 120th min of infusion-79. 1 $\pm$ 7.75, in 24 hours-69.2 $\pm$ 17.9, $p < 0,05$), only in 48 hours it's content returned to initial level (29.9 $\pm$ 0.1). Insuitability of use of long-term glucose infusion (6, 12, 18 or 24 hours) and appropriateness of it's repetition in 48 hour or 72 hour were showed of above mentioned data.

Some authors have achieved a decrease in tumor pH using a 40% glucose solution. Our investigations showed that pH values were almost the same in either a 20% or 40% glucose solution was administered, although differences in the

dynamics of tumor acidification were observed. When a 40% glucose solution was infused, the time required for pH stabilization to begin was halved and the rate of decrease of the pH was slightly increased. Moveover, in contrast to a 20% solution, the 40T glucose solution induced more osmotic effects and more lactatemia. During a 180 min. infusion of the 20% solution the average diuresis was 4.4 ± 1.1 ml, whereas with a 40% solution it was 8.2 ± 0.9 ml with a consequently greater loss of antitumor drugs, salts and water.

Blood insulin content was evaluated under i.v. infusion of 20% glucose solution in rats Guerin carcinoma. It was observed that insulin content increased almost 2-fold at 60-120th min. of glucose infusion indicating the physiological reaction of pancreas. Insulin level was decreasing to it's initial value in 1 hour after cessation of the 120th min. infusion and in 1.5-2 hours after the 240th min. one.

On the basis of the above mentioned data we have proposed the schedule of IHG as follows: a 20% glucose solution was infused i.v. at a rate of 60-80 mg/kg per min. for 1.5-2 hr.

It was observed that pH of some experimental tumors decreased significantly and selectively under this IHG schedule (Table 1)ñ The pH measurements were performed using the specially developed tissue pH microelectrode (M. Sidorenko, our institute). Corning-glass pH electrode with .25-0.4 mm diameter has a sensitive section 4-10 mm long. The sensitivity of electrode is 55-60 mV/pH at 37 degrees C, drift it's indications in the buffer up to 0.005 pH/hr.

The degree of acidification appeared to be more pronounced in the carcinoma than in the sarcoma. Tumor pH decreased significantly within 60-90 min. of glucose infusion and then remained stable. Further administration of glucose did not change the tumor pH level. Infusions both of saline or 20% mannitol solution did not change the tumor pH.

It is interesting to note that the pH drops rather rapidly during the initial period of infusion; in most cases the pH started to drop at the 3rd to 5th min. of glucose administration. When the infusion was complete (120 min.), the pH value remained at the same level for 4-5 hr., then started to rise, and at the 24th hour after cessation of glucose infusion it remained stable at around 5,95 ± 0.3.

In tumors in which pH was not decreasing as significantly (in average to 6.2), the pH started to increase in 1-1.5 hours after cessation of glucose infusion and in 24 hours it was almost normal.

After the i.p. 40% glucose solution injections at a dose of 7.2 g/kg, pH, Guerin carcinoma decreased quite rapidly and resulted in such low values as may be seen under i.v. schedule. However, the follow dynamic of pH was something different. If the average initial pH was 6.6

(6.37-6.74) in 35-40 min. after of i.p. glucose injection-5.55 (5.1-5.90), in 1 hr-5.70 (5.54-5.89), in 2 hr-5.94 (5.75-6.03) in 3 hr-6.25 (6.08-6.45), in 24 hr-6.47 (6.25-6.68), (see Figure 1)

Table 1. pH in tumors and in some normal tissues under IHG

| Tissue | Initial pH | Terminal pH |
|---|---|---|
| Rat: | | |
| Guerin carcinoma | 6.73- 0.05 | 5.51 - 0.08 |
| Pliss lymphosarcoma | 6.68 - 0.08 | 5.66 - 0.27 |
| Yoshida sarcoma * | 7.07 - 0.15 | 6.24 - 0.12 |
| DS-carcinosarcoma * | 7.02 - 0.14 | 6.08 - 0.22 |
| 7,12 DMBA Mammary Ca. | 6.70 - 0.08 | 6.10 - 0.09 |
| glyoblastoma (strain 101.8) | 6.73 - 0.20 | 5.92 - 0.24 |
| muscle | 7.35 - 0.40 | 7.33 - 0.60 |
| brain | 7.20 - 0.10 | 7.25 - 0.17 |
| mouse | | |
| sarcoma 180 | 6.79 - 0.90 | 6.38 - 0.13 |

* With collaboration of Dr. P.G. Reitnauer (Dresden, GDR)

IHG schedule that was developed by Yarmonenko (1981) may prevent such rapid pH increase. By means of this method we have obtained the following results: Guerin carcinoma pH decreased below 6.0 and it remained unchanged for about 3-4 hours and then started to increase. In general, the acidification value was not as low as under i.v. glucose infusion.

The mentioned data about tumor acidification at the different IHG schedules pointed out that i.v. glucose infusion has preference with comparison to i.p. ones. This data supports the opinion of Vaupel, Okunieff (1988) concerning the table use of the i.p. glucose injections for studying the IHG effects.

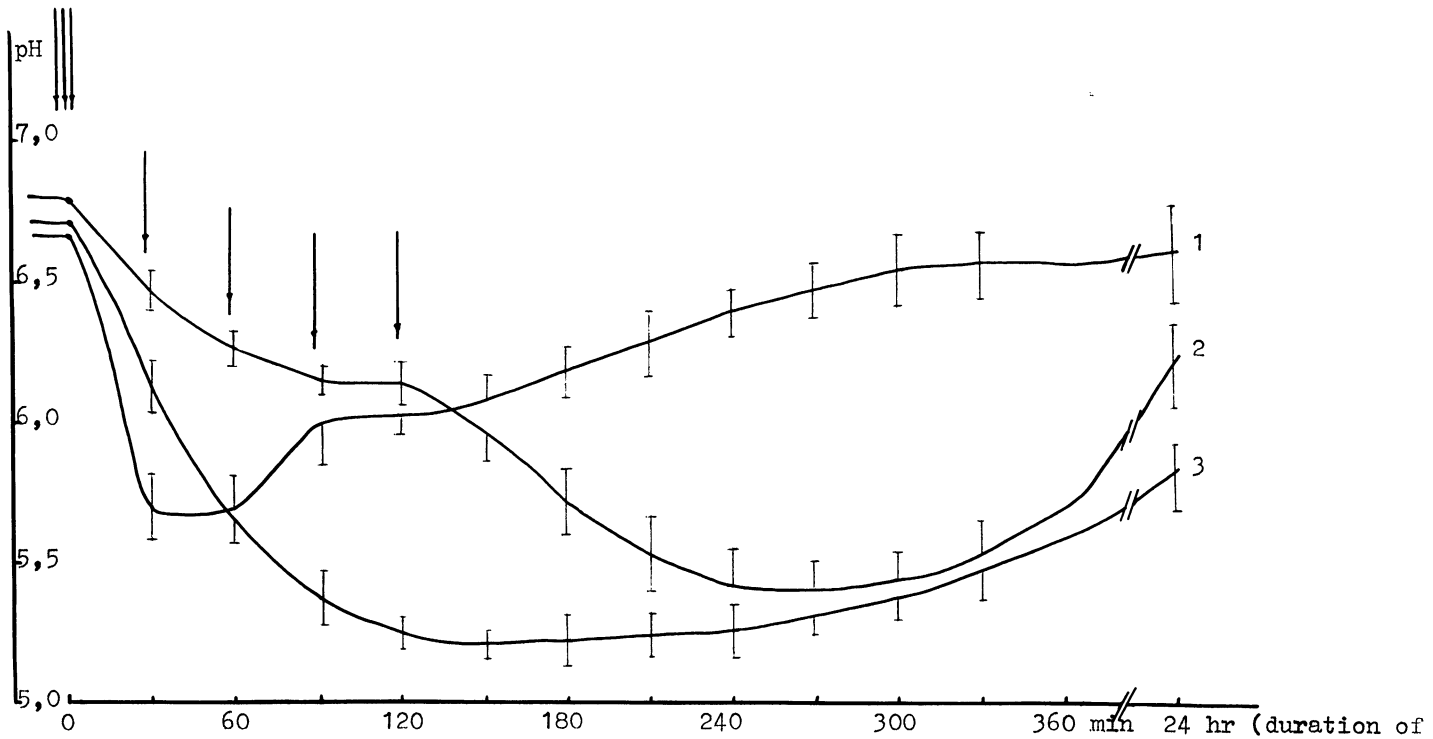


Fig. 1. Tumor pH under different schedules of IHG in rats bearing Guerin carcinoma.

1 - single i.p. injection of 40% glucose solution (7,2 g/kg)

2 - repeated i.p. injections of 40% glucose solution with 30 min interval (1-3 injections 2,6 g/kg, 4 and 5 - 1,3 g/kg)

3 - constant i.v. infusion of 20% gluvose solution ( 80 mg/kg per min)

It is known that blood flow rate in a tumor is very important for successful heating. We determined a blood flow rate in Guerin carcinoma by means of the hydrogen clearance method.

It was determined that the blood flow rate was increasing at the initial stage of glucose infusion by 152%, then it decreased (in some experiments up to the zero values). The scattering in values of blood flow rate in a tumor may be explained by tumor heterogeneity and it's vascular network. We divided a tumor into several groups: 1st-a marked blood flow increase and lack of it's decrease (or unsignificant presence) below the initial level; 2nd-moderate blood flow and it's considerable subsequent decrease (down to the zero value); 3rd-absence of a blood flow increase in a registration zone and relatively rapid (at 40-80 th min) it's complete blocking, (see Figure 2).

In a two-way analysis of variance, we carried out, it accurately showed that the observed dynamics in a tumor depends upon the initial reaction of a tumor's vascular network on glucose. If at the IHG initiation a blood flow rate in a tumor is increasing, later, at the 240th min. it is decreasing very slowly. If at the IHG initiation a blood flow rate increase is unsignificant or absent, at the 200-240th min. it practically ceases. The latter may be explained by the fact that under a poor blood flow in tumor lactate is accumulating. It may enhance blood flow ihibition according to Ardenne's data (1978).

Increase in viscosity of blood by the alteration in physical and chemical properties of erythrocytes and trombocytes under glucose influence at high concentration is one of the blood flow inhibition mechanisms (Calderwood, Dickson, 1980). Rise in osmotic pressure during glucose infusion in the tumor results in osmotic diuresis and intratissue hypotension is also considered (Urano, Kim, 1983). Calderwood, Dickson (1980) believe that the primary IHG effect on a tumor is blood flow inhibition which results in lactate accumulation in the tumor tissue and pH decrease. Confirming their view, the authors present their own data that firming their view, the authors present their own data that blood flow in a tumor is decreasing high rapidly both after i.p. glucose injection and after i.v. infusion. Tumor pH decreases less rapidly and reaches it's minimum in the 3-4th hour.

This concept appears to be too categorical of primary significance in the IHG effect on a tumor pH decreases and blood flow inhibition accelerates a pH decreases and enhances a glucose acidification.

In our experiments we used the above mentioned IHG method. In most cases pH starts to decrease within 8-12 min. after glucose infusion is initiated. Naturally, in

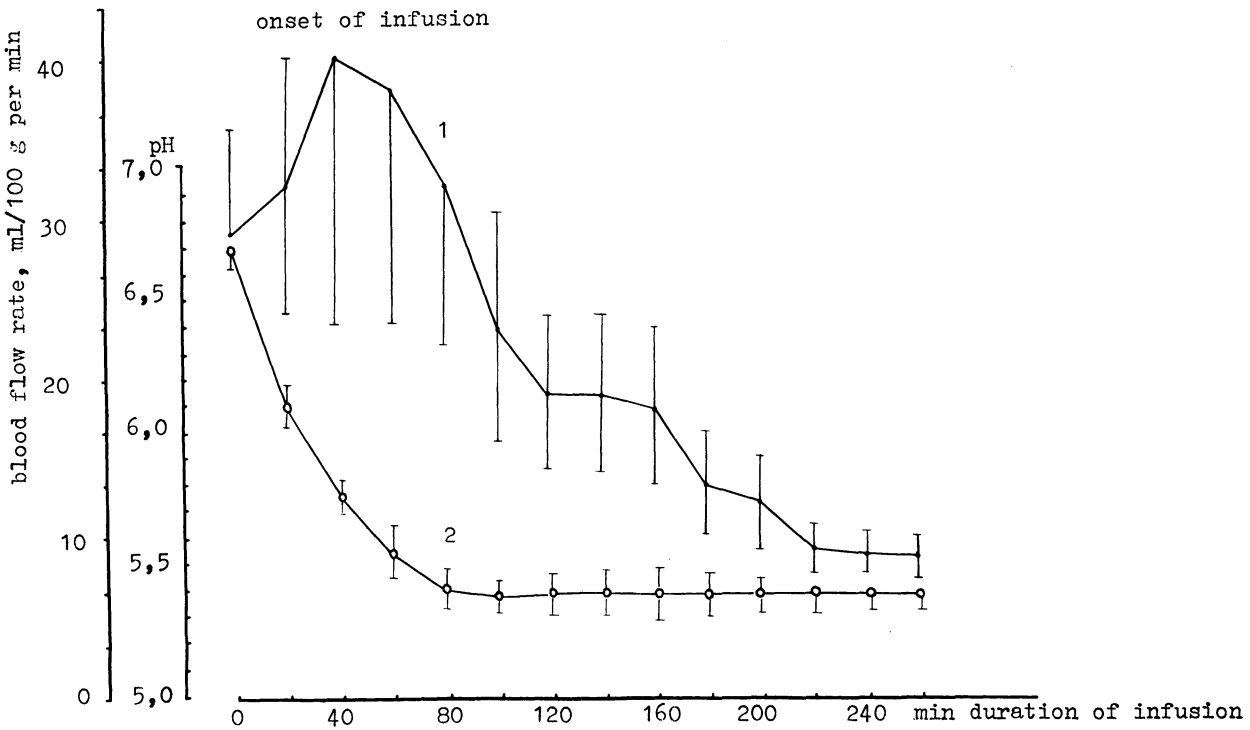


Fig. 2. Blood flow ( 1 ) and pH ( 2 ) of Guerin carcinoma.

tumors of the 3rd group, the pH started to decrease within 5-7 min. which was much more rapid than in the tumors of the 1st group. It appears that a primary link in a tumor pH decrease during IHG is because tumor tissue glycolysis and the activity of it's key enzymes. Glucose infusion results in formation of a large amount of lactate and the pH decrease under high glycolysis activity. As a blood flow inhibition starts it promotes a lactate accumulation in the tumor and the pH decreases further. Complete blood flow inhibition is a major reason for the fact that in many tumors in the 24 hours after glucose infusion a pH remains below it's initial value.

Tumor acidification under IHG depends on the blood flow. Evidence shows that under IHG the blood flow is inhibited only in a tumor itself. However the normal tissues remain practically unchanged. In DS-carcinosarcoma in rats, initial blood flow rates were as follows:-0.815 $\pm$ 0.085 (rel. U), at the 180th min. of glucose infusion-0.13 $\pm$ 0.09. In the muscle this index was the following: initial -2.25 $\pm$ 0.31; at 180th min.-2.43 $\pm$ 0.47.

Increase in tumor hypoxia while inhibiting blood flow was expected. Confirmation with rats bearing the Guerin carcinoma occurred. $PO_2$ values were determined by the polarography method.

In 35% of the tumors during the initial period of glucose infusion the $pO_2$ increased by 20-25% (at an average by the 45th min.). At the 110th min. (35-120) of infusion the $pO_2$ values in both tumors where the initial increase of $pO_2$ was not observed has been decreasing by 50-75% from the initial. In some cases zero values were obtained.

By correlation analysis a considerable heterogeneity of tumor tissue was found: there are zones in which the $pO_2$ is significantly dependent on a blood flow and zones where the $pO_2$ shows a definite independence. It's interesting to note that in muscle the unconsiderable $pO_2$ increase in 15-25 min. after IHG initiation. Further $pO_2$ reverted to the initial level and remained stable.

The $pO_2$ value decrease in a tumor tissue under IHG is caused by the lower oxygen supply in a tumor due to blood flow inhibition and lowering a dissolved oxygen concentration in a tumor. Increase of hypoxia in a tumor under IHG confirmed a great advisability of tumor radiation before a hyperglycemia (Yarmonenko et al., 1981) and a need of hyperthermia application immediately after glucose infusion.

In 1973 Ardenne von has suggested that the hyperglycemia may cause the effect of tumor cells synchronization. We had shown that number of the tumor cells in $G_2$ -phase was increasing 4-fold and in S-phase

almost 2-fold under our IHG schedule. Moreover, our results indicated a considerable delay in the $G_2$ to M, and an activation in $G_1$ to S and in the $G_0$ to S transition under these conditions. On that basis we have suggested the stimulation effect of IHG on transition the tumor resting cells into proliferation compartment. This was confirmed by the evaluation of a growth fraction: control-55,8% after 60-min glucose infusion-82.6% after 90-min. one-95.6%.

It should be pointed out on the results of our experiments with mannitol, that it is not metabolized by the cells. At the same time it was shown that the osmotic effect of 20% mannitol solution was similar to 20% glucose solution. But the modification of tumor cell cycle was absent under infusion of mannitol. With this knowledge, we have supposed that IHG modifies the tumor cell kinetic due to acidification, improved nutrition and other changes in the microenvironment of tumor cells.

It is important to note that neither mitotic activity nor proliferative activity of small intestine cells and bone marrow cells were changed under IHG, whereas the labeled index of the promyelocytes and myelocytes was increasing unsignificantly (by 4.5%).

We have also observed that IHG did not change the labeled index of the liver cells either under the normal situation or after a hepatectomy.

The obtained results demonstrated that glucose synchronized the tumor cells selectively, increasing the cell numbers in $G_2$-and S-phase of a cell cycle.

Thus, it was evaluated that under IHG the tumor pH and $pO_2$ values are decreasing selectively, a blood flow in inhibited and the cell numbers in S-phase is increasing two-fold. All the above mentioned alterations are favorable for the enhancement of the tumor cells thermosensitivity; and support the idea about the use of IHG for enhancement of the HT effect, (see Table 2)

Some authors oppose this suggestion reasoning that the data about the increase of the tumor glucose content and energy level under IHG, protects the cells against heating (Calderwood, Dickson, 1980; Haveman, Hahn, 1981; Henle et at., a984). Response to the accuracy of the given discussion could be proved conclusively by experiments studying IHG influence on an antitumor hyperthermia effect.

Guerin carcinoma and Pliss lymphosarcoma were treated by means of "Luch-2" machine (2450 MHz). The heating was given immediately after the IHG and maintained for 1 hour. The tumor temperature was 41 $\pm$ 0.05 degrees C or 43 $\pm$ 0.05 degrees C. The thermometry was performed by means of perturbing copper-constantan thermocouples inserted into the

Table 2 Indices of Tumor Metabolism During IHG

| Index | Initial Value | On 90th min of IHG |
|---|---|---|
| pH (U) | 6.73 - 0.05 | 5.54 - 0.10* |
| Tumor lactate content (microM/g) | 31.70 - 1.40 | 61.20 - 3.70* |
| glycemia (mM/l) | 4.10 - 0.20 | 23.60 - 3.70* |
| lactatemia (mM/l) | 3.40 - 0.25 | 6.30 - 0.40* |
| $O_2$ tension value (kPa) | 3.45 - 0.90 | 0.90 - 0.60* |
| blood flow rate (ml/100 g per min) | 28.10 - 7.80 | 8.70 - 5.00* |
| cell numbers in S phase (relative value) | 0.217 - 0.005 | 0.396 - 0.01* |
| tumor glucose content (mM/kg) | 0.02 - 0.002 | 3.50 - 0.25* |
| content in tumor of: | | |
| ATP | 1.40 - 0.10 | 2.20 - 0.10* |
| ADP (micro M/g) | 1.15 - 0.10 | 1.10 - 0.10 |
| AMP | 0.30 - 0.10 | 0.32 - 0.04 |
| $P_i$ (mg/g) | 8.50 - 0.30 | 6.10 - 0.30* |
| energy charge of tumor tissue | $3.2.10^{-3}$ M | $3.8.10^{-3}$ M |

* The difference between the mean values for control and IHG (p 0.05) was significant.

tumor perpendicular to the electrical field. Rats bearing subcutaneous tumors in size 1.5-2.0 cm were used for therapeutic experiments.

It was observed that time doubling (TD) of Guerin carcinoma was: control-2.5 ± 0.3 days; 41 degrees C-60 min.-2.6 ± 0.3; 43 degrees C-60 min.-4.0 + 0.6; IHG + HT 41 degrees C-5.6 ± 0.7; IHG + HT (43 degrees C)-7.6 ± 0.2 (p é 0.05). TD of Pliss lymphosarcoma was: control-1.9 ± 0.4 days; 43 degrees C-60 min.-4.3 ± 0.8; IHG + HT (43 degrees C)-5.75 ± 0.1 (p é 0.05). It should be noted that HT carrying out at 41 degrees C (60 min.) after IHG, provided obtaining TD for the Guerin cardinoma by 1.6 days more in comparison with HT at the regime of 43 degrees C (60 min.) (See Figure 3).

The obtained results clearly demonstrate the enhancement HT antitumor effect under IHG, which conforms with the results of Urano, Kim (1983); and contradicts the data of Dickson, Calderwood (1979) and Shah et al. (1983). To explain this situation we indicated the considerable difference between our experiment and those of Dickson, Calderwood (1979) and Shah et al. (1983) by the two indices, i.e.: pH value and glucose content in a tumor tissue (see Table 3). In our study tumor pH was significantly lower than that in the studies of these authors. This fact is very important because enhancement of the cytotoxic HT effect under low pH was demonstrated (Kim et al., 1980).

The opponents of an IHG + HT combination consider glucose to be a peculiar "protector" for the tumor cells against heating. The studies where the tumor cells have been incubating with glucose at different concentrations, and were HT treated, confirmed the cited suggestion (Kim et al., 1980). But our schedule indicates that glucose does not accumulate in such amounts that it significantly decreases HT cytotoxic effect. In the studies of the above mentioned authors, glucose content in a tumor varied from 5 up to 20 mmol/l. In a study of Henle et al. (1984) titled "Protection Against Thermal Cell Death in Chinese Hamster Ovary Cells by Glucose, Galactose or Mannose", it's concentration was 100-300 mmol/l. In our studies, a glucose content in tumor under IHG was from 0.5 up to 3.5 mmol/l. Glucose in such amounts does not prevent cytoxic heating effect (Kim et al., 1980). It should be mentioned that Hahn's view (1974), was that glucose in general plays no role in the tumor's thermosensitivity.

It is probable, also, that the glycemia level has no direct significance in the realization of HT antitumor effect, as with almost the same levels in our studies, and the ones of Dickson, Calderwood (1979), Shah et al. (1983) opposite results were obtained. Unfortunately, in the study of Urano, Kim (1983) the glycemia level, tumor pH, and tumor glucose content data were absent.

Shah et al. (1983), explaining absence of enhancement of the HT effect under the IHG, point out that glucose, retarding tumor blood flow may prevent formation of the immunological relationships in host-tumor. But can one seriously think about a considerable immunomodulating effect of a blood flow inhibition under the IHG?


Fig. 3. Time doubling of tumor size of rats treated as follow:
1 - non, 2 - 41°C, 60 min, 3 - 43°C, 60 min, 4 - 41°C, 60 min + IHG,
5 - 43°C, 60 min + IHG.

Table 3 Relationship Between Methods of Hyperglycemia Induction and Changes of Some Indices Before Heating of Tumors

| Treatment | indices before HT: Tumor pH | glycemia mM/l | tumor glucose cont. mM/kg | tumor | ref. |
|---|---|---|---|---|---|
| i.p. injection of glucose (6 g/kg body wt) 60 min before the initiation of HT (water bath,42 C, 45 min) | 6.63 | 30.0 | 10.0 | Yoshida sarcoma | Dickson, Calderwood 1979 |
| i.p. injection of glucose (6 g/kg) 60 min before the initiation of HT (water bath, 43 C, 120 min) | 6.60 | 40.0 | 17.0 | Walker 256 carcinoma | Shah et al 1983 |
| i.v. infusion of glucose was (80 mg/kg per min for 90 min) immediately followed by HT (2450 MHz, 43 C, 60 min) | 5.70 | 25-30 | 2.5 - 5.0 | Guerin Carcinoma | Our works |

It also has been indicated on appearance of metastases and intensification of metastases growth under a combination of IHG + HT (Shah et al., 1983) the authors consider that the tumor blood flow rate would rise under 120 min. HT; and tumor cells might interfere with blood circulation. Such an explanation may be quite acceptable for the experiments presented in papers mentioned above. But under our IHG schedule, blood flow remains inhibited for 24 hours; this may prevent the process of interference of tumor cells. The data of Shah et al. (1983) in general are difficult to explain, as in the other studies under the i.p. glucose injections, the intensification of metastasizing process was not observed (Zavrid et al., 1986; Istomin, Furmanchuk, 1988; ando et al., 1987). Probably, the biological peculiarities of the different tumors should be considered; specifically: activity of their glycolysis and the blood vessels reactivity which is of primary significance in formation of tumor response to IHG. Low pH and inhibited blood flow are the major manifestations of the IHG effect on a tumor, promoting the successful realization of the HT anti-tumor effect.

There is however, data (Hahn, 1984) reporting that thermosensitivity of some cells decrease in the long-term incubation under low pH. But under a drastic and sharp pH decrease which, incidentally, occurs under IHG, the cells sensitivity to pH is enhanced.

In some studies a direct correlation between an increase of cell energy charge and enhancement of their thermo-resistance was observed (Haveman, Hahn, 1981; Gerweck, 1985). However, an energy charge of tumor tissue under our IHG schedule did not practically increase (see Table 2). It indicates that energy level in tumors under IHG, cannot change thermosensitivity.

The findings that glucose at a concentration of 1-5 mM does not decrease HT antitumor effect was confirmed by experiments with the L 1210 leukemia cells. The cells were incubating in 199 medium ($4 \cdot 10^5$ cells in 1 ml of medium), with the different glucose concentrations and heating at 43 degrees C for 30, 60 and 90 min. After heating the cells have been i.p. inoculated into the $F_1$ recipients ($1 \cdot 10^5$ cells/mouse). It was shown that glucose even at a concentration of 10 mM results in the enhancement of HT cytotoxic effect. Survival time of the recipients, after inoculation of the cells heated with glucose, was enhanced significantly (see Figure 4).

Efficacy of thermochemotherapy was also enhanced IHG. The rats with Guerin carcinoma were treated: with IHG (our schedule) immediately followed by HT (43 degrees C, 60 min.). Thiophosphamide was given i.p. at a dose of 2 mg/kg at the 60th min. of glucose infusion. The dose of drug was 3 mg/kg in rats that were not treated with combined method. Treatments were repeated 2 times with a 2 day interval. TD of tumor increased two-fold when thermotherapy with drug was used after the IHG (see Table 4).

The data of the different authors and our own, clearly indicated that low tumor pH enhances a cytotoxic hyperthermia effect considerably. From these results, it is

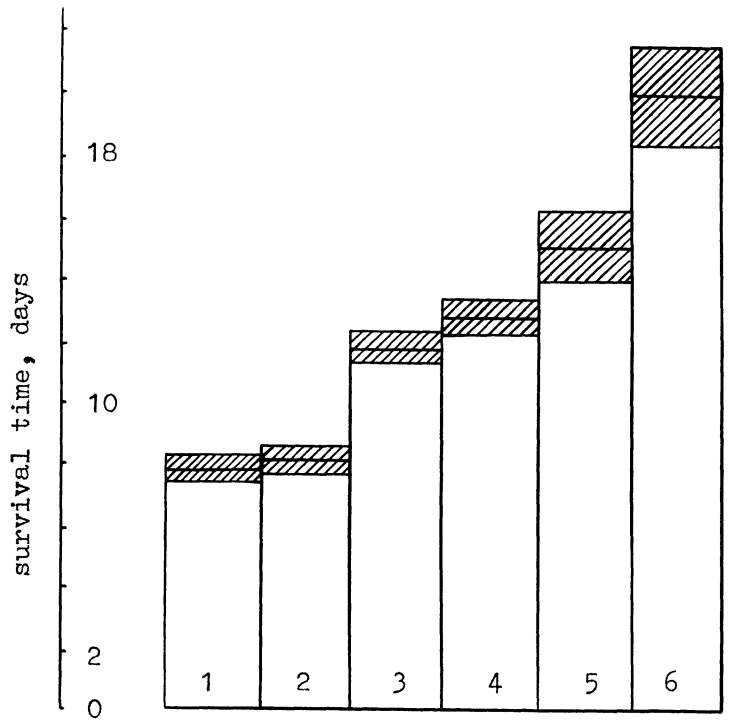


Fig. 4. Survival time of $F_1$ recipients after L 1210 cells inoculation. Intact donor cells have been treated: 1 - non, 2 - 43°C,30 min, 3 - 60 min, 4 - 90 min, 5 - 43°C, 60 min + glucose, 5 mM, 6 - 60 min + 10 mM.

suggested that the extremely low tumor pH may provide the more higher thermosensitizing effect. The mechanism for the regulation of internal pH in cells, however, prevent them from achieving maximal acidification. A greatly important regulating system is Na+/H+ exchange in plasma membranes. Activation of this mechanism due to the lowering of intracellular pH results in the increase in intracellular pH. Hence, the use of an inhibitor for Na+/H+ exchange system must prevent cell alkalyzation and intensify heating effect. Several authors have already reported the thermosensitizing effect of the diuretic drug, amiloride, inhibiting the Na+/H+ exchange activity (mijakoski et al., 1986; Ruifrok, Konings, 1987; Kim et al., 1988). No animals bearing the experimental tumors were used in these studies, however.

In our experiments we used rats bearing Guerin carcinoma, and the diuretic drug, triampur, containing triamteren, inhibiting the Na+/H+ antiport system (Macfie et al., 1981). Triampur was injected i.p. at a dose of 11.2 mg/kg body weight during i.v. glucose infusion, or after i.p. injection, when tumor pH remained stable or started to rise. It was shown that under these conditions, tumor pH appeared not to increase during 4-5 hours (see Figure 5). Under different IHG schedules we have not observed such dynamics of tumor acidification. The obtained results indicated the ability of triampur to amplify the process of tumor acidification, and encouraged the use of this inhibitor of Na+H+ antiport system for enhancement of HT effect. Table 5 shows the influence of HT treatment (43 degrees C, 60 min) on Guerin carcinoma when it was applied immediately after the combination of i.v. IHG + i.p. triampur. As can be seen, using this combined treatment resulted in an increased TD by a factor of 2. It must be noted that TD of tumors treated with IHG + HT (43 degrees C, 60 min.) was similar to TD after treatment with IHG + triampur + HT (41 degrees C, 60 min.). The obtained data demonstrated the efficacy of the Na+/H+ antiport system inhibitor in combination with IHG to enhance HT antitumor effect. We think that triampur and other drugs with similar effect on Na+/H+ exchange may be useful as a hyperthermic sensitizer in clinical cancer treatment.

The results of our experiments and clinical work, convincingly showed the enhancement of HT effect due to modification of some biological indices of tumor tissue by means of IHG. It is very important to note that IHG effects (pH and $pO_2$ drop, blood flow inhibition, cell cycle modification) were observed in tumors only, not in normal tissues. Hence, the combination of HT + IHG gives us a unique method for selective treatment for the most tumors results to become a high therapeutic gain.

Table 4

Thermochemotherapy Results of Rats Bearing Guerin Carcinoma

| Schedules | Index | | | |
|---|---|---|---|---|
| | Response (%) | | | Time Doubling of Tumor Size (days) |
| | Complete Regression | Partial | No Change | |
| drug (D) | 17.0 | 17.4 | 65.6 | 4.4 +/- 0.4 |
| IHG | 0.0 | 0.0 | 100.0 | 2.9 +/- 0.5 |
| HT | 0.0 | 25.0 | 75.0 | 4.0 +/- 0.6 |
| IHG + D | 50.5 | 37.5 | 12.0 | 7.8 +/- 0.6 |
| HT + D | 45.0 | 10.0 | 45.0 | 5.8 +/- 0.5 |
| IHG + D + HT | 75.0 | 25.0 | 0.0 | 12.3 +/- 0.9 |

Table 5. Time doubling (TD) of guerin carcinoma treated with combination of HT, IHG and injection of triampur.

| Rat groups | TD ( days ) |
|---|---|
| controle | 2.5 ± 0.3 |
| HT (41°C, 60 min) | 2.6 ± 0.3 |
| HT (43°C, 60 min) | 4.0 ± 0.6 |
| IHG + HT (41°C, 60 min) | 5.7 ± 0.5 |
| IHG + HT (43°C, 60 min) | 7.6 ± 0.2 |
| IHG + triampur + HT ( 43°C, 60 min ) | 7.8 ± 0.3 |
| IHG + triampur + HT ( 43°C, 60 min ) | 12.6 ± 0.6 |

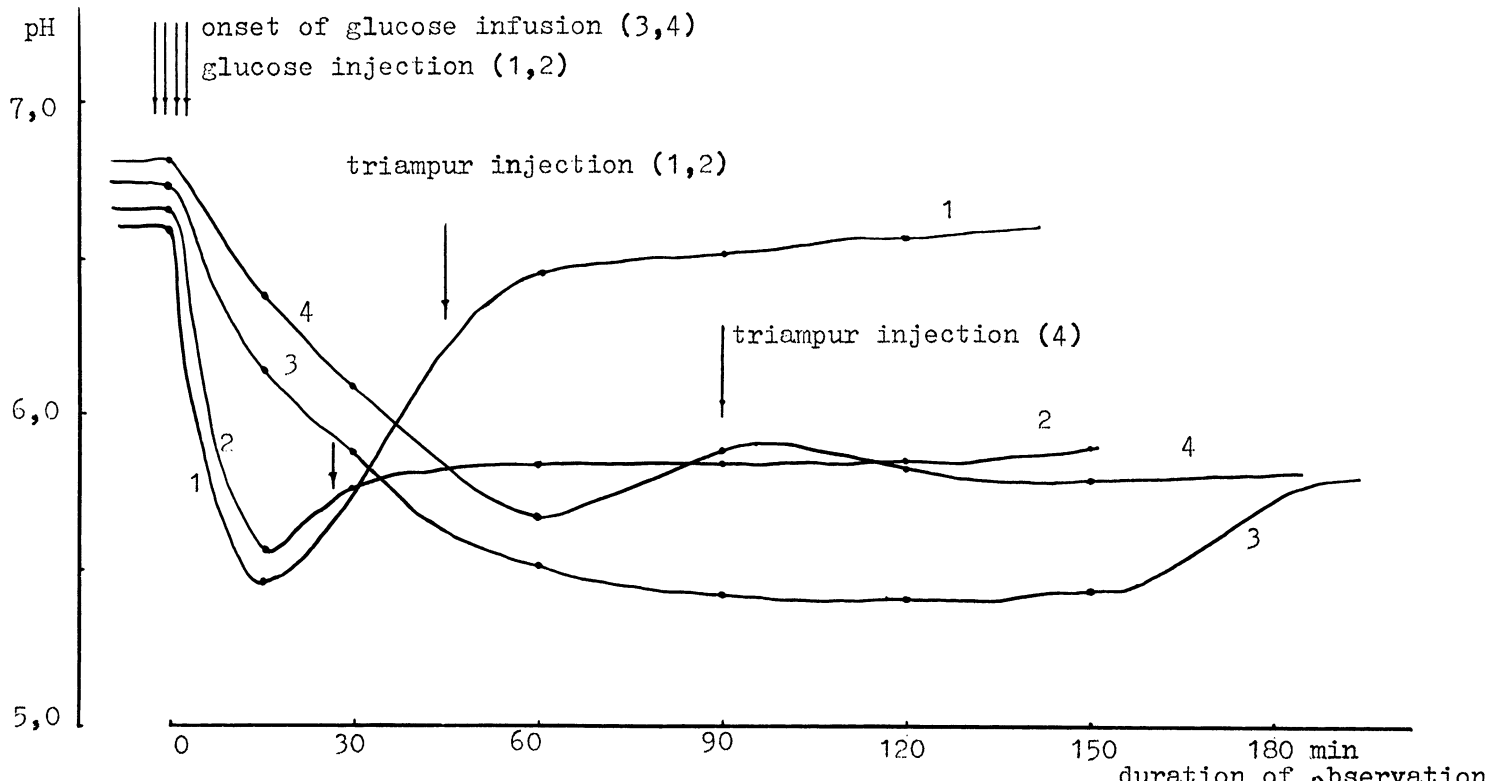


Fig. 5. pH-dropping curves of individual animals bearing Guerin carcinoma. Curves 1 and 2 - i.p. injection of 40% glucose solution (7,2 g/kg), curves 3 and 4 - i.v. infusion of 20% glucose solution (80 mg/kg per min).

REFERENCES

1) Bhuyan, B.K., et al., Cancer Res., vol. 37, p.3780-3784, 1977.

2) Freeman, M.D., et al., Cancer Res., vol. 45, p 2291-2300, 1980.

3) Kim, S.H., et al., Cancer Res., vol. 40, p 3459-3462, 1980.

4) Haveman, J., Hahn, G.M., J. Cell Physiol., vol. 107, p 237-241, 1981.

5) Wike-Hooley, J.L., et al., Hyperthermia and hyperglycemia in oncology, Kiev: Naukova Dumka, 1987.

6) Zavrid, E. et al., Hyperthermia and hyperglycemia in oncology, Kiev: Naukova Dumka, 1987.

7) Ardenne von M., Rieger, F., Naturforsch, F., vol. 21B, S.472-482, 1966.

8) Ardenne von M., Adv. Pharmacol. Chemother., vol. 10, p 339-380, 1972.

9) Dickson, J.A., Calderwood, S.K., J. Nat. Cancer Inst., vol. 63, p 1371-1381, 1979.

10) Jarmonenko, S. et al., Med. radiology, N 2, p 46-50, 1981.

11) Vaupel, P.W., Okunieff, P.G., Cancer Res., vol. 48, p. 7102-7106, 1988.

12) Ardenne von M., Cancer Therapy Hyperther. Radiat., Munich: Schwarzenberg, p 96-104, 1978.

13) Calderwood, S.K., Dickson, J.A., Cancer Res., vol. 40, P. 4728-4733, 1980.

14) Urano, M., Kim, M.S., Cancer Res., vol. 43, p 3041-3044, 1983.

15) Ardenne von M., Naturwiss., vol. 60, p 483-484, 1973.

16) Henle, K.J., et al, Cancer Res., vol. 44, p 5499-5504, 1984.

17) Shah, S.A., et al., Cancer Lett., vol. 19, p 317-323, 1983.

18) Hahn, G.M., Cancer Res., vol. 34, p. 3117-3123, 1974.

19) Zavrid, E., et al., Exper. Oncology, vol. 8, p 57-59, 1986.

20) Istomin, Ju., Furmanchuk, A., Med. radiology, N 5, p. 22-25, f1988.

21) Ando, K., et al., Int. J. Hyperther., vol. 3, p 453-457, 1987.

22) Hahn, G.M., Cancer Res., vol. 44, Suppl. 10, p 4733, 1984.

23) Gerweck, L.E., Cancer Res., vol 45, p 3408-3414, 1985.

24 Mijakoski, J., et al., Cancer Res., vol. 46, p 1840-1843, 1986.

25) Ruifrok, A.C.C., Konings, A.W.T., Int. J. Radiat. Biol., vol. 52, p 385-392, 1987.

26) Kim, G.E., et al., Int. J. Radiat. Oncol. Biol. Phys., vol. 15, Suppl. 1, p 175, 1988.

27) Macfie, H.L., et al., Drug Intel. Clin. Phar., vol. 5, p 94-99, 1981.

# THE BIOLOGICAL RESPONSES TO HEAT

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The therapeutical application of heat, in any manner obtained(radiofrequency, microwave, ultrasound hyperthermia), causes progressive physiopathologic modifications to the tumoral mass.These consist in structural damages sufficiently known(18). The heating of a neoplastic mass usually brings about impairments of enzymatic cellular processes, with an increase of oxygen consumption, interesting anoxic or partially anoxic cells.These enzymatic impairments imbalance the normal homeostatic equilibrium,leading to cell death(55). The thermal washout mechanisms, which protect the normal cells, for certain temperature limits, are missing for the neoplastic cells placed in different environment.The nutritive supply to the tumor microenvironment largely depends on an inefficient and inelastic vascular system (8). The temperature increase, beyond an optimal shoulder, 42.5°C, creates higher oxygen consumption, increase of microsomal activity and of anaerobic glycolysis, leading to two different processes: (A)increased production of lactic acid with consequently pH decrease,(B)a drastic reduction of ATP formation. The reduced availability of energy, due to high temperatures,impairs the enzymatic repair system of tumor DNA molecules.Therefore there is a decrease of RNA and protein synthesis.All these metabolic modifications, induce a physicochemical change of tumoral extracellular environment and microcirculation (52). The extracellular pH progressively decreases, with a secondary reduction of metabolic exchanges.Inside the tumor capillaries, the hematic viscosity increases. This is due to RBC stiffening,to an increased formation of fibrinogen gel and leucocytes-platelets adhesion, leading to intravascular coagulation(55).The hyperthermia affects,also,the permeability of capillary endothelium, producing a peritissular oedema.This worsens the neoplastic metabolic activity.These circulatory changes, above all reduces the blood flow,already irregular and insufficient in the central zone of the tumor mass. The damages to the molecular structures of the nucleus,with progressive breakdown of the membrane activity, may carry to the cellular death. The target of hyperthermia is the central zone of the tumoral mass, which is spontaneously more or less hypoxic (6). These hypoxic zones are less sensitive to

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radiotherapy,and to chemotherapy.Only some drugs are active(i.e Mitomycin-C, Adriamicin)and the different tumoral circulation determines the ability of the pharmacological agents to reach in vivo the cell,and the effectiveness of the treatment (23). Most of the current modalities in cancer treatment:surgery,radiotherapy,chemotherapy,immunotherapy,have had limited success in the treatment of solid tumors. Therefore,two or more of these modalities ,used in combination with hyperthermia may increase tumor response and host cure rate (See fig.1). The cellular target of radiotherapy and chemotherapy is radically different from that of hyperthermia,and the sequential use of their combination may carry to complete eradication of a tumor mass,as has been recently documented. Radiotherapy and chemotherapy,at the usual recommended doses,create a lot of undesired effects,which may limit their utilization.The association with hyperthermia,a treatment devoid of great side effects,can decrease the therapeutical doses of XRays and chemotherapy,determing a good response with less collateral consequences. An interesting utilization of hyperthermia would be the association with immunostimulant and immunomodulant therapies. This approach may be particular useful for older patients,where the use of chemo and Xrays are not possible at the recommended dose. Different studies,have shown that some metabolic modifications during hyperthermia may increase the sensitivity to heat,improving the tumor kill (10). These metabolic manipulations are not applied in a systematic way in humans. In our laboratory,we are studying doses and schedules to enhance this thermosensitivity (See fig. n.2).

## THE CENTRAL ROLE OF HYPOXIC CELL

It has been recognized by oncologists for more than a quarter of century that hypoxic cells, present in most solid tumors,may be critical for the successful treatment of cancer. In particular hypoxic cells are resistant to:ionizing radiations,photodynamic therapy and large majority of chemotherapeutic drugs.However, recently it has been suggested that hypoxia may be a weapon which can be used against the tumor (5).It has been,indeed,demonstrated by many authors that hypoxic cells are responsive to specific drugs and are very sensitive to heat treatment(20).Hypoxia is generally due to the inadequacy of vascular beds supporting the tumor.The chronic or transient deficiency of blood flow,results in the development of chronic or acute hypoxia. Chronic hypoxia(DIFFUSION LIMITED),is thought to develop because the proliferation of tumor cells is sufficiently rapid that the vasculature cannot develop at corresponding rate.Tumor cells close to microcapillary are fairly well oxygenated and when oxygen remains sufficient cell division occurs. However,the oxygen tension decreases with distance from the capillary because of cell respiration and gradually falls to a level insufficient to sustain cell division.Oxygen deprivation is responsible for the area of necrosis which usually develops about 150-200$\mu$ m from the nearest blood vessels (35). Solid tumors may also contain regions of transient hypoxia(PERFUSION LIMITED),caused by temporary interruption of the blood flow through individual blood vessels within the tumor (44). Transient perfusional hypoxia can be caused by

COMBINATION MODALITIES
FOR CANCER CELL DESTRUCTION

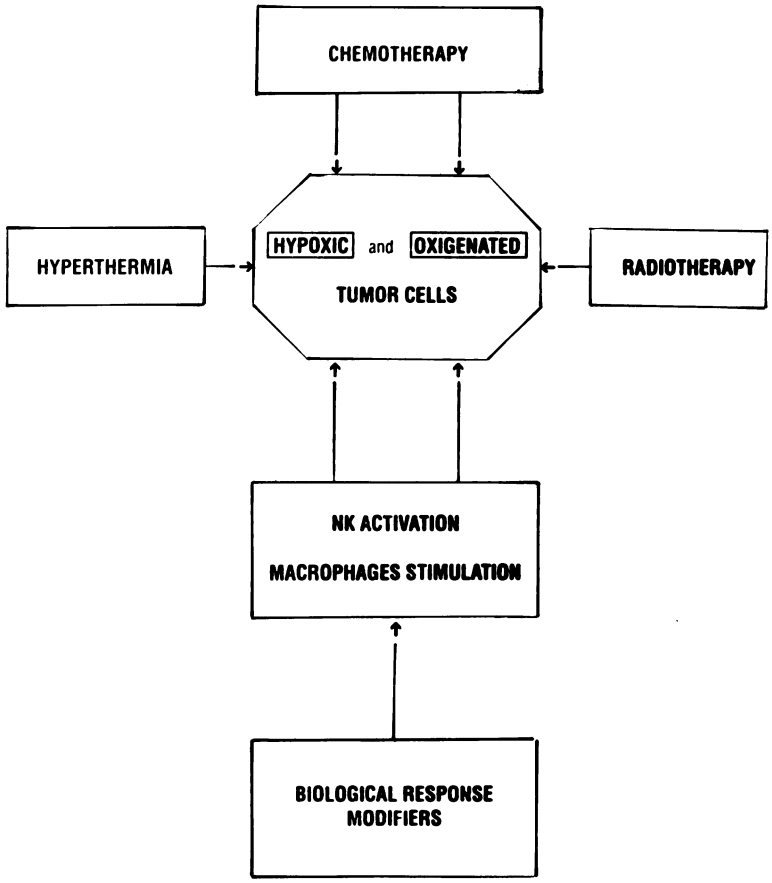


Figure 1

**BIOLOGICAL RESPONSE TO HEAT - SENSITIZING FACTORS**

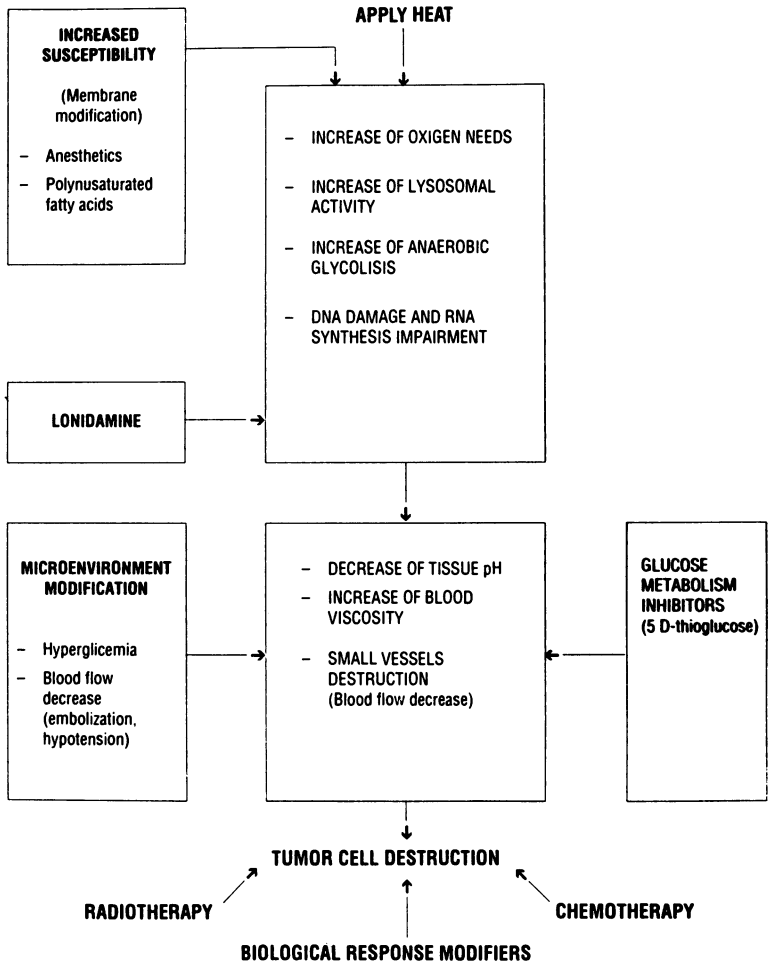


Figure 2

hypotension,clotting, embolization, chemoembolization. Arterial ligation in the tumor area brings to stable perfusional hypoxia. Although the mechanism of hypoxia may be different,there is evidence that the resultant hypoxic cells at the interface region between the well oxygenated tissue and the necrotic areas, can remain potentially viable and clonogenically active for a considerable period(15).Many cells,in this region,suffer from hypoxia,lack of nutrients, lack of cellular energy and relatively low intra and intercellular pH. Their energy metabolism is based on anaerobic glycolysis and their proliferation halted in G1-G0 cycle phase.Chemotaxis of hypoxic cells towards better environments has been suggested by ROCKWELL, and recently confirmed (44).These cells resume active proliferation and cause a tumor relapse. One of the peculiar charactheristic of these cells, demonstrated using an histochemical reaction for LDH isoenzyme,and specific staining methods, is an accumulation of lipids of non homogeneous composition. It has been revealed a peripheral halo of neutral lipids,and a central core of unsatured lipids.The accumulation of lipids is supposed to be caused by activation of phospholipases,followed by hydrolysis of the mitochondrial membrane,and accumulation of multilamellar bodies,often containing damaged organelles. Such autophagic processes could provide a survival mechanism for hypoxic cells.The presence of colonies of hypoxic cells coming from the edge of necrosis towards blood vessels,and the existence of hypoxic cells inside,may suggest that these cells are the most prone to metastasis(15)(See fig.3,4).This agrees with the opinion of NICOLSON,who suggests that competitive environment may be selective for variants with higly metastatic property (38).Our clinical observation, that no metastatic spread is observed in some early-stage tumor patients,3-6 years after treatment with localized hyperthermia,supports this hypothesis, that hypoxic cells,very heat-sensitive,are an important component of the hematogenous spread of tumor.This last consideration dramatically shows,how important is the presence of viable hypoxic cells in human cancer. The detection of this fraction, in humans, is not easily performed, but recent photometric and fluorescent techniques promise a way for quantifiyng oxygenation of solid tumors. The search for a specific therapy able to kill a great quantity of hypoxic cells are illustrated in table 1.

Table 1 APPROACHES TO TREATMENT OF HYPOXIA AND HYPOXIC CELLS

| | |
|---|---|
| INCREASED OXYGEN DELIVERY | -transfusion- |
| | -hyperbaric oxygen- |
| | -per-fluorochemicals- |
| | -altered Hgb affinity- |
| HYPOXIC CELL SENSITIZIERS | +-thiol modification-porfiromycin- |
| BIOREDUCTIVE AGENTS | -misonidazole- |
| | -porfiromycin- |
| | -5-thio-D-glucose- |
| CHEMOTHERAPEUTIC AGENTS | -mitomycin-c- |
| | -porfiromycin- |
| | -adriamycin- |
| | -nitrosureas- |
| | -bleomycin- |
| HYPERTHERMIA | |

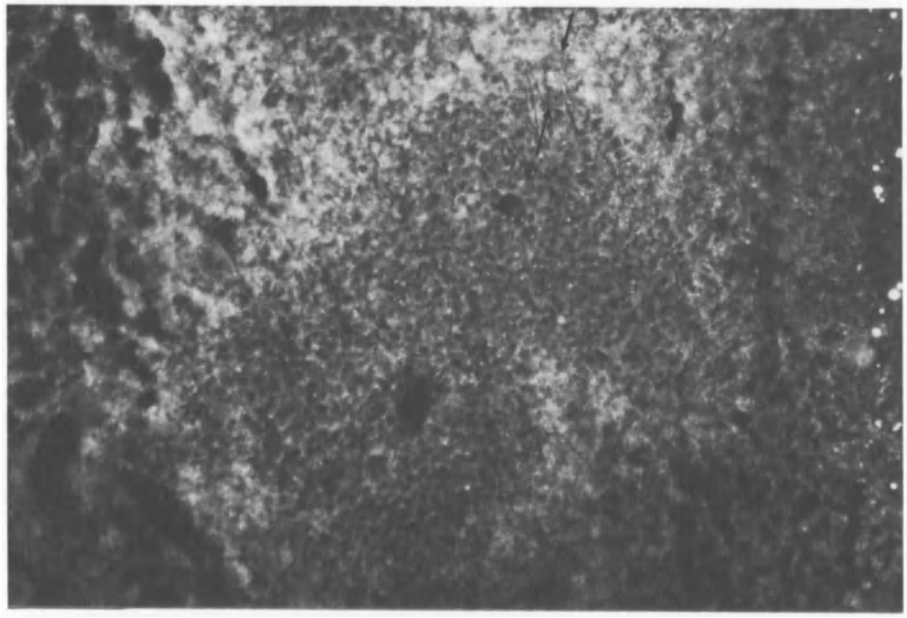

Fig.3 Ehrlich carcinoma,12th day after inoculation of Ehrlich ascites cells in the muscle parenchyma. Nile Red (fluorescence) staining. A cuff-like pattern, with an average radius of 150μm, is observed. Hypoxic cells occupy a 1-2 cell layer at the periphery of the cuffs (edge of necrosis)(arrows). They are recognized by hyghly fluorescent intracellular droplets of neutral lipids.

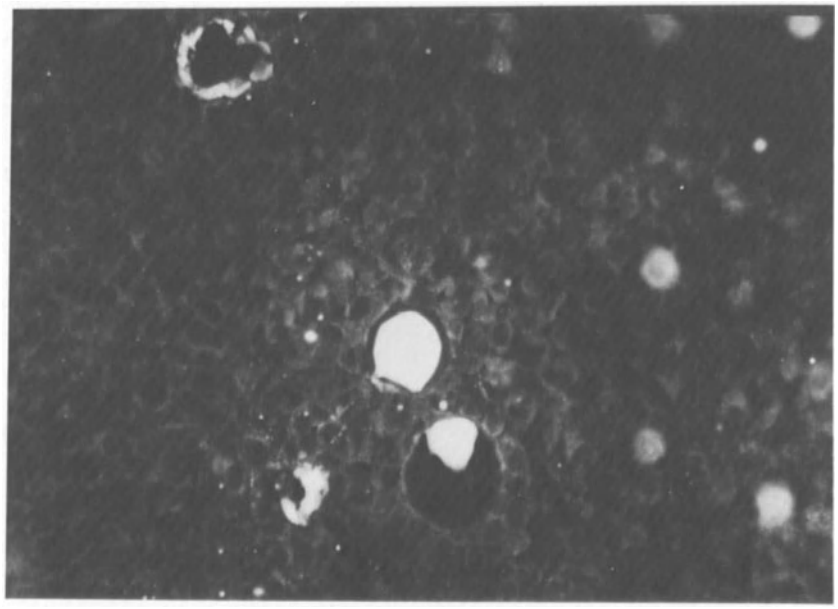

Fig.4 Ehrlich carcinoma,15th day after inoculation. Nile Red fluorescence. Tentacular formation of cells visualized by intense intracellular fluorescence of neutral lipids, and thus presumed to be hypoxic, arrive from the edge of necrosis in the direction of blood vessels, clogging them with lipid .

As shown in table 1, hyperthermia may be combined with some other means,and is potentially applicable to the two different hypoxias. The biological reason, for expecting a bigger effect of the heat on the tumors than in normal tissues is related to the poor vasculature of tumor.It is not the hypoxia per se that sensitizes cells to the heat ,but rather does it the combination of hypoxia,nutrient deficiency and acidosis which is charateristic of tumor cells distant from the vasculature(9). Tumor blood vessels are not able to respond to hyperthermia,by increasing their blood flow and therefore dissipating efficiently the heat,when locally applied. A further advantage results because tumor blood vessels actually collapse after moderate heat treatments, and the perfusion remains poor for at least 24 hs, leading to ischemia and nutritional death of many tumor cells(28).It must be indiced that in our personal experience, a tumor temperature of 50°C, maintened for 30 minutes or more, caused massive necrosis of the tumor mass,without any documented damage to healthy cells.This means that the repair system of the normal tissues(vascular reactivity,unaffected glucose metabolism pathways, efficiency of intracellular enzymes,namely DNA repair system) is sufficiently able to protect,within certain limits,the healthy tissues surrounding the tumor. A right application of heat can increase the kill of more tumor cells reducing the relapses. In our series of inoperable tumor patients, we obtained a certain number of complete documented tumor eradication,using in combination hyperthermia plus chemotherapy or radiotherapy.Therefore we can say that tumor cure is possible also in cases usually intractable by chemo or radiotherapy (51).

## BIOLOGICAL AND PHYSICAL MANIPULATIONS TO ENHANCE HEAT EFFECT

As outlined by COLEMAN,the environmental conditions,such as pO2,pH,and the nutrient concentration may be much more critical to cell's response to therapy,than are the intrinsic charactheristics of the cells(4). Different means can be used to increase the susceptibility to heat and to obtain modifications of the tumor microenvironment. We will describe the theoretical and pratical approaches on the following pages.

### Membranes modifications

Membrane integrity,the efficiency of Na-K pump,the selective or passive drug (chemo) intake,may play a role in metastatic spread. Membranes are one of the target organ to heat.We can increase the susceptibility using n3 fatty acids supplementation.

### n3 fatty acids supplementation

Tumors require fatty acids as oxidative substrates,for replacement of membrane lipid component and to manifacture the new membranes needed for growth and cell division.The fatty acids necessary for these purpose are obtained either by synthesis from glucose,or they can be supplied by the host as

free fatty acids(FFA) and for a less extent by triacylglycerols contained in plasma lipoproteins(50). Biological membranes consist of a lipid bilayer composed of phospholipids and cholesterol,with proteins embedded in this lipid bilayer.Proteins have important cellular functions,such as:receptor,transporter,enzyme. Their activity is modulated by the physical state of lipid bilayer.About 35-40% of fatty acyl chains normally making up the membranes are saturated,the remainder are unsatured and contain 1 and 6 double bonds.Any change in composition of the lipid bilayer may affect the normal barrier functions and the responsivness of the cells (36).It is possible that the charactheristic of neoplastic cell may change sufficiently to alter its growth and increase it sensitivity to certain forms of therapy,with metabolic manipulation . Most efforts to manipulate the composition of cell membranes have been obtained with polyunsatured fatty acids(PUFA), which cannot be synthetized de novo and therefore are obtained from nutrition (49).There are two main classes of PUFA:the n6 or plant derived class and the n3 or fish oil derived (45).PUFA manipulations change lipid bilayer fluidity and consequently the phase.transition temperature of proteins. This change in fluidity might have important consequences for neoplastic cells, influencing the uptake of nutrients ,of chemotherapeutic agents,and the heat sensitivity (for complete review of this aspect see Spector 1985,1987). GUFFY examined the response to heat of L1210 leukemic lymphoblasts enriched in culture with various fatty acids.Following enrichment cells were transferred to a standard medium ,heated and then tested for cytotoxycity by a clonogenic assay.Cells enriched in 22:6 medium, where more heat-sensitive than with other unsatured acids.The effect was greater at 42°C and dependent on the degree of the enrichment. These observations are in accordance with other experiments, where the enrichment in PUFA was accompanied by a change in fluidity(17).Enrichment of Escherichia Coli cells with linolenic acid(18:3) showed that cells were incapable of growth at temperature above 40°C,whereas cells supplemented with oleic acid(18:1)could grow at temperatures over 45°C (30). These findings were confirmed by others working on different cell lines:mammary carcinoma,ascite cells in culture and mouse fibroblasts. The lesson we may learn from these experiments is that the enrichment with PUFA is easily obtained, but the number of double bonds is critical,as is the physical state(49,50). Interesting is the use of n3 derivates,for their selective killing activity on human cancer cells.This effect is probably due to increased hydroperoxide production and PGs modification(2,3).In summary,changes in membrane fatty acid composition can influence the sensitivity of neoplastic cells to heat, but,the molecular mechanism responsible for this phenomenon is unknown.We are studying the effect of these PUFA on the tumor relapse and on the production of lymphokines, which seem negatively affected by these fatty acids(26).

## Anesthetics

Local anesthetics , expecially procaine and lidocaine, are membrane active drugs.The first observations on in vitro interaction between lidocaine and hyperthermia was reported by YATVIN et AL (10).They obtained a survival time threefold increased over control,injecting lidocaine into the tumor,

before heat treatment at 42-43.5°C.Similar results were reported by other authors. Some, found ,heat sensitization to lidocaine administrated e.v. at dose within the therapeutic range for control of arrythmias(43).Recent studies point to a direct action of anesthetic on membrane fluidity and membrane proteins interaction.This is confirmed by the works of KONINGS,who showed that procaine inhibits or delays thermotolerance ,a phenomenon not seen with PUFA (30). We use systematically lidocaine intratumorly and in metastatic nodules locally treatable.

## Microenvironment Modification

The tumor microenvironment is mainly the interstitial fluid between the malignant cells and the tumor microvasculature(24). The properties of the interstitial fluid is dependent on tumor blood flow,which can modify the tissue microenvironment,the pH,the oxygen tension and the nutritional status (48). The changes in microenvironment affect thermal sensitivity of the cells,their thermotolerance,their response to Xrays and drug cytotoxicity. These, heat induced,alterations in vascular function and microenvironment affect the fate of the tumor within the treated target volume. Inside the same tumor, the distribution of vasculature and the blood flow are heterogenous and complex,and appears less than in normal tissue. As outlined by JAIN, tumor blood flow is proportional to the pressure difference between the arterial and venous sides of tumor vasculature and inversely proportional to viscous and geometric resistance (23). Tumor blood modifications are the major key to changes in tumor microenvironment,and may be attained by hyperglicemia,blood flow decrease and pH sensitizing drugs(32).

## Induced Hyperglicemia

Malignant tumors metabolize carbohydrates mainly by anaerobic glycolisis,even under aerobic conditions, because they lack of certain enzymes and carrier systems.The oxidation of glucose stops at the stage of pyruvic acid, which may be metabolized to lactic acid,or entering in the mithocondria,to acetyl CoA.This, is used as precursor of lipid biosynthesis or for energetic substrates -ATP- (52). In the tumors anaerobic glycolisis is dominating, but general statements must be made cautiously,even if the glucose metabolism remains the most prominent metabolic difference between oxic and hypoxic cells(1).Under hypoxic condition,glucose is utilized rather inefficiently in anaerobic glycolisis and more glucose is needed to produce the same amount of energy than in aerobic condition. Hyperglicemia stimulates glycolisis and local decrease in tumor blood flow, producing an increase in lactic acid.Lactic acidosis leads to decrease in tumor extracellular pH, and tumor sensitization to the effects of hyperthermia, owing to activation of lisosomal enzymes (55). Decrease in tumor blood flow results from both systemic and local mechanisms.Systemic mechanisms include a reduction and redistribution of cardiac output, a "steal phenomenon" inhibiting tumor blood flow (23). Experimental observations

have shown that the reduction of tumor blood flow precedes the pH change and both are dependent on the glucose dose,lasting some hours(4-6 hs) after injection (23). Local mechanisms include increased red blood cells rigidity, white blood cells and platelets adhesion, increased interstitial pressure(24).ASHBY described results of continuos measurement of pH, in vivo, and the effect of raising blood sugar in melanoma nodules.The pH fall was associated with elevated blood sugar and occured very shortly after the start of dextrose infusion (10-40 minutes) (1).Same results have been obtained by Russian clinicians, who are using glucose infusion during hyperthermia,just from 1975(46,57).Dextrose infusion is usually performed with 10-20% solution(500-1000ml),half an hour before treatment, and will continue for all the treatment time.

## Blood Flow Decrease

A method to obtain an increase of the heat effect, is to lower perfusion pressure.The decreased blood flow renders the normally oxygenated cells acutely hypoxic.In this situation, depletion of nutrients ,accumulation of waste products and pH change might all contribute to tumor response to heat . It's possible to obtain decreased perfusion pressure using two methods: (A) using vasodilatators or vasoconstrictors;(B) clamping the major artery supplying the tumor.Tumor vasculature is a composition of newly formed vessels and of the host vessels surviving in the tumor. Tumor vasculature is a high resistance bed with no contractile capacity, for the absence of adrenergic innervation(21,23).So tumor blood flow may be decreased using vasoconstrictor drugs as adrenaline. Experimental observations,however, showed that the decrease of blood flow was a passive one, depending on blood flow decrease through surrounding normal tissues. Similarly,vasodilatator drugs, reducing blood pressure, may reduce tumor blood flow, but such effect is compensated by physiologic responses,as increase in heart rate. The most apparent difficulty in employing such drugs is the variability of response of tumor blood flow and on absence of a model(24). A more promising approach is the occlusion of the major artery supplying the tumor.There are experimental and clinical evidences for the use of this technique (21). The concomitant administration of hyperthermia and local intraarterial chemotherapy has shown an impressive response with no adverse effects.In the liver tumor, this association is somewhat effective if the lesions are large and few. The theoretical reasons for this enhancement of chemotherapy and hyperthermia assiociation are:(a)the occlusion of the artery supply renders the tumor relatively ischemic enhancing the heat effect;(b)the reduction in circulation prevents the rapid reabsorption of chemotherapeutic agents;(c) the killing power of chemotherapeutic drugs is not only related to concentration but also to the duration of contact with tumor cells. We use this technique for tumor not resectable ,and for tumor locally treatable, with injection of chemotherapeutic drugs inside the tumor mass.In this way we obtain a higher concentration in the tumor and regional lymph nodes as comfirmed by experimental studies with radioctive labelled bleomycin injected into mouse breast cancer (31).

## Glucose inhibitors and energy

As outlined, tumor cells obtain their energy supply predominantly by glucose .Inhibition of .this pathway induces lactate levels increase with extracellular pH decrease,enhancing thermal sensitivity. SONG and KIM,using 5-thio-d-glucose, an inhibitor of glucose metabolism and transport, demonstrated a decrease in energy production and a reduction of viability of hypoxic cells (47). A combination between 5-thio-d-glucose and hyperthermia may enhance considerably the cell killing.In our laboratory, we are testing different concentrations of the molecule,hoping in an increased heat effect.

Lonidamine (1-(2,4-dichlorobenzyl)-1-h-indol-3-carboxylic acid),is a selective inhibitor of aerobic glycolisis (13). In an experimental study, lonidamine reduced the oxygen consumption in both"normal"and neoplastic cells.This molecule inhibits selectively the mitochondrial bound hexokinase which is absent in normal differentiated cells.The net effect is a decrease in energetic substrates (ATP)rendering the cell anoxic. SILVESTRINI et al,reported a thermal enhancement in vitro and in vivo of lonidamine at low concentration. KIM reported a futher potentiation at lower pH conditions. HAHN reported a more effective inhibition when the drug was used before and after an exposure to Xrays (19).The mechanism responsible for this effect is not known,a reasonable hypothesis is that recovery from Xrays application is a energy requiring process.These results may have important therapeutical applications. Undesired effect of the drug, used at the concentration of 450-1200 mg/day are: nausea, vomiting,cramps arrhytmias;its use is difficult in the elderly.

## Biological Response Modifiers(BRM) and thermoimmunotherapy

Host immune system and tumor growth- Cancer is a disease of the host.The dissemination and the growth of tumor cells are dependent at last partially on the host immune system(39).As outlined by FIDLER,metastasis are the major cause of death from cancer and of treatment failure(12). Tumor-cell resistance to conventional therapeutic modalities, is the single most important reason for the lack of success in treating cancer. A method to circumvent the cellular heterogeneity and to eradicate the resistant tumor cells is to stimulate the host immunitary system.Tumor growth is the result of an equilibrium between the host defense system and the ability of tumor cells to escape this control, using blocking mechanisms.Malignant transformation is accompanied by membrane changes, with gain and loss of surface antigens, which influence cell-cell interaction within the host.The majority of cancer cells are heterogeneous, genetically unstable, subjet to phenotypic change and expressing consequently a weak host immune response(11).The host immune

defense against tumors is cell mediated. T cells play a large role in this process because they can adoptively transfer tumor immunity and cause tumor regression in a major histocompatibility complex(MHC) restricted fashion.Natural killers(NK) ,macrophages and in certain circumstances Bcells have an antitumor effect.Another potent antitumor effector phenomenon,Lymphokine Activated Killers(LAK),have the capacity to lyse NK-resistant tumor cells in on MHC-unrestricted fashion(37).The increase in knowledge about tumor immunology and biothecnology has opened new treatment modalities . Biotherapy can be effective alone or in association with surgery, chemotherapy, radiotherapy and hyperthermia.As outlined by OLDHAM, biotherapy can have activity not only on imperceptible disease or a tumor mass less than $10^6$cells, but also on a clinically apparent disease(39).Different are the immunomodulators used.WE will describe the followings and the interaction with hyperthermia.

## Thymic extracts

Thymic extracts are a family of polypetides , responsible for the normal maturation of the various subclasses of T lymphocytes.The complex process of thymocytes differentiation involves a number of independent steps, regulated by a specific thymic extract. Some thymic extracts have shown immunorestorative effects on T cells in different clinically immunodeficiency states.A study on 200 neoplastic patients treated with immunotherapy and hyperthermia have shown an increase in the total number of lymphocytes,an alteration of the ratio helper/ suppressor and a decrease of opportunistic infections (42). Several clinical trials with thymic extracts, following radiation therapy to head, neck and lung, have shown a reconstitution of T cell function.The same results have been obtained in patients receiving cyclic combination chemotherapy and thymic extracts (56).

## Interferons

Interferons are small biologically active glycoproteins, with antiviral , antiproliferative and immunomodulatory activities.They are classified as interferon :alpha,beta,gamma An important effect is the antiproliferative action,with increase of all cell cycle phases and prolongation of the overall generation time.Interferons have demonstrated a differentiative effect and an increased expression of HLA antigens.This increased surface antigens expression may play an important role in tumor control and in metastasis diffusion (14). Clinical trials have documented that the response is dose dependent and that not all tumors are responsive. Another interesting application is the intralesional treatment. This holds promise for an association with hyperthermia for malignancies as melanoma, breast, bladder cancer and topically for cervical carcinoma in situ (56).

## Lymphokines

Among the different soluble mediators, produced by activated macrophages, Interleukin -2 (IL-2) has gained a great relevance for tumor treatment. IL-2 is a 15Kd immunoregulatory glycoprotein, which incubated with human lymphocytes generates a population of cells capable of lysing fresh tumor cells in vitro. This phenomenon ,termed LAK, distinguishs these cells from cytotoxic T lymphocytes and from NK cells. LAK cells are cytolytic for a broad range of fresh and cultured human tumors, do not attack normal cells and are not HLA restricted in their specificity. LAK cells constitute a heteregeneous population, in part derived from Tcells and NKcells (37).Extensive animal studies, have demonstrated that LAK cells cause the regression of established tumors and inhibit the growth of metastasis. The animal model has indicated some consequences for maximum effect on humans. These are: 1)cells should be cultivated in high dose IL2 for 3-4 days before infusion, 2)a calculated dose at least $10^{11}$ LAK cells would probably be necessary for treating human tumors, 3)the mechanism of LAK action in vivo is not perfectly known. The current hypothesis is that activated cells migrate to tumor sites, expand in situ under the influence of exogenous IL2 and destroy tumor cells either directly or indirectly by cytolysis(29).In a study done by ROSENBERG and MULE' on selected patients not responsive to convenctional therapy,it has been obtained with adoptive transfer of LAK cells plus IL2 or IL2 alone a complete (10%) or partial regression (20%) of advanced metastatic cancer(37).The toxicity associated with LAKcell-IL2 therapy is substantial. The most severe effects appear related to dose and to the method of IL2 administration(29). MULE'and ROSENBERG have obtained a reduction of doses, of toxic effects and an increase of antitumor activity, combining IL2 with IFN alpha, monoclonal antibodies,tumor infiltrating lymphocytes, TNF, and other cytokines.In our opinion an association hyperthermia, IFN,IL2 or Thymic hormones may promise beneficial effects on cancer patients.

## Non specific immunomodulators

The CALMETTE-GUERIN-BACILLUS, under certain conditions of dose and time, leads to macrophages activation(14).The results of large number of studies are controversial, but controlled trials have shown an increase of second remissions and prolongation of survival in leukemic patients. Local administration seems to give relatively reproducible results (i.e. intravescical administration in bladder cancer) (39). CIMETIDINE,H2 receptor antagonist, induces enhanced cell-mediated immunity and shows a synergistic effect with interferon in myeloid leukemia, myelodysplastic syndrome and melanoma (39). LEVAMISOLE,inhibits T-suppressor cells and induces a significant prolongation in breast cancer and myeloid leukemia. CORYNEBACTERIUM PARVUM can activate macrophages and is usually associated with thymic extracts, influencing positively the disease trend. BIOSTIM, a glycoprotein obtained from Klebsiella Pneumoniae, stimulates natural cytotoxicity and IL1 production by large granular lymphocytes. In vitro it enhances neutrophil, T,B and

macrophage functions. It protects against bacterial and viral infections, but is unable to correct the NK cell function deficit in patients with large tumor mass(39).MURAMYL DIPEPTIDE (MDP): is a bacterial peptidoglycan derivative.It augments T cell activity and macrophage release of O2 and reduces tumor metastasis in animals. FIDLER using MDP with interferon and MAF encapsulated in liposomes succeded to activate macrophages in vivo.Following intravenous administration, the majority of liposomes (80-90%) are taken up by reticuloendothelial cells in the liver, spleen, lymph nodes, bone marrow and circulating monocytes.The in situ activation of macrophages result from the direct interaction of liposomes with macrophages.Activated macrophages discriminate between normal or tumorigenic cells. This discrimination is species specific, independent of transplantation and tumor associated antigens. Host macrophages recognize,lysed tumor cells and play a role in determining the outcome of tumor metastasis (12). ZINC can regulate the T4/T8 ratio and in immunocompromised cancer patients in remission, normalises the helper function and improves the NK activity(33).VIT A and its analogs RETINOIDS affect the proliferation and differentiation of many normal and malignant cells.They augment the cytotoxic and helper T cells numbers and functions, and inhibit prostaglandin synthesis by host tumor activated macrophages. VITAMIN E and SELENIUM augment the resistance to infections and increase the activity of T helper (27).

## IMMUNOLOGICAL ASPECTS OF HYPERTHERMIA

Experimental data show the regression of metastasis following curative heating of the primary tumor.The reverse process has been demonstrated (7).When the tumor cells are pre-heated the tumor graft in animals is difficult, probably because of an increased expression of the suface antigens on the tumor cells. In some instances a local immunologic reactivity with lympho-plasmacytic infiltration and increased complement dependent cytotoxicity has been documented in heated tumors. DICKSON has shown a difference between local tumor heating and total body hyperthermia.He obtained a decreased survival in animals treated by local heating followed by total body hyperthermia (7). This tumor growth enhancement may be dependent from NK cells activty depression.Observations in this sense have been documented incubating lymphocytes at 39°-40°C for more than 4 hs(40).Not all the T cell lineage is equal responsive to heat.The NK heat damage appears not to be reparable, where Tcells seem easier repared. This study may explain the increase of frequency of distant metastasis in some animal tumor system, but the conditions seem more applicable to total hyperthermia than to local heating. In man,many regressions of distant metastasis after treatment of the primary tumor are documented. As outlined by GREENSTEIN "the key to cancer problem lies in the host-tumor relantionship". Since the characteristics of growing tumor are continually changing and the primary tumor may influence the number and the rate of growth of its metastasis, heat therapy must be viewed as a perturbation of this dynamic state(16).The host defense system may influence the response to

heat.Hyperthermia combined with BRM, induces : NKcells activation in vivo, LAK cells induction and increase of IL1, IL2, IFN alpha in the peripheral blood.This improves immune reactivity and can deter the development of infections and collateral effects caused by the disease and therapies, amplifying the documented local immunological changes caused by hyperthermia (See fig.5). Clinical study on thermoimmunotherapy ( with combination between heat and interferons, thymic extracts or bacterial derivatives) were undertaken in advanced prostatic cancer , melanoma, breast cancer and other neoplasms. The available results indicate that the number of responders is higher in groups treated with immunotherapy than those treated with other modalities(41,54). Although the observations are mostly uncontrolled and some randomized trials still in progress, many authors suggest that thermoimmunotherapy offers a real alternative for advanced neoplasms and opens a new field for experimental and clinical investigation.

## CONCLUSIONS

Based on actual observations,hyperthermic treatment of the tumor can be used in combination with chemotherapy,irradiation and immunotherapy.This association is based on strong bilogical rationale.Hyperthermia alone seems not able to eradicate the tumor mass,but in synergism with chemo, radio and immunotherapy has an enhancing effect for its selectivity on acidotic,hypoxic,radioresistant cells and tumor abnormal vasculature (22,34).The heat has marked effects on the tumor structure, lysing the cancer cells, destructing the neoformed capyllaries and boosting the immunitary response.The hyperthermia may be combined with different therapeutic modalities in the following ways:

CHEMOTHERAPY:a) generalized
b) selective intra-arterial
c) local intralesional application

RADIOTHERAPY:a) external beams
b) local treatment(brachytherapy)

IMMUNOMODULATION:a) active aspecific
b) adoptive
(active aspecific are: thymic extracts,bacterial extracts, adoptive are:interleukines,cytokines,LAK)

It may also be used with hormonotherapy(breast and prostate cancers) potentiated by metabolic drugs(i.e. glucose inhibitors),by membrane modifier substances(anesthetic,PUFA) or by metabolic manipulations tending to decrease the tumor pH. One of the most interesting application of hyperthermia is its use as neoadjuvant presurgical cytoreductive therapy. In our experience (411 patients treated in the period 1980-1989,affected by inoperable tumors) the 4.3% had a radical cure,the 51.8% had a clinical response (documented total regression, partial or minor response,long term stabilization of disease lasting more than 18 months).It must

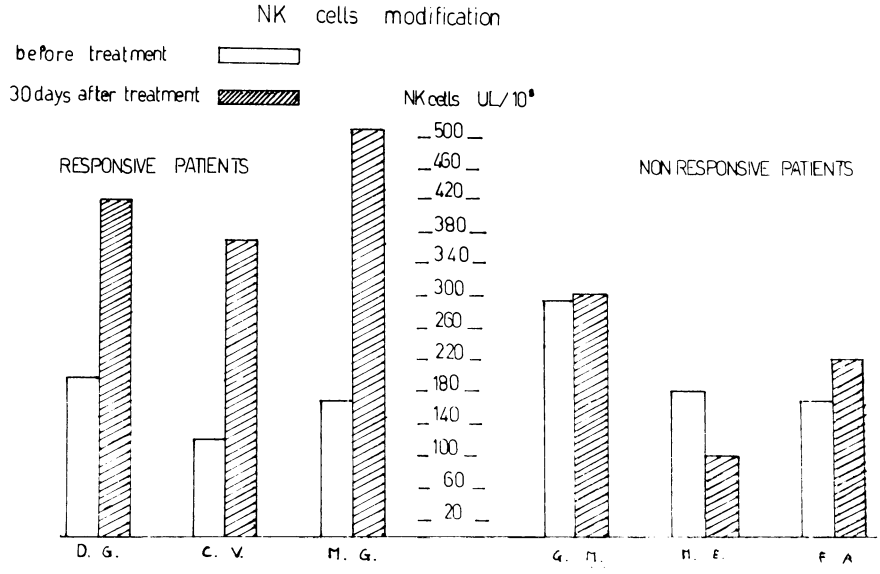


Fig.5 LOCALIZED HYPERTHERMIA PLUS IMMUNOTHERAPY(i.m.TP5)
In the fig.5 is illustrated the enhancing effect of hyperthermia plus thymic extracts on NK cells. In the responsive patients it has been obtained a 100% increase 30 days after treatment.

be noted that the treated cases were poorly sensitive to the conventional therapies(41). The utilization of the hyperthermia, with selective chemotherapy,local radiotherapy and with Biological Response Modifiers may overcome: 1)the adverse effects increasing the doses of chemotherapy and radiotherapy 2) the early appeareance of resistant clones. Our retrospective study shows that the biological factors negatively correlated with the response to therapy are: high alpha 2 glycoprotein level > 1.0g/l;high sedimentation rate > 50/1h;low level of total lymphocites < 500/mc.; T helper/T suppressor imbalance ratio < 1.2.All the patients with these hematological data failed to obtain remission.

Our suggestions about hyperthermia use in 1990 are : hyperthermia must be used in association with other modalities. Its use as palliative treatment in preterminal disease states should be avoided. The schemes utilizing hyperthermia plus chemo or radiotherapy could be used in advanced stages. In the elderly, in preoperative neoadjuvant utilization, and in localized disease, hyperthermia could be advantageously combined with immunotherapy, in order to obtain a stabilization of the disease, with good performance status. In some instances a complete tumor eradication can be documented.

REFERENCES

1) B.S. Ashby: pH Studies in Human Malignant Tumors.Lancet,i:312-315 1966

2) M.E.Begin,U.N.Das,G. Ells,D.F. Horrobin:Selective Killing of Human Cancer Cells by PUFA. Prostaglandins Leukotrienes and Medecine,19: 177-186,1985

3) M.E.Begin,G. Ells,D.F, Horrobin:PUFA and Induced Cytotoxicity Against Tumor Cells and Its Relationship to Lipid Peroxidation. J.N.C.I,80:188-194,1988

4) C.N.Coleman:Hypoxia in Tumors:A Paradigm For The Approach To Biochemical and Physiologic Heterogeneity. J.N.C.I, 80:310-317,1988

5) J. Denekamp:Physiological Hypoxia and Cancer Therapy. Aggiornamenti Di Radiobiologia. Vigo Curti Ed.1984

6) M.W. Dewhirst:Will Hyperthermia Conquer The Elusive Hypoxic Cell? Radiology,137:811-817,1980

7) J.A. Dickson,S.A. Shah:Immunologic Aspects of Hyperthermia. pp487-543 in "Hyperthermia in Cancer Therapy"K. Storm ed.G.K. Publ 1983

8) T.E. Dudar, R.K. Jain:Differential Response of Normal and Tumor Microcirculation to Hyperthermia. Cancer. Res.,44:605-612,1984

9) B. Enami,G.H. Nussbaum,R.K. TenHaken, W.L. Hughes:Response of Capillary Blood Flow and Structure to Local Tumor Heating.Radiology, 137:805-809,1980

10) R. Engelhardt:Hyperthermia and Drugs.Recent Results in Cancer Research, 104:137-204,1988

11) M. Feldman,L. Eisenbach:Genes Controlling The Metastatic Phenotype.Cancer Surveys,7:555-572,1988

12) I. Fidler:Macrophages and Metastasis.A Biological Approach to Cancer Therapy : A Presidential Adress.Cancer. Res,45:4714-4726,1985

13) A. Floridi,M.G. Poggi,M.L. Marcante,B. Silvestrini,A. Caputo,C. De Martino:Lonidamine a Selective Inhibitor of Aerobic Glycolysis of Murine Tumor Cells. J.N.C.I.,66:497-499,1981

14) K.A. Foon:Biological Response Modifiers: The New Immunotherapy. Cancer Res, 49:1621-1639,1989

15)I. Freitas,P. Pontiggia,S. Barni et al:Histochemical and Ultrastructural Probes for Crhonic Hypoxia.Some Hints for The Chemotaxis of Hypoxic Cells. Submitted for Publ.

16) J.P. Greenstein:"Biochemistry of Cancer" Academic Press 1955

17) M.M. Guffy,J.A. Rosenberg,Z. Simon,G.P. Burns: Effect of Cellular Fatty Acids Alteration on Hyperthermic Sensitivity in Cultured L 1210 Murine Leukemia Cells.Cancer. Res,42:3625- 3630,1982

18) J. Halle,L.R. Roizin-Towle:Biological Effect of Heat.Cancer.Res,44 :4708s-4713,1984

19) G.M. Hahn,I.VonKersen,B.Silvestrini:Inhibition of Recovery from Potentially Lethal Damage by Lonidamine.Br.J.Cancer,5o:657.660, 1984

20) T.S. Herman,B.A. Teicher,M. Jackson,J.Clark,G. Svensson,C.N. Coleman :Rationale for The Use of Local Hyperthermia with Radiation . Hyperthermia Therapy and Selected Anticancer Drugs in Locally Advanced Human Malignancies.Int.J.Hyperthermia,4:143-158,1988

21) B.S.A. Hill,J. Denekamp:The Effect of Vascular Occlusion on Thermal Sensitization of Mouse Tumor Br.J.of Radiology,51:997- 1002,1978

22) S.A. Hill,J. Denekamp:Therapeutic Benefit from Combined Heat and Radiation .Recent Results in Cancer Research,109:28-35,1988

23) R.K. Jain:Determinants of Tumor Blood Flow,A Review.Cancer. Res. 48:2641-2658,1988

24)R.K. Jain,K. Ward-Hartley:Tumor Blood Flow-Characterization Modifications in Hyperthermia.IEE Transactions and Sonic and Ultrasonics , 31:504-526,1984

25) M. Janak,S. Szmigielski:Alteration of Immune Reactions by Whole Body and Local Hyperthermia in Normal and Tumor

Bearing animals. Review of Own 1976-1980 Experiments. Br.J.Cancer,45:122s-126s,1982

26) R.A. Karmali: n-3 Fatty Acids and Cancer.Journal of Internal Medecine , 225:197s-200s,1989

27) L. Kiremidjian,G. Stotzky:Selenium and Immune Response. Environmental Research,42:277-303,1987

28) S.H. Kim,J.H. Kim,E.W. Hahn:Enhanced Killing of Hypoxic Cells by Hyperthermia. Br.J. of Radiology,48:872-874,1975

29)H.G. Klein,S.F. Leitman:Adoptive Immunotherapy in The Treatment of Malignant Disease. Trasfusion,29:170-178,1989

30) A.W.T. Konings:Membranes as Target for Hyperthermic Cell Killing.Recent Results In Cancer Research.109:9-22,1988

31) H.H. LeVeen,P.R. Rajogopalan,I. Vujic,R.P. Gabien,P.H. Brien,R. Hutto,P. Pontiggia,N. D'Ovidio,R. Pethig ,D. Armitage:Radiofrequency -Thermotherapy,Local Chemotherapy and Arterial Occlusion in The Treatment of Non Resectable Cancer. The American Surgeon,50:61-65, 1984

32) G.C. Li:Thermal Biology and Physiologic Clinical Hyperthermia.Current State and Future Needs. Cancer. Res,44:4886s-4893s,1984

33) G. Mathe' et al. : The Effect of Zinc on Normal and Neoplastic T-Lymphocites Proliferation. Med. Oncol&Tumor PharmacoTher,2:203-210,1985

34) M. Molls,E. Scherer:The Combination of Hyperthermia and Radiation :Clinical Investigation. Recent Results in Cancer Research,104: 110-136,1987

35) J.E. Moulder,S. Rockwell:Tumor Hypoxia :Its Impact on Cancer Therapy . Cancer Metastasis Reviews,5:313-341,1987

36) C.E. Mountford,L.C. Wright:Organization of Lipids in The Plasma Membranes of malignant and stimulated cells :A New Model.TIBS 13:172 -177,1988

37) J.J. Mule',S. Rosenberg:Immunotherapy with Lymphokines Combination.Important Advances in Cancer and Oncology 1989

38) G.L. Nicolson:Tumor Cell Instability,Diversification and Progression to Metastatic Phenotype:from Oncogene to Oncofetal Expression. Cancer. Res,47:1473-1487,1987

39) R.K. Oldham:Biotherapy :General Principles. "Principles of Cancer Biotherapy" R.K. Oldham ed. Raven Press.1987

40) M. Onsrud:Effect of Hyperthermia on Human Natural Killer Cells. Recent Results in Cancer Research,109:50-57,1988

41) P. Pontiggia,A.Introzzi:The Therapeutic Enhancement caused by Hyperthermia. Medecine Biologie Environment,16:499-506,1988

42) P. Pontiggia:Il Dilemma Terapeutico Dei Tumori Maligni. Federazione Medica,4:235-244,1989

43) H.I. Robins,J.S. Slattery,T.A. Lange,M.B. Yatvin: Systemic Lidocaine Enhancement of Hyperthermia-Induced Tumor Regression in Transplantable Murine Tumor Models. Cancer.Res,43:3187- 3191,1983

44) S. Rockwell:Hypoxic Cells as Target for Cancer Chemotherapy. "Development of Target Oriented Anticancer Drugs"Y.C.Cheng ed. Raven Press 1983

45) H.M. Sinclair:Essential Fatty Acids in Perspective.Human Nutrition. Clinical Nutrition,386:245-260,1984

46) V. Shapot:Biochemical Aspects of Tumor Growth. MIR Publ.MOSCOW,pp:44-50,1980

47) C.W. Song,J.J. Clement.S.V. Levitt:Cytotoxic and Radiosensitizing Effects of 5-thio-D-glucose on Hypoxic Cells. Radiology,123:201-205, 1977

48) C.W. Song:Effect of Local Hyperthermia on Blood Flow and Microenvironment, a Review. Cancer.Res,44:4721s-4730s,1984

49) A.A. Spector,C.P. Burns:Biological and Therapeutical Potential of Membrane Lipid Modification in Tumors. Cancer. Res,47:4529-4537, 1987

50) A.A. Spector,M.A. Yorek:Membrane Lipid Modification ,Composition and Cellular Function, Journal of Lipid Res,26:1015-1035,1985

51) C. Streffer, D. vanBeuningen: The Biological Basis for Tumor Therapy by Hyperthermia and Radiation. Recent Results in Cancer Research, 104:24-70,1987

52) C. Streffer:Metabolic Changes During and after Hyperthermia. Int. Journal of Hyperthermia,1:305-319,1985

53) C.D. Stubbs, A.R. Smith:The Modification of Mammalian Membrane PUFA Composition in Relation To Membrane Fluidity and Function. Biochimica et Biophysica Acta,779:89-137,1984

54) S. Szmigielski, S. Zaboklicki,J. Gil,J. Jeljaszewicz,G. Pulverez: Inhibition of Lewis Lung carcinoma in Mice by Local Microwawe Hyperthermia Combined with Immunomodulating Propionibacterium Granulosum KP45. Cancer Immunology Immunotherapy,16:151-156,1984

55) P. Vaupel,F: Kallinowski:Physiological Effects of Hyperthermia. Recent Results in Cancer Res,104:71-110,1987

56)J. Wagstaff,M. Kees:Lymphokines and Cytokines. Cancer Chemotherapy and Biological Response Modifiers.Annual 10 H.M. Pinedo,D.L. Longo,B.A. Chabner ed. Elsevier 1988.

57)S.P. YARMONENKO :RADIOBIOLOGY of Humans and Animals. MIR Pub: MOSCOW, pp:362-366,1988.

# PERSPECTIVES AND HOPES FOR THE 1990s

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Today the management of malignant tumors is to apply an integrated approach to the particular disease using hyperthermia alone and in combination with other modalities.

In fact thermotherapy, particularly associated with radio-,chemo- and immunotherapy, represents a valid aid in cancer therapy, especially when the traditional therapies have failed or can no longer be applied.

On the other hand, the use of immunomodulating drugs (interleukin, interferon, monoclonal antibodies), allows the physician to utilize the immune system in the treatment of cancer. Hyperthermia has no negative effects on the immune system.

The goal of cancer therapy to prolong life of patients, killing or reducing the neoplasm with a complete or partial remission, maintaining the general conditions and the "performance status" of patients for a long period, is particularly important for a good quality of life.

In these last years the progress in the fields of experimental and clinical hyperthermia show significant improvement in the technical equipment and in their applicative methods, in thermosensitizing drug use, in patient selection and in the appropriate timing to give the hyperthermia therapy, although sure and effective protocols are not ready.

We think the most promising and qualifying points of hyperthermia in the next decade are these three:

1) The stabilization of malignant tumors
2) Intraoperative hyperthermia
3) The possibility to evaluate the temperature in deep tumors by the non-invasive magnetic resonance imaging system.

In regards to the first consideration, it is interesting to give prominence to the fact that we can

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obtain these responses after hyperthermia treatment: no response, complete or partial remission, stabilization of the tumor. In regards to this last response, we would like to emphasize the importance of these results: the possibility to obtain an arrest in tumor growth in 35 to 40 percent of treated patients with a good "performance status" and a good quality of life, with a follow up from six months to several years, represents without a doubt a new aspect in the response to cancer therapy. One must consider that these cases had no longer responded to other modes of therapy.

The exact pathogenetic mechanism of this phenomenon is not yet clear (maybe the immunological system is involved); however, patients that no longer respond to radiation or chemotherapy can live after hyperthermia in good condition despite the presence of very significant residual disease. In particular, in our experience, the stabilization of hepatic tumors (especially secondary from digestive tract), lung tumors and pelvic tumors are numerous.

We were pleasantly amazed at the possibility for us to operate previously inoperable tumors, which now can become sealed and operable because of hyperthermia, and in some cases prolong life and life's quality. We think traditional therapies could also be administered in association with hyperthermia when more treatment is non-effective alone or because of too much immunodepression.

In regard to the second point, we emphasize the importance to obtain the maximum level of therapeutic hyperthermia inside the tumor (6); the problem of reaching 41.5 degrees centigrade to 42 degrees centigrade is not always solved especially in patients in which the tissues between the generators and tumor represent an excessive impediment to the heat penetration. The interstitial applicators and microwaves, or radio-frequency intraoperatory electrodes, applied from many authors [Emami (2), Kushiro (4)], allow to be more detectable in reaching deeper tumors (lung, pancreas, bile duct).

In the future, if a surgeon will extend the indications to the pre-intra or post-surgical treatment with associated therapies [La Veen (8)], we think a big step in the control of tumoral growth will be made. Surgery must be included more and more in the context of a multidisciplinary treatment, in which the extirpation of an operable tumor will be a fundamental step. Now, considering that the neoplastic disease is characterized of a systemic involvement of the entire organism, it will be necessary to intervene with other associated treatment (3). If the tumor appears to be inoperable only at the time of exploration, it is possible to suggest a cytoreduction with implant of intraoperative radiation and hyperthermic applicators so that the local complete control of the tumor may be possible at this time.

However, on the other hand, the advantage of the intraoperative insertion of applicators will be doubtly useful: the reaching of the neoplastic time with therapeutic

temperatures through the applicators will be accompanied by the possibility to insert thermometers inside also or beside the tumor. Without doubt this method is a warranty of reaching effective temperatures in many organs.

In regard to the third point about the non-invasive amelioration of temperature measurement, recent studies [Charny (1), Le Bihan (5)], suggest the very first interesting results: the association of hyperthermia and magnetic resonance imaging system. This system consists of a modified mini-annular phased array designed for the heating of human limb tumors and a whole-body magnetic resonance imaging system. Temperature images are obtained non-invasively with high accuracy (0.5 degrees centigrade resolution using two 3.5 minute scans), using images of molecular diffusion. The equipment for heating has been modified to be compatible with the magnetic resonance unit by removing all of its original ferromagnetic parts and rewiring it. The compatibility of the modified system was tested using a phantom made of a doped acrylamide gel. Temperature MR images agreed very well with temperature measurements obtained from fiber-optic probes placed inside the gel. The temperature resolution was found to be better than 0.5 degrees centigrade with a spatial resolution better than 1 cm. The possibility to realize simultaneously an immediate comparison of temperature and of images, now still in experimental stage, offers a perspective for measuring the temperature also in deep tumors. Maybe many problems have divided physicians and physicists till now and have limited an adequate development of hyperthermia in the world will be sourmounted with this method.

In conclusion, really hyperthermia is an effective tumoricidal agent in man and animals. However, many questions remain with respect to how to best utilize this modality of treatment [Skibba (10)]. What disease should be treated and when, what technique should be used and should some modifiers be used in conjunction with heat, additive therapy, or increasing the tolerance of normal tissues., are all questions for future research. How we can make the results predictably better and which is the effort to make a treatment in the best manner on a single patient is the subject of this conclusion.

High tumor temperatures must be reached in less than 30 minutes and sustained for 2 1/2 to 3 hours. The aim of the hyperthermia users must be to kill off the cancer in the first new treatments. The number of thermosensitizing drugs is increasing, and methods to make the tumor more sensitive to heat have found interesting application. The specific absorption rates of the cancer can be dramatically increased by the injection of ferrite, a powered ceramic. Such ferrites are used in microwave ovens to convert microwaves to heat. Powered ferrite is suspended for injection into the cancer with a needle and syringe along with chemotherapy.

In regards to drugs which prevent glucose entry into cancer cells, these drugs lower the cells' vitality and make them susceptible to chemotherapy. The life of the cell is

directly correlated to glucose's utilization, and so the sensitivity to heat and to chemotherapic drugs will increase if intracellular pH and metabolic induced suffering will be present. Glucose blockers suppress cellular repair of sublethal injury caused by heat and chemotherapy. Multidrug resistance to chemotherapeutic agents is caused by an active membrane pump which ejects these poisons from the cells. The pump utilizes ATP, but cancers deprived of glucose can not sustain intracellular ATP. It is easy to understand how these drugs can be the drugs that will aid future therapy [La Veen (7)].

At least, we remember differential temperatures between normal adjacent tissue and cancer during R.F. heating is usually 3 degrees to 4 degrees centigrade. This can be increased by selective reduction of the blood flow through the cancer. Temporary or permanent occlusion (9) of the artery supplying the region selectively reduces blood flow through the tumor because the perfusion pressure drops distal to the occlusion. Blood ceases to flow through the high resistance vascular network of the cancer. In this manner the embolized tumor is more sensitive to heat and this fact represents undoubtly another interesting tool to realize a more effective thermotherapy.

REFERENCES

1) Charny C.K. et al.: Med. Phys., 15: 17, 1988. Emami B. et al.: Interstitial thermoradiotherapy in the treatment of recurrent/residual malignant tumors. Am. J. Clin. Onc. (CCT) 7, 699-704, 1984.

3) Koga S. et al.: Total-body hyperthermia and chemotherapy, Vol. III, Chap. 8, Hyperthermia in Cancer Treatment, CRC Press, 1986.

4) Kushiro H. et al.: Lecture, XII International Symposium on Clinical Hyperthermia - Rome, 1989.

5) Le Bihan D. et al.: Mapping of temperature using IVIM MR Imaging of diffusion: application to hyperthermia. SMRM Meeting, 1988.

6) Lele P.P.: Lecture, XII International Symposium on Clinical Hyperthermia, Rome 1989.

7) Le Veen H.H.: "Optimizing Hyperthermia" Magistral lecture, XII International Symposium on Clinical Hyperthermia, Rome 1989.

8) Le Veen H.H. et al.: Radiofrequency thermotherapy, local chemotherapy and arterial occlusion in the treatment of non-resectable cancer. Am. Surg., 50, 61, 1984.

9) Madoule P. et al. Chemio-embolisation: principles and perspectives, Microencapsulation, 1, 21-25, 1984.

10) Skibba J.L. et al.: Hyperthermia of liver: Vol. III, Ch. 3, Hyperthermia in Cancer Treatment, CRC Press, 1986.

# CONTROL OF THE PHYSICAL PARAMETERS IN LOCAL ELECTROMAGNETIC HYPERTHERMIA

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## ABSTRACT

The evaluation of the clinical results in experimental applications of local electromagnetic hyperthermia requires strict control of the physical parameters of the treatment and adhesion to close medical protocols. Development and application processes in close cooperation between physicians and electromagnetic experts are reported and discussed, on the basis of experimental and theoretical research work. Attention is concentrated on a 434 MHz apparatus, where the fundamental physical limits have been identified and the operating features in the clinical practice have been evaluated. The cases eligible for treatment were chosen according basically to the limitations posed by depth, volume and steep temperature gradients. Several cases are reported mainly in association with radiotherapy. Lines of technical improvement are suggested, mainly based on the development and optimization of lower-frequency applicators.

## INTRODUCTION

Several clinical and engineering results must be pursued and ascertained before the promise of electromagnetic hyperthermia as a simple and agile technical aid for treating localized lesions with very minor or no invasivity can be effectively implemented. First and most fundamental of all, the therapeutic efficacy is to be analyzed and the effective potential ascertained. Effects have been observed and defined. Yet a larger basis of data possessing adequate homogeneity is required, both from the medical point of view (seat and histotype) and from the electromagnetic point of view (mainly control of temperature profile and SAR, Specific Absorption Rate). It would further be desirable that follow-up data be available in addition to the short-term response. Correlation and exchange of data among several clinics is essential, both for the above considerations and for the limitations of the method that presently withhold the number of cases eligible for treatment.

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## BASIC CLINICAL PHILOSOPHY

Our basic research effort concentrated on the evaluation of the therapeutic effect. To this purpose, the choice was to treat superficial or moderately deep lesions, for easy and reliable clinical evaluation and also for acceptable physical control (temperature distribution and measurement). The basic characteristics of a suitable apparatus were set down in collaboration between of physicians and engineers. Minimal medical specification can be stated as follows:

- depth of the lesion: from surface to a few centimeters;
- treated volume a few tens of $cm^3$;
- minimum temperature across the treated volume 42°C with a maximum temperature of 45°C;
- temperature monitoring: at least body surface and center of the mass, with multiple reading points along a line desirable (say. four temperature readings along the same line, to increase temperature information with minimal invasivity);
- temperature control: within ⌋0.2 °C;
- surface cooling: limited to about +5°C at the body surface.

It was agreed that the subcutaneous temperature could not be guaranteed by design and that it should be decided separate monitoring on a case-by-case basis according to anatomical considerations. In defining the limits of the volume to be treated, the macroscopic limits of the tumor are mainly observed, but the actual extent of the malignant tissue is usually larger and ill-defined. This must be also taken into account when planning a possible treatment.

Being the method in an experimental phase, only tumors not treatable with conventional methods were considered eligible, or those where the conventional therapies failed. It was stated that damaging healthy tissue was not acceptable. Additionally partial treatment of the malignant volume was considered not desirable.

On the basis of the above considerations the investigation was aimed at producing treatments where the physical parameters could be controlled as closely as possible so that the clinical evaluation was correlated to an identified and reproducible set of parameters.

## THERMAL ANALYSIS AND THEORETICAL LIMITATIONS

The basic theoretical limits of temperature distribution can be obtained with a simplified thermal/electromagnetic model. The real world is far more complicated, yet the model permits to enlighten orders of magnitude and physical trends of the phenomenon.

The electromagnetic excitation of the tissue is assumed to correspond to uniform plane wave at normal incidence. The electromagnetic fields of all practical applicators markedly differ from the above scheme, but the basic features of the power deposition process are expected to be maintained in the model, with exclusion of possible edge effects. Experimental observations on phantoms permit to detect such effects and give a semi-quantitative evaluation[1].

Further simplifying assumptions are as follows: (a) plane, homogeneous, isotropic tissue of infinite depth; (b) distributed blood perfusion, uniform in space and invariable with respect to temperature; (c) electric and thermal conductivity uniform and invariable; steady-state

conditions (time invariance). Thermal, electric and blood-perfusion data correspond to muscle tissue according to data derived from the literature[2,3]. The surface temperature is assumed in the following example as the minimum allowable value (T= 5°C), higher temperatures giving rise to more superficial temperature distributions.

The behavior of the temperature profiles is shown in Figure 1. There a peak temperature of 47°C is given, although in our treatment a limit of 45°C was assumed. An accepted trend appears in the literature towards somewhat increased tumor core temperature while keeping safe limits (42 or 43°C[4]) in the normal tissues. The value of 47°C is however adopted here for better evidence and to give a picture of the best obtainable results.

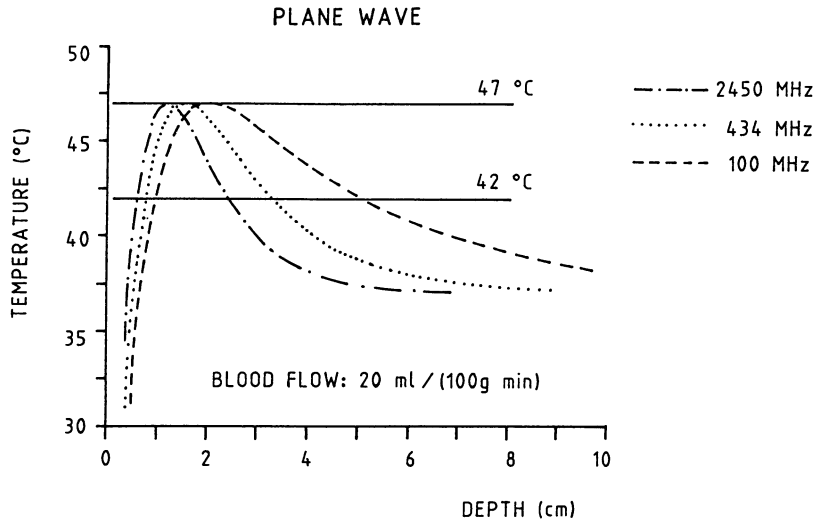


Figure 1. Temperature profiles vs. depth for various operating frequencies; maximum and minimum temperatures for treatment are also shown. Surface temperature 5°C.

The intercept of the field profiles with the T = 42°C isothermal line gives the hyperthermic range (HR) as the difference of the deep limit (DL) at 42°C and the superficial limit (SL) at the same temperature. These interesting quantities are shown vs. frequency in Figure 2, together with the isothermal line at the peak (47°C) temperature. It is seen that the SL line is relatively insensitive to frequency, as could be expected. HR decreases increasing frequency thus suggesting to adopt as low a frequency as technically feasible, compatibly with applicator dimensions, weight and maneuverability. Notwithstanding the extreme simplification of the analysis, the data in the Figures give the correct order of magnitude of the obtainable depths, as demonstrated by a comparison with our *in vivo* observations at 434 MHz and by reference to recent Manufacturer's data. Also advanced analyses existing in the literature offer confirmations.

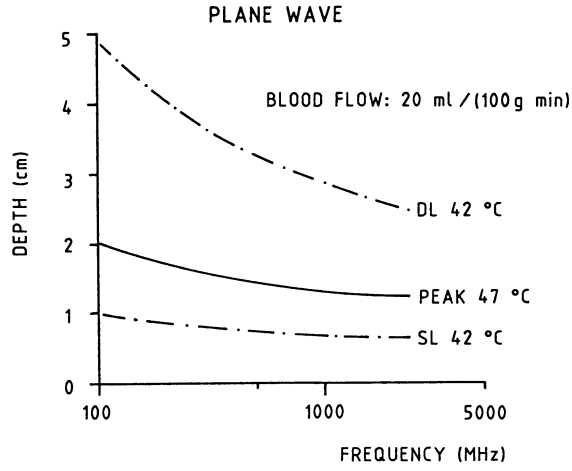


Figure 2. Depth vs. frequency of the peak temperature (47 °C) and deep (DL) and superficial (SL) limits at 42°C. Surface temperature 5°C.

## CLINICAL EXPERIENCE AND DISCUSSION

The basic hyperthermia system used in our clinical applications was especially developed following the medical specifications and the ongoing indications of the medical experience. A frequency of 434 MHz (allocated to I.S.M. equipment) was chosen as a compromise between obtainable depth and operational agility. Technical effort is presently being expended towards obtaining lower-frequency applicators of practical operability for treatment of larger volumes. Yet the 434 MHz apparatus is capable of offering stable and practical clinical operation within its technical limits. These, for what depth, volume and anatomical site are concerned, are those dictated by fundamental physical limits and correspond to those of any state-of-the-art apparatus operating at the same frequency. The applicators were of the radiative type (circular open waveguide). The waveguide is excited by an antenna, with *fixed* matching. No matching is required in operation and the reflected power level is negligible (typically less than 10%). Inside circulating water and integral bolus (contact bag) make good thermal and electromagnetic contact to the patient. Highly efficient cooling is obtained with cold-water circulation in large-section thermally-insulated hoses. Two pressure trimming devices are provided: one mechanical (axial positioning of the applicator) and one hydraulic (pressure volume trimming). Incident and reflected power monitors have analog display. An ON/OFF power control was added to the regular step or continuous regulation. This was of the non-latching push-button type for fast short-term interruption of the power flow and revealed itself of great practical value both for controlling a fast initial temperature rise and for an intervention undetected by the patient.

Apart from experimental applications on animals, the clinical observations are based on 316 applications on 48 superficial or moderate-depth lesions in 31 patients.

Seat and histotype were subdivided as follows: 5 patients with cutaneous or subcutaneous melanoma metastases; 5 patients with thorax-wall

recurrences or supra-clavear lymphonodal metastases from breast carcinoma; 2 patients with soft-tissue sarcoma; 14 patients with lateral-cervical metastatic lymphadenopathies (4 of thyroidal origin, 2 of rino-pharingeal, 7 of laryngeal origin and 1 of lingual origin); 1 patient with local recurrence from thyroidal carcinoma; 1 with maxillary-sinus carcinoma and 3 with pluri-recurrent spino-cellular carcinoma of the skin.

For the evaluation of dimensions and infiltration into adjacent tissues, CAT or xerography were performed in addition to the clinical examination.

Each treatment cycle is constituted of 6 applications in 2 weeks, for a time duration of 45 to 60 min. each, with temperatures in the range 42 to 45°C and intervals of 48 to 72 hours between treatments.

Hyperthermia (HT) was used alone on 8 lesions associated with radiotherapy (RT) in 26 lesions and associated with chemotherapy (CT) in 14 lesions. In association with RT two treatment schemes were performed adopting multi-fractioning of the total dose (4000 rad) into 1 or 2 daily treatments of 400 or 200 rad respectively, repeated 5 times per week for 2 weeks. HT was applied within 1 hour of RT for a total of 6 treatments in the two weeks (48 to 72 hour intervals). CT was associated with HT by intra-lesional or systemic or infusional administration (infusional only for limbs) using various cytostatic drugs in relation to the specific histotype sensitivities.

Temperature monitoring was by means of two or three probes inserted into the tumor tissue and surroundings. Probe insertion was by means of 16 G catheter needles.

Clinical response subdivided according to histotype and treatment modalities is shown in Table I. Variable amounts of reduction of the treated volume are observed and frequently reduction of local symptoms (when present) such as pain, dysphagia, dyspnea.

Table I. Therapy and response vs. histology

| HISTOLOGY | Totals | HT | HT+RT | HT+CT | CR | PR | NR | DP | NE |
|---|---|---|---|---|---|---|---|---|---|
| Melanoma | 7 | 1 | 1 | 5 | | 4 | | 1 | 2 |
| B.C. | 19 | 6 | 12 | 1 | 3 | 9 | 6 | 1 | |
| S.C. | 3 | | | 3 | | 1 | 1 | 1 | |
| H.N.C. | 17 | 1 | 13 | 3 | 2 | 5 | 6 | | 4 |
| Sa. | 2 | | | 2 | | | 1 | 1 | |
| Totals | 48 | 8 | 26 | 14 | 5 | 19 | 14 | 4 | 6 |

**B.C.** = Breast recurrence or metastasis; **S.C.** = Skin Spino-cellular Cancer; **H.N.C.** = Head and Neck Cancer; **Sa.** = Sarcoma; **CR** = Complete Response; **PR** = Partial Response (reduction > 50%); **NR** = Non Response (reduction < 50%); **DP** = Disease Progression (Size increase); **NE** = No Evaluation; **HT** = Hyperthermia; **RT** = Radiotherapy; **CT** = Chemotherapy.

Therapeutic effects (Table II) appear more frequently associated with RT. Synergic activity between HT and RT suggests the possibility to lower the maximal RT doses thus reducing serious hangover from radio-lesions. HT alone does not appear to produce a significant therapeutic effect.

Table II. Response vs. therapy

| THERAPY | CR | PR | NR | DP | NE | Totals |
|---|---|---|---|---|---|---|
| HT | | 2 | 5 | | 1 | 8 |
| HT+RT | 5 | 13 | 6 | 1 | 1 | 26 |
| HT+CT | | 4 | 3 | 3 | 4 | 14 |
| Totals | 5 | 19 | 14 | 4 | 6 | 48 |

**CR** = Complete Response; **PR** = Partial Response (reduction > 50%); **NR** = Non Response (reduction < 50%); **DP** = Disease Progression (Size Increase); **NE** = No Evaluation; **HT** = Hyperthermia; **RT** = Radiotherapy; **CT** = Chemotherapy.

The apparatus has demonstrated to be reliable and suited to application also onto difficult anatomical sites. The integral-cooling applicator lends itself to easy electrical and mechanical matching to the volume to be treated and the cooling is effective in preventing thermal superficial damage.

Hyperthermic treatments have demonstrated to be devoid of specific morbidity, therefore no limits are seen for repetition.

It is not worth to perform a rigorous statistical analysis due to the limited number of patients and the dishomogeneity of treated cases in relation to tumor type and seat. It is however possible to derive considerations emerging from single-case evaluations as briefly pointed out above.

## CONCLUSIONS

The fundamental physical limitations of electromagnetic hyperthermia have been discussed. Limits of depth and volume reduce the number of cases eligible for treatment. Preliminary treatment procedure have been determined and the results permit to observe the presence of a clinical response. Our clinical observations are essentially based on a 434 MHz system developed according to technical criteria stemming from joint medical and physical experience.

Further work is needed to analyze the real therapeutic effectiveness. Some margin exist for enlarging the space limitations (depth and volume of the treated mass) through the use of lower frequencies and larger applicators and for improving temperature monitoring and control (multiple probes). The 434 MHz apparatus was a good compromise for treated volume and ease of use. A larger statistical basis, however, can only be obtained through the association of several Clinics, to overcome the limitations of the number of cases eligible for treatment. This essential cooperation can give definite results only by the use of (1) well-defined common protocols and (2) effective and homogeneous

hyperthermic apparatuses having rigorously controlled physical parameters of treatment. Technological improvements can offer some possibility of increasing the number of patients treatable with hyperthermia. Compact radiative applicators working around 100 MHz are presently under development and they should be made ready for clinical use in the near future.

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REFERENCES

1. D.Andreuccetti, M.Bini, A.Ignesti, R.Olmi and R.Vanni: "A tissue-equivalent material for broad-band electromagnetic dosimetry studies" Proc. 9.th Colloque *Hertzian Optics and Dieletrics* Pisa (Italy), 2-4 September 1987, pp.62-65;
2. C.C.Johnson and A.W.Guy: "Nonionizing electromagnetic wave effects in biological materials and systems", Proc. IEEE, vol. 60, 1972, pp. 692-718;
3. G.M.Hahn: "Blood Flow"; in: "Physics and Technology of Hyperthermia", S.B.Field and C. Franconi Ed., NATO ASI Series No.127, M.Nijhoff Publishers, Dordrecht, Boston, Lancaster 1987, pp. 441-447;
4. Kapp, P.Fessenden, T.V.Samulski, M.A.Bagshaw, R.S.Cox, E.R.Lee, A.W.Lohrbach, J.L.Meyer and S.D.Prionas: "Stanford University institutional report. Phase I evaluation of equipment for hyperthermia treatment of cancer"; Int. J. Hyperthermia, vol. 4, 1988 p. 77;

# EXPERIMENTAL USE OF EXTENSIVE PRE-COOLING OF SUBCUTANEOUS FATTY TISSUES IN RADIOFREQUENCY CAPACITIVE HEATING

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## INTRODUCTION

For regional deep heating a number of non-invasive electromagnetic devices have been developed using either quasi-static or radiative methods to transfer the energy to the patient. They comprise the Annular phased array (AA), capacitive systems, magnetic coils, coaxial - and ridged waveguide systems. Generally the devices with a circumferential E-field are expected to be more favorable to obtain deep heating. However, current experience (1,2,3,7,9,11,12,15,19,20) indicates that there is no system available yet, which is able to induce adequate heating at all tumor locations in depth. Therefore continuation of development and clinical evaluation of the prototype systems is necessary in order to obtain a range of "site specific" hyperthermia systems.
An advantage of radiofrequency (RF) capacitive heating systems over other deep heating devices is their simplicity and easiness to handle. Due to the relatively small applicators used, good access to the patient during treatment is provided and the whole applicator set-up causes no systemic stress. Well-known disadvantages of RF capacitive systems are: excessive heating at the edges of the electrodes and preferential heating of the subcutaneous fat tissue. However, with better designed applicators and by choosing the proper size of the plates in relation to the body diameter (16) the energy distribution can be improved. The preferential heating of the fat layer may be adequately countered (1,7,13) with efficient precooling, provided that the fat layer does not exceed a thickness of 2 cm. Secondly, the use of the saltwater bolus (1 % NaCl) also minimize the edge effects. Under these conditions Japanese researchers (5,7,8,14,21) have demonstrated that deep heating with capacitive systems is feasible in clinical situations. Temperatures above 42 °C at the deepest spot of the tumour (>7 cm) can be obtained in about 60 % of the treatments (1,14), which seems to be comparable to results as reported (4,6,9,11,12,15,19,20) for the AA system. In contrast with the AA the RF capacitive system can be used for the treatment of lung tumors. Tachibana et al. (22) reported to obtain temperatures of 43 °C in lung tumors in 5 out of 6 patients. The general conclusions from these investigations is that RF capacitive heating may be developed into a valuable hyperthermic method for a number of selected situations. As, Japanese patients generally have less subcutaneous fat than the European or American patients, the limitations of this method should, however, be well kept in mind.

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MATERIAL AND METHODS

All experiments have been performed with a 13.56 MHz HTM3000P system (Tecnomatix, Belgium). This system is equipped with standard, ridged, rectangular capacitor plates of 10x10 $cm^2$, 15x20 $cm^2$ and one multiconnector plate of 15x20 $cm^2$; RF-generator with maximum RF-power output of 1200 W; automatic impedance tuning device, cold water circuit integrated in the applicators; and a continuous temperature measuring system using RF-filtered thermocouples.

Phantom Experiments

For each applicator configuration the specific absorption rate (SAR) distributions were measured in muscle equivalent phantoms of 40x30 $cm^2$ and a thickness of 10 or 20 cm. The muscle material used was composed of 3 W% (weight %) agar, 0.33 W% formaldehyde and 0.32 W% NaCl in deionized water. The electrical conductivity is estimated to be 0.6 S/m at 27.12 MHz (10). The SAR distribution was derived from the temperature distribution, which was measured after 3 minutes of heating with a RF input power of 500-900 W. After removing the capacitor plates and splitting the phantom, the temperature distribution of the exposed surface was measured within 30 seconds after termination of the heating by means of an AGA infrared camera interfaced to a personal computer (23).

Animal experiments

To test the in-vivo deep heating feasibility of this system the hindquarters of pigs were first cooled for 30 minutes at ± 5 °C. Hereafter RF-heating was administered for 1.5 - 2 hours with the large (15x20 $cm^2$) capacitor plate electrodes. In total 4 experiments were performed. During the whol experiment the animals were fully anaesthetized. Temperatures were measured either with the thermocouples supplied with the HTM3000P or with fiberoptic thermometry systems (TP4 Clinitherm and ASEA fiberoptics, FT1110). In each experiment 9 to 12 catheters, insertion length 8 to 15 cm, were used to acquire temperature information of the surface tissues as well as the tissues at a relative depth of 1/4, 1/2 and 3/4 of the total thickness. A schematic representation of the experimental set-up is given in figure 1. The thermometry probes were pulled back manually through the catheters with intervals of 1 cm ("thermal mapping") to obtain information on temperature distribution.

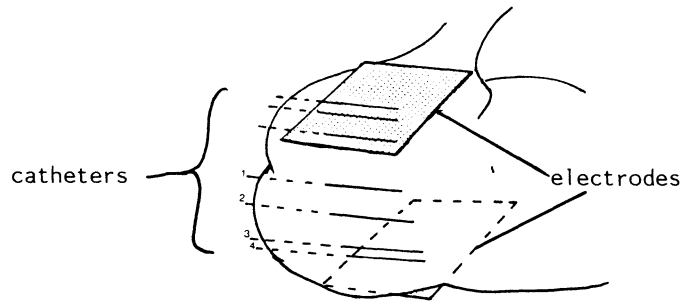


Figure 1.

Schematic representation of the applicator and thermometry set-up during heating of the hindquarters of the pig.

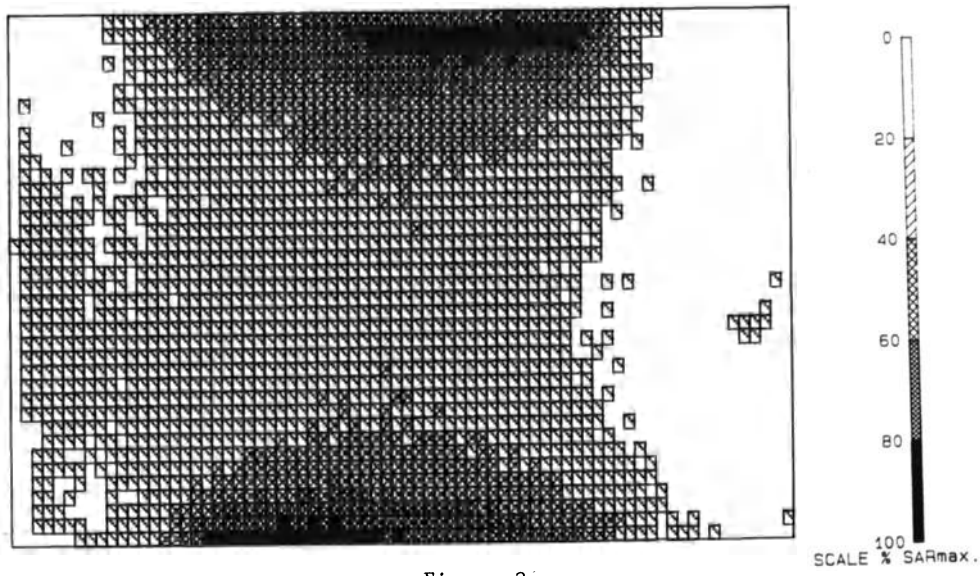


Figure 2.

Relative SAR distribution in the vertical cross-section plane at the center of the HTM300 (15x20 $cm^2$) capacitor plate electrode. Frequency 13.56 Mhz; net input power 900 W for 3 minutes; phantom size 30x20 $cm^2$, width 40 cm.

## RESULTS AND DISCUSSION

### Phantom experiments

For the small capacitor plates the effective treatment area (relative SAR>50%) directly below the electrodes equals the plate size (100 $cm^2$). Although the heating is inhomogeneous with the highest SAR-values located at the side opposite to the cable connection, there is no edge effect noticeable. The effective treatment area decreased to 75 $cm^2$ and approx. 35 $cm^2$ at a depth of 2.5 cm and 5 cm respectively; total phantom thickness was 10 cm. The SAR measurement for the large applicator (15x20 $cm^2$) with a phantom thickness of 20 cm showed again: no edge effect and maximum SAR values opposite to the cable connection. However, for this applicator the effective treatment area is smaller (75%) than the plate size (300 $cm^2$). Figure 2 shows the SAR distribution in the vertical cross-section at the center of these large capacitor plates. The distribution shows that the SAR drops rapidly with increasing depth and at the center of the phantom the relative SAR is decreased to 30 % of the maximum SAR at the surface. No significant difference between a single connector and a multi connector applicator was observed. Reduction of the effective treatment area to 30%, was noticed when a bad contact between the plate and the phantom exist. In the clinical situation this problem can be solved by using a salt water bolus bag attached to the applicator. The plastic bolus bag can to some extent follow the body contour.

### Animal experiments

In the first three experiments in the same animal (weight of the pig increasing from 35 to 65 kg over time) good deep heating, after 30 minutes of pre-cooling at 10 °C, was obtained easily. The fat thickness of the pig was between 8 and 17 mm during experiment 1 and 2 and between 8 and 25 mm during the third experiment. Figures 3 and 4 give the temperature-time profile at different sensor depths located at the central axis between both capacitor plates, for the first and third experiment

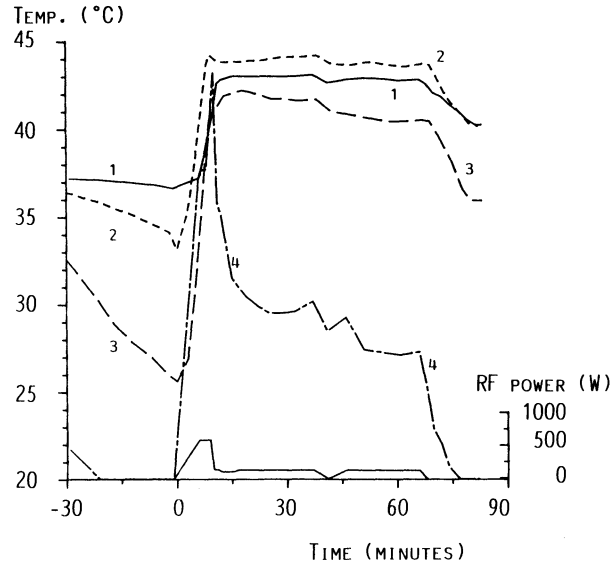


Figure 3. Temperature-time profile, for the first pig experiment, at the catheters 1, 2, 3 and 4.

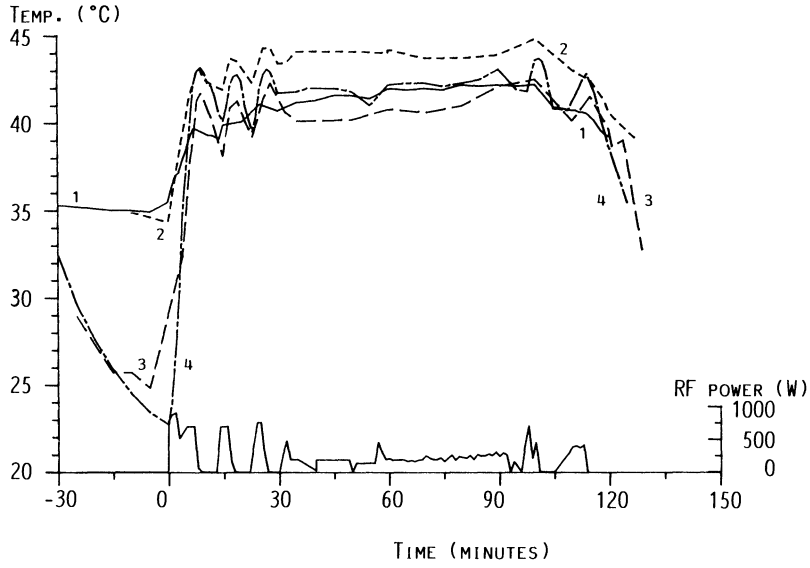


Figure 4. Temperature-time profile, for the third pig experiment, at the catheters 1, 2, 3 and 4.

respectively. In the first experiment (figure 3), animal weight only 35 kg, the superficial temperatures remained much lower then those in the third experiment (figure 4), where the animal weighed 65 kg. In all three experiments a large volume at depth could be heated to therapeutic temperatures (T>42 °C). Toxicity was minor to the superficial tissues directly below the electrodes illustrating the effect of skin-cooling. Second degree burns developed during two experiments at the skin between both hindlegs. Development of these burns could be prevented by placing an additional salt water bolus between the legs.
The fourth animal experiment was less successful. In this pig it was not

possible to induce a symmetric temperature increase at the lower and upper capacitor plate. A large third degree burn developed below the upper capacitor plate, caused by the high temperatures, 46 °C for approx. 1-1.5 hours, at this location. The temperatures at the other side (the side on which the animal was lying) were much lower and reached about 44 °C for the same time. It was observed that the burns started at the site of catheter insertion below the electrode and continued along the track of the catheters within the subcutaneous fat.

## CONCLUSIONS

The HTM3000P system using standard rectangular capacitor plate electrodes with skin-cooling to 10-15 °C is able to induce deep pelvic heating in pigs with a fat layer of 1 to 2 cm. The third degree burn which developed during the fourth animal experiment is a direct result of the high muscle temperature 46 °C during 1.5 hour. This high temperature in the upper hindquarter may have been caused by the superficial catheters. The better conducting - fluid cylinder around the catheter track may have formed a short circuit for the RF energy through the fat tissue resulting in a higher energy deposition around these catheters.
Our clinical data, to be published elsewhere, are, however in contrast with these animal data. Despite extensive cooling of the skin and subcutaneous fat to 10-15 °C for a period of 30 minutes, it was not possible to reproducibly induce deep therapeutic heating within pelvic tumours with the prototype HTM3000P system. Although all six patients treated had a fat thickness of less than 2 cm the increase of tumor temperature at depth was limited by high temperatures at the fat-muscle interface and did not reach therapeutic levels.
In order to obtain improved cooling of the superficial tissues a new cooling unit with a better water bolus, lower water temperatures and higher flow rates within the bolus is presently being developed by Tecnomatix.

ACKNOWLEDGEMENTS: This work is supported by The Netherlands Cancer Foundation "Koningin Wilhelmina Fonds", project RRTI 87-9.

## REFERENCES

1 Abe M., Hiraoka M., Takahashi M., Egawa S., Matsuda C., Onoyama Y., Morita K., Kakehi M. and Sugahara T., 1986, Multi-institutional studies on hyperthermia using an 8 MHz radiofrequency capacitive heating device (Thermotron RF-8) in combination with radiation for cancer therapy, Cancer 58: 1589-1595.

2 Corry, P.M., Jabboury, K., Kong, J.S., Armour, E.P., McCraw, F.J. and Leduc, T., 1988, Evaluation of equipment for hyperthermia treatment of cancer, Int. J. Hyperthermia, 4: 53-74.

3 Egawa, S., Tsukiyama, I., Akine, Y., Kajiura, Y., Ogino, T. and Yamshita, K., 1988, Hyperthermic therapy of deep seated tumors: Comparison of the heating efficiencies of an APA and a capacitively coupled RF system, Int. J. Radiation Oncology Biol. Phys., 14: 521-528.

4 Emami B., Perez C., Nussbaum G. and Leybovich L., 1984, Regional hyperthermia in treatment of recurrent deep-seated tumors: preliminary report, In: Hyperthermic Oncology I, Ed. J. Overgaard, Taylor and Francis, London, 605-608.

5 Fukuhara T., Suzuki Y., Tsutsumi S., Nakajima T., Ito K., Kida A., Fukuda H., Sugimoto K., Izumi A. and Onoyama Y., 1986, Hot-spot in human body during RF hyperthermic treatment, In: Hyperthermic Oncology Japan, Proc. 3th JSHO, Ed. Y. Onoyama, Mag Bros Inc.: 81.

6 Gibbs F.A., Sapozink M.D., Settles K.S. and Stewart J.R., 1984,

Regional hyperthermia with an APA in the experimental treatment of cancer, IEEE Trans. Biomed. Eng. BME-31: 115-119.

7 Hiraoka M., Jo S., Akuta K., Nishimura Y., Takahashi M. and Abe M., 1987, Radiofrequency capacitive hyperthermia for deep-seated tumors. I. Studies on thermometry, Cancer 60: 121-127.

8 Hiraoka M., Jo S., Akuta K., Nishimura Y., Takahashi M. and Abe M., 1987, Radiofrequency capacitive hyperthermia for deep-seated tumors. II. Effects of thermoradiotherapy, Cancer 60: 128-135.

9 Howard G.C., Sathiaseelan V., King G.A., Dixon A.K., Anderson A. and Bleehen N.M., 1986, Regional hyperthermia for extensive pelvic tumours using an APA applicator: a feasibility study, Br. J. Radiol. 59: 1195-1201.

10 Ishida, T. and Kato, H., 1980, Muscle equivalent agar phantom for 13.56 MHz RF-induced hyperthermia, Shimane J. Med. Sc., 4: 134-140.

11 Issels R.D., Mueller M., Tihing K., Nagele A., Wadepohl M. and Willmans W., 1987, Thermal dose distribution in human pelvic tumours during regional hyperthermia, Int. J. Hyperthermia 3: 570.

12 Kapp D.S., Fessenden P., Samulski T.V., Bagshaw M.A., Cox R.S., Lee E.R., Lohrbach A.W., Meyer J.L. and Prionas S.D., 1988, Stanford University institutional report. Phase I evaluation of equipment for hyperthermic treatment of cancer, Int. J. Hyperthermia 4: 75-115.

13 Kato H., Hiraoka M., Nakajima T. and Ishida T., 1985, Deep heating characteristics of an RF capacitive device, Int. J. Hyperthermia 1: 15-28.

14 Nishimura Y., Hiraoka M., Jo S., Akuta K., Nagata Y., Takahashi M. and Abe M., 1986, Radiofrequency capacitive hyperthermia combined with radiation for lower abdominal and pelvic deep-seated tumors, In: Hyperthermic Oncology Japan, Proc. 3$^{th}$ JSHO, Ed. Y. Onoyama, Mag Bros Inc.: 371.

15 Pilepich M.V., Myerson R.J., Emami B.N., Perez C., Leybovich L. and Von Gerichten D., 1987, Regional hyperthermia: a feasibility analysis, Int. J. Hyperthermia 3: 347-351.

16 Reddy N.M.S., Maithreyan V., Vasanthan A., Balakrishnan I.S., Bhaskar B.K., Jayaramn R., Shanta V. and Krishnamurthi S., 1987, Local RF capacitive hyperthermia: thermal profiles and tumour response, Int. J. Hyperthermia 3: 379-387.

17 Samulski T.V., Kapp D.S., Fessenden P. and Lohrbach A., 1987, Heating deep seated eccentrically located tumors with an APA system: A comparitive clinical study using two annular operating configurations, Int. J. Radiation Oncology Biol. Phys. 13: 83-94.

18 Sapozink, M.D., Gibbs, F.A., Thomson, J.W., Eltringham, J.R. and Stewart, J.R., 1985, A comparison of deep regional hyperthermia from an annular array and a concentric coil in the same patients, Int. J. Radiation Oncology Biol. Phys. 11: 179-190.

19 Sapozink, M.D., Gibbs, F.A., Gibbs, P. and Stewart, J.R., 1988, Phase I evaluation of hyperthermic equipment-University of Utah Institutional report, Int. J. Hyperther. 4: 117-132.

20 Shimm, D.S., Cetas, T.C., Oleson, J.R., Cassady, J.R. and Sim, D.A., 1988, Clinical evaluation of hyperthermia equipment: The university of Arizona Institutional report for the NCI hyperthermia equipment evaluation contract, Int. J. Hyperther. 4: 39-51.

21 Song C.W., Rhee J.G., Lee C.K.K. and Levitt S.H., 1986, Capacitive heating of phantom and human tumors with an 8 MHz radiofrequency applicator, Int. J. Radiation Oncology Biol. Phys. 12: 365-372.

22 Tachibana S., Ozeki M., Kaito N., Shimomura T., Sobajima T., Tsuji H., Harumari H., Orino T. and Takeuchi A., 1986, Hyperthermia for lung cancer combined with conventional therapies, In: Hyperthermic Oncology Japan, Proc. 3$^{th}$ JSHO, Ed. Y. Onoyama, Mag Bros Inc., 333.

23 Van Deursen J.B.P. and Van Rhoon G.C., 1988, A low-cost interface for upgrading an AGA thermograph, Biomed Meas Inform Contr 2: 10-13, 1988.

# FOUR ELEMENT COMPUTER CONTROLLED 432 MHz PHASED ARRAY HYPERTHERMIA SYSTEM

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## INTRODUCTION

A phased array hyperthermia system using four applicators has been developed to treat tumors at mid-depths from the body surface. The operation frequency is 432 MHz. The system consists of four independent but otherwise fully coherent channels. The applicators are water loaded waveguide cavities of 2.9 X 5.8 $cm^2$ aperture. In order to model the system and to operate it as a phased array system electromagnetic and optimization theory techniques have been used.

The phased array system consists of three sub-units:
a) Coherent signal generator
b) Amplifier unit
c) Computer system

The system block diagram is shown in Fig. 1. The four coherent signals are generated at 28 MHz. The output of a master oscillator operating at 28 MHz is divided into four channels each one introducting a digital phase (4 bit) and amplitude (3 bit). These digital phase and amplitude values are controlled by a software program developed on a personal computer. The phase shifters are realised by employing coaxial lines while $\pi$-type resistive networks are employed for attenuators in conjuction with electromechanical relays. After setting the phases and amplitudes on each channel the four signals are up-converted to 432 MHz by employing mixing circuits; Then each signal is amplified up to 50 W and driven to each applicator to illuminate the tissue medium to be heated. The incident and reflected wave amplitudes are monitored on a continuous basis. The applicators are of water loaded type open waveguides described in detail in Ref. [1]. Indirect cooling is employed to lower the temperature and to protect the skin.

## PREDICTION OF THE SYSTEM OPERATION

For quality assurance and treatment planning purposes analytical techniques have been employed concerning:

a) the prediction of the power deposition from the four array elements.
b) temperature distributions within the tissue medium
c) optimal control of the phase and amplitudes.

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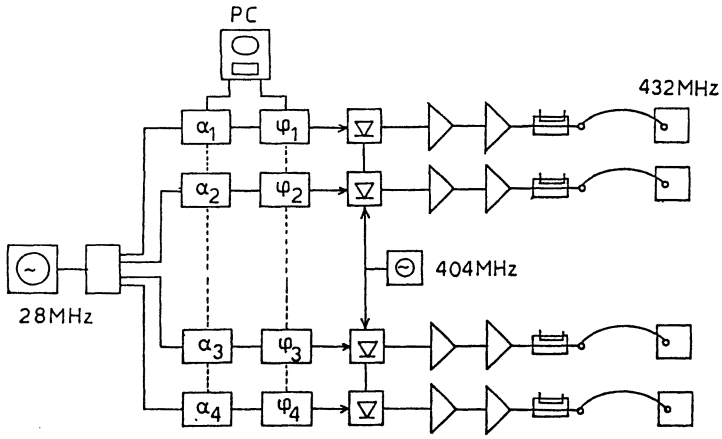
*In treating the electromagnetic problem to predict the power deposition of applicators the canonical problem of the radiation from an open aperture waveguide towards a stratified planar layered medium is solved rigorously. Then an approximate superposition principle is employed to compute the electromagnetic field inside tissues subject to the illumination of more than one applicators.*

*In computing the steady-state temperature distributions a Green's function theory is employed. To this end the temperature at an arbitrary point $\underline{r}$ inside the tissue medium to be heated is determined to be*

$$T(\underline{r}) = T_b + \frac{\sigma}{2} \sum_{i=1}^{4} \sum_{j=1}^{4} \sqrt{P_i P_j}\, e^{j(\phi_i - \phi_j)} \iiint_{\text{Tissue volume}} \Gamma_\theta(\underline{r},\hat{r}) \underline{G}(\hat{r}/\underline{r}_i) \cdot \underline{G}^*(\hat{r}/\underline{r}_j) d\hat{r} \qquad (1)$$




BLOCK DIAGRAM OF THE PHASED ARRAY SYSTEM

**Fig. 1.**

*where $\sigma$ is the tissue electric conductivity, $T_b = 37^oC$ is the normal human body temperature, $\sqrt{P_i}$ and $\phi_i$ $(i=1,2,3,4)$ are the amplitude and phase of the i'th array element respectively, $\Gamma_\theta(\underline{r},\hat{r})$ is the thermal Green's function and $G(\underline{r}_1,\underline{r}_2)$ is the electric field at the point $\underline{r}_1$ due to an applicators its center being at $\underline{r}_2$. Defining the system vectors*

$$\bar{P} = [\sqrt{P_1}, \sqrt{P_2}, \sqrt{P_3}, \sqrt{P_4}]^T \qquad (2)$$

$$\bar{\phi} = [\phi_1, \phi_2, \phi_3]^T \qquad (3)$$

with $\phi_4 = 0$ (reference phase) and $\bar{\underline{A}}(\underline{r},\bar{\phi})$ being a 4 x 4 matrix eq. (1) can be rewritten as follows

$$T(\underline{r},\bar{P},\bar{\phi}) = T_b + \frac{\sigma}{2}\,\bar{P}^T.\bar{\underline{A}}(\underline{r},\bar{\phi}).\bar{P} \qquad (4)$$

Based on eq. (4) we formulate the following optimization problem for selecting power and phase to the i'th antenna:

$$\min_{\bar{P},\bar{\phi}} \{ \sum_{j=1}^{m} (T(\underline{r}_j,\bar{P},\bar{\phi})-T_0)^2 : T(\underline{r}_k,\bar{P},\bar{\phi}) < T_1, \quad k = m+1,\ldots,M\} \qquad (5)$$

Where points $\underline{r}_j$, $j = 1,\ldots,m$ lie inside and points $\underline{r}_k$, $k = m+1,\ldots,M$ lie outside the tumor area, $T_0$ is the target temperature inside the tumor and $T_1$ is an upper bound for the temperature outside the tumor region.

The optimization problem is solved by the penalty function method:

$$\min_{P,\phi} \{ \sum_{j=1}^{m} (T(\underline{r}_j,\bar{P},\bar{\phi})-T_0)^2 + \mu \sum_{k=m+1}^{M} (\max(0,T(\underline{r}_k,\bar{P},\bar{\phi})-T_1)^2\}$$

Unconstrained minimization of the penalty function is carried out by a stabilized Newton method. Small violations of the constraints $T(\underline{r}_k,\bar{P},\bar{\phi}) \leq T_1$ of the original problem may be considered tolerable, therefore the penalty parameter $\mu$ need not be increased to very high values.

REFERENCES

1. N.K. Uzunoglu, E.A. Angelikas, P.A. Cosmidis, "A 432 MHz local hyperthermia system using an indirectly cooled, water-loaded waveguide applicator" IEEE Trans Microwave Theory Tech, vol. MTT-35, pp. 106-111, 1987.

# RESULTS OF DEEP BODY HYPERTHERMIA

# WITH LARGE WAVEGUIDE RADIATORS

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## INTRODUCTION

The purpose of this report is to present results of work in progress on hyperthermia treatment planning and clinical application of a four-applicator phased array hyperthermia system for heating of large and deep-seated tumors.

## MATERIAL AND METHODS

### Apparatus

The basis of the system is a home-build four aperture array system of large water-loaded waveguide radiators excited in the $TE_{10}$ mode and operating at a frequency of 70 MHz. The aperture size of each applicator is 20x34 cm. Three applicators have been placed inside a supporting frame and the fourth has been installed under a motorized patient support coach. Figure 1. shows a patient in position within the apparatus. Four waterboluses are necessary between the patient and the applicators. The waterbolus upon the lower applicator is closed and fits inside the mattress of the coach, the other three are open and hanging below the upper, and in front of the lateral applicators. In the beginning of the treatment the supporting frame is moved to one side of the coach and the patient can easily be positioned on the waterbolus on top of the coach. At this stage the position of the thermometry catheters with respect to the applicator can be checked by radiography. The supporting frame can subsequently be moved over the patient and the tabletop with patient is axially shifted and lifted until the patient makes an optimal contact with the upper bolus. Then the lateral applicators are moved inwards until proper contact is made with the sides of the patient. The four boluses can be chilled or warmed by circulating the water through heat exchangers, which are under thermostatic control.

The 70 MHz RF system consists of a four-channel generator feeding four power amplifiers (Henry Radio), which can deliver a maximum power of 500 W each. The waveguide radiators are matched to the amplifiers by double slug tuners. By changing the relative amplitude and phasing, it is possible to steer the heating pattern to a certain extent. Phase shifts have been realized by changing lengths of cable.

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Fig. 1. The clinical setup of the four aperture array system

Treatment-planning

Extensive simulations of energy depositions in rectangular and elliptical phantoms have been performed with a 2d- computational model (1). The software has been recently installed on an IBM PS/2 model-80. Temperature- and E-field measurements have been performed within the above mentioned phantoms. An early comparison of measured and calculated data for the rectangular phantom showed a good overall agreement, provided attention was given in the calculation matrix to reflections at the applicator apertures. Good results with the calculations have been obtained if a layer of 4 cm of water behind the 'applicator line' was taken into account. Besides some enhancement of the E-field in the forward direction, the whole interaction pattern fitted better the experimental results (2). The pre-treatment calculations, based on CT-information of actual patients, indicate good possibilities for obtaining an optimal heat absorption pattern which depend on a proper choice of position, amplitude and phase of each applicator.

Clinical methods

From september 1987 through september 1988, 26 patients were treated with mainly two opposite waveguide radiators. These patients had large tumours in the abdomen/pelvis (17), groin (2), mamma (5) or extremities (2). The treatment of the breast was made possible due to the relatively short axial length of the aperture of 20 cm. Patient characteristics are shown in tabel 1. All patients had histological confirmation of advanced, recurrent, or persistent malignant tumors with or without the presence of metastatic disease. Heat treatments were scheduled once weekly for a maximum of five treatment sessions. Continuous temperature monitoring was accomplished by placing one or more catheters into the tumor. Additional catheters were placed into the rectum, bladder, and vagina, in the case of pelvic treatments. Intratumoral temperatures were registered by multi-sensor thermocouple probes. Maximum, minimum and median values of the temperature within the target area were averaged for each patient over the actual given number of sessions. The Thermal Isoeffect Dose has been calculated as the number of equivalent minutes at 43 °C (3).

Table 1. Patient characteristics

| | | | | | |
|---|---|---|---|---|---|
| Sex: | Male | 10 | Age: | Median | 65 years |
| | Female | 16 | | Range | (32-87) |
| | | 26 | | | |
| Performance: | | | PA: | adeno carc | 15 |
| | WHO Scale-0 | 10 | | melanoma | 3 |
| | -1 | 6 | | sq c carc | 3 |
| | -2 | 8 | | so t sarc | 1 |
| | -3 | 2 | | other | 4 |
| Treatment localization: | | | Tumor volume: | | |
| | abd/pelvis | 17 | | median 400cm3 | |
| | groin | 2 | | range (125-1000) | |
| | mamma | 5 | | | |
| | extremity | 2 | | | |
| Prior therapy: | | | New total RT dose: | | |
| | no therapy | 6 | with prior RT 10-30 Gy | | |
| | surgery | 8 | no prior RT: | | |
| | radiotherapy | 4 | abd/pelvis | | 60-68 Gy |
| | surg. + radioth. | 5 | melanoma | | 24-27 Gy |
| | surg. + chemoth. | 3 | mamma | | 70 Gy |

RESULTS

Hyperthermia planning

In figure 2. calculated energy depositions are shown for a typical patient with a rectum tumor treated by two opposite applicators. The

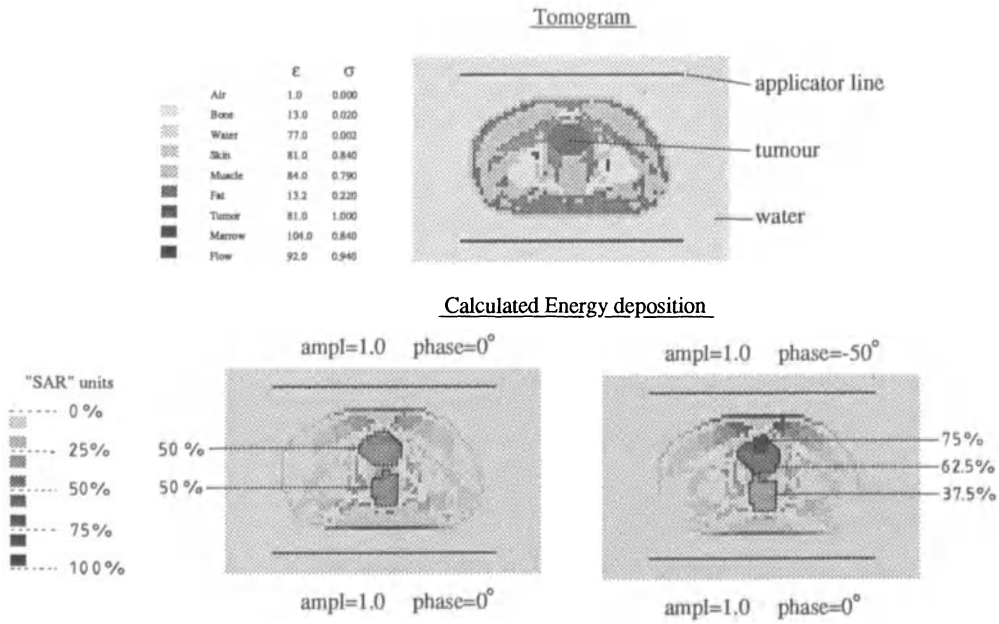


Fig. 2. Calculated energy distribution: effect of phase delay

Table 2. Thermometry of A) abdomen/pelvic and B) breast cancer patients

A)

| Pat nr | Nr of htses | Nr of appl. | Aver power | Av.Pow. on time | Nr of prbs | Nr of sens | Aver. Min T | Aver. Max T | Aver. Med T | Av.min teq43 | Av.max teq43 | Av.med teq43 | Av.med t:T>41 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 2 | 500 | 59.6 | 1 | 5 | 40.9 | 43.0 | 42.1 | 1.7 | 18.7 | 21.1 | 28.8 |
| 2 | 4 | 2 | 424 | 54.8 | 2 | 6 | 40.5 | 42.1 | 41.3 | 0.3 | 3.9 | 1.0 | 2.3 |
| 3 | 4 | 2 | 480 | 56.5 | 2 | 8 | 40.2 | 41.7 | 40.6 | 0.5 | 5.4 | 0.8 | 0.3 |
| 4 | 4 | 2 | 380 | 58.8 | 2 | 8 | 41.1 | 43.6 | 41.7 | 1.2 | 35.8 | 4.3 | 25.1 |
| 5 | 2 | 2 | 458 | 62.5 | 1 | 5 | 39.9 | 45.4 | 43.9 | 0.1 | 151.0 | 33.5 | 47.0 |
| 6 | 3 | 2 | 340 | 44.0 | 2 | 6 | 39.4 | 42.3 | 39.9 | 0.0 | 7.5 | 0.1 | 0.0 |
| 7 | 5 | 2 | 390 | 61.8 | 1 | 5 | 42.0 | 44.5 | 42.8 | 3.1 | 124.1 | 34.2 | 52.8 |
| 8 | 3 | 4 | 720 | 56.0 | 2 | 10 | 41.3 | 43.2 | 41.8 | 6.3 | 20.2 | 4.7 | 26.7 |
| 9 | 4 | 4 | 410 | 48.0 | 2 | 9 | 40.5 | 41.0 | 40.3 | 0.6 | 2.0 | 1.4 | 11.5 |
| 10 | 3 | 2 | 280 | 52.3 | 1 | 7 | 41.0 | 43.4 | 42.0 | 1.5 | 18.5 | 7.3 | 21.7 |
| 11 | 5 | 2 | 440 | 58.6 | 2 | 10 | 39.9 | 42.6 | 41.4 | 0.2 | 10.1 | 2.9 | 5.2 |
| 12 | 2 | 2 | 300 | 59.5 | 1 | 6 | 41.6 | 42.0 | 41.9 | 1.6 | 5.8 | 4.2 | 32.3 |
| 13 | 5 | 2 | 300 | 47.6 | 1 | 5 | 39.0 | 39.7 | 39.4 | 0.0 | 0.2 | 0.1 | 0.0 |
| 14 | 3 | 2 | 250 | 62.0 | 1 | 5 | 39.9 | 41.0 | 40.3 | 0.5 | 1.5 | 0.7 | 0.0 |
| 15 | 4 | 2 | 340 | 60.3 | 1 | 5 | 40.3 | 40.8 | 40.5 | 1.8 | 3.4 | 2.6 | 11.8 |
| 16 | 5 | 2 | 340 | 57.0 | 1 | 5 | 40.8 | 41.3 | 41.0 | 0.8 | 1.1 | 1.2 | 5.2 |
| 17 | 5 | 2 | 230 | 49.6 | 1 | 4 | 39.7 | 40.7 | 40.1 | 0.5 | 1.6 | 1.1 | 5.9 |
| Nr pat.s: | | AVG | 387 | 55.8 | 1 | 6 | 40.5 | 42.2 | 41.2 | 1.2 | 24.2 | 7.1 | 16.3 |
| 17 | | STD | 113 | 5.4 | 0 | 2 | 0.8 | 1.4 | 1.1 | 1.5 | 42.6 | 10.9 | 16.3 |
| Nr sess: | | MAX | 720 | 62.5 | 2 | 10 | 42.0 | 45.4 | 43.9 | 6.3 | 151.0 | 34.2 | 52.8 |
| 66 | | MIN | 230 | 44.0 | 1 | 4 | 39.0 | 39.7 | 39.4 | 0.0 | 0.2 | 0.1 | 0.0 |

B)

| Pat nr | Nr of htses | Nr of appl. | Aver power | Av.Pow. on time | Nr of prbs | Nr of sens | Aver. Min T | Aver. Max T | Aver. Med T | Av.min teq43 | Av.max teq43 | Av.med teq43 | Av.med t:T>41 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 2 | 345 | 62.0 | 2 | 6 | 38.7 | 43.3 | 41.0 | 0.0 | 28.6 | 1.1 | 8.0 |
| 2 | 5 | 2 | 320 | 56.0 | 2 | 9 | 40.5 | 43.6 | 42.0 | 0.6 | 27.1 | 5.5 | 28.8 |
| 3 | 5 | 2 | 280 | 59.4 | 1 | 7 | 40.4 | 44.6 | 43.0 | 1.7 | 102.1 | 35.0 | 49.8 |
| 4 | 5 | 2 | 280 | 61.8 | 2 | 7 | 38.4 | 43.3 | 40.5 | 0.0 | 27.7 | 0.9 | 8.0 |
| 5 | 5 | 2 | 330 | 61.6 | 2 | 11 | 39.6 | 43.0 | 41.0 | 0.5 | 32.4 | 4.4 | 18.4 |
| Nr pat.s: | | AVG | 311 | 60.2 | 2 | 8 | 39.5 | 43.5 | 41.5 | 0.5 | 43.6 | 9.4 | 22.6 |
| 5 | | STD | 27 | 2.3 | 0 | 2 | 0.9 | 0.6 | 0.9 | 0.6 | 29.3 | 12.9 | 15.6 |
| Nr sess: | | MAX | 345 | 62.0 | 2 | 11 | 40.5 | 44.6 | 43.0 | 1.7 | 102.1 | 35.0 | 49.8 |
| 21 | | MIN | 280 | 56.0 | 1 | 6 | 38.4 | 43.0 | 40.5 | 0.0 | 27.1 | 0.9 | 8.0 |

amplitude distribution along the two applicator lines has been assumed to be cosine shaped. The calculations indicate an improvement of percentage values for the SAR contour surrounding the tumor from 50 to 62.5 % with the application of a phase delay of 50 degr. for the upper applicator as compared with the situation of equal phases for both.

## Temperatures Achieved

In table 2. the thermometry results are shown of 17 abdomen/pelvic and 5 breast cancer patients treated mainly with two opposite applicators. In 10/17 patients with tumours in the abdomen/pelvis, an average maximum temperature was achieved of 42 °C or higher. The median time averaged over the actual given treatment sessions with all sensors within the tumor above 41 C ranged between 0 and 53 min, with a mean of 16 min. The mean of the average minimum temperatures was 40.5 ± 0.8 °C. In this group of patients we obtained only a rough idea about the effect of phase steering, but it was not applied in a controlled way. On the contrary, looking back we assume that for some patients (from nr 12 and higher) there was no optimal phase relation adjusted between the antennae. Applications with two opposite waveguide radiators did not give matching problems, however, measurements with all four radiators were hindered by reflections due to

mutual coupling. In a few of the pelvic cancer patients we tried to use the lateral applicators in addition to the upper and lower antennae. The results were not encouraging. A significant increase in temperature in centrally localized tumors was not observed. Further study of the phase problem might give better results. In all breast cancer patients average maximum temperatures were achieved of 43 °C or higher. However, the average minimum temperatures were relatively low (mean: 39.5 °C). These low temperatures were reached near the surface, since it appeared to be difficult to heat tissue layers near the surface and at depth at the same time. This has been attributed to too much skin-cooling, which is required for heating at depth. Similar problems were met with heating of large tumors in the groin.

Treatment results

The number of treatments administered per patient was 5 for the breast cancer patients and ranged from 3 to 5 for the pelvic cancer patients. The average power on time was close to the aimed treatment time of one hour. The radiotherapy schemes are shown in table 1. In the abdomen/pelvic cancer patients 43 % had pain as the main complaint. In 24 % complete improvement, in 43 % mayor to mild improvement and in 24 % no improvement of the main complaints has been obtained. Three months after treatment complete response was obtained in 3 (14 %), partial response in 3 (14 %) and stable disease in 9 (43 %) of the 21 abdomen/pelvic cancer patients.

## DISCUSSION

The four aperture array hyperthermia system is a loco-regional heating device, which has shown potential for the treatment of deep-seated tumors in abdomen/pelvis, groin, breast and extremities. However, there is still much to be learned, especially about the proper use of all four applicators and even about the situation with only two applicators. Much insight in the problem of phase steering has been obtained with the help of computer treatment planning calculations of absorbed power patterns. The contribution of the lateral applicators for the pelvic cancer patients has been relatively small in effect as compared to the contribution of the anterior and the posterior applicator. However, calculations for four applicators indicate the possibility of further optimization. The proper use of E-field probes and phase measuring equipment is obviously critical in a study such as this, and will be further improved in the near future. The early results for two applicators with an experimentally controlled phase delay are promising.

## REFERENCES

(1) Van den Berg, P.M., De Hoop, A.T., Segal, A., and Praagman, N., 1983, A computational model of the electromagnetic heating of biological tissue with application to hyperthermic cancer therapy, IEEE Transactions on Biomedical Engineering, 30, 797 - 805.

(2) Delfini, M.G. and Van Dijk, J.D.P., 1989, Comparison between temperature distribution measurements in phantoms and SAR calculations, submitted to the Int.J.Hyperthermia.

(3) Hand, J.W., Lagendijk, J.J.W., Bach Andersen, J. and Bolomey, J.C., 1989, Quality assurance guidelines for ESHO protocols, Int.J.Hyperthermia, 5, 421 - 428.

# SUPERFICIAL AND INTRAURETHRAL APPLICATORS FOR MICROWAVE HYPERTHERMIA *

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## INTRODUCTION

In this paper we present the results of the experimental examinations of our recent applicators i.e., an applicator based on a cylindrical waveguide for treatment of superficial cancers, and an intracavital one based on a monopole antenna.

The practical realization of the first of the aforementioned applicators was preceded by a thorough analytical study, described in details in [1,2]. As we shall show here, the practical and theoretical results are in very good agreement. The applicator employs a $TM_{01}$ electromagnetic wave mode which features axial symmetry, and hence the symmetry of radiated. Moreover, $TM_{01}$ mode provides a very high utilization of the radiating aperture. These two properties make our applicator superior to widely used rectangular waveguides both in the convenience in use and its electromagnetic behavior.

The other of the herein presented applicators is designed for treatment of neoplastic and non-neoplastic diseases of a prostate. Increasingly more demanding treatment requirements yield, as it may be observed in literature, increasingly more complex applicator constructions. In contrast to this tendency, in this paper we propose quite a simple construction which nonetheless provides good electromagnetic properties. Therefore, we shall describe this device in details, in order to make its wide use feasible.

The practical experiments were performed in a standard way, we used a thermographic camera[3] to observe the distribution of energy radiated into a muscle phantom. A special mixture of agar-agar, sodium-chloride, water and alcohol was developed to provide phantom with suitable electromagnetic, thermal and mechanical properties. The radiation timing, and the power level were carefully selected to ensure that the thermographic image would closely reflect the distribution of the dissipated electromagnetic power.

## SUPERFICIAL APPLICATOR

The practical realization of the applicator was preceded by thorough theoretical and numerical examinations. The objective of these studies was to evaluate the electromagnetic field distribution in a tissue model. It was also desirable to predict the existence of edge effects and find the way to suppress them, to determine the role of each component of the field, to determine the dependence

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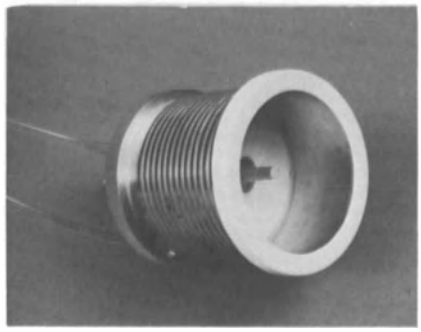
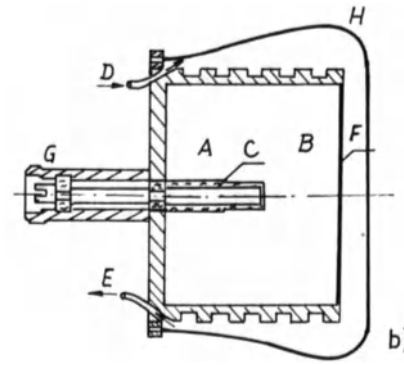


a)




b)

**Figure 1. The superficial applicator. a) General view (cooling system removed). b) The schematic construction: A–feeding and matching coaxial lines, B–cylindrical waveguide, C–teflon sheath, D,E–cooling agent inlet and outlet, F–teflon foil, G–connector, H–plastic foil**

of the shape of radiated field on the ratio of the aperture radius to the wavelength in tissue, and finally to compute the antenna impedance. The results of this study [1,2] were applied in the design of the prototype fig.1a.

The construction of the applicator is sketched in fig.1b. It comprises a cylindrical waveguide section and several sections of coaxial lines. The duo-dielectric coaxial lines serve matching purposes and launch the $TM_{01}$ mode into the cylindrical waveguide.

The applicator presented here can work at three frequencies. However to ensure a single mode operation and proper radiation conditions the applicator is filled with double-distilled water when used with 433 MHz e.m. wave, with alcohol at 915MHz, while a special teflon ring is inserted into the waveguide at 2450MHz.

We shall now present distributions of the power dissipated in muscle tissue obtained by means of computer simulation and compare them with experimental results obtained in a muscle phantom. First, we shall consider the applicator working with 433 Mhz wave. The results of computer computations are plotted in fig.2a, while the thermographic image of the same configuration is presented in fig.2b. In both figures we take into consideration a cross–section parallel to the symmetry axis. The close resemblance of both figures is evident. The electromagnetic power is absorbed mainly along the symmetry axis. This is because at this frequency the dominating field component is $E_z$[1]. In fig.2c we present the thermal image of the phantom surface (a plane perpendicular to the symmetry axis). Though the symmetry of the absorbed power is not perfect (owing to not fully homogeneous phantom structure), the figure confirms the $TM_{01}$–mode nature of the excited electromagnetic field.

In figs.2d,2e,2f we illustrate the behavior of the radiator at 2450MHz frequency. Again the theoretical – fig.2d, and practical – fig.2e results turn out to be in good agreement. Note however, that on the contrary to 433MHz case, the absorbed power is now concentrated at a certain distance from the symmetry axis. This is well illustrated in fig.2f and results from $E_r$ field component domination in shaping of the radiated field at this frequency [2]. In [2] we have shown that working with 2450MHz is advantageous when the applicator is separated from the muscle-like tumour by a fat layer.

The most homogeneous distribution of the radiated power and the best utilization of applicator aperture may be obtained at 915MHz. The results of computer simulation at this frequency are drawn in fig.3. Up to now, we have no experimental data for this frequency, yet the experience gained during work with previous two frequencies let as assume that also in this case the theoretical data give us a good prediction. It is worth noticing that in all three cases we have obtained a very good utilization of the aperture.

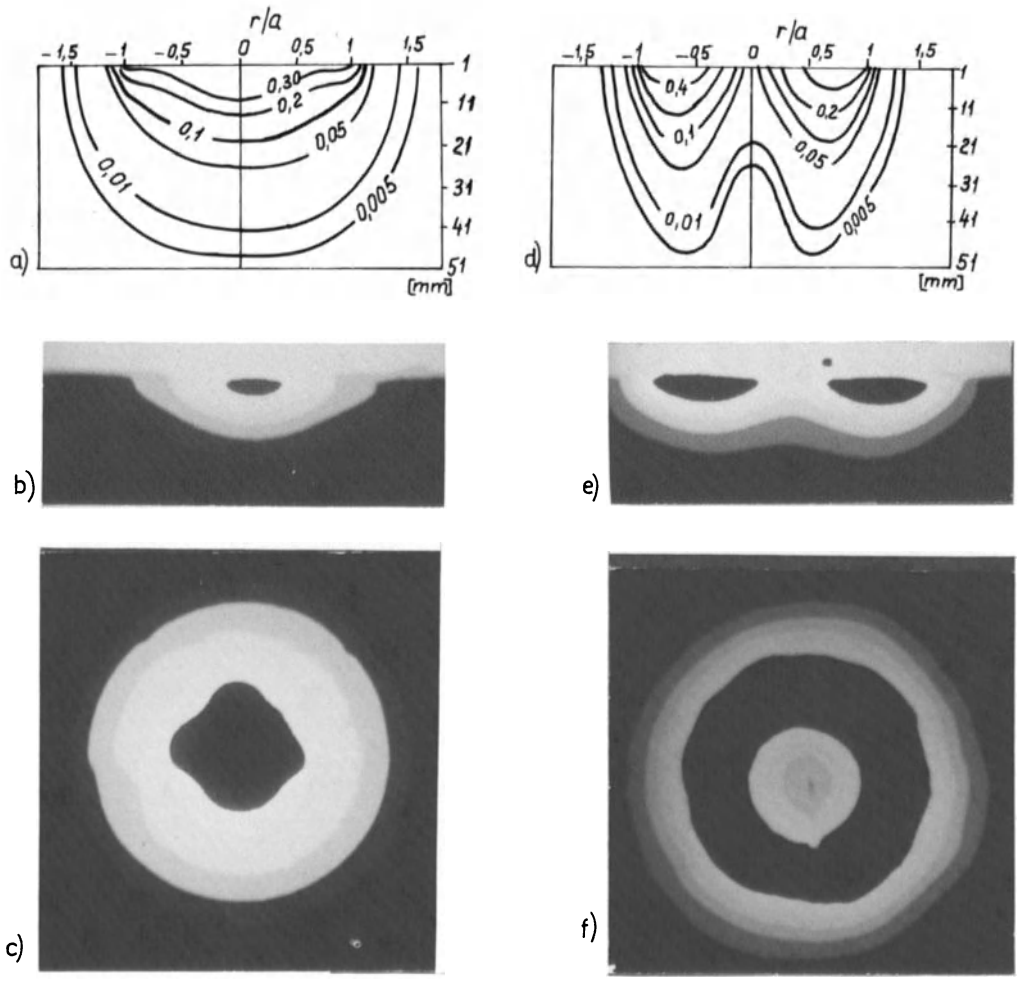



**Figure 2. Thermal characteristics of superficial applicator radiating into muscle tissue. a), b), c) – at 433MHz; d), e), f) at 2450MHz. a),d) results from computer simulation — normalized distribution of absorbed power ($\sigma|E|^2$); a – applicator radius, r – distance from the symmetry axis. b), c), e), f) – thermographic images: b), e) same cross-section as in a) and b) respectively; c), f) thermal images of the phantom surface. Note: The lighter the area, the higher the temperature, except for inner black areas which have the highest temperature.**

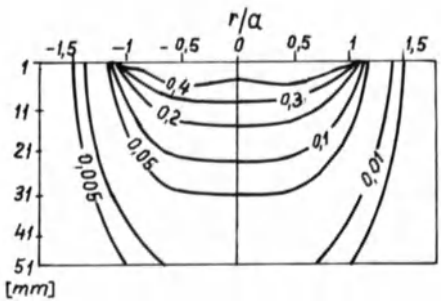



**Figure 3. Normalized distribution of absorbed power ($\sigma|E|^2$) in muscle tissue at 915MHz — computer simulation. Other parameters as in fig.2.**

## INTRAURETHRAL APPLICATOR

The intraurethral applicator (fig.6) designed to work at 2450MHz, has the form of a 230mm long cylinder with a step diameter discontinuity and a typical microwave SMA connector at one end. The overall construction is stiff and coated with teflon.

The thinner cylinder is destined to be introduced into a prostate. It comprises two sections: the first 40mm forms an active, radiating area, the remaining 20mm, which may be slightly bend, simplify the insertion procedure.

The dimensions quoted above results from medical rather than technical requirements and thus may be slightly varied to meet particular demands. In order to localise the applicator in the proper place of the prostate one needs to perform a through–rectum palpation to find the position of the diameters step (see fig.4).

It is worth noticing that a large proportion of the thick part of the applicator is surrounded by lossy biological tissue. We took advantage from this fact to simplify the construction —

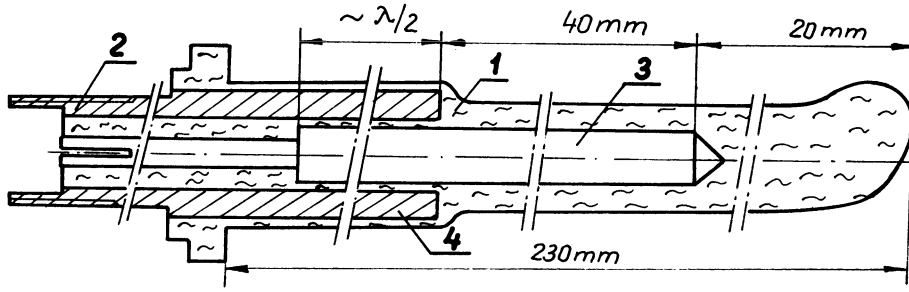



Figure 4. Simplified construction of the intraurethral applicator: 1–teflon insulation, 2-SMA connector, 3-radiating monopole, 4-feeding line outer conductor

we resigned from complicated circuits used in short, rectal applicators [5] where they were indispensible, since they cut waves excited on external surfaces of feeding lines, and were used for matching. Instead we connected the feeding line and the monopole with a section of low impedance line about $\lambda/2$ long — fig.4. This line serves two purposes. First, it stiffens the construction and prevents it from an accidental bending at the beginning of the monopole. Secondly, it enables circuit matching, since a minute change of length of such a section results in a large transformation of the imaginary and only a slight one of the real part of the impedance with which the line is terminated. Thus, the normalized impedance of the monopole in an excitation plane need only have the real part close to the unity, with the imaginary part being almost arbitrary.

The equivalent circuit observed from the plane of the monopole excitation is drawn in fig.5. The radiation impedance is a sum of radiation impedances of the monopole and of the surface of

the feeding line – $Z_B$. Since the power launched to the monopole is to be substantially higher than that radiating from the surface of the feeding line, the real part of $Z_B$ must exceed $\mathcal{R}e\{Z_A\}$. Both $Z_B$ and $Z_A$ can be easily found using the theory developed by King[4]. For example, if we assume $a$=7mm we have from King's theory $Z_A$=0.25-$j$0.14 for $h_a$ > 30mm. Therefore, almost entire power excited on the external surface of the feeding line would be attenuated on the distance

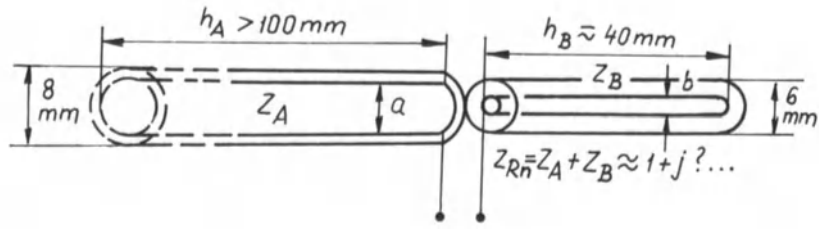



Figure 5. Equivalent circuit of the intraurethral applicator: $Z_{Rn}$–normalized radiation impedance, $Z_A$–radiation impedance of the feeding line outer conductor, $h_a$–its length, $a$–its diameter; $Z_B$–radiation impedance of the monopole of length $h_b$ and diameter $b$.

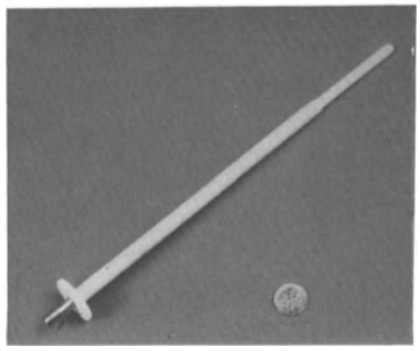


Figure 6. Intraurethral applicator.

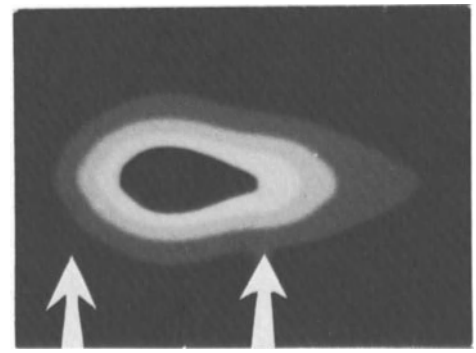


Figure 7. Thermographic images of intraurethral applicator in a muscle phantom. Arrows mark the monopole.

shorter than 30mm. To have the system matched we require $\mathcal{R}e\{Z_B\}$=0.75, which can be easily achieved putting $h_B$=40mm and $b$=3mm.

In fig.8 we present the theoretical and measured frequency characteristics of the reflection coefficient of the applicator. These results confirm the appropriateness of the analytical approach which we have applied.

The results of thermographic experiments are shown in fig.7. Two arrows were used to mark the monopole. It is clearly visible that the radiation from the external surface of the feeding line (outside the left arrow) is sufficiently small.

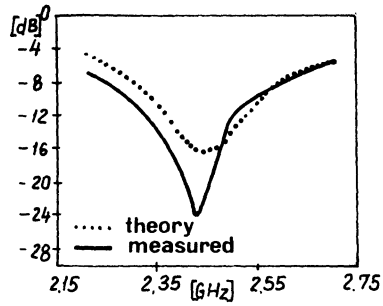



Figure 8. Theoretical and measured characteristics of reflection coefficient of the intraurethral applicator.

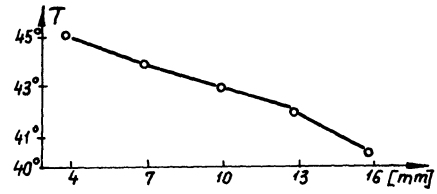



Figure 9. Temperature versus distance from monopole axis measured during experiment on a dog.

Final experiments were carried out on a dog. The laparotomy was performed followed by incision of the urine bladder through which the applicator was inserted into the prostate. Three thermistor probes have been placed in the prostate alongside the radiating area and at 1mm distance from the applicator surface. Seven wats of electromagnetic power were applied. Once the thermal conditions became stabilized (after about 10 min.) we started to withdraw one of the probes in order to measure the radial temperature distribution. The results depicted in fig.9 indicate relatively deep penetration of applied power.

## CONCLUSION

The results of the experimental test of the superficial applicator which we have presented, encourage us to proceed to the clinical stage of investigations. We are also preparing experiments at 915MHz where the results of the numeric modelling were most promising.

The intraurethral applicator is already being clinically tested. We are also preparing its flexible version with a special device for facilitation of the proper localization in a prostate.

## REFERENCES

[1] E.Okoniewska et al., "Cylindrical Flange-Waveguide Applicator for Microwave Hyperthermia Treatment", *IEE Conf. publ. 274*, Fifth ICAP-87, York, U.K. 1987.

[2] E.Okoniewska et al., "Relative, $\sigma|E|^2$ Heating Patterns in Multilayered Biological Tissue Exposed to a Cylindrical Waveguide Applicator", *IEE Conf. publ. 301*, Sixth ICAP-89, Coventry, U.K. 1989.

[3] T.C.Cetas, "Physical Models (Phantoms) in Thermal Dosimetry" in *"Hyperthermia In Cancer Therapy"*, edited by F.K.Storm, G.K. Hall Medical Publ., Boston 1983.

[4] R.W.P.King, G.S.Smith, *"Antennas in Matter"*, MIT Press, Cambridge Ma., USA 1981.

[5] P.Dębicki, "An Inside–Contact Applicator for Microwave Hyperthermia", abstracts of XXI General Assembly of URSI, pp.71-72, Florence 1984.

# TRIPAS : A TRIAPPLICATOR SYSTEM WITH RELOCATABLE 'HOT SPOT' AT TISSUE DEPTH

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## ABSTRACT

Solving the problem of heat focusing and standardization of the clinical application of hyperthermia requires a mathematical prediction model. The model should include the medium constitutive parameter, and be able to predict positioning of the microwave applicators to optimize treatment planning and provide for reproducible treatment set-up. We present a configuration of 3 applicators subtended by an equilateral triangle in order to target and relocate a 'hot spot' for improved treatment of deep tumors. A simple geometric analysis is illustrated. The microwave beam absorption profile, from the three power sources, was obtained from phantom studies depicting the radiative heat pattern for the triapplicator system (TRIPAS). A complex mathematical model was developed to demonstrate interaction of the beams in the medium.

It was observed empirically that under coherent propagation in the near field electromagnetic (EM) waves tend to add at the center, while varying the propagation axial focal length caused a relocation of the summing focal points.

Mathematical prediction correlated very well with the phantom studies. SAR values above 100 W/kg were achieved at 12.5 cm phantom depth, creating a relocatable 'hot spot' at the concentric foci of the 3 air cooled horn microwave applicators operating at 300 MHz.

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## INTRODUCTION

The problem of microwave deep heating has been extensively analyzed by several authors (Andersen 1984, Johnson et al. 1985, and Turner and Kumar 1982). Although some devices are available for regional deep hyperthermia applied to different areas of the body, the problem of insufficient energy penetration persists. Heretofore this has been only partially solved by multiple phased array applicator configurations (Bach Anderson 1984, Gee et al. 1984, Samulski et al. 1987 and Sato et al. 1986). The rationale behind these techniques is the in-phase, coherent addition of electromagnetic (EM) energy from several strategically located sources. The selection of a source frequency lower than 300 MHz theoretically enhances deeper penetration while paradoxically limiting localization of the energy to a defined tumor volume. In prior publications ( Bicher et al.1979,1980, 1982, 1984, 1985, 1986) we have described the use of single or parallel opposed (POPAS) 300 MHz air cooled dielectric loaded microwave applicators. The purpose of this paper is to define the characteristics, mathematical rationale and the penetration of EM energy of a triapplicator system (TRIPAS) focused on a deep target in muscle phantom experiments. A mathematical model is presented based on synthesizing EM waves propagated in the biomedium, using its constitutive parameters, the incident energy and effective wavelength to predict and graphically demonstrate the convergent 'hot spot', and to relocate the target within the concentric configuration of the three applicators.

## MATERIALS AND METHODS

### Device Construction

The TRIPAS system 2* is based on the use of three air cooled dielectric loaded applicators, 20x22 cm in aperture, which have been previously described (Bicher et al 1985). The system operates at 300 MHz, and is driven by fixed frequency generator capable of output of up to 1000 watts. The three applicators are mounted on a graduated circular stand (see Figure 1) by the use of movable brackets. This allows the applicators to be placed in close proximity to the skin overlying the target area in a convergent fashion. The applicators are attached to brackets by universal ball joints, which allow angling to conform to the body surface.

### In vitro Experiments

In vitro experiments were conducted by placing applicators on the three sides of an equilateral triangular phantom box built of plexiglass, measuring 41.5 cm per side. The applicators were placed flush against each side, with aperture focal points converging at the center of the triangle.

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2* Equipment supplied by HBCI Medical Group, Inc., 14427 Chase Street #203 Panorama City, Ca 91402 USA

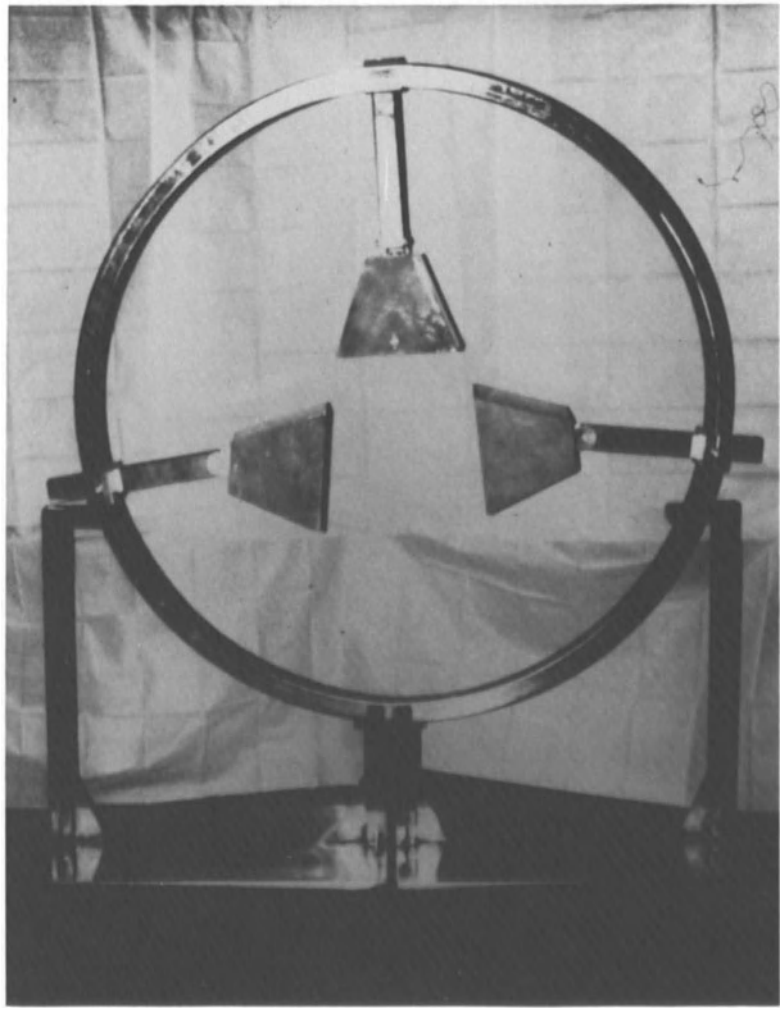

Figure 1. TRIPAS Applicator Stand
Construction of TRIPAS system. Note graded stand supporting 3 concentric relocatable air cooled horn applicators. For explanation see text.

The distance from the center of the triangle to the midpoint of each aperture was 12.5 cm. The phantom was split in a plane perpendicular to one applicator, to allow placement of microthermocouples and liquid crystal thermochromic paper (LCP) between the applicators (see Figure 2). Changes in the color of the thermochromic sheet indicated the thermal field and point of wave convergence. Colors ranged from black (22 C) through red to blue (30 C), making heat field pattern clearly discernible. The composition of the muscle equivalent phantom material was that tabulated by Stuchly and Stuchly (1980), having a dielectric constant of 50-58 F/m and conductivity of 0.909-0.952 S/m at 300 MHz.

SAR Determination

Determination of the power deposition pattern within the phantom involved monitoring 27 precisely positioned microthermocouples in rectangular matrix on the LCP. The triangular split phantom was used to place sensoring devices in the central plane, normal to one of the three radiating apertures. The LCP gave the additional advantage of an overall picture of the heat pattern induced. The specific absorption rate (SAR) from each sensor was determined using the formula: SAR = 4184*c* ($\Delta T/\Delta t$ ) W/kg [17,18], where c is the specific heat capacity of muscle tissue phantom (0.86 cal/gm/deg. C), T is the rise in temperature above normalized temperature (deg. C), and t is the period of time the volume was exposed to the microwave energy. The microwave field was applied to the phantom for 60 seconds prior to each reading. Peak temperature changes induced were 2.6 deg.C at 4cm depth, 0.7 deg C at 8cm depth, and 1.8 deg at the summation point 12.5cm deep. A longer duration of exposure to the microwave field was required to provide the thermocontours simulated in Figure 2.

Model Derivation

The complex propagation constant of EM energy in a medium is readily derivable from hyperbolic wave (Helmholtz) equations using Maxwell's fundamental wave equation ;

$$\nabla \times \bar{E} = - i\omega\mu \bar{H} \qquad \text{.....1)}$$

$$\nabla \times \bar{H} = ( \sigma + i\omega\mu ) \bar{E} \qquad \text{.....2)}$$

for a stratified heterogeneous medium (single propagation constant)

$$\nabla\times\nabla\times\bar{E} = \nabla(\nabla\cdot\bar{E}) - \nabla\cdot\nabla\bar{E} =$$

$$-\omega\mu\omega(\sigma+i\omega\varepsilon)\bar{E} \qquad \text{.....3)}$$

from Maxwell's continuity law :

$$\nabla\cdot\bar{E} = 0 \qquad \text{.....4)}$$

$$(\nabla^2 - \gamma^2)\bar{E} = 0, \qquad \text{.....5)}$$

$$\gamma^2 = \omega^2\mu\varepsilon - i\omega\mu\sigma \qquad \text{.....6)}$$

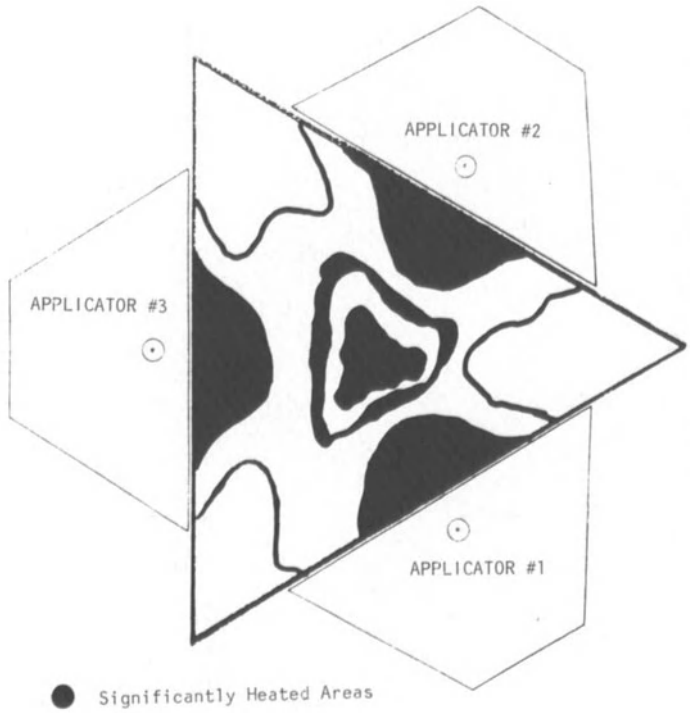


Figure 2. TRIPAS - Muscle Phantom Heating Pattern
Positioning of the applicators for a typical measurement of temperature increment using thermochromic paper. Note the high intensity thermal field in front of each applicator, at the central convergent area and within a circle of positive interference surrounding it.

Eq.(5) specifies the wave equation while Eq.(6) specifies the complex propagation constant. The general solution of Eq.(5) for a homogeneous case is given :

$$\bar{E}y = Ae^{(-\gamma z)} + Re^{(\gamma z)} \quad \ldots\ldots\ldots\ldots\ldots 7)$$

for both forward and backward propagation on z-axis, where A and R are constants described by the medium and excitation characteristics. The complex propagation constant, Eq.(6), can be broken down into its real and imaginary parts :

$$\gamma = \alpha + i\beta \quad \ldots\ldots\ldots\ldots\ldots 8)$$

where $\alpha$ (alpha) describes the attenuation factor, expressed in terms of the medium's constitutive parameters :

$$= w(\mu\varepsilon/2)^{1/2}\left[\left((1 + (\sigma/\varepsilon w)^2\right)^{1/2} - 1\right)^{1/2} \quad \ldots\ldots 9)$$

and where $\beta$ (beta) describes the phase factor, expressed in terms of the medium's constitutive parameters :

$$= w(\mu\varepsilon/2)^{1/2}\left[\left((1 + (\sigma/\varepsilon w)^2\right)^{1/2} + 1\right)^{1/2}\right] \quad \ldots\ldots\ldots 10)$$

A harmonically excited, linearly polarized plane wave traveling in a medium or media with known constitutive parameters can be described.

$$\bar{E}y = Er.e^{(-\alpha z)}\, e^{i(wt-\beta z)} \quad \ldots\ldots\ldots\ldots\ldots\ldots 11)$$

Thus precomputing Eq. (9) and Eq. (10) respectively, Eq. (11) can be easily computed and graphically simulated.

Plane Wave Interactive Simulation

The prediction of the spatial heating effect on deep seated neoplastic tissue is mathematically tractable, and can be deduced from Eq.(11). It is well established that a radiating source is most efficient when the physical parmeters of its aperture are comparable to the wavelength of the source being used (Guy et al. 1974). Thus, the structure of the applicator and the source wavelength dictate in part, the distribution of an EM field profile. Studying the behavior of a plane wave in an excited medium with its characteristic complex propagation constant, does produce observable interaction of electromagnetic waves within KD the medium (Guy 1971), Kantor and Cetas 1977). The selection of these variables as shown by this simulation, depicts its potential value in understanding electromagnetic interaction with biological media. This is further enhanced by the use of 3D graphical analysis. For an approximate linearly polarized plane wave, an axial E-field could be

written :

$$Er = \left(1/(\gamma r) + 1/(\gamma r)^2 + 1/(\gamma r)^3 + 1/(\gamma r)^n\right)e^{(-\gamma r)} \quad \ldots\ldots\ldots 12)$$

where r is written in rectangular coordinate system :

$$r = ((x-x')^2 + (y-y')^2 + (z-z')^2)^{1/2} \quad \ldots\ldots\ldots\ldots\ldots 13)$$

and where n is an integer.

2.6. TRIPAS

Three applicators are configured around an encircled equilateral triangle to target and relocate the calculated 'hot spot' in order to achieve significant heating in the clinically desired area. Simulation of the analytical solution, Eq. (11), for the plane electromagnetic wave propagated in the medium is then implemented.
The rationale behind the design of TRIPAS is not different from the theory of wire antennae. Applicator apertures are considered as planar collections of small electric ( or magnetic) dipoles, each of which radiates complex energy waveforms as the source to impinge on the tissue individually. A monotone excitation is modelled in the biomedium plane by convoluting ( superimposing) plane wave solutions on a surface expressions :

$$Ey = Er.\ e^{(-\alpha\ (x^2 + y^2))^{1/2}}.e^{(-\ (x^2 + y^2)\ 1/2)} .\cos(6.238(x^2 + y^2)^{1/2}/\ ) \quad \ldots\ldots\ldots\ldots\ldots 14)$$

(see notation for definition parameter).

It is presupposed that small electric (or magnetic) dipoles radiate complex energy waveforms, the energy contributions of which are spatially superimposed and summed over individual contributions at the point where they meet in the biomedium when in phase, while negating each other when out of phase.

TRIPAS Analysis

Assumptions

1) A convergent linearly polarized beam axially propagates on the positive z-axis from a strategically located source.

2) Each beam simulates the characteristic excitation and the medium's complex propagation constant.

3) Heterogeneous layers are stratified to homogeneous layers to enhance paractical single parametric values for the medium dielectric constant and conductivity.

4) Each applicator is designed for 300 MHz, EM energy absorption is enhanced by electrical dipoles characteristics in Rayleigh region
( Kritikos et al. 1976 ($\beta R < 2D^2/\lambda_{eff}$)).

5) A muscle phantom with dimensions consistent with the effective wavelength of 300 MHz is selected to enhance constructive interference. (This is derived from Nilsson, P. et al 1985.

Figure 3 shows the simplified analytical geometry which illustrates the convergence of the beams from three dipoles on the vertices of an equilateral triangle. The point of maximum summation, or the point of minimum substration, is located at the center of an encircled equilateral triangle resulting from the three tangential dipoles (Figure 3, case 1). Assuming the point of convergence as the aperture focal point (R) in the medium, R is selected to be proportional to the effective wavelength of the medium to meet Rayleigh region such as:

$$R < D^2 / \lambda_{eff} \quad \text{.....15}$$

Target Relocation

The relocation of the target 'hot spot' is postulated by angular tilting of two adjacent apertures so as to display a constructive focal point within the quadrants of the circle. Figure 3, cases 2 to 4, display theoretical results of tilting a pair of adjacent applicators. The tilting angle of the pair of adjacent applicators can be derived from simple geometrical analysis:

$\Theta$ ( theta ) = arcsin ( L/R - 1) .....16)

where L is the distance from the third aperture to the perpendicular line of adjacent tilted applicators. In other words knowing the precise depth of a tumor in a strategical preconfiguration of TRIPAS, a constructive interference is postulated to give differential heating effect resulting in the formation of a 'hot spot. In order to demonstrate the feasibility of this postulation, 3D graphic simulations and thermographic split muscle phantom experiments were implemented as previously described (see 2.1 -2.3).

Graphic Analyses

For computer simulation of dipoles plane wave, Eq (14), three element sources were located 120 degrees apart around a rectangular co-ordinated circle with its center as the origin
( Figure 3, case 1) Two commercial 3D graphics packages (3*, 4*) were invoked to implement the computer simulation. A fortran 77 routine was written on main-frame to a priori compute the and valves, with the medium's constitutive parameters: relative permittivity, conductivity and the excitation frequency.

These were passed into the 3D Curve package to generate a data file. The flexible Golden Surfer package was invoked to produce the 3D output response of the simulations. Selection of the co - ordinate system can be obtained from :

(0,r), (r,-rsin30), (-r, -rsin30).....17)

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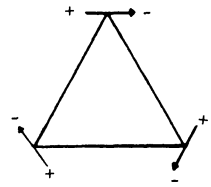
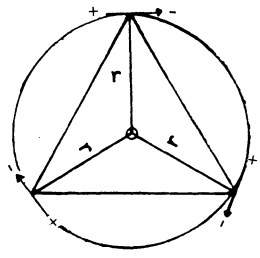
3* Curve Three - D West Coast Consultants, 4202 Genesee Ave, Suite 308, San Diego CA 92117

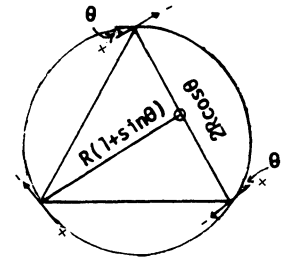
4* Surfer Golden Software Inc., 807 14 Street, P.O.Box 281 Golden CO. 80402



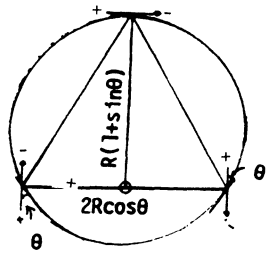
Basic Dipoles Configuration



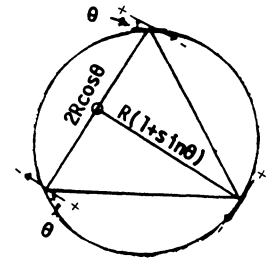

Case 1




Case 2




Case 3




Case 4

Figure 3. TRIPAS Analysis - Postulated Target Relocation Arrows indicate dipoles strategically located around encircled equilateral triangle.
Case 1: Dipoles sum their plane - wave contributions at the center.
Case 2: Pair dipoles rotated at their tangential mid - points by theta to enhance relocation of summation point (target) at the RHS of circular segment.
Case 3. Pair dipoles rotated at their tangential mid - point by theta to enhance relocation of summation point (target) at the Bottom circular segment.
Case 4. Pair dipoles rotated at their tangential mid - points by theta to enhance relocation of summation point (target) at the LHS circular segment.

Three dipoles circularly located at the above rectangular co- ordinates with center (0,0). The valve (r) is matched to (R), the axial focal length, though (r) can be arbitrarily selected. Each co - ordinate goes into Eq. 4, respectively :

$$\bar{E}y = Er.e^{(-\alpha((x-0)^2 + (y-12.5)^2)^{1/2}} .e^{(-\beta((x-0)^2 + (y-12.5)^2)^{1/2}} .\cos(6.283((x-0)^2 + (y-12.5)^2)^{1/2}/\lambda_{eff}) \quad \ldots\ldots 18a)$$

$$\bar{E}y = Er.e^{(-\alpha((x+12.5)^2 + (y-6.3)^2)^{1/2})} .e^{(-\beta((x+12.5)^2 + (y-6.3)^2)^{1/2})} .\cos(6.283((x+12.5)^2 + (y-6.3)^2)^{1/2}/\lambda_{eff}) \quad \ldots\ldots 18b)$$

$$\bar{E}y = Er.e^{(-\alpha((x-12.5)^2 + (y-6.3)^2)^{1/2})} .e^{(-\beta((x-12.5)^2 + (y-6.3)^2)^{1/2})} .\cos(6.283((x-12.5)^2 + (y-6.3)^2)^{1/2}/\lambda_{eff}) \quad \ldots\ldots 18c)$$

for r = R = 12.5 creating the plane wave superimposed on the traveling surface wave ( see Figure 3a and Figure 5 )

## Results

### SAR measurement

The results of the SAR measurements indicate that the temperature increases obtained were greatest close to each radiating aperture, and then lessened substantially, until a zone of summation is reached, where they create a 'hot spot'. The SAR measurements at the 12.5 cm 'hot spot' closely approached those measured at the near field of the aperture (see Figure 4).

### Phantom Temperature Determination

Thermal responses visualized on the liquid crystal sheets showed three different zones of preferential heating :A 'hot spot' was located close to each applicator. A second clearly defined heat zone appeared at the center of the phantom, at the point of beam interaction. Both of these zones are about 6 cm in diameter. A third heat zone is circumferential about midway between the peripheral and central hot spot ( see Figure 2), about 3.4 cm wide ( one quarter of the effective wavelength of 13.4 cm at 300 MHz in the muscle phantom).

### Simulation

The results of the simulation of Eqs. 18a - c are displayed 3-dimensionally in Figure 5. The inputs for computing the complex propagation constant and its components ($\alpha$ , $\beta$ ) simulated in the muscle tissue excited at

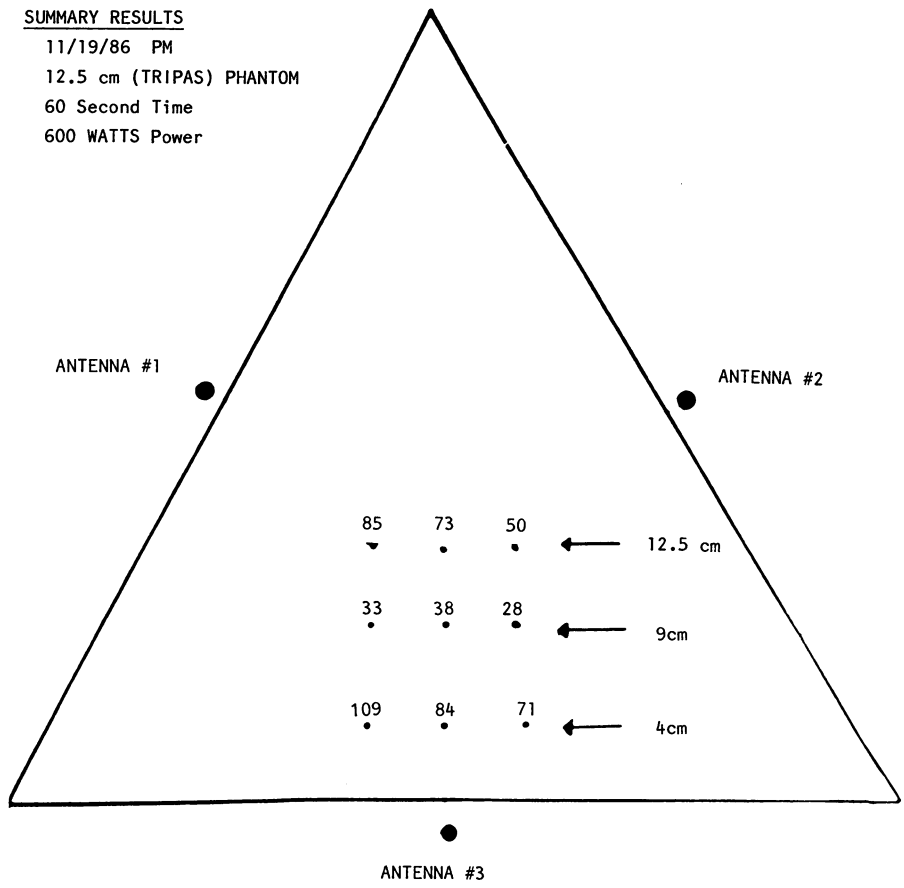



Figure 4. TRIPAS - SAR Profiles

SAR (specific absorption ratio) measured in a triangular muscle phantom, using 300 MHz at 600 Watts equally split among three equivalent applicators (dimension,design) for 60 seconds. SAR's in the area of summation approximate those near the front of the applicator aperatures.

300 MHz, were : permittivity value of 54 F/m and conductivity value of 1.07 S/m, with free space permeability value of 12.57x10 H/m and the permittivity of free space as 8.854x10 F/m. These parameters were appropiately scaled as input variables into the graphic package for the data file. The selection of effective wavelength ($\lambda$eff) can be calculated theoretically, but in this case was extrapolated from the muscle phantom heating pattern studies. A typical value of $\lambda$eff= 13.4 cm was used ( see SAR measurements ).

The dispersive effect of the plane wave was accomplished by the addition of electromagnetic plane wave solution with surface expression ( see Eqs.(14) and (15)). Figure 5 displays the 3D output surface plot of the simulation. The letter marker A,B and C indicate the origin of the dipoles , strategically located at 120 degree separation in circular configuration. The arrow marker points to the central summation ' hot spot'. The main features highlighted by these 3D plots are : 1) high intensity singularity in front of each simulated aperture, 2) rapid decay in amplitude intensity due to generation of destructive interference from subtractive surface waveforms, 3) at even greater depth a second high intensity area due to additive interference causing a peaking standing wave, 4) followed by subtractive interference creating an intensity valley and 5) finally producing the theoretically predicted summation at the centrally located ' hot spot'. The high intensity peak at the aperture (source) is a common observation in experimental and theoretical modellings (Bach Andersen 1984, Gee et al .1984, Kantor and Cetas 1977), that accounts for near- field effects. The surface dispersive effect is predominant at the viewing angle in this simulation. The amplitude steering (modulation) of the target 'hot spot' is implemented by the inclusive term E Eqs. 18a-c. It should be noted that the above surface plot is in very good agreement with experimental observations as described earlier, (comparing Figures 2 and 5). Note, this specifically applied to case 1 of Figure 3 relating to the theoretical postulation.

## Discussion

The present results make use of a mathematical model of radiating dipoles to predict the coherent and noncoherent interference of three converging electromagnetic wave sources interacting within biological media to produce a relocatable 'hot spot'. The muscle-phantom experimental results in part verified this postulation.

Previous studies (Bach Andersen 1984,Turner and Kumar 1982, Wait 1986),solved domain-integral equations for electric field vector potential as a result of induced magnetic current elements derived from fundamental Maxwell's classic microscopic equations, provided versatility of implementing the medium heterogeneity. Turner and Kumar (1982,'84) used Huygen's principle to simulate a horn-type applicator as an array of point-source dipole radiators, in order to predict the heating patterns and performance of the

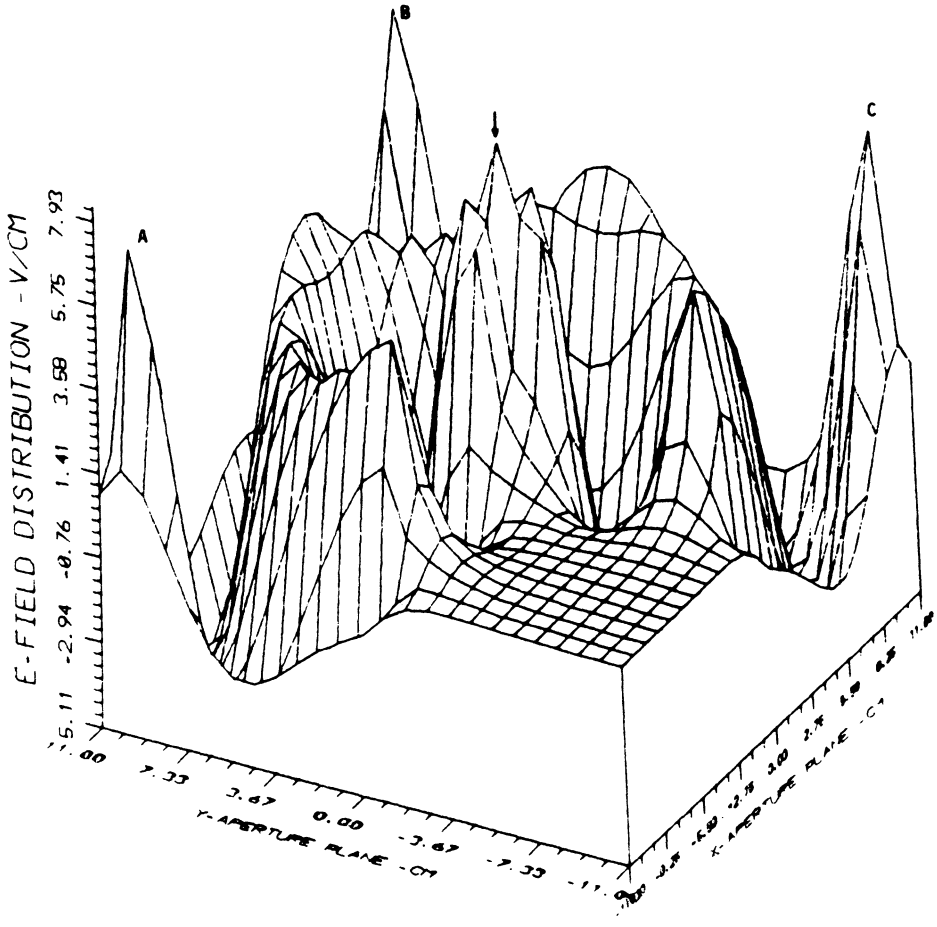



Figure 5. TRIPAS - Simulation

3D surface plot displaying pattern of split 300MHz microwave beams into muscle phantom.
Note:1) the area of central convergence indicated by arrow(as postulated in Fig. 3 case 1), 2) high intensity thermal field in front of each applicator (A,B, and C) and 3) circular area of positive interference at approximate 1/8 wavelength depth between the applicator and the convergent center.

aperture. Such mathematical model was limited in its ability to spatially predict the interaction of harmonically excited plane waves in biological medium. Johnson et al. (1984,'85) and Sato (1986) analyzed circular compact arrays simulating resonance conducting plates as magnetic current sources. A mathematical integration over the aperture surface yields field distribution. This computational method is limited by the convergence or divergence of the numerical technique used for the integration.

Bach Andersen (1984) modelled an array of dipoles to explicitly define radiative antenna gain for power density at a given point relative to the total input power. This concept was used to analyze radiative apertures and their penetration capability in biological tissue. This author (Bach Andersen 1984) further showed the theoretical synthesis of one- dimensional convergent beams meeting at a focal point to produce constructive interference.He pictorially demonstrated that two waves traveling in opposite directions produce either an additive coherence or subtractive noncoherence effect. The present investigation followed the same theoretical postulation.

For cylindrical geometry the large number of waves add to produce power intensity convergence at the axis (Lin 1982). This central cylindrical focusing is generally applied in regional heating of cylindrical tissue, (Samulski et al., 1987 and Turner 1984), but lacks the flexibility to relocate a localized 'hot spot'.

Nilsson et al. (1985) tilted two applicators at 45 degrees. They reported a distinct heating pattern when both the radiative source had no phase relationship, while constructive and destructive interference were observed when both applicators were excited in phase coherently.

Gee et al. (1984), Bach Andersen (1984) and Ling et al (1984) presented theoretical and experimental models of near field phased array focusing analyzed in 3D plane wave profiles. The alternative use of an interactive plane waves model might ease the limitations inherent in the above simulation techniques.

Earlier, we reported (Bicher et al 1985) an experimental parallel opposed system that achieved therapeutic temperatures in the 42 to 43 degree Celsius range at 8 cm depth in both muscle equivalent phantom and in pigs 20-30 cm thick. In other papers (Bicher et al 1982, '84, '86) we defined the clinically usable penetration using one air cooled applicator operated at 300 MHz to be 5 cm. Using POPAS (two opposed applicators operated in phase), we were able to heat therpeutically to a depth of 8 cm. By placing one applicator anteriorly and the other posteriorly, treatment of tumors at moderate depth, in thorax, abdomen and pelvis, have proved successful.

Our results of in vitro experiments and mathematical simulation ( Figure 3, case 1) clearly show effective heating at a depth of 12.5 cm using 3 converging microwave

beams from standard 300 MHz external applicators. The flexibility of the system (TRIPAS) and the ability to change the incident angle of the beams to relocate the ' hot spot' within the target volume, make the system extremely practical for the treatment of deep seated tumors, without the need to heat an entire body region as is required with current phased array systems which operate at lower frequencies. Positioning of the air gap applicators around the patient is quick and easily reproducible. These applicators have poor coupling however, so there is some energy loss. In the present system this is compensated by the use of a high power generator operating at fixed frequency (300 MHz), capable of 300 watts per applicator. Thus a 900 watts generator for TRIPAS will be required for clinical use. Such a device has already been built and is operational in our laboratory.

The good correlation between our mathematical predictions and phantom studies validates the theoretical approach used. The ability of the TRIPAS system to predict the location of the 'hot spot' within the treatment field makes computer planning of the applicators positioning in the clinical situation feasible.

Nomenclature

D = applicator aperture dimension (cm)
Er = axial spatial electric field magnitude (v/cm)
-
Ey = electric field complex vector (v/cm)
f = frequency (MHz)
-
H = magnetic field omplex vector (A/cm)
i = imaginary operator (Sq.root (-1))
n = numerical approximate integer for axial E - field
L = distance from the aperture to target - tumor (cm)
R = axial aperture focal length (cm)
r = graphical radius
x,y,z = rectangular coordinate system
$w = 2\pi f$ ; angulart frenquency (rad)
$\alpha$ = attenuation factor (Np/m)
$\beta$ = phase factor (rad/m)
$\sigma$ =conductivity of the medium (S/m)
$\varepsilon$ = relative dielectric constant
$\mu$ = free space permeabiliti (H/m)=
$\gamma$ = complex propagation constant (H.Np/m)
$\lambda$ eff = effective wavelength (cm/ cycle)
$\Theta$ (theta) = tilting axial aperture angle (deg)
$\nabla$ = vector differential operator
$\nabla^2$= laplace operator
$\Delta$ = variable incrememt

REFERENCES

1) Bach Andersen, J; 1984, Eletromagnetic heating - a review. Proceedings of the 4th International symposium on Hyperthermia Oncology, Aarhus, Vol. 2, ed. J. Overgaard (London, Philadelphia: Taylor & Francis, 1984), pp. 113 - 128.

2) Bach Andersen, J; 1984, Theoretical limitation on radiation into muscle tissue. International Journal of Hyperthermia, 1, 45 - 55.

3) Arcangeli, G., Lombardini, P.P., Lovisolo G. A., Marsiglia G. and Piattelli, M., 1984, Focusing of 915 MHz electromagnetic power on deep human tissue: a mathematical model study. IEEE Transactions Biomedical Engineering, BME - 31, 47 -54.

4) Bicher, H. I., Sandhu, T. S., and Hetzel, F. W., 1979, Hyperthermia as a radiation adjuvant : An effective fractionation regime. International Journal of Radiation, Oncology, Biology. Physics. 5,171.

5) Bicher, H.I., Sandhu, T.S., and Hetzel, F.W., 1980, Hyperthermia and radiation in combination. International; Journal of Radiation, Oncology, Biology, Physics, 6, 867 - 870.

6) Bicher, H. I., Hetzel, F. W., and Sandhu, T. D.S., 1982. Results of a phase I/II clinical trial of fractionated hyperthermia in combination with low dose ionizing radiation. Advance Experimental Medical Biology, 157, 87 - 97.

7) Bicher, H. I., 1984, The Set up of the state of art Hyperthermia clinic - therapeutic, ethical, and practical considerations. Frontiers in Radiation Therapy Oncology, 18, 153 - 161.

8) Bicher, H. I., and Wolfstein, R. S., 1984, Microwave hyperthermia as an adjuvant to radiation therapy. Summary experience of 208 multifraction treatment cases. Hyperthermia Oncology, vol. 1, ed. by J. Overgaard (London, Philadelphia : Taylor & Francis, 1984),pp. 363 -366.

9) Bicher, H. I., Moore, D. W., and Wolfstein, R. S., 1985, Air cooled 300 MHz Applicators used in a parallel opposed phased (POPAS). International Journal of Radiation, Oncology, Biology, Physics, 11,SI, 217.

10) Bicher, H. I., and Wolfstein, R. S., 1986, Microwave hyperthermia as an adjunct to radiation therapy: Summary experience of 256 multifraction treatment cases. Interational Journal of Radiation, Oncology, Biology, Phisics 12,1667 - 1671.

11) Bicher, H. I., and Wolfstein, R. S., 1986 Local hyperthermia for deep tumors - experience with three techniques. Journal of Microwave Power, 21, 103.

12) Gee, W., Lee, S., Bong, N. K., Cain, C. A., Mittra, R., and Magin, R. L., 1984,Focused array hyperthermia applicator: therory and experiment. IEEE Transactions. Biomedical; Engineering, BME - 31, 38-46.

13) Guy, A W., 19781, Electromagnetic fields and relative heating patterns due to a rectangular aperture source in direct contact with bilayered biological tissue. IEEE Transactions. Microwave Theory and Technique, MTT -19, 214 - 223.

14) Guy, A. W., Lehmann, J.T., and stonebridge, J.B., 1974, Therapeutic application of electromagnetic power.Proceedings of the IEEE, 22,55 -75.

15) Guy, A.W., Lehmann, J.F., stonebridge, J.B., and Sorensen, C.C., 1978, Development of a 915 MHz direct contact applicator for therpeutic heating of tissues. Ieee Transactions. Microwave Therory and Techniques, MTT - 26, 550 -556.

16) Johnson, C.C., Durey C.H., and Plenk, H. P., 1977, Experimenwith 915 MHz hyperthermia treatment of malignant tumors. Journal of Bioengineering. 1, 511 - 530.

17) Johnson,R.H., James, J. R., Hand, J.W , 1984, Hopewell, J. W, Dunlop, P.R.C, and Dickson, R.J., New low- profile applicators for local heating of tissues. IEEE Transactions. Biomedical Engineering, BME - 31, 28 - 37.

18) Johnson, R. H., Andrasic, g., Smith, D. L., and James, J. R., 1984, Field penetration of arrays of compact applicators in localized hyperthermia. International Journal of Hyperthermia 1, 29 -43.

19) Kantor, G., 1981, Evaluation and survey of microwave and radiofrequency applicators. Journal of Microwave Power 16, 135 - 150.

20) Kantor, G., and Cetas, T. C., 1977, A comparative heating pattern study of direct - contact applicators in microwave diathermy. Radio Science, 12, 6S, 111 - 120.

21) Kritikos, H. N., Foster, K. R. and Schwan, H. P., 1981, Temperature profiles in spheres due to electromagnetic heating. Journal of Microwave power, 16 (3&4), 327 - 344.

22) Lin,J.C.,Kantor G., and Ghods, A., 1982, A class of new microwave therapeutic applicators. Radio science, 17 5S, 1195 - 1235.

23) Ling, H., Lee, S.W., and Gee, W., 1984, Frequency optimization of focused microwave hyperthermia applicators. Proceedings of the IEEE, 72, 224 -225.

24) Nilsson, P., Larsson,T., and Persson, B., 1985, Absorbed power distribution from two tilted waveguide applicators. International Journal of hyperthermia, 1, 29 -43.

25) Samulski, T. V., Kapp, D. S., Fessenden, P., and Lohrbach, A., 1987, Heating deep seated eccentrically

located tumors with an annular phased array system: A comparative clinical study using two annular array operating configuration. International Journal of Radiation, Oncology, Biology, Physics, 13, 83 - 94.

26) Sato, G., Shibata, C., Sekimukai, S., Wakabayashi, H., and Mitsuka, K., 1986, Phase - controlled circular array heating equipment for deep - seated tumors: Preliminary experiments. IEEE Transaction. Microwave Theory and Techniques MTT - 34, 520 - 524.

27) Turner, P. F., and Kumar, L., 1982, Computer solution for applicator heating patterns. National Cancer Institute Monograph, 61, 521 - 523

28) Turner, P. F., 1984, Regional hyperthermia with an annular phased array. IEEE Tranaction. Biomedical Engineering, BME - 31, 106 - 119.

29) Wait, J. R., 1986, Analysis of the radiation leakage for a Four - aperture phased - array applicator in hyperthermia therapy. IEEE Transactions, Microwave Theory and Techniques Mtt - 34 539 541.

# NON-INVASIVE MEASUREMENTS IN HYPERTHERMIA: RADIOMETRY AND PREVISIONAL THERMOMETRY

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## INTRODUCTION

The development of Hyperthermia as a therapeutic method is closely related to the possibilty to control and quantify hyperthermic treatment in the portion of a biological body heated. For this purpose, the thermometer would have resolution and precision between 0.1°C and 0.2°C, because of the great dependence of the biological response to the temperature increase; suitable spatial resolution, between 5 and 10 mm, cause of the necessity to work in large temperature gradients; ability to follow the temporal variations of temperature with a temporal resolution of 1 second and a dimensions below 16 gauge to minimize trauma for patients. Actually the thermometry system used in the routine consist of thermistors, thermocouples and optical fibers. All these system are invasive: they give punctual temperature measurements in the whole tissue area and are traumatic for the patient.To avoid these disadvantages in our institute two non invasive methods have been tested: they're a microwave radiometer, which measures the emitted intensity of bodies, that is directly related to their temperature and a simulation program, which calculates the temperature behavior of an heated tissue by the solution of the bio-heat equation. The results of our tests show that the thermometric technique, based on radiometer, gives temperature measurements that are to be considered as a mean among the ones obtained in the whole tissue area and not global at all; on the other hand the calculation of the heat produced by metabolism and over all of the heat taken away by the blood flow. With the intent to verify the goodness of the program we have simulated 22 hyperthermic treatments comparing the real temperature, obtained by optical fibers inserted in the tissue area during heating, with the simulated one. In parallel it was made a comparison between invasive measurements, with optical fibers, and non invasive ones, revealed by radiometer.

*Consensus on Hyperthermia for the 1990s*
Edited by H. I. Bicher *et al.*
Plenum Press, New York, 1990

## MATERIALS AND METHODS

### Hyperthermic system

The available system is the SAPIC SV03/A, made by Aerita-lia, Gruppo Sistemi Avionici ed Equipaggiamenti, Caselle Torinese. It allows all the services needful for local hyperthermic treatments in Tumor therapy: the electromagnetic (EM) energy generation, its delivery in patients, the measure and the registration of the temperature data. It has been carefully tested and it has been used in routine since September 1983; it can be used for superficial and for average depth hyperthermia with invasive and non invasive applicators. The system is completely administered by a computer which tests the power to deliver, the temperature reached into the heated body and the cooling temperature. This latter helps to increase patient's tolerance during heating. The system has two operative working ways: the first is the remote: all phases are administered by HP 310 computer, whose software is loaded on 3 1/2" floppy disc. Dialogue with operator is managed through keyboard,color display is provided with touch screen and plo tte r.Automatic power control allows fixing temperature behavior of tumoral tissue and verification of security of treatment to support patient to eventual overheating. All treatment parameters are visible on computer monitor for the entire treatment duration:so you can change a parameter to optimize treatment. The second working way is the local one: parameter control and regulation are completely committed to operator's experience because computer and pheripheric units are totally unfitted. All system components control unity includes a hardware logic that prevents wrong commands on operator behalf and manages by itself conditions that can damage treatment security and that can happen. However it can recognize the syntetizator for radiofrequencies (RF) signal generation in HF band At last the system has a screened cabin with the intent to avoid surrounding pollution from EM radiation and in particular from magnetic fields generated from them. The cooling system is a closed circuit hydraulic system composed of a peristaltic pump, a thermostatic bath, a water pillion and some flexible little tubes for connection. It's possible to maintain patient's surface to constant temperature between 10 and 40°C by circulation of distilled water in the pillion based between applicator and a patient. This system helps to optimize coupling between applicator and surface to treat. We can manage the cooling system by computer for all its parameters except for the flux range that must be manually regulated. The available antennas have different shape and work at different frequencies, so they can be used according to the various clinical needs. The range of interest in most all cases is between 434 and 915 MHz (microwaves), but when lesions are deeper than 3-4 cm, we use an applicator which works in RF range at a standard frequency (27.12 MHz). For temperature control we use some sensors made by LUXTRON CO (Mountain View, CA USA). They give temperature measurement that are free from error due to undesired fluctuations and whose range is between -5 and 80 C on the scale portion useful for Hyperthermia. They

allow us to have a maximum of 8 measurements at the same time in the tissue area, with an accurancy of 0.2°C, using four single sensors (0.25 mm of diameter) and one multiple sensor (four points).

Microwave radiometer

The microwave radiometer is a receiver of EM radiation: it has low noise added, high sensivity, wide band and it is able to work with three different frequencies (1.5, 2.5 and 3.5 GHz ) respectively . Unfortunately the large wave length microwave at these frequencies allows a loss in spatial resolution. The available radiometer is made by ODAM: it has originally been made to work at two frequencies but then it has been modified to work at three frequencies. This change has been made because the resolution power of instruments is related to the frequency number; on the other hand its increase allows a big advance in build prices. The radiometer grounds itself on the principle that all bodies at temperature above absolute zero (0 K) emit an EM radiation that is directly related to the temperature itself. The relationship between temperature and EM radiation intensity is expressed by Plank equation, which can be approched to the Railegh-Jeans equation for microwave range:

$$I(V_i) = \frac{2\pi K \tau V^4}{C^3}$$

If we express light emitted intensity in terms of brilliance temperature, then we can deduce physic temperatures at microwave frequency. In fact, brilliance temperature, measured by radiometer with a specific frequency, is represented by the integral:

$$T_{b(V)} = \int_0^L K_p(v,x)\, T(x)\, dx$$

This is a temperature profile integral weighted along X axis perpendiculary to skin surface. In this expressed K is the weighing function, p is the vertical or horizontal polarization and L is the depth over which there aren't any brilliance temperature contributions: the expression is represented by a first class Fredholm integral whose weighing function K can be obtained for every layer in close form, solving Maxwell equations and chosing suitable outline conditions.Actually we don't know any mathematical algorithm which allows us to solve this equation and to obtain physical temperature from brilliance . Then we can base our measurement on brillance temperature only: this means that if heating would be homogeneous the temperature is the real one. The dishomogeneity of tissues composing biological body causes a different heating in the volume "seen" by radiometer; the values obtained by these measurements would represent the average of all the temperatures in tissue only. About the available instrument, it uses antennas which can be connected to a power generator: so they can deliver power and receive emitted radiation almost at the same time. The conversion from receiving to delivering antenna can be made through a switch. We have two different antennas: the bigger one is able to work better at lower frequencies, while the smaller one is more useful for higher frequencies.

## Simulation program

The simulation program calculates the temperature behavior of heated tissue by the solution of the bio-heat equation; the initial parameters on which it grounds are the heating tissues and the applicator used for heating. This program has been developed by a collaboration between Electronic and Automatic Department of Engineering, University of Ancona and Aeritalia, Gruppo Sistemi Avionici ed Equipaggiamenti, Caselle Torinese. The first step is to divide an ecographic or TC image in discrete areas and to obtain data related to each elements dielectric and thermic constant. Then calculation of irradiated field in free space is made; this means applicators characterization and SAR ( Specific Abortion Rate) determined for a non homogeneous body. So it's possible to calculate biological body temperature distribution after a certain heating time, according to the power delivered by the applicator,the heat taken away by the blood flow, the metabolic heating and then the heating exchange with external atmosphere. Under these calculations, the program simulates temperature evolution during map heating. Evolution time is real: on video you can follow isothermal modifications and a time-temperature graph on some points of the map. The useful parameters are: kind of applicator used, heating tissue distribution and up to date and temperature data calculated. If the temporal step is small, results are precise and elaboration time increases: chosing temporal step between 15-30 seconds, we can obtain good precision and thermography can be brought up to date in real time. As in the real treatment, a power control is made. This parameter is varied to maintain temperature of the warmest point at a previously fixed value (treatment temperature).

## RESULTS

### Comparison between invasive and previsional techniques

Using simulation program 21 hyperthermic treatments have been made: data related to sensors position, treatment time, frequency to be used and cooling temperatures have been inserted in the program to be used. The simulation gives useful information about global behavior in tissue: the thermographic maps evolution is seen through a computer monitor. Moreover you can obtain any information about thermal behavior of some selected points in heated tissues. The effectiveness of this program is verified through a comparison between graphics that show temperature behavior really measured by sensors during the hyperthermic treatment and graphics obtained by simulation. In both cases an automatic power control has been made: so hot spots are quite the same but it's important to underline how correspondent are the reference points in both treatments (real and simulation ones). These results confirm the real effectiveness of simulation programs tested. In Table I data related to the 21 simulation are presented: we define GOOD CORRESPONDENCE (GC), a correspondence greater than 60% between compared points, DISCRETE CORRESPONDENCE (DC), a

correspondence greater than 30% but smaller than 60% and BAD CORRESPONDENCE (BC), every other correspondence smaller than 30%. In some cases the tumor area could be near large blood vessels: because of the coarse approximation made, the simulation program doesn't take into account properly the perfusion phoenomena. In other cases the TC section used to design the anatomical map doesn't correspond to the central treatment plane, causing a different position in the target volume of the reference points. That's why in these cases the correspondence is bad. In Table II the number of reference points used in simulation is shown: this one is never quite equal to the number of sensors used for real treatment control; in fact, in the simulation we use only a section of the whole volume heated in the real treatment.

Comparison between invasive and non-invasive technique

This kind of comparison has been preceded by an experimentation about instrument characteristics and responses as function of heating tissue depth.The first tests consisted of cooling and heating of a thermostatic bath and then of a muscle simulated phantom of about 3 cm of depth. This one showed that device response was good and its resulting characteristic is quite linear. To test the brillance temperature behaviour as a function of depth, we heated a portion of the phantom of 2 cm in depth at a temperature of 40°C. Then we posed some phantom layers of 1 cm in depth at a temperature of 39°C, over the initial portion and measured the brilliance temperature. This measure is quite reliable because the difference between the two temperatures guarantee a certain time before conduction phoenomena : the time is enough to reveal brilliance temperature. In both tests made temperature is a constant during all the emitted brilliance temperature measurement: this situation can be obtained because of the short measuring time of the radiometer. Problems come when heat distribution is non-homogeneous: in it's actual use, the device is able to reveal a temperature which isn't real, but is a mean among measured temperatures in the whole heated volume. Experimental tests were made on phantom and also on patients: they show a brillance temperature that follows the temperature increase but whose value is more or less an average between invasive and superficial temperature value. This technique can't be considered as effective actually because it can't allow an immediate control of hot spots that rise during hyperthermic treatment. We can only have useful information about quite superficial treatments: in these cases depth is small (less than 2cm) to allow the hot spots detection, even if it's quite approximate.

CONCLUSION

Experimentations of these two non-invasive techniques, chosen as an alternative method to contro temperature instead of optical fibers, show their limits.

Since we can't obtain a temperature profile into heated tissue because of the unknowing of good calculation

Table 1. RESULTS OF PROVISIONAL THERMOMETRY

| NUMBER OF SIMULATED TREATMENT | GC | DC | BC |
|---|---|---|---|
| 21 | 8(38.1 %) | 8(38.1 %) | 5(23.8 %) |

GC = Good Correspondence (> 60 %)
DC = Discrete Correspondence (> 30 %, <60 %)
BC = Bad Correspondence (< 30 %)

TABLE 2. NUMBER OF SENSORS

| * | ** | *** | CORRESPONDENCE |
|---|---|---|---|
| 12 | 4 | 2 | GC |
| 4 | 4 | 2 | DC |
| 7 | 5 | 2 | BC |
| 11 | 7 | 1 | GC |
| 12 | 6 | 6 | DC |
| 10 | 5 | 1 | DC |
| 6 | 6 | 6 | BC |
| 10 | 5 | 4 | DC |
| 7 | 3 | 1 | DC |
| 12 | 6 | 4 | GC |
| 7 | 4 | 3 | BC |
| 10 | 6 | 5 | GC |
| 10 | 7 | 3 | BC |
| 3 | 3 | 2 | GC |
| 12 | 5 | 0 | BC |
| 12 | 4 | 0 | DC |
| 12 | 7 | 2 | DC |
| 12 | 6 | 2 | DC |
| 12 | 9 | 4 | GC |
| 12 | 6 | 1 | GC |
| 12 | 7 | 2 | GC |

* Total number of sensors used for real treatment
** Number of sensors useful for comparison with invasive thermometry
*** Number of invasive sensors used for comparison with invasive thermometry

algorithm, then we can use radiometer only for superficial tumor temperature measurements. On the other hand, the interest in the radiometer pushes us to continue this kind of experimentation. A five band radiometer is under study: an increase in band numbers can allow a better spatial and temporal resolution of the instrument and at the same time, it can give a greater number of dates for temperature profile calculation. About the simulation program, we have obtained quite good results, even if the thermometric system doesn't guarantee full effectiveness because of the coarse approximation made in the bio-heat equation solution about power delivered by metabolism and power taken away by the blood flow. To determine a mathematical tridimensional algorithm for blood flow perfusion a lot of studies have been developed. Unfortunately complexity of such methods makes it impossible to be elaborated; so the solution of the bio-heat equation in its correct form isn't permitted. The intent of our institute is to use simulation before every hyperthermic treatment to optimize treatment itself. Knowing temperature distribution into points for thermometry will improve thermal control during heating.

REFERENCE

1) Audone B, Bolla L, Delogou A and coll: Dosimetry and applicators in RF and microwave hyperthermia. Strahlentherapie: 159-367, 1983.

2) Audone B, Bolla L, and coll: Microwave and RF computerized system for hyperthermic oncology. Proc 4Th Int Symp on Hyp Onc: 715-717, Aahrus, 1984.

3) Cerri G, De Leo R: Waveguide applicators for biomedical application: an accurate analysis of power coupling in homogeneous biomedical media. Int Wroc Symp on El Comp, 1984.

4) Hand JW: Thermometry in Hyperthermia. Prooc 4Th Int Symp on Hyp Onc: 299-308, Aahrus, 1984.

5) Plancot M, Prevost B, Chive M and coll: a new method for thermal dosimetry in microwave hyperthermia using microwave radiometry for temperature control. Int J Hyp 1, 9-19, 1984

6) Strohben JW, Roemer RB: A survey of computer simulation of hyperthermia treatment. IEEE Trans, vol BME-31:136-149, 1984.

# LOCAL HYPERTHERMIA FOR SUPERFICIAL AND MODERATELY DEEP TUMORS--FACTORS AFFECTING RESPONSE

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## INTRODUCTION

Many reports in the peer review medical literature on the clinical application of hyperthermia over more than a decade have contributed to recognition of its safety and efficacy, with a synergistic effect in combination with radiation and/or chemotherapy. (1-36)

Mechanisms of hyperthermia effect on tumor while sparing normal tissue have been well evaluated, including interaction with ionizing radiation (37-48). Tumor cells are more sensitive to heat than normal cells. More significant however, because of physiological factors, tumor tissue is far more affected by application of heat inducing energy than is normal tissue. Tumor cells distant from capillaries undergo anaerobic metabolism and thus have lower pH. Hyperthermia impairs microcirculation in tumors, thus further increasing tumor acidity, and decreasing tumor oxygenation (37-40, 42, 46). Hyperthermia cytotoxicity is maximal in the S phase of the cell replication cycle (41,45,47). Since radiation and chemotherapy are least effective on cells in the S phase and induce S phase in cells undergoing sublethal injury, there is a rational basis for expecting greater tumor destruction when these modalities are combined with hyperthermia.

Most groups, including our own, have employed clinical protocols with intervals of 3 days between hyperthermia treatments, in tacit or stated deference to the well established phenomenon of thermotolerence, which has been well described in laboratory studies that demonstrate resistance of treated cells to a second heat insult over a period of time (43,44).

Our experience over the past four years, on which this report is based, suggests that thermotolerence may not be a definitive factor in the clinical use of hyperthermia in certain situations. In the current studies we undertook to evaluate the factors affecting response rate of superficial and deep tumors using different fractionation regimes of both irradiation and hyperthermia.

*Consensus on Hyperthermia for the 1990s*
Edited by H. I. Bicher *et al.*
Plenum Press, New York, 1990

## MATERIALS AND METHODS

Single air gap microwave applicators operating at 915 or 300 MHz with power up to 400 Watts* were used for hyperthermia treatment of tumors up to 5 cm from the surface. For deeper tumors two such 300 MHz were used, parallel opposed and operated in phase (POPAS) at power up to 800 watts (36,49). Thermometry was done throughout each treatment session using two triple junction copper-constantan microthermocouples (100 micron)*. Temperature readings were obtained at 4-5 minute intervals with power off, to prevent microwave interference artifact, recorded on a modified computer system that also controls the power on-off cycle. Thermocouples were placed in the tumorous and normal tissue.

Each hyperthermia treatment lasted up to one hour, with the goal of achieving minimum tumor temperature of 42 degrees C for at least 30 minutes. Hyperthermia treatments were given either twice a week over 5 weeks, as previously reported, or daily for 5 weeks. Since September of 1987 all patients have been given daily hyperthermia treatments.

All treatment was given under FDA approved protocols, and all patients accepted for treatment signed the appropriate consent form. Eligible patients had advanced primary cancer (36 patients), post treatment local recurrence (54 patients), or metastatic disease (82 patients).

For patients receiving radiation therapy, this treatment was given within 2 hours of hyperthermia, either before or after. Total radiation dose and fractionation depended on normal tissue tolerence. Patients treated with the goal of cure received the same full course of radiation therapy that would be considered appropiate if no adjunct hyperthermia were given. Patients on chemotherapy received the drug dosage schedule appropiate to their individual problem, at the discretion of the referring medical oncologist. Chemotherapy was given in conjunction with as many hyperthermia treatments as practical and as close as possible to hyperthermia, optimally by infusion continuing through the hyperthermia session.

This report analyses results of hyperthermia treatment of 299 tumors fields in 172 evaluable patients treated since September, 1984. Of these, 178 were superficial, treated with a single applicator, and 121 were treated with POPAS. Some superficial treatments (15/144) and 40% of POPAS treatments (48/121) were given with no adjunct radiation therapy; almost all of these patients did receive chemotherapy. Tumor response was evaluated by physical examination where feasible, precisely measured in 2 dimensions with estimated tumor depth, and in deeper tumors by x ray, CT or MRI. Response was graded as complete (CR) when there was no residual tumor, partial (PR) if tumor regression exceeded 50%, stable (SD) when there was less regression or no progression of tumor within two months following treatment.

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RESULTS

Overall results are shown in Table 1. Side effects were minimal, noted in less than 10% of patients. Superficial and POPAS treatments were equally well tolerated (Table 2). Patients did perspire quite freely during POPAS treatments, kept comfortable with damp cloths and drinks. Core temperature did not rise significantly (less than 2 degrees C) even after consecutive treatment of two fields given over more than 2 hours. Radiation induced skin reaction was never more than anticipated for the same dose given without hyperthermia. No patient discontinued hyperthermia treatment because of toxicity or subjective intolerence.

Table 1

Summary Results

227 Patients, 172 evaluable, 299 Fields

| | Response | |
|---|---|---|
| CR (complete) | 113 | (38%) |
| PR (50% or more) | 123 | (41%) |
| SD ( No growth at 2 months) | 29 | (10%) |
| NR ( No response) | 34 | (11%) |

| Complications | |
|---|---|
| Thermal Burns | 16 ( Healed fully) |
| Ulceration | 3 ( Healed fully) |
| Pneumothorax | 2 ( Hospitalized 1 day) |
| Ileus | 1 ( Hospitalized 3 days) |

Table 2

Summary Results

| Response | Superficial (N=178) | POPAS (N=121) |
|---|---|---|
| CR(%) | 89 (50) | 24 (20) |
| PR(%) | 66 (37) | 57 (47) |
| SD(%) | 10 (6) | 19 (16) |
| NR(%) | 13 (7) | 21 (17) |

Abbreviations: CR, complete; PR, partial; SD, stable disease; NR, no response

Patients with objective tumor regression less than 50% but no progression at 2 months following treatment were considered to have stable disease (SD), as also suggested by others (35). Most with SD had palliation and improved clinical status. The category of SD was established because many patients, 16% of those treated with POPAS, obviously benefited from treatment but without significant objective decrease in tumor size.

Table 3

Results By Radiation Dose

| | Superficial | | | POPAS | | |
|---|---|---|---|---|---|---|
| | 30Gy(+) (N=83) | 20Gy(-) (N=67) | 0 (N=28) | 30Gy(+) (N=35) | 20Gy(-) (N=38) | 0 (N=48) |
| CR(%) | 45(54) | 37(55) | 7(25) | 16(46) | 3 (8) | 5(10) |
| PR(%) | 32(39) | 20(30) | 14(50) | 14(40) | 18(47) | 25(52) |
| NR+SD% | 6 (7) | 10(15) | 7(25) | 5(14) | 17(45) | 18(38) |

Abbreviation: (+), or more; (-), or less; CR, complete response; PR, patrial response; NR, no response; SD, stable disease

Table 4

Results By Hyperthermia Dose

| A. | Superficial | | POPAS | |
|---|---|---|---|---|
| | (N=78) | (N=76) | (N=31) | (N=29) |
| CR(%) | 43(55) | 31(41) | 4(13) | 5(17) |
| PR(%) | 30(38) | 28(37) | 19(61) | 9(31) |
| NR+SD(%) | 5(7) | 17(22) | 8(26) | 15(52) |

| B. | Subsequent results using POPAS, 25 Heat (N=61) |
|---|---|
| CR (%) | 15(24) |
| PR (%) | 29(48) |
| NR+SD (%) | 17(28) |

Abbreviations: CR, complete response; PR, partial response; NR, no response; SD, stable disease

Tumor response was improved when hyperthermia was combined with higher dose of ratiotherapy, as in our previous series (31). The SD-NR rate was 2-3 times greater for tumors receiving 20Gy or less compared to those treated with 30Gy or more (Table 3). Tumor response was also found to be related to the number of hyperthermia fractions (Table 4A) The NR rate was reduced by over half with more hyperthermia treatments. Because of the results of this comparison we now routinely give daily hyperthermia treatments, and results have remained essentially the same (Table 4 B).

Unexpected but gratifying was the finding that POPAS treated lesions given low dose radiation versus no radiation had equivalent tumor response rates. (Table 3). Since nearly all patients receiving no radiation were on a chemotherapy regimen designed for optimal combination with hyperthermia, based on published research (29,30,32,33,34,50,51) one would like to ascribe the results in POPAS patients treated without radiation to the adjunct effect of chemotherapy on hyperthermia. However, in superficial tumors treated without radiation were relatively poor, little better than published reports of tumor response to hyperthermia alone (8,9,17,19,21).

In analyzing results by tumor site, we found that CR rate in superficial tumors was nearly threefold better than in POPAS treated lesions, while SD-NR rate was correspondingly less (Table 2). Much of this difference is accounted for by results in treatment of breast adenocarcinoma (primary and recurrent), a category that included over half of all superficial lesions and had by far the best results (Table 5). Indeed, at least 50% response rate (CR&PR) was as good in tumors of pelvis, chest and pancreas (Table 6) as in superficial lesions exclusive of breast (Table 5).

Table 5

Superficial Hyperthermia Results

| Site | # | CR | (CR+PR%) | PR | SD | NR |
|---|---|---|---|---|---|---|
| Breast | 91 | 60 | (95) | 26 | 3 | 2 |
| Head + Neck | 39 | 15 | (71) | 15 | 1 | 8 |
| Other | 48 | 14 | (81) | 25 | 6 | 3 |
| Total | 178 | 89 | (87) | 66 | 10 | 13 |

Toxicity: 10 Skinburns, 3 Ulcerations
Abbreviation: CR, complete response; PR, partial response
NR, no response; SD, stable disease

The group of patients treated for potential cure is of particular interest. This group comprises only 22 patients with advanced primary or local postoperative recurrence, excluding those with pancreas adenocarcinoma (Table 7). Ten had breast cancer, 7 with untreated primary tumors including 5 measuring 10 cm. or more 3 with palpable axillary or supraclavicular adenopathy and 3 with untreated post mastectomy chest wall recurrence. Except for 2 with postoperative recurrent cervical nodes, all patients required at least two hyperthermia treatment fields to

Table 6

Hyperthermia Results with POPAS

| Site | # | CR | (CR+PR%) | PR | SD | NR |
|---|---|---|---|---|---|---|
| Pancreas | 14 | 1 | (71) | 9 | 2 | 2 |
| Liver | 28 | 3 | (46) | 10 | 6 | 9 |
| Other Abdomen | 8 | 1 | (50) | 3 | 2 | 2 |
| Pelvis | 32 | 9 | (78) | 16 | 5 | 2 |
| Lung | 14 | 6 | (79) | 5 | 2 | 1 |
| Other Chest | 25 | 4 | (72) | 14 | 2 | 5 |
| Total | 121 | 24 | (67) | 57 | 19 | 2 |

Toxicity: 6 Skin burns, 2 Pneumothorax, 1 Ileus
Abbreviations: CR, complete response; PR, partial response; SD, stable disease; NR, no response

cover their extensive disease. Seventeen of the 22 (77%) had CR. Twelve are alive, 10 at least 1 year, all but one free of local recurrence. Of 20 patients evaluable for at least 1 year, 8 (40%) have no evidence of disease. Only 1 patient of 17 with CR has had recurrence within the treatment field following definitive combined treatment.

For 150 patients, 87% of the total evaluable, the treatment goal could only be palliation, local tumor control and possible prolongation of life. In such cases treatment

Table 7

Patients Completing Definitive Treatment

| Patient | Tumor site/stage/type | | | Treatment Gy | Heat | Resp. | Status |
|---|---|---|---|---|---|---|---|
| IO 63 F | O Ph | T3N1 | Squ | 70 | 2/W | C | 10m rec |
| HC 70 F | Neck | Rec | An | 60 | 2/W | N | 1.5m rec |
| IB 56 F | Br | T4N? | Ade | 50 | 2/W | C | 44m NED |
| AC 58 F | PI | T3No | Squ | 70 | 2/W | C | 46m NED |
| BS 48 F | Br | T3N? | Inf | 50 | 2/W | P | ? |
| MC 56 F | Lung | T4No | OC | 40 | 2/W | C | 4m met |
| JS 32 M | O Ph | T2No | AC | 60 | 2/W | C | 38m NED |
| DL 50 F | Br | T4No | Ade | 50 | 2/W | P | ? |
| LD 41 F | Br | Rec | Ade | 64 | 2/W | P | ? |
| CM 64 M | Neck | Rec | Squ | 72 | 2/W | C | 22m NED |
| LF 54 F | Br | Rec | Inf | 60 | 5/W | C | 26m NED |
| WM 61 F | Br | Rec | Ade | 50 | 5/W | C | 23m NED |
| DJ 49 F | Br | T4N1 | Inf | 70 | 5/W | C | 7m NED |
| JT 74 F | N Ph | T3N1 | Squ | 50I | 5/W | C | 13m NED |
| FA 61 F | Br | T4N2 | Ade | 66 | 5/W | C | 17m NED |
| RE 60 F | Lung | T2N2 | Ade | 66 | 5/W | C | 13m NED |
| JF 73 M | Lung | T1N2 | OC | 55 | 5/W | C | 3m met |
| JR 58 F | Br | T4N1 | Ade | 60 | 5/W | C | 14m met |
| LW 79 F | Br | T4No | Ade | 50 | 5/W | P | 12m rec |
| SL 52 M | Ton | T3N1 | Sq | 70 | 5/W | C | 8m met |
| NS 66 M | Pros | T2N? | Ade | 40+33I | 5/W | C | 9m NED |
| GH 62 M | Esop | T2NO | Squ | 60 | 5/W | C | 4m met |

Abbreviations: O Ph, oral pharynx; Br, breast; PI, piriform sinus; N Ph, nasal pharynx; Ton, tongue; Pros, prostate; Esop, esophagus; I, Implant; W, Week; Rec, recurrence; NED, no evidence of disease, Met, metastasis; Squ, squamous cell; Ana, anaplastic; Inf, inflammatory OC, oat cell; AC, adenocystic; Ade, adeno.

failed in 18 (12%), who did not have at least stable disease with palliation. Of the 117 patients for whom significant tumor regression was achieved (CR&PR), 90 are evaluable for at least 6 months.

Of 31 patients with CR 20 (65%) lived at least 6 months with no local recurrence; and 12 (39%) are alive 6 to 38 months after treatment (Table 8) including 3 with no evidence of disease. Of 60 patients with PR 17 (28%) lived 6 months or more without regrowth of treated disease; and 10 (17%) remain alive 6 to 48 months following treatment (Table 9).

When regrowth occurs, manifested by change in size on examination or x-ray, recurrent symptoms, and/or increase in

Table 8

Patients Treated with Limited Goal
Complete Response Duration

| Status | Total (N=31) | 25 Heat (N=18) | 10 Heat (N=13) | 30Gy(+) (N=17) | 20Gy(-) (N=14) |
|---|---|---|---|---|---|
| No rec min 6m(%) | 20(65) | 3(72) | 7(54) | 8(47) | 12(86) |
| Alive min 6m(%) | 12(39) | 10(56) | 2(15) | 5(29) | 7(50) |

Abbreviations: (+),or more; (-), or less; m, months; rec, recurrence; min, minimum

Table 9

Patients treated with limited Goal
Partial Response Duration

| Status | Total (N=60) | 25 heat (N=41) | 10 heat (N=19) | 30 Gy(+) (N=27) | 20 Gy(-) (N=33) |
|---|---|---|---|---|---|
| No rec min 6m(%) | 17(28) | 13(32) | 4(21) | 5(19) | 12(36) |
| Alive min 6m(%) | 10(17) | 9(27) | 1(5) | 2 (7) | 8(24) |

Abbreviation: (+), or more; (-), or less; m, months; rec, recurrence; min, minimum

tumor markers such as CEA, the tumor is retreated. For example: A 46 year old male with 60 pound weight loss over one year and progressive intractable abdominal pain finally developed obstructive jaundice and underwent by-pass surgery for unresectable pancreatic carcinoma with liver invasion and regional adenopathy. It was recommended that he have no treatment. After further deterioration over 2 months, he was too weak to walk when hyperthermia was started in combination with 5-fluorouracil. Palliation and improvement in his general condition during 5 weeks of daily hyperthermia treatment allowed him to return to work. CT scan showed 75% regression of the primary mass, normal liver and complete regression of adenopathy. He continued to function well for over a year during which time two additional courses of hyperthermia combined with 5-FU were required. His last course of hyperthermia, this time given along with 45 Gy was less effective clinically; however, CT

scan 6 minutes later showed no tumor. Serial retreatment with hyperthermia has not resulted in toxicity, except probably in a 72 year old male with liver metastasis and mesentric adenopathy secondary to colon adenocarcinoma, who twice developed ileus.

## DISCUSSION

We have herein reported results obtained in 172 patients treated by one group, employing various techniques of external microwave hyperthermia. Best results have been obtained in the group of patients, previously disussed, that we were able to treat definitively (Table 7), as reported by other groups (15,27).

Treatment results in superficial lesions appear to have been much better than in deep lesions using POPAS (Table 2). Analysis of results by radiation dose, however, shows no significant difference in those tumors receiving 30 Gy or more (Table 3). More superficial than deep tumors received a higher dose of irradiation, respectively 47% (83/138) vs 29% (35/121). Response in deep tumors improved with higher radiation dose (Table 3). Independent of radiation dose tumor response was significantly better when more hyperthermia treatments were given (Table 4), which calls into question the applicability of thermotolerance in clinical practice.

The phenomenon of thermotolerance has been well established in the laboratory by in vitro and in vivo experiments (43, 44). Both normal and cancer cells and tissue have been found to resist a second heat insult for over a month following initial heating. It is far from clear, however, what effect thermotolerance has along with the many other factors that affect tumor response to hyperthermia in clinical practice. Most clinics, including our own until recently (3,4,31), have empirically given hyperthermia treatment on a twice a week basis. Even with weekly treatment only the first hyperthermia session should be fully effective if thermotolerance were a predominant operating factor.

With this paradox in mind we have tried giving hyperthermia treatment daily 5 days per week and have found significantly better tumor response using daily treatment. The number of superficial and deep tumors that failed to regress by at least 50% (NR+SD) was 32/105 (31%) with biweekly treatments as compared to 13/109 (12%) following daily hyperthermia treatment (Table 4). These results provide further demonstration that thermotolerance is much less important in clinical hyperthermia practice than other factors as yet poorly defined. Tumor heating is never homogeneous, and it is likely that the inhomogeneities vary from one hyperthermia treatment to another; thus inhomogeneous heating may actually be advantageous, by heating cells that have not developed thermotolerance. Since the development of thermotolerance has been shown to be

partially inhibited at low pH (53), and tumors have low pH further reduced by hyperthermia, thermal response of tumor relative to normal tissue may be enhanced by daily heat treatment.

Other groups have used daily hyperthermia treatment successfully. Moffat et al (30) and Falk et al (54) reported on thermochemotherapy in 178 patients with unresectable hepatic neoplasm given "1 to 25 treatment courses (median 6 courses)---of 1-5 consecutive daily sessions of 75-120 min. thermotherapy---". Hornback et al (55) treated stage IIIB cancer of the cervix with irradiation given 150-200 cGy per day to 40Gy and "each patient was exposed to 40 to 45 minutes of heat after each external radiation treatment". Corry et al (24) gave one to three courses of hyperthermia for superficial tumors, that "consisted of one hour treatment on three successive days of each week". Falk et al (39) treated pancreatic cancer with "two to three consecutive daily sessions every 2 weeks" along with chemotherapy. Hornback et al (6) treated a hemangiosarcoma of the scalp with "3000 rad at 200 rad/day, each treatment followed immediately by 20 minutes of heat". Earlier they (5) reported on 21 patients given 3-6000 rads at 100-200 rads/day with "20 minutes of microwave radiation to the local tumor area immediately prior to the prescribed dose of ionizing radiation".

Marmor and Hahn (22) compared tumor response between matched tumors in the same patient with multiple superficial lesions given 2-6000 rads in 200-400 rad fractions. "One of the matched nodules was given hyperthermia (43°C) for 15 minutes before and 30 minutes after each radiation fraction". While the number and frequency of neither hyperthermia nor radiation treatments were otherwise specified, at least some patients must have been treated daily. " Seven of 15 patients had an improved response in the tumor that received hyperthermia", and the difference was more apparent in those given low-dose radiation (2- 4000 rad).

Streffer et al (56) found that the development of thermotolerance was suppressed in human melanoma cells given factionated heat combined with ionizing radiation: "thermotolerance is apparently not a significant factor after combined treatment schedules which are used in clinical tumor therapy". Arcangeli and Nervi (23) gave weekly or twice weekly hyperthermia to separate tumors in the same patient for 5 weeks. "When comparing equal RT fractionation schedules(i.e. daily fractions of 2Gy) tumor TER increased from 1.83 to 2.2 when the number of equal HT treatments ( i.e. 43.5 degrees Celsius for 45 minutes) was doubled from 5 to 10. The skin TER, in contrast, appeared to decrease with increasing number of fractions." They (23) concluded that:" Our data does not seem to evidentiate any thermotolerance induction in human tumors, at least by using clinical treatment schedules like those employed in this study, although the problem can only be clarified with more clinical data". Our data supports these conclusions.

Analysis of response duration in our current series (Tables 8,9) raises serious questions regarding the use of radiation therapy. When response (CR or PR) was achieved with 20Gy or less,including patients given no radiation, duration of response was more prolonged than with a higher radiation dose. The numbers of patients surviving at least 6 months with no local recurrence were, respectively, 24/47 (51%) vs 13/44 (30%). Comparable figures for 25 vs 10 hyperthermia treatments were, respectively 26/59 (44%) and 11/32 (34%). For those patients still alive at the time of this writing (minimum 6 months) with or without local recurrence the figures are even more striking: less vs more radiation, 15/47 (32%) vs 7/44 (16%); more vs fewer hyperthermia treatments, 19/59 (32%) vs 3/32 (9%). We know of no other study that has analyzed results in this way. Does palliative irradiation impair immune response so much as to adversely affect survival? Does hyperthermia enhance immune response so much that more treatments improve survival? These questions remain unanswered and require further investigation.

REFERENCES

1) Arcangeli G, Barni E, Cividalli A, et al : Effectiveness of microwave hyperthermia combined with ionizing radiation; Clinical results on neck node metastases Int J Radiat Oncol Biol Phys 6: 143-148, 1980

2) Arcangeli G, Cividalli A, Nervi C, et al : Tumor control and therapeutic gain with different schedules of combined radiotherapy and local external hyperthermia in human cancer.
Int J Radiat Oncol Biol Phys 9: 1125-1134, 1983

3) Bicher H I, Sandhu T : Results of a phase I/II clinical trial of fractionated hyperthermia in combination with low dose ionizing radiation. In Bicher H I, Bruley D (Eds): Hyperthermia. New York, Plenum Press 1982 pp 87-89

4) Bicher H.I, Sandhu T, Hetzel F: Hyperthermia and radiation in combination : A clinical fractionation regime. Int J Radiat Oncol Biol Phys 6: 867-870, 1980

5) Hornback NB, Shupe RE, Homayon S, et al : Preliminary clinical results of combined 433 MHz microwave therapy and radiation therapy on patients with advanced cancer. Cancer 40: 2854-2863, 1977

6) Hornback NB, Shupe RE, Shidnia H, et al : Radiation and microwave therapy in the treatment of advanced cancer. Radiology 130: 459-464, 1979

7) Johnson R, Sandhu T, Hetzel F, et al : A pilot study to

investigate the therapeutic ratio of hyperthermia 41.5 - 42.0 C and radiation. Int J Radiat Oncol Biol Phys 5: 947-954 1979

8) Kim J, Hahn GM, Benjamin F: Treatment of superficial cancer by combination hyperthermia and radiation therapy. Clin Bull 9:13-16, 1979

9) Manning M, Cetas T, Miller R, et al: Clinical hyperthermia: Results of a phase I clinical trial employing hyperthermia alone or in combination with external beam or intersitial radiotherapy. Cancer 49: 205-216, 1982

10) Oleson J, Manning M, Heusinkveld R: Hyperthermia by magnetic induction.II. Clinical experience with concentric electrodes. Int J Radiat Oncol Biol Phys 9: 549-556, 1983

11) Oleson J, Manning M, Sim D, et al: A review of the University of Arizona human clinical hyperthermia experience. Front Radiat Ther Onc 18:136-143, 1984

12) Sapozink M, Gibbs F, Gates K, et al: Regional hyperthermia in the treatment of clinically advanced deep seated malignancy: Results of a study employing an annular array applicator. Int J Radiat Oncol Biol Phys 10: 775-786, 1984

13) Sapozink M, Gibbs F, Thomson J, et al: A comparison of deep regional hyperthermia from an annular array and a concentric coil in the same patients. Int J Radiat Oncol Biol Phys. 11: 179-190,1985

14) Scott R, Johnson R, Kowal H, et al: Hyperthermia in combination with radiotherapy; a review of five years experience in the treatment of superficial tumors. Int J Radiat Oncol Biol Phys 9: 1327-1333, 1983

15) Scott R. Johnson R, Story K, et al: Local hyperthermia in combination with definitive radiotherapy: increased tumor clearance, reduced recurrence rate in extended follow up. Int J Radiat Oncol Biol Phys 10: 2119-2123, 1984

16) Storm K, Silberman A, Ramming K, et al: Clinical thermochemotherapy: A controlled trial in advanced cancer patients. Cancer 53: 863-868, 1984

17) U R, Noell K, Woodward K, et al: Microwave induced local hyperthermia in combination with radiotherapy of human malignant tumors. Cancer 45: 638-646, 1980

18) Fazekas JT, Nerlinger B S : Localized hyperthermia adjuvant to irradiation in superficial recurrent carcinomas: A preliminary report on 46 patients. Int J Radiat Oncol Biol Phys 7: 1457-1463, 1981

19) Dunlop P R C, Hand J W, Dickinson R J, et al: Combined radiation and hyperthermia in clinical practice. Int J Hyperthermia 2: 39-50, 1986

20) Luk K H, Francis M E, Perez C A, et al: Combined radiation and hyperthermia: Comparison of two treatment schedules based on data from a registry established by the radiation therapy oncology group (RTOG). Int J Radiat Oncol Biol Phys 10: 801-809, 1984

21) Field S B, Bleehen N M: Hyperthermia in the treatment of cancer. Cancer treatment reviews 6: 63-94, 1979

22) Marmor J B, Hahn G M: Combined radiation and hyperthermia in superficial human tumors. Cancer 46: 1986-1991, 1980

23) Arcangeli G, Nervi C: The lack of clinical evidence of tumor thermotolerance after some schedules of combined heat (HT) and radiation (RT). In Overgaard J (ed): Hyperthermia Oncology 1984 Vol. 1. Taylor and Francis, London and Philadelphia, 1984, pp231-235

24) Corry P M, Barlogie B, Tilchen E J, et al : Ultrasound-induced hyperthermia for treatment of human superficial tumors. Int J Radiat Oncol Biol Phys 8: 1225-1229, 1982

25) Sapozink M D, Gibbs F A, Egger M J, et al: Abdominal regional hyperthermia with an annular phased array. J Clin Oncol 4: 775-783, 1986

26) Oleson, J R, Sim D H, Conrad J, et al: Results of a phase I regional device evaluation: Microwave annular array versus radiofrequency induction coil. Int J Hyperthermia 2: 327-336, 1986

27) Arcangeli G, Benassi M, Cividalli A, et al: Radiotherapy and hyperthermia. Analysis of clinical results and identification of prognostic variables. Cancer 60: 950-956, 1987

28) Hiraoka M, Jo S,, Akuta K et al: Radiofrequency capacitive hyperthermia for deep seated tumors. II. Effects of thermoradiotherapy. Cancer 60: 128-135, 1987

29) Falk R E, Moffat F L, Lawler M, et al: Combination therapy for resectable and unresectable adenocarcinoma of the pancreas. Cancer 57: 685-688, 1986

30) Moffat F L, Gilas T, Calhoun K, et al: Further experience with regional radiofrequency hyperthermia and cytotoxic chemotherapy for unresectable hepatic neoplasia. Cancer 55: 1291-1295, 1985

31) Bicher H I, Wolfstein R S, Lewinsky B S, et al:

Microwave hyperthermia as an adjunct to radiation therapy: summary experience of 256 multifraction treatment cases. Int J Radiat Oncol Biol Phys 12:1667-1671, 1986

32) Hahn G M: Hyperthermia to enhance drug delivery. In Rational Basis for chemotherapy, Alan R. Liss, N Y, 1983, pp 427-436

33) Hahn G M: Interaction of hyperthermia and drugs: Treatments and probes. Natl Cancer Inst Monogr 61: 317-323, 1982

34) Engelhardt R: Hyperthermia and drugs. Recent Results Cancer Res 104: 136-203, 1987

35) Storm F K, Scanlon E F, Baker H W, et al: Tumor stabilization after hyperthermia: an important criterion of response to thermal therapy. J Sur Oncol 34: 143-149, 1987

36) Bicher H I, Wolfstein R S: Local hyperthermia for deep tumors experience with three techniques. In J Microwave Power, symposium summaries: 1986,pp 103-104

37) Bicher H I, Hetzel F: Effects of hyperthermia on normal and tumor microenvironment. Radiology 137: 523-530. `980

38) Emami B, Nussbaum G H, TenHaken R K, et al: Physiological effects of hyperthermia: response of capillary blood flow and structure to local tumor heating. Radiology 137: 805-809,1980

39) Bicher H I, Mitagvaria N P: Circulatory response of malignant tumors during hyperthermia. Microvascular Res 21:19-26, 1981

40) Song C W, Kang M S, Rhee J G, et al: The effect of hyperthermia on vascular function, pH and cell survival. Radiology 137: 795-803, 1980

41) Li G C, Evans R G, Hahn G M: Modification and inhibition of repair of potentially lethal x-ray damage by hyperthermia. Rad Res 67: 491-501,1976

42) Dewhirst M W, Ozimek E J, Gross J, et al: Will hyperthermia conquer the elusive hypoxic cell? Implications of heat effects on tumor and normal tissue microcirculation. Radiology 137: 811-817. 1980

43) Urano M, Maher J, Kahn J, et al: Studies on fractionated hyperthermia in experimental animal systems III. uneven daily doses. Int J Radiat Oncol Biol Phys 9:717 72 - 1983

44) Urano M, Kahn J: Differential Kinetics of thermal resistance (thermotolerance) between murine normal and tumor tissue. Int J Radiat Oncol Biol Phys 12: 89-93, 1986

45) Mivechi N F, Hofer K E: Evidence for separate modes of action in thermal radiosenitization and direct thermal cell death. Cancer 51 : 38-43,1983

46) Bicher H I, Mitagvaria N P: Changes in tumor tissue oxygenation during microwave hyperthermia- clinical relevance. In Overgaard J (ed): Hyperthermia Oncology 1984 1: Taylor & Francis, London and Philadelphia, 1984, pp 169-172

47) Vaupel P, Kallinowski F: Physiological effects of hyperthermia. Recent Results Cancer Res 104: 71-109, 1987

48) Corry P M, Robinson S, Getz B S: Hyperthermia effecte on DNA repair mechanisms. Radiology 123:475-482,1987

49) Bicher H I, Moore D, Wolfstein R S: Air- cooled 300MHz applicators used in a parallel opposed phased system (POPAS). Int J Radiat Oncol Biol Phys 11: S1:217, 1985 (abst)

50) Marmor J B: Interactions of hyperthermia and chemotherapy in animals Cancer Res 39:2269-2276, 1979

51) Hahn G M: Potential for therapy of drugs and hyperthermia. Cancer Res 39:2264-2268, 1979

52) Kapp D S: Site and disease selection for hyperthermia clinical trials. Int J Hyperthermia 2: 139-156,1986

53) Goldin E M, Leeper D B: The effect of reduced pH on the induction of thermotolerance. Radiology 141: 505-508, 1981

54) Falk R E, Newhook L, Moffat FL, et al: Thermochemotherapy for unresectable hepatic cancer. Recent Results Cancer Res. 100: 315-320, 1986

55) Hornback N B, Shupe RE, Shidnia H, et al: Advanced stage IIIB Cancer of the cervix treatment by hyperthermia and radiation. Gyn Oncol 23: 160-167, 1986

56) Streffer C, Van Beuningen D, Marbach J: Thermotolerance: A barrier for the use of hyperthermia in tumor therapy ? Int J Radiat Oncol Phys 8:109 (abst.),1982

# ULTRASOUND AND THE BLOOD-BRAIN BARRIER

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## ABSTRACT

High intensity focused ultrasound was employed to modify the permeability of the normal feline and canine blood-brain barrier (BBB) to a circulating vital dye - Evans blue (EB). The threshold doses (W sec/cm$^2$) for focally increasing the permeability of the BBB in white matter (WM) and gray matter (GM) were as follows: internal capsule (WM) - 340 to 680; thalamus (GM) - approximately 1326; and caudate nucleus (GM) - 2284 to 2952. In the presence of supralesioning doses of ultrasound, the cross sectional area occupied by the EB was consistently greater than that of the attendant nonhemorrhagic lesion - thus suggesting that BBB changes may be inducible at sublesioning doses. These findings, in conjunction with those of others, suggest that high intensity focused ultrasound may have a role in the treatment of brain tumors based on cell destruction by two

Key Words: Blood-brain barrier, brain tumor, neurosurgery, stereotactic surgery, ultrasound.

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mechanisms: (a) direct, by the ultrasound and (b) indirect, by an antineoplastic agent which is delivered via an ultrasonically modified BBB.

## INTRODUCTION

There are only a few reports of the effect of high intensity focused ultrasound on the blood-brain barrier (BBB) (1, 2, 3). The application of supralesioning doses of high intensity pulsed focused ultrasound to the brains of craniectomized cats increased the permeability of the BBB to a large molecular complex (trypan blue bound to serum albumin) and to 32P; although neuronal and glial cells were destroyed in the gray and white matter targets, the vasculature was morphologically normal (2). In two other similar studies which utilized supralesioning doses of ultrasound (1, 3), it was observed that the induced permeability to trypan blue was reversible; by 72 hr postradiation, the trypan blue no longer exited the vascular compartment in the area of the lesion; thus, the integrity of the modified barrier was restored. Based on these observations for supralesioning doses of ultrasound (the greater resistance of vascular cells to damage and the reversibility of barrier permeability), additional studies employing supra- and sublesioning doses on normal and neoplastic brain seem warranted. We report the results of modifying the permeability of the normal feline and canine BBB with high intensity focused ultrasound; these results include the threshold doses and cross-sectional areas occupied by the BBB permeability marker Evans blue. Also, the potential application of high intensity focused ultrasound, in conjunction with antineoplastic agents, for treating brain tumors is discussed.

## MATERIALS

Two adult mongrel cats (3.9 $\pm$ 0.8 kg) and four adult mongrel dogs (11.3 $\pm$ 1.9 kg) were used in this study. The animals were housed in the approved quarters of the Indiana University School of Medicine and cared for in accordance with the standards established by the Animal Welfare Acts as described in the Guide for the Care and Use of Laboratory Animals.

A stereotactic system was employed for cranial roentgenography, positioning the radiating transducer and blocking in the in-situ brain in stereotactic space.

An M469 Instruments for Industry High Power (5 kW, 65 dB gain) Wide Band (1.0 to 100 MHz) Water Cooled Amplifier was used to drive the focused ultrsound transducer (1.0 MHz piezoelectric element coupled through a quarter-wavelength thick oil layer to a lucite Fresnel lens, 12.7 cm diameter, 13 cm focal length) which had half-power (3 dB) beam dimensions of 2.0 mm and 12.0 mm for the transverse and longitudinal axes, respectively.

Evans blue (EB), a vital dye with a molecular weight of 961 daltons, which binds to circulation albumin (12 molecules of EB per molecule of albumin) with a molecular weight of

69,000 daltons to form a hydrophilic complex, was used as an in-vivo marker for detecting changes in BBB permeability.

## METHODS

Both white and gray matter were examined because of differences in vascularization, ultrasound lesion threshold and the pathological conditions which occur in them. The one white matter (WM) and two gray matter (GM) radiation targets were the internal capsule, and the thalamus and caudate nucleus, respectively. The stereotactic coordinates for these targets were determined from cranial structures which were directly and/or roentgenographically visualized.

Requisite for determining the ultrasound intensity at the target was the tissue path distance (d) traversed by the ultrasonic beam. This distance was obtained by measurement from the cranial roentgenograms.

The cats and dogs were anesthetized with sodium pentobarbital (33 mg/kg, IV), intubated and placed in the stereotactic headholder. The dura was exposed via a large bilateral craniectomy. The ultrasonic transmission medium (Ringer's solution, pH 7.0, 37 degrees C) was maintained in the temperature controlled head pan which was coupled to the scalp with a wire clamp. Approximately 15 min prior to radiation, EB (2% filtered, 1.5 ml/kg, 37 degrees C) was infused (1.2 ml/min, IV, Harvard Pump). With a temporal separation of 2.5 min, the targets (interanl capsules, thalami and caudate nuclei) were radiated. Sixty min postradiation, the anesthetized animal, which had remained mounted in the head holder, was heparinized (5,000 u, IV) and sacrificed with saturated KCl (15 ml bolus, IV). With the animal remaining in the headholder, the ascending aorta was cannulated via the left ventricle, and the brain was perfused with Ringer's solution (2 L, pH 7.0). Two frontal plane cuts, which were parallel to the central axis of the ultrasound beam, were made. The block of brain containing the radiated targets was removed from the cranium and frozen sections (20 μm thick) were obtained; the cut surface of the frozen brain was photographed to document the location of the EB. Alternating sections were stained with hematoxylin and eosin for cells and with luxol fast blue for fibers. BBB threshold dose for EB ($T_{EB}$) was defined as the lowest dose for which EB could be detected in the 35 mm color slides. Lesion (LX) threshold dose ($T_{LX}$) was defined as the lowest dose for which a gross or light microscopic lesion (i.e., abnormality in neuronal soma or fiber, glia or vasculature) could be detected in the stained sections. The cross-sectional areas of the EB and LX were determined with a Supergrid digitizing tablet (Summagraphics Corp).

Ultrasound dose, defined as the product of intensity ($W/cm^2$) and radiation time (sec), was employed to describe the amount of ultrasound delivered to the target because it could be compared to doses reported in other studies. The peak ultrasound intensity at the target site (I) was determined by the method of Fry (4) and the peak ultrasound intensity in a free-field ($I_0$) was determined by the method of Dunn et al (5).

RESULTS

To determine the precision of the ultrasonic radiation system, the preselected voltage was compared to the delivered voltage (fractional transducer driving voltage) and the preselected dose was compared to the delivered dose for each of the 33 radiations. The mean difference between the preselected and delivered voltages (volts peak-to-peak (Vpp))

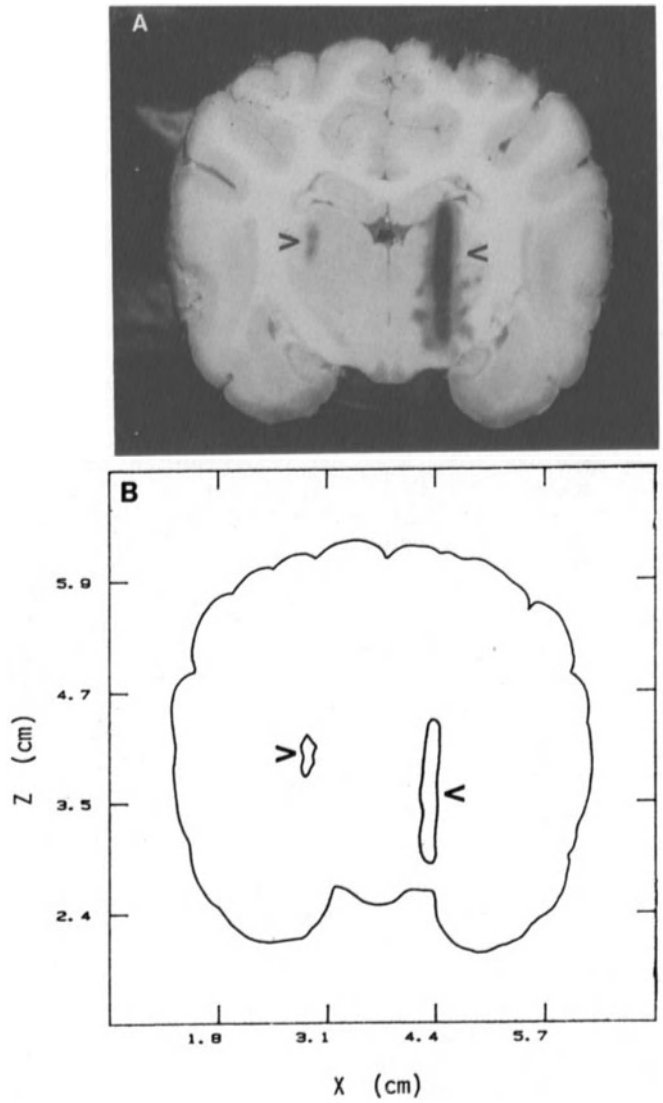


Fig 1. Frozen brain section (A) and corresponding digitized section (B) from dog (D4549); left hemisphere on the left side. Arrowheads indicate areas of Evans blue in the thalami (gray matter).

was -0.03% (range -6% to +6%). The mean difference between the preselected and delivered doses (W sec/cm$^2$) was +0.3% (range -10% to +11%).

A modified BBB was detected by observing EB in frozen sections (Figs 1A and 2A) and quantified by determining the cross-sectional area occupied by the EB (Figs 1B and 2B).

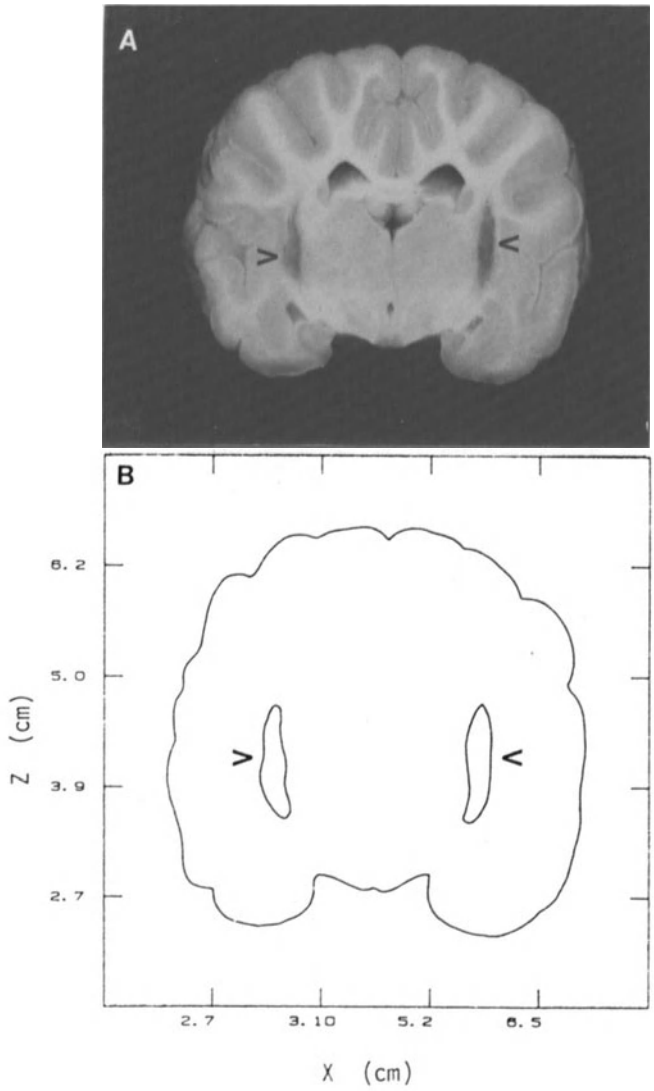


Fig 2. Frozen brain section (A) and corresponding digitized section (B) from dog (D4596); left hemisphere on the left side. Arrowheads indicate areas of Evans blue in the internal capsules (white matter).

Histologic lesions were detected by gross and light microscopic examination of stained sections (Fig 3A) and quantified by determining the cross-sectional area occupied by the lesion (Fig 3B). Histologic sections were obtained only from brains with EB present. Although freezing artifact

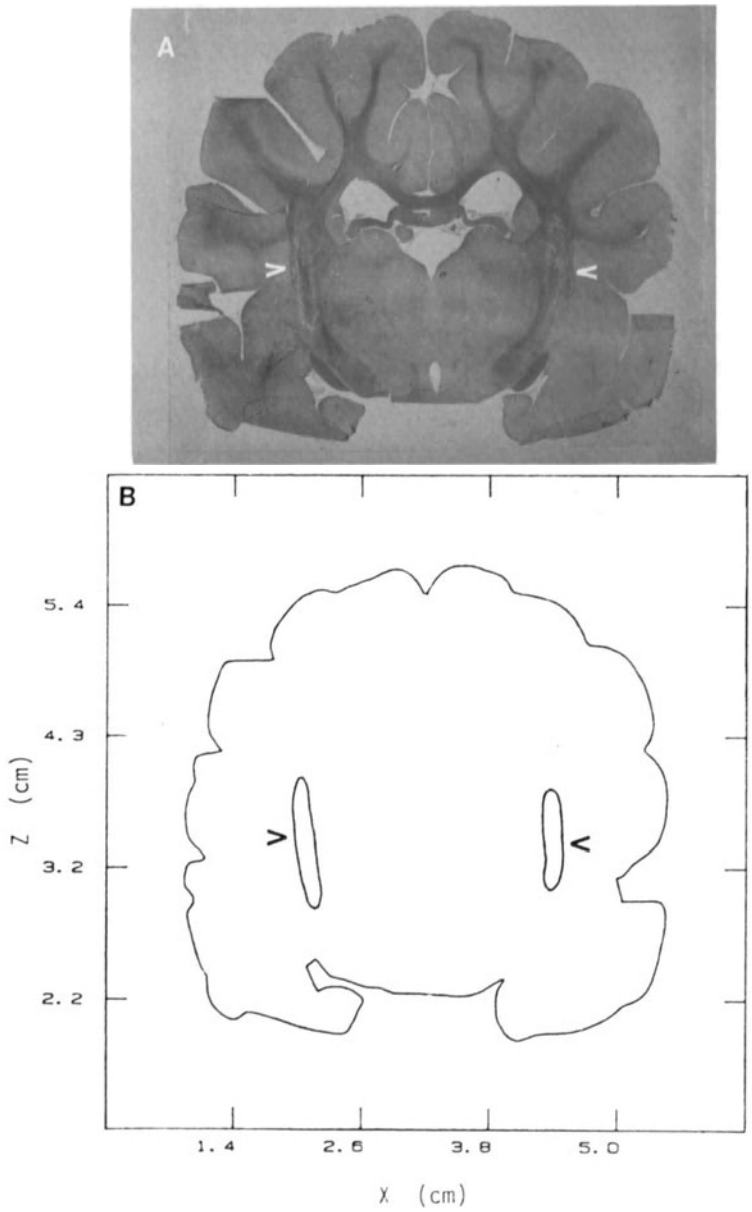


Fig 3. Myelin stained brain section (luxol fast blue) (A) and corresponding digitized section (B) from dog (D4596); left hemisphere on the left side. Arrowheads indicate areas of lesions in the internal capsules (white matter).

was severe and prevented a light and microscopic characterization of neuronal and glial cell changes, no hemorrhage was observed grossly or microscopically in the stained sections.

The threshold doses for modifying the permeability of the BBB to EB were as follows: for the internal capsule, between 340 and 680 W sec/cm$^2$ (Fig 4); for the thalamus, approximately 1326 W sec/cm$^2$ (Fig 5); and for the caudate nucleus, between 2284 and 2952 W sec/cm$^2$ (Fig 6). Based on gross and light microscopic examination of sections, no hemorrhage was detected in the radiated targets.

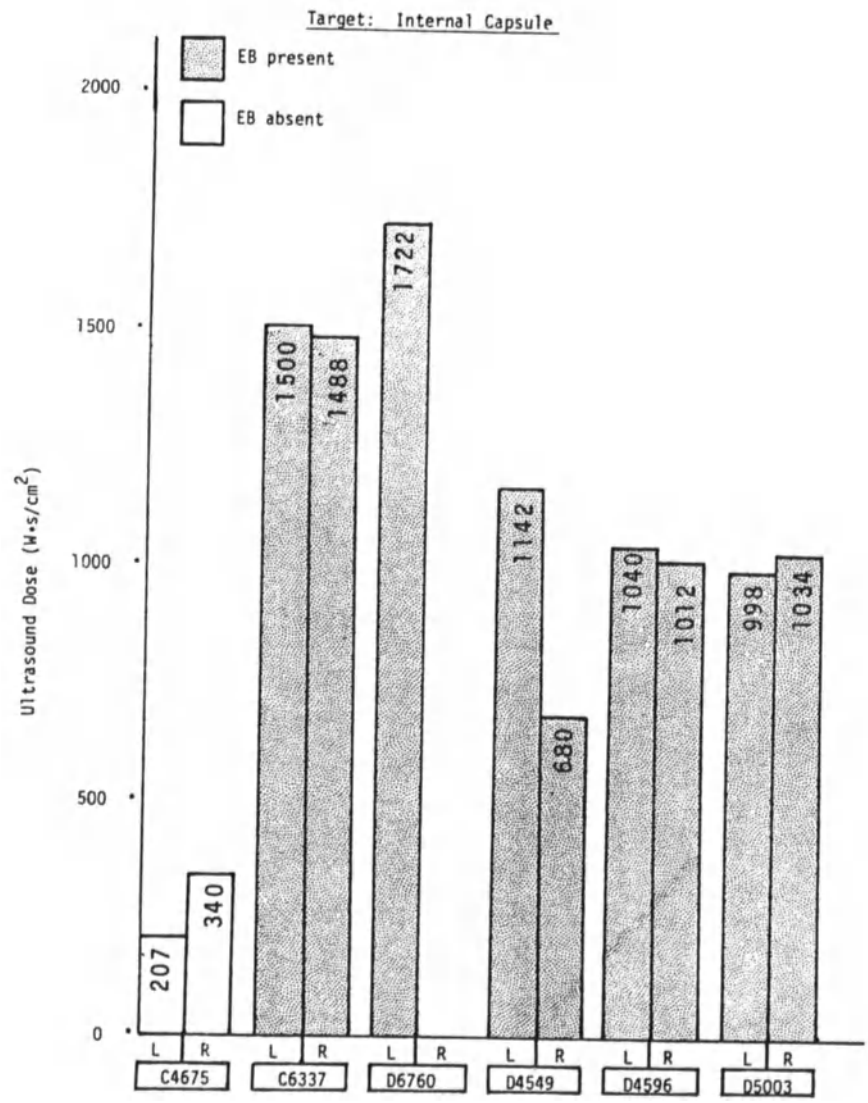


Fig 4. Bar graph for the internal capsule (white matter) indicating animal identification number, presence or absence of Evans blue (EB) and corresponding ultrasound dose; L - left, R - right, C - cat and D - dog.

The areas of BBB permeability and of histologic lesion, both as a function of radiation dose, were determined for two dogs (Table 1). Because the circulation times for EB were different, interdog comparisons of permeabilities were not made. The following intradog trends appeared to be present for white matter (internal capsule) and gray matter

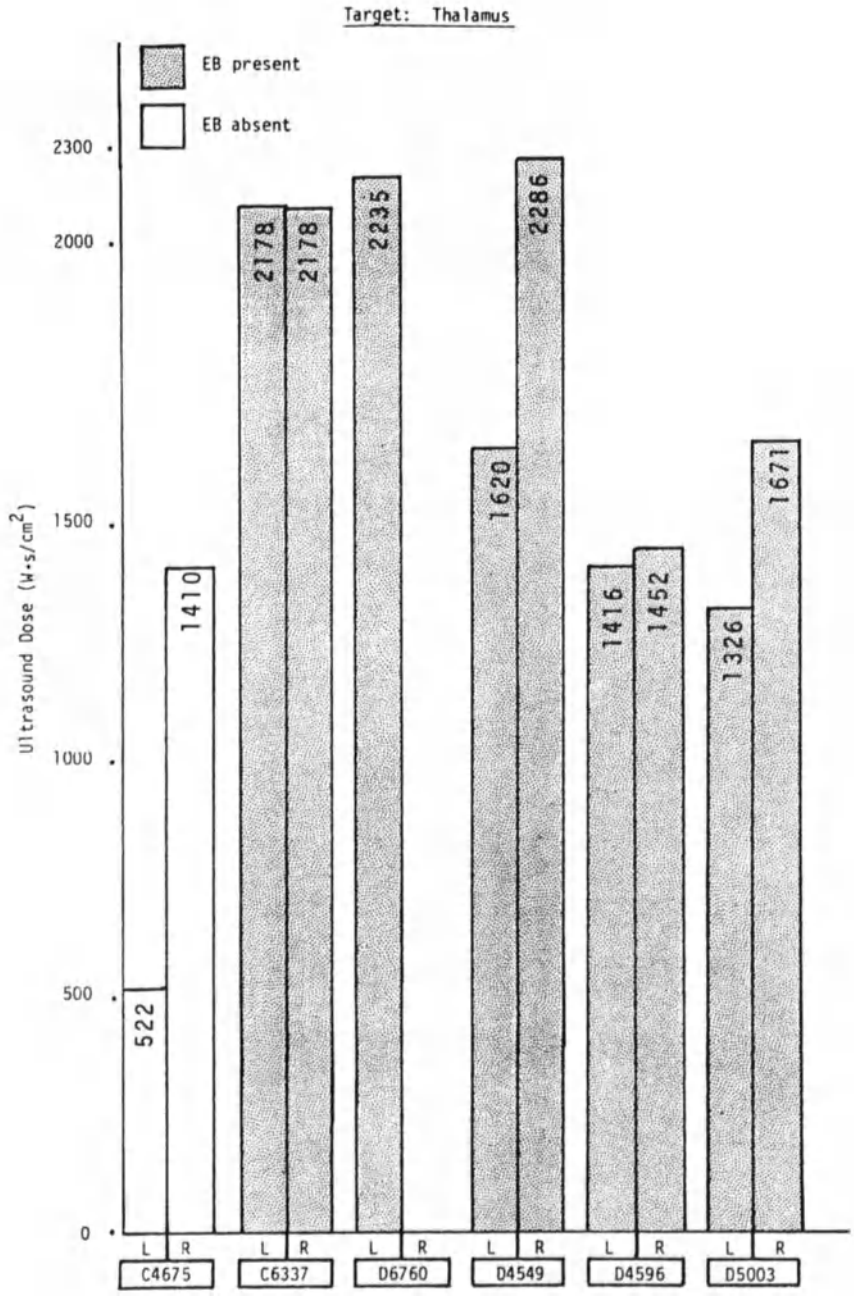


Fig 5. Bar graph for the thalamus (gray matter) indicating animal identification number, presence or absence of Evans blue (EB) and corresponding ultrasound dose; L - left, R - right, C - cat and D - dog.

(thalamus): (a) as the dose was increased, the areas of EB and lesion were increased; (b) for a specific dose, the area of EB was greater than that of the lesion; and (c) for the same target, an increase in dose resulted in a greater increase in EB area, and an even greater increase in lesion area (e.g., for the internal capsule of dog D4549, a 68% increase in dose resulted in a 172% increase in EB area, and a 510% increase in lesion area).

## DISCUSSION

Good precision obtained for the preselected vs delivered voltage to the transducer (mean difference of -0.03%, range -6% to +6%) and for the preselected vs delivered dose to the target (mean difference of +0.3%, range -10% to +11%). Good precision is important because small differences in the preselected vs delivered voltage (Vpp) can result in large differences in the preselected vs delivered dose; this situation obtains because the dose is a partial function of the square of the peak-to-peak voltage ($(Vpp)^2$) (4,5).

For each radiation, the area occupied by the EB was greater than that of the LX (Table 1) - thus suggesting that BBB changes may have been induced in the perilesional area without attendant cell damage; however, interstitial diffusion of EB cannot be ruled out as a mechanism contributing to its extra-lesional location. Bakay et al (2) applied transdural high intensity pulsed focused ultrasound (2.5 MHz, 30 - 50 pulses of 0.4 sec duration at one pulse per sec, intensity approximately 600 $W/cm^2$) to the cat brain and reported that the area occupied by 32P was greater than that of the trypan blue. The increased distribution of 32P was attributed to its smaller size and thus more ready egress from the vascular compartment. The few other reports of ultrasound induced modification of the BBB were based on using trypan blue as a macroscopic indicator of histologic lesion location and did not include a quantitative assessment of barrier changes (e.g., dose-response curves) (6,7).

Lesion threshold dose for WM (internal capsule), which corresponded to that for BBB threshold dose, was between 340 and 680 W sec/$cm^2$ (Fig 4). A dose of 680 W sec/cm2 (I = 340 $W/cm^2$; t = 2.0 sec) resulted in a WM lesion of 0.52 $mm^2$ (Table 1; D4549). Lesion threshold dose for GM, which corresponded to that for BBB threshold dose, was approximately 1326 W sec/$cm^2$ for the thalamus (Fig 5) and between 2284 and 2952 W sec/$cm^2$ for the caudate nucleus (Fig 6).

For tumors implanted in animals and treated with high intensity focused ultrasound, decreased tumor volume and increased survival time have been reported (8,9,10). The decrease in volume was attributed primarily to direct ultrasound destruction of tumor cells and perhaps secondarily to an increased immune response. Fry and Johnson (8) reported a significant increase in survival time for hamsters with ultrasonically (1.11 MHz, 907 $w/cm^2$, 7 sec) treated medulloblastomas (subcutaenous implantation). Goss and Fry (9) reported a significant decrease in tumor volume for rats

with ultrasonically (4 MHz, 1600 w/cm$^2$, 4 sec) treated Yoshida sarcoma (subcutaneous implantation). Kishi et al (10) reported a significant increase in survival time for mice with ultrasonically (944 kHz, 1600 W/cm$^2$, 4 sec) treated murine glioma (subcutaneous implantation); also, although there was no signficant increase in survival time for mice with ultrasonically (944 kHz, 1000 W/cm$^2$, 2 sec) treated murine glioma (parietal lobe implantation), histological examination revealed focal destruction of the radiated tumors. In summary, these animal studies employed an ultrasound dose range of 2000 - 6400 W sec/cm$^2$ for destroying

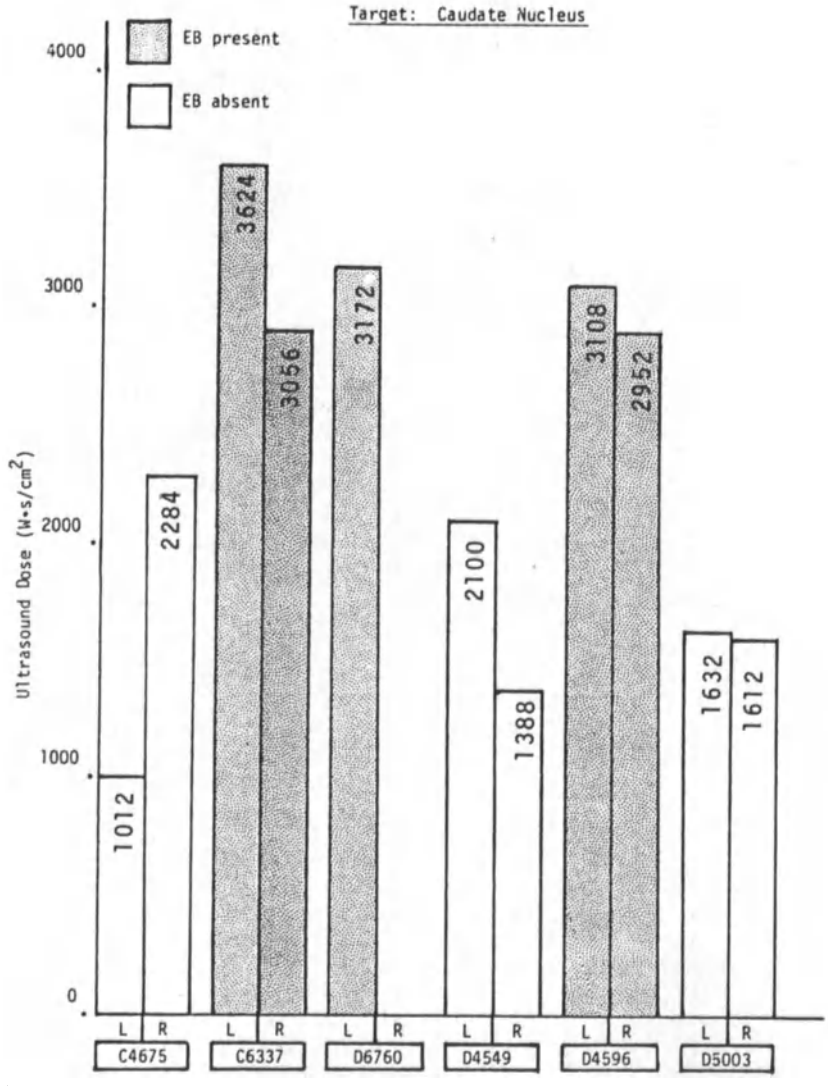


Fig 6. Bar graph for the caudate nucleus (gray matter) indicating animal identification number, presence or absence of Evans blue (EB) and corresponding ultrasound dose; L - left, R - right, C - cat and D - dog.

Table 1

Ultrasound Dose and Areas of Evans Blue and Lesion

| Dog ID No. | Target | Dose ($W \cdot sec/cm^2$) | % Increase In Dose | $Area_2$EB ($mm^2$) | % Increase in Area EB | $Area_2$LX ($mm^2$) | % Increase in Area LX | % Increase in Area EB vs. LX |
|---|---|---|---|---|---|---|---|---|
| | Lt IC | 1142 | | 4.24 | | 3.17 | | 34 |
| D4549 | | | 68 | | 172 | | 510 | |
| | Rt IC | 680 | | 1.56 | | 0.52 | | 200 |
| | Lt TH | 1620 | | 7.24 | | 2.52 | | 187 |
| D4549 | | | 41 | | 1073 | | 946 | |
| | Rt TH | 2286 | | 84.90 | | 26.36 | | 222 |
| | Lt IC | 1040 | | 30.94 | | 21.94 | | 41 |
| D4596 | | | 3 | | 1 | | 21 | |
| | Rt IC | 1012 | | 31.39 | | 18.08 | | 74 |
| | Lt TH | 1416 | | 67.14 | | 12.00 | | 460 |
| D4596 | | | 3 | | 29 | | 76 | |
| | Rt TH | 1452 | | 86.93 | | 6.83 | | 1173 |

Abbreviations: Lt (left); Rt (right); IC (internal capsule); TH (thalamus); EB (Evans Blue); and LX (lesion).

subcutaneous and parietal lobe implanted tumors (medulloblastoma, sarcoma and glioma). However, no determination of a threshold dose, which may be significantly less than 2000 W sec/cm$^2$, for tumor destruction was addressed. For the study reported herein, the minimum dose that resulted in focal destruction (and increased BBB permeability) of normal canine brain gray matter was 1326 W sec/cm$^2$ (thalamus; Fig 5). It is of great importance that the threshold dose(s) for destroying brain tumors and modifying the permeability of the peritumoral BBB be determined.

Currently, the recommended treatment of supratentorial malignant gliomas consists of maximal tumor resection, followed by whole-brain and coned-down radiotherapy plus chemotherapy with a nitrosourea (e.g., BCNU, MW 214) (11); however, median survival time is only approximately 12 months. Experimentally, two promising chemotherapeutic agents are monoclonal antibodies (MAbs) (12) and immunotoxins (13); e.g., the MAbs IgM (MW 1,000,000) and IgG F(ab')2 fragments (MW 100,000) (14), and the protein toxin ricin (MW 65,000) (13). Delivery of these agents to brain foci, at systemically nontoxic doses, requires increasing the BBB permeability (12). This report describes the focal modification of the normal BBB to the EB - Albumin complex (MW 80,000); additional studies are required to determine if the permeability can be increased with ultrasound for the larger MW immunologic agents.

In summary, it appears that high intensity focused ultrasound may have the potential for treating brain tumors based on cell destruction by two mechanisms - direct, by the ultrasound and indirect, by an antineoplastic agent which is delivered via an ultrasonically modified BBB.

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REFERENCES

1) Astrom, K.E., Bell, E., Heidensleben, E.: An experimental neuropathological study of the effects of high-frequency focused ultrasound on the brain of the cat. J. Neuropath. Exp. Neurol. 20, 484, 1961.

2) Bakay, L., Hueter, T.F., Ballantine, H.T., Sosa, D.: Ultrasonically produced changes in the blood brain barrier. Arch. Neurol. Psych. 76, 457, 1956.

3) Ballantine, H.T., Bell, E., Manlapaz, J.: Progress and problems in the neurological applications of focused ultrasound. J. Neurosurg. 17, 858, 1960.

4) Fry, W.J.: Intense ultrasound in investigations of the central nervous system. Advances in Biological and Medical Physics 6, 281,1958.

5) Dunn, F., Averbuch, J.J., O'Brien, W.D.: A primary method for the determination of ultrasonic intensity with the elastic sphere radiometer. Acustica 38, 58, 1977.

6) Ballantine, H.T., Hueter, T.F., Nauta, W.J.H., Sosa, D.M.: Focal destruction of nervous tissue by focused ultrasound: Biophysical factors influencing its application. J. Exp. Med. 104, 337, 1956.

7) Hueter, T.F., Ballantine, H.T., Cotter, W.C.: On the problem of dosage in ultrasonic lesion making, in Ultrasound in Biology and Medicine. Washington, DC, American Institute of Biological Sciences, p 131-155, 1957.

8) Fry, F.J., Johnson, L.K.: Tumor irradiation with intense ultrasound. Ultrasound Med. Biol. 4, 337, 1978.

9) Goss, S.A., Fry, F.J.: Cancer therapy with intense ultrasound, in Proc. N. Am. Hyperthermia Group, 1983.

10) Kishi, M., Mishima, T., Itakura, T.: Experimental studies of effects of intense ultrasound on implantable murine glioma, in Proc. 2nd European Congress Ultrasonics Med., p 28-33, 1975.

11) Shapiro, W.R., Green, S.B., Burger, P.C., Mahaley, M.S., Selker, R.G., VanGilder, J.C., Robertson, J.T., Ransohoff, J., Mealey, J., Strike, T.A., Pistenmaa, D.A.: Randomized trial of three chemotherapy regimens and two radiotherapy regimens in postoperative treatment of malignant glioma. J. Neurosurg. 71, 1, 1989.

12) Lippitz, B.E., Bigner, D.D.: Monoclonal antibodies in diagnosis and treatment of brain tumors. Surgical Rounds, March, 43, 1989.

13) Zovickian, J., Johnson, V.G., Youle, R.J.: Potent and specific killing of human malignant brain tumor cells by an antitransferrin receptor antibody-ricin immunotoxin. J. Neurosurg. 66, 850, 1987.

14) Neuwelt, E.A., Barnett, P.A., Hellstrom, I., Hellstrom, K.W., Beaumier, P., McCormick, C.I., Weigel, R.M.: Delivery of melanoma-associated immunoglobulin monoclonal antibody and Fab fragments to normal brain utilizing osmotic blood-brain barrier disruption. Ca. Res. 48,4725,1988.

# CLINICAL EXPERIENCE WITH LOCAL HYPERTHERMIA IN ROTTERDAM

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In the Rotterdam Cancer Center, local hyperthemia has been applied clinically since 1979. Experience has lead to the development of a 433 MHz multi-applicator multi-generator system for heating superfically located tumors. Temperatures are measured using multi point optical fiber probes within closed tip catheters. Hyperthermia is generally given in combination with a series of radiotherapy. At present, 360 patients have been treated on a total of 440 fields (over 3000 treatment sessions). Various analyses of the results achieved have been performed. The findings indicate how and when HT can be useful.

## Analysis of the influence of "minimum hyperthermia dose" on response

One hundred and twelve patients with various carcinomas were treated with radiotherapy and hyperthermia, using non-invasive techniques, and had evaluable tumor responses. Radiotherapy dose ranged from 13-70 Gy (except for one patient receiving hyperthermia alone) with a mean of 28.6 Gy. The combined treatment was primarily aimed at giving palliation; 79% of the patients had received previous irradiation of the same area. Hyperthermia was given twice weekly following radiotherapy. From the temperature data collected, 12 different parameters expressing the hyperthermia "dose" were derived. The various parameters for both treatment modalities, i.e. radiotherapy and hyperthermia, and some of the tumor parameters were statistically evaluated with respect to their influence on tumor response. Only the first field treated in each patient was included in the analysis. The overall response rate was 87% including 33% complete response. The complete response rate increased with increasing radiotherapy total dose, i.e. from 23% (14-25 Gy) and 36% (28-36 Gy) to 62% (>38 Gy). A positive correlation between the tumor temperature parameter representative if the coldest spot in the tumor, and the level of response was also found. Response level appeared to be determined to a considerable

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extent by radiotherapy total dose as well as tumor volume. The correlation between response level and the minimum hyperthermia dose parameters persisted, however, after correction for the influence of tumor volume and radiotherapy total dose (14).

Results of 8 x (4 Gy plus hyperthermia) in recurrent breast cancer. We have previously reported the results of reirradiation (RT) plus hyperthermia (HT) in recurrent breast cancer (15). The schedule 8 x (4 Gy plus HT) in 4 weeks was applied to 28 patients in this series, resulting in a complete response (CR) rate of 61%. The CR rate was significantly higher in small tumors ( $< 15 cm^3$ ;93%) than in larger tumors ($> 15 cm^3$ ;23%). Since the earlier analysis, 80 more fields in 71 patients with recurrent breast cancer have been treated with the same schedule. The mean radiation dose applied to the same field previously was 45.2 Gy. Tumor response was evaluable in 54 fields. CR was achieved in 83% of the fields, which is considerably higher: 71% (2p 0.016). Although the areas treated were large (one third of the fields were over 300 $cm^2$), the acute toxicity was mild and generally gave no clinical problems. In only one patient a radiation ulcer developed in the axilla, at a site where already before treatment, severe telangiectasis existed due to the previous radiotherapy (50 Gy).

## DISCUSSION

The finding of a dose-effect relationship for the hyperthermia dose parameter respresentative of the coldest spot within the tumor is essentially in agreement with findings of other investigators who have applied the concept of "minimum" hyperthermia dose (1,8,9). In our opinion the observation of such a dose-effect relationship for hyperthermia in clinical material in which so many factors influence the outcome of therapy is very supporting. For clinical practice this finding means that thermometry should be performed at as many sites as possible and that it is worthwhile to strive for tumor temperatures as high as possible.

Analysis of the results in patients with recurrent breast cancer in previously irradiated areas has shown that a treatment schedule of 8 x ( 4 Gy plus HT) in 4 weeks is safe,effective and well tolerated and thus provides good local palliation. With radiotherapy alone at relatively low doses (30-35 Gy), CR rate varying from 37 to 88% (2,4,5,6,10,11,12,13,15). With the radiotherapy schedule of 8 x 4 Gy alone, used in the RTOG 81-4 study, the CR rate achieved in patients with breast adenocarcinoma was 33% (11),which is significantly lower than the 61% achieved with the 8 x (4Gy plus hyperthermia) schedule in our early patient series (Fisher's exact 2p 0.014).
The improved results in our later series probably reflect the development and use of a better heating technique, i.e. of a multi-applicator multi-generator system operating at 433 MHz. These improved results with the better heating

system illustrate the importance of adequate heating. The results of the later series confirm the conclusions of our previous analysis (15): 8 x (4 Gy plus hyperthermia), twice weekly, is a safe and effective locally palliative treatment for patients with breast cancer recurring in previously irradiated areas.

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REFERENCES

1) Dewhirst MW, Sim DA, Sapareto s and Connor WG (1984). Importance of minimum rumor temperature in determining early and long-term response of spontaneous canine and feline tumors to heat and radiation. Cancer Research 44, 43-50.

2) Dragovic j, seydel HG, Sandhu T, Kolosvary A and Blough J (1989). Local superfical hyperthermia in combination with low dose radiation therapy for palliation of locally recurrent carcinoma. J. Clin. Oncol. 7, 30-35.

3) Gonzalez Gonzalez D, Dijk JDP van and Blankk LECM (1988). Chestwall recurrences of breast cancer: results of combined treatment with radiation and hyperthermia. Radiotherapy and Oncology, 12, 95-103.

4) Hofman P, Lagendijk JJW and Schipper J. (1984). The combination of radiotherapy with hyperthermia in protocolized clinical studies. In: Hyperthermic Oncology 1984 vol. I Summary papers J. Overgaard,ed. Taylor and Francis, London and Philadelphia, 379-382.

5) Jo s, Hiraoka M, Akuta K, Nishimiura Y, Nagata Y, Takahashi M and Abe M (1987). Clinical results of thermoradiotherapy for breast cancer. In:Proceedings of the 3rd annual meeting of the Japanese Society of Hyperthermic Oncology, 339-340.

6) Kapp DS, Fessenden P, Cox, R, Bagshaw MS, Lee ER, Prionas SD and Lohrbach A (1988). Combined hyperthermia (HT) and radiation therapy (XRT) in the treament of local regional (LR) recurrences from adenocaracinomas of the breast. Presented at the 5th International Symposium of Hyperthermic Oncology Kyoto, Japan.

7) Kjellen E, Lindholm CE and Nilsson P (1988). Radiotherapy in combination with Hyperthermia in recurrent or metastatic mammary carcinomas. Presented at the 5th International Symposium on Hyperthermic Oncology, Kyoto, Japan.

8) Luk KH, Pajak TF, Perez CA, Johnson RJ, Conner N and Dobbins T (1984). Prognostic factors for tumor response after hyperthermia and radiation. Hyperhermic Oncology vol.I. Summary papers. J. Overgaard, ed. Taylor and Francis, London and Philadelphia, 353-356.

9) Oleson JR, Sim DA and Manning MR (1984). Analysis of prognostic variables in hyperthermia treatment of 161 patients. Int. J. Radiation Oncology Biol. Phys. 10, 2231-2239.

10) Perez CA, Kuske RR, Emami B and Fineberg B (1986). Irradiation alone or combined with hyperthermia in the treatment of recurrent carcinoma of the breast in the chest wall. A nonrandomized comparison. Int. J. Hyperthermia 2, 179-187

11) Perez CA, Gillespie B, Pajak T, Hornback NB, Emami B and Rubin P (1989). Quality assurance problems in clinical hyperthermai and their impact on therapeutic outcome: a report by the Radiation Therapy Oncology Group. Int. J. Radiation Oncology Biol. Phys. 16, 551-558.

12) Seegenschmiedt MH, Brady LW, Sauer R, Karlsson UL, Herbst M, Tobin R, radiation therapy for superficial chestwall recurrences. Presented at the 8th meeting of the NAHG.

13) Storme G and Berge D van den (1988). Personal communication.

14) Zee J van der, Putten WLJ van, Berg AP van den, Rhoon GC van, Wike-Hooley JL, Broekmeyer-Reurink MP and Reinhold HS (1986). Retrospective analysis of the response of tumors in patients treated with a combination of radiotherapy and hyperthermia. Int.J. Hyperthermia 2, 337-349.

15) Zee J van der, Treurniet-Donker AD, The SK, Helle PA, Seldenrath JJ, Meerwaldt JH, Wijnmaalen AJ, Berg AP van den, Rhoon GC van, Broekmeyer-Reurink MP and Reinhold HS (1988). Low dose rerradiation in combination with hyperthermia: a palliative treatment in combination with breast cancer recruing in previously irradiation areas. Int.J. Radiation Oncology Biol. Phys.15, 1407-1413.

# WHOLE BODY HYPERTHERMIA EXPERIENCE IN BREAST CANCER AT AMERICAN INTERNATIONAL HOSPITAL

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One hundred thirty nine patients with metastatic breast cancer were seen at American International Hospital during the years 1982-86. Forty two evaluable patients received whole body hyperthermia (WBH) via the the heated water blanket technique of Barlogie et al[1] and of Larkin et al[2] with chemotherapy. Our 1985 research protocol outlined a plan of initial chemotherapy for up to two months, with WBH added in poor responders. We attempted to achieve a core body temperatures of 42.2° C for two hours. Chemotherapy began 18 hours after completion of WBH. This was done to utilize WBH under optimum conditions, and to gain experience with a wide range of chemotherapy drugs, each given in combinations and doses chosen to be optimum for patient care independant of WBH. These studies were primarily intended to evaluate the safety of both forms of therapy given sequentially.

Eligibility criteria included: histologically proven malignancies, with life expectancy > 12 weeks, Zubrod performance status <= 3, age > 15 years, serum creatinine < 2 mg/dl, pulmonary vital capacity > 75% of predicted, and an absence of brain metastases. Patients with prior radiotherapy to the spine were accepted, but we preferred to exclude those with radiotherapy to the spine within 90 days. Cardiac screening is important, for patients with significant cardiac disease are unlikely to tolerate the stresses of WBH. Electrolyte abnormalities should be corrected and the electrocardiogram should not evidence a significant arrhythmia. Consultation with cardiologists, and special studies, may be needed to help determine eligibility for WBH.

We have recently completed a more comprehensive review of our evaluable patients with breast cancer who began therapy at AIH from 1982 - 1986, including 42 patients who received WBH and chemotherapy and 44 who received only chemotherapy. Proportional hazards statistical analysis[3] identified that survival depended upon Zubrod performance status, tissue hormone receptor (Estrogen and Progesterone), number of prior chemotherapy regimens that were previously

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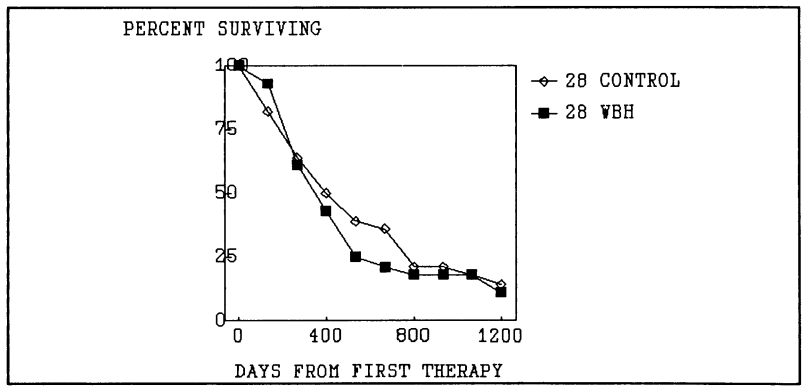
Table 1. Comparisons of median time to tumor progression (TTP) and median survival in patients with and without WBH who were matched for clinical risks.

| GROUP | Number of pairs | TTP(days) Control | TTP(days) WBH | Survival(days) Control | Survival(days) WBH |
|---|---|---|---|---|---|
| Total group | 28 | 165 | 169 | 398 | 364 |
| Liver Metastases | 8 | 92 | 179 | 203 | 228 |
| Visceral Mets. | 18 | 116 | 153 | 279 | 239 |
| No Prior CH | 11 | 290 | 292 | 457 | 487 |
| Failed Prior CH | 15 | 80 | 157 | 318 | 239 |
| ER and PrR + | 7 | 290 | 179 | 638 | 487 |
| ER and PrR- | 4 | 165 | 117 | 279 | 339 |
| Perf.Status >2 | 6 | 28 | 117 | 235 | 153 |

failed, and infiltration of tumor into visceral sites. These factors were independent of the effects of WBH.

In this group including extensively pretreated patients, 38 patients responded either completely, partially, or minimally. A logistic regression model[4] of response identified an increased chance of response with a positive estrogen receptor assay (p=0.0031) or with the use of doxorubicin (p=0.031). A decreased chance of response was noted in patients with prior chemotherapy for metastatic disease (p=0.012) or in patients with visceral metastases (p=0.02).

Chart 1. Median survival in 28 WBH and 28 risk-matched control patients who received doxorubicin chemotherapy.




Clinical data of 28 evaluable patients who received WBH and doxorubicin were matched with data of 28 patients who received only doxorubicin in an effort to identify strengths and weaknesses of WBH. Risks in both groups were matched strictly with parameters of ECOG performance status, number of prior chemotherapy regimens failed, tissue hormone receptor status (estrogen and progesterone receptor), and the presence of visceral disease. Multiple subgroup analyses could then be done on matched pairs, but as expected, no statistical inference could be obtained with such small

groups. Nevertheless, these studies produced several interesting results, shown in Table 1.

From this we conclude that overall groups had similar response durations and survivals, in spite of the fact that WBH patients were selected on the basis of not having an early response to chemotherapy, Chart 1. There seems to be a prolongation of TTP in patients with high risk characteristics such as liver metastases, failing prior chemotherapy, and having poor performance status. The sample groups were too small for meaningful statistical analysis and further patient accrual will be necessary. These results could not be correlated with the doxorubicin dose intensity calculated in mg/$M^2$/wk.

Chart 2. Median time to tumor progression in 28 WBH and 28 risk-matched control patients who received doxorubicin chemotherapy.

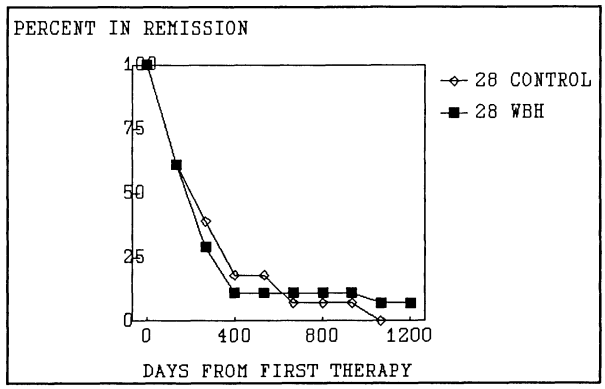
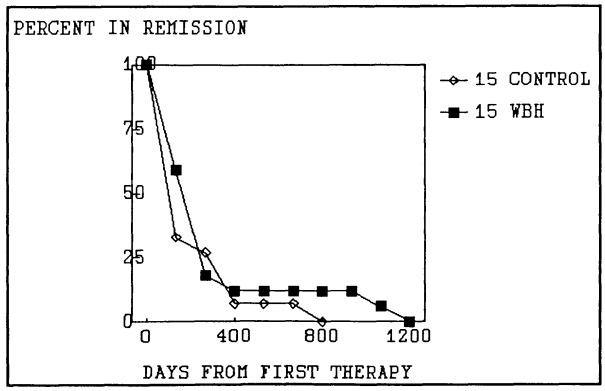


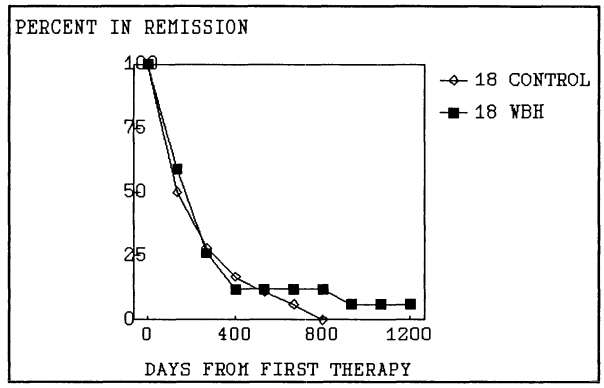

Chart 3. Median time to tumor progression in 15 WBH and 15 risk-matched control patients who had failed at least one prior chemotherapy regimen, and who then received doxorubicin chemotherapy.




Calculations of the thermal dose according to the algorithm of Houston Dewey[5] were measured, standardizing to eqivalent minutes of therapy at 42oC. Thermal doses among 40

patients were normally distributed around a mean of 306 minutes, S.D.= 46, range 190 to 433, and median 299 minutes. The thermal dose had no association with either tumor response or with survival. With our procedure, there was little variation in thermal doses between treatments, with most variation occurring due to slower heating phases evident in patients with a higher total body mass.

Chart 4. Median survival in 18 WBH and 18 risk-matched control patients with visceral metastases, all of whom received doxorubicin chemotherapy.




Several distinct benefits for WBH were suggested in these retrospectively matched groups. The effects were not evident in the overall groups because of inclusion of some good risk patients with positive hormone receptor tests or with previously untreated patients whose initial chemotherapy was relatively effective. The design of the studies to include only patients without an excellent response after initial chemotherapy may well underly these conclusions. Current studies for breast cancer patients include randomized prospective trials to clarify the role of WBH, including trials for patients with, and without prior doxorubicin therapy.

CONCLUSION

The responses of patients with metastatic breast cancer who received WBH and chemotherapy have not correlated with thermal dose intensity or with doxorubicin dose intensity. This suggests that other mechanisms of activity may be involved. Nevertheless, clinical remissions may be achieved in patients whose disease is refractory to multiple chemotherapy regimens, and transient benefit and pain control can provide individual patients with new hopes. Our studies continue, and include additional studies of immune function during and after WBH, as well as prospective randomized trials to delineate further the clinical role of WBH.

REFERENCES

1. B. Barlogie, P. M. Corry, E. Yip, et al, Total Body Hyperthermia with and without Chemotherapy for Advanced Human Neoplasms, Cancer Res. 39:1481 (1979).

2. J. M. Larkin, W. S. Edwards, D. E. Smith, and P. J. Clark. Systemic Thermotherapy: Description of a Method and Physiologic Tolerance in Clinical Subjects, Cancer 48:3155 (1977).

3. M. Friedman, The Use of Ranks to Avoid the Assmuption of Normality Implicit in the Analysis of Variance, J. Amer. Statist. Assn. 32:675 (1937).

4. D. R. Cox, Regression Models and Life Table, J. Roy. Statist. Soc. Series B 34:187 (1972).

5. W. C. Dewey, Interation of Heat with Radiation and Chemotherapy, Cancer Res. 44s:4714 (1984).

# NEW CLINICAL ASPECTS OF WHOLE BODY HYPERTHERMIA

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Recorded evidence for the secretative healing properties of fever can be discovered as early as within the Greek legends of Parmenides and Hippocrates. Thru time, this phenomenon repeatedly appeared within the published reports of such well known European physicians as Thomas Sydenham. These findings continued to gather substantiation from the numerous experiments performed during a time span extending from the end of the last century, until the advent of scientifically-oriented medical education.
Prior to the development of antibiotics, infectional diseases were commonly treated with hyperthermia. Interestingly, the highest success rates achieved with this form of therapy were those attained in cases of gonnorhea (70 to 75%). The significance of hyperthermia in the treatment of infectious disease was overshadowed, however, by the published report of the famous Viennese psychiatrist Wagner Jauregg on the successful healing of patients with the central nerveous form of syphilis through vaccination with malaria tertina pathogens. Though the positive clinical results achieved with hyperthermic treatment could be scientifically proven through diversified laboratory animal investigations, it´s significant use has been all but forgotten by modern medicine.

Up until the turn of the 19th century an "era of hyperthermic treatment" prevailed. It was during this time that the work of Bruns, (in 1887), appeared directing initial attention to yet another aspect of pyretotherapy, the treatment of tumors. William Coley is actually attributed with being the first to encompass hyperthermia within a cancer treatment protocol, whereby advanced stadium cancer patients were administered a suspension of attenuated streptococci, resulting in bouts of approximately 40 °C fever for over a period of 24-36 hours. He later switched to a mixture of bacterial toxins. In his initial findings Coley reported astounding cases of remission, but his data could not be duplicated by other investigators due to the complicated and difficult production process of a standardized bacteria culture possessing a constant toxicity.

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Consequentially, numerous further investigations sought yet other methods to artificially overheat the human body. Once again, these studies could report impressive results in individuals, but the patient collective was so small that it could only be viewed as an ongoing list of case reports rather than an systematically organized clinical investigation. There is also no found evidence that these attempts were carried by an experienced clinician over an extended period of time. And so it appears that the original enthusiasm expressed for clinically applied hyperthermia had dimmed. Evidently, all of the reported attempted investigations had involved whole body hyperthermia. This is not to say that perhaps other further studies did not exist in this area, but their results were never published.

In 1953 H. Nauts, the daughter of William Coley further carried out her father's work using the Coley-toxin. Her results were published as a retrospective study of 1200 patients over a time span of 35 years in which she described an astonishing survival rate of 60%. Nauts postulated a direct connection between response rate, and the degree as well as the length of fever. In 1971, a paper from Miller appeared in Cancer describing his experiences using Coley's-toxin in combination with classic cancer therapy in achieving an overall 5 year survival of 64%. These results distinguish themselves essentially from those of other studies.

There is a difference of active and passive warming. An active warming in that the body itself takes over rising temperature production, whereby all of the physiological functions are forced to strongly react. Sedation of the patient would only lead to a halting of the process. It becomes clear at the same time that controlled regulation of a rising temperature level is impossible to dictate, and is left strictly up to coincidence. In most of the early investigations the maximally achieved level of fever was mucher lower than 41.8° C, which has been experimentally proven necessary for a suitable hyperthermic effect. In cases of passive warming, it is sensible to sedate the patient in order to protect against any possible pathophysiological overreactions. Perhaps an explantation for the particularly good response achieved with autohyperthermia is the yet unclarrified, pathophysiological feedback or control mechanism it induces, through which a tumor is biologically attacked. Methods for active warming are described by W. Coley and M. von Ardenne.

In sharp contrast to active warming, the procedure of temperature increase, maintenance and length of duration during passive warming can be accurately regulated as well as reproduced in later instances. Various methods for passive heatings are described and successfully tested in animals and patients in the last 30 years. First was Pettigrew who placed the patients in melted wax. At the same time v. Ardenne used a water bath. More modern concepts were decribed by Pomp and others who used a chamber with different radiation energys. Bull et al are using hot air in a Clinitron-bed or special water suits. Robbins reported about a radiation chamber where patients could undergo whole body hyperthermia without anesthesia. Especially his results are very promising. All this heating systems are

using external warming. A different way was described by parks who used an extracorporal circuit for internal warming. This method is used in our clinic too.

Before going on any further, there is a brief list of the potential target areas where hyperthermia exercises it´s direct and indirect effects, while here lies an additional explantation for the success or failure of total body hyperthermia.
An essential factor is the transpositioning of the circulation within the entire organism with total body hyperthermia. At lower body temperatures levels at the beginning of treatment, the tumor capillary bed is well supplied with blood. This alters drastically, however, following initiation of hyperthermic therapy in that the tumor experiences a drastic reduction in blood flow. Parallel to this, a relative hypoxia occurs, which is further aggravated by a warmth-stimulated energy requirement. In cases of extended exposure, the pH level drops in the face of hyperacidity, and the compensation mechanisms of the body become overexhausted. The result is stasis of the tumor perfusion.
The point of attack to the basal membrane is just as complex. Regardless of the amount or type of lipid and protein composition, an increase in membrane viscosity occurs, and with that, increased membrane permeability. Both effects are directly responsible for an impressive interstitial edema, which can also be observed within healthy tissue, but is reversible there after a short period of time. The effect of hyperthermic therapy at the cellular level is even more complicated and has been already described in detail elsewhere.
Several studies have demonstrated a postive reaction within the immune system to total body hyperthermia. Patients from our own investigation showed a sustained increase in T-lymphocytes. Yet another fascinating subject is the impact of the psyche on tumor treatment. This aspect is already undergoing intensive investigation. It was most impressive to observe the notable improvement in mood and attitude of those patients who had previously undergone unsuccessful classic tumor treatment, once they had become a part of our hyperthermic treatment program.
All of these elements can be tied into the impressive results delivered by hyperthermia. This corresponds, as well, to the positive findings reported by various groups investigating the impact whole body hyperthermia exerts on tumors.

What remains, however, are still-active parameter tumor cells which have managed to avoid being sufficiently damaged. Though tumor cells appear to be highly sensitive to hyperthermia, it is more important to recognize that healthy organs are capable of protecting themselves through autogenic feedback or control mechanisms (for example the various buffer systems in blood). Tumor cells who manage to situate themselves close to healthy tissues may actually find protection in this refuge. This hypothesis places several results of cell culture research in question, but serves as the best possible explantation for the phenomenon of rapid tumor recurrence in humans and experimental animals following single hyperthermic therapy. The post hyperthermic phase, involving subtherapeutic temperatures, presents an ideal growth milieau for tumor cells, and for this reason,

hyperthermic treatment alone, even at such extremely temperature levels of 42.5° C and 43° C, is solely inadequate in tumor treatment. Those remaining active parameter cells represent a clearly suitable target for concomitant cytostatic and/or radiational therapy. The question is how would cystostatic medications react in the presence of such high temperatures within the human organism ? Would they become prematurely inactivated ? The maximum core temperature for whole body hyperthermia in men has been defined as 41.8 ° Celsius for a long time. This temperature can be tolerated by patients without temperature related major problems 6 hours and longer - as is shown in literature.

Encouraged by the work of Leon Parks an interdisziplinary group was established in 1981 in our hospital to start a pase I research project for extracorporal induced whole body hyperthermia. Our patients had been treated before ineffectually by chemotherapy, surgery, or radiation. Survival time was exspected to bee under 6 months. The Karnofski-index was between 60-80%. Most patients suffered from tumor induced pain.

A firt protocol was defined as follows: Plateutemperature of 41.8° C for 6 hours. Induction of hyperthermia via extracorporal circuit as quick as possible (time 35 min - 70 min; shunt-flow 1200 - 1600 ml/min). Additional chemotherapy was given in all therapies in a normal dose during heating phase ( 39.0 °C ). On-line temperature monitoring with precision of 1/10 °C in the urinary bladder, esophagus, rectum, pulmonary arterial blood, inspired air and blood supply. Given balanced anesthesia and artificial ventilation during whole procedure. Invasive monitoring of cardiovascular reaction, pulmonary funktion, and blood serum contents very close until some days after treatment. Patients had been in ASA groups II - IV.
Using the 1. protocol we treated 20 patients in 44 wbh of 4 different groups: 1. Colorectal carcinoma, 2. gastric carcinoma, 3. malignant melanoma, 4. various tumors. Our overall response rate was about 27 % together with pain relieve and augmented Karnofski-index. Exspected livespan was prolonged by improved quality of live. Histological investigationes also showed necrosis of the peripheral tumor zones.

The cardio-vascular changes in men associated with wbh are published in various reports. An increase of cardiac output is attracting notice as almost essentially which sometimes achieve more than the triple of baseline value. At the same time the heart rate is rising to value about 130 bpm. Stroke volume is more closely due to cardiac output can duplicate itself and alternate regulary with the heart minute volume. At the same time the total vascular resisitance and the pulmonary vascular resisitance decrease until to 50% of baseline value. Thereby the cardiac work of the left and the right heart is able to maintain in normal value. The resulting work-load by WBH corresonds to light until to intermidiate activity. That can be reason for observing heart-failures at no time. The pulmonary function is closely due to blood circulation. The augmented basal metabolism and the physical load leads to an increase in oxygen consumption and carbon dioxyde production. At the same time an

increase of the of the intrapulmonary right-left-shunt is occuring until to 30 %. As a result of the high cardiac output the availability of oxygen increases likewise, that the oxygen extraction rate continues almost constantly; therefore it results no oxygen debt. The high carbon dioxyde production requires a duplication of the respiratory minute volume. Thus pulmonary functions must be controlled continuosly. During wbh an extreme vasodilatation occurs with an fluid and electrolyte shift requiring an adapted infusion protocol of about 350 ml/$m^2$ bs crystal fluid with special components. Similar effects are described by other authors.
But our clinical results in tumor destruction in patients were not like described in literature for animals and cell culture. Therefore in following protocols we varied the time of the plateau-temperature for research in time-temperature-relationship. The time of plateau-temperature was changed for different groups: 10 h (n = 2), 4 h (n = 17), 2 h (n = 36). Due to the small groups of patients in each time-protocol no statistically comparable results could be drawn. But our feeling is that a plateau-time of 2-4 hours is adequat for tumor-cell-killing in wbh.

In the last years a lot of groups have been founded to work in the field of whole body hyperthermia in animals and men. The number of patients who have been treated will be some thousends now. But there is only little report in literature. This may be due to some facts; The absolute number of comparable patients and tumors in each group is very small. There are no randomised studies. It is more a long list of case reports. All patients have been treated before unsuccessfully by other therapies. Therefore the results in clinical wbh are not really comparable to those of the classical tumor therapies. The try to compare these with the laboratory work in animals and especially cell culture will show worse results. There is a feeling of a missing link. Ethical reasons will prevent to use some of the results of laboratory research in men.

Concerning of temperature effects in men you can find various reports of even higher temperature in fever, heatstroke, and other diseases where the patients regain their health. Working with an extracorporal-induced hyperthermia model as we do, you will find overshooting temperatures ( 0,2 - 0,4 ° C ) in different organs very often. These findings are never accompanied by serious damage. In early work with rabbits and guinea pigs we we were able to reach core temperatures up to 43 ° C without clinical problems and detectable serious histological damage. Above 43 ° C all animals died within some minutes or at last during the cooling phase.
With this knowledge we started a wbh-protocol with a 2 hours plateau and a core temperatur of 42,2 ° C for 10 treatments. All our tests showed no differences in cardio-vascular action, pulmonary function, and blood samples to the former treatments. Encouraged by these results in a second protocol we again raised the core temperature to 42.6 °C at the same duration ( n = 4 ). Clinically we observed an extensively augmented cardiac output syndrome 3.5 times of baseline value and a prolonged awakening after anesthesia, which

could possible be the effect of a of slight brain edema. After one day in the ICU the outcome was normal. There was no need for prolonged artificial ventilation.
In a third protocol ( n = 10 ) temperature was again set to 42.6° C for a 2 h period. After 1 h the temperature was again raised to 43 ° C for a maximun of 15 min. The second heating time was almost between 30 - 45 min. This maximum core - temperature in our protocols had been associated by an increased cardiac electric instability at least after 20 min. For this reason we had to stop the heating. Clinical patients developed a brain edema lasting for some hours longer than under the wbh - treatment with 42.6 ° C. But almost one to two days after treatment patients react quite normal, only a few direct temperatur related defects could be detected. These are related to the light brain edema lasting for some days and an even bigger raise in liver enzyms in the plasma than after normal wbh.

In our opinion these findings are: patients are under general anesthesia which protect them from overshooting pathophysiologic reactions as an example barbiturate anesthesia is used beneficial for the therapy of traumatic brain edema. Close control of cardio-vascular action and the direct possibility to react on oncoming problems asshures the neccessary circulation. With artificial ventilation the oxygen availibility and carbon dioxyd elemination is sufficient. Changes in blood values, due to the extra-intra cellular shift can be treated immediately.
Working with wbh without anesthesia which is possible with the machine developed by I. Robbins and his group is very striking. It is very simple, needs only a small number of personal and therefore saves a lot of costs. But anesthesia is not harming a patient under wbh as it is pointed out in literature very often. Anesthesia means a better control of physical reactions and so is very helpful. With our protocol we could treat patients in a reduced physical state. For example one of them had 2 cardiac infarctions in his history. A lot of them had no physical training for months because of tumor related pain.

But anyway we could not find significantly more tumor destruction with the extreme core - temperature than under a normal wbh - protocol. Therefore we came back to the saver protocol with a plateau-temperature of 42.2 ° C - 42.5 ° C. Further inverstigations with a larger number of patients will show if a time-temperature relationship as known by basic research has an effect for cancer patients as well. It could be that with a different and more successful adjuvant chemotherapy the protocol with the extreme temperature may be used again. With our protocol now we feel safe in wbh treatment even when temperatures above 42 ° C are aspired. Our experience comes from 116 whb in 50 patients with a total temperature plateau time of about 457 hours. The number and the severity of side effects is comparably low in this radical therapy. It is the aim to communicate that 43 ° C are able in man during wbh at least for a period of 30 min and that the side-effects are not the protocol limiting feasibility. In the light of these preliminary results, the rationale for regional hyperthermia treatment should be reconsidered, because wbh is the only hyperthermic procedure, where the tumor and occult metastases can be heated.

# THERMAL INDUCTION AND TEMPERATURE CONTROL IN THE HYPERTHERMIC ANTIBLASTIC REGIONAL PERFUSION WITH EXTRA CORPOREAL CIRCULATION

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## ABSTRACT

An optimal treatment temperature choice is based on a compromise between the higher values desirable for best synergic effects between heat and drugs and the lower values required to avoid drug inactivation and tissue damage. Temperature uniformity throughout the limb under treatment favors approaching the highest temperature compatible with the above compromise. Several modifications have been introduced in the methodology during our experience consisting of more than 100 perfusions. The induction of true hyperthermic temperatures (41.5-41.8 °C) is achieved both heating the perfusate and directly heating the limb by means of a warm-water circulation blanket, thus insuring an optimal thermal flow to the superficial tissues. Devices in the extra-corporeal circuit have also been adopted to obtain a fast and accurate perfusate temperature control. The following results can be obtained: (a) a short delay time is required to reach the therapeutic temperature range; (b) the perfusate temperature is maintained at a value close to 40 ±0.5 °C during the whole hyperthermic phase of the treatment; (c) the various districts of the limb are maintained at substantially homogeneous hyperthermic temperature.

## INTRODUCTION

One of the fundamental problems in the optimization of the method was the definition of the thermal range where the limb heating was to be contained[1,2,3]. In fact, high temperatures ( >42 °C) are desirable for their damaging potential against the malignant cells, but these high temperatures render the treatment complex and hazardous for their toxic effects on the healthy tissues and for the thermal inactivation of the cytostatic drugs[4,5,6].

As a compromise, temperatures in the range 41 to 42 °C have proved to be able to enhance the therapeutic effects both for their synergic effects with the drugs and because heat favours a better tissue perfusion, whereas the inactivation effects remain within acceptable limits.

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On the basis of the previous assumptions, the regional isolated hyperthermic-antiblastic perfusion has entered the clinical application stage for the treatment of limb tumours in advanced loco-regional phases. It is however still subject to technical and methodological improvements[4,7,8,9,10,11]. Several modifications have been adopted in our operating procedure and the following some improvements mainly related to heating optimization and temperature control will be presented and discussed.

## CLINICAL OBSERVATIONS AND REMARKS

When inducing hyperthermia by means of the heated perfusate, one can observe that at the start of the perfusion the limb is at a relatively low temperature, usually below 36 °C, hence extended time duration is needed to reach the hyperthermic range. Additional considerations are suggested by the temperature readings at the level of muscle, subcutaneous and skin of the arm/forearm or thigh/leg respectively, as follows. The temperature in the arterial line of the circuit must be maintained 2 or 3 °C above the target temperature. Subsequently, during the steady-state phase, this temperature gradient can be reduced but is still present. Furthermore, the various parts of the limbs tend to exhibit highly dishomogeneous temperature distributions, with muscle tissues reaching higher values with respect to subcutaneous which in turn is at a higher temperature than the skin. Temperature differences are more evident at the thigh and especially in patients having thick adipose tissue. Higher temperatures are reached more rapidly in the distal rather than in the proximal districts.

The above observations are important in that the therapeutic effect is to act in the entire limb, and especially on the surface tissues when the lesions are localized at the cutaneous and/or subcutaneous level (melanoma, spinocellular carcinoma etc...).

When considering, additionally, that the high-temperature cytostatic inactivation occurs even in the circuit and that the synergism between hyperthermia and drug is the more effective the higher the temperature (below the inactivation value), it follows that optimal temperature distributions must be carefully planned in respect of the above conditions, with temperature monitoring in the arterial line of the circuit and temperature-gradient monitoring in the various anatomical districts of the limbs.

Thus, on one side, reaching temperatures as high as 41.5-41.8 °C at the surface tissues (where the lesions are present) is desirable for best therapeutic effects but, on the other side, higher temperatures must be accepted in the deep tissues and in the perfusate with the known undesirable effects of tissue damage (which is at the basis of the compartmental syndrome) and of the increased thermal inactivation of the drugs. In other words, containing the temperature level in the deep tissues and in the circuit at values not higher than 42 °C causes limiting the maximum subcutaneous and cutaneous temperature to 38-40 °C.

## TECHNICAL PROCEDURE AND RESULTS

We discuss here our heat-induction criteria aimed at improving safety and effectiveness and the technical implementation means developed during our clinical experience of more than 100 perfusion[10,9].

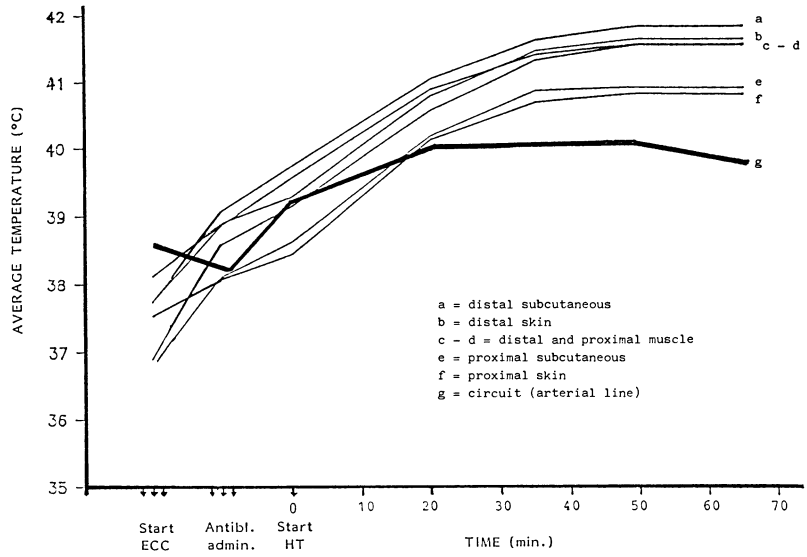


Figure. 1 Behavior of the temperature distribution vs. time. Average in 22 treatments. Lower limb.

During the vascular isolation phase and the insertion into the extra-corporeal circuit the limb is heated to 38 °C (as measured at the subcutaneous) by means of a thermostatic water blanket wrapped around the entire limb. The prime is heated to 39 °C. At the beginning of the perfusion, after controlling the leakage, the cytostatic drug is administered (when more than one is used, time intervals of 10 min. occur between one administration and the next). The subcutaneous temperature is maintained at 38-39 °C for 10 minutes. Subsequently this temperature is raised up to 41.5-41.8 °C both by the heat supplied by the blanket and by increasing the perfusate temperature. The warm-water blanket is used both as an external heat source and as a thermal "fly-wheel" to stabilize the temperature and to reduce heat dispersion to the ambient. The procedure outlined in this paragraph is especially relevant for obtaining a good temperature uniformity across the various compartments (skin, subcutaneous and muscle) while maintaining the perfusate at an optimal temperature (maximum compatible with thermal inactivation). The combined action of the two heat sources is in fact capable of a reasonably homogeneous heating of the superficial and deep tissues, of reducing the temperature differences between distal and proximal districts and, above all, optimal temperatures can be obtained at skin and subcutaneous while maintaining the circuit temperature at values close to 40 °C (±0.5 °C) throughout the hyperthermic phase of 50-60 minutes duration (Fig. 1).

Care is taken, additionally, of implementing the known technical procedures to obtain adequate flow and effective heating of the perfusate. A flow of 300 to 600 ml/min is maintained in the upper limb and 800 to 1200 ml/min in the lower limb, according to limb volume. Catheter needles as large as 16F gauge for the arteries and 22F gauge (upper limb) or 24F gauge (lower limb) for the veins have been successfully used. The needle tips are positioned as proximal as possible in the common femoral vessels. Our extra-corporeal circuit setup (schematic in Fig. 2) employs an oxygenator (Dideco D700 Hi-Flex) which incorporates a heat exchanger

having ample thermal-exchanger surface. This permits flexible operation and fast temperature rise. A thermostatic bath (Dideco D613 variograde) regulates the heat-exchanger water, with fast rise and good temperature stability.

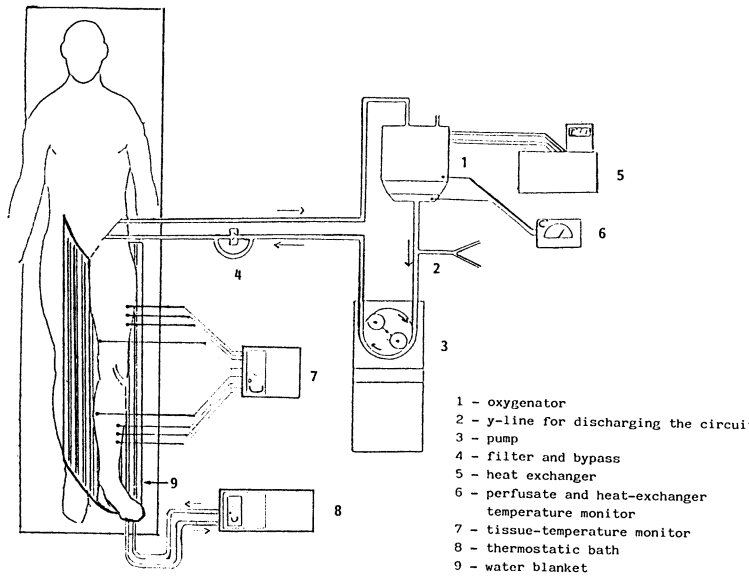


Figure. 2 Extracorporeal circulation setup and temperature monitoring.

The external thermal source consists of a thermal-exchange unit with temperature monitoring and control, feeding water to the thermal blanket (Heto-Physthern).

Limb-temperature monitoring is by means of needle or plate probes placed on the skin, in the subcutaneous tissue and in the muscle. Two triplets are used, one located at the median third thigh (arm) and the other at the median of the leg (forearm). Two additional plate probes are located at the skin surface of the medial face of the thigh and leg where the possible occurrence of overheating was detected in our technique.

## CONCLUSIONS

Our heat-induction modalities have proven through extended clinical application to be able to produce satisfactory results: (a) short heat-induction times are needed to reach the target temperature; (b) the perfusate temperature never exceeds 40.5 °C in the circuit throughout the hyperthermic phase; (c) low thermal gradients are obtained across the various compartments (muscle, subcutaneous and skin). The present results and analysis suggest the technical feasibility of some improvement in the thermal administration control, thus coming even closer to the target temperature distribution.

## REFERENCES

1. Cavaliere R., Ciocatto E.C., Giovanella B.C.: "Selective heat sensitivity of cancer cells", Cancer, **20**, 1351-1381, 1967;

2. Hahn G.H.: "Hyperthermia and Cancer", G.H. Hahn Ed., Plenum Press, New York, 1982;
3. Giovanella B.C., Stehlin J.S. jr, Morgan A.C.: "Selective lethal effect of supranormal temperatures on human neoplastic cells", Cancer Res., **36**, 3944-3950, 1976;
4. Stehlin J.S. jr., Giovanella B.C., De Ipolyi P.D., Muenz L.R., Anderson R.F.: "Results of hyperthermic perfusion for melanoma of the extremities", Surg. Gynec. Obstet. **140**, 339-348, 1975;
5. Natali P.G., Zupi G., Greco C., Cavaliere R., Giacomini P., Ferrone S,: "Effect of Hyperthermia and neoplastic drugs on cell survival and antigenic profile of two human cell lines", Proc. of 13th Intern. Congress of Chemotherapy, H.Khspitzy and K.Karrer Eds., Verlag Egerman H. A/1170, pp. 246/9-246/12, Wien 1983;
6. Vaglini M., Ammatuna M., Nava M., Prada A., Rovini D., Santinami M., Cascinelli N.: "Regional Perfusion at high temperature in treatment of stage IIIA - IIIAB melanoma patients", Tumori **69**, 585-588, 1983.
7. Ghussen F., Nagel K., Groth W. Muller J.M., Stutzer H.: "A prospective randomized study of regional extremity perfusion in patients with malignant melanoma", Ann. Surg., **200**, 764-768, 1984;
8. Krementz E.T., Carter R.D., Sutherland C.M., Muchmore J.H.: "Chemotherapy by regional perfusion for limb melanoma", Ann. Surgeon, **53**, 133-140, 1987;
9. Kroon B.B.R.: "Regional isolation perfusion in melanoma of the limbs: accomplishments, unsolved problems, future", Eur. J. Surg. Oncology, **14**, 101-110, 1988;
10. Krige J.E.J., King H.S., Stover R.M.: "Prophylactic Hyperthermic limb perfusion in stage I melanoma", Eur. J. Surg. Oncology, **14**, 321-326, 1988;
11. Pace M., Filomena A., Manetti R., Galli A., Macera Mascitelli E.: "Perfusione regionale ipertermico-antiblastica in circolazione extracorporea nei melanomi degli arti: indicazioni e tecnica", Arch. Atti Soc. It. Chir., 90° Congr., vol. I, pp. 367-383, Roma (Italy) 1988;
12. Pace M., Filomena A., Manetti R., Galli A., Macera Mascitelli E.: "Improvements in the technique of isolated regional perfusion for melanoma of the extremities", XXVI World Congress of the Intern. College of Surg., Vol. VIII, pp. 265-269, Milan (Italy) 1988;

# CENTRED RADIO-FREQUENCY HYPERTHERMIA

# IN SOLID TUMOURS

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## INTRODUCTION

Nowadays hyperthermia through radio or microwaves, even if to be perfected, has aroused much interest and has been widely applied abroad both in western and eastern countries, but not in Italy where it has still little diffusion.

## MECHANISM OF ACTION OF MICROWAVES

We do not know which the mechanism of action of microwaves is. We must still think that the heating, noxious to tumours, is caused by the same mechanism which is employed in microwave ovens where an endogenous heating, autonomous fron the tissues, is obtained. We can think that the neoplastic masses are heated because the waves of the electromagnetic field, which surrounds them, are not absorbed and the not absorbed energy is turned into heat. Different kinds of equipment are employed. Some of them are based on cooled, malleable plates which are put in contact with the body where the neoplasn has been found: in this case radio-waves are generally used; they are led to depth with several devices, trying to strike and go through the tumorous masses. Besides there are machines which are not directly in contact with the patient and which are used especially for superficial tumours because the penetration of microwaves is smaller than that of radio-waves. A computer has been applied to some machines; it permits, within certain limits, the radiations to be addressed in a better way, which is indispensable in this therapy considering that the maximum penetration, which varies from tissue to tissue, is usually of 10-15 cm. According to Hain Dicher and Ralph Wolfstein patiences' thermal tolerance does not seem to be a clinically predominant factor and we agree with this assertion. As far as the damaging of the neoplastic mass is concerned, the number of the heartz employed is not as important as the heat obtained and generally a considerable effect cannot be attained over 1000 Mhz. We seem to have several positive effects with 15 Mhz, 250 Mhz, 500 Mhz. In employing microwaves, the treatment may cause two complications of little importance: very slight cutaneous burns, which we have observed three times out of over one thousand applications and slight necroses in the subcutaneous adipose tissue.

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## ACHIEVED RESULTS AND FUTURE AIMS

The diffusion in the world of centred thermotherapy is due to an increase, although slow, in positive results. As well as to a progressive reduction of symptomatology, especially of pain, we aim to the stop of the neoplastic diffusion or to the decrease in volume of the tumorous mass or to its disappearance. For the results we have achieved, see table I [illegible]. From these tables we can notice that the results are different according to the kind of tumours and sex but, above all, to the patient's general conditions at the beginning of the treatment. We feel that we do not only have to treat the tumour but we have to treat the neoplastic sufferer since the denfeces of his organism have to dominate the neoplasm, which is generally discovered very late even when it is said to have started recently. It is evident that we can achieve better results with patients in better conditions. When the general conditions are so worn down to make us think that the recovery is impossible, the success is very limited or null: however it has clearly resulted that some patients, who were thought to be near their ends, were able to get great benefit and even satisfactory local and general conditions. Unfortunately we cannot distinguish a priori these cases from the ones which cannot attain any results.

## BEHAVIOUR OF THE VARIOUS KINDS OF NEOPLASM AND OF REPETITIVE LESIONS

Considering that we have not enough knowledge yet to tell which neoplasms respond better to thermotherapy, we can affirm that the responce depends on the patient's general conditions and that the lesions which respond the least are the pulmonary ones, rather well the hepatic ones, better the glandular ones and the ones in the lower abdomen; of course the more superficial are the lesions the better we can treat them.

## MODIFICATIONS CAUSED BY THE THERAPY IN NEOPLASTIC MASSES

Thermotherapy causes, in all the strata from the cutis to the depth, an increase in the hematic flux, a real vascular congestion, which persists even after several days, as it has been noticed during operations performed after a period of three-ten days from the end of the therapy. However the considerable bleeding in all the strata does not hinder the operation since the hemostasis is easy and quick and there are no post-operative suffusions. Except for experimental reports on induced tumours, which have a different significance, we can already affirm, on the basis of our observations, that neoplastic masses, when the applications are efficacious, can undergo a more or less accentuated necrosis as we can see from histological observations made before and after the treatment at a distance of a few days. This necrosis is very clear in pulmonar neoplasms; in fact, when the patient coughes, he ejects fragments of neoplastic tissue. The modifications in the neoplastic tissue caused by thermotherapy are well evident in a histological observation made on an open-air biopsic taking made before a destroying operation which takes place after three applications of thermotherapy. Patient D.V.A. was afflicted with an infiltrating mammary carcinoma. She had not been subjected to any other treatments (chemotherapy, radiant therapy, etc.). The conclusions of our Consultant Anatomist-Pathologist Doctor Ariele Saragoni are the followings: "Morphologic cellular alterations are compatible with "cellular damages" caused by physical and chemical agents. In fact every cellular and stromal alterations, but above all cellular, is paradigmatic and related to lesions from physical and chemical agents, as it is described in literature. So the difference between the two histological reports, considering the short lapse of time between the first and the second operation during which three applications of thermotherapy took place, is due to alterations caused by physical and chemical agents and certainly by the thermo-

therapy itself. In the end, as a final result, we may have a definitive sclerosis of the tumorous mass, as it is evident in LeVeen and his collaborators' observations.

## WHEN THERMOTHERAPY MUST BE EMPLOYED

First of all, thermotherapy can be employed preventively, before the operation, in order to limit the expantion of the neoplasm or of small isolated cellular masses. Applications of thermotherapy are also useful after the operation to consolidate the recovery and destroy residual neoplastic cellular masses. We have had to modify the criterion of choice of the patients for the therapy because on one hand we have noticed that subjects in an apparently terminal stage may, in rare cases, have such benefit to be able to lead a long, active life; on the other hand we have had the most insistent requests, sometimes difficult to reject, from relatives and patients who, conscious of the terminal stage, wanted to try thermotheraphy as a last attempt. Therefore we have divided the patients into three groups:
i) patients with little or no hope. In this case thermotherapy is employed only with an adjuvant or palliative intent. Before starting the treatment we tell the patients that they will riceive four applications and that the treatment willbe continued only if there are signals of improvement, which is improbable and unforeseeable.
2) curative treatment: to be tried on patients in fairly good or good conditions, even if it is impossible to foresee a priori how good the results will be. We start from six applications which will be increased in number according to the results achieved, deciding case by case.
3) preventive treatment before or after the operation, as we have described above.
After a few months, another few applications of thermotherapy are advisable (booster therapy) in order to consolidate or improve the recovery. The number and the rate of the applications of the booster or consoliding therapy must be decided case by case on the basis of the clinic course.

## COMBINED THERAPIES

While we always combine an immunizing therapy based on thymus extracts, especially "Timunox", in innocuous and unprejudicial quantities and in suitable doses administered with time intervals according to our experience, we are really careful about using interferon and similar preparations and very hesitating about employing interleukyns, which only in considerable doses, surely toxis,can do good. We frequently associate antimitotic substances and/or radiant theraphy. Both of them are employed in small doses, as it has been proved that they have a considerable therapeutical efficacy and no negative effects, when associated to thermotherapy. We can achieve better results when chemotherapy or or radiant therapy is applied within sixteen hours after the applications of thermotherapy (1). We think that it is a mistake to administer normal doses of antimitotic substances and rafiant theraphy, whose toxicity is increased by thermotherapy, because in this way the advantages of thermotherapy are annulled.

## THE FIFTH WEAPON AGAINST TUMOURS

As far as we know, we have to affirm that the main weapon for demolishing tumoural masses is the surgical therapy. It must be applied, when possible, even when the surgeon cannot foresee a priori if the operation will be radical or not. Other weapons are chemotherapy and radiant therapy, pro-

1) Experimental research: Dewhirst et al. I984 - Gilette I985.

vided that they are used in doses which are not toxis so that they cannot destroy the patient's physical resistances. They can be employed especially on young subjects and combined with thermotherapy, which enables us to reduce enourmously the dosages. The fourth weapon is immunotherapy which aims to increase the physical defences. In this treatment we employ thymus derivatives which are administered in moderate doses with suitable intervals in order to avoid that the considerable primitive increase in the number of T lymphocytes could be followed by a fall with a consequent difficulty in restoring the right number. We have tried interferon too, but the results are still very questionable, so we agree with the ones who think that these substances are not useful in low doses and may be dangerous in high doses, since they are toxis. In short, there is no doubt that thermotherapy, with the machines we employ, is innocuous, that is it has no appreciable negative effects, and there is no doubt that, in a high number of patients, the advantages can range from the disappearance of fever and pain to the stabilisation or disappearance of the neoplasm. We have to consider that there can be a total recovery in a certain percentage of the cases or a stabilisation with a sensation ot total wellbeing, which may last for years. However only time can confirm the results achieved even when the patient feels good and no neoplastic symptom can be noticed. As a conclusion we insist on the fact that we do not only have to treat the illness but enable the patient's organism to counteract neoplastic tissue modifications. According to the results stated in every countries, 30-40 cancer patients out of 100 recover with normal therapies already in use, the remainind 60-65 die from primitive or secondary lesions; it is in these cases that thermotheraphy may give results, even with patients almost without hope.

SUMMARY: BAZZOCCHI G. AND COLL. "CENTRED RADIO-FREQUENCY THERMOTHERAPY IN SOLID TUMOURS"

After some notions on the mechanisms of action of radio-waves on solid tumours, the treatise illustrates the results achieved in the first 125 cases of malignant tumours treated with this method, its results are definitely encouraging, even in cases apparently with no hope. It also describes briefly the histologic modifications induced by this therapeutic method on neoplastic masses and discusses the criteria of the directions of thermotherapy, alone or combined, which is obviously the fifth weapon against tumours.

Table 1. RESULTS ACHIEVED

JUDGEMENT PARAMETERS EXPRESSED IN SCORES (each of the following parameters includes all the previous ones)

no improvement = score 0
disappearance of fever = score 1
disappearance of pain = score 2
improvement of coenaesthesia-ponderal increase = score 3
well-being and resumption of human relations = score 4
decrease in the volume of the neoplasm = score 5
disappearance of the neoplasm = score 6

TREATED CASES: 125 (64 males, 61 females)

| REPEATED THERAPY | DIVISION ACCORDING TO AGE |
|---|---|
| once = 33 patients | from 0 to 30 = 3 pat. (1 m., 2 f.) |
| twice = 4 pat. | from 30 to 50 = 25 pat. (10 m. 15 f.) |
| three times = 1 pat. | over 50 = 97 pat. (53 m., 44 f.) |

SUBD. ACC. TO HISTOLOGIC TYPES: carcinoma = 94 cases, sarcoma = 4, neoplasm SNC = 5, lynphoma = 3, melanoma = 1, leukemia = 2, bony metastasis of unknown origin = 1, prostatic hypertrophy = 15.

DIVISION ACCORDING TO ORGAN OR SYSTEM

| ALIMENTARY SYSTEM: 28 pat. | BREAST: 23 pat. | LUNGS: 16 pat. |
|---|---|---|
| sc. 0 = 3 cases | sc. 0 = 3 cases | sc. 0 = 7 cases |
| sc. 1 = 4 cases | sc. 1 = ------- | sc. 1 = 1 case |
| sc. 2 = 5 cases | sc. 2 = 1 case | sc. 2 = 5 cases |
| sc. 3 = 7 cases | sc. 3 = 7 cases | sc. 3 = 2 cases |
| sc. 4 = 2 cases | sc. 4 = 3 cases | sc. 4 = ------- |
| sc. 5 = 2 cases | sc. 5 = 1 case | sc. 5 = ------- |
| sc. 6 = 1 case | sc. 6 = ------- | sc. 6 = ------- |
| prophylaxis = 2 cases | prophylaxis = 7 cases | |
| prophylaxis neg. = 2 cases | prophilaxis eff. = 1 case | |

| URINARY SYSTEM = 9 pat. | GENITALS = 14 pat. (2 m., 12 f.) | |
|---|---|---|
| | 2 MALES | 12 FEMALES |
| sc. 0 = 1 case | sc. 0 = ------ | sc. 0 = ------- |
| sc. 1 = ------- | sc. 1 = ------ | sc. 1 = 1 case |
| sc. 2 = 1 case | sc. 2 = ------ | sc. 2 = 3 cases |
| sc. 3 = 3 cases | sc. 3 = 1 case | sc. 3 = 2 cases |
| sc. 4 = 1 case | sc. 4 = ------ | sc. 4 = ------- |
| sc. 5 = 3 cases | sc. 5 = ------ | sc. 5 = ------- |
| sc. 6 = ------- | sc. 6 = 1 case | sc. 6 = 2 cases |

| LYMPHOMATA: 3 pat. | SARCOMATA: 4 pat. | OTHERS: 4 pat. |
|---|---|---|
| sc. 0 = 1 case | sc. 0 = 1 case | sc. 0 = 1 case |
| sc. 1 = ------- | sc. 1 = 1 case | sc. 1 = ------- |
| sc. 2 = ------ | sc. 2 = ------ | sc. 2 = ------- |
| sc. 3 = ------ | sc. 3 = ------ | sc. 3 = ------- |
| sc. 4 = 1 case | sc. 4 = 1 case | sc. 4 = 1 case |
| sc. 5 = 1 case | sc. 5 = 1 case | sc. 5 = 2 cases |
| sc. 6 = ------ | sc. 6 = ------ | sc. 6 = ------- |

| CENTRAL NERVOUS SYSTEM: 5 pat. | PROSTATE: 19 pat. hyperthrophy: 15 pat. | K. prostate: 4 pat. |
|---|---|---|
| sc. 0 = 1 case | sc. 0 = ------ | sc. 0 = ------ |
| sc. 1 = ------ | sc. 1 = 1 case | sc. 1 = ------ |
| sc. 2 = ------ | sc. 2 = 1 case | sc. 2 = ------ |
| sc. 3 = 3 cases | sc. 3 = 2 cases | sc. 3 = ------ |
| sc. 4 = 1 case | sc. 4 = 4 cases | sc. 4 = 3 cases |
| sc. 5 = ------ | sc. 5 = 7 cases | sc. 5 = 1 case |
| sc. 6 = ------ | sc. 6 = ------ | sc. 6 = ------ |

INFLUENCE OF THE PRESENCE OF REPETITIVE LESIONS

| PRIMITIVE NEOPLASMS: 64 pat. | PRES. METASTASES: 60 pat. |
|---|---|
| sc. 0 = 6 cases | sc. 0 = 12 cases |
| sc. 1 = 2 cases | sc. 1 = 6 cases |
| sc. 2 = 7 cases | sc. 2 = 9 cases |
| sc. 3 = 9 cases | sc. 3 = 18 cases |
| sc. 4 = 9 cases | sc. 4 = 7 cases |
| sc. 5 = 16 cases | sc. 5 = 3 cases |
| sc. 6 = 2 cases | sc. 6 = 3 cases |
| prophylaxis = 16 cases | prophylaxis = 2 cases |
| not val. = 1 case | |

RESULTS IN CONNECTION WITH THE PATIENT'S CONDITIONS AT THE BEGINNING OF THE THERAPHY

| PROPHYLAXIS: 14 pat. (before and after the operation) | CURATIVE TH.= 52 pat. | ADJUVANT TH.= 58 pat. |
|---|---|---|
| | sc. 0 = ------ | sc. 0 = I8 cases |
| | sc. 1 = 1 case | sc. 1 = 8 cases |

| | | |
|---|---|---|
| prophylxis | sc. 2 = 4 cases | sc. 2 = 12 cases |
| neg. = 1 case | sc. 3 = 15 cases | sc. 3 = 12 cases |
| not val. = 1 case | sc. 4 = 10 cases | sc. 4 = 6 cases |
| | sc. 5 = 17 cases | sc. 5 = 2 cases |
| | sc. 6 = 4 cases | sc. 6 = ------- |

OVER-ALL RATING OF THE RESULTS OF THERMOTHERAPY ON THE FIRST 125 CASES

sc. 0 = 18 cases (14,4%)
sc. 1 = 8 cases ( 6,4%)
sc. 2 = 16 cases (12,8%)
sc. 3 = 27 cases (21,6%)
sc. 4 = 16 cases (12,8%)
sc. 5 = 19 cases (15,2%)
sc. 6 = 4 cases ( 3,2%)
prophylaxis = 16 cases (12,8%)
not val. = 1 case ( 0,8%)

BIBLIOGRAPHY


Audone B., Bolla L., Marone G., "An automatic hyperthermia system for cancer treatment", Locoregional High-Frequency Hyperthermia and Temperature Measurement, edited by G.Bruggmoser, W.Hinkelbein, R.Engelhardt, M.Wannenmacher, pag. 47, I989.

Bazzocchi G. and Coll., "Radiofrequency Thermotherapy in the Treatment of Cancer. Early Personal Results", XII Int. Sympsium on Clinic Hyperthermia, Roma, 27-29 April 1989.

Hand W.H., "Heat delivery and thermometri in clinical hyperthermia", Recent Results in Cancer Research - Hyperthermia and the Therapy of Malignant Tumors, edited by C.Steffer, pag. 1, 1986.

Kim J.H., Hahn G.M., Antich P.P., "Radiofrequency hyperthermia for clinical cancer theraphy", in The Third Intern. Cancer Therapy Symp., Colorado, 1980.

Kogelnik H.D., "Recent Results in Cancer Research", Vol. IOI, Springer-Verlag Berlin-Heidelberg, I986

LeVeen H.H., Pontiggia P, "Radiofrequency thermotherapy in the treatment of cancer", in Pro. Intern. Symp. on Biomedical Thermobiology, Strasbourg, I98I.

LeVeen H.H., Pontiggia P., Colombo W., Introzzi A., "Le radiazioni termiche nel trattamento dei tumori", Fed. Med. XXXIX-8, I986

Mathé G., Zadra M.F., "La malattia minima residua impercettibile e le nostre possibilità di cura dei tumori maligni", Fed. Med. XLII-4, I986.

Molls M., Scherer E., "The combination of hyperthermia and radiation: clinical investigations", Recent Results in Cancer Research - Hyperthermia and the Ththe Therapy of Malignant Tumors, edited by C.Steffer, pag. 127, I987.

Pigliucci G.M., Foggi G.M., Sartori S., Ogier C., "Le radiazioni termiche nel trattamento dei tumori. Aspetti biologici", Fed. Med. XXXIX-8, I988.

Pontiggia P., Ogier C., "La termoterapia localizzata nel trattamento delle neoplasie", L'Arcispedale S.Anna, Ferrara, Vol. XXXIV,1?1984.

Steffer C., Van Beuningen, "The biological basic for tumor therapy by hyperthermia and radiation", Recent Results in Cancer Research -Hyperthermia and the Therapy of Malignant Tumors, edited by C.Steffer, pag. 24, I987.

Szmigielski S., Jeljaszewic J.,Pulverer G., "Thermo-immunotherapy of advanced neoplasm. A concept and preliminary results", Biomed Pharmacoter, 16, 151, 1987.

# LOCAL HYPERTHERMIA FOR DEEP TUMORS

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Hyperthermia for superficial tumors has been considered standard treatment since 1984, based on multiple reports showing synergistic therapeutic advantage in combination with radiotherapy compared to irradiation alone (Table 1). Results of external hyperthermia treatments for deep tumors combined with irradiation or chemotherapy have shown significant improvement over controls (24). While side

Table 1

COMPLETE RESPONSE TO IRRADIATION (RT) ALONE VERSUS (RT) AND HYPERTHERMIA (HT) FOR SUPERFICIAL TUMORS

| AUTHOR | EVALUABLE PATIENTS. | RT ALONE | RT AND HT |
|---|---|---|---|
| Arcangeli (1) | 163 | 38% | 74% |
| Scott (2) | 62 | 39% | 87% |
| U (3) | 14 | 14% | 86% |
| Kim (4,5) | 238 | 39% | 72% |
| Overgaard (6) | 101 | 39% | 62% |
| Corry (7) | 33 | 0 | 62% |
| Hiraoka (8) | 33 | 25% | 71% |
| Li (9) | 124 | 29% | 54% |
| Shidnia (10) | 185 | 33% | 64% |
| Perez (11) | 154 | 41% | 69% |
| Van Der Zee (12) | 71 | 5% | 27% |
| Steeves (13) | 90 | 31% | 65% |
| Dunlop (14) | 86 | 50% | 60% |
| Goldobenko(15) | 65 | 86% | 100% |
| Muratkhodzhaev (16) | 313 | 25% | 63% |
| Lindholm (17) | 85 | 25% | 46% |
| Valdagni (18) | 78 | 36% | 73% |
| Emami (19) | 116 | 24% | 59% |
| Marmor (20) | 15 | 7% | 47% |
| Gonzales (21,22) | 46 | 33% | 50% |
| Sugimachi (23) | 129 | 52% | 80% |

*Consensus on Hyperthermia for the 1990s*
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Plenum Press, New York, 1990

effects of deep treatment have not been greater than with superficial hyperthermia, tolerance of regional deep heating has been poor, with treatment limiting pain and/or systemic stress in most patients (25,26). Local deep treatment on the other hand has been well tolerated (27,28).

In this paper results of local deep treatment using parallel opposed microwave applicators are analysed, including a phase I study of 25 cases previously reported (27).

## MATERIALS AND METHODS

Patients had advanced primary cancer, post treatment recurrence or metastatic disease. Of 121 evaluable treatment courses 73 had hyperthermia combined with radiotherapy and 48 with chemotherapy.Dosages of irradiation varied widely, depending on normal tissue tolerance and treatment goal. Chemotherapy drug and dosage were appropriate to the case, determined in cooperation with the medical oncologist, with consideration of maximal interaction with hyperthermia and minimal side effects.

Hyperthermia was delivered by two 300 MHz external applicators, parallel opposed and operated in phase (POPAS)* at power up to 800 watts evenly divided (29). Applicators are standard design 300 MHz 20x23cm horn waveguide with air gap coupling. Prior to clinical studies appropriate in - vitro and in-vivo studies were performed. Therapeutic temperatures were obtained centrally in phantoms and pigs up to 20cm thick. A flow of cooled air through an aperture in the applicator enhanced homogeneity of the heat field.

Hyperthermia was given twice a week in early clinical trials, more recently five days per week, over five weeks or throughout the radiotherapy course if longer. Standard radiotherapy fractionation was used, with treatment 5 days per week within 2 hours of hyperthermia, either before or after. One or two fields were treated each day; usually two fields were required to adequately cover large tumors or extensive disease in liver and lung. Each hyperthermia treatment was given for one hour, with the goal of achieving 42 degrees Celsius minimum tumor temperature for 30 minutes. Thermometry throughout each session utilized two triple junction copper-constantan microthermocouples (100 micron)* inserted in tumor when feasible and in normal tissue or on skin surface. Temperature readings were obtained at 4 - 5 minute intervals with power on - off cycle controlled by a computer system that also recorded a permanent temperature record.

Each patient was treated within FDA approved protocol guidelines and signed the appropiate consent form.

## RESULTS

At least partial response was obtained in 67% overall, 86% with hyperthermia combined with at least 30 Gy

* HBCI Medical Group, Inc., Panorama City, Ca 91402

irradiation, 55% with less irradiation, and 62% with chemotherapy. Impact of radiation dose on complete response rates was more dramatic : 46% with more radiation versus 8 - 10 % for low dose or no radiation (Table 2).Patient tolerance was excellent. No patient discontinued treatment because of iatrogenic signs of symptoms. Thermal burns occurred in 5% at some time during the treatment course, usually at the first or second hyperthermia session presumably because the patient was not yet fully aware of the danger. Thermometry is not sufficient to avoid burns, and we must rely on the patient to inform us as soon as pain develops. Two patients had pneumothorax, secondary to thermocouple insertion requiring 1 day in hospital. One patient with extensive abdominal disease developed ileus, at first suspected to be bowel obstruction, possibly an effect of hyperthermia. Patients generally perspired profusely. Core temperatures did not rise significantly, never more than 2 degrees Celsius and usually much less.

Table 2

POPAS Response by Radiation Dose

| Radiation | # | CR(%) | PR(%) | NR+SD(%) |
|---|---|---|---|---|
| 30Gy (+) | 35 | 16(46) | 14(40) | 5(14) |
| 20Gy (-) | 38 | 3(8) | 18(47) | 17(45) |
| 0 | 48 | 5(10) | 25(52) | 18(38) |
| TOTAL | 121 | 24(20) | 57(47) | 40(33) |

COMPLICATIONS : 6 Skin Burns, 2 Pneumothorax, 1 Ileus

Results were significantly better with a greater number of hyperthermia treatments. CR plus PR was 73% after 25 treatment sessions versus 48% after 10 treatments (Table 3), discussed further elsewhere (30).

Table 3

POPAS Response by Hyperthermia Dose

| Dose | # | CR(%) | PR(%) | NR+SD(%) |
|---|---|---|---|---|
| 25 | 92 | 19(21) | 48(52) | 25(27) |
| 10 | 29 | 5(17) | 9(31) | 15(52) |

Results were equivalent for chest, pelvic and pancreatic tumors, in the 70% CR + PR range, relatively less (47%) for other abdominal lesions (Table 4).

Table 4

POPAS Results by Site

| Site | # | CR | PR | (CR+PR) | SD | NR |
|---|---|---|---|---|---|---|
| Thorax | 39 | 10 | 19 | (74%) | 4 | 6 |
| Pancreas | 14 | 1 | 9 | (71%) | 2 | 2 |
| Other Abd. | 36 | 4 | 13 | (47%) | 8 | 11 |
| Pelvis | 32 | 9 | 16 | (78%) | 5 | 2 |
| TOTAL | 121 | 24 | 57 | (67%) | 19 | 21 |

The types of lesions treated in the chest are shown in Table 5. Complete local control was obtained in 3 of 4 patients treated by full dose radiation with curative intent, 2 lung and 1 esophagus. One patient with adenocarcinoma metastatic to mediastinum remains free of disease at two years, while the others died from metastatic disease. Of the 29 patients given 39 courses of hyperthermia treatments for thoracic lesions, 5 of 10 with primary lung cancer lived over one year; as did 6 of 18 others given palliative treatment. Metastatic lung lesions represented the majority of treated thoracic disease ( Table 5). Patients usually had bilateral disease progressive despite other previous treatment. Hyperthermia was given over five weeks along with 1500 rad in 15 fractions to the predominantly involved lung. The other lung was then usually treated, depending on response to the initial course. At least 50% tumor regression was achieved in 76% of 21 treatment courses given for lung metastasis.

Table 5

Thoracic Cancer, Response by Cell Type

| Type | # | CR | PR | SD | NR |
|---|---|---|---|---|---|
| Adenocarcinoma, Lung | 7 | 2 | 2 | 2 | 1 |
| Squamous Cell Ca, Lung | 2 | - | 2 | - | - |
| Oat Cell Ca, Lung | 4 | 4 | - | - | - |
| Squamous Cell Ca Esophagus | 1 | 1 | - | - | - |
| Mesothelioma | 2 | - | 2 | - | - |
| Sarcomas | 2 | - | - | 1 | 1 |
| Lung Metastasis | 21 | 3 | 13 | 1 | 4 |
| Total | 39 | 10 | 19 | 4 | 6 |

Except for pancreatic primaries, treated abdominal lesions were metastatic, mostly in liver ( Table 6). Of 9 patients given 14 treatment courses for pancreatic adenocarcinoma, all with liver and/or nodal spread, 3 survived 1-2 years following the initial treatment course. All but one had at least partial response and profound palliation. The one patient who survived 2 years had 3 treatment courses, 2 with 5 FU infusion and the last with 45 Gy irradiation. CT Scan showed complete resolution of the left lobe liver invasion and adenopathy after the first course, and of the primary after thermoradiotherapy. He died of sepsis; no cancer was found at autopsy. The clinical course was striking in this 48 year old man. Pain, extreme weakness and anorexia reversed within two weeks of initiation of thermochemotherapy, completely alleviated at completion of the course; after which he went on a two week hiking trip. He completly regained his 80 pound weight loss and lived a normal life for 18 months. The other 28 patients with other abdominal tumors, mostly liver metastasis, had 36 treatment courses. Response was often of short duration but prolonged by additional treatment, most often by thermochemotherapy. Radiotherapy was given with only 14 of 50 abdominal hyperthermia treatment courses. Interestingly response was much better in metastatic disease treated with chemotherapy, or in a few cases with hyperthermia alone, than radiotherapy (10-20 Gy): 4CR + 10PR (58%) versus 2PR (25%) respectively.

Table 6

Abdominal Cancer

| Type | Patients | Courses |
|---|---|---|
| Adenocarcinoma, Pancreas | 9 | 14 |
| Hepatocellular Ca | 1 | 1 |
| Liver Metastasis | 20 | 28 |
| Mesothelioma | 1 | 1 |
| Peritoneal Mets, Ovary | 2 | 2 |
| Lymph Node Mets | 4 | 4 |

One Patient with ovarian cancer developed ascites 8 months after partial regression of pelvic recurrence, with complete palliation using hyperthermia combined with 2400 rad in 100 rad daily fractions to the pelvis. Treated with hyperthermia alone to two abdominal fields, ascites was completely controlled until her death 13 months later.

As in the chest and abdomen, nearly all pelvic treatments were for palliation after failure of intensive previous treatment. Significant palliation was achieved in 90% of

pelvic cases, including most of those patients whose tumors remained stable. Complete response was obtained in 9/32(28%) (Table 7), all in smaller tumors, less than 5cm; but most lesions were quite bulky, in the 10-15 cm range. While lesions that showed CR remained controlled, patients treated palliatively generally died at 4 to 8 months post treatment. One lymphoma patient and one prostate patient remain alive at 18 and 23 months respectively, the latter free of disease.

Table 7
Response by Cell
Pelvic Cancer,

| Type | # | CR | PR | SD | NR |
|---|---|---|---|---|---|
| Rec. Colon | 13 | 3 | 5 | 4 | 1 |
| Rec. Ovarian | 6 | 2 | 4 | - | - |
| Sarcomas | 5 | - | 4 | - | 1 |
| Prostate | 3 | 2 | 1 | - | - |
| Lymphoma | 3 | 2 | 1 | - | - |
| Melanoma | 1 | - | 1 | - | - |
| Rec. Endomet. | 1 | - | - | 1 | - |
| Total | 32 | 9 | 16 | 5 | 2 |

## DISCUSSION

The treatment of metastatic disease, particulary to lung and liver, represents a major problem in cancer management. Chemotherapy is not effective in most patients. Because of sensitivity of normal tissue, radiotherapy can be given effectively to only small areas of the liver and lung. Hyperthermia has been shown to have no apparent effect on normal tissue and to achieve at least temporary control of metastasis in the majority of cases. Local Hyperthermia can, however have no effect on the overall course of metastatic disease, and the disappointingly short survival despite good local tumor response only confirms this obvious fact.

For post radiation recurrence and chemotherapy failure, hyperthermia remains the sole treatment option. Excellent palliative results have been obtained in treatment of recurrent disease in chest, abdomen and pelvis, as earlier demonstrated for recurrent superficial tumors.

Most locoregional external hyperthermia treatment for deep tumors has been for recurrent or metastatic disease, by other groups as well as ourselves. Untreated advanced

primary cancer represented 18% of the patient population in a multi-institutional study reported by Storm et al (31),29% in a multi-institutional study reported by Petrovich et al (32), and 17% in this report. No authors have analyzed results in these patients separatly. In our series all patients with a treatment goal of cure had complete regional control; however this included only 5 patients, 2 free of disease at 20-25 months. (Prostate and lung adenocarcinoma) and 3 who died of metastasis (2 oat cell, lung; squamous cell carcinoma, esophagus).

Most deep hyperthermia treatment has been regional, employing either magnetic induction at 13.5% MHz with concentric electrodes (Magnetrode, Henry Medical Electronics, Inc., Los Angeles,CA) or an annular phased array (BSD Medical Corporation,Salt Lake City, UT). Of 756 deep tumors treated with the annular array 6% had CR, 26% PR(31). With the annular array 353 treated tumors showed 10% CR, 17% PR (32). In our series results of local deep hyperthermia treatment were 20% CR and 47% PR.

RF capacitive heating (Thermatron FR - 8, Yamamoto Vinyter Co., Osaka) is being used extensively in Japan for local deep heating. This equipment employs an 8 MHz RF generator feeding two opposed disc electrodes. Experience has been reported by Hiraoka et al (33) in only 40 patients. They now exclude patients with subcutaneous fat over 2 cm, after 4 patients, all with thicker fat layer developed fat necrosis. Otherwise treatment was quite well tolerated. Response was 15% CR, 47% PR. These patients had no previous irradiation and received at least 30 Gy with hyperthermia.

All authors (31-33) and including our current series found significantly better results when hyperthermia was combined with standard radiotherapy, at least 30 Gy (Table 8). Results using hyperthermia combined with low dose irradiation, up to 30 Gy (31,32) or 20 Gy in our series were little better than with no irradiation (Table 9). Surprisingly combination with chemotherapy was not shown to improve response rates as compared to hyperthermia alone (Table 9). Specifics of chemotherapy type and administration were not discussed in any of these reports. The most common adjunct chemotherapy we have used is 5 FU by intravenous infusion, 250 mg over 24 hours on each day of hyperthermia. Our results with chemotherapy were equivalent to results with low dose radiation, better than could be anticipated with hyperthermia alone. A recent report comparing results of 5 FU given by infusion to the more common bolus technique (34) found objective tumor response to be respectively 30% and 7% and with far less toxicity using infusion. This marked advantage for infusion may well carry over to improved results in combination with hyperthermia.

Tumor stabilization (SD) for at least 2 months was achieved in 16% in our series, and for at least 3 months in 15% in the series reported by Storm(31). Both groups

Table 8

Results With and Without Standard Irradiation

| Author | # | Treatment | | Results | | Total | |
|---|---|---|---|---|---|---|---|
| | | 30Gy(+) | No RT | HT or HT+RT | | CR% | PR% |
| | | | | CR% | PR% | | |
| Storm (31) | 960 | 32% | | 20 | 40 | | |
| | | | 57% | 3 | 20 | 9 | 28 |
| Petrovich (32) | 353 | 39% | | 21 | 36 | | |
| | | | 26% | 2 | 29 | 10 | 39 |
| Bicher | 121 | 29% | | 40 | 46 | | |
| | | | 40% | 10 | 52 | 24 | 57 |
| Hiraoka (32) | 40 | 100% | | 15 | 47 | 15 | 47 |

Table 9

Results with Hyperthermia alone, with Chemotherapy, and with low dose Radiation

| Author | HT alone | | | HT+chemo | | | HT+low RT | | |
|---|---|---|---|---|---|---|---|---|---|
| | (#) | CR | PR | (#) | CR | PR | (#) | CR | PR |
| Storm (31) | (142) | 3% | 20% | (405) | 3% | 20% | (107) | 5% | 33% |
| Petrovich(32) | (47) | 2% | 34% | (42) | 2% | 26% | (121) | 3% | 42% |
| Bicher | | | | (48) | 10% | 52% | (38) | 8% | 47% |

consider this an important criterion (35) separate from progressive disease (NR) both clinically and physiologically. Most patients with SD have either or both significant palliation and improved general condition. Extensive central necrosis is usually noted in stable tumors on CT and pathologic examination of tumors excised following treatment. Lack of tumor regression despite massive cell kill most likely relates to collapse of tumor microvasculature, one of the main mechanisms of thermal effect (36,37).

Local deep hyperthermia is easily tolerated, far better than regional treatment. A comparison of the annular array with capacitive heating in the same 13 patients with abdominal or pelvic tumors showed no significant difference in tumor temperatures; however duration of treatment using the annular array was limited by increase in body temperature and pulse rate (38). Comparison of the two regional heating devices in the same 23 patients with abdominal or pelvic disease showed treatment limiting factors in 7/14 with pelvic tumors treated by the annular array (AA) and 13/14 using the Magnetrode (CC). The authors concluded that "the AA is superior to the CC for pelvic treatment and that both devices have limitation in abdominal treatment" (25).

A single report in the medical literature compares results of definitive radiotherapy alone or combined with hyperthermia for deep tumors. Hornback et al (24) treated 33 patients with stage III B cervix carcinoma each receiving 25 MeV photon beam irradiation and two Cesium insertions to a total minimum tumor dose of 65 Gy at the lateral pelvic wall. Eighteen of the 33 also received hyperthermia on a daily basis 10-15 minutes following each radiation fraction, using a device with two anterior and two posterior 434 MHz microwave antennae. Acute radiation toxicity was the same with or without hyperthermia, "and the addition of heat caused minimal discomfort". Tumor response was subjectively thought to be more rapid in the hyperthermia group. Complete local control without recurrence was achieved in 8/15 (53%) after irradiation alone versus 13/18 (72%) after combination of irradiation and hyperthermia. Median survival in the two groups was 26 and 36 months respectively.

In contemplating these results what can be said as to the appropiate use of hyperthermia for deep tumors? Hyperthermia for deep tumors has been sufficiently evaluated that it is ready now to be considered standard therapy. Hyperthermia offers palliation, hope and improved quality of life when other treatment options have been exhausted. For patients with untreated advanced primary cancer hyperthermia combined with standard radiotherapy offers a significantly better chance for local control. The use of combination treatment should be strongly considered before giving radiotherapy without hyperthermia. Local hyperthermia can be given for tumors in any body region safely and with excellent patient tolerance, with equivalent tumor response rates in thorax, abdomen and pelvis, in the 70% range in our series.

REFERENCES

1) Arcangeli, G; Benassi, M; Cividalli, A; et al: Radiotherapyand hyperthermia. Analysis of clinical results and identification of prognostic variables. Cancer 60 : 950 - 956, 1987.

2) Scott, RS; Johnson, RJR; Story, KV; et al: Local hyperthermia in combination with definitive radiotherapy: Increased tumor clearance, reduced recurrence rate in extended Follow - up. Int. J. Radiat. Oncol. Biol. Phys. 10 : 2119 - 2123, 1984.

3) U, R; Noell, T; Woodward, KT; et al : Microwave - induced local hyperthermia in combination with radiotherapy of human malignant tumors. Cancer 45: 636 - 646, 1980.

4) Kim, JH; Hahn, EW; Antich, PE: Radiofrequency hyperthermia for clinical cancer therapy. Natl. Cancer Inst. Mongr. 61: 339 -342, 1982.

5) Kim, J H; Hahn, E W; Ahmed, SA; et al: Combination hyperthermia and radiation therapy for malignant melanoma. Cancer 50: 478 - 482, 1982.

6) Overgaard, J : The current and potential role of hyperthermia in radiotherapy. Int. J. Radiat . Oncol. Biol. Phys. 16: 535 - 549,1989

7) Corry, Pm; Barlogie, B; Tilchen, EJ; et al: Ultrasound induced hyperthermia for the treatment of human superficial tumors. Int. J. Radiat. Oncol. Biol. Phys. 8: 1225 - 1229, 1982

8) Hiraoka, M; Jo, S; Dodo, Y; et al : Clinical results of radiofrequency hyperthermia combined with radiation in the treatment of radioresistant cancer. Cancer 54: 2898 - 2904, 1984

9) Li, RY; Wang, HP; Lin, SY; et al : Clinical evaluation of combined radiotherapy and thermotherapy on carcinoma of the breast. Clin. Oncol. 12: 73 - 76, 1985

10) Shidnia, H; Hornback, NB; Shupe, RE; et al : Correlation between hyperthermia and large dose per fraction in treatment of Malignant Melanoma (Abstr.) Int. Clin. Hyperthermia Soc. Meeting, Lund Sweden, 1987.

11) Perez, Ca; Kuske, RR; Emani, B et al : Irradiation alone or combined with hyperthermia in the treatment of recurrent carcinoma of the breast in the chest wall. A nonranomized comparison. Int. J. Hyperthermia 2: 179 - 187, 1986

12) Van Der Zee, J; Van Rhoon, GC; Wike - Hooley, JL; et al: Clinically derived dose effect relationship for hyperthermia with low dose radiotherapy. Br. J. Radiol. 58: 243 - 250, 1985

13) Steeves, RA; Severson, SB; Paliwal, BR; et al: Matched - pair analysis of response to local hyperthermia and

megavoltage electron therapy for superficial tumors. Endocurietherapy / Hyperthermia Oncol. 2: 163 - 170, 1986

14) Dunlop, PRC; Hand, JW; Dickinson, RJ; et al : An assessment of local hyperthermia in clinical practice. Int. J. Hyperthermia 2: 39 - 50, 1986.

15) Goldobenko, GV; Durnov, LA; Knysh, VI; et al : Experience in the use of thermoradiotherapy of malignant tumors. Med. Radiol. 32: 36 - 37, 1987.

16) Muratkhodzhaev, NK; Svetitsky, PV; Kochegarov, AA; et al: Hyperthermia in therapy of cancer patients. Med. Radiol. 32: 30 - 36, 1987.

17) Lindholm, CE; Kjellen, E; Nilsson, P; et al : Microwave - induced hyperthermia and radiotherapy in human superficial tumors. Clinical results with a comparative study of combined treatment versus radiotherapy alone. Int. J. hyperthermia. 3: 393 -411, 1987.

18) Valdagni, R; Amichetti, M; Pani, G: Radical radiation alone versus radical radiation plus microwave hyperthermia for N3 neck nodes: A prospective randomized clinical trial. Int. J. Radiat. Oncol. Biol. Phys. 15: 13 - 24, 1988

19) Emami, B; Perez, CA; Konefal, J; et al: Thermoradiotherapy of malignant melanoma. Int. J. Hyperthermia 4: 373 - 381, 1988

20) Marmor, JB; Hahn, GM; Combined radiation and hyperthermia in superficial human tumors. Cancer 46 : 1986 - 1991, 1980.

21) Gonzalez ,D ; Van Dijk, JDP; Blank, LECM; et al : Combined treatment with radiation and hyperthermia in metastatic malignant melanoma. Radiother. Oncol. 6: 105 - 113, 1986

22) Gonzalez Gonzalez, D; Van Dijk, JDP; Blank, LECM; et al : Chest wall recurrence of breast cancer : Results of combined treatment with radiation and hyperthermia. Radiother. Oncol. 12:95 - 103: 1988

23) Silberman,AW; Rand, RW; Krag, DN; et al : Effect of localized magnetic - induction hyperthermia on the brain. Cancer 57: 1401 - 1404, 1986

24) Hornback, NB; Shupe, RE; Shidnia, H; et al: Advanced stage III B cancer of the cervix treatment by hyperthermia and radiation. Gyn. Oncol. 23 ; 160 - 167, 1986.

25) Sapozink, MD; Gibbs, FA; Thomson, JW; et al: A comparison of deep regional hyperthermia from an annular array and a concentric coil in the same patients. Int. J. Radiat. Oncol. Biol. Phys. 11: 179 - 190, 1985

26) Pilepich, MV; Myerson, RS; Emami, BN; et al: Regional hyperthermia assessment of tolerance to treatment. Int. J. Radiat. Oncol. Biol. Phys. 14: 347 - 352, 1988

27) Bicher, HI; Wolfstein, RS : Local hyperthermia for deep tumors - Experience with three techniques. J. Microwave Power 21: 21st International Microwave Power Symposium Summaries, 103 - 104, 1986

28) Hiraoka, M; Jo, S; Akuta, K; et al: Regional hyperthermia for cancer. Am. J. Surgery 143: 586 - 590, 1982

29) Bicher, HI; Moore, DW; Wolfstein RS: Air - cooled 300 MHz applicators used in a parallel opposed phased system (POPAS). (Abstr.) Int. J. Radiat. Oncol. Biol. Phys. 11: S1: 217, 1985

30) Bicher, HI; Wolfstein, RS: Local superficial and deep hyperthermia - Factors affecting tumor response and patient survival (Abstr). Presented at 37th Annual Meeting of the Radiation Research Society, Seattle, Wash. 3/18 - 23/ 1989

31) Storm, K; Baker, HW; Scanlon, EF; et al: Magnetic induction hyperthermia. Results of a 5 year multi - institutional national cooperative trial in advanced cancer patients. Cancer 55: 2677 - 2687, 1985

32) Petrovich, Z; Langholz, B; Gibbs, FA; et al: Regional hyperthermia for advanced tumors : A clinical study of 353 patients. Int. J. Radiat. Oncol. Biol. Phys. 16: 601 - 607, 1989

33) Hiraoka, M; Jo, S; Akuta, K; et al: Radiofrequency capacitive hyperthermia for deep - seated tumors. II. Effects of thermoradiotherapy. Cancer 60 : 128 - 135, 1987

34) Lokich, JJ; Ahlgren, JD; Gullo, JJ; et al: A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: A Mid - Atlantic Oncology Program study. J. Clin. Oncol. 7: 425 - 432, 1989

35) Storm, KF; Scanlon, EF; Baker, HW; et al: Tumor stabilization after hyperthermia: An important criterion of response to thermal therapy. J. Surg.Oncol. 34: 143 - 149, 1987

36) Bicher, HI.; Hetzel, FW; Sandhu, TS; et al: Effects of hyperthermia on normal and tumor microenvironment. Radiology 137: 523 - 530, 1980

37) Bicher, HI; Hetzel, FW; Sandhu TS: Physiology and morphology of tumor microcirculation in hyperthermia. In Storm, FK (ed): Hyperthermia in Cancer Therapy. Year Book Medical Publishers, 1985,pp 207 - 222.

38) Egana, S ; Tsukiyana,I; Akine, Y; et al: Hyperthermia therapy of deep seated tumors: Comparison of the heating efficiencies of an annular array applicator and a capacitively coupled radiofrequency system. Int. J. Radiat. Oncol. Biol. Phys. 14, 521 - 528, 1988.

# THERMORADIOTHERAPY OF DEEP SEATED TUMOURS OF THE PELVIS WITH THE APA AND SIGMA APPLICATOR

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## INTRODUCTION

Clinical and experimental studies have shown the potential benefit of hyperthermia combined with radiotherapy (Overgaard 1989). Concerning superficial tumours clinical studies confirm that the combined treatment with both modalities increases the rate of complete and partial responses. However, when compared with superficial tumours heating of deep seated malignancies is more difficult. At present the most reliable method of external deep hyperthermia is the application of electromagnetic-wave hyperthermia which can be performed with the commercially available BSD 1000 (APA) and 2000 (SIGMA) devices (BSD Medical Corporation, Salt Lake City, USA). This procedure deposits power throughout a large volume of tissue including the tumour and surrounding normal tissues (Howard et al., 1986, Kapp et al., 1988; Molls et al., 1989; Petrovich et al., 1989; Pilepich et al., 1988). The clinical results of regional hyperthermia are presented with respect to the following four topics:

1. Tumour temperatures and thermal dose
2. Normal tissue temperatures
3. Toxicity
4. Response

## MATERIALS AND METHODS

12 patients with extensive pelvic tumour mass were considered suitable for the combined treatment. The age ranged from 26 to 71 years with a mean of 50 years. The tumour volume ranged from 118 to 1320 cc. with a mean of 366 cc. Patients had locally advanced tumours considered to be unlikely to respond to standard therapy. The majority of patients had pelvic recurrences of rectal (n=7) or cervical cancer (n=2). 2 patients with locally advanced sarcomas of the pelvis and 1 patient with a non resectable pancreatic carcinoma were also considered suitable. In 5 patients the actual radiation dose was low (30-36 Gy) due to previous irradiation. In one patient no further irradiation could be performed. In this case hyperthermia was combined with chemotherapy (5-FU). In 6 patients definitive radiotherapy with doses in a range from 50 to 60 Gy could be performed. The daily tumour dose was 180 to 200 cGy.
A total number of 83 regional hyperthermia treatments was performed with the Annular Phased Array (APA) of the BSD-1000 device or the SIGMA applicator of the BSD-2000 device. Heat was given immediately after irradiation twice weekly on a Monday-Thursday or Tuesday-Friday basis.

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Using APA equipment a partial array activation was chosen. In this configuration the power was delivered to the two adjacent quadrants that were closest to the tumour and best surrounded the target volume. Using Sigma equipment the treatment parameters (frequency, phase and power balance of the four amplifier channels) were optimized to achieve maximization of the focussing at the target zone as anticipated by the computerized pretreatment planning. Only in centrically located tumours all quadrants were activated. The operating frequency in the range of 60 to 80 MHz was selected to give a minimal percent reflected power. The duration of the heating period ranged between 26 and 110 min. with a mean of 74 min. Temperatures were measured with Bowman probes. They were inserted into normal and tumour tissues along blind ended Teflon-catheters under control of computed tomography or ultrasound. Further sites for temperature measurements were the rectum, vagina, urinary bladder, perineal and paraproctic fat, and the mouth for monitoring of changes in core temperature. The temperature distribution in the tumour, the perineal fat and the rectum was recorded along the length of the implanted catheter by thermal mapping. This procedure was performed every 15 minutes after the beginning of the hyperthermia treatment.
Thermal doses were calculated as minutes equivalent at 42.5 °C (min/eq/42.5 °C) for the tumour centre using a standard formula (Sapareto and Dewey, 1984). In this formula a "Thermal Equivalence Factor" was used, which relates time at various temperatures in the range from 40 to 45.5 °C to equivalent time at 42.5 °C. Thermal dose was added for all treatments and recorded as a total TD.

## RESULTS AND CONCLUSIONS

1. Tumour temperatures:
Total thermal dose was recorded in 11 patients, while in one patient, treatment temperatures were low and no significant TD accumulated. In the 11 patients with a recorded thermal dose, TD was > 50 <100 (n=4), 100 <200 (n=2) and 200 min in (n=5). These data indicate, that in single treatments in 5 of 12 patients we were able to maintain 42.5 °C within the tumour for a time period of 30 min. Petrovich (1989) recorded no significant TD in 42% of patients and a significant TD > 200 min only in 10% of the patients.

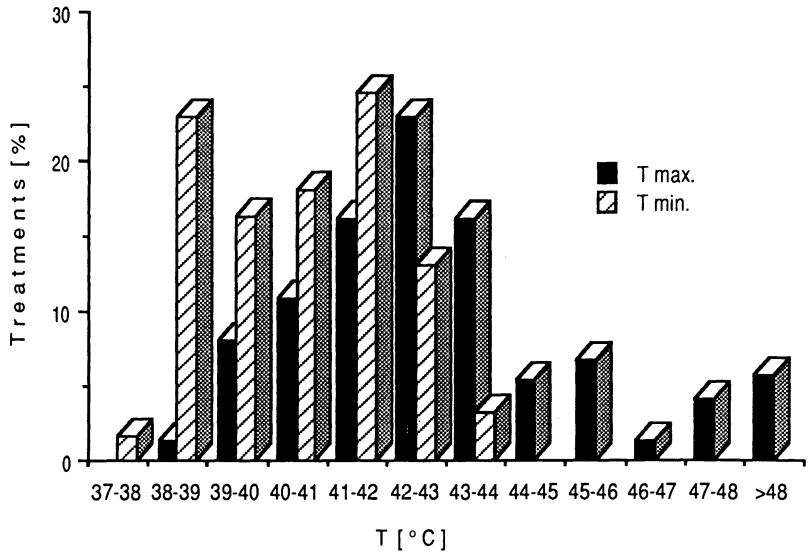


Fig. 1. Maximum and minimum tumour temperatures in 83 treatments

Other investigators (Howard et al., 1989) recorded heat doses in excess of 5 min (eq 43.0 °C) only in 41% of treatments. It might be that the observed relatively high heat doses in some of our patients are the result of long hyperthermia sessions.
Overall in our series maximum tumour temperatures from 42 to 51.2 °C were reached in 62% of the treatments (Fig. 1). The mean maximum temperature ± SD of the tumours was 43.3 °C ± 3.0 (range 38.8-51.2 °C), the mean minimum temperature ± SD was 40.3 ± 1.5 °C (range 37.8-43.5 °C). In 60% of the treatments we were able to achieve temperatures in the tumour centres 42.5 °C. However, only 60% of the points in which temperatures were measured showed temperatures of 41.0 °C. This demonstrates that the APA and SIGMA applicator is capable of heating deep seated tumours of the pelvis. But as previously reported (Kapp et al., 1988) it was very difficult to obtain uniform heating of the tumours, even if phase and power steering was used to achieve maximization of focussing at the target zone.

2. Normal tissue temperatures:
Regional hyperthermia for deep seated pelvic malignancies is frequently limited by pain probably related to excessive heating of normal tissues in the pelvic field. The mean maximum temperature ± SD of the vagina, the rectum and the bladder were 40.8 ± 1.2 °C (range 39.1-42.6 °C), 40.9 ± 1.6 °C (range 37.5-43.0 °C) and 40.5±1.6 °C (range 37.4-44.7) respectively (Table 1). The mean maximum systemic temperature ± SD was 37.7 °C ± 0.7 °C (range 36.8-39.9 °C). The highest normal tissue temperatures occured in the perineal and paraproctic fat with a mean maximum temperature ± SD of 42.6 ± 1.1 °C (40.7-46.0 °C). The maximum temperatures in the perineal fat were 1 to 2 °C higher than in the bladder or the rectum.
The most common sites for normal tissue overheating which have been reported in the literature (Howard et al., 1986; Pilepich et al., 1988; Samulski et al., 1987) were the bladder and the rectum. Overheating of these organs can

Table 1. Mean maximum normal tissue temperatures

| Localization | T max. ± SD | (range) |
|---|---|---|
| Bladder | 40,5 ± 1,6 °C | ( 37,4 - 44,7 ) |
| Rectum | 40,9 ± 1,6 °C | ( 37,5 - 43,0 ) |
| Perineal fat | 42,6 ± 1,1 °C | ( 40,7 - 45,1 ) |
| Vagina | 40,8 ± 1,2 °C | ( 39,1 - 42,6 ) |
| Systemic | 37,7 ± 0,7 °C | ( 36.8 - 39.9 ) |

be avoided by performing bladder cooling at the time the intracystic temperature reaches more than 42.5 °C. Furthermore overheating of faecal masses could be minimized by pretreatment preparation ensuring an empty rectum. However, the application of regional hyperthermia in the pelvis can be limited by high temperatures in the perineal fat, especially when indicated by pain or local discomfort.
It is the first time that the perineal fat is recognized as a critical tissue in deep hyperthermia. This was possible as the catheters for temperature measurements were inserted from the perineal region over a long distance of perineal, paraproctic and presacral fat into the tumour. The excessive temperature increase of the perineal fat in patients with eccentrically located tumours is a result of the maximization of the E-field to the tumour bearing area. The axial dimension of the E-field is determined by the length of the applicator. In future it will be possible to limit the E-field in the axial direction exclusively to the region of the tumour. Furthermore,

the observed high maximum temperatures in the tumour and the perineal fat is mainly the result of the blood flow situation. The estimates of blood flow from analysis of the measured temperature curves following the completion of a tumour heating session reveal a significant difference between tumour and normal muscle tissue and the perineal fat and normal muscle tissue (mean washout tumour: 7.7 ml/100 g-min; mean washout perineal fat: 7.5 ml/100 g-min; mean washout muscle: 28.2 ml/ 100 g-min). In the clinical situation the relatively low blood flow in the tumours and the perineal fat was an important cause in achieving high maximum temperatures in these tissues when compared with the low maximum temperatures * SD in muscle (mean 39.8 °C ± 0.7 °C).

3. Toxicity:
In most of the treatments the temperatures achieved were limited by the acute toxicity of the treatment (Table 2). There were 80 instances of some form of toxicity which limited treatment. During some treatments patients complained of more than one side effect, necessitating power reduction or treatment termination. Acute toxicity was of short duration and usually subsided within 1 hour of treatment. In 20 cases the treatment limiting toxicity was the general discomfort of the patient. The second most common problem, occuring in 19 treatments, was localized discomfort or pain within the pelvis. It relieved by a reduction of power. Other recorded treatment-limiting factors were anxiety and pain not related to heating. Unacceptable tachycardia or rise in core temperature limited only 5 treatments respectively.
On two occasions small burns were recorded predominantly in the upper thigh area in patients treated to the pelvis.
In one patient severe pain of the sacroiliac region was noted during the

Table 2. Acute treatment limiting toxicity in 83 treatments

| Toxicity | No.of times recorded |
|---|---|
| Generalized discomfort | 20 |
| Pelvic discomfort or pain | 19 |
| Anxiety | 12 |
| Pain not related to heating | 13 |
| Rise in core temperature (oral temperature >39 °C) | 5 |
| Tachycardia pulse > 140 | 5 |
| Hypertension: diastolic pressure >100 mm Hg | 1 |
| Hypotension: systolic pressure <100 mm Hg | 5 |

hyperthermia session. The pain was tolerable with small reduction of applied power and the duration of pain was approximately 24 h after treatment. 48 h after treatment we observed a large perineal hematoma.
Another patient with a large presacral recurrence developed a gluteal necrosis which was related to tumour invasion and hyperthermia induced tumour regression.

These observations underline the necessity of careful patient and tumour site selection in deep heating trials as described by other investigators (Kapp et al., 1988; Petrovich et al., 1989; Pilepich et al., 1988). Patients who are very debilitated, have a low pain threshold.

Others who have serious cardiopulmonary, hepatic, or arthritic disease, are not likely to be able to tolerate deep regional heating. Patients with necrotic, ulcerated lesions, overlaying or invading a major vessel or critical structure are at risk to develop hemorrhages or fistuli, if the tumour is responsive to treatment.

4. Response:
There has been a documented follow-up of disease in 12 patients. 9 patients are alive between 2 months and 1 year after treatment. 3 patients died due to tumour progression. In 4 of 12 patients a reduction in tumour volume greater than 50% has been demonstrated by CT. In 7 patients tumour has remained static for periods from 2 months to 1 year. In one patient we observed a tumour progression under treatment. In view of the advanced nature of the disease the main aim of our treatments was palliation. A very good palliation with relief of severe pain was achieved in 4 patients, moderate palliation in further three patients and in three patients no control of symptoms could be obtained (2 patients had no symptoms at the beginning of treatment).
Palliation with relief of severe pain occured even among patients who showed static disease on the CT-scan. Local tumour response to therapeutic modalities is most commonly addressed on the basis of tumour regression. In one clinical study (Hiraoka et al., 1987), however, it was shown that tumour response to thermoradiation cannot be fully assessed with this method. Histopathologic examinations and follow up observations revealed remarkable effects in a considerable number of tumours that did not exhibit regression but showed a clear low density area in the tumour on the posttreatment CT scan. This low density area seems to be partly attributable to destruction of the tumour microvasculature and partly to direct killing of cancer cells by heat. In two patients we have seen a clear low density area in the tumour (more than 50%) but no reduction in tumour volume (NC). In this situation CT is disadvantageous in that it cannot disclose complete necrosis of the tumour.
Our observed response rate is in agreement with the published results of other investigators (Howard et al., 1986; Petrovich et al., 1989). In view of the advanced nature of the disease and the heavily pretreated patients these results are remarkable. Most patients were treated to maximum levels of tolerance. In future, improved heating technology as well as some control over local regional blood flow may be required to achieve substantial increases in intratumoral temperatures and to reduce the acute toxicity of the treatment. Additionally phase-II-studies with selected previously untreated patients have to confirm the potential benefit of radiotherapy plus hyperthermia in deep malignancies of the pelvis.

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**References**
1. Hiraoka, M., Akuta, K., Nishimura, Y., Nagata, Y., Jo, S., Takahashi, M., Abe, M., 1987, Tumor response to thermoradiation therapy: Use of CT in evaluation. Radiology 164:259.
2. Howard, G. C. W., Sathiaseelan, V., King, G. A., Dixon, A. K., Anderson, A., Bleehan, N. M., 1986, Regional hyperthermia for extensive pelvic tumors using an annular phased array applicator: A feasibility study. Br. J. Radiol. 59: 1195.

3. Kapp, D. S. , Fessenden, P. , Samulski, T. V. , Bagshaw, M. A. , Cox, R. S. , Lee, E. R. , Lohrbach, A. W. , Meyer, J. L. , Prionas, S. D. ,1988, Stanford University Institutional Report. Phase I evaluation of equipment for hyperthermic treatment of cancer. Int. J. Hyperthermia 4:75.
4. Molls, M. , Feldmann, H. J. , Baumhoer, W. , Sack, H. , 1989, Radiotherapy and regional hyperthermia: Thermal profiles and treatment results, in: "Proceedings of the 5th International Symposium on Hyperthermic Oncology", Kyoto, Japan, in press.
5. Overgaard, J., 1989, The current and potential role of hyperthermia in radiotherapy. Int. J. Radiat. Oncol. Biol. Phys. 16:535.
6. Petrovich, Z. , Langholz, B. , Gibbs, F. A. , Sapozink, M. D. , Kapp, D. S. , Stewart, R. J. , Emami, B. , Oleson, J. , Senzer, N. , Slater, J. , Astrahan, M., 1989, Regional hyperthermia for advanced tumours: A clinical study of 353 patients. Int. J. Radiat. Oncol. Biol. Phys. 16:601.
7. Pilepich, M. V. , Myerson, R. J. , Emami, B. , Perez, C. A. , Straube, W. , VonGerichten, D.,1988, Regional hyperthermia - Assessment of tolerance to treatment. Int. J. Radiat. Oncol. Biol. Phys. 14 :347.
8. Samulski, T. V. , Kapp, D. S. , Fessenden, P. , Lohrbach, A. W. ,1987, Heating deep seated eccentrically located tumors with an annular phased array system: A comparative clinical study using two annual array configurations. Int. J. Radiat. Oncol. Biol. Phys. 13 : 83.
9. Sapareto, S. A. ,and Dewey, W. C. ,1984, Thermal dose determination in cancer therapy. Int. J. Radiat. Oncol. Biol. Phys. 10:787.

# INTRACAVITARY HYPERTHERMIA COMBINED WITH H.D.R. AFTERLOADING IRRADIATION IN VAGINAL RECURRENCES OF CERVICAL CARCINOMA

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Hyperthermia has been shown to be effective for treatment of superficial tumors, expecially when combined with radiation and chemotherapy.
It appears that certain tumor sites in hollow viscera or cavities such as gastrointestinal, gynecological and genitourinary systems can be treated with an intracavitary technique, thus eliminating some of the pitfalls of external hyperthermia applications as described by Gibbs, et al.(1).
The technique implies that the heating sources are closely contacted to the target volume.
We will report on our preliminary experience with this technique.
After initial measurements in phantom, three patients were treated, in our department, using a protocol combining intracavitary hyperthermia and brachytherapy.
Technique and results are presented here.

## METHODS AND MATERIAL

### Patients

Three patients affected by vaginal recurrences of cervical carcinoma were treated with intracavitary microwave hyperthermia combined with radiation.
They were hystologically proven squamous cell carcinoma.
Details of the patients are given in tab.I.
Two patients have had radical hysterectomy for cervical cancer two years earlier. They received pelvic external beam radiotherapy previously and both received intracavitary radiotherapy. In conjunction with Ir192 irradiation the intracavitary microwave applicator was inserted.
The other one, who was previously irradiated for cervical stump cancer, 9 months earlier, at dose of 46 Gy, was treated only with 192Ir irradiation combined with intracavitary hyperthermia.

### Radiation

External beam radiotherapy was delivered with 8 MV photons

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TAB.I Summary of the patients treated for vaginal recurrences of cervical carcinoma.

| Case No | Age | Vaginal site | Previous treatment | Present treatment | Treatment toxicity | Complications | Response | Present status |
|---|---|---|---|---|---|---|---|---|
| 1 | 68 | Upper third | surgery (Meigs) 2years | ExtRt 45Gy/25fr. Brachy 7Gy/3fr. Ht 41.5 Cx3 | none | Mild cystitis | CR | Alive NED/4months |
| 2 | 66 | Apex | surgery (Meigs) 2years | ExtRt 45Gy/25fr. Brachy 7Gy/3fr. Ht 43 Cx3 | local pain | Proctitis, bleeding vag. | CR | Alive NED/3months |
| 3 | 75 | Cervical stump and whole vag. | surgery (subtotal for fibroma) ExtRt 60-Co 9months | Brachy 40Gy/20fr. Ht 42 Cx4 | local heat | Bleeding cystitis | PR | Dead for intercurrent disease/3months |

ExtRT: External beam radiotherapy; CR: Complete response; Ht: Hyperthermia;

Brachy: Brachytherapy; PR: partial response; NED: No evidence of disease.

of Linac trough 4 orthogonal portals. A total of 45 Gy over 5 weeks was delivered at 1.8 Gy/fraction.
Endocavitary radiotherapy was given with a remote afterloading system (GAMMAMED IIi) using a single high active 192Ir source(370 GBq).
7 Gy at 0.5 cm from the source was delivered at weekly intervals , for a total of 3 fractions,in two patients. One patient received another brachytherapy schedule (2 Gy x 5 /week) for a total of 40 Gy.

Hyperthermia

Heat treatment was given with LUND SCIENCE HYPERTHERMIA SYSTEM 4010 using 915 MHz microwaves generator with 200 W of power output and a separate thermometry system (ATS 100) with thermistor probes (0.75mm). An intracavitary applicator inserted in a perspex vaginal obturator with an outer diameter of 20 mm and 130 mm length, having one central channel, was used.
The obturator with the applicator and the thermistors attached to its surface was inserted into vagina and closely contacted to its walls. About six thermistors were positioned alongside the applicator, from the tip to the introitus of vagina.
Recently we acquired an applicator, originally designed for hyperthermia treatment of prostate tumors, with a peripheral channel for the antenna. This applicator of 20 mm diameter and 180 mm length has a cooling system by circulating tap water.Thus we can heat delimited portions of the vagina sparing the surrounding normal tissues.
The tumor temperatures registered by the thermistors were 41-45 C during the 45-60 min. of the treatment. Heat was given weekly within 30 min of the 192Ir irradiation.

Experimental apparatus

We characterized the performance of the two applicators in tissue equivalent material. The phantom material was composed of the following:60% saline (2.5% NaCl, 97% H2O );22.5% sugar and 17.5% Tx150 (superstuff) (Nilsson 84) (2). The muscle material was contained in two halves of a 20cmx20cmx 15cm bisected polystyrene box (0.5 cm thick) with a central hole for the applicator. After a brief (60-120 seconds) period of high power (40 watts) exposure the model wasseparated and a thermogram was taken by infrared camera (AGA 782 Thermovision). Microwave frequency at 915 MHz was used. The surface temperature before microwaves exposure was recorded too.

RESULTS AND CONCLUSIONS

Qualitative informations on the heating patterns were obtained by taking polaroid photographs of the phantom model thermograms. The heating patterns varied with the applied frequency (915 MHz) around the two applicators,
For both applicators,approximately 40 W of power for 2 min.

were necessary to produce a temperature rice of 1 C in the tissue equivalent phantom about 3 cm away from the applicator surface. In the applicator having a central hole for the antenna the heat distribution was relatively uniform and symmetrical around it. An asymmetrical heat distribution has been acheived for the second device. The active section was from the tip and down 6-7 cm for the two antennas. Heating patterns of these applicators were necessary informations to select a proper probe for the treatment of tumor at a particular site.

Two patients developed vaginal recurrences of cervical carcinoma after previous (2 years earlier) radical abdominal hysterectomy (Meigs technique) causing vaginal discharge. They received radical radiotherapy to the pelvis followed by three weekly intracavitary radiothermotherapy. The patients are alive with normal appearance of vagina and negative biopsy and pap smear.

The other patient presented a cervical stump and vaginal recurrence of cervical stump cancer previously irradiated. The patient was treated with palliative dose of brachytherapy (40 Gy, 20 fractions in 4 weeks) and hyperthermia (for a total of 4 sessions at weekly intervals). The treatment resulted in symptomatic relief (pain, vaginal bleeding) but she presented residual disease at 2 months into follow up. The patient expired 1 month later from an intercurrent disease. There has not been sufficient length of follow up time to allow a report of the efficacy of this treatment.

All the patients tolerated the hyperthermia sessions, lasting 45-60 min. There were sensations of local heating or pain , but all patients completed the planned treatment. Subsequent speculum examination did not reveal acute thermal damage.Our patients have not the early radiation reactions from the bladder, intestines or vaginal mucosa so far accentuated. We conclude that this modality of treatment can be given safely in combination with radiotherapy.

REFERENCES

1 Gibbs F., Peacok. L., Settles K., Stewart J.
Preliminary experience with induction of deep regional abdominal hyperthermia using the BSD annular phased array applicator system.
Radiation research, 1982, 91, 423

2 Nilsson P.
1984 Phd Thesis, Lund University, Sweden

# LOCAL MICROWAVE HYPERTHERMIA AND BENIGN PROSTATIC HYPERPLASIA INDUCED BLADDER OUTLET OBSTRUCTION

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## INTRODUCTION

Hyperthermia (HT) has been used as a treatment for cancer since last century, both alone or combined with chemotherapy and radiotherapy. The rationale supporting the application of heat in cancer therapy is based on the following data: 1.) HT increases tumor sensitivity to radiation and it can be cytotoxic itself 2.) HT increases the rate of neoplastic necrosis induced by chemotherapy 3.) hypoxia cells are sensitive to HT while are resistant to radiations 4.) solid tumors are heat reservoirs due to poor blood supply 5.) tumors previously irradiated can be submitted to HT with no additional damage to normal tissue 6.) heat inhibits the repair of radiation induced damage to DNA 7.) neoplastic cells are more sensitive to heat (42-44 °C) than normal cells. This experimental and clinical evidence contributed to the progressive broadening in the use of HT. In 1982 Yerushalmi and associates (1) reported the first series of prostate cancer patients submitted to selective prostatic HT. Since then, several authors have reported experience with local HT for prostatic diseases (2.3). This study reports our series of 100 benign prostatic hypertrophy (BPH) submitted to local prostatic HT and evaluated at the six month follow-up date by clinical, histological and ultrastructural studies.

## MATERIAL AND METHODS

From November 1987 to December 1988, 199 BPH patients underwent treatment with local prostatic HT. This study deals with the first 100 patients evaluated at the six month follow-up date. Thirty patients had an indwelling catheter and 70 complained of severe bladder outlet obstruction symptoms. The mean age of patients was 64.9 years (range 54-81 years). The system used to deliver HT (Prostathermer 99D) was developed by Yerushalmi and associates (1). Briefly, it is composed of a 915 MHz microwave source a rectal heat applicator with a cooling system for the interior wall of the rectum, a urethral probe to measure prostatic temperatures and a computer system for data analysis. Hyperthermia was delivered in 60 minute long sessions, once a week in patients younger than 70 years and twice a week in older patients and in case of an indwelling catheter. The entire therapeutic course lasted 5 weeks. Calculated

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interprostatic temperature was 42 ± 0.5 °C. Treatments were performed on an outpatient basis as neither anesthesia nor sedation were used even when patients with an indwelling catheter underwent combined treatment with HT and cyproterone acetate (50 md/day for the entire duration of treatment); the remaining 13 patients were submitted to HT monotherapy. Patients were evaluated pre- and post-operatively by subjective scoring of symptoms, physical examination, complete biochemistry, intravenous pyelogram, trans-rectal ultrasound of the prostate, rectoscopy, uroflowmetry and evaluation of rest volume. Nuclear magnetic resonance (NMR) was performed in 15 patients. A questionnaire was used to evaluate subjective symptoms: answers were scored from 1 to 9, the highest value corresponding to the best clinical condition and vice versa. Twenty-five patients presented with a clinical suspicion of prostate cancer: a transperineal biopsy was performed before starting the treatment and at the one month follow-up date. Specimens were studied by light and transmission electron microscopy (TEM). Selection criteria was strictly observed: HT was administered only to patients at poor operative risk, young patients refusing post-surgical retrograde ejaculation and patients refusing surgery because of personal motivations. Only 55% of the patients evaluated for treatment with HT were considered as eligible and entered the study. Patients were seen at the one week, one month, three month and six month follow-up dates.

## RESULTS

Subjective (frequency, nocturia and urgency) and objective (maximum and medium urinary flow) parameters were studied in 70 non-catheterized. Subjective symptoms were improved in 57 (81.4%) patients while in 13 (18.6%) patients HT did not modify the clinical picture. Table 1 shows pre- and post-operative subjective scoring of symptoms. Table 2 reports uroflowmetric data: the increase in urinary flow rate and the decrease of rest volume after HT were statistically significant ($p < .001$) in 54 (77.1%). The improvement of at least four of the considered parameters, were used to define the treatment as succesful.

Table 1 Pre- and post-hyperthermia subjective symptomatology in 70 non-catheterized patients

| | PRE | POST |
|---|---|---|
| Frequency | 4.0 | 6.5 |
| Urgency | 3.4 | 5.5 |
| Nocturia | 4.3 | 6.5 |

Scoring: from 1 = worst to 9 = best.

Table 2. Pre-and post-hyperthermia objective parameters in 70 non-catheterized patient.

| | Pre Median + SEM | Pre Median + SEM | P+ |
|---|---|---|---|
| Maximum flow | 7.4 +/ -0.4 | 9.8 +/ -0.2 | <.001 |
| Medium flow | 3.3 +/ -0.2 | 4.6 +/ -0.2 | <.001 |
| Rest Volume | 115 +/ -14.4 | 35 +/ -8.7 | <.001 |

P + Wilcoxon Rank Sum Test

According to these criteria, 70% of the patients were considered as having a succesful result. Among the patients with an indwelling catheter, 14 out 17 (82.3%) treated by combined HT and CPA and 8 out of 13 (61.5%) submitted to HT monotherapy, could void spontaneously. The mean rest volume in patients who had the catheter removed was 75 and 50 ml in the two groups, respectively. Statistical analysis was not performed in these two sub-groups due to the limited number of patients. Post-operative transrectal ultrasound of the prostate did not show any definite modifications: only in some cases a slight tendency to hypoechogenicity was seen, possibly due to an increase in the fluid fraction of the gland. The same finding was confirmed by NMR when performed. Both histological and ultrastructural studies demonstrated the integrity of cell membranes, the presence of lymphocytic infiltrates, interstitial edema, dilation of intraprostatic vessels and ducts. The latter finding could confirm the patterns evidenced by ultrasound and NMR. No tessutal and cellular irreversible lesions could be detected. Complications included urinary tract infection (3 cases), transient urethral bleeding (12 cases), transient hematuria (5 cases), hemospermia (8 cases).

## DISCUSSION

Almost 200 BPH patients were submitted to LH at our institution. The patient selection was the real clue for achieving satisfactory results. We believe that surgery is still the first choice therapy for obstructed BPH patients, being successful in 95% of cases. At our institution we suggest the patient to undergo local prostatic HT only in case of poor operative risk or absolute refusal of surgical treatment. Alternative options are transurethral balloon dilation of the prostatic urethra (5) and endoprostatic prostheses (6). Results reported are still preliminary and a definite conclusion cannot be drawn yet. Presently local prostatic HT has been used in large series of BPH patients:

several authors reported satisfactory results, i.e. 80% catheter removal and 70% amelioration of both subjective and objective symptoms. Different therapeutic protocols as regarding the number and duration of sessions, intraprostatic temperatures and systems for delivering HT, do not allow for a correct evaluation and comparison of data achieved. In our series of patients, a 70% success rate thus slightly lower to data reported in the literature. At our institution, at least 4 out of the 6 parameters evaluated must be improved in order to consider the treatment as successful. We deem that we will increase our success rate by increasing the number of local prostatic HT sessions as we usually notice the clinical improvement after the first 4-5 sessions. We have recently started a protocol with 10 HT sessions also in young patients without a catheter but it is also a safe therapy and TEM could demonstrate for the first time the integrity of cellular membranes after HT (Figure 1).We conclude that LH is a safe and effective treatment for BPH patients not considered as suitable for surgical therapy. If the results achieved in our experience were confirmed at long-term follow-up, a revolutionary therapeutic proposal could be suggested: prophylaxis of BPH-induced bladder outlet obstruction by therapy with HT performed before the onset of clinical symtomatology.

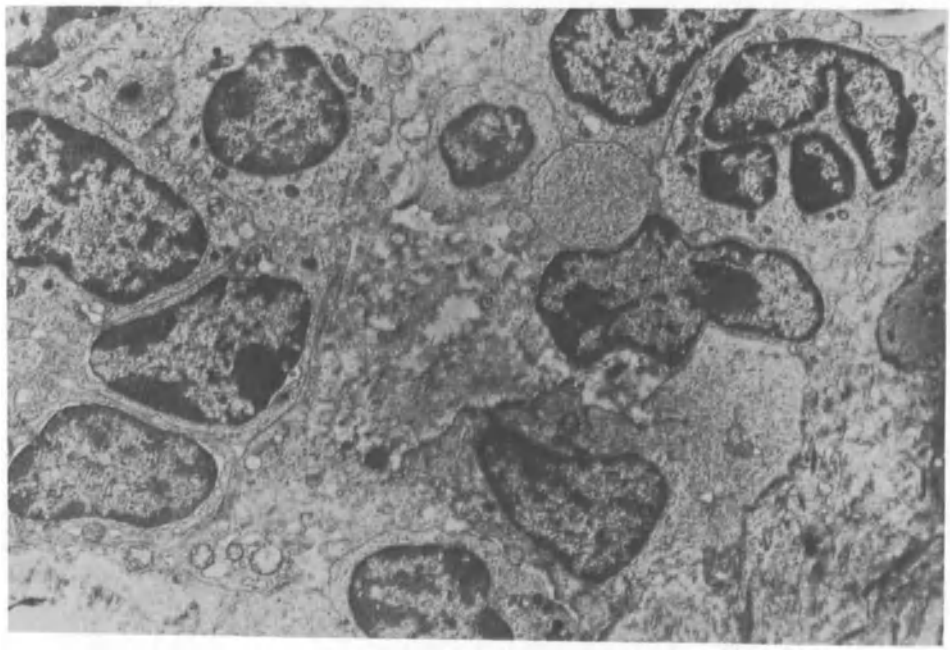

Figure 1. Prostatic biopsy taken at the post -HT one month follow-up date. Mononucleated inflammatory cells infiltrate the prostate; intra cellular membranes are well preserved (TEM 6400X)

REFERENCES

1) Yerushalmi, A., Servadio, C., Leib, Z., Fishelowitz, E., Rakowsky, E., Stein, J.A., Local Hyperthermia for treatment of carcinoma of the prostate, 1982, Proatate, 3:623.

2) Servadio, C., Leib, Z., Diseases of prostate treated by local microwave hyperthermia, 1987, Urology, 30:97.

3) Lindner, A., Golomb, J., Siegel, Y., Lev, A., Local Hyperthermia of the prostate gland for the treatment of benign prostatic hypertrophy and urinary retention, 1987, Br J Urol, 60:567.

4) Peters, C.A., Walsh, P.C., The effect of nafarelin acetate, a LHRH agonist, on benign prostatic hyperplasia, 1987, N Engl J Med, 317:599.

5) Reddy, P.K., Wasserman, N., Castaneda, F., Cataneda Zuniga, W, Transurethral balloon dilatation of prostate for prostatism : preliminary report of nonsurgical technique, 1987, J Endourol 1:269.

6) Garbit, J.L., Blitz, M., Bomel, J., Pin, P., Paillot, J., La prothese endoprostatique de Fabian, 1988, J Urol (Paris), 94:265.

# THERMORADIOTHERAPY OF PELVIC TUMOURS

## WITH THE BSD 2000: first clinical results

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### Introduction

The start of clinical hyperthermia reminds of the development of radiotherapy. Both had some difficulties to reach localized deep processes. Only highvoltage irradiation allowed to pass the hindering skin. For a long time hyperthermia was only capable to heat tissue within a deepness of 3 to 5 cm. Since the use of electromagnetic waves effective heating of deep seated tumours seemed possible. A great number of patients is now treated successfully with the annular phased array system (4). The BSD 2000 device with the sigma-applicator expresses a technical improvement and promises advanced local hyperthermia technique (6, 7). Does this more sophisticated new system now allow to perform a safe and distinctive hyperthermia treatment in any region deep in the body? Our first clinical observations with radiotherapy and hyperthermia may lead to some answers.

### Methods

Patients with inoperable, large and central necrotic tumours in the pelvis and/or abdomen were treated with radiotherapy and deep local hyperthermia. The advanced local extension seemed to be resistant to other conventional treatment schedules. Photons with energies of 6-MeV up to 45-MeV and electrons up to 45-MeV were used. The daily fraction was 180-200 cGy, modulated by the initial treatment volume. A total dose of 45-50 Gy was given in 4 to 5 weeks; including a boost to shrinked tumour masses. The hyperthermia was produced with the BSD 2000 device and the sigma applicator (BSD Medical Corporation, Salt Lake City, Utah, USA). The temperatures were measured with calibrated Bowman-probes to get an appropriate accurracy (1).

After diagnosis 1 to 3 teflon-catheters were implanted intraoperatively in the already known tumour region. A CT-control afterwards was performed. Always just before the treatments additional catheters were placed in the rectum, the bladder and - in female patients - in the vagina. Also the measurement of the skin in the treatment field and if possible of critical points was done. During the treatment the probes were moved along the catheters. This allowed to detect inhomogeneities and their correction whenever possible. External and invasive e-field probes measured the

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electromagnetic field distribution. The positioning of the patient in the applicator could be controlled. During the treatment changing of frequency and amplitudes was followed by a focused energy without extensive manipulations on the patient. Heart rate and blood pressure were monitored every 5 minutes. The aim was to reach 42,0°C to 42,5°C whenever possible. In large masses in the abdomen we thought 41,5°C to be sufficient. So complications with fixed small bowel should be avoided. If possible hyperthermia was given once or twice a week within half an hour after irradiation. CT-scans were repeated after 2000/4000 cGy and every three months. Additional analgetics were never given out of principal reasons.

Radiobiological examinations of irradiated tumours is a method initiated and performed in our institution (2). As a routine during the implantation of the catheters a new biopsy was taken. The living tumour cells were cultivated and treated under same conditions.

**Results**

We report of our first experiences with 3 patients.

Table 1

**SUMMARY OF PATIENTS**

| | W.R. | U.P. | R.L. |
|---|---|---|---|
| age (years) | 47 | 47 | 67 |
| first symptom | pain | pain | stenosis subileus |
| primary | sarcoma | colon | cervix |
| stage | III ($T_3N_0M_0$) | Dukes D | III |
| treated region | pelvis | abdomen + pelvis | pelvis |
| localization | excentric | excentric | central |
| tumour volume (CT) | 16x24x11 cm | 18x10x10 cm | 7x7x5 cm |
| tumour control | CR | PR | PR |
| survival (months) | 4.0 | 4.0 | living |

The average time from the first symptom to diagnosis was 7 months. One patient presented a large slowly growing cancer with distant metastasis. In this palliative situation over more than two years no causal therapy was performed. In two situations an initial transverso-sigmoidostomia was needed. Intraoperative there was only a biopsy possible. No previous chemotherapy was initiated. Two patients died of distant metastasis meanwhile. A local tumour control was clinically achieved.

Large tumours are measured radiologically, a remarkable regression after 2000 cGy is shown.

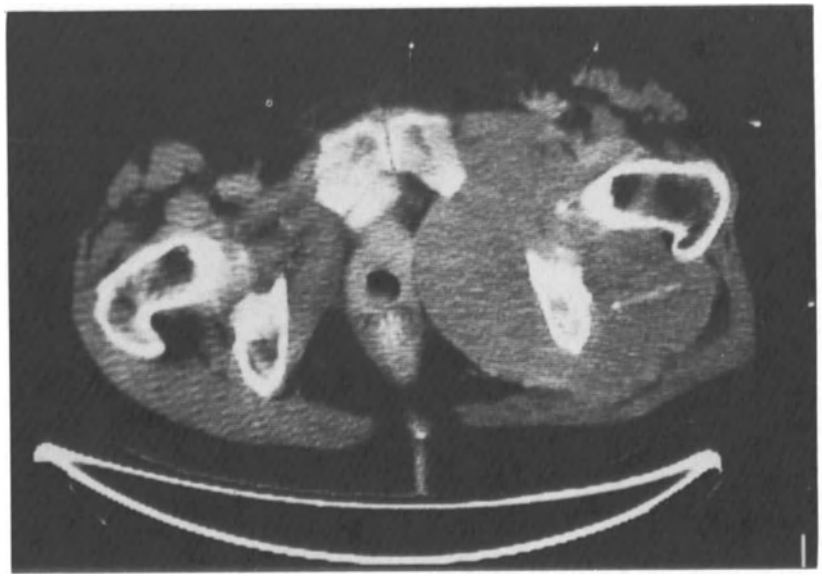
Table 2

**CT-results of combined treatment (RT - HT)**

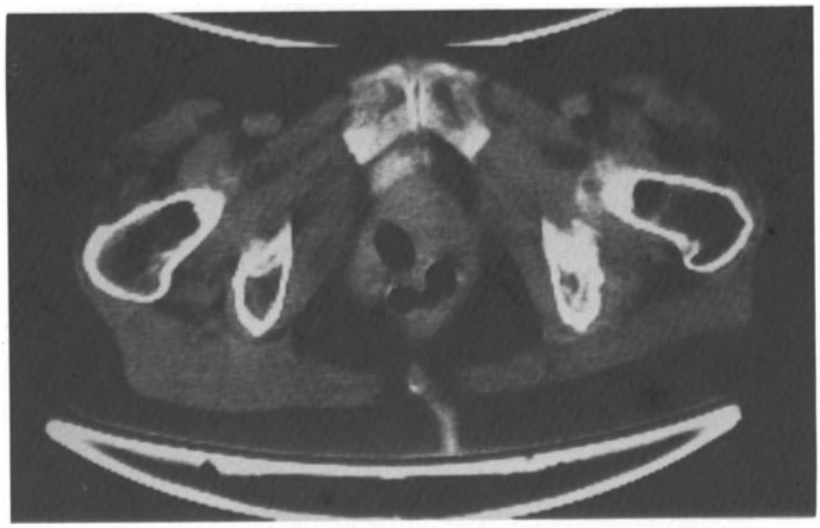
| | W.R. | U.P. | R.L. |
|---|---|---|---|
| **mean volume (cm)** | | | |
| initial | 16x24x11 cm | 18x10x10 cm | 7x7x5 cm |
| 2000 cGy | 10x 9x 9 cm | 18x11x 9 cm | 7x7x4 cm |
| 4000 cGy | - | 14x10x 9 cm | 5x5x3 cm |
| 3 months | - | 14x 8x 8 cm | 4x4x3 cm |
| **inhomogeneities** | | | |
| initial | - | + | + |
| 2000 cGy | - | + | ++ |
| 4000 cGy | - | ++ | ++ |
| 3 months | - | ++ | ++ |
| **central necrosis** | | | |
| initial | - | + | + |
| 2000 cGy | - | ++ | + |
| 4000 cGy | - | ++ | + |
| 3 months | - | ++ | ++ |
| **calcification** | | | |
| initial | - | - | - |
| 2000 cGy | - | + | - |
| 4000 cGy | - | ++ | - |
| 3 months | - | +++ | + |
| **commentary** | **CR!** (no tumour visible) | **PR!** | **PR!** |

A rapidly intratumoural calicification was observed after treatments. Radiologically a complete remission and two partial regressions are documented. Photograph 1a and 1b show the complete remission of a large sarcoma (case W.R.). Note the implanted temperature probes. On photograph 2a and 2b the extensive calcification of an adenocarcinoma (case U.P.) can be seen.

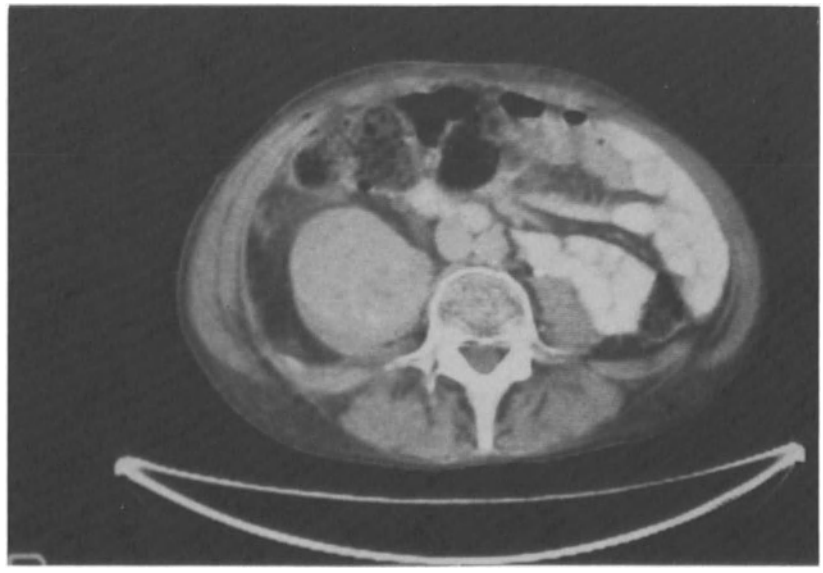
The average time of hyperthermia sessions was 77 min. In one patient large masses were found in the abdomen and retroperitoneal down to the pelvis, therefore carefully 41,5°C were given. The total time is 83 min.



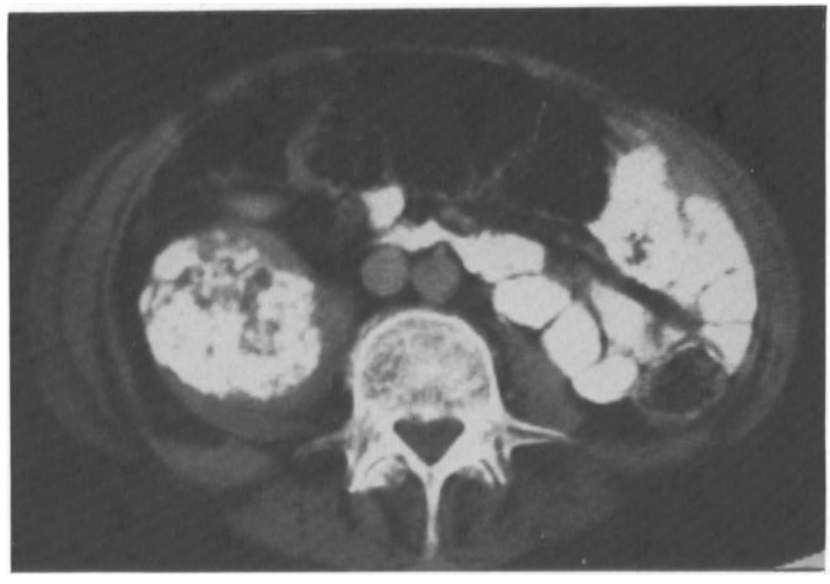
Photograph 1a. Computertomography of the pelvis of case W.R.: Large sarcoma of the left pelvis with infiltration of the muscular structures and the bones. Note the implanted temperature probes.



Photograph 1b. Case W.R.: Radiologically complete remission after 4400 cGy and 5 hyperthermia treatments.



Photograph 2a. Large retroperitonal-abdominal tumour mass (U.P.). Situation before the onset of the combined treatment.



Photograph 2b. Computertomographic findings three months after 6 hyperthermia sessions and 4000 cGy: Extensive calcification of the known adenocarcinoma.

Table 3
**"HEAT DOSE"**

| | W.R. | U.P. | R.L. |
|---|---|---|---|
| tot.min. 41,5°C | 205 | 83 | 192 |
| tot.min. 42,0°C | 150 | 0* | 99 |
| tot.min. 42,5°C | 71 | 0* | 21 |

*) not intended

42,0°C could be measured over 150 min. respectively 99 min., or 42,5°C over a period of 71 min. respectively 21 min. Cooling of the bladder was necessary in two patients, no complications were observed.

Heat sensations most occurred on predicted localizations as in head and neck, along masses of bones, intratumoural and in anatomical narrows parts of the body.

Table 4
**LOCALIZATION OF HEAT SENSATIONS**

| | W.R. | U.P. | R.L. |
|---|---|---|---|
| in the treatment field | + | + | + |
| beyond the treatment field | + | + | + |
| back/spine | + | - | - |
| sacrum/ileum | + | + | + |
| perineum | + | - | + |
| inguinal | + | - | - |
| tibial/foot | + | - | - |
| head + neck | + | + | + |
| cicatrice | - | + | - |

All patients presented after the treatment a moderate erythema of the skin according to the treatment ports of radiotherapy, we never saw an ulceration or blisters. No other complications were revealed. The surgical implantation of the catheters avoided infections or bleedings in the best possible way.

A extraordinary relief on pain and specific local tumour symptoms was seen. A mobilisation of two patients was followed by their dismission at home. Clinically the local tumour control remained.

Table 5
**CLINICAL RESULT**

| | W.R. | U.P. | R.L. |
|---|---|---|---|
| Karnofsky | +30 % | +30 % | +40 % |
| relief of symptoms | 90 % | 85 % | 100 % |
| relief of pain | 90 % | 90 % | - |
| mobilisation | 100 % | 100 % | initial |
| local tumour control | yes | yes | yes |

**Discussion and remarks**

In the first clinical observations the new system had to prove its capability and feasibility. We never saw a break-down of the security system and alarm controls in all performed simulations with phantoms and under the clinical conditions. An advantage was the relatively easy focusing of the energy center in the patient without extensive manipulations or positioning. Therefore a clear enhancement of temperatures in the tumour region was obtained.

A separation is soon seen: (Fig. 1a) Intratumoural (and especially in bad perfused regions) the intended temperatures are rapidly realized. In the rectum, bladder and vagina there is only a moderate temperature rising. This effect was allways seen in the first treatment sessions. The radiologically proven tumour shrinking lead to the application of more power with less differences of the monitored temperatures. (Fig. 1b) Here the preventional bladder cooling was performed.

Table 6

**ANALYSIS OF TREATMENT-INTERRUPTIONS/STOP**

| | W.R. | U.P. | R.L. |
|---|---|---|---|
| **clinical** | | | |
| - stress | - | 1x | 1x |
| - pain | - | 1x | 1x |
| - heat | - | 1x | - |
| - systemic temperature | - | - | - |
| - cardial/pulmonal | - | - | - |
| - bolus pressure | - | 1x | - |
| **technical** | | | |
| - inadequate temp. measurement | - | 1x | - |
| - defect bolus | - | - | 1x |

Extensive scannings of the probes detected influences of anatomical structures as bones, air filled bowels, large vessels and necrotic zones. The reported heat sensations were partially predicted and partially measured. Conclusively also the following corrections are registered. Clinical the physical enlargement of the body with sodium chloride-filled plastic water bags was the most effective help.

In one treatment situation the patient reported of an intensive pain in the cicatrice (lapratomy). Because the scar continued deep into the body, also changes of frequency, power amplitudes and excessive bolus cooling did not success. Once an intratumoural/intraperitoneal removement of a temperature probe was detected, for security reasons the treatment stoped. The same thing we report for technical reasons. The defect bolus could be repaired easily and the following day a successful hyperthermia treatment was done.

The influence of local hyperthermia to the whole body was moderate and could be managed easily. A greater problem was the bolus pressure.

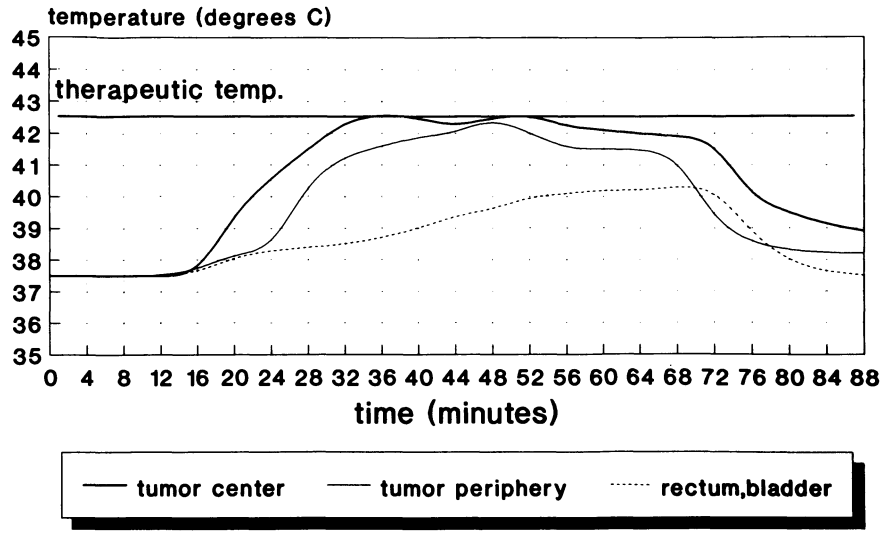



Figure 1a. Temperature profile of the first treatment (case W.R.). Distinct realization of the intended temperatures intratumoural (central and periphery). Note the moderate temperature rising in the surrounding tissue (rectum, bladder).

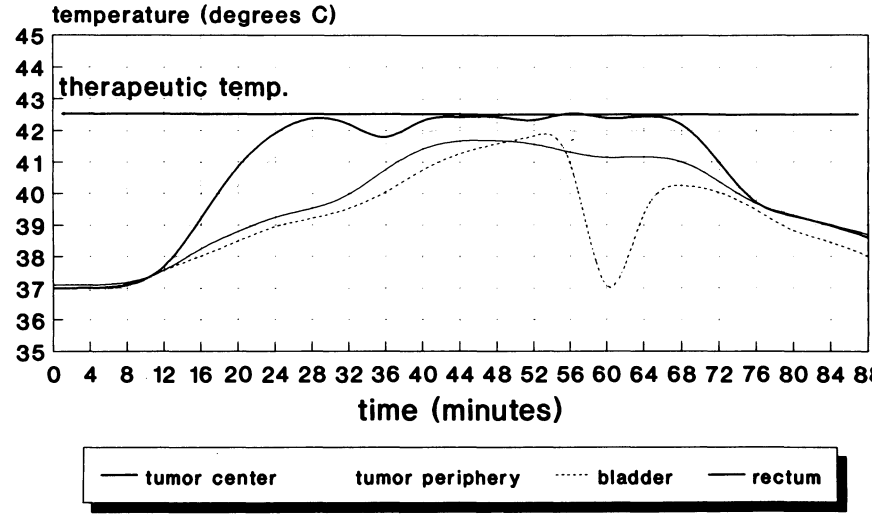



Figure 1b. Temperature profile of the fifth treatment (case W.R.). The radiologically proven tumour shrinking lead to the application of more power with less differences of the monitored temperatures. Here the preventional bladder cooling was performed (dotted line).

Those patients who suffered initially from heavy pains reported a stronger pressure or even more pain. This was one reason for a treatment stop. Regressions in the CT-scans after the combined treatments are even more impressive compared to the initial tumour size. Especially the undifferentiated sarcoma regressed clearly after 2000 cGy. Inhomogeneities and central necrosis are more visible after the treatment.

We think that the observed rapid calicification could be specific for the effectiveness of the combined treatmentmodality (7). The obtained volume reductions asked for the parallel in vitro investigations with living cells of the same tumour. In our institution cell cultivation of irradiated tumours is regularly performed. It has been demonstrated that only the combined treatment could achieve a local tumour control (3). This clearly points out the successful treatment.

**Conclusions**

With the BSD 2000 device improvements of technical use and clinical applications could be seen. The question was if the sophisticated system also produces sufficient heat in any wanted region in the pelvis or abdomen. Our clinical results are encouriging so far. Combined with external radiotherapy this efforts lead to a distinctive enhancement of radiosensibility. Impressive clinical success was the result.

Parallel to physical and clinical investigations the in vitro simulation of the treatment under same conditions revealed the effectiveness of the treatment modality. More clinical studies are therefore needed to consolidate the new local treatment approach.

**References**


1) Bowmann R.R.:
A probe for measuring temperature in radiofrequency treated material
IEEE Trans MTT, 43-45, January 1976

2) Burkard W., Mindek G., Schwegler N., Notter M., Podvinec M., Stoll W.:
Radiobiological experiments for improvement of radiotherapy: flow cytometric investigations on short-term cell cultures of human tumour tissues
Kärcher K.K., Ed.: Progress in Radio-Oncology IV, ICRO, Vienna, 1988

3) Burkard W., Schwegler N., Notter M.:
Radiosensitizing effect of hyperthermia in radioresistant tumours
Comparative studies in vivo and in vitro (in Press)

4) Petrovich Z., Emami B., Astrahan M., Langholz B., Luxton G.:
Regional hyperthermia with BSD 1000 annular phased array in the management of recurrent deep seeted malignant tumours
Strahlentherapie & Oncologie 163, 430-433, 1987

5) Turner P.F.:
Technical aspects of the BSD 2000
Essen University and BSD Medical Corp. Symposium
Essen, Germany, April, 29-30, 1988

6) Turner P.F.:
Operational and clinical aspect of the BSD 2000
Essen University and BSD Medical Corp. Symposium
Essen, Germany, April 29-30, 1988

7) Schwegler N., Notter M., Burkard W.:
Hyperthermie - ein neues Element in der Krebsbehandlung
Schweiz. Rundschau für Medizin PRAXIS Nr. 34, 1989

# RADIOSENSITIZING EFFECT OF HYPERTHERMIA IN RADIORESISTANT TUMOURS

## COMPARATIVE STUDIES IN VIVO AND IN VITRO

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### Introduction

The prospect of a successful introduction of hyperthermia in the field of radiooncology increased during the last few years as a result of the technical realization of selective and controlled heat applications in well defined and also deep seated target volumes. This lead to extensive radiobiological studies on this subject (10). Possible side effects as the development of "thermotolerance", an adaptive reaction of the tissue (2, 4, 6, 8, 9), as well as cytotoxicity of heat treatments were discussed (1,5).

Unfortunately the results of the majority of these studies are hardly representative for the clinical situation. Most of the in vitro studies are done with animal cells. Regarding to the experiments based on human tumour cells it is of importance that the established cell lines often represent a selection of cells with an atypical chromosome frequency distribution compared to the original tumour in vivo. In addition hyperthermia experiments often exceed clinically relevant values regarding the duration (more than 1 hour) or the temperature range (more than 43°C) and for combined treatments radiation doses often pass over the therapeutic limits.

Radiobiological examinations of tumour tissue obtained from actually treated patients is a method initiated by the headmaster (N.S.) and performed routinely in the radiotherapy departement of Aarau. Especially in the field of combination of radiation treatment with local hyperthermia of the tumour tissue, we just started a series of comparative studies of in vivo and in vitro reaction of the same patients tumours, in order to get a better knowledge of synergistic effects and their dependence upon possible adaptive reactions (7).

### Methods

To assure the transferability of in vitro investigations to the clinical situation our experiments were based on short term cultures of human tumour cells derived directly from surgical specimens of tumours in man (3). The cells were cultivated in the radiotherapy departement and then

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treated with the same modalities and the same technical equipment as the patient. Irradiation was performed with a linear accelerator (6-MeV-Photons) using a clinical fractionation of 200 cGy. The total dose was the same as administered in the patient. For technical reasons heat applications for cell cultures were performed by circulating air. Temperature measurements are monitored with Bowman probes and the BSD 2000 device (figure 1).

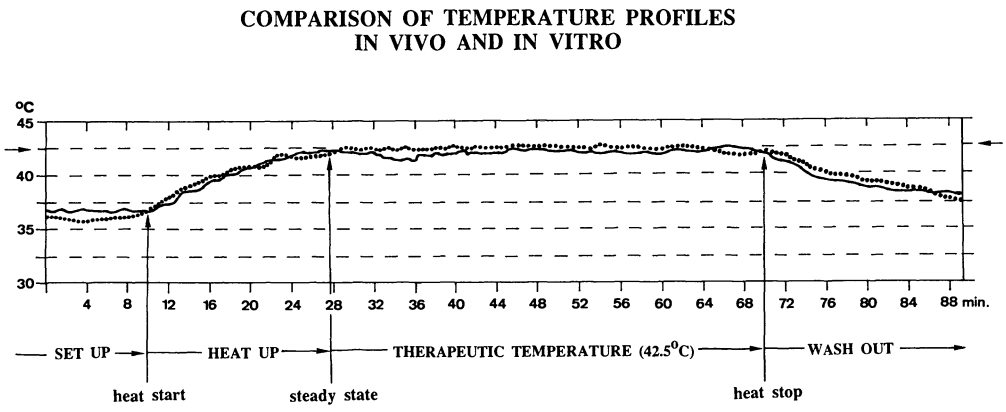



Figure 1

This allowed an exact in vitro simulation of the in vivo temperature course in the tumour. So the treatments of patients and cell cultures of the specimen of their tumours were made under the same clinical conditions. The efficacy of the combined treatment modality could be compared with other treatment forms as radiotherapy or hyperthermia alone.

To assure the results and to get further information concerning cell kinetics during and after the treatment a second series of investigations was initiated using an established human cell line (HuTu80) derived from an adenocarcinoma of the duodenum. The cell line was choosen because of its similar karyology compared to most of the adenocarcinomas we checked for chromosome number: About 70 % of the cells show the normal 2n number of 46 chromosomes and the other cells are slightly aneuploid with chromosome numbers between 43 and 48.

The cells were treated as follows:

- no treatment
- 5x irradiation per week (200 cGy; 6-MeV-Photons)
- 2x hyperthermia per week (42,5°C during 45 minutes)
- 5x hyperthermia per week
- 5x irradiation and 2x hyperthermia per week
- 5x irradiation and 5x hyperthermia per week

In each case series of 3,5 million cells were treated during 5 weeks. To quantify the extent of cellular damage the number of vital cells and their

growth characteristics in clonal assays were determined. These tests were done weekly during this time and after the end of the treatment period.

**Results**

We first investigated a successfull therapy of an inoperable undifferentiated sarcoma of the pelvic region of a 48-year old woman who suffered from progredient invalidity. The combination of radiotherapy by 6-MeV-Photons with hyperthermia applications twice a week lead to a surprising rapid shrinking of the tumour mass. After a radiation dose of only 4000cGy

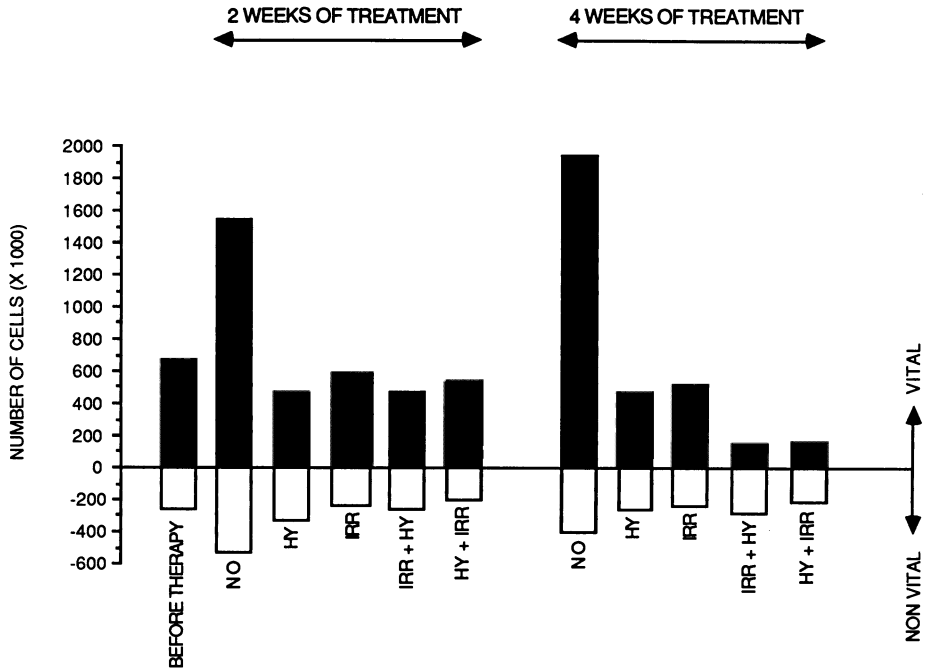


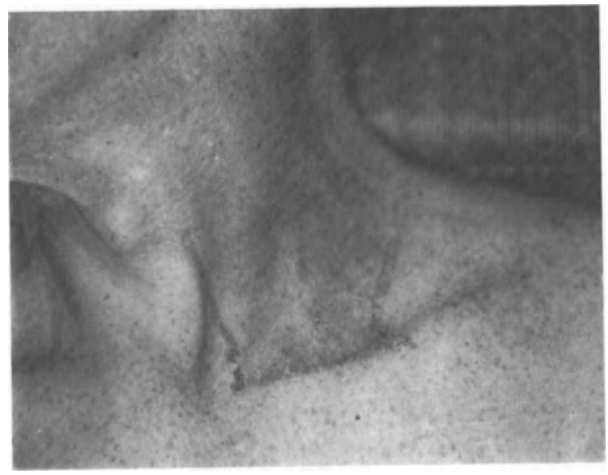

Figure 2

the tumour had disappeared and was no more detectable by computer tomography. The patient got free from pains.

By means of cell cultures derived from tumour tissue which was surgically removed before the beginning of the described therapy it was possible to repeat the very successfull therapy under in vitro conditions and to compare its efficacy with other treatment forms as radiotherapy or hyperthermia alone. Figure 2 shows the changes in cell number of the cultivated sarcoma during the therapy, depending on the treatment mode.

In the beginning of the therapy no significant differences between the combined treatments and the monotherapies can be noted, that means the tumour cells are inactivated to a similar extent. With progression of the therapies definite differences regarding the regression of tumour cells could be seen, showing the gain in effectiveness of the therapy by combining the radiation treatment with fractionated hyperthermia.

The behaviour of the cells surviving the therapy and showing vital properties is of special interest (see figure 3).



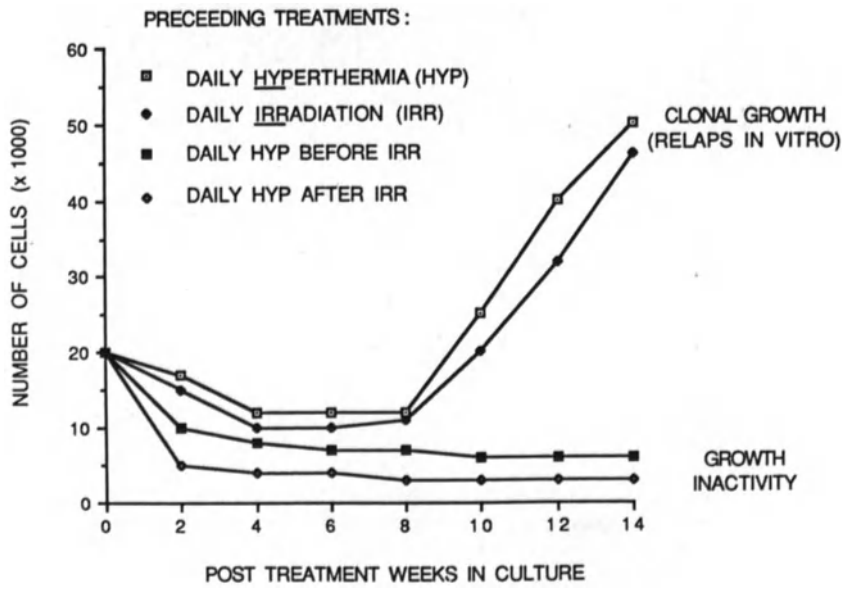



Figure 3

An initial reduction of the cell number occurs following all treatment forms, due to the death of damaged cells. A stabilization of the cell number is reached after 4 weeks, but cultures which received monotherapeutic treatments (radiotherapy or hyperthermia alone) developped some weeks later the ability to regrowth clonally, representing an in vitro manifestation of a relapse situation. In contrast no growth was noticed after the combined treatments.

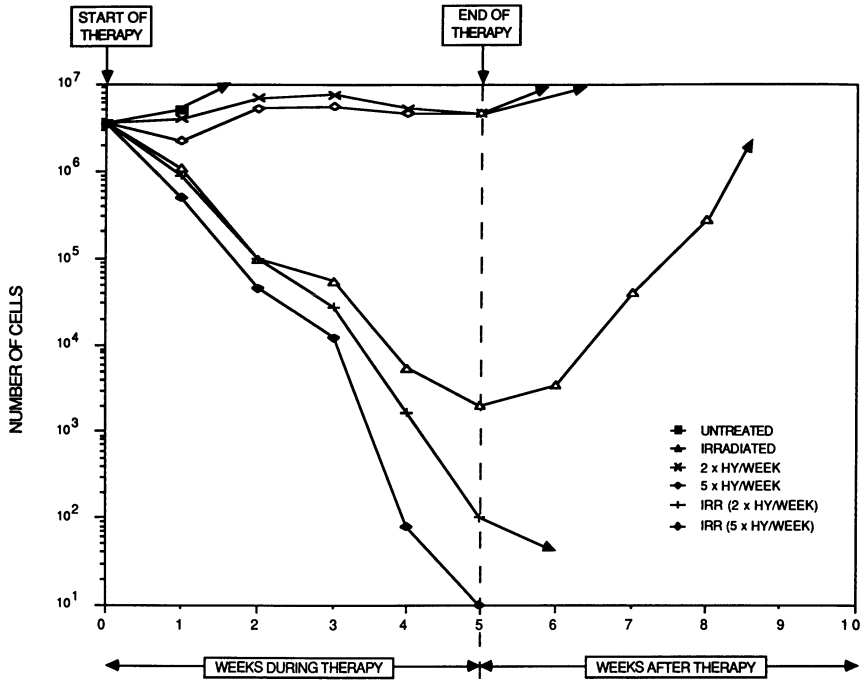



Figure 4 shows the results obtained with the cultivated adenocarcinoma (HuTu80).

Whereas the untreated cells grew exponentially, the cultures treated by hyperthermia alone showed a reduced growth and finally a stabilization of the cell number at the same level of about 4,5 million cells, independent on the number of treatments per week. At the end of the treatment cells continued to grow exponentially, comparable to non treated cells. Both the irradiated and the combined treated cultures showed initially a comparable loss of cells, followed by an accelerated regression in the combined treated cultures. The few cells surviving the combined treatments lost their vitality during the posttherapeutic period, whereas the cells treated with radiation alone showed regrowth after the end of therapy. This observation correlates to experiences of in vivo therapy, where radiation doses of 4000 - 5000 cGy are not destructive for adenocarcinomas.

Our preliminary results of clonal assays indicate that doses at least between 7000 and 8000 cGy are needed to destroy entirely these human tumour cells by radiation alone, whereas after the combination of radiation treatment with hyperthermia the same result is obtained already after 4000 - 5000 cGy, that means approximatively 70 % of the regular dose.

**Discussion**

Comparative studies of in vivo and in vitro treatments of tumour cells may help to enhance the knowledge about the combination of radiotherapy and hyperthermia. Especially in successfull therapies of advanced local tumour extensions rises the question if either irradiation or hyperthermia alone could have lead to the same clinical result. The simultaneous performed treatments with patients and cultivated tumour cells obtained from biopsies of the same patient before the onset of the combined treatment ought to find possible answers. The observed advantage of the combined treatment modalities in our cell investigations will need further studies with different tumour types and more different tests to quantify the possible dose modifying effect for combined therapies. If one considers the used clinical conditions (42-43°C), factors as cytotoxity of hyperthermia per se and "thermotolerance-effects" may influence the result. Therefore the clonogenic properties of cells treated with hyperthermia twice per week or five times per week were tested (figure 5).

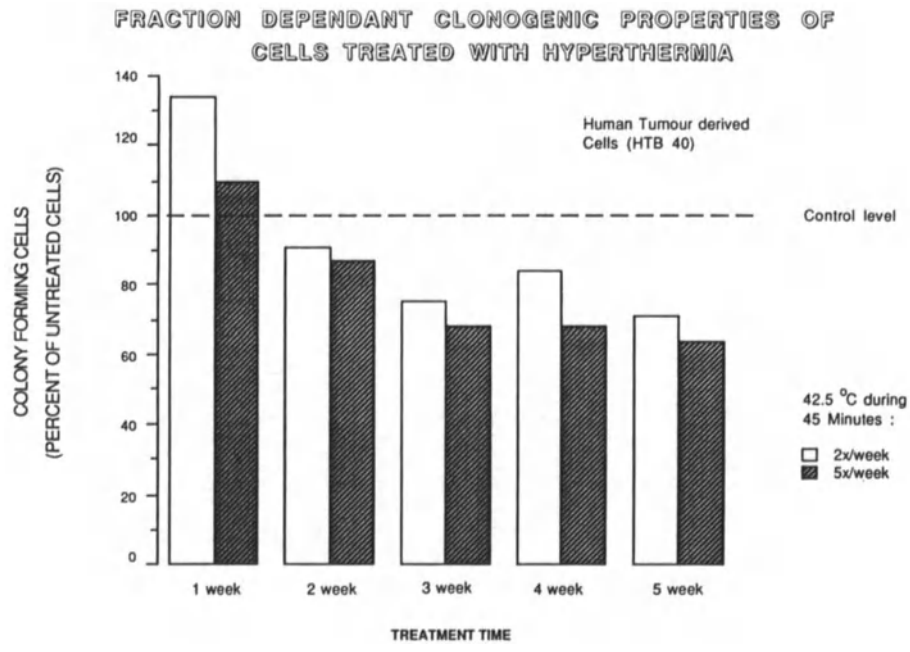



Figure 5

During the first week of treatment the hyperthermia resulted in a slight activation of cell proliferation, registered in an augmentation of the portion of colony forming cells. In a broad interpretation this observation could be understood as thermotolerance. However with the continuance of hyperthermia treatments, for instance from the second week, the content of

clonogenic cells decrease and reaches values below those of untreated cells. The same tendency is seen regarding the growth characteristics of the cells at different moments of fractionated hyperthermia as shown in figure 6.

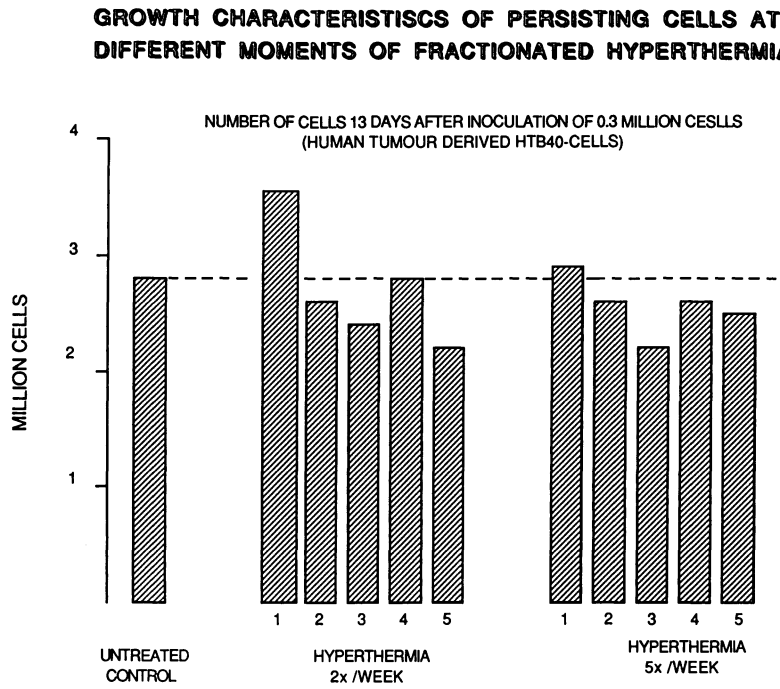



Figure 6

No significant changes are seen between cells treated twice or five times per week, for instance no thermotolerance was indicated by the more frequent applications. The combined treatments with hyperthermia and radiation show a reduced survival of tumour cells already beginning with the onset of treatment.

The result that fractionated hyperthermia combined with radiotherapy did not lead to a certain "thermoresistance", even if heat treatments were given as frequent as five times per week, corresponds well to other observations (11). The question remains if under clinical conditions five hyperthermia sessions are tolerable, especially under the aspect that a similar result may be obtained with two treatments per week.

**Conclusions**

We think that the use of heat as a radiosensitizing agent could be a successful modality especially for the radiation therapy of supposed less radiosensi-

ble tumours. There is hope that further comparative investigations between in vivo and in vitro reactions on the same patient's tumour, including flow cytometric and electron microscopic methods, will help to create a solid basis for an optimal utilization of non toxic heat treatment as radiosensitizing agent and therewith will lead to an improvement of local regional treatment forms.

**References**


1) Breipohl W., van Beuningen D., Ummels M., Streffer C., Schönfelder B.:
Effect of hyperthermia on the intestinal mucosa of mice
Verh Anat Ges 77: 567-569 (1983)

2) Burdon RH.:
Heat shock proteins
In: Overgaard J. (ed) Hyperthermic oncology, vol II;
Taylor and Francis, London, pp 223-230 (1985)

3) Burkard W., Mindek G., Notter M., Podvinec M., Schwegler N., Stoll W.:
Radiobiological experiments for the improvement of radiotherapy: Flow cytometric investigations on short-term cell cultures of human tumour tissues
In: Kärcher KH. (ed) Progress in Radio-Oncology IV, ICRO Austria, Wien (1988)

4) Dewey WC., Esch JL.:
Transient thermal tolerance: cell killing and polymerase activities
Radiat Res 92: 611-614 (1982)

5) Hume SP.:
Experimental studies of normal tissue response to hyperthermia given alone or combined with radiation
In: Overgaard J. (ed) Hyperthermic oncology, vol II;
Taylor and Francis, London, pp 53-70 (1985)

6) Lunec J., Cresswell SR.:
Heat-induced thermotolerance expressed in the energy metabolism of mammalian cells
Radiat Res 93: 588-597 (1983)

7) Schwegler N., Notter M., Burkard W.:
Hyperthermie - Ein neues Element in der Krebsbehandlung
PRAXIS, Schweiz. Med. Wochenschrift Nr. 34 (1989)

8) Sciandra JJ., Subjeck JR.:
Heat shock proteins and protection of proliferation and translation in mammalian cells
Cancer Res 44: 5188-5194 (1984)

9) Sciandra JJ., Gerweck LE.:
Thermotolerance in cells
In: Watmough DJ. Ross W. (eds) Hyperthermia - Clinical and Scientific Aspects; Blackie and Son Ltd., Glasgow, pp 99-120 (1986)

10) Streffer C., van Beuningen D.:
The biological basis for tumour therapy by hyperthermia and radiation
In: Streffer C. (ed) Hyperthermia and therapy of malignant tumours (Recent Results in Cancer Research Vol 104), ISBN 3-540-1720-5;
Springer, Berlin (1987)

11) van Beuningen D., Streffer C., Pelzer T.:
Radiosensitization of exponential and plateau phase cells
Strahlentherapie 161: 552 (1985)

# HYPERTHERMIA AND HYPERGLYCEMIA IN THE TUMORS THERAPY

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Despite considerable progress made in the field of hyperthermia, problems still exist. Resolution of which should improve the possible application of hyperthermia and improve it's results. The following scheme including all the possible variants for solving the problem of optimizing the hyperthermia method:

1. Development of heating devices which should be improved by the two means: to improve the existing devices and development of the new equipment.

2. Enhancement of antitumor and radio- and chemosensibilizing hyperthermia effects: modification of a tumor cells membrane, decreasing of tumor pH and pO2, inhibition of intratumoral blood flow.

This paper will not report the technical problems but will discuss the biological aspects of hyperthermia optimization.

At the present time it would hardly be possible to obtain uniform heating of a tumor. Therefore there is a danger of tumor underheating decreases the efficacy of hyperthermic method. To avoid this danger, enhancement of a tumor tissue thermosensitivity might be quite promising. This could be obtained by using one of the above mentioned biological approaches.

Hyperthermic effects are known to be the highest at low pH level or hypoxia; which creates deficiency of nutrients and energy, slow blood flow and increase in cell numbers in S-phase of the cell cycle.

The most promising method of the thermosensibilization according to some authors (M. von Ardenne, 1972; Zhavrid et al., 1987; Osinsky et al., 1988; Yarmonenko et al., 1988) is induced hyperglycemia. Our experiments confirm this idea.

It was shown before that the tumor pH drops selectively under induced hyperglycemia (IHG). Different authors who

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used the various methods for induction of hyperglycemia, the tumor pH was decreased in the following ways: 5.50-6.46 in the rats, 6.15-6.22 in the mice and 6.29-6.55 in the patients (Wike-Hooley et al., 1984).

Taking into account the above data, application of the IHG, for the enhancement of the hyperthermic efficacy, is quite promising. Some authors, however, do not support application of IHG. They state that during IHG the tumor glucose contents and energy level will increase (Haveman, Hahn, 1981; Dickson, 1982; Shah et al., 1983). In the experiments in vitro, the tumor cells turned out to be thermoresistant. Opponents of the IHG studied the problem in vitro only. Several experiments were carried out with the intraperitoneal glucose injections into animals for inducing the hyperglycemia. This schedule however, is not only clinical ones but even could not produce conditions in a tumor similar to those under intravenous method of IHG.

We have developed the original method for hyperglycemia induction: intravenous infusion of the 20% glucose solution at a rate of 60-80mg per min. for 1.5-2h. Such schedules of IHG were determined to be the most optimal for a thermo- and chemosensibilization of the tumors.

Using our method of the IHG we obtained a considerable selective pH decrease in the different experimental tumors:

| Tumors (rat) | Initial pH | Final pH |
|---|---|---|
| Guerin carcinoma | 6.73 ± 0.05 | 5.51 ± 0.08 |
| Pliss lymphosarcoma | 6.68 ± 0.08 | 5.66 ± 0.27 |
| Yoshida sarcoma | 7.07 ± 0.15 | 6.24 ± 0.12 |
| DS-carcinosarcoma | 7.02 ± 0.14 | 6.08 ± 0.22 |
| 7, 12 DMBA mammary carcinoma | 6.70 ± 0.08 | 6.10 ± 0.09 |
| Glyoblastoma (strain 101.3) | 6.73 ± 0.20 | 5.92 ± 0.24 |
| (Mouse) Crocker sarcoma | 6.79 ± 0.09 | 6.38 ± 0.13 |

Under such conditions the other indices, that are important for modification of the thermosensitivity, were also altered. It was observed that the pO2 value decreased from 5 to 0.5 kPa, the tumor cell numbers in the S-phase increased by a factor of 2, blood flow rate decreased by a factor of 2 to 3 (sometimes to zero ml/100g per min.). Tumor energy charge and glucose content remained relatively unchanged: 3.2 10 3.8 10 M and 0.1---3mM, respectively. These values are not comparable with those presented in the experiments mentioned above (Haveman, Hahn, 1981; Dickson, 1982; Shah et al., 1983).

It was noted that antitumor effects of microwave hyperthermia was increased significantly under the two year survival was not observed.

Some dogs with soft tissue sarcomas were treated with microwave local hyperthermia (2450MHz, 60 min.) and intratumoral injection of methotrexate (0.5mg/kg) at 45-50 min. of heating. This combined treatment was used once per week during a 1-3 week period. After 10 days the dogs were operated on. Temperature distribution was as follows: skin-50.9 degrees C, tumor center-43.5 degrees C, under tumor-40.9 degrees C. Complete regression in 17% cases and partial regression in 83% were obtained. Mean time to progression was 610 (60-1117) days. Methotrexate dose was decreased by 1.5-2 fold.

Combined treatment methods, using a local hyperthermia, in 106 patients with the head and neck tumors were approved. Tumors of the following sites were diagnosticated: mucosa oral cavity-35 patients, tongue-34, upper jaw-13, salivary gland-10, lower lip-6, hairy part of head-8.

According to the TNM classification, the tumors were in the following stages: $T_2N_{0-1}M_0$-21, $T_3N_0M_0$-24, $T_3N_{1-2}M_0$-36, $T_4N_{1-2}M_0$-25.

Histologically, in 93 patients squamous cell carcinoma was determined, in 12 patients-adenocarcinoma and in 1 patient-an undifferentiated cancer was observed.

Radiation was given in 5 daily fractions per week. The fraction size was 2.5Gy. Total dose was 30Gy. Chemotherapy and HT were given after the radiation fraction of twice per week. (On the 3rd and the 5th days). HT was to be started within 20-25 minutes after the radiation and 10-15 min. after the drug injection. Patients were treated with microwave units operating at 915 Mhz at 42-44 degrees c, for 45-60 min. Methotrexate at a dose of 15-25 mg was injected into the tumor or into a corresponding external carotid arteries. The total dose of drug was 60-100 mg.

In the next week the treatment was repeated. Within 3-4 weeks all the patients with stage II of the disease and patients with stages III-IV had considerable tumor regression those with more than 50% volume were operated on. In the remaining cases, within 3 weeks the second course of thermoradiochemotherapy, was administered similar to the first one, followed by surgery.

Temperature was measured by means of thermocouples which were inserted directly into the tumor and under it. The latter were incorporated into the 0.5 mm syringe needless.

Data of the thermoradiochemotherapy results are demonstrated in Table 1.

The best immediate direct results were obtained during the treatment of patients with a spread tumor process, alteration of tumor cells microenvironment.

Guerin carcinoma and Pliss lymphosarcoma were treated by means of " Lunch-2" machine (2450 MHz). The heating were given immediately after the IHG and maintained for 1 hour.

The tumor temperature was 41 0.5 or 43 0.5 °C. The thermometry was performed by means of perturbing copper-constantan thermocouples that were inserted into the tumor perpendicular to the electrical field. Rats bearing subcutaneous tumors in size 1.5 - 2.0 cm were used for therapeutic experiments.

It was obtained that time doubling ( TD ) of Guerin carcinoma was: control- 2.5 +/- 0.3 days;41 C-60 min- 2.6

Table 1. Treatment result of the patient with the head and neck tumors

| Indices | Stages of Disease (patient numbers are shown in brackets) | | |
|---|---|---|---|
| | $T_2 N_0 M_0$ | $T_3 N_{0-1} M_0$ | $T_4 N_{1-2} M_0$ |
| tumor reaction (%) | 21 | 60 | 25 |
| complete regression | 85.7(50.5)* | 28.3 (0)* | 8.0(0)* |
| partial regression | 14.3 | 45.0 | 48.0 |
| no change | --- | 26.7 | 44.0 |
| progression | --- | --- | --- |
| time to progression (months) | 10-21 | 8-10 | 6-7 |

*-complete regression rate in patients treated with the radiation alone.

+/- 0.3; 43 C - 60 min - 4.0 +/- 0.6;IHG + hyperthermia (HT) 41 C- 5.6 +/- 0.7;IHG + HT (43 °C) - 7.6 +/- 0.2 (p 0.05). TD of Pliss lymphosarcoma was: control - 1.9 +/- 0.4 days; 43 C - 60 min - 4.3 +/-;IHG +HT ( 43° C) - 5.75 +/- 0.1)p 0.05).

Efficacy of thermochemotherapy was also enhanced under IHG. The rats with Guerin carcinoma were treated with IHG (our schedule) immediate followed by HT (43° C, 60 min). Thiophosphamide was given intraperitonealy at a dose of 2 mg/kg at the 60th min of glucose infusion. The dose of drug was 3 mg/kg in rats that were not treated with combined method. Treatments were repeated for 2 times with 2 days interval. TD of tumor increased 2- fold when thermotherapy with drug was used after the induced hyperglycemia control - 2.5 +/- 0.3 days; HT - 4.3 +/- 0.6; IHG- 2.9 +/- 0.5; drug

- 4.4 +/- 0.4; drug +IHG-7.8 +/- 0.6; drug+ht- 5.8 +/- 0.5; IHG+drug+HT-12.3 +/- 0.9 ($P_{7-6}$ and $P_{7-5}$ 0.05).

Complete regression in tumor volume was obtained after the combined treatment in 75% cases, after application of thiophosphamide alone in 35%, after combination of drug+HT- in 50%, after combination of IHG + drug - 60%.

Treatment efficacy of the dogs with the spontaneous mammary gland tumors in the II-IV stages of a disease combined therapy with IHG was studied (with collaboration A. Hassan, Ph.D.). Chemotherapy alone or in combination with a IHG was applied once in 10 days with 2-3 times repetition. A 20% glucose solution was intravenously infused at a dose of 60-80 mg/kg per min and a methotrexate was slowly injected at dose of 0.5mg/kg for 60-70 min beginning from a glucose infusion. In 10 days, when the drug therapy was completed, the majority of the animals were operated on,the largest tumors were removed.

The dog survival time was as follows: after a surgical treatment alone- 258.8 days (60-500): after drug treatment- 410.4 (90-794); and after combined treatment - 602.8 (260-1038). More than 2 years following the drug treatment, 13% of the dogs were alive and after combined therapy 35% animals continued to live. After a surgical treatment, corresponding to $T_2$ . In patients with a spread tumor process, corresponding to $T_3$ , a complete regression of neoplasia was obtained in 28.3%, in patients with $T_4$ - in 8%. Partial tumor regression or stabilization of a process was observed in 27% patients. In patients with stage IV partial decrease of tumor size was indicated in 80% cases. It is important to note that the our results were obtained under lowering of total dose of radiation by a factor of 1.25. Serious complications during the combined treatment were not observed. Only the patients with the oral cavity mucosa tumors showed some more expressed radiation reactions similar to epitheliiti.

The above mentioned results and literature data gives us the possibility to make the following concluding remarks:

-HT is a significant modality in combined treatment of some tumors

-HT could be applied in the clinics in combination with radio- or chemotherapy only

-IHG enhances the HT effect considerably

-IHG is of independent significance as a chemo-radiosensibilize

-IHG/chemotherapy relationship depends on the antitumor drugs affinity to specific tumors
-Development of tests for tumor sensitivity to HT and IHG

-Optimization of thermoradiochemotherapy methods occurs in clinical settings

REFERENCES

1) Ardenne M. von. Adv. Pharmacol. Chemother., vol. 10, p.335-380, 1972.

2) Zavrid E., Osinsky S., Fradkin S., Hyperthermia and hyperglycemia in oncology, Kiev: ND, 1987.

3) Osinsky S., Gusev A., Bubnovskaja L., Abstr. of VIIth Int. Symp. Hyperther. Oncol., Kyoto, 1988.

4) Yarmonenko S. et al., ibid.

5) Wike-Hooley J.L., Haveman J., Reinhold H.S., Radiother. Oncology, vol. 2, P. 343-366, 1984.

6) Haveman J., Hahn G.M., J. Cell Physiol., vol.107.P. 237-242, 1981.

7) Dickson J.A., Nat. Cancer Inst. Monogr., vol 61. P. 544-547, 1982.

8) Shah S. A., Jain R.K., Finney P.L., Cancer Lett., vol.19, P.317-323, 1983.

# ENHANCING THERMORADIOTHERAPY EFFICACY BY HYPERGLYCEMIA

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The tumorcidal effect of hyperthermia (HT) and especially of combined use of HT and radiation-thermoradiotherapy (TRT) may be substantially increased by induced hyperglycemia (HG). In this respect we have shown: (1) that the proper scheduling of TRT with HG is very important and (2) that the therpeutic effect is highly dependent on HG-induced changes in certain physiological processes, such as inhibition of blood flow, increasing hypoxia and anaerobic glycolysis and decreasing pH in tumors. The first results of clinical use of HG have been also received. A search for the most efficient scheme of TRT with HG (Fig.1) was based on the following principles: (1) J.Overgaard (1982) and some others demonstrated the advantages of tumor heating several hours after irradiation; (2) according to our studies (S.P.Yarmonenko et al., 1981), the induction of HG, especially after irradiation, selectively increases the antitumor effect of irradiation; (3) M. von Ardenne, P.G. Reitnauer (1980) and many others demonstrated that the tumoricidal effect of HT was increased under hyperglycemic conditions due to a decreased pH and blood flow. Taking into consideration all these data we proposed that the following sequence of treatment should be most effective: irradiation followed by HG and then by HT (the last line on Fig. 1).

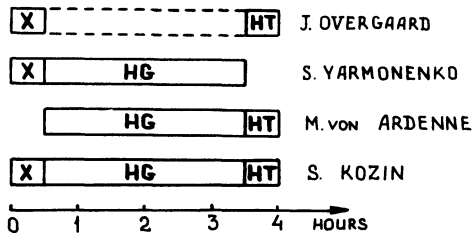


Figure 1. Logical scheme of the combined use of X-irradiation (X), HG and HT.

This hypothesis has been confirmed in experiments on mice bearing solid Ehrlich carcinoma (Fig.2) (S.V.Kozin et

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40GY. HG was induced by 5 i.p. injections of glucose (total dose of 10.4 g/kg) during 2 hours. Tumors were heated in a water bath (at 43 °C) for 30 minutes. The induction of HG in all experiments was initiated 3 hours before HT. Tumors were irradiatied at one of the following time points:

(1) 4 hours before HG, (2) immediately prior to HG, (3) between HG and HT, (4) 0.5 hour after HT, or (5) 2.5 hours after HT. For comparison, seperate groups of mice were treated with only one or with any two of these three modalities (HG,HT or irradiation). Treatment efficacy was assessed by the following end-points: tumor growths delay and the cure rate for mice during 120 days of post-treatment

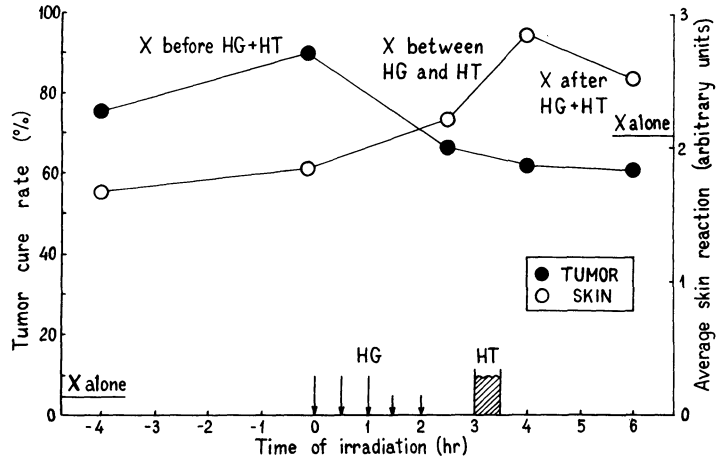


Figure 2. Ehrlich tumor and skin response to X-irradiation (x = 35 Gy) given at various times relative to HG and HT.

observations of the skin adjacent to the tumor were estimated 15-25 days after irradiation using a graded scale. As shown in Fig. 2, all schemes of polyradiomodification were highly effective and the highest cure rate (90%) was achieved when irradiation alone cured only 4% of animals, and in combination with only HG or only HT - no more than 20% (data not presented here). Similar results concerning the relative effectiveness of the studied regimens were obtained with lower doses of irradiation using tumor growth delay end-point.

Fig. 2. also demonstrates that irradiation-induced skin reactions were not enhanced by HG plus HT when only these motalities were applied after irradiation.

So we can make a conclusion that the best results are provided with the schedule of polyradiomodification when irradiation precedes HG and HT.

In other experiments we have estimated the therapeutic gain of this the most effective scheme (Table 1).

Table 1. The efficacy of TRT with HG

| Experimental conditions | Number of mice | Cured % | Skin reaction |
|---|---|---|---|
| 40 Gy | 20 | 45 | 3,58 |
| 30 Gy | 30 | 0 | 2,07 |
| 30 Gy + HG | 30 | 7 | 1,97 |
| 30 Gy + HT | 26 | 23 | 2,01 |
| 30 Gy + HG + HT | 38 | 82 | 2,08 |
| 20 Gy + HG + HT | 20 | 60 | - |
| 10 Gy + HG + HT | 39 | 33 | - |

It is evident that for irradiation combined with subsequent HG plus HT the therapeutic gain factor was approximately 3 compared to irradiation alone, and over 2 compared to TRT. What are the mechanisms by which HG increases the effectiveness of TRT? We think that the most significant factors are selective inhibition of blood flow and decrease of pH in tumors. As shown in figure 3, both the blood flow and pH for examined Ehrlich tumors were already substantially diminished under the influence of HG before the beginning of HT. When HT followed HG, the tumor microcirculation was retarded synergistically more than 10-fold and wasn't completely restored for at least 1-2 days.

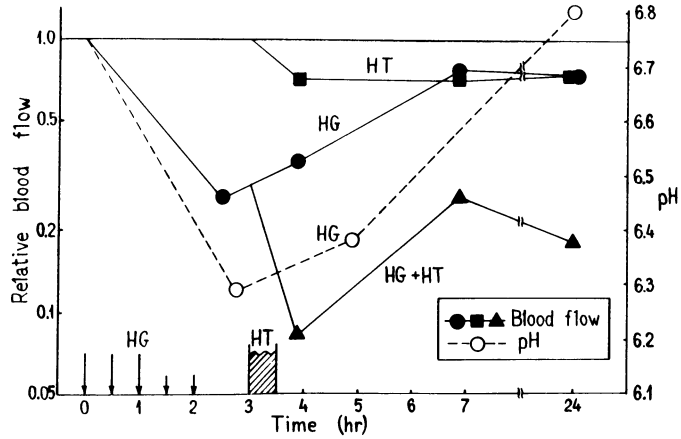


Figure 3. Decrease of blood flow and pH in Ehrlich tumors after treatment with HG and/or HT

Such changes in tumor blood flow played an important role in relative effectiveness of the polyradiomodification regimens we performed. The pronounced decrease of microcirculation under the influence of HG and HT in all cases took part in the enhancement of tumoricidal effect. But when irradiation was employed after HG and HT, the decrease of oxygenation due to retardation of the blood flow also produced a negative radioprotective effect. Therefore, the highest efficacy of post-irradiation use of HG plus HT becomes quite obvious.

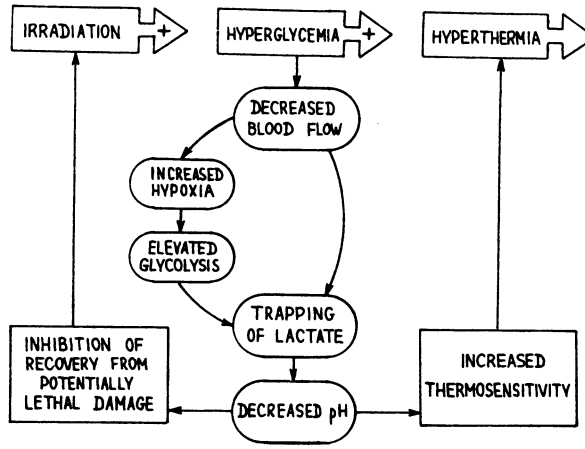


Figure 4. Processes occurring in irradiated tumors under the influence of HG before and during HT.

Fig.4 shows for this regimen the general idea of HG-induced physiological processes occurring in irradiated tumors before and during HT which enhance the efficacy of TRT. First HG inhibits the already inadequate tumor blood flow. Consequently, tumor hypoxia increases, and the lactate, formed in the process of anaerobic glycolysis, gets trapped. As a result,the intratumor pH level decreases. all these phenomena not only increse tumor thermosensitivity but probably also inhibit reparation of potentially lethal radiation lesions. Besides, the therapeutic effect of the combined HG and HT after irradiation is highly dependent on the prolonged changes in tumor microenvironment after the end of treatment. Significant long -term retardation of tumor blood flow is a key factor here.

This concept suggests that any methods for additional increasing of inhibiation of tumor blood flow may enhance the efficacy of TRT combined with HG. At present this problem is under study. The importance of post-irradiation inhibition of tumor blood flow is illustrated in table 2. In these experiments a tourniquet was applied for one hour to block the blood circulation. When clamping was used immediately after irradiation or after irradiation with subsequent HG, the tumoricidal effect was enhanced significantely. Applying the tourniquet 2 hours after irradiation proved to be ineffective. This fact justifies the assumption that the post-irradiation suppression of the blood circulation inhibits the reparation of potenially lethal damage. Similar results were obtained when we used a vasoconstrictive agent mexamine (5-methoxytrytamine), instead of clamping. At present we have preliminary results concerning the combined use of HG and vasoactive drugs (mexamine, hydralazine) in TRT. As shown in Table 3, the

Table 2. Effect of clamping (C) on the cure rate of Ehrlich tumors (20 - 30 mice in each group).

| Experimental conditions | Cured % |
|---|---|
| 25 Gy | 3 |
| 25 Gy+HG | 18 |
| 25 Gy+C (in 5 min) | 31 |
| 25 Gy+C (in 120 min) | 9 |
| 25 Gy+HG+C | 81 |

administration of mexamine (40mg/kg) i.p. between HG and HT leads to a two-fold increase in the cure rate of tumor bearing mice. Permanent tumor regression was observed in 67% of the animals after only 10Gy irradiation combined with three modifiers, while with irradiation alone, only 45% of mice were cured after receiving 40Gy, a dose which produced severe skin reactions (see Table 1).

We suppose that the evaluation of the degree of tumor blood flow decrease may be used for predicting the efficacy of HG in TRT for individual patients.

Fig 5 describes the ralationship between the treatment efficacy for individual mice and the degree to which the tumor blood flow rate was evaluated by determining the rate of clearance of Xe injected intratumorally. The data rather varied for individual animals but there was an evident trend showing that tumor growth delay increased as the blood flow was progressively, inhibited. Substantial improvement of the antitumor effect of irradiation was observed when the blood circulation was diminished 5-10

times or more. It is clear that in addition to the blod flow suppression a HG-induced drop in the intratumoral pH level is also important for enhancing TRT efficacy.

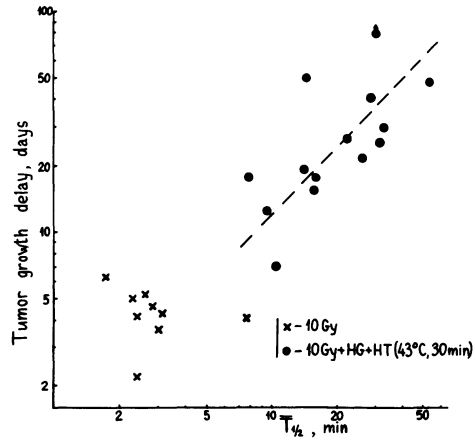


Figure 5. The correlation between tumor growth delay and inhibition of blood flow (half-time of Xe clearance).

Table 4. Glycolytic activity of Ehrlich and human tumor cells under hypoxic conditions.

| Tumor cells | Number of tumors | G, $\frac{nM\ lactate}{10^6\ cells \cdot sec}$ |
|---|---|---|
| Ehrlich (model) | 25 | 10.9 ± 1.3 (9.8÷14.0) |
| Human lung | 31 | 9.1 ± 4.9 (5.6÷23.4) |
| Human esophagus | 8 | 9.1 ± 5.1 (3.9÷17.5) |
| Human stomach | 14 | 5.0 ± 3.2 (1.7÷8.8) |

This problem is being investigated now. One of the factors which determine the pH decrease, may be glycolytic activity of tumor cells. In order to evaluate the potential of HG for clinical use, we have started investigating the glycolytic activity of various human tumor cells under anoxic conditions (Table 4). Tumor cells suspension were prepared from biopsy specimens by enzymatic digestion resulting in yields of viable cells, as determined by trypan blue dye exclusion, of between 10 and 3.10 cells per gramm wet weight of tissue. Cells were incubated under anoxic conditions at 37 C in a balanced salt solution containing 0.4% glucose, and glycolytic activity was estimated by a decresase in pH and lactate accumulatio normalized to time and cell concentration.

Table 5. Efficacy of HG in the preoperative radiotherapy (R) at lung squamous cell carcinoma (III stage) (MINSK)

| Treatment | Number of patients | Survival, % | | |
|---|---|---|---|---|
| | | 1 year | 2 years | 3 years |
| R | 17 | 66.7 | 19.0 | 19.0 |
| HG+R | 16 | 71.4 | 35.7 | 35.7 |
| R+HG | 18 | 68.7 | 60.2 | 38.3 |

Table 6. Response of recurrent rectum cancer to radiotherapy (R) with HG and/or HT (MOSCOW).

| Treatment | Number of patients | Response | | | |
|---|---|---|---|---|---|
| | | CR | PR | MR | NR |
| R | 12 | - | - | - | 12 |
| R+HG | 20 | - | 10 | 5 | 5 |
| R+HT | 40 | 4 | 21 | 12 | 3 |
| R+HG+HT | 20 | 3 | 9 | 6 | 2 |

Table 7. Efficacy of TRT with HG at esophagus cancer ( TASHKENT).

| Treatment | 3-years survival, % |
|---|---|
| TRT | 11.0 |
| HG+TRT | 21.0 |

To date, results have been collected for human lung, esophageal and stomach tumors. Cells derived from lung squamous cell carcinoma, lung adenocarcinomas and esophageal carcinomas exhibited rather high glycotic activity, similar to Ehrlich carcinoma used as a model, whereas this activity for gastric carcinoma cells was 2 times lower.

In conclusion we report the first of the HG use in some radiooncologycal centers in the USSR, which coincide, to a certain extent, with our experimental findings. Table 5. shows that for advanced lung squamous cell carcinoma induction of HG, especially after irradiation, improved the efficially of preoperative radiotherapy. Table 6. demonstrates a similar effect for recurrent rectal cancer.

But for these tumors no difference between TRT and TRT plus HG was observed. However, as shown in Table 7, HG was rather effective at TRT against esophageal carcinoma.

REFERENCES

1) Ardenne von M., Reitnauer p.G., Jpn. J. Clin. Oncol. 10:31-48.1980

2) Kozin S.V., Sevastyanov A.I., Med. Radiol. 32:N1, 60-62, 1987.

3) Kozin S.V., Voloshina E.A., Vinskaya N.P., Yarmonenko S.P., Med. Radiol. 34:N2, 67-72, 1989.

4) Overgaard J., Nat. Cancer Inst. Momgr., N 61, 325-332, 1982.

5) Yarmonenko S.P., Shapot V.S., Voloshina E.A., Gorzhanskaya E.G., Dyuskaliev Zh.D., Krimker V.M., Med. Radiol. 26: N2, 46-50, 1981.

# INFLUENCE OF HYPERTHERMIA ON EXPERIMENTAL VIRAL INFECTIONS IN VITRO

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## Summary

The effects of hyperthermia (39 and 41 C) on virus multiplication in vitro (HSV-1, VSV on Vero cells and EMC on L929 cells) have been explored. The cells were infected with HSV-1, VSV or EMC viruses and heated to 39 or 41 C before, during or after the viral infection. The titer of the virus show that temperature of 41 C acts as an inhibitory agent on HSV-1 and VSV virus replication in Vero cells.

Key words: hyperthermia, viral infection, HSV-1, VSV, EMC virus

## Introduction

The use of hyperthermia in treatment has a long tradition mentioned in medical reports of ancient greek physicians (Streffer,1987). The more recent use of hyperthermia started in the late of 60's. Temperature higher than 42 C acts as a cytotoxic agent on mammalian cells since cells die after heating in a time-temperature and cell cycle depèndent manner (Kase and Hahn,1976).

According to present knowledge, this cell killing effect of hyperthermia is based on multiple mechanisms leading to heat induced lesions. One of the main cellular targets for hyperthermia is the cell membrane (Lepock, 1982). Changes are induced also in the intracellular membrane structures and in the cytoskeleton. In addition, hyperthermia affects DNA as well as RNA replication and protein synthesis (Schlesinger, 1986). Recently, hyperthermia is a matter of concern in cancer treatment, though many problems of the therapy remain unsolved (Naruse at el, 1986). Influence of hyperthermia on viral infections has to be investigated.

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Therefore, it seemed interesting to extend our studies to the influence of hyperthermia on viral infections. For the present experiments DNA and RNA viruses in experimental infections in vitro were chosen.

## Materials and Methods

Viruses: Herpes simplex virus type 1 (HSV-1) and vesicular stomatitis virus (VSV) were maintained in Vero cells. Stock virus titer was 10 TCID50 for HSV-1 and 10 TCID50 for VSV virus. EMC virus was maintained in L929 cells and its titer was 10 TCID50. The infectivity of the viruses were measured by 10-fold dilution method.

Cell cultures: The continous cell lines Vero and L929(mice fibroblasts) were passaged in Parker or Eagle's medium (respectively) with 5% fetal calf serum.

Experimental design: Cell cultures in tubes, forming monolayer were infected. Before or after infections cells were heated at 39 or 41 C by immersion of the culture tubs into a temperature controlled water bath, then transferred to temperature 37 C. For the infections, stock of the viruses at the dose of 0.01 TCID50 per tube was used. The virus was allowed to adsorb onto the cells for 1 hour at 37 C, then the cell cultures were washed with PBS and exposed to 39 or 41 C in the water baths in various experimental schemes as follows: A-cells were heated for 3 hours before infection, B-cells were heated for 3 hours after infection, C-cells were heated for 3h before and 3h after infection. D-cells were heated from 4th to 6th hour after infection (3 hours), B-cells were heated for 24 hours after infection. The titer of the virus was measured every 24 hours during our 72 hour of observation period. Experiments were triplicated and average of the data are presented.

Statistic method: For statistical analysis of the results the test described by Lorenz (Lorenz, 1960) was used and p values less than 0.05 were considered significant.

## Results and Discussion

In Table 1, the titer of HSV-1 virus during the 72 hour post infection period are presented. The results clearly show that, there was no inhibition of HSV-1 virus multiplication in temperature 39 C, even when the infected Vero cells were heated for 24 hours after infection. Yet, incubation for 24 hours in 41 C (Table 1, scheme E) completely inhibited the virus multiplication during 24 hours post infection. The differences between the virus titer in control and tested groups were also significant 48 and 72 hours post infection (Table 1). It should be noted that studies on cell viability based on incorporation of trypan blue which had been made directly after 24-h heating and after further 24-h cultivation of noninfected cells, both Vero and L929 at 37 C, showed no significant increase in the number of dead cells as compared to control cells which were cultivated at 37 C all the experimental period.

Table 1. Multiplication of HSV-1 in VERO cell cultures exposed to temperature of 39 or 41 C

| Temp.: | 39 C | | | 41 C | | |
|---|---|---|---|---|---|---|
| Time : | 24h | 48h | 72h | 24h | 48h | 72h |
| Schedule | HSV-1 Titer (log TCID50/ml) | | | | | |
| A | 3.39 | 5.39 | 4.0 | 3.0 | 5.69 | 3.69 |
| B | 3.69 | 6.0 | 4.69 | 2.69 | 5.39 | 4.0 |
| C | 3.0 | 6.0 | 4.0 | 2.69 | 5.0 | 3.69 |
| D | 3.87 | 6.39 | 4.69 | 3.0 | 6.0 | 4.69 |
| E | 2.69 | 5.0 | 3.0 | N.D.* | 2.87* | 2.0* |
| CONTROL temp.37 C | 3.69 | 6.69 | 4.0 | 3.69 | 6.69 | 4.0 |

Experimental schedule: A-cells were heated for 3h before infection; B-3h after infection; C-3h before and 3h after infection; D-cells were heated from 4th to 6th hour/for 3h/ after infection at 39 or 41 C; E-cells were heated for 24h after infection.

(N.D.)- Not Detected (titer 1)
(*)-significant, p 0.01

Table 2. Multiplication of VSV in VERO cell culures exposed to temperature of 39 or 41 C

| Temp.: | 39 C | | | 41 C | | |
|---|---|---|---|---|---|---|
| Time : | 24h | 48h | 72h | 24h | 48h | 72h |
| Schedule | VSV Titer (log TCID50/ml) | | | | | |
| A | 5.69 | 6.0 | 4.69 | 5.69 | 5.39 | 5.0 |
| B | 5.39 | 5.0 | 4.87 | 5.69 | 5.0 | 4.69 |
| C | 5.69 | 5.0 | 5.0 | 4.87+ | 5.0 | 5.69 |
| D | 5.39 | 5.0 | 4.87 | 5.69 | 5.69 | 5.0 |
| E | 5.0 | 5.39 | 5.0 | 3.0* | 3.69* | 4.87 |
| CONTROL tem.37 C | 6.39 | 6.0 | 5.69 | 6.39 | 6.0 | 5.69 |

Experimental schedule: A- cells were heated for 3h before

infection; B- 3h after infection; C- 3h before and 3h after infection; D- cells were heated from 4th to 6th hour(for 3h) after infection at 39 or 41 C; E- cells were heated for 24h after infection.

-Significant: +/p 0.25, */p 0.01

Table 3. Multiplication of EMC virus in L929 cell cultures exposed to temperature of 39 or 41 C

| Temp.: | 39 C | | | 41 C | | |
|---|---|---|---|---|---|---|
| Time : | 24h | 48h | 72h | 24h | 48h | 72h |
| Schedule | EMC virus Titer(log TCID50/ml) | | | | | |
| A | 2.69 | 5.69 | 4.87 | 2.87 | 5.69 | 4.69 |
| B | 3.0 | 6.0 | 4.69 | 2.69 | 6.0 | 4.69 |
| C | 3.69 | 6.0 | 4.0 | 3.0 | 5.69 | 4.87 |
| D | 3.39 | 5.87 | 4.39 | 3.0 | 5.69 | 4.69 |
| E | NOT TESTED | | | | | |
| CONTROL temp.37 C | 3.0 | 6.39 | 5.0 | 3.0 | 6.39 | 5.0 |

Experimental schedules: A-cells were heated for 3h before infection; B- 3h after infection; C- 3h before and 3h after infection; D- cells were heated from 4th to 6th hour (for3h) after at 39 or 41 C, E- not tested

The inhibitory effect of 41 C on VSV virus multiplication in Vero cells was also observed (Table 2). In these experiments heating at 41 C, for 3 hours before and 3 hours after infection resulted in significant decrease of virus titer 24 h after infection(4.87 to 6.39 log TCID50 tested to control group, respectively). But after further incubation (48 and 72h) significant differences were not detected (Table 2, scheme-C). The similar effects were observed when the VSV-virus infected cells were heated for 24 h at 41 C (Table 2, scheme-E). In this case the long (till 48 h after infection) inhibitory effect on viral multiplication was observed.

In EMC virus L929 infected cells there were no significant differences between the titer of the virus in the control kept at 37 C or tested groups kept at either 39 or 41 C in all experimental schedules (Table 3).

The data from ours experiments indicate that temperature of 41 C applied 24-hour after infection may inhibit multiplication of DNA(HSV-1) and RNA(VSV) viruses in infections in vitro. Hyperthermia applied for shorter period (3 hours) before or after infection showed little, if any, effect. Our results seem to indicate that

hyperthermia directly interferes with the virus replication cycle in vitro and that it is a virus, temperature and time-temperature dependent process.

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References

Kase, K.R., Hahn, G.M.(1976): Comparison of some response to Hyperthermia by normal human diploid and neoplastic cells from the same origin. Europ. J. Cancer, 12, 481-491.

Lepock, J. R.(1982): Involvement of membranes in cellular responses to hyperthermia. Rad. Res. 92, 841-853.

Lorenz, R. J.(1960): Utersuchungen uber die Dosis-Wirkungs-Beziehung und die Genauigkeit bei Virustitrienungen. Arch. Ges. Virusforsch. 10, 560-570.

Naruse, S., Higuchi, T., Horikawa, Y., Tanaka, C., Nakamura, K., and Hirakawa, K. (1986): Radiofrequency hyperthermia with successive monitoring of its effect on tumors using NMR spectroscopy. Proc. Natl. Acad. Sci. USA, 83, 8343-8347.

Schlesinger, M.J. (1986): Heat shock proteins; the search for functions. J. Cell of Biology, 103, 321-325.

Streffer, C. (1987): pp. 3-5. In Springer-Verlag Berlin Heidelberg (Ed.): Hyperthermia and the malignant tumors.

# IMMUNOTHERAPEUTICAL STRATEGIES IN CANCER PATIENTS

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## INTRODUCTION

Individuals bearing tumors belong to the category of immunocompromised subjects. In fact, these patients exhibit multiple deficits of their immune responsiveness either in terms of acquired immune response or in terms of phagocytic functions[1]. The above immune dysfunctions may depend on several factors[2] such as:

i. Suppressive factors released by the same neoplasia;

ii. A primary condition of immune alteration in a given subject which may be responsible for the development of the neoplasia. In this respect, individuals affected by congential immunodeficiencies or secondary immune deficits (e.g. transplanted patients or uremic subjects) undergo cancer with higher frequency than patients who had no clear evidence of immune alterations before the discovery of cancer;

iii. Chemotherapy and radiotherapy represent an additional cause of immune suppression in neoplastic patients.

The cancer-related impairment of immune responsiveness could account for the increased susceptibility to infections especially in leukemic individuals[3]. In this regard, subjects with neoplasia succumb because of fulminat sepsis rather than for the disease progression[3]. In the light of these concepts, immunomodulating agents represent appropriate drugs to be associated to conventional treatments in cancer patients in order to potentiate the depressed immune system.

## IMMUNOMODULATORS

Immunomodulating agents are substances able to increase immunodepressed functions either in vitro or in vivo[4]. On the other hand, these compounds are not effective in the presence of a normal immune responsiveness or of other conditions such as bone-marrow aplasia in which there is a lack of immunocompetent cell progenitors[4].

In general terms, immunomodulators can be divided into three main groups, as shown in Table 1.

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TABLE 1. Immunomodulating Agent Classification

a) BACTERIAL PRODUCTS:
   - Bacillus Calmette Guerin (BCG)
   - Corynebacterium parvum
   - Gram-positive Bacteria
   - Gram-negative Bacteria

b) IMMUNE-DERIVED SUBSTANCES:
   - Cytokines (Tumor Necrosis Factor, Interferons, Interleukins)
   - Thymic Hormones

c) NEUROPEPTIDES:
   - MET-ENKEPHALIN
   - SUBSTANCE P

Immunomodulators of bacterial origin have been used since long ago and, in particular, BCG has represented one of the first immunomodulating attempt in cancer patients[5]. However, because of its side effects and the introduction of less toxic drugs, BCG is no longer administered in neoplastic subjects. Gram-positive and Gram-negative bacteria as immunoadjuvants will be discussed in the following sections.

Immune-derived drugs represent the most widely used agents in the management of immunocompromised patients. Just recently, administration of interleukin-2 in combination with Lymphokine-Activated-Killer (LAK) cells has given good results in some cases of melanoma[6]. At the same time, other clinical trials have been undertaken with tumor necrosis factor (TNF), a monokine which causes tumor lysis[7]. However, further studies are required to establish the optimal dosage and eliminate side effects due to these cytokines.

Thymic hormones will be illustrated in the next sections and emphasis will be given to a synthetic compound, the thymopentin (TP-5).

Finally, neuropeptides are substances released by the central and peripheral nervous system, which possess several biological effects, even including immunomodulating properties[8]. For instance, met-enkephalin has been used in the treatment of patients with Acquired Immunodeficiency Syndrome (AIDS)[9]. In addition, substance P, which is an enhancer of immune functions (e.g. stimulation of the gut-associated-lymphoreticular tissue)[8] should be taken into consideration as an immunomodulator, even in the case of localized bowel tumors.

## GRAM-POSITIVE BACTERIA

These microorganisms possess a cell wall with several immunogenic and immunomodulating constituents[10]. Therefore, gram-positive organisms when inoculated in a susceptible host are able to positively modulate the immune response. In this respect, recent studies have pointed out the immunomodulating activities of dietary lactic acid bacteria (LAB) (e.g. L. bulgaricus and S. thermophilus). The influence of LAB on the host immune system are illustrated in Table 2.

TABLE 2. Immunobiological Effects of LAB

- Increase of IgG2a serum levels in mice
- Stimulation of murine lymphoid follicles
- Augmentation of resistance to infections in mice through:
  a) Enhancement of Peyer patches' antibacterial activity;
  b) Accumulation of macrophages in the sites of infection;
  c) Proliferative responses of splenocytes
- Adherence to human peripheral blood T lymphocytes
- Induction of interferon-gamma release from human lymphocytes

The interaction of LAB with human peripheral blood T lymphocytes may lead to the release of cytokines such as interferon-gamma[11]. In fact, in vitro stimulation of lymphocytes with LAB in the presence of concanavalin A induces release of IFN-gamma and augments the activity of natural killer cells [11]. Similar results have been obtained by administering LAB in normal human volunteers (unpublished results). Therefore, such an enhancement of the interferon-natural killer cell system support the immunomodulating role of these bacteria and their potential use in cancer patients.

GRAM-NEGATIVE BACTERIA

These organisms possess in the outer membrane of their cell wall a major component, the so-called lipopolysaccharide or endotoxin[12]. Lipid A represents the biologically active entity of the entire molecule and its immunological activity is expressed in Table 3.

TABLE 3. Main Effects of Lipopolysaccharide on the Immune System

- Induction of B lymphocyte mitogenis responses
- Adjuvanticity
- Cytokine release: Interleukin-1, Tumor Necrosis Factor, Colony Stimulating Factor, Interferons
- Natural Killer cell stimulation
- Enhancement of polymorphonuclear cell and monocyte phagocytosis and killing
- Augmentation of leukocyte migration inhibitory activity

Recently, in advanced cancer patients, we have carried out a clinical trial based on the i.v. administration of a rough mutant of Salmonella minnesota, the strain R 595 (Re). This gram-negative bacterium has the lipopolysaccharide represented by three molecules of chetodeoxyoctonate (KDO) covalentely linked to the lipid A[12]. Acetic acid treatment of Re bacteria leads to the KDO removal and exposure of lipid A to the bacterium surface[13]. In 20 patients with cancer at the final stage,four courses of acetic acid bacteria have been administered at the dose of 0.5, 1, 2 and 4 ugs with an interval of two weeks between each cycle[13,14]. This immune regimen was free of side effects, but led to a recovery of depressed immune functions such as phagocytosis and killing bypolymorphs and monocytes, leukocyte inhibiting factor release and natural killer cell cytotoxicity[13,14].

At the moment, Re bacteria seem to be very effective immunomodulating drugs and in progress studies will evaluate their clinical efficacy in cancer patients.

## TP-5 MODULATION OF THE IMMUNE SYSTEM

TP-5 (Italfarmaco, Milan, Italy) is a synthetic compound composed by five aminoacids which represent the biologically active part of the thymopoietin[15]. Some of the major effects of TP-5 on the host immune response are listed in Table 4.

TABLE 4. Modulation of the Immune Response by TP-5

- Enhancement of cytotoxic lymphocyte precursor units
- Early T cell differentiation
- Influence on proliferative responses and PHA-induced interleukin-2 production
- Release of interferon-gamma
- Improvement of natural killer cell activity

According to our experience, thymopentin is a strong immunomodulator in patients with lepromatous leprosy, since s.c. administration of this drug (50 mg every second day for 5 wks) normalized the inverted CD4+/CD8+ ratio and augmented the interferon-gamma release by peripheral blood lymphocytes[16]. A similar therapeutical protocol has been applied to a group of cancer patients with ovary and cervix malignancies in advanced stage and out of convetional therapy[17]. In this case, TP-5 was very effective in the conversion of the altered CD4+/CD8+ ratio, less efficacious in the recovery of phagocytosis and killing and unable to increase the production of interferon-gamma. At the same time, no side effects were noted and in some patients who received further cycles of the hormone a clear-cut improvement of the above immune parameters was noted (unpublished results).

## CONCLUDING REMARKS

Modern biotechnology has led to the development of new immunomodulating drugs such as recombinant cytokines. Some of these mediators are now under clinical trial and, it is

likely that the interleukin cascade will represent in a near future the most appropriate approach for combined treatment in cancer patients. On the other hand, bacterial products are still valid immunomodulators for their capacity to elicit good immune responses via cytokine release. At the present, therapy with interleukins (e.g. interleukin-2 and LAK cells) is very expensive for the Institutions and therefore these immunomodulators cannot be routinely administered. Therefore, gram-positive and gram-negative organisms, as inducers of cytokines, are efficacious products for the enhancement of the immune response in cancer patients. As far as thymic hormones are concerned, they are potent immunomodulators but the limitation for their use is the availability as commercial drugs which is restricted to a few countries.

In conclusion, despite many studies on immunomodulators, further investigations are required to establish appropriate therapeutical strategies , even including the association of more immunomodulators in order to enhance the antitumor effects.

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REFERENCES

1. Nelson, D.S., Nelson, M., Farram E., Inoue Y. Cancer and subversion of host defences. Aust. J. Exp. Biol. Med. 59: 229 (1981).
2. Penn I. Depressed immunity and the development of cancer. Clin. Exp. Immunol. 46: 459 (1981).
3. Easmon, C.S.F. and Gaya,H. (Eds.). The second international symposium on infections in the immunocompromised host. Academic Press, London (1983).
4. Fudenberg, H.H., and Smith, L. Immunomodulation and immunotherapy: an overview of biologic and synthetic agents and their effects on the immune system. EOS J. Immunol. Immunopharmacol. 1: 3 (1981).
5. Bast, R.C., Zbar, B., Borsos, T., and Rapp, H.J. BCG and cancer. N. Engl. J. Med.290: 1458 (1974).
6. Rosenberg, R.A. The adoptive immunotherapy of cancer using the transfer of activated lymphoid cells and interleukin-2. Semin. Oncol. 13: 200 (1986).
7. Tanneberger, S., Lenk, H., Müller,V., Ebert, J., and Shiga T. Human pharmacological investigation of a recombinant human tumor necrosis factor preparation. A phase I trial and preliminary results of a phase II trial, in: "Tumor Necrosis Factor/Cachectin and Related Cytokines", Bonavida, B., Gifford, G.E., Kirchner, H., and Old, L.J., eds., p. 205, Karger, Basel (1988).
8. Covelli,V., and Jirillo, E. Neuropeptides with immunoregulatory functions: current status of investigation. Funct. Neurol. 3: 253 (1988).
9. Gupta, S. Immunotherapy of human immunodeficiency virus infection. EOS J. Immunol. Immunopharmacol. 8: 231 (1988).

10. Hamada, S., and Slade H.D. Biology, immunology and cariogenicity of Streptococcus mutans. Microbiol. Rev. 44: 331 (1980).
11. De Simone,C., Jirillo, E., Bianchi-Salvadori, B., Baldinelli, L., Tzantzoglou, S., Di Fabio, S., Grassi, P.P., and Vesely, R. Stimulation of host resistance by a diet supplemented with yogurt, in: "Immunomodulators and Non-Specific Host Defence Mechanisms Against Microbial Infections, Masihi, K.N., and Lange, W., eds. p. 279, Pergamon Press, Oxford (1988).
12. Jirillo, E., Michalek, S.M., and McGhee, J.R. Lipopolysaccharide regulation of the immune response: LPS effects on lymphoreticular cells and mucosal immune responses. EOS J. Immunol. Immunopharmacol. 4: 21 (1984).
13. Jirillo, E., Miragliotta, G., Caretto, G., Cedola, M.C., Nappi, R., Sansone, L.A., Galanos, C., Antonaci, S. Relationship between immune system and gram-negative bacteria. Acid-treated Salmonella minnesota R 595 (Re) enhances immune responsiveness in patients with gynaecologic malignancies. Int. J. Immunopharmac. 8: 881 (1986).
14. Jirillo, E., Munno, I., Pellegrino, N.M., Grassi, P.P., Maffione, A.B., Catino, A.M., Jirillo, A., and Antonaci, S. Immunotherapeutic approach to cancer and leprosy patients, in: "Molecular Aspects of Human Disease", Gorrod, J.W., Albano, O., and Papa, S, eds. p. 240, Ellis Horwood Limited, Chicherster, England (1989).
15. Sztein, M.B., and Goldstein, A.L. Thymic hormones. A clinical update. Springer Semin. Immunopathol. 9: 1 (1986).
16. Munno I., Pellegrino, N.M., Fumo, G., Barbieri, G., Polimeno, G., De Filippis, V., and Jirillo,E. Effects of a synthetic extract (thymopentin) on the immune system of lepromatous leprosy patients. Cytobios 52: 167 (1987).
17. Munno, I., Pellegrino, N.M., Di Vagno, G., Nappi, R., and Jirillo, E. Effect of thymopentin pentapeptide (TP-5) on the immune system of patients with gynecological malignancies. EOS J.Immunol. Immunopharmacol. 7: 117 (1987).

# COLEY TOXINS - THE FIRST CENTURY

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## EDITORIAL COMMENT

We are pleased to be able to present this summary of the first century of Dr. Coley's toxins prepared by Helen Coley Nauts. We believe you will agree that this is an objective scientific presentation particularly when one considers the criticism, frequently unfounded, which has been given Coley's work in the past. Those reading this chapter and working in hyperthermia will find many parallels and similarities with their frustrations and peer criticisms as noted herein. Comparable modes of action will be found for those proposed to explain the benefits of hyperthermia. Possibly with today's more detailed understanding of the intricate complex immune system, a logical basis can be offered for the "dramatic cures" which in themselves caused skepticism, and Coley's results can be better understood. A study of Coley's life's work will lead to a better understanding of the problems, obstacles and potential solutions we have in hyperthermia and also I suspect that it will help our morale to realize that we are not the first or only ones to have criticisms heaped upon sound and meticulous work. It is gratifying that in recent years Coley is being recognized as the pioneer of cancer immunology.

In January, 1893, W.B. Coley, a young New York surgeon treated his first case of cancer with the mixed bacterial toxins of Streptococcus pyogenes and Bacillus prodigiosus (now known as Serratia marcescens). This bedridden male, aged 19, had an inoperable sarcoma of the abdominal wall and pelvis (16x13 cm.) involving the bladder with incontinence. Only biopsy had been performed. Injections in or near the tumor caused reactions up to 40oC. or more and complete regression occurred in four months. He remained well until death from a heart attack 26 years later (1, case 1).

In 1939, we began our analysis of Coley's method to

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answer the following questions: 1) Was there sufficient clinical and experimental evidence to justify the conclusion that the method had therapeutic value? 2) If so, what factors governed success or failure? 3) Why did the method not achieve wider recognition? 4) If the conclusions to these questions warranted further study we asked ourselves what can be done to make the Coley toxins consistently effective in most types of neoplastic disease (2). Some factors or data that seemed vital to success or failure were identified:

1. Variability of preparations used

No comprehensive text book on the method had been published by Coley, although he was working on one at the time of his death in 1936. He made every effort to obtain unequivocal diagnosis by eminent pathologists from the beginning. However he did not recognize the great importance of obtaining potent, stable preparations of the mixed toxins to avoid variability from different formulae or from batch to batch (1,2). Coley had no bacteriological training and relied on others to made the preparations.

The first observation brought out by our long term study was that at least 13 different preparations had been used during Coley's active years, (1892-1936) of which three were considerably more potent than the rest, Buxton VI, Tracy X and Tracy XI. Unfortunately the first two commercial preparations Parke, Davis & Co., IX and XII were very weak and the English preparation (Lister Institute VIII) was even weaker, so very few English surgeons achieved success (1,2). These weaker preparations did not produce adequate febrile reactions (3, Fig.1).

In 1902 a patient with recurrent inoperable lymphoma of the axilla reported to Coley that it took eight minims of the Parke Davis IX to give the same febrile reaction as 1/4 minim of the Buxton VI (6, page 79). Despite such a clearcut case, Coley does not seem to have attempted to remedy the situation, and may have been unaware of this problem until 1911 when he gave a lecture at Guy's Hospital in London and he discussed it briefly in his response to the vote of thanks, ending with the remark "success depends on the preparation..." Dr. Coley, in London in 1911, following recognition at Guy's Hospital for his work stated, "I am greatly obliged to you, gentlemen. What I have heard led me to believe that British audiences were cold, but I have never in my own country received such a hearty reception as you have given me today. Sir Alfred Fripp tells me that you have tried the fluid in the hospital. The trouble has been that a different preparation is sometimes used. Mr. Mansell Moullin of the London Hospital had five successful cases, and he said he got his successes with the fluid obtained from the Cornell Laboratory (Buxton VI). Middlesex Hospital had had three failures but in the fourth, they used the Buxton VI and the growth regressed to 1/14 in a few weeks...."

Finally in May 1915, Tuholske of St. Louis wrote him about a case of extensive sarcoma of the pharynx and nasopharynx with almost complete obstruction -- a tracheotomy was imminent (1, case 23, p. 68-72; 3, case 56, p. 131-135). Even massive doses of Parke Davis XII had had no effect at

all. Coley then sent him the Tracy XI (see below for details relating to technique). This case made him contact Parke Davis and get them to work more closely with Tracy and so Type XIII was made considerably more potent than XII (1,2).

## 2. Techniques of Administration

The Tuholske case illustrates the importance of a number of factors. First, the danger of stopping the injections too soon, even if complete regression has occurred. Although complete regression occurred in six weeks, the disease recurred on the opposite side in about three months with evidence of brain metastases. Second, the injections for the recurrence were given subcutaneously or intramuscularly in the deltoid or scapular regions with very poor absorption. Not until given in the abdominal wall did good febrile reactions occur, and the recurrence disappeared, but the symptoms of brain metastases persisted. The patient went into coma for 3-1/2 weeks. No further injections were given. With supportive treatment he regained consciousness and made a complete recovery. He remained well until death of a coronary occlusion 33 years later (3, case 56, p. 131-135). Although Coley published 143 papers or monographs on his method between 1893 and 1936, (8) he seldom gave sufficient detail on methods of administration; i.e., site, dosage, frequency, duration, and the desired optimum febrile reaction.

### a. Site and Dosage of Injection

The type of reaction elicited depended on two things. The site of injection and the dosage. Injections given intramuscularly or subcutaneously remote from the tumor, required much larger doses to elicit a reaction of 101oF. or more than did an injection into the tumor or in a vascular tissue or intravenously. In the early years Coley used intratumoral injections into different parts of the tumors. These elicited not only fever but tumor necrosis factor and an inflammatory reaction all of which were more effective in causing destruction of the tumor thus imitating an erysipelas infection. (The most dramatic "spontaneous" regressions of cancer occurred during and following acute erysipelas injections which produce a more intensive inflammatory reaction than any other infection).

We found only one case treated by Senecal with intraperitoneal injections -- a huge ovarian carcinoma with widespread metastases in the peritoneum and ascites. Very dramatic regression occurred in four weeks. The case became operable and recovered. She remained well 27 years (1, pp 89-42, 8, pp 85-41).

Intravenous injections were not used by Coley until about 1925 and usually some intramuscular injections were given first. Very much smaller doses were needed, and these caused no inflammatory reactions. They were well tolerated.

Fowler in 1898 recognized the importance of site of injection. When given subcutaneously, the intensity of the general reaction varied with the dosage and site used. With subcutaneous injections a larger dose was required to produce

the desired reaction, whereas a few drops were sufficient for the intravenous route. "The vascularity of the tumors explains the ease with which a reaction can be produced by Coley's method of interstitial injections, the latter being quite analogous, if not identical with the intravenous method" (cited by Moullin, 9).

X-rays and radium were discovered in 1895, only a year after Coley read his first important paper before the American Surgical Association (8). He was one of the first surgeons in New York to use x-rays in his practice having persuaded Memorial Hospital in 1901 to procure an x-ray machine, paid for by one of his wealthy patients. He read his first report on the work before the American Surgical Association in June 1902 (11). The growing enthusiasm for both x-ray and radium quickly overshadowed Coley's method before it had been properly standardized or its mode of action understood. Coley, anxious to prove that his toxins had a systemic rather than a local reaction such as x-ray, radium and surgery, stopped using intratumoral injections about 1906, and not until a year or two before he died did he come to realize the mistake he had made.

b. Frequency of Injections

In the early years Coley and other surgeons gave injections daily or every other day at first, which appeared to be more effective than less frequent injections, especially when treating inoperable cases. One surgeon who routinely used the Coley toxins in both his operable and inoperable cases, Calkins of Watertown, New York, gave the injections daily or every 48 hours for about six months, then twice weekly with occasional intervals of rest for another six months (12 pp. 42-43 & 53). Injections were given as an outpatient after the first two weeks. Calkins achieved 80% five year survival in using this technique over a 32 year period. Matagne also often gave injections daily. (See below).

Many surgeons, such as the Mayo brothers, did not wish to get involved in such long term therapy, so they advised the family physician who had referred the case to the Mayo Clinic to administer the injections after the patient returned home. As a result a considerable number of Mayo Clinic cases were successfully treated.

c. Duration

Coley did not recognize the importance of duration of treatment, especially in the inoperable cases until 1926 when Christian and Palmer succeeded in curing a reticulum cell sarcoma of the tibia recurrent in the stump after amputation with extensive metastases near the umbilicus and in the left inguinal region. In discussing this case in 1927 Coley stated "I am quite willing to admit that, had the patient been under my care, he would probably not have been alive today. ...I am almost certain that I should not have continued the treatment after three months when not only had no improvement been noticed, but marked increase had taken place in the metastatic

tumors and especially in the recurrent tumor of the stump (from 17-31 inches). In the second place I am quite sure that I should not have dared to increase the dose to such a large amount (2 cc). However, it was not until these large daily doses were given that the improvement continued until all the tumors had disappeared. ....I feel that many of the past failures might have resulted otherwise had larger doses and more frequent injections been given" (1, case 19, p. 84-89).

Our end result studies beginning in 1946 have shown that, if the injections were given for six months, 80% of inoperable sarcoma of soft tissues survived 5-88 years. (3)

In osteogenic sarcoma when the Coley toxins were given as an adjuvant to surgery for at least three months 85% survived and were traced up to 53 years later. Three other cases so treated died 4-13 years later of late recurrence or metastases, i.e., prolonged survival. If given for less than three months 36-43 percent survived. This was considerably better than the 10-15 percent survival from amputation alone in that period (4, Figure 2, p. 10).

d. Type of Febrile Reaction

Coley did not sufficiently appreciate the benefit of producing febrile reactions averaging 39o-40oF.) from the beginning of treatment. This did not occur if they used small doses intramuscularly or subcutaneously remote from the tumor, or when the very weak products were used. This factor was more important in treating inoperable cases. For example, in the soft tissue sarcoma 60 percent of the inoperable cases, whose reactions averaged 102o-104oF. with chills, were traced well 5 to 88 years, as compared to only 28 percent of those having reactions below 102oF. and no chills (3, Figure 2).

3. Stage (Operable vs Inoperable Cases)

In treating operable cases as an adjuvant to surgery very few surgeons began the treatment prior to surgery. This is unfortunate because preliminary toxin therapy, even for only a brief course, can counteract the immunosuppressive effects of anesthesia and surgery, due to stimulation of cytokines such as interferon, interleukins, tumor necrosis factor and others. In cases of amputation, it counteracts the psychic stress of losing a limb, which is also immunosuppressive.

The first physician to recognize that operable cases might benefit was Matagne of Brussels, Belgium. He first began using the Coley toxins in inoperable cancers, having observed a dramatic case cured following an erysipelas infection reported by Bidlot in 1891 (7, Ref. #39). In 1896 he gave a rather brief incomplete description of his inoperable cases and was soon criticized by a commission charged with examining it (14). This did not deter him from continuing his clinical studies and over the next 57 years he published 12 more papers interrupted by two world wars (8, Ref. #265-277).

In 1902 he reported on the use of Coley toxins in operable cases as a means of preventing recurrence, the first

physician to do so (14,15,16). In 1905 he presented a series of these operable cancers in which he had administered the toxins before operation, usually for four or five weeks, in some cases for three months. (17).

One extensive recurrent inoperable malignant melanoma of the upper arm in the region of the humeral artery with metastases in the axilla, received injections in the recurrent tumor for seven months in 1902 with rapid but incomplete regression of the recurrent mass, but the axillary metastases did not regress. Shoulder joint disarticulation was then performed. There was no further recurrence and the patient remained well over 41 years after onset (15,16,17).

Matagne prepared his own Coley toxins using the effective Buxton VI formula (17, p. 1389). He gave injections daily into the tumor beginning with a dose of 5 cg., gradually increasing by 2.5 cg. each day or every other day until a febrile reaction of 39o-39.5oC. or 40oC. was elicited. The reaction usually consisted of a violent chill which began 30 minutes after injection and lasted 30 minutes. Dosage was increased to 10 mg. in some cases, in others to 30, 40 or even 50 cg.

The first surgeon to save a limb by using Coley toxins following surgery was Owens in 1894 in a highly vascular giant cell tumor of the proximal tibia following curettage. This was the first case of giant cell tumor in which the limb was saved (19). In two-thirds of the giant cell tumor cases involving long bones, the limb was saved by the use of curettage and toxin therapy combined, in some cases, with radiation. The remarkable regeneration of bone destroyed by the tumor following toxin therapy was especially evident in Series A, Cases 8,11,13,26 and 40 (22).

Coley began using his toxins in operable cases as early as 1895 (20). The first published case was an osteogenic sarcoma of the femur. The patient remained free from recurrence or metastases for 53 years. (4, Case 1, Table 1). Other American surgeons soon followed, including Ochsner in 1915 and Calkins in 1917, for breast carcinoma and sarcoma (6) and Meyerding of the Mayo Clinic for osteogenic sarcoma and Ewing's sarcoma of bone.

4. Early Criticism: Limiting Types of Tumors Treated

Between 1891 and 1896 Coley published 16 papers describing his method (8, references 42-64). Editorials began to appear in 1894 with the first negative report entitled "Failure of the Erysipelas Toxins" (21). In 1895 Abbe, the President of the New York Surgical Society, suggested that the method be tried in different hospitals: a fair proposal, but no enthusiasm was shown. It is a great pity that such a plan was not carried out, but it is not surprising that a group of surgeons were reluctant to pursue a medical approach to the treatment of cancer. In October 16, 1895 Coley read a paper before the New York State Medical Association entitled: "The indications for the non-operative treatment of tumors. The value of Toxins". This was published in reprint form in 1896

(19) and caused more concern among surgeons and a few more negative reports (5).

To editorials, mostly unfavorable, Coley responded objectively on December 29, 1894, "That a few physicians in a very limited number of cases with indifferent preparations of the toxins have failed to obtain good results will not... have great weight in the minds of the scientific portion of the profession in determining failure or success of this method of treatment of sarcoma" (20).

In 1915 Harmer of Boston published a critical report on 134 microscopically proven cases treated by Coley's toxins (21). He concluded that they are of value in certain cases of inoperable sarcoma. Although his conclusions were somewhat negative he noted that in a small number of cases they produced striking relief of pain. Coley's many surgical friends in England and in the United States urged him to limit his use of the toxins to
sarcomas since the early experiences with his weaker preparations in advanced carcinomas or melanomas had not proved successful.

Other surgeons had successfully treated metastatic cervical carcinoma (1, Case 5), extensive metastatic breast carcinoma, (1, Case 14, 15; 23, p. 232) giant cell tumor of vertebrae (1, Cases 6, 20), recurrent malignant melanoma (1, Case 17), ovary, metastatic (1, Cases 25, 30; 12, Cases 20, 21). These reports encouraged Coley to use his method on many other neoplasms; testicular cancer (24), breast cancer (23), lymphoma and Hodgkin's disease (25,26), malignant melanoma (27), multiple myeloma (28), Ewing's sarcoma (29), neuroblastoma (30), colon cancer (31) and renal cancer (32) with a great many remarkable results (1,33).

5. Animal Experiments

In 1907 Beebe and Tracy published the results of their studies (34) in which they stated: "The striking results attained (by Coley) in an increasing number of cases have diverted the attention to the possible significance of the Bacillus prodigiosus in the preparation. ...It seemed important to study with some care the effect of inoculation, not only with the Bacillus prodigiosus (now known as Serratia marcescens) but with other bacterial toxins as well.....with the hope of determining the rationale of this method of treatment, and if possible of placing it on a more scientific basis". They used B. prodigiosus, Streptococcus pyogenes, Staphylococcus aureus and Bacillus coli communis (now known as Escherichia coli; also the mixture known as Coley toxins. They treated with these preparations lymphosarcoma grown in dogs transplanted from a spontaneous tumor.

They reported that B. prodigiosus alone and Streptococcus pyogenes alone as well as the mixture of these two (the Coley toxins) caused regression of this tumor in the dogs. Staphylococcus aureus given into the tumors caused fever to 105.9oC., but no real regression. E. coli in one dog caused steady regression; all tumors disappeared in five weeks. They used different sites of injection. The intratumoral

injections caused much more rapid regression (34). In conclusion they stated that, "though the action is chiefly local, it is at the same time something more than this for it was repeatedly observed that tumors at a distance from the site of injection undergo regression simultaneously while in one dog all the injections were given remote from the tumor" (34).

6. Radiation and Toxin Therapy

Ewing became medical director of Memorial Hospital about 1915 and he was an ardent advocate of radium, a large supply of which had been given to the hospital by Dr. Douglas. At that time Coley had been appointed Chief of the Bone Tumor Service. Ewing insisted that every single ward case should receive radium or x-ray prior to amputation and, despite the fact that Coley believed this to be a dangerous protocol, he had to comply. In 1927 Coley published the end results (35). Not a single patient so treated had survived, while in his private patients to whom he gave the toxins following surgery 50 percent had survived. If injections were given for three or more months, 85 percent survived four - 40 years later (4). The Mayo Clinic also achieved 50 percent five year survival in their toxin-treated cases, while other surgeons here and abroad were curing only 10-15 percent with amputation alone.

Matagne, as well as Coley, became interested in utilizing radiation in his practice and he acquired some radium at considerable expense which he used especially in epitheliomas. Like many other surgeons he felt constrained to use it to justify the expense incurred, as a result fewer patients were treated with the toxins thereafter.
Coley treated one of his first cases with x-ray therapy in 1901. He irradiated an inoperable lymphosarcoma of the cervical, axillary and mediastinal nodes which was producing dyspnea and edema of the lower extremities. Marked regression and increased mobility had occurred following toxin therapy alone, but then control was lost and the patient became bedridden, with severe dyspnea. X-ray was then given 4 to 6 times weekly causing remarkable regression in three weeks and complete disappearance in six months. No further toxins were given after radiation was begun. The disease then reactivated with hundreds of pea to egg-sized nodules over the entire body. Death occurred in June 1904, 5-1/2 years after onset. Coley believed the radiation had lowered the resistance of this patient to her tumor (6).

In contrast, an eight year old boy had had amputation for a fungating Ewing's sarcoma (later regarded as a reticulum cell sarcoma) of the fibula with metastases to the inguinal and iliac lymph nodes. Toxins (Tracy XI) were begun immediately after amputation and given two months with marked reactions. Soon after the injections were stopped a 15 cm. mass in the iliac fossa and lung metastases developed. All disappeared after one radium pack to the groin (10,109 mch). The child remained well and free from disease until death from an emergency appendectomy 15 years later (36, 37).

## 7. Radiation Protection

About 1958 a number of investigators began reporting on the protective effect of bacterial endotoxins against radiation injury (39-41). This occurred if injections were given prior to the radiation, optimally 24 hours before.

Thomson (1962) reported "bacterial endotoxins prepared from Salmonella typhosa, Escherichia coli, Serratia marcescens and Proteus morganii all promoted hematopoietic recovery when given before or after whole body radiation. Post radiation infection is appreciably reduced, hematopoietic tissues regenerate and survival is enhanced" (39).

Ainsworth, of the Cellular Biology Branch of the U.S. Naval Radiobiological Defense Laboratory, San Francisco, published several reports in one of which he noted that low pyrogen doses are known to produce a more rapid rise in resistance than larger doses, i.e., 50 mcg. was not as effective as 2 mcg. of pseudomonas in increasing survival time to lethal radiation (40). At our request in 1962 Ainsworth screened the Coley toxins as a potential radioprotectant on x-irradiated mice. In this experiment the smaller dose was more protective. Data from this unpublished work follows:

Effect of Coley Toxins on Survival of Irradiated Mice

Method: Typhoid-paratyphoid vaccine was used to compare Coley's Toxins since previous data had shown that TAB is highly effective in reducing the radiation mortality in mice. The mice used were CF1 females, 100 days old, weighing 19-24 grams. Total body x-irradiation was delivered from a 250 kv Westinghouse x-ray machine operated at 15 ma and a distance of 40 inches. Added filtration consisted of 0.5 mm Cu and 1 mm Al.

## 8. Modes of Action

In addition to radiation protection and potentiation of tumor response, the Coley toxins produce fever which we know is beneficial, they also stimulate the reticuloendothelial system, activate macrophages, increase hematopoiesis, increase production of prostacyclin, endogenous interferon, endorphins, tumor necrosis factor, interleukins and growth factors (44, 45). Interleukin-1 for example, induces a profound hypoferremia which assists the patient in withholding iron from the cancer cells. Weinberg has summarized some of the numerous papers in regard to this subject including Torrance et al. (45a,45b).

Certain infections such as erysipelas (13) and the Coley toxins also stimulate wound healing (1) and regeneration of bone destroyed by tumor (23). For example, a case of an extensive giant cell tumor of the proximal femur with complete destruction of 17 cm. including the neck and trochanter with pathologic fracture, recovered under Coley toxins in or near the tumor given for 7-1/2 months combined with two radium treatments. The first was given after one week of toxins,

the second seven weeks later. Complete regression of the tumor and regeneration of the hip joint occurred, with 14 cm. shortening; the patient remained well until death 45 years later of coronary occlusion. A second case involved the neck, both trochanters, with complete destruction of the acetabulum and ischium and pathologic fracture. He received one radium treatment (9000 mch) three days before toxins were begun. Complete regression occurred. The limb was kept in traction and the hip joint and femur regenerated without shortening (22, Case 20, pp. 46-50). In another giant cell tumor of the distal radius involving the ulna, the extensive tumor regressed completely under toxins alone and the bone regenerated with perfect function (22, Case 36, pp. 53-57).

9. Dramatic Cures Promote Skepticism

One form of criticism occurred as a response to dramatic cures. Richardson of Boston in reporting a remarkable case he had referred to Coley in 1893 stated: "Skepticism may be so extreme that carefully observed cases are thrown out for one reason or another, though I cannot but think chiefly for the reason that they were successful. In this case Dr. Garland and myself at the time of operation made the diagnosis as hopeless malignant disease of the abdominal wall. Dr. Whitney made a careful microscopic examination of the tumor and reported it as fibrosarcoma". In October 1893 she received local injections daily into the tumor for six weeks with marked reactions. Within two weeks improvement was very evident. The patient's general condition suffered but little and she was up and about most of the time. She returned home for Christmas, a second four week course was given in January. Richardson concluded: "The tumor, though as large as a child's head, disappeared. ....If a cure by means other than surgical is from the very fact of cure, declared sufficient proof of a mistaken diagnosis. There seems little use in presenting evidence. ....The curative influence of micro-organisms upon malignant growth, whether during the course of an accidental wound infection, or under the influence of deliberate toxin injection is a hopeful indication of far-reaching possibilities for good". (1, Case 2, pp. 22-25; 3, Case 3, pp. 3, pp. 51-53).

In July 1920 Codman, who had organized the Bone Sarcoma Registry in 1920 with Ewing and Bloodgood of Johns Hopkins, wrote Coley, "You have probably more living cases than any man in the world. That your treatment has a profound systemic effect I have no question but I am inclined to attribute the successful cases to errors in diagnosis. Yet I must admit you have more to your credit than anyone else".

In May 1934 Codman was Chairman of a Bone Sarcoma Symposium held at Memorial Hospital, and summarized Coley's paper on Ewing's sarcoma of bone stating. "This paper will give great satisfaction to Dr. Coley's many friends who have admired his courageous, tireless fight to overcome the skepticism of his colleagues. ...His six registered cases of five year cures are alone enough to sustain his argument. ....Just as it seemed quite justifiable for the Memorial

Hospital during the last decade to test out the value of radiation alone in inoperable cases or in patients opposed to operation, so it seems even more indicated that some great clinic should try out Coley's toxins during the next decade. Unquestionably they produce a profound constitutional effect. ...It is time for some great hospital to apply its laboratory resources to the wholly justifiable and distinctly hopeful purpose of giving this treatment a fair trial under favorable conditions. Certainly, in cases of Ewing's tumor one would hardly feel justified in not recommending the use of the toxins. The question of whether also to give radiation is the difficult one. Dr. Coley quite logically suggests that the combination of the two may be a bad one, for the rapid destruction caused by irradiation may open up channels for further invasion. Radiation in small amounts stimulates lymphocytosis, and its use in this way was advocated in 1918 for the treatment of malignant tumors by Dr. James B. Murphy, of the Rockefeller Institute, after some very convincing experimental work on animals. Large amounts of radiation, on the contrary, destroy the lymphocytes whose formation it at first stimulates. A series of cases treated by the toxins without the concomitant use of radiation, in which the blood reactions are carefully followed, might prove of great value. There must have been many, many cases in the past treated by these toxins on the hit or miss principle by the family doctor, without careful study, such as is possible in the modern clinic. Yet under these disadvantageous circumstances, occasional miracles have occurred and in Coley's own undiscouraged hand these miracles have not been infrequent". (46)

10. The Coley Toxins after Coley

After Coley's death in 1936, his son Bradley L. Coley, M.D., became Chief of the Bone Tumor Service and he and his associate Higinbotham continued to produce excellent results with the toxins in Ewing's sarcoma, reticulum cell sarcoma and some osteogenic sarcomas of bone. B.L. Coley and Higinbotham served in the Army from 1942 to 1946. Upon their return, chemotherapy became a priority. The Medical Director, Rhoads, without consulting the Bone Tumor Service, wrote Parke Davis & Company in 1950 telling them to stop making the toxins. A typical administrator's callused and unethical act against a number of patients who were receiving them. For a time Rhoads had the toxins made at Sloan-Kettering Institute (SKIxiv) and eight reticulum cell sarcomas were successfully treated with this product, combined with x-ray therapy in some cases. In inoperable cases with metastases the limb was saved in all these, one was a Mayo Clinic case (37).

In 1939 we began a half century of study on Coley's work. We found the factors affecting success were very concrete as outlined above, but they had been largely ignored by Coley and most of the other men using the method. Matagne was an exception. He made his own toxins so they did not have to be shipped and lose potency in transit before the days of airmail, and he administered them wisely. In 1953 we founded Cancer Research Institute, to provide incentive and

support for investigators in this field of cancer immunology. The first two investigators we funded were Johnston and Havas at Temple University, Philadelphia, Pennsylvania, Johnston's laboratory prepared the Coley toxins for clinical use and for a special study at New York University (50,51). Havas also prepared the toxins but did not use them clinically (52,53).

Between 1954 and 1963 a number of physicians and surgeons became aware of our studies, requested reprints and obtained toxins (Johnston XV). We also sent them detailed directions for administration and toxin therapy record sheets to facilitate analysis of results. Successful results were obtained by several including Johnston (50, 51). Rank in Texas, breast cancer (23); Fowler, in Connecticut, multiple myeloma (54) and colon cancer (31), and Nicholson in Philadelphia, reticulum cell sarcoma (37).

Following the tragedy of thalidomide in Europe in 1963, the Kefauver bill was passed enabling the Food and Drug Administration (FDA) to establish very stringent regulations regarding clinical trials of new drugs. Though the Coley toxins were 70 years old, the FDA ruled it was a new drug requiring special permits and endless red tape to use it clinically, hence all those who were using it stopped. Despite this terrible blow we continued to assemble data and to edit and to publish 22 monographs after 1963 (8). We also read papers at 11 international cancer conferences (8).

One study was undertaken with an F.D.A. protocol at Memorial Sloan-Kettering Cancer Center in June 1976 by Kempin et al (50). Patients with advanced non-Hodgkins lymphoma were randomized to receive or not receive one subcutaneous injection of mixed bacterial vaccine (MBV) five days before each cycle of chemotherapy. There were 40 nodular poorly differentiated lymphoma, five nodular mixed lymphoma, three nodular histiocytic lymphoma, Stage II, five cases, Stage III, 23 cases, Stage IV, 21 cases. Radiation therapy was given to initial areas of bulky disease or to nodal or extranodal sites responding only partially to chemotherapy. The MBV treated patients had a higher rate of complete remission (73 vs. 44 percent) longer duration of remission ($p=0.005$) and longer survival ($p=005$).

The product they used was made in Germany by Bayer, and they used only subcutaneous injections at infrequent intervals which caused little or no febrile reactions. Despite these factors the MBV did improve remission and survival, although a few years later the survival curves merged due to late recurrences. Thus the study was not continued, although the authors had reported in 1981 that the study strongly suggested a potential role for MBV therapy in the management of these tumors (50).

Another physician working independently in Milwaukee, Wisconsin, began in 1972, after having worked in a regional burn unit for nine years where he treated 3,000 cases with a mixed bacterial vaccine he had made up to prevent the virulent

infections these patients develop. His vaccine made isolation and antibiotics unnecessary. In 1972 he decided to treat his cancer patients with the vaccine which was made in his own laboratory. By 1986 he reported on 139 patients. They received BCG, transfer factor and MBV -- all were advanced cases that had failed under chemotherapy or refused it (54). His results suggested that combined immunotherapy is well tolerated and safe and that it had a salutary effect on a number of patients. His best results were in lung cancers and in operable breast cancer given the treatment as an adjuvant to surgery: none of the 30 cases so treated has died (55).

## 11. PRELIMINARY RESULTS OF MIXED BACTERIAL VACCINE IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA AS ADJUVANT

In 1981 Guo, a pediatric oncology surgeon at Beijing Children's Hospital became interested in using the method for his patients. He also was impressed by a case of very extensive sarcoma of the thigh that had recovered following a severe staphylococcus infection with complete recovery (44). He also had read our first monograph (1) which was in his Medical School Library. He wrote us and we provided the end result studies on pediatric malignancies, the directions as to preparing and administering the toxins and kept in close touch. We went to China in October 1983 and brought Guo to New York that fall, so he could visit Memorial Sloan-Kettering Cancer Center's excellent pediatric division. We emphasized the need for more space in the ward, an area for play and more nurses. We also stressed the need to allow the mothers to be with their children as much as possible to reduce stress which is immunosuppressive. We provided the toxins prepared by Havas in Philadelphia for part of the time.

In the past 7-1/2 years he has treated 49 cases in children. Of the inoperable cases receiving ten or more injections, eight were successful, traced up to eight years after onset. There were 16 inoperable failures, of whom six received 7 to 10 injections - too brief a period.

Of the 22 operable cases, only two have died, one of which was recurrent when toxins were begun. Twelve have remained free from disease up to six years after onset, seven more recent cases remain well and one equivocal case is probably cured but awaits a "second look".

Guo is the first surgeon since Matagne in Belgium to use the toxins before as well as after surgery. His results indicate how successful this technique can be: No primary operable case has died. He used injections intratumorally, intradermally over the tumor site, and intramuscularly (57).

Guo has also treated a considerable number of adults in another hospital, but it is too soon to evaluate the results.

In Shanghai at our suggestion, Tang a very well-known liver cancer surgeon, has been using the Coley toxins provided by Havas of Temple University. From May 1985 to December 1987

patients received hepatic arterial ligation plus cannulation for 30-40 consecutive days, including 12 cases with second stage resection, and 34 patients with palliative resection. These cases were randomized. The controls did not receive mixed bacterial toxins (as the Coley toxins are now called, MBV). One group had cis-platin, one group had MBV and radiation, one group had all three. The patients receiving MBV had 47.8 percent survival versus 35 percent for the controls. In the second look resection 9/25 survived versus 3/20 in the controls. Remarkable lymphocyte infiltration was found in the tumor specimen after second look resection in the MBV cases. (Zhao You Tang, Hai Yan Zhou, Gang Zhao, Li Mian Chai, Ji Zhen Lu, Kang Da Liu, Havas, H.F., Nauts, H.C.).

It may be of interest to some readers that there is currently a clinical trial testing the effect of mixed bacterial vaccines (MBV) upon the immunocompetence of patients with far advanced cancers and its toxicity and potential benefits to the patient. The vaccine has been registered with the Food and Drug Administration and the principal investigator, Dr. Rita Axelrod, possesses IND #BB-2016 for testing this drug. The protocol is quite broad and general so as to maximize the accrual of patients; however, in order to qualify the patient must have failed all standardized treatment modalities or be unacceptable for such management. The patients studied to date, with the exception of one patient, has shown acceptable toxicity to the vaccine and a number have shown gratifying improvement in some of their immune parameters. Dr. Axelrod is located at Temple University Medical School, Philadelphia, PA.

## 12. Conclusions

We believe that the factors outlined in this review indicate why the Coley Toxins never acheived wider recognition. Since the mistakes of the past are now more clearly understood, there is a real opportunity to organize cooperative studies. Not only for inoperable cancers but as an immunoadjuvant to potentiate the response to the usual modalities and protect against their immunosuppressive effects. The Chinese studies have already shown that this is possible.

REFERENCES


1. Nauts, H.C., Fowler, G.A. & Bogatko, G.H.: A review of the influence of bacterial infection and of bacterial products (Coley's toxins) on malignant tumors in man. Acta Medica Scandinavia 145: Supplement 276. Stockholm, April 1953.

2. Nauts, H.D., Swift, W.E., & Colery, B.L.: The treatment of malignant tumors by bacterial toxins, as developed by the late William B. Coley, M.D., reviewed in the light of modern research. Cancer Research 6:205-216, 1946.

3. Nauts, H.D.: Beneficial effects of immunotherapy (bacterial toxins) on sarcoma of the soft tissues, other than lymphosarcoma. End results in 186 determinate cases with microscopic confirmation of diagnosis - 49 operable, 137 inoperable. Monograph #16. Cancer Research Institute, New York, 1975.

4. Nauts, H.D.: Osteogenic sarcoma: End results following immunotherapy (bacterial vaccines) 165 cases, or concurrent infections, inflammation or fever, 41 cases. Cancer Research Institute, Monograph #15. New York, 1975.

5. Coley, W.B.: The treatment of cancer. Guys' Hosp. Gaz. 26:7-14, 1911.

6. Nauts, H.C., Fowler, G.A.: End results in lymphosarcoma treated by toxin therapy alone or combined with surgery and/or radiation (87 cases) or with concurrent bacterial infection (14 cases). Monograph #6, New York Cancer Research Institute, Inc.*, New York, 1969.

7. Nauts, H.C.: The beneficial effects of bacterial infections on host resistance to cancer. End results in 449 cases. A study and abstracts of reports in the world medical literature (1775-1980) and personal communications. Cancer Research Institute, Monograph #8, 2nd Edition, 1980.

8. Nauts, H.C.: Bibliography of reports concerning the experimental or clinical use of Coley toxins (Streptococcus pyogenes and Serratia marmarcescens) 1893-1989. (394 references, including 143 by W.B. Coley). Published 1975,1977,1980,1982,1984,1985,1986,1987,1988,1989.

9. Moullin, C.M.: The treatment of sarcomata by the injection of mixed toxins (Coley's fluid). Brit. M.J. London 2: 451, 1898.

10. Coley, W.B.: Treatment of inoperable malignant tumors with toxins of erysipelas and the Bacillus prodigiosus. Trans. Amer. Surg. Assn. 12: 183-212, 1894.

11. Coley, W.B.: The influence of the Roentgen ray upon the different varies of sarcoma. Trans. Amer. Surg. Assn. 20:308-309, 1902. (Also in Med. News. 81:542-545.

12. Nauts, H.C.: Beneficial effects of acute concurrent infection, inflammation, fever or immunotherapy (bacterial toxins) on ovarian and uterine cancer. Cancer Research Institute, Monograph #17. New York. 1977. (122 pp.) and conference with Calkins, 1943.

13. Matagne, H.: Premiers essais de traitement des tumeurs malignes inoperables par les toxines de Coley: un cas de cancer gueri. Gaz. Med. Liege 8: 401-402, 1896. (See also: Rapport de la commission qui a ete chargee d'examiner le memoire de M. le Dr. H. Matagne, a Bruxelles, initule: Premiers essais traitement des tumeurs malignes inoperables par les toxines de Coley; un cas de cancer gueri. Bull. Acad. Roy. de Med. de Belg. (Brussels) 10: 275-278, 1896.

14. Matagne, H.: Traitement des tumeurs malignes inoperable par l'erysipele et par les toxines de Coley. Press Med. Belge, (Brussels) 51:603-607; 624-629; 633-639, 1899.

15. Matagne, H.: Les toxines de Coley employees dans le but de prevenir la recidive du cancer. Press Med. Belge 54:1-3, 1901.

16. Matagne, H.: Presentation de cancereux gueris par les toxines de Coley, employees conjointement avec intervention chirurgicale. Presse Med. Belge. 57:173-179, 1905.

17. Matagne, J.H.J.: Vers la guerison du cancer. Le Scalpel 104:504-544. 1951; and 106: 1387-1395, 1953.

18. Owens, J.F.: The use of toxins in the treatment of sarcoma, particularly of operable cases. Illinois State Med. Soc. Trans. (Chicago) 218-277, 1897. (also New Orleans, M. & S. J. 50:1-8, 1897.

---

*Name changed to Cancer Research Institute Inc. in 1973.

19. Coley, W.B.: The indications for the non-operable local treatment of tumors. The value of Toxins. Concord, N.H. Rep. Press Assn., 1896. (read before the New York State Medical Association, October 16, 1895. Reprint)

20. Editorial: J.A.M.A. Dec. 15, 1894; Coley's reply Jan. 5, 1895.

21. Harmer, T.W.: A study of the efficiency of mixed toxins (Coley) in inoperable sarcoma. A critical analysis of 134 microscopically proven cases. Boston M. & S. J. 172: 331-338; 373-377; 411-416;440-448, 1915.

22. Harmer, T.W.: Remarks upon the effects observed in the use of the mixed toxins (Coley) in certain cases of sarcoma. Boston M. & S. J. 171: 253-261, 1914.

23. Nauts, H.C.: Giant Cell Tumor of Bone: End results following immunotherapy (Coley toxins) alone or combined with surgery and/or radiation (66cases), or with concurrent infection (4 cases). Monograph #4, 2nd Edition, Cancer Research Institute, Inc., New York, 1975.

24. Nauts, H.D.: Immunological factors affecting incidence prognosis and survival in breast cancer. Part I: Factors affecting host resistance to breast cancer and therefore its incidence and response to treatment. Part II: The immunopotentiating effects of concurrent infections, inflammation or fever. Part III: Immunotherapy, effects of bacterial vaccines. Cancer Research Institute. Monograph #18, New York, 1984.

25. Coley, W.B.: Cancer of the testis; containing a report 64 cases, with special reference to 12 cases of cancer of the undescended testis. Trans. South Surg. & Gyn. Assn. 26:17-67, 1914. (Also in Ann. Surg. 62:40-73, 1915).

26. Coley, W.B.: Primary neoplasms of the lymphatic glands including Hodgkin's disease. Ann. Surg. 63:35-70, 1916.

27. Coley, W.G. & Hoguet, J.B.: Melanotic cancer; with a report of ninety cases. Trans. Am. Surg. Assn. 34:319-383, 1916.

28. Coley, W.B.: Multiple myeloma. Ann. Surg. 93:77-89, 1931. (Also in Trans. Amer. Surg. Assn. 48:489-514, 1931).

29. Coley, W.B.: Endothelioma, or Ewing's tumor. Am. J. Surg. 27:7-18, 1935.

30. Fowler, G.A. & Nauts, H.D.: The apparently beneficial effects of concurren infections, inflammation or fever and of bacterial toxin therapy on neuroblastoma. New York Cancer Research Institute, Inc., Monograph #11, New York, 1970.

31. Fowler, G.A.: Beneficial effects of acute bacterial infections or bacterial toxin therapy on cancer of the colon or rectum. New York Cancer Research Institute, Monograph #10, New York, 1969.

32. Nauts, H.C.: Enhancement of natural resistance to renal cancer: Beneficia effects of concurrent infections and immunotherapy with bacterial vaccines. New York Cancer Research Institute, Inc. Monograph #12, New York, 1973.

33. Nauts, H.C.: Bacterial products in the treatment of cancer: past, present and future. In Bacteria and Cancer, eds. J. Jeljaszewicz, G. Pulverer & W. Roszkowski, Proc. International Colloquium on Bacteria and Cancer, Cologne, Germany, March 16-18,1982. London/New York, Academic Press, 1982, pp. 1-25.

34. Beebe, S.P. & Tracy, M.: The treatment of experimental tumors with bacterial toxins. J.A.M.A. 49: 1493-1498, 1907.

35. Coley, W.B. & Coley, B.L.: Primary malignant tumors of the long bones; end results in one hundred and seventy operable cases. Ann. Surg. 13: 779-836, 1926; 14: 63-141, 1927.

36. Coley, W.B.: Endothelioma, or Ewing's tumor. Am. J. Surg. 27:7-18, 1935.

37. Miller, T.N. & Nicholson, J.T.: End results in reticulum cell sarcoma of bone treated by toxin therapy alone or combined with surgery and/or radiation (47 cases) or with concurrent infection (5 cases). Cancer 27: 524-548, 1971.

38. Coley, W.B.: Diagnosis and treatment of bone sarcoma. Glasgow, M.J. 126:49-86; 128-164, 1936.

39. Thomson, J.F.: Radiation protection in mammals. Reinhold Publishing Corporation, New York, p. 178, 1962.

40. Ainsworth, E.J. & Forbes, P.D.: The effect of Pseudomonas pyrogen on survival of irradiated mice. Radiation Research 14:767-774, 1961, and personal communications.

41. Ainsworth, E.J.: The effect of pyrogen dose in radiation protection. Radiation Research 14:446-447, 1961.

42. Coley, W.B.: The treatment of inoperable sarcoma by bacterial toxins (the mixed toxins of the streptococcus of erysipelas and the Bacillus prodigiosus). Practitioner (London) 83:589-613, 1909. (Also in Proc. Royal Soc, Med., Surg., Sect. 3:1-48, 1909-1910. (Abst. in Brit M.J. 2: 144-145, 1909).

43. Coley, W.B.: The treatment of cancer. Guys' Hosp. Gaz. 26:7-14, 1911.

44. Nauts, H.C.: Immunology of Cancer -- The Pioneer Work of Coley. Presented at: International Symposium on Endotoxin: Structural Aspects and Immunobiology of Host Responses, Riva del Sole, Giovinazzo (Bari), Italy, May 29 - June 1, 1986.

45. Old, L.J.: Tumor Necrosis Factor. First identified because of its anti-cancer activity, the factor is now recognized to be one of a family of proteins that orchestrate the body's remarkable complex response to injury and infection. Scientific American: 59-75, May, 1988.

46. Coley, W.B.: Office records 1892-1936.

47. Coley, W.B.: The treatment of malignant inoperable tumors with the mixed toxins of erysipelas and Bacillus prodigiosus, with a brief report of 80 cases successfully treated with the toxins from 1893-1914. Brussels, M. Weissenbruch, 172 p., 1914.

48. Coley, W.B.: Prognosis and treatment of giant-cell sarcoma, based on a further study of end results in 69 cases. Ann. Surg. 86:641-665, 1927.

49. Coley, W.B.: End results in Hodgkin's disease or lymphosarcoma treated by mixed toxins of erysipelas and Bacillus prodigiosus, alone or combined with radiation. Trans. Amer. Surg. Assn. 46:331-357, 1928. (also in Ann. Surg. 88:641-667, 1928).

50. Johnston, B.: Clinical effects of Coley's toxins. I. A controlled study. Cancer Chem. Repts. 21:19-41, 1962.

51. Johnston, B.: Clinical effects of Coley's toxins. II. A seven-year study. Cancer Chem. Repts. 21:43-68, 1962.

52. Havas, H.F., Grosbeck, M.E. & Donnelly, A.J.: Mixed bacterial toxins in the treatment of tumors. I. Methods of preparation and effects on normal or Sarcoma 37-bearing mice. Cancer Res. 18:141-148, 1958.

53. Havas, H.F. & Donnelly, A.J.: Mixed bacterial toxins in the treatment of tumors. IV. Response of methylcholanthrene-induced, spontaneous, and transplanted tumors in mice. Cancer Res. 21(1):17-25, 1961.

54. Nauts, H.C.: Multiple Myeloma: Beneficial effects of acute infections or immunotherapy (bacterial vaccines). Cancer Research Institute, Inc., Monograph #13, New York, 1975.

55. Kempin, S. et al: Improved remission rate and duration in nodular non-Hodgkin's lymphoma (NNHL) with the use of mixed bacterial vaccine (MBV). Trans. Amer. Soc. Clin. Oncol. 22:514, 1981.

56. Kempin, S. et al: Combined modality therapy of advanced nodular lymphomas (NL). The role of non-specific immunotherapy (MBC) as an important determinant of response and survival. ASCO extract, May, 1983.

57. Zheren, G. & Nauts, H.C.: Pilot study of mixed bacterial vaccine (MBV) on pediatric cancers: 57 cases. In manuscript.

58. Tang, Z. et al: Experimental and clinical studies on mixed bacterial vaccine in the treatment of primary liver cancer as adjuvant. In manuscript.

59. Waisbren, B.A., Sr.: Observations on the combined systemic administration of mixed bacterial vaccine, Bacillus Calmette-Guerin, transfer factor, and lymphoblastoid lymphocytes to patients with cancer, 1974-1985. J. Biol. Response Modifiers 6:1-19, 1987, and Personal Communications, 1977-1989.

45a. Weinberg, E.G.: Iron withholding: a defense against infection and neoplasia. Physiological Reviews 64:65-102, 1984.

45b. Torrance, J.D., Charlton, R.W., Simon, M.O. et al: The mechanism of endotoxin induced hypoferraemia. Scand. J. Haematol. 21:403-410, 1978.

# GENERATION OF NON MHC RESTRICTED CYTOTOXIC IMMUNE RESPONSES: EFFECTS OF "IN VITRO" HYPERTHERMIC TREATMENT

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## INTRODUCTION

Hyperthermia represents a promising approach to the therapy of neoplastic diseases. Application of local hyperthermia to a tumor has been suggested as a method to selectively enhance the action of chemotherapeutic agents at the tumor site (1). Moreover evidence suggests that hyperthermia by itself or in conjunction with X rays produce an increase in the cure rates of human tumors (2). Many studies support the hypothesis that the plasma membrane is a critical target for heat cell inactivation. Hyperthermia has been reported to induce structural changes in cell membranes (3-5). The mechanisms involved in hyperthermic action have been reviewed extensively (6,7) but little is known about the effects of hyperthermic treatment on immune response. As many recent data enlight the effectiveness of adoptive immunotherapy protocols in the control of neoplastic diseases (8,9), we sought to investigate the feasibility of a combined-therapeutic approach including hyperthermia and adoptive immunotherapy. In this line we studied the effects of hyperthermic treatment on basic immune responses, such as anti T3-Ti triggering induced lymphoproliferation, and the generation of non MHC-restricted cytotoxic activity against tumor cells.

## MATERIALS AND METHODS

### PBMC preparation and heat treatment

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood samples from healthy donors by gradient centrifugation according to standard methods. Cells were then washed twice and resuspended in RPMI medium (GIBCO, Grand Island, New York, USA) supplemented with Glutamine (2 mM),10% pooled AB serum and antibiotics (complete medium). When indicated, separated PBMC were heat treated in a

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precision water bath (Haake D8, West Germany) for the appropriate timing. Cells were then washed, adjusted at the indicated concentrations in complete medium and used as detailed in individual experiments.

Proliferation assays

PBMC were cultured in complete medium at $10^6$ /ml final concentration in 0.2 ml in 96 round bottom microwell trays (Nunc, Denmark). All tests were performed in triplicate. Lymphocyte activation through T3-Ti complex triggering was induced by a mitogenic anti T3 monoclonal antibody (Mab), CBT3G ,gently provided by F.Malavasi, (Turin,Italy).Ascitic fluid was added to the cultures at 1:1000 final dilution. Lymphoproliferative response was assessed by 3H thymidine incorporation according to standard methods on day three of culture.

Generation of LAK cells and cytotoxicity tests

In order to generate IL-2 activated killer cells, PBMC were cultured at $10^6$ /ml concentration in complete medium in the presence of 100 units /ml recombinant IL-2 (courtesy of Dr. Garotta, Roche, Basel,Switzerland) in 24 wells plastic trays (Costar, Cambridge, USA) for seven days. In parallel cultures IL-2 induced proliferation was also measured by 3H thymidine incorporation on day seven. Cytotoxic activity of LAK cells was assayed by a 4 hours 51Cr release test as previously detailed (10). Briefly, NK sensitive (K562) or NK resistent (1301) target cells were labelled by a 1 hour incubation with 150 µCi of a 51Cr solution (0.1 mCi: 298.08mCi/ml)washed and added to serially diluted effector cells in 96 wells round bottom microtrays. After a 4 hours culture at 37 C. in humidified atmosphere, supernatants were collected and radioactivity was measured by a gamma counter. Specific lysis was calculated according to the standard formula.

Immunofluorescence studies

In immunofluorescence studies resting or cultured PBMC were labelled with FITC conjugated monoclonal antibodies as indicated in the following section, (Beckton Dickinson, Mountain View, USA) according to standard protocols. Cells were then washed and samples were analyzed by FACS Scan (Ortho, USA).

Interferon assay

Day 7 supernatants of IL-2 stimulated and control PBMC cultures were assayed for Interferon gamma (IFN-gamma) content by inhibition of Sindbis virus hemagglutinin yield after a single growth cycle as previously detailed (11).

**RESULTS AND DISCUSSION**

In preliminary experiments we tested the effects of heat treatment on viability of PBMC and we found that 42.5 C heat treatment prolonged up to 3 hours doesn't significantly affect PBMC viability while after 18 hours of treatment a 30% cell mortality, as assessed by dye exclusion, was observed.

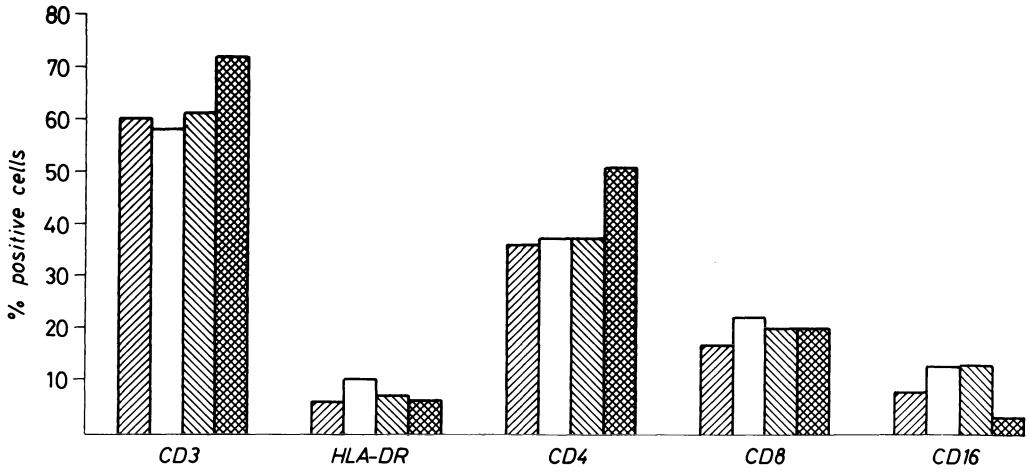


**Fig.1. Effects of hyperthermic treatment on the viability of PBMC subsets.**

PBMC were separated and heat treated (42.5 C) for ○ hour (▨), 2 hours (▥), 3 hours (▩) or 18 hours (▩). Cells were stained with specific FITC conjugated Mabs according to standard protocols. Data are expressed as percent of cells positive for the indicated Mab.

We next sought to determin whether a selective heat induced toxicity on specific PBMC subpopulation could be observed. As shown in figure 1 heat treatment prolonged up to 3 hours

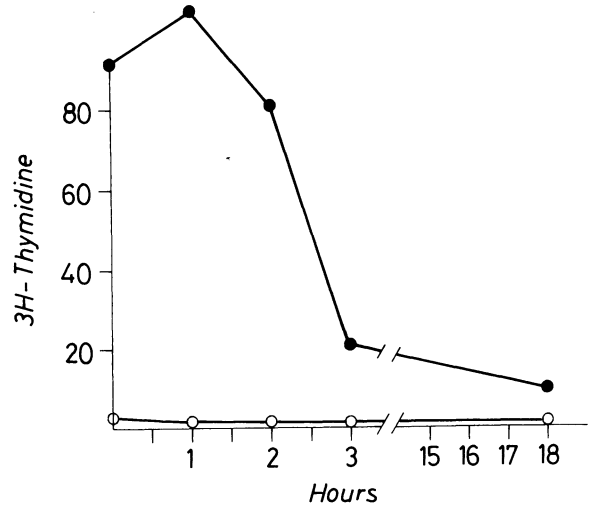


**Fig.2. Effects of hyperthermic treatment on anti T3 induced PBMC proliferation.**

Separated PBMC were treated for the indicated times at 42.5 C temperature. Cells were cultured in the presence (●), or absence (○) of a 1:1000 dilution of a mitogenic anti CD3 Mab containing ascitic fluid. Lymphoproliferation was assessed by 3H thyimidine incorporation on day 3 of culture.

did not induce selective loss of CD3, DR, CD4, CD8 or CD16 positive cells. On the other hand after 18 hours treatment we observed a significant reduction of CD16 positive cells, a cell subset including a large part of non MHC restricted cytotoxic effector cells.

In subsequent experiments we assayed the effects of heat on the PBMC activation induced by triggering of the antigen receptor complex by monoclonal antibodies (12). As shown in figure 2 we found that hyperthermic treatment prolonged up to 2 hours did not adversely affect anti T3-induced lymphoproliferation, while longer treatments markedly depressed antigen receptor mediated PBMC activation.

We then assayed the capacity of recombinant IL-2 used at 100 U/ml to induce activation of 42.5 C pretreated PBMC. As shown in figure 3 (panel A), proliferation induced by IL-2 was indeed sligthly increased in heat treated PBMC when hyperthermic treatment was applied no longer than 3 hours. On the other hand, after 18 hours treatment we observed a marked decrease of IL-2 induced PBMC proliferation. Generation of non MHC restricted cytotoxic activity against tumor cells induced by IL-2 was not affected by previous hyperthermic treatment. As shown in figure 3(panel B) cytotoxic activity against K562 target cell line was practically unchanged in heat treated PBMC. A slight

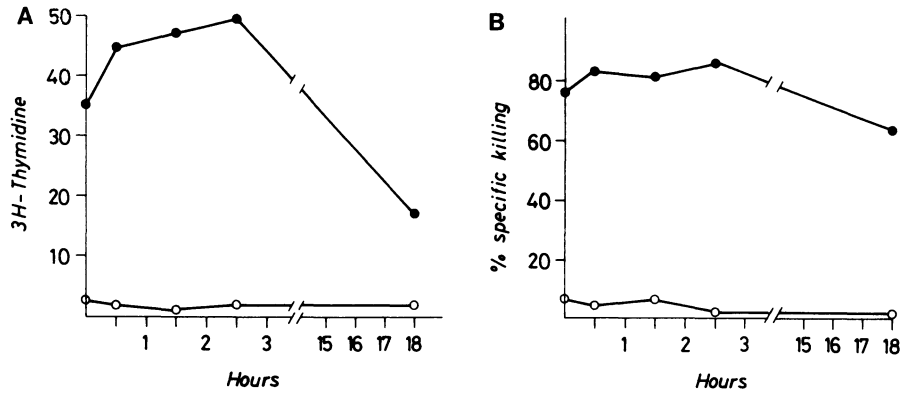


**Fig.3. Effects of hyperthermic treatment of PBMC on IL-2 induced proliferation and generation of cytotoxic activity.**

Separated PBMC were treated at 42.5 C for the indicated times. Recombinant IL-2 (100 U) was then added (●) or not (○) and cultures were prolonged for 7 days. Proliferation was assessed by 3H thymidine incorporation (panel A). 7 days cultured cells were also assayed for cytotoxic activity against K562 target cells. Data are reportedas percent specific killing (panel B).

reduction was observed in 18 hours pretreated PBMC. Similar results were obtained by using as target an NK resistant cell line (1301).

Among the soluble factors whose production is induced in stimulated PBMC a special role is played by IFN-gamma. Indeed

it has been shown by us and others that block of IFN-gamma effects by specific monoclonal antibodies adversely affect the generation of non MHC restricted cytotoxic activity induced by IL-2 or microbial antigen (13,14). We thus assayed the production of IFN-gamma induced by IL-2 stimulation in heat treated PBMC. We observed that heat treated PBMC produced significantly higher amounts of IFN-gamma than untreated cultures with a peak production in 3 hours treated PBMC. IFN-gamma production, however, sharply decreases in 18 hours treated PBMC.

Taken together our data emphasize the apparent lack of immunotoxicity of hyperthermic treatment prolonged up to 2-3 hours. These data also enlighten the possibility of associating hyperthermia and adoptive immunotherapy, at least in some phases of the treatment, such as lymphoapheresis, in that heat treated PBMC seem to be fully competent for the generation of LAK effector cells. A further step will be the assessement of the role, if any, of the immune system in the clinical effectiveness of hyperthermic treatment in cancer patients.

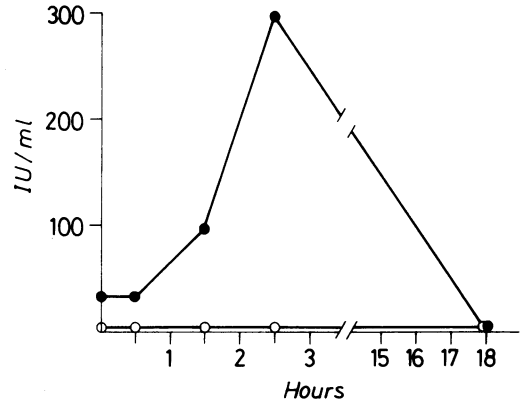


**Fig.4. Effects of hyperthermic treatment on IL-2 induced production of IFN gamma by PBMC.**

PBMC were heat treated (42.5 C), cultured in complete medium in the presence (●) or absence (O) of 100 U rIL-2. Supernatants were collected on day 7 of culture and assayed for IFN gamma activity.

## REFERENCES


1. Magin,R.L.,Cysyk,R.L.,Litterst,C.L..Cancer Treat.Rep.,64, 203,(1980).
2. Van der Zee,J.,Van Rhoon,G.C.,Wike-Hooley,J.L..Brit.J. Radiol.,58,243,(1985).
3. Bass,H.J.,Moore,J.L.,Coakley,W.T..Int.J.Radiat.Biol.,33, 57,(1978).
4. Lin,P.S.,Wallach,D.F.H.,Tsai,F..Proc.Natl.Acad.Sci.U.S.A., 70,2492,(1973).

5. Coss,R.A.,Dewey,W.C.,Bamburg,J.R..Cancer Res.,39,1911, (1979).
6. Spiro,I.J.,Denman,D.L.,Dewey,W.C..Radiat.Res.,89,134, (1982).
7. Anghileri,L.G.,Marcha,C.,Crone-Escanye,M.C.,Robert,J..Eur. J.Cancer Clin.Onc.,21,981,(1985).
8. Rosenberg,S.A..J.N.C.I.,75,595,(1985).
9. Grimm,E.A.,Wilson,D.J..Cell.Immunol.,94,568,(1985).
10. Ausiello,C.M.,Sorrentino,V.,Ruggiero,V.,Rossi,G.B.,Immunol. Lett.,8,11,(1984).
11. Stanton,G.J.,Langford,M.J.,Dianzani,F..Methods Enzymol., 78,351,(1981).
12. Alcover,A.,Ramarli,D.,Richardson.N.,Chang,H.-C.,Reinherz, E.L.. Immunol.Rev.,97,1,(1987).
13. Herberman,H.B..In:Vilcek J.and De Maeyer,E.eds." Interferon and Immune system".Elsevier,Amsterdam.Pp.61. (1984).
14. Ausiello,C.M.,Spagnoli,G.C.,Antonelli,G.,Malavasi,F., Dianzani,F.,Casciani,C.U.,Cassone,A..J.Biol.Reg. Homeost.Agents,1,59,(1987).

# RESPONSES OF IMMUNE SYSTEM TO HYPERTHERMIA

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The evidence of time dependent, heat induced impairment of immune functions is unequivocal from in vitro studies. While earlier investigations at moderately elevated temperatures led researchers to postulate that in the clinical set-up hyperthermia might in fact stimulate the immune system. the growing number of studies at more relevant temperatures provide no support for this concept. For review of the temperatures see Skeen et al, 1986. Since the importance of immune reactions in tumor control after hyperthermia has been firmly established in immunodeficient mice (Alfieri et al, 1981) the relevance of in vitro data to the situation in inpatients undergoing hyperthermia remains to be determined. The clinical situation is obviously more complex and it has proven in the past to be difficult to correlate patients immunological parameters with clinical outcome in cancer patients.

In spite of this problem, modification of the immune response has gained increased attention in the recent years as the possible practical approach to the adjuvant treatment of cancer patients. The hope for eventual development the treatment modality of the clinical value lies both in modulation of the specific and nonspecific immune response. Among mechanisms of the specific immune response under current consideration are monoclonal antibodies and tumor infiltrating lymphocytes (TILS), especially after genetic manipulation (S. Rosenberg, 1989, personal communication).

Among cells mediating non-major histocompatibility complex (MHC) restricted (non-specific) cytotoxicity special attention is devoted to lymphokine-activated killer (LAK) cells and activated macrophages.

Some of the cells involved may not only mediate tumor cytolysis by themselves but may release molecules with anti-tumor activity like tumor necrosing factor-alpha and lymphotoxin. Hyperthermia may be put into this context in order to determine under what circumstances and in what way it may modify naturally occurring phenomena of anti-neoplastic nature.

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Alternatively, hyperthermia may be used in combination with any of the various forms of adoptive immunotherapy.

The essential problem is that the precise mechanisms of the antitumor action of the immune system are not always clear especially in the in vivo conditions. For instance, it is unknown how Interleukin-2 induces remissions in some cancer patients. The role played by LAK cells is not well understood either, especially because in spite of being capable of killing tumor target cells in vitro, LAK cells do not home in the tumor in vivo.

To the best of our knowledge there are no studies reported which deal with the issue of how increased temperature affects TIL'S, in vitro or in vivo in animal or human tumors. The effect of hyperthermia on macrophages activated to express tumor-specific cytolytic function is poorly understood. No data are available on the effects of hyperthermia as an adjunct to the immunotherapy with recombinant Interleukin-2 (rIl-2), alpha and gamma interferons, tumor necrosing factor-alpha and lymphotoxin.

All the above subjects are open for investigation in both animal and human models. The only study related to above questions available at this time is on the effects of hyperthermia on human LAK and NK cells functions, and is published by McLaren and Olkowski elsewhere in this volume.

In parallel with their earlier studies on the effects of hyperthermia or the mitogenic response of human lymphocytes, the data suggests that mild hyperthermia delivered in less than 60 minutes time stimulates MHC non-restricted lymphocyte cytotoxicity while higher temperatures above 43°C and prolonged hyperthermia (over 60 min.) results in the severe functional impairment.

It is of interest that immune modulators, such as rIl-2, rIl-1 alpha interferons, tumor necrosing factor alpha, administered to cancer patients in Phase I & II clinical trials, produce FEVER. One may speculate that the antitumor activity of above molecules may be related to hyperthermia induced this way.

It has to be pointed out that while diversity of immune cells and molecules may play an important role in the tumor control, hyperthermia by itself may affect different tumors in a variety of ways depending on their rate of growth, vascularization, expression of HLA, dependence on the specific growth factors, including possible expression of receptors for molecules utilized as inducers of immune cells, such as rIl-2. (Aughileri, 1986).

In conclusion, recent advances in experimental and clinical immunotherapy of cancer require extensive update of the knowledge regarding the combination of hyperthermia with newest immunomodulators to increase the cure rate of malignant disease.

REFERENCES

1. Alfiere, A.A., Hahn, E.W., and Kim, J.H.
-- Role of cell mediated immunity in tumor eradication by hyperthermia.
Cancer Res. 41, 1301, 1981

2. Anghileri, L.J. - Role of tumor cell membrane in hyperthermia. In "Hyperthermia in Cancer Treatment."
Eds. L.J. Anghileri and J. Robert
Vol. 1, 1, 1986, CRC Press, Boca Raton, Fla.

3. Skeen, M.J., McLaren, J.R., and Olkowski, Z.L.
Influences of Hyperthermia on Immunological Functions.
Ibid., Vol. 1, 94, 1986

# PERSPECTIVES FOR THE COMBINED USE OF PHOTODYNAMIC THERAPY AND HYPERTHERMIA IN CANCER PATIENT

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Chemotherapy and/or radiotherapy widely used in the last decades for cancer treatment are frequently barely effective on tumor growth and metastatic spread. Years of disappointing results, at least for the large majority of human solid tumors, induced the search for more active treatments. Photodynamic therapy (PDT), a relatively new method, has been tested for the treatment of a certain number of chemoresistant cancers, sometimes successfully (1). Recent in vitro and in vivo experiments suggest that the combination of hyperthermia (HT) and PDT can increase the therapeutic effect of these two therapies when used in combination (2,3,4).

The presence of molecular oxygen is essential for the success of PDT, since single oxygen and other oxygen-derived species are the main cytotoxic agents in this treatment modality. When the oxygen concentration in the medium is low, the damaging effect of photosensitization on cells is remarkably reduced or even nil. This raises serious problems in the clinical use of photosensitization for treatment of cancer, since most solid tumors contain cells living under extremely low oxygen pressures. To understand fully the extent of protection from PDT that hypoxia confers on tumors cells, the following points should be considered: (a) the oxygen concentration in the air at sea level is about 0.24 mM and it's partial pressure is 152 mmHg: (b) in arterial blood, the oxygen concentration is about 0.12 mM (78 mmHg); (c) in normal tissues $p(O_2)$ of about 40 mmHg are found (63.1 mM) ; (d) in tumors $0 < p(O_2) < 40$ mmHg has been determined. The number of cells living and in some cases proliferating in $p(O_2)$ values lower than 5 mmHg (7.89 mM), i.e. the "hypoxic fraction", varies widely with tumor type and growth site. Hypoxic cells may reoxygenate after the destruction of oxygenated cells by therapy and resume active proliferation, thus causing local tumor growth.

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It was observed that if PDT is immediately followed by HT, a superadditive (synergistic) potentiation is obtained. Some hypothesis have been put forth, but a satisfactory explanation for such synergism has not been proposed. In this work, through analysis of various aspects of the two therapies is performed and a possible interaction mechanism is derived. This analysis moreover explains the specific treatment sequence requested to obtain a synergistic potentiation (that is, PDT prior to, and not vice versa).

## MECHANISM OF ACTION AND EFFECT ON TUMOR VASCULATURE

PDT is primarily based on the preferential uptake and retention of some porphyrins by tumor (and, in general)by all tissues characterized by a high proliferation rate with respect to most normal tissues. A differential porphyrin distribution is observed at tumor level: the extracellular compartment and, in particular, the vascular area, macrophages and necrotic areas contain higher drug concentrations than the tumor cells themselves(5). (N,B.The vascular endothelial cells of tumors,where the highest percentage of drug concentration is found, have a proliferation rate that exceeds what is found in normal tissues by a factor ususally greater than 20). After specific light activiation of the porphyrin-containing tumor, a highly cytotoxic species,singlet oxygen,is produced due to porphyrin sensitized photodynamic action. Several hours after PDT tumors show evident hemorrage,congested vessels, platlet thrombi and lysed tumor cells. The main factor that contributes to tumor destruction by PDT seems to be the stoppage of blood flow and the resultant ischemia of the tumor due to collapse of the vasculature(6).When HT is performed before PDT, the vascular modification caused by heat drastically decreases light penetration in the tumor; the efficiency of PDT is therefore highly impaired.

## MICROENVIRONMENTAL OXYGEN AND pH RELATED EFFECTS

Tumors usually contain a high proportion of hypoxic cells that may be resistant to PDT (7).The hypoxic cells, due to their high glycolisis, live in an acidic environment, and therefore are very heat sensitive. There may thus be a complimentary effect of HT and PDT, in terms of the destruction of this population, such as the one that exists between HT and Radiotherapy (8). PDT causes a sharp reduction of PO2, due to the abrupt reduction for blood flow, and no significant shift of PH, since it does not evoke any metabolic stimulation (9). If HT is performed after PDT on already badly perfused environment ulteriorly hinders the efflux of heat from the tumor;the temperature differential between the tumor and the surrounding, well perfused, normal tissues will therefore ultimately increase. This may be a further parameter contributing to the synergism between PDT and HT, and again to the observed sequence importance. The increase in acidity and the

decrease in nutritional supply during HT increases the thermal damaging effect on the surviving tumor cells. An effect that has not so far deserved due attention is the influence of the heat-induced vascular alterations on the normal tissue and to the tumor. As a matter of fact, the increase inblood flow and in vascular permeability observed concentration there;on the other hand,vascular status in the tumor may decrease drug delivery,therefore decreasing the therapeutic index(7).

## CELLULAR RELATED EFFECTS

The macroscopical effects of PDT and HT on the vasculature,presumably the main responsible for the observed synergism,are due to collapse of the tumor peculiar endothelia (10). The microscopical effects that condition the response not only of the tumor endothelial cells but also of macrophages and the tumor cells themselves, worth therefore to be considered. The cellular damaging effects of PDT and HT will now compare recalling that cell activity requires selectivity of membrane permeability, and that reproductive capability is assured by the integrity of the nucleus and of the intracellular organelles.

### Effect of PDT

The cellular response to PDT is dependent on several experimental parameters (e.g. composition,concentration and mode of delivery of the sensiltizer, incubation time, oxygen concentration, photoactivatin energy, presence of serum proteins(11). Furthermore, cytotoxicity is due to the unspecified action of free radicals, that are produce after attack of singlet oxygen to electron rich areas of biomolecules (e.g. conjugated double bonds). It is thus rather difficult to evaluate the initial lesion, since free radicals initially formed in a well defined localization soon initiate chain reactions spreading damage all over the cell.

Electron micropsy, EM, is an almost ideal technique to observe damage onset and spreading. Although some papers describe the EM picture of a cell after PDT, they refer to different experimental conditions. One, however, deserves mentioning since it describes not only damage but also the structural modifications that suggest an attempt of repair. (12). The main effects observed are: (a) proportionally, much less proliferating and more degenerating and dying cells than before PDT; (b) soon after PDT,enlargement of cellular interspace, reduction of cell volume, diminution and shrinkage of microvilli and appearance of pseudopodia;(c)part of the membrane destroyed with cytoplasm shed outside; (d) vacoules of different dimensions, the larger of which occupy almost half of the cell; (e) loose atypical and no more compact, filaments in the cytoplasm; (f) immediately after PDT, blurred mitochondrial membranes, some shrunk mitochondria with indistinct cristae filled with

high density material and vacuoles, some electron density particles; as the time after PDT increases, fewer particles and more vacuoles in the mitochondria; (g) rough endopasmatic reticulum, RER, filled with high density material (h) formation of an increased number of Golgi complexes connected to several vacuoles; (i) synthesis of new lysosomes that pile up in the concave part of the nucleus; (l)rounded up nuclei with decrease electron density and decreased granular euchromatin; heterochromatin attached to the nuclear membrane; (m) several mitosis containing degenerated mitochondria,dilated ER vacuoles;(n) centrioles and condensed chromosome still seen in the mitosis without spindle fibers.Membranes, and, in particular, the plasma membrane, are the most important cellular targets of PDT.The mainmolecular effects of PDT at membrane level are: i oxidation of unsaturated liquids and cholesterol, and (ii) crosslinking and oxidation of some -SH groups of proteins. These chemical alterations lead in turn to a marked inhibition of the physiological activities of the membrane, and, in particular, of passive and active transports; this effect shows a marked correlation with porphyrin-induced loss of cell viability(13). It was observed, in particular:(a) enhancement of ANS binding, evidentiating increased membrane hydrophobicity;(b) inhibition of thymidine,nucleoside and main acid transport,that in part explains the inhibition of DNA and protein synthesis;(c)increased permeability to chromate, to K (in pat due to the oxidation of the -SH groups), and to actinomycine D; (d) inhibition of membrane enzymes (e.g. 5-nucleotidase, alkaline phosphatase, Na-K ATPase); (e) inhibition of cappine of antibodies and CON A agglutionation, which are properties that require normal fluidity of the plasma membrane and integrity of cytoskeleton; and (f) that cholesterol impregnation of leukemic cell membranes induces resistance to PDT. It should be mentioned that the cellular structures surrounded by membranes communicate dynamically with each other apparently by means of a continuous interchange in membrane fragments. Therefore, peroxidation initiating at the plasma membrane may easily propagate to other membranuous compartments. Mitochondria are one the PDTs main targets: Essential functions (e.g. respiration, oxidative phosphorilation and Ca membrane transport)are affected since the activity of enzymes such as cytochrome C oxidase and succinate dehydrogenase, and ATPase is a well known effect of photo sensitization that ultimately causes the release of hydrolytic enzymes. After PDT, leakage of acid phosphatase, B-glycoronodase, Arylamidase, B-galacrosidase and B-N-acetylglucosdaminidase was indeed reported. In particular, damage to the lysosome of endothelial cells after porphyrin sensitization in vivo causes stasis of blood, aggregation of cells, intravascular hemolysis and irreversible damage. Isolated DNA can be photodynamically damaged by porphyrins: alkali-labile sites and single and double strand breaks were reported. Photodynamic crosslinking of DNA to protein is also induced. When whole cells are submitted to PDT,DNA single strand breaks also

occur,at significantly lower rates than than with x-rays. The chemical nature of the strand breaks induced by PDT seem to be different than those produce by x-rays. These lesions are not always easily repaired,particularly when high light doses are used. The ability of DNA to serve as a template to DNA dependent RNA synthesis is impaired. Crosslinking of DNA proteins is also observed with whole cell irradiation. As concerns the repair processes after PDT, the following observations were made:(a)prophyrin sensitization rapidly inhibits viral and mammalian DNA polymerasees; (b) cells are able to accumulate and recover from PDT damage in a way similar to the accumulation and recovery from x-ray damage, "Elkind repair"; and, (c) PDT is followed by synthesis of stress proteins, similar to heat shock proteins or to the protein synthesized in response to glucose deprivation and chronic anoxia. These morphologic and biochemical effects were extensively reviewed (14).

The sensitivity to photodynamic inactiviation increase as the cells progress in their cell cycle from G1 to mid S phase (15). The particular susceptibility during the S phase was attributed to a peculiar condition of reduced microviscosity of plasma membrane damage in this phase. Although the speculation about a possible correlation between sensitivity to PDT and membrane viscosity seems quite plausible, another fact deserves consideration: in the S phase the DNA molecule is in a conformation fully extended for replication,and therefore particularly vulnerable to attack by external agents. These agents might be, for instance, the hydrolytic peroxidation. The effects of PDT on the nuclear structures do confirm the vulnerability of DNA, but it has not yet been clarified how the damage to the nucleus depends of the cell cycle phase. The damage bought to the DNA molecule may hinder its ability to act as a template for RNA synthesis, therefore impairing the synthesis of proteins with repair function.

Effects of Hyperthermia

An EM study that very exhaustively depicts the onset and propagation of damage induced by HT was published years ago (16). It was observed in this work that, during the first few days after treatment, the cellular morphology of nonmalignant tumor cells (e.g. fibroblasts and endothelial cells) was only slightly and reversibily damaged, while the malignant cells showed massive destructive alterations. This study also points out that the presence of a hypertrophic Golgi area,contemporary to the formation of larger lysosomes, indicates that a high lysosomal activity may be primarily important in heat induced cytotoxity.

In the EM study above reported, concerning the study of a tumor in vivo the thermal effect on the endothelial cells was irrelevant with respect to the effect on the malignant cells and, in particular, reversible. However, since HT is followed by occlusion of blood flow, damage to the vasculature should also play an important role in tumor

control. In effect, it was recently reported that, in vitro, the capillary endothelial cells are more heat sensitive than fibroblasts; at temperatures normally used in HT,damage results in cell death, demonstrable already at 30 min after initiation of a 44 C/30 min dose. In particular, capillary endothelial cells stimulated to proliferate are even more heat sensitive than quiescent endothelial cells. Since the tumor vasculature contains a significantly higher proportion of proliferating endothelial cells than the normal vasculature, the enhanced thermal sensitivity of the tumor neovasculature with respect to that of the host tissue is easily explained(6).The heat induced lethality results from the combined effect of several lesions to key structures in the cell, as it happens with PDT. For the sake of clarity, they will be again presented separately.

The plasma membrane, which is the first cellular structure where heat energy is deposited, contains lipids and proteins highly susceptible to thermal damage. The membrane morphology after HT, observed with scanning EM, shows a rapid and irreversible transformation: from ordinarily smooth it acquires a cobblestone morphology; most microvilli disappear and those that remain clump into a cap(17). It was long supposed that cytotoxicty was due to an irreversible increase in membrane fluidity, but it was later demonstrated that there is no correlation between thermal cell killing and membrane liquid fluidity (18). On the other hand, the activiation energy required for cell killing indicated a protein target for thermal damage. Heat has moreover a differential effect on the Na-K-ATPase: the capacity to bind ouabain decreases, while the ATP hydrolyzing acitivity on the membrane inner surface retains most of the original acitivity (20). This shows that, within the same protein that acts as sodium-potassium pump, two different regions are deputed to the mentioned properties, and each of these is influenced differently by heat.

One of the main mechanism also proposed for cell killing by HT consists in the cell digestion by hydrolytic enzymes released after lysosome rupture. This assumption is based on the synthesis of an increased number of lysosomes, and of an increased proteolytic enzyme activity after HT (21).Moreover, tumor cell lysosomes seem more heat-labile than normal cell lysosomes. However:(a) neither lysosomal damage by photosensitization with neutral red, nor intralysomial inhibition of hydrolases by trypan blue, modifies the onset of inhibition of respiration of tumor cells by HT(22);and,(b) agents that modify the lysosome membranes, either by labilizing them (retinol) or by stabilizing them (hydrocortisone), do not alter the response of the cells to HT(23). These experiments indicate that lysosomal damage by itself may not cause cell death, although it may substantially contribute to it.

The proteins of the mitochondrial membrane undergo structural transitions at hyperthermic temperatures. The marked morphological alterations of mitochondria, that are

illustrated by EM, have a functional equivalent as inhibition or depression of the oxidative metabolism (24) with consequent decrease in ATP levels(25). Anaerobic glycolysis in tumors, on the other hand, seem not to be very affected by heat, and thus represents the only energy producing pathway in heated cells. Glucose deprivation, in effect, highly increases the heat induced toxicity(26). The increased importance of glycolysis causes an increase in acidity in tumor cells and in extracellular space in the tumor tissue. In these conditions, the activity of the lysosomal acid hydrolases may be intensified. It was already mentioned that heat affects the number and distribution of the "primary stimulus" receptors of the cell located in the plasma membrane. Heat besides affects "secon message" substances, such as $Ca^{++}$, $H^{+}$, and cyclic AMP, increasing their intracellular concentration. It was suggested that the increase in cell volume during and after HT is due to osmotic changes resulting from H ion flux(27). A recent speculation, moreover, proposes that HT induces alterations in the information flow through the cell, that starts at the outer receptors of the plasma membrane,continues in the internal signal effectors and later disrupts the cytoskeleton and inhibits protein and DNA synthesis(25).Therefore, heated cells are unable to complete cytokinesis, they become tetraploid, and loose their reproductive integrity(28).

## CONCLUSIONS

In spite of these premises, most researchers involved in PDT clinical practice have not yet faced the fact that tumor regrowth due to spared hypoxic cells can easily be foreseen and should thus be expected after a single PDT dose. Since this event seriously hampers PDT and since established procedures are readily available to overcome hypoxia, clinicians should not ignore the interest and potentiality of the association of such methods with PDT in clinical experimentation. A detailed analysis of the biological effects of PDT and HT explains why this combination should be complementary if PDT is performed before HT. In effect, while PDT affects the tumor vasculature in the first place, and only secondarily the well-oxygenated tumor cells, HT primarily destroys the tumor cells living in an acidic, nutritionally deprived, environment (that are, moreover, hypoxic), and, to a lesser extent, also the tumor vasculature. If HT is performed before PDT, a synergism is not obtained, since HT causes a sharp decrease in tumor oxygenation (oxygen is one of the PDT"fuels") capillary collapse and hemorrhagic phenomena, that hinders light penetration in the tissues. On the contrary, PDT before HT favors HT; in effect, the deterioration of the tumor blood flow induced by PDT further hinders heat dissipation by blood circulation, and therefore increases the temperature differential between the tumor and the normal surrounding tissue. Also at the cellular level the damaging effects of PDT and HT reinforce each other, particularly at membrane

and nuclear levels. The overall mutagenicity of the combination PDT+HT, not yet determined, may not be negligible, although, separately, each of these therapies seems to be much less dangerous than equivalent doses of x-rays. Particular caution is requested to this specific point.

REFERENCES

1) T.J. Dougherty:Photodynamic Therapy. In G.YORI and C.PERRIA eds, photodynamic Therapy of Tumors and Other Diseases. Libreria Progetto, Padova,1985

2) S.M. WALDOW, B.W. HENDERSON, T.J. Dougherty: Potentiation of Photodynamic Therapy by Heat: Effect of Sequence and Time Interval Between Treatments in Vivo. Lasers. Surg. Med. 5:83-94, 1985

3) T.S. Mang, T.J. Dougherty: Time and Sequence Dependent Influence of In Vivo Photodynamic Therapy Survival by Hyperthermia. Photochem. Photobiol 42:533-540, 1985.

4) P.C. LEVENDAG et al.: Interaction of Interstitial Photodynamic Therapy and Interstitial Hyperthermia in a Rat Rabdomiosarcoma. Abstracts in Biological effect of Nonionizing Electromagnetic Radiation, 37-38, 1989

5) P. BUGELSKY, C.W. PORTER, T.J. DOUGHERTY: Autoradiographic Distribution of Hematoporphyrin Derivative in Normal and Tumor Tissue of The Mouse. Cancer. Res 41:4606-4612, 1982

6) J. DENEKAMP: Endothelial Cell Proliferation as a Novel Approch to Targeting Tumor Therapy. Br. J. Cancer 45:136-139, 1982

7) P. VAUPEL: Hypoxia in Neoplastic Tissue. Microvasc. Res. 13:399-408, 1977

8) J.T. LEITH, R.C. MILLER, E.W. GERNER, M.L. BOONE: Hyperthermic Potentiation: Biological Aspects and Applications to Radiation Therapy. Cancer 39:766s-779s, 1977

9) H.I.BICHER, F.W. HETZEL, P. VAUPEL, T.S. SANDHU: Microcirculation Modifications by Localized Microwave Hyperthermia and Hematophorphyrin Phototherapy. Biblioth. Anat. 20:628-632, 1981

10) P.VAUPEL, S. FRINAK, H.I.BICHER: Heterigenous Oxygen Partial Pressure and ph Distribution in CH3 Mouse Mammary Carcinoma. Cancer. Res. 41:2008-2013, 1981

11) D.R. DOIRON, C.J. GOMER: Porphyrin Locaslization and Treatment of Tumors, Alan R. Liss Ed. 1984

12) N. AI-LAN, P. OIONG-OIAN: Studies on Hematophorphyrin-

photosensitized Effect on Human Cancer Cells in Vitro: TEM and SEM observations. In : Methods in Phorphyrin Photosensitization, edited by D. Kessel, Plenum Press, 117-122, 1985

13) D. KESSEL: Effect of Photoactivated Porphyrin at The Cell Surface of Leukemia L1210 Cells. Biochemistry 16:3443-3449, 1977

14) I. FREITAS: Photodynamic Therapy and Hyperthermia:Common and Complementary Effects. Medecine Biologie Enviornment 14:93-111, 1986

15) T. CHRISTENSEN, J.MOAN, E. WIBE.R. OFTEBRO:Photodynamic Effect of Haematoporphyrin Thoughout The Cell Cycle of The Human Cell Line NHIK 3025 Cultivated in VItro. Br. J. Cancer 39:64-68,1979

16) J.OVERGAARD: Ultrastructure of Murine Mammary Carcinoma Exposed to Hyperthermia in Vivo. Cancer Res. 36:983.985,1976

17) P.S. LIN, F.H. WALLACH, S. TSAI: Temperature Induced Variations in The Surface Topology of Cultured Lymphocytes Are Revealed by Scanning Electron Microscopy. Proc. Nat. Acad. Sci. USA 70:2492-2496,1973

18) J.R. LEPOCK, P. MASSICOTE-NOLAN, G.S. RULE,J. KRUV:Lack of COrrelation Bewteen Hyperthermic Cell Killing, Thermotolerance, and Membrane Lipid Fluidity. Radiat. Res. 87:300-313,1981

19) D.B. LEPE:Molecular and Cellular Mechanisms of Hyperthermia Alone or Combined with Other Modalities. In: Hyperthermic Oncology, 1984, edited by J. Overgaard, Taylor and Francis, London, pp 9-40,1984

20) R.L. ANDERSON,G.M. HAHN:Differential Effects of Hyperthermia on the Na, K ATPase of Chinses Hamster Ovary Cells. Radiat. Res. 102:314-323,1985

21) G.M. BARRATT,E.D. WILLS: The Effect of Hyperthermia and Radiation on Lysosomal Enzyme Activity of Mouse Mammary Tumors. Eur. J. Cancer 15:243-250, 1979

22) C. TURANO,A. FERRARO,R. STROM,R. CAVALIERE, A. ROSSI FANELLI: The Biochemical Mechanism of Selective Heat Sensitivity of Cancer Cells III. Studies on Lysosomes. Eur. J. Cancer 6:67-72,1970

23) R. CAVALIERE, E.C. CICCATTO,B.C. GIOVANELLA, C, HEIDELBERGER,R.O. JOHNSON, M. MARGOTTINI, B. MONDOVI, G. MORICA, A. ROSSI FANELLI:Selective Heat Sensitivity of Cancer Cells. Biochemical and Clinical Studies. Cancer 20: 1351-1381,1967

24) K.G. HOFER, B. BRIZZARD,M.G. HOFER:Effect of Lysosomal Modification on the Heat Potentiation of Radiation Damage and Direct Heat Death of BP-8 Sarcom Cella. Eur. J. Cancer 15:1449-1457,1979

25) S.K. CALDERWOOD,E.A. BUMP, M.A. STEVENSON, R. GONZALES-MENDEZ, E. SHUI, E. VAN KERSEN, G.M. HAHAN:INvestigation of Adenylate Energy Charge, Phosphorylation Potential, and ATP Concentration in Cells Stressed With Starvation and Heat. J. Cell. Physiol. 124:261-268, 1985

26) S.H. KIM, J.H. KIM, E.W. HAHN, N.A. ENSIGN: Selective Killing of GLucose and Oxygen Deprived HeLa Cells by Hyperthermia. Cancer Res. 40: 3459-3462, 1980

27) P.N. YI, C.S. CHANG, M. TALLEN, W. BAYER, S. BALL:Hyperthermia induced Intracellular Changes in Tumor Cells. Radia. Res. 93: 534-544, 1983

28) R.A. COSS, W. C. DEWEY, J.R. BAMBURG: Effect of Hyperthermia on Dividing Chinese Hamster Ovary Cells and on Microtubules in Vitro. Cancer Res. 42: 1059-1071,1982

# ASSESSMENT OF COMBINED THERMORADIOTHERAPY IN RECURRENT OF ADVANCED CARCINOMA OF THE BREAST

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During 1980 to 1983, 40 patients with advanced or locally recurrent breast cancer were treated with the thermo-radiotherapy. Ten of these were primary cases and 30 recurrent. In 23 patients who had 2 to 3 lesions, Altogether there were 64 lesions treated. Combined thermo-radiotherapy was applied to the bigger lesions (42 in number) and irradiation alone to the small ones (22 in number). Average size of lesions treated with combined therapy was 38.7 $cm^2$ and that by irradiation alone was 12.1 $cm^2$.

In 20 patients of this series previous irradiation (40-65 Gy) were given.

Hyperthermia was given by using microwave 2450 MHz and 915 MHz, tiwce a week, 15-30 minutes following irradiation. Intratumoral temperature was maintained at 41-44°C for 40 minutes. A average of 11.6 sessions of heat was given.

Radiotherapy was given after conventional freactionation technic 2-2.5 Gy/day x 4-5/week to reach a total dosage of 20-80 Gy (average 47 Gy). Notwithstanding the size of tumor treated with combined therapy was 2 to 3 times larger than that treated by radiotherapy alone, the CR rate, of lesions after combined therapy was significantly better than radiation alone, 64.3% V.S. 36.4% ($P<0.01$).

The authors believe that the optimal size of tumor for hyperthermia using microwave 2450 MHz or 915 MHz may be less than 5-6 in diameter.

## INTRODUCTION

During the period from 1980 to 1983, 40 patients with advanced or locally recurrent breast cancer were treated with thermo-radiotherapy. We report the clinical results obtained using 2450 MHz and 915 MHz microwave generators.

## MATERIALS AND METHODS

The age of the patients ranged from 34 to 78 years with a median of 56 years. In 10 patients with primary breast cancer the lesion were classi-

Keywords: Hyperthermia Breast cancer

fied as clinical stage III ($T_4$ 8 case, $T_{2-2}$, $N_2$ 4, $N_3$ 1, $N_o$ 5) with huge ulceration in 4 and fixation to chest wall in 3. The other 30 patients had recurrent breast cancer following surgery and irradiation (40-65 Gy). The size of recurrent lesions ranged from 2.5 to 9.5 cm in diameter. Nine patients had diffuse lesions on the chest wall. In 23 patients there were 2 to 3 lesions. Altogether there were 64 lesions treated. Combined thermo-radiotherapy was applied to 42 lesions with larger size and irradiation alone to 22 smaller lesions. Average size of tumors treated with combined therapy was 38.7 $cm^2$ in area and irradiation alone was 12.1 $cm^2$

Hyperthermia was given by using microwave of 2450 MHz and 915 MHz, twice a week, 15-30 minutes after irradiation. Temperature were monitored by a thin needle inlaid with 6 equidistantly apart thermocouple sensors. The needle was placed into the central part of the tumor through the skin. Intratumoral temperature was maintained at 41-44°C for 40 minutes. An averge of 11.6 sessions of heat was given.

Radiation therapy was delivered after conventional freactionation technic 2-2.5 Gy/day x 4-5/week to reach a total dose of 20-80 Gy (average 47 Gy). Tumor sites of post-radiation recurrences would not tolerate much further irradiation. Therefore, under these circumstances lower dosage in the vicinity of 40 Gy was given.

RESULT

Assessment of therapeutic effect was made by evaluating clinical and X-Ray evidences. Complete disappearance of tumor maintained for 2 months duration was designated as CR. Those cases in which tumor reduced to more than half its original size are classified as PR, and those equal or less than half reduction in size as NR.

In 64 lesions, 42 were treated using combined thermo-radiotherapy, 22 irradiation alone.

Average size of tumors treated by combined therapy was 2 to 3 larger than those by irradiation alone. However, the CR rate in combination with hyperthermia was 64.3% versus 36.4% with irradiation alone.(see Table 1). Leaving alone the 12 primary braast tumors or larger lesions, of the 52 recurrent lesions of chest wall again for better CR rate was observed in lesions treated by combination therapy 73.3% as compared with 36.4% of those by radiation alone (see Table 2).

The patients in this series were followed-up for 6-37 months. Twelve patients are alive, with no evidence of disease in 9.

Table 1 Response of 64 Lesions to Irradiation V.S. Irradiation Plus Hyperthermia

| Treatment | No.of Lesion | Average size ($cm^2$) | Average Dose (Gy) | Response (%) CR | PR | NR |
|---|---|---|---|---|---|---|
| RT+HT | 42 | 38.7 | 48 | 64.3 | 33.3 | 2.3 |
| RT | 22 | 12.1 | 47 | 36.4 | 50 | 13.6 |

$X^2 = 15.65 \quad P < 0.01$

Table 2 Comparison of Response of 52 Chest Wall Recurrent Lesions to Two Methods

| Treatment | No.of Lesion | Average Size ($cm^2$) | Average Dose(Gy) | Response (%) CR | PR | NR |
|---|---|---|---|---|---|---|
| RT+HT | 30 | 28.9 | 42 | 73.3 | 23.3 | 3.3 |
| RT | 22 | 12.1 | 47 | 36.4 | 50 | 13.6 |

$X^2 = 27.64 \quad P < 0.01$

DISCUSSION

Combined thermoradiotherapy is currently a well accepted modality for treatment of cancers. Local hyperthermia combined with radiation therapy has resulted in a 52-68% complete remission rate which is significantly superior to either modality alone[1-3]. This series of breast cancer thermoradiotherapy has achieved similar good result with a far better CR rate than that of irradiation alone (64.3% VS. 36.4%).

Management of recurrent breast cancer in irradiated area is rather difficult. Twenty of the 40 patients in this series had prior irradiation in the range of 40-65 Gy. With the help of hyperthermia lower dosage of irradiation averagely 47 Gy, 16 of the 20 patients had their lesions achieved CR. Thermoradiotherapy, therefore, might be of choice in the treatment of recurrent breast cancer which failed irradiation.

To our experence[4-5], the size of tumor affects therapeutic effectiveness. The CR rate in tumor with size of 28.9 $cm^2$ was 73.3% which is significantly better than 47.5% for tumor sized 37 $cm^2$. We believe that the optimal size of tumor for hyperthermia using microwave 2450 MHz or 915 MHz may be less than 5-6 cm in diameter.

REFERENCES


1. J.Van der Zee, et al Results of Hypertermia in combination with Reirradiation in Patients with Recurring Breast Cancer. ABSTRACTS, 5th International Symposium on Hyperthermic oncology In Kyoto 1988. P72.
2. CA perez, et al Irradiation and Hyperthermia in the Treatment of Recurrent or Locally Advanced carcinoma of the Breast Chest Wall. Ibid.
3. E.Kjellen, et al. Radiotherapy In combination with Hyperthermia In Recurrent or Metastatic Mammary carcinomas. Ibid.
4. Zhang Tian-ze, et al, Combined Radio-hyperthermotherapy in the Treatment of Superficial malignancies Clinical oncology, 9: 135, 1982, (Chinese).
5. Li Rui-ying, et al, Effect of Radiation combined with hyperthermia in the Treatment of Superficial cancer in 90 patients, Clinical oncology 12(1): 12, 1985 (Chinese).

# LOCAL HYPERTHERMIA FOR TREATMENT OF ADVANCED PROSTATIC CARCINOMA : PRELIMINARY RESULTS

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## INTRODUCTION

Since ancient ages, heat administration was used as a therapeutical modality for several indications. Together with the anti-inflammatory, the anti-cancer cytotoxicity seems to represent the main effect of hyperthermia. Following the biologic rationale for using heat in the treatment of cancer, various technical devices were designed for heat administration in cancer patients.

The great majority of these machines (often using radiofrequency capacitive modalities) were designed to be theoretically effective towards the most various types of (deep and superficial) tumors.

Differently, the Prostathermer* (PT) we used for this study, as well as the other (recently realized) similar machines (5, 8), represent peculiar (perhaps unique) devices specifically designed for therapy of (not only neoplastic) diseases of a single organ.

Extracorporeal (suprapubic and perineal) and intracavitary (transurethral and transrectal) routes of administration can be used for heating prostate. Intracavitary ones appear to be obviously preferable. At the moment, the (theoretically better) transurethral way has to be considered potentially dangerous and then still experimental. Accordingly to the choice of the transrectal way, microwave radiation seemed to represent the best heating modality of the prostatic tissue.

Here described are the preliminary results obtained by local deep microwave hyperthermia (LDMwh) in stage $T_3$-$T_4$, $M_{0-1}$ prostatic carcinomas.

## MATERIALS AND METHODS

14 patients (pts), aged 16-78 years (average: 70.5) with histologically proven prostatic carcinoma entered this study.

All pts were suffering from $T_3$ tumors, excepting one "accidental" $T_2$ lesion.

Key words: Microwaves, Intrarectal hyperthermia, Prostatic cancer

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As for the grade, the 14 pts were listed as follows:
- 2 $G_1$
- 8 $G_2$
- 3 $G_3$
- 1 $G_x$

In the pre-LDMwH period, all pts were examined in the standard fashion, including prostate digital palpation, fine needly aspiration biopsy, chest film, intravenous pyelogram, bone scan, CT abdominal scan, hormonal profiles, serum prostatic acid phosphatase and specific prostatic antigen determination. The diagnostic work-up specifically related to LDMwH consisted of:

1) a minimal ano-rectoscopy (up to 10 cms from the sphincter);
2) a simplified urodynamic evaluation (urinary flow and post-voiding rest determination) and a
3) transrectal ultrasound study (TRUS).

TRUS (performed through a real time rotating probe) was considered essential to obtain exact measurament of prostatic diameters and rectal wall thickness, necessary to plan the device computer.

5 pts suffered from a chronic urinary retention, bearing an indwelling urethral catheter.

No pts showed marked pyelo-ureteric dilation.

4 pts were suffering from a $M_1$ disease; they all presented bone metastasis, confirmed by plain x-tomography.

No pts showed (clinically evident) chest or liver metastasis.

With no regard to the CT scan evaluation, all pts were considered $N_x$.

The prostate was heated through 915 Mhz. delivered by a probe, inserted in the rectal cavity.

A continuous flow system was used to cool the rectal mucosa by means of cold liquid. Appropriately placed thermocouples on the applicator and in the prostatic urethra (through a specially designed Foley catheter) provided a continuous measure of rectal and urethral temperatures. Calculated prostatic temperature, energy delivery and leakage were also available on the monitor screen, during the treatment. The device is scheduled in such a way as to automatically shut off during treatments in case of exceeding maximal pre-fixed safety values. A thermal equivalent dose (TED) calculation was used as a specific tool for theoretical estimate of heat quantity received by the prostate with regard to the time-temperature relationship.

LDMwH was administered by means of a Prostathermer System Model 99D twice weekly in 10 sessions (each lasting about 60 minutes), on an out-patient basis. During treatments the highest possible prostatic temperature was reached within the range of 42-44°, within limits of pts' tolerance

All pts were receiving hormonal therapy (LH RH agonist or orchidectomy + anti-androgenic drugs) since a minimum period of six months when starting LDMwH, with the exception of a single pt (who personally chose to directly undergo both hormonal and hyperthermic treatments. Fig. 2).

The standard LDMwH diagnostic work-up was repeated at 1, 3 and 6 months follow-up periods after LDMwH.

## RESULTS

1 pt was lost to the follow-up. 2 pts dropped out the treatment, because of intolerance of LDMwH. In one case, LDMwH treatments were interrupted because of impossibility of operators to get and maintain appropriate prostatic temperatures. 1 pt hasn't yet reached 1 month of follow-up.

Of 9 evaluable pts:
1 pt died during the LDMwH course because of cancer progression.
4 pts have a follow-up longer than 6 months, 2 pts longer than 3, 2 pts longer than 1 month.

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* BIODAN MEDICAL SISTEM LTD. KIRYAT WEIZMANN. REHOVOT ISRAEL.

Concerning morphological evaluation: 3 pts showed a marked reduction of the prostatic size, 3 pts a slight reduction, 3 pts no reduction at all.

Excellent results were obtained in the pt who personally chose to contemporarely ungergo both hormonal and hyperthermic treatments, with stage reduction from $T_3$ to $T_2$ tumor. (Fig.2).

In all pts a (various grade but henerally clearly evident) improval of glandular echographic pattern was shown the TRUS follow-up examinations.

Urodynamic evaluation showed: imoroval in 5 pts, steadiness in 3 pts, worsening in 1 pt.

Above all, all 4 pts of the catheter-bearing group were relieved of their indwelling catheters and resumed voiding (1 pt for 3 months only).

For this group of pts, the average follow-up is 10 months (range 4-16).

In no case, could we demonstrate disappearance or reduction of bone metastasis.

## DISCUSSION

LDMwH seems to play a role in therapy of advanced prostatic carcinoma.

Furthermore, it probably shows a synergetic effect with chemotherapy but mainly with radiotherapy. Excellent results obtained in one case of (simultaneously realized) hormonal and hyperthermic treatments could claim for a real synergetic effect of these therapies in prostate cancer (Fig.2).

Radio-resistant hypoxic, "nutrients deprived", "low pH" and "S phase" tumoral cells tend to be sensitive to heat.

Hyperthermia also seems to be able to inhibit the repair of radio-induced DNA injury.

In this preliminary series, radiotherapy was not associated to hyperthermia in order to assess the real effectiveness of LDMwH.

We deem that a (low energy) LDMwH treatment of prostatic benign diseases (hyperplasia and prostatitis) can be performed without any temperature measurement. We agree that a complete and exact prostatic thermometry is practically impossible.

In our experience, we also noticed that the thermometric urethral catheterization mainly limit tolerance of LDMwH.

In spite of the above considerations, we personally believe that some sort of thermometric evaluation, even invasive and approximate, should be considered necessary during (high energy) LDMwH treatments for prostatic cancer.

On the other hand, we can affirm that LDMwH was fairly well tolerated, de spite 2 pts who spontaneously chose to interrupt the therapy.

Severe complications have been described following LDMwH, e.g.: urethral strictures and mainly recto-prostatic fistulas (probably related to incorrect indication to the treatment with LDMwH) (3).

In our series urethral catheterization was often responsible for mild urinary side-effects (discomfort, dysuria, hematuria, urethral bleeding, etc.) but no real complications occurred because LDMwH.

## CONCLUSIONS

Transrectal heat delivering should be considered the best (available today) route for heating the prostate.

In our hands, LDMwH seemed to be effective in prostatic cancer therapy. LDMwH seems to be fairly safe and well tolerated, easy to perform and to repeat.

By virtue of the synergetic effect of LDMwH with radiotherapy, the association of these two therapeutical modalities could become the "treatment of choice" for locally advanced prostatic tumors.

Limited diffusion of LDMwH claims for further (controlled, perspective, randomized and possibly multicentric) studies in order to assess its real validity in the field of prostatic diseases therapy.

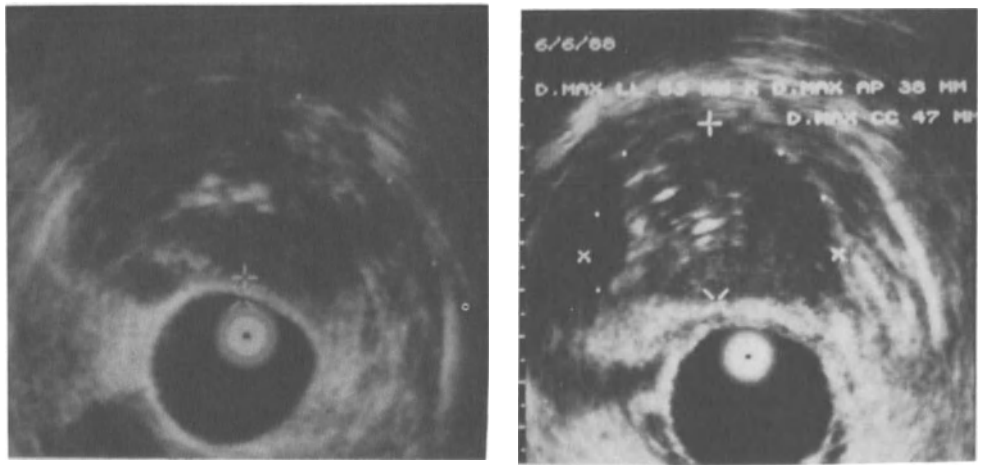


Fig. 1. Case 1. On the left: pre-treatment TRUS. On the right: 3-months follow-up TRUS. See: reduction of the mass, marked improvement of the glandular echo-pattern and creation of hypo-echogenic areas (Oedema?, necrosis?).

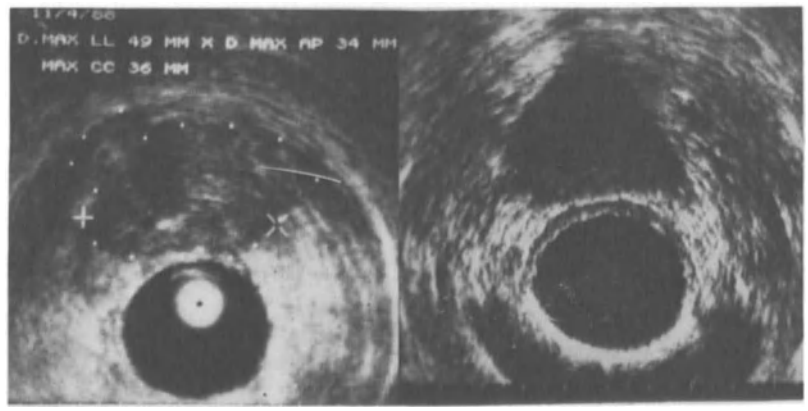


Fig. 2. Case 2. On the left: pre-treatment TRUS. On the right: 6-months follow-up TRUS. See: remarkable reduction of the mass, with lowering of the clinical stage for $T_3$ to $T_2$ tumor in a pt who personally chose to simultaneously undergo both hormonal and hyperthermic treatments.

REFERENCES


1) Bichler K.H., Strohmaier:
Histological effects of local hyperthermia in prostatic cancer.
Lecture book XXVI World Congress of the I.C.S., Milan July 3rd-9th 1988, p. 1503.

2) Mendecki J., Friedenthal E., Botsein C.:
Microwave applicators for localized hyperthermia treatment of cancer of the prostate.
Int. J. Radiat. Oncol. Biol. Phys., 6: 1583, 1980.

3) Rigatti P., Maffezzini M., Guazzoni G., Montorsi F., Consonni P., Colombo R.:
Local microwave Hyperthermia in advanced prostate cancer.
XII International Symposium on Clinical Hyperthermia, Rome, April 27th-29th, 1989. Abstracts book p. 69.

4) Rigatti P., Colombo R., Trabucchi E., Radice F., Guazzoni G., Montorsi F.:
Light and electron microscopy in prostates submitted to local microwave hyperthermia.
See 3, p. 113.

5) Servadio C., Leib Z.,:
Hyperthermia in the treatment of prostate cancer.
The Prostate 5: 205, 1984.

6) Servadio C., Leib Z., Lev A.:
Further observations on the use of Local Hyperthermia for the Treatment of Diseases of the Prostate in Man.
Eur. Urol., 12: 38, 1986.

7) Servadio C., Leib Z., Lev A.:
Further studies on the use of local hyperthermia for the treatment of diseases of the prostate.
J. Urol. 137: 388A, 1987.

8) Szmigielski S., Zielinski H., Stawarz B., Gil J., Sobezynski J., Jeljaszewiez J., Pulverer G.:
Local Microwave Hyperthermia in Treatment of Advanced Prostatic Adenocarcinoma.
Urol. Res. 16, 1, 1988.

9) Szmigielski S., Zielinski H., Stawarz B., Gil J., Debieki P.:
Local intrarectal hyperthermia in treatment of neoplastic and non-neoplastic diseases of prostate.
See 3, p. 109.

10) Szmigielski S., Jeljeszewiez J., Pulverer G.:
Thermoimmunotherapy of advanced prostatic and breast neoplasm.
See 3, p. 73.

11) Yerulshalmi A., Servadio C., Fishelowitz Y., Leib Z., Rakowsky A., Stein J.A.:
Local hyperthermia for treatment of carcinoma of the prostate. A preliminary report.
The Prostate, 3, 623, 1982.

12) Yerushalmi A., Shani A., Fishelovitz Y., Arielly J., Singer D., Levi E., Katnelson R., Rakowsky E., Stein J.A.:
Local microwave hyperthermia in the treatment of carcinoma of the prostate.
Oncology 43; 209, 1986.

# AN OVERVIEW OF THE ROLE OF RADIATION THERAPY AND HYPERTHERMIA IN TREATMENT OF MALIGNANT MELANOMA

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## ABSTRACT

From January, 1970 until December, 1987, a total of 188 malignant melanoma lesions in 92 patients were treated at the Department of Radiation Oncology, Indiana University Medical Center, Indianapolis, Indiana. Response was evaluated in 181 evaluable lesions treated by radiation alone and radiation plus hyperthermia to assess differences in response to a total dose, dose per fraction and overall time of treatment, as well as effects of adjunctive hyperthermia treatment.

Different fractions of radiation, ranging from 100 cGy to 1000 cGy, were used. Local hyperthermia was administered for one hour following radiation treatment using microwave with different frequencies. The tumor temperature was also monitored during treatment.

With a radiation dose of less than 400 cGy per fraction, and complete response rate (CR) was 34% (16/47) and the objective response rate (OR) was 62% (29/47). When hyperthermia was added, the complete response rate rose from 34% to 70%. With a dose of more than 400 cGy per fraction, the CR was 63% (48/77), and OR was 95% (73/77). When hyperthermia was added, the complete response rate rose from 63% to 77%.

## INTRODUCTION

Malignant melanoma is a neoplasm that is commonly regarded as poorly responsive to conventional fractionation of radiation therapy. For many years it has been considered to be a radioresistant tumor and despite some encouraging results with radiation therapy over the past 50 years, a pessimistic approach is often taken and conventional fractionation radiation therapy has often been given by default.(2, 3, 10, 14, 27) Recently, there has been a great deal of interest in the treatment of malignant melanoma with

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large fractions of radiation. There are also many encouraging results in the response of malignant melanoma to hyperthermia in combination with radiation therapy. (5, 7) In vitro and in vivo studies have suggested that malignant melanoma should be treated with a large dose per fraction of radiation therapy. (1, 7, 29, 30)

HISTORICAL PERSPECTIVE

Wolfelder, 1929, (34) and Evans and Leucutia, 1931, (6) reported better results with radiation therapy than with surgery for patients with malignant melanoma.

Hellriegel (15) reported the treatment results of 259 melanoma patients treated from 1935 to 1960. The five year control rate was 62% for radiation alone and 38% for locally excised followed by radiation; however, one-third of the patients did not have histopathology proven malignancy.

Weitzel, (35) 1970, reported on 47 primaries of malignant melanoma treated with electron beam therapy, utilizing fractionated doses of 300-1000 rad. Most of the patients received 500 rad per fraction for a total dose of 10,000-12,000 rad. Forty-nine percent showed objective response, and 3% showed no response. Hilaris el al., (16) 1963 in a study of 73 patients reported a 57% improved response to metastatic malignant melanoma with conventional fractionation of radiation therapy compared to 53% for other metastatic tumors treated.

Hornsey, (17, 18) 1978, in a retrospective study, reviewed 52 melanoma patients treated in the United Kingdom. The author noted the response to a fraction of 400-800 rad was significantly better than the usual fractionation of 200-299 rad.

Overgaard reported on 49 cutaneous and lymph node metastases on 36 patients in a non-randomized study comparing 400 cGy fraction verses 800 cGy fraction. There was a correlation between the tumor response and dose per fraction independent of total dose; the response was significantly better with 800 cGy per fraction when compared with 400 cGy per fraction. In his review of literature in 1986, he found 48% CR with no correlation between total dose, treatment time and modification of nominal single dose. However, there was a significant relationship between dose per fraction and response 59% CR for dose 4 Gy, versus 33% CR for doses per fraction 4 Gy. He also noted that the tumor response was further improved in 134 additional cases receiving adjuvant hyperthermia in which a thermal enhancement ratio (TER) of 2.0 was observed. (22-26)

Strauss, 1981, reported the result of 83 sites on 43 melanoma patients treated with fractionation of 600-800 cGy, which resulted in 80% overall response. (32)

Kim, et al., showed a good correlation between tumor volume, tumor control, and dose of radiation per fraction. (19-21)

Habernalz, (11, 12) comparing fractionation and total

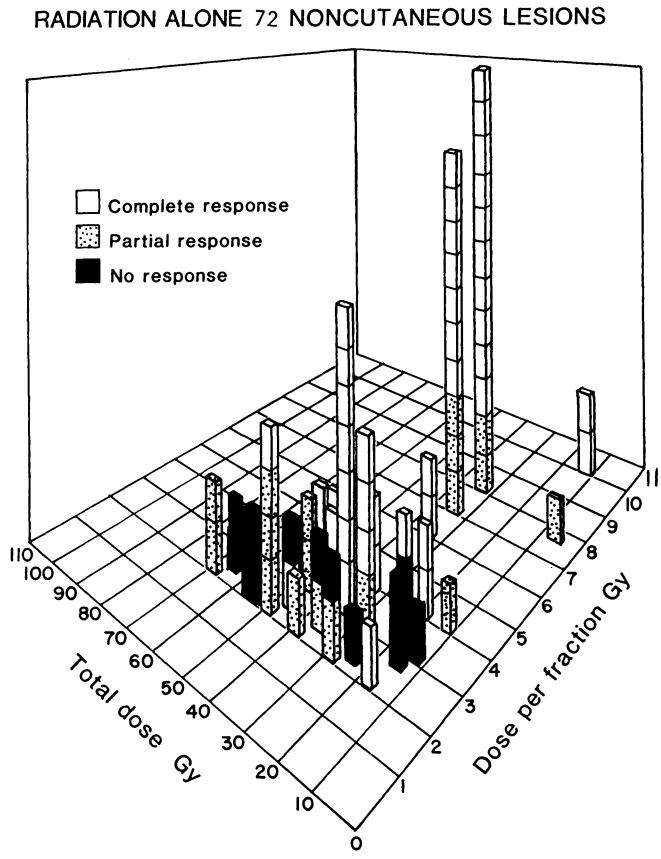



Fig. 1. Radiation alone 72 Noncutaneous Lesions

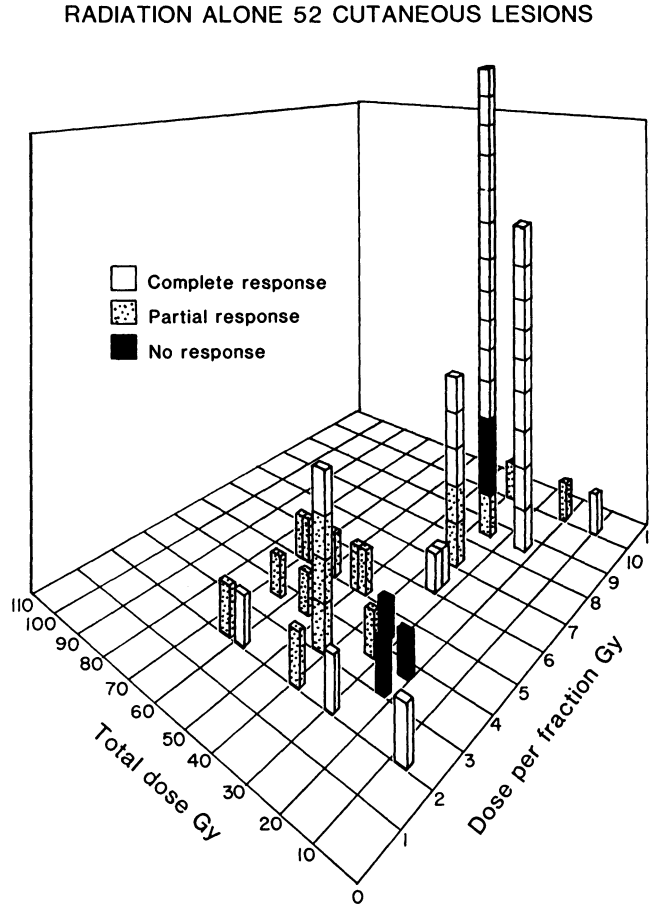



Fig. 2. Radiation Alone 52 Cutaneous Lesion.

tumor dose used at four different centers, reported a complete response rate of 50%. Doss, (8) 1982, found an overall response rate of 37% in 41 lesions in 25 patients treated with conventional fractionation. When the dose per fraction was increased to 400 cGy the overall response rate increased to 67%. Shen, et al., (30) demonstrated that the human melanoma cell line responds more favorably to a single large dose of 800 cGy once a week than to smaller fractions of 200 cGy 5 times per week. (Table 1).

## MATERIAL AND METHODS

188 sites in 92 patients were treated using the following doses of radiation therapy: a) 200 cGy daily for a total of 30 fractions in 6 weeks, b) 600 cGy twice a week times 6 in 17 days, c) 730 cGy once a week times 5 in 28 days and, d) 830 cGy times 4 in 20 days. 1 lesion was treated with 100 cGy times 2 fractions, 2 lesions were treated with 900 cGy times 3 fractions, and 5 lesions were treated with 100 cGy times 3 fractions. Several anatomical areas were treated and included lesions involving the eyes, nose, skin, subcutaneous area and lung. Equipment used included 180 kV x-ray machine with 0.5 mm copper filter, cobalt 60 teletherapy unit, and 4 MeV linear accelerator with electron beams ranging from 7 MeV to 28 MeV.

Lesions larger than 2 cm were treated with a combination of radiation therapy followed by 60 minutes of local heat. Heat treatments were given with either a 433 MHz unit, a 915 MHz unit, or a 2450 MHz unit.

Intratumor temperatures were measured with a minimum of one probe per lesion; in some lesions up to four probes were used. Several thermometry systems were used such as Bailey instruments, Vitek, and lately, the fiberoptic system provided by Clini-therm. Hyperthermia was started no later than one-half hour after radiation therapy for one hour. The intratumor temperatures varied from 39.8 degrees C to 43.2 degrees C. This temperature was reached normally within ten minutes. In some cases a skin cooling device was used. In two patients the treatments were interrupted, one due to a thermal lesion and another by the patient's request.

## RESULTS

Two lesions in one patient received only two treatments; both lesions failed treatment. Five lesions in one patient were treated with heat alone, with five out of five having objective response. Both of the above patients were excluded from this study. 181 lesions in 90 patients were evaluated in this study.

124 lesions, because of location or size being less than 2 cm, were treated with radiation alone. 57 lesions were treated with radiation with doses greater than 400 cGy

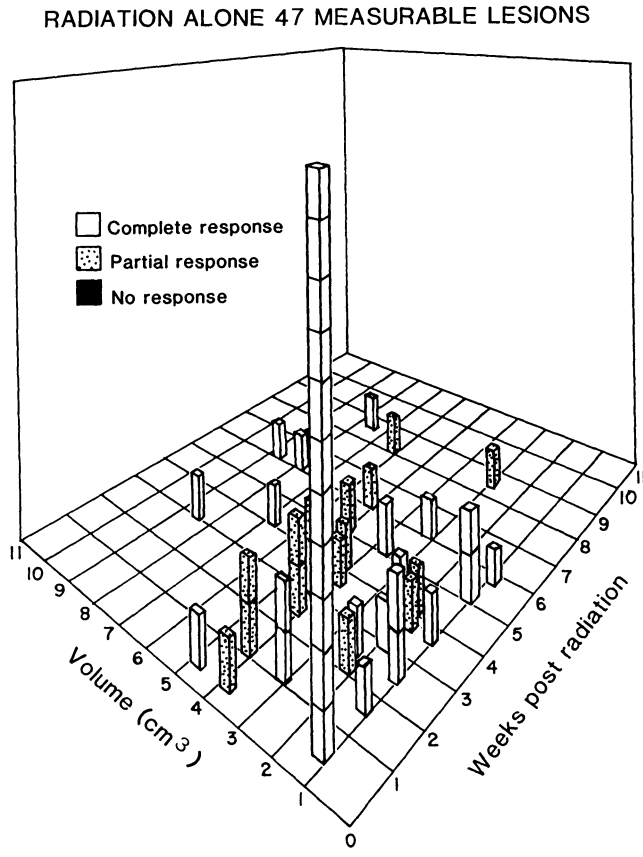



Fig. 3. Radiation Alone 47 Measurable Lesions

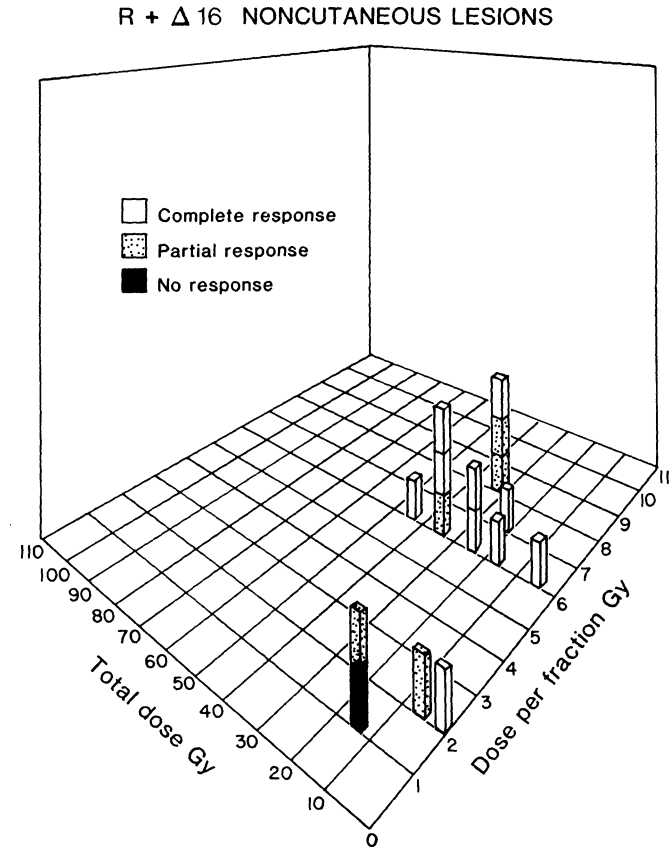



Fig. 4. Radiation Plus Hyperthermia 16 Noncutaneous Lesions

and one hour of hyperthermia;the results being 70% CR and 90% or 47 of the 57 lesions were treated with radiation with doses greater than 400 cGy and one hour of hyperthermia the results being 77% CR and 100% OR. Two lesions failed radiation therapy alone and were treated a second time with the same dose of radiation and hyperthermia. The lesions were controlled for six and eight months until the patients died of widespread disease. There was no unusual skin reaction in those patients( Tables 1 and 2)

Table (1)

RESPONSE' TO CUTANEOUS
MALIGNANT MELANOMA TO X-RAY THERAPY

| | <400 cGy Fractionation | | | >400 cGy Fractionation | | |
|---|---|---|---|---|---|---|
| Authors | # of Lesions | CR% | OR% | # of Lesions | CR% | OR% |
| Hornsey | 57 | 39 | 65 | 37 | 54 | 81 |
| Overgaard | 17 | 6 | 35 | 32 | 34 | 81 |
| *Overgaard | | 33 | | | 59 | |
| Lobo | 21 | 52 | 67 | | | |
| Habermalz | 110 | 32 | 54 | 111 | 45 | 83 |
| Doss | 32 | 28 | 39 | 9 | 67 | 67 |
| Strauss | 45 | | 38 | 36 | | 88 |
| Kim (R) | 19 | 32 | | 35 | 54 | |
| Kim (R + Δ) | 16 | 56 | | 29 | 76 | |
| Present Series(R + Δ) | 10 | 70 | 90 | 47 | 77 | 100 |
| Present Series (R) | 47 | 34 | 62 | 77 | 62 | 95 |

CR = Complete Response
OR = Objective Response

*Based on extrapolation of data on 618 patients.

Table 2

RESULT OF TREATMENT OF MALIGNANT MELANOMA
(RADIATION AND HYPERTHERMIA)

| | ⟨400 cGy | | ⟩400 cGy | | |
|---|---|---|---|---|---|
| Response | X | X + | X | X + | total |
| CR | 34%<br>16/47 | 70%<br>7/10 | 63%<br>48/77 | 77%<br>36/47 | 59%<br>107/181 |
| OR (CR+PR) | 62%<br>29/47 | 90%<br>9/10 | 95%<br>73/77 | 100%<br>47/47 | 87%<br>158/181 |

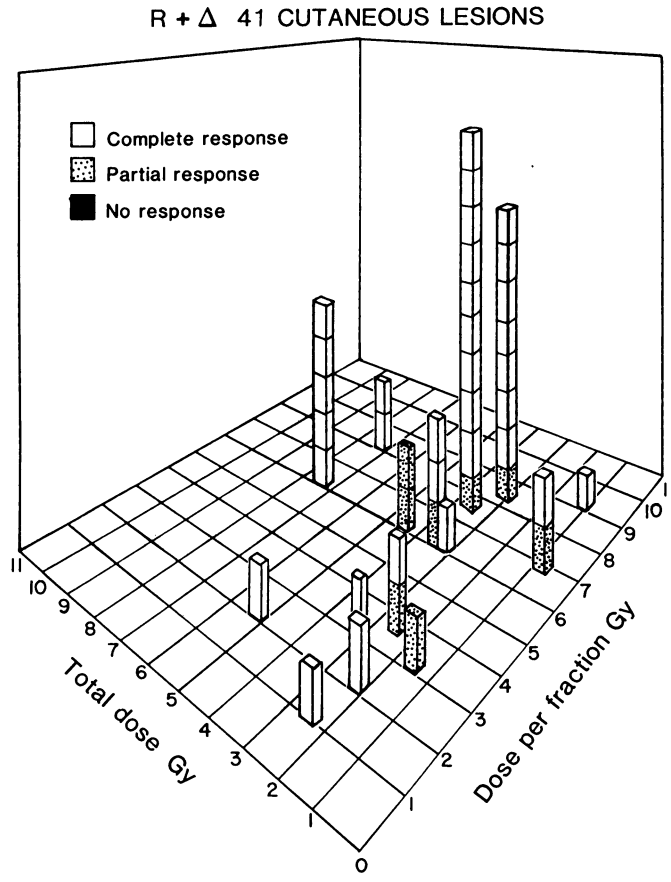



Fig. 5. Radiation Plus Hyperthermia 41 Cutaneous Lesions

## DISCUSSION

Based on laboratory, animal and clinical studies, there has been support for the use of large fractionation in treatment of malignant melanomas. (5, 9, 29, 30, 32, 33) With the adjunctive use of hyperthermia, a dose less than 400 cGy per fraction of radiation can be given and it is expected to produce a gain over radiation alone. This regimen may be used when large fractions of radiation cannot be given, such as to the spinal cord, central nervous system, or abdomen. It seems the thermal gain factor of hyperthermia is not so pronounced when using a dose greater than 400 cGy per fraction of radiation therapy, but the result is not inferior to the radiation therapy alone. Furthermore, hyperthermia appears to make it possible to give an additional course of radiation therapy.

Table 3-4 Shows distribution of response in 124 lesions treated radiation alone notice high distribution response with higher fraction not related to total dose.

Table 5 Shows there is no apparent relation between the tumor volume and response time.

Table 6-7 Shows distribution of response in 57 lesions. All except one responded to treatment even though the response distribution is higher with high fraction, but there is no definite pattern in regard to fractionalation.

Table 8 As in x-ray alone, there is no relation between the size of lesion and time to respond.Pictures Portal treatment with same total dose (830 x 4 cGy) 1 & 2 (1) once a week and same dose in (2) two fractions a day.

Due to use of different thermometry equipment, correlation between thermal dose and response to treatment was not made. The long term effect of this fractionation has not been studied in detail. We also have observed that patients treated with a split course of radiation therapy with a four hour interval in large fraction regimens tolerate radiation therapy better than those treated by a single fraction, without losing the effect of tumor control (Pictures 1 and 2). A randomized study on effects of radiation plus heat, and split courses versus single fractionation, is recommended.

## SUMMARY

1. Malignant melanoma lesions can be treated effectively with large fractions of radiation.
2. In areas that large fractions of radiation cannot be given, small fractions plus hyperthermia offers equally good results.
3. A large single fraction of radiation can be split into two fractions with a four hour interval with no adverse effect on control of the tumor and with better normal tissue tolerance.

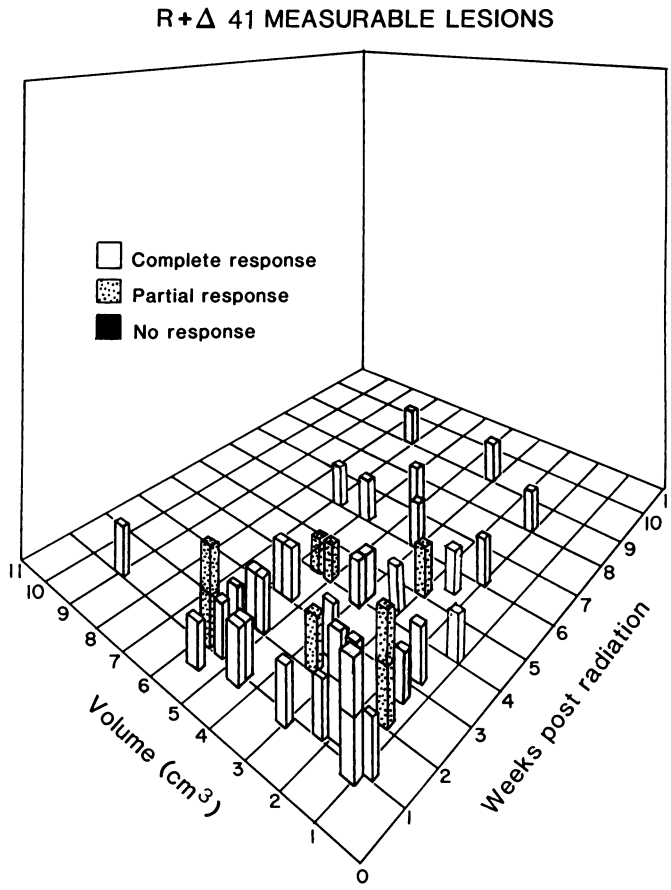
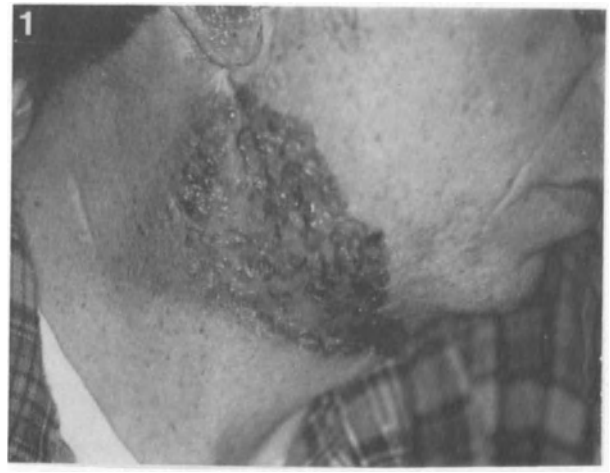
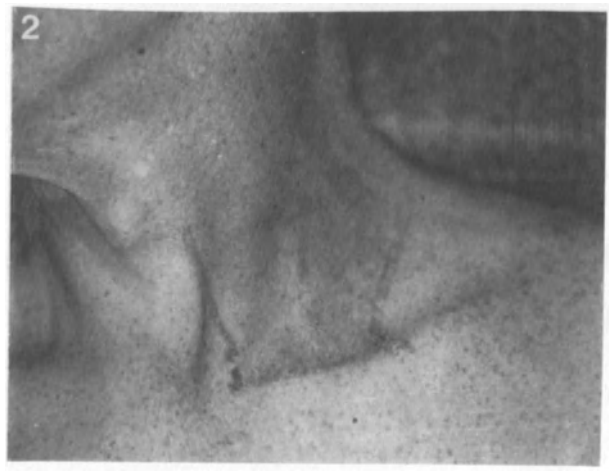



Fig. 6. Radiation Plus Hyperthermia 41 Measurable Lesions




Pictures 1 and 2

REFERENCES

1) Barranco SC, Romsdahl MM, Humphrey Rm: The Radiation Response of Human Malignant Melanoma Cells Grwon in Vitra. Cancer Res; 31-830-833, 1971.

2) Chaoul H, Greineider K; Die Behandlung des Malignen Melanoms mit der Rontgen Nahbestrahlung. Strahlentherapie; 56:40-49, 1936.

3) Cooper JS, Kopf AW, Bart RS: Present Role and Future Prospects for Radiotherapy in the Management of Malignant Melanomas. J. Dermatol Surg Oncol; 5(2): 134-139, 1979.

4) Creagan ET, Cupps Re, Ivins JC, Pritchard DJ, Sim FH, Soule EH, O'Fallon Jr: Adjuvant Radiation Therapy for Regional Nodal Metastases from Malignant Melanoma. Cancer; 42(5): 2206-2210, 1978.

5) Emami B, Perez CA, Konefal J, Pilepich MV, Leyovich, L: Thermoradiotherapy of Malignant Melanoma. Int J Hyperthermia; 4:373-381, 1988.

6) Evans WA, Leuctia T: The Treatment of Melanotic Tumors of Skin Pigmented and Malignant Melanoma. Am J Roentgenol; 26:236-256, 1931.

7) Dewey, DL: The Radiosensitivity of Melanoma Cells in Culture. Br J Radiol; 44:816, 1971.

8) Doss, LL, Memula N: The Radioresponsiveness of Melanoma. Int J Radiation Oncology Biol Phys; 8:1131-1134, 1982.

9) Fletcher GH, BArkley HT Jr, Shukovaky LJ: Present Status of the Time Factor in Clinical Radiotherapy. J Radiol; 55:745-751, 1974.

10) Greineder K, Neumann W: Neue Ergebnisse uber die Nahestrahlung des Malignen Melanoms. Strahlentherapie; 66:89-95, 1939.

11) Habermalz HJ, Fischer, JJ: Radiation Therapy of Malignant Melanoma. Cancer; 38:2258-2262, 1976.

12) Habermalz H: Irradiation of Malignant Melanoma Experience in the Past and Present. Int J Radiation Oncology Biol Phys; 7:131-133, 1981.

13) Hall E: Time, Dose and Fractionation in Radiotherapy. Radiobiology for the Radiologist. 2nd ed., Hagerstown MD: Harper and Row; 273-290, 1978.

14) Harwood AR, Cancuart F, Fitzpatrick PJ, Brown T: Radiotherapy in Nonlentiginous Melanoma of the Head and Neck. Cancer; 48:2599-2605, 1981

15) Hellriegel W.: Indikation und Ergebnisse der perkutanen. Stahlenbehandlung des Malignen Melanoms. Strahlentherapie; 149 (1) 1-20, 1975.

16) Hilaris BS, Raben M, Calabrese AS, Phillips RF, Henschke UK: Value of Radiation Therapy for Distant Metastases from Malignant Melanoma. Cancer; 16(6): 765-773, 1963.

17) Hornsey S: The Radiosensitivity of Melanoma Cells in Culture. Br J Radiol; 45:158, 1972.

18) Hornsey S: The Relationship between Total Dose, Number of Fractions and Fraction Size in the Response of Malignant Melanoma in Patients. Br J Radiol; 51-905-909, 1978.

19) Kim JH, Hahn EW: Clinical and Bilogical Studies of Localized Hyperthermia. Cancer Res. 39:2258-2261, 1969.

20) Kim JH, Hahn EW, Tokita N: Combination Hyperthermia and Radiation Therapy for Cutaneous Malignant Melanoma. Cancer 41-2143-2148, 1978.

21) Kim JH, Hahn EW, Sultan AA: Hyperthermia and Radiation Therapy for Melanoma. Cancer; 50, 478-482, 1982.

22) Lobo PA, Liebner EJ: Radiotherapy in the Management of Malignant Melanoma. Int J Radiation Oncology Biol Phys; 7:20-26, 1981.

23) Overgaard J: Radiation Treatment of Malignant Melanoma. Int J Radiation Oncology Biol Phys; 6:41-44, 1980.

24) Overgaard J: The Role of Radiotherapy in Recurrent and Metastatic Malignant Melanoma: A Clinical Radiological Study. Int J Radiation Oncology Biol Phys; 12:867-872, 1986.

25) Overgaard J: Some Problems Related to the Clinical Use of Thermal Insoffect Dose. Int. J. Hyperthermia; 3(4):329-336, 1987.

26) Overggaard J: The Current and Potential Role of Hyperthermia in Radiotherapy. Int J. Radiation Oncology Biol Phys; 16:535-549, 1989.

27) Rappaport AH, Phillips TL: Local Control of Malignant Melanoma by Radiation. Int J Radiation Oncology Biol Phys (Supplement 2); 2:126, 1977.

28) Sapareto S: Thermal Dose Determinations in Cancer Therapy. Int J Radiation Oncology Biol Phys; 10:787-800, 1984.

29) Shen R, Hornback N, Shidnia H, Lu L, Broxmeyer H: Comparative Effect of Changes in Irradiation Dosage and Timing on the Growth in Vitro of Two Human Melanoma Cell Line. Int J Radiation Oncology Biol Phys submitted May 29, 1988.

30) Shen R, Hornback N, Shidnia H, Lu L, Montebello J, Brahmi Z: A Comparison of Lung Metastasis and Natural Killer Cell ACtivity in Daily Fractions and Weekly Fractions of Radiation Therapy on Murine B16a Melanoma. Radiation Res; 114, 1988.

31) Storck H, Ott F: Verlauf and Therapie der Malignen Melanoma. Schweiz Med Wochenschr; 106(51):1871-1877, 1976.

32) Strauss A, Dritschilo A, Nathanson L, Piro AF: Radiation Therapy of Malignant Melanomas: An Evaluation of Clinically Used Fractionation Schemes. Cancer: 1262-1266, 1981.

33) Trott KR, VonLieven H, Kummermehr J, Skopal D, Lukacs S, Braun-Falco O: The Radiosensitivity of Malignant Melanomas Part I: Experimental Studies. Int J Radiation Oncology Biol Phys; 7:9-13, 1981.

34) Wolfelder H: Welche Behandlung Bietet Die Best Heilungsaussicht Bei Melanosarkoma. Roentgenpraxis; 1:19-27, 1929.

35) Weitzel G: Die Strahlenbehandlung des Melanomas. Schweiz Med Wschs; 100:982-987, 1970.

# CLINICAL RELEVANCE OF HEAT SHOCK PROTEINS

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## THE HEAT SHOCK RESPONSE AND THE HEAT SHOCK PROTEINS

A sudden increase of the physiological temperature (heat shock) elicits in cells a specific sequence of biochemical events designated, on the whole, as "heat shock response"[1]. Since this response occurs whenever the cells are exposed to adverse, stressing, conditions this behaviour is also generically referred to as stress response.

A relevant phenomenon, characterizing the heat shock response, is a transient modification of the gene expression pattern. Such a changeover, which involves regulatory mechanisms, acting both at transcriptional and at translational level, leads to a selective increase in the synthesis of a restricted goup of proteins, called Heat Shock Proteins (HSPs) or Stress Proteins (SPs).

One of the main features of these proteins is their high degree of conservativity, as shown by the structural homology among each group of SPs synthesized in different cells, both of prokaryotic and eukaryotic type as well as of invertebrate and vertebrate origin. It is also notewhorthy that different, unrelated, stressing agents, such as ethanol, arsenite, metals, aminoacid analogues, induce the synthesis of identical SPs.

The HSPs are classified into three groups, on the basis of their molecular weights:

1) High molecular weight group. The major constituent of this group is the 90 KDa HSP: this protein is synthetized at rather high levels in normal unstressed cells, but its synthesis appreciably increases after exposure to stress.

2) 70-72 KDa group. This set includes several structurally related proteins with slight differences in their isoelectric points, and with different regulation. The so-called heat shock cognate proteins are constitutively expressed and their relative rate of synthesis is only moderately increased, or even unaffected, by exposure to stress. Other proteins of this group, undetectable in unstressed cells, become a major product of synthesis following stress exposure.

3) Low molecular weight group. It is a very heterogeneous one among the various organisms. In Drosophila, there are four related species, ranging in size from approximately



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22 to 30 KDa. In yeast, chicken and mammalian cells, however, only one protein species of 28 KDa can be observed. This 28 KDa HSP may be resolved into several isoforms, some of which are phosphorylated.

Fig. 1 shows the 1-D/SDS-PAGE pattern of the proteins synthesized in a human melanoma cell line before and after a hyperthermic exposure at 42 °C, for 1 hr. By comparing the two lanes, the first one corresponding to the unstressed cells (lane 1) and the other corresponding to the heated cells (lane 2), the presence of the three above-mentioned HSPs is clearly apparent.

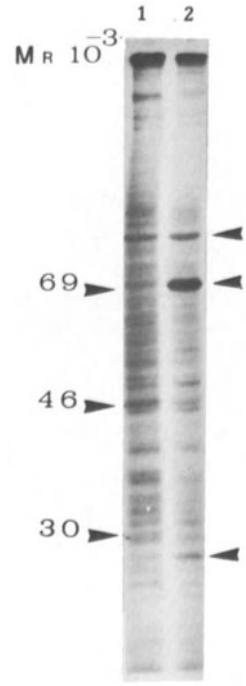


Fig. 1 One dimension (SDS-PAGE) analysis of labelled proteins synthesized in M-10 human melanoma cells.
LANE 1: Control cells, maintained and labelled at 37 °C.
LANE 2: Heat-shocked cells, incubated at 42 °C for 1 hr, returned to 37 °C for 1 hr and then labelled.
Labelling was performed by replacing culture medium with 1 ml of leucine-free MEM, supplemented with 100 µCi of 3H-leucine (142 Ci/mMole). After 60 min of incorporation, monolayers were washed with ice-cold Hanks' buffer and cells were scraped in a detergent-containing buffer. Cell lysates were clarified by centrifugation and used for polyacrylamide gel electrophoresis in the presence of SDS, according to Laemmli[2]. The protein markers used to calibrate the relative mobilities in the gel are reported on the right side of the figure. The arrows indicate the three main groups of HSPs.

In 2-D gel electrophoresis (Fig. 2) the 70 KDa HSP resolves into a cluster of radioactive spots with similar molecular weights but with different I.P. A magnified picture of this cluster of proteins is shown in the insert on the right side of the fluorographs: the different constitutive level of synthesis and the different heat inducibility of the HSPs belonging to the 70 KDa group can be easily appreciated.

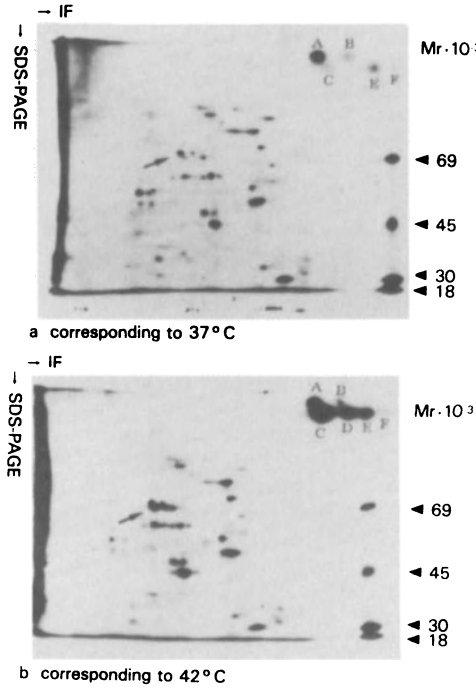


Fig. 2 Two-dimension (IF/SDS-PAGE) analysis of pulse-labelled proteins synthesized in M-10 human melanoma cells.
Upper panel: Control cells, maintained and labelled at 37 °C.
Lower panel: Heat-shocked cells, incubated at 42 °C for 1 hr, returned to 37 °C for 1 hr and then labelled.
Labelling was performed by replacing culture medium with 1 ml of methionine-free MEM supplemented with 100 μCi of 35S-methionine (1220 Ci/mMole). Cell lysates were analyzed by a two-dimension (IE/SDS-PAGE) separation technique, carried out according to O' Farrel[3]. The positions of the protein markers used to calibrate the relative mobilities in the second dimension polyacrylamide gels are reported on the left side of the figure. The arrows point to the 70 KDa HSPs group. Inserts (upper right corner) represent a magnification of the region indicated by the arrows. A,B,C,D,E and F refer to individual spots, corresponding to different proteins belonging to the 70 KDa HSP group.
(From Ferrini and al.[4] with permission)

The fact that the HSP are highly conserved in the whole animal kingdom indicates that these proteins play a key role in the cellular viability. However, in spite of the great amount of experimental works carried out on this matter, the biological functions of HSPs (both of those constitutively expressed and of the stress-induced ones) are still undefined. It has been found, however, that some HSPs specifically bind themselves with other proteins. This behaviour has been demonstrated for the 90 KDa HSP, which is able to bind with some retrovirus-coded transforming proteins and with estrogen receptors. As for the 70 KDa HSP, it has been observed an association with actin, fatty acids and with small molecules like ATP. The significance of these specific interactions, however, is still unclear.

Studies performed both in vivo and in vitro demonstrated that the cells which survive a sub-lethal hyperthermic exposure acquire the capacity of withstanding a subsequent, otherwise lethal, heat challenge: this transient state of decreased thermosensitivity is defined as thermotolerance. On the basis of several experimental evidences, it has been suggested that the induction of HSPs synthesis is the crucial event triggering the onset of thermotolerance. According to this hypothesis, the prominent biological function of HSPs is to protect intracellular target/s, critical for cell survival, against heat-induced damages, thus increasing the cell resistance to heat cytotoxicity.

The thermotolerance represent a serious obstacle in the clinical applications of hyperthermia, either when this treatment is applied in fractionated doses or when the hyperthermia is administered for an extended period of time. The possible involvement of HSPs in the acquisition of thermotolerance, therefore, is both of biological and of clinical interest.

The current literature data and the researches performed on this subject in our laboratory will be presented herein, with emphasis on the possible clinical use of the experimental results.

## COINCIDENCE BETWEEN HSPs INDUCTION AND DEVELOPMENT OF THERMOTOLERANCE

The hypothesis of a correlation between HSPs synthesis and thermotolerance is supported by the finding that both the induction and the repression of HSPs synthesis are coincident, respectively, with the development and the decay of thermotolerance[5,6] . Parallel changes between the rate of HSPs synthesis and the resistance to heat cytotoxicity have been also documented during the embryonic development of Xenopous,[7] Sea Urchin[8] and Drosophila[9].

It is notewhorthy that the thermotolerance can develop not only after the heat treatment but also after the exposure to other stressing agents able to induce an enhanced SPs synthesis. This positive correlation has been verified in a large number of different and completely unrelated agents, including ethanol, heavy metals and inhibitors of energetic metabolism[10] .

In some cases, however, the association between the induction of SPs and the onset of thermotolerance does not seem to occur. As demonstrated by Landry and Chrétien[11] , cultured Morris epatoma cells exposed to sodium arsenite do not develop any thermotolerance, even if this drug is an

efficient inducer of SPs. On the contrary, it was found that cells treated with 2,4-dinitrophenol become thermotolerant, even if this compound does not induce SPs synthesis[12]. Moreover, Li and Laszlo[13] found that Chinese hamster fibroblasts treated with canavanine (an aminoacid analogue of arginine) actively synthetize HSPs: nevertheless these cells, instead of developing any thermotolerance, become very sensitive to a subsequent heat challenge. In this case the incorporation of the analogue into newly synthetized proteins is responsible for the synthesis of altered proteins, including the SPs, unable to perform their specific functions.

To obtain more compelling evidences for a cause-effect relationship between SPs synthesis and thermotolerance, a number of experiments has been performed to ascertain whether the thermotolerance could be expressed when the production of HSPs was impaired or fully suppressed. In procaryotes, it has been found that the expression of the thermotolerance requires the protein synthesis; in eukaryotic cells, instead, several data indicate that the inhibition of the protein synthesis by cycloeximide or by puromycin does not hamper cell's ability to develop thermotolerance[14].

## INTRACELLULAR LEVEL OF HSPs AND THERMOTOLERANCE

The aim of the above discussed researches was to verify the correlation, or the lack of correlation, between the stress-promoted increase in the rate of SPs synthesis and the development of thermotolerance. In other studies, however, attention has been focused on the intracellular concentration of HSPs. In fact, it is conceivable that the intrinsic heat-sensitivity of a given cell (that is its own thermoresistance, in absence of any stress) may be correlated with the intracellular content of the constitutively expressed HSPs, while the increased transient resistance against heat (that is the acquired thermotolerance) develops when the intracellular concentration of these proteins suddendly increases.

The available experimental data, however, are still contradictory. In some heat-resistant variants of hamster ovary cells the constitutive level of HSPs appears to be much higher than in the parental strain[15]. By contrast, in other cell lines adapted to grow at supranormal temperatures the intracellular content of HSPs is not different from that measured in the respective original strains, growing at 37 °C[16]. In mouse embryo cells transformed by SV-40 the amount of the constitutively expressed HSPs is higher than that observed in untransformed cells; besides, their rate of synthesis can be further stimulated by exposure to heat. Nevertheless, these virus-transformed cells are more sensitive to heat and develops a reduced degree of thermotolerance in comparison with the untransformed ones[17].

The relationship between the cell survival after exposure to heat and the intracellular level of individual HSPs has been carefully studied by Li and Mak in Chinese hamster ovary cells[18]. It was found that the intracellular concentration of the 70 KDa HSP progressively increases during development of the thermotolerance, attains a plateau when the thermotolerance reaches its maximum value, then slowly decreases to the basal level, along with a reduction of the thermotolerance.

## CELLS UNABLE TO EXPRESS HSPs DO NOT DEVELOP ANY THERMOTOLERANCE

In the course of a comparative study performed in our laboratory on several murine tumors, we found that an Ehrlich tumor and an MC sarcoma were unable to express HSPs both in response to heat and after treatment with a number of stressing agents. This finding prompted us to ascertain whether the two ascitic tumors were also unable to develop any thermotolerance. For this purpose, the cells collected from tumor-bearing mice were divided into four groups and treated as follows:

1) Cells maintained at 37 °C (controls)
2) Cells exposed at 42 °C for 1 hr (conditioning treatment)
3) Cells exposed at 45 °C for 30 min (hyperthermic challenge)
4) Cells treated at 42 °C for 1 hr, returned to 37 °C for 2 hrs and then further exposed at 45 °C for 30 min.

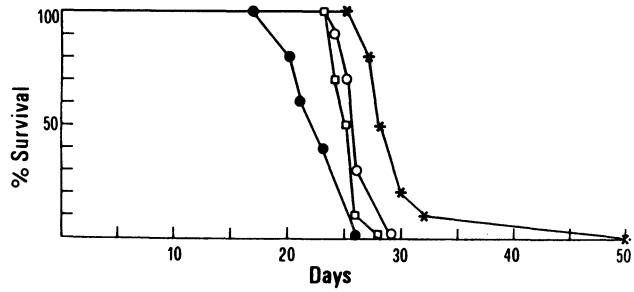


Fig. 3 Death rate of mice inoculated i.p. with an HSPs-producing tumor (sarcoma 180)

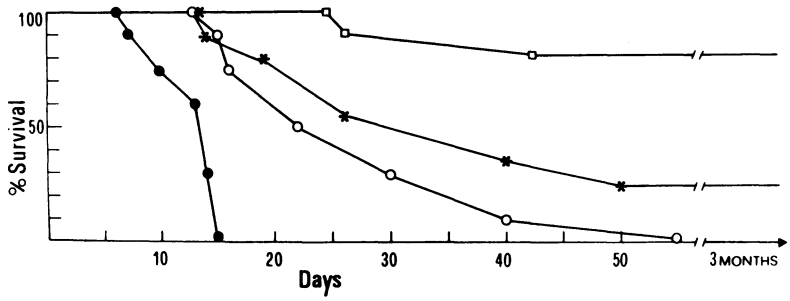


Fig. 4 Death rate of mice inoculated i.p. with an HSPs-unresponsive tumor (MC sarcoma).

Before beeing reinoculated into the recipient animals, tumor cells were treated as follows:
● -- No treatment, cells maintained at 37 °C.
○ -- Incubation at 42 °C for 1 hr.
* -- Incubation at 45 °C for 15 min.
□ -- Incubation at 42 °C for 1 hr and, 2 hrs later, at 45 °C for 15 min.
Ten animals were used for each experimental group. (From Mattei and al.[19] with permission).

Following these treaments the cells were re-inoculated into the abdomen of the recipient mice ($1x10^6$ cells/animal), using 10 animals for each experimental group. Parallel experiments were carried out with other murine tumors in which the ability to synthetize SPs was preliminary ascertained.

On the basis of the death rates, as reported in Figures 3 and 4, it appears that the behavior of unresponsive tumor cells is very different from that observed in the HSPs-producing ones. In the latter case, when the animals were re-inoculated with cells first conditioned by a mild hyperthermic exposure and then subjected to a 45 °C challenge, their death rate was significantly higher than that of the animals inoculated with cells treated at 45 °C only (Fig 3). Therefore, the exposure at 42 °C, which promotes in these tumors an enhanced synthesis of HSps, induces also a thermotolerant state, able to reduce the effect of the subsequent hyperthermic challenge. On the contrary, it appears (Fig. 4) that about 30% of the MC sarcoma-bearing mice were still alive up to three months after the inoculation with cells treated first at 42 °C for 1 hr and then at 45 °C for 30 min. Since these values exceed those found in mice inoculated with cells solely treated at 45 °C, it is evident that the damaging effects exerted by the two hyperthermic treatments were summing up in HSP-unresponsive cells. The MC sarcoma cells, therefore, appear to be both unresponsive for HSPs induction and unable to elicit thermotolerance.

In agreement with these data, other Authors demonstrated that, both in bacteria and in yeast, mutants unable to synthesize the 70 KDa HSP in response to stress are also unable to develop any thermotolerance[20].

## THERMOTOLERANCE, HSPs SYNTHESIS AND SOD ACTIVITY

Experiments were also performed to study a possible relationshp among HSPs synthesis, induction of thermotolerance and activity of the enzyme superoxide dismutase (SOD). This research has been undertaken because it has been recently demonstrated that an exposure to stressing conditions gives rise to harmful superoxide radicals, responsible for lipids peroxidation, enzymes degradation, DNA damage and production of other reactive species[21]. In eukaryotic cells, these superoxide radicals are inactivated by a SOD-catalyzed reaction, which converts them into hydrogen peroxide. Some experimental data suggest that the ability of a given cell to inactivate the stress-generated radicals may be crucial for its survival in adverse conditions[22].

Experiments were carried out in our laboratory by submitting cultured human melanoma cells to three different well-known stressing conditions: 1) Exposure to a supranormal temperature 2) Treatment with canavanine ( aminoacid analogue of arginine) 3) Treatment with disulfiram (an inhibitor of metal and disulphide-dependent enzymes, like SOD). Preliminarly, we demonstrated that all these treatments were able to induce in this cell line a full complement of HSPs. Afterwards, the experimental conditions ( dosage, exposure time and reincubation either at normal temperature or in drug-free medium) were carefully adjusted in order to obtain nearly equivalent intracellular amounts of HSPs in the cells treated with these three stressing agents. Following the stressing treatments, cells were both assayed for SOD activity and

subjected to an hyperthermic challenge, in order to check the onset of the thermotolerance.

It was observed (see Fig. 5) that the heat-treated cells (1 hr at 42 °C plus 2 hrs of reincubation at 37 °C) became thermotolerant and increase their SOD activity up to 30% of the controls. In cells incubated with canavanine (0,3 mM, 18 hrs of treatment without any further incubation in drug-free medium) the level of SOD activity was practically unmodified in respect to controls, but the cells were not only unable to express any thermotolerance but they also become extremely heat-sensitive. In disulphiram-treated cells ( 2,5 mM, 1 hr of treatment followed by 2 hrs of reincubation in drug-free medium) the SOD activity was severely reduced (-40% ) in comparison with controls, while the thermosensitivity was unmodified.

Therefore no correlation can be demonstrated among HSPs induction, SOD activity and development of thermotolerance.

## CONCLUSIONS

At the present time it is difficult to draw any definite conclusions from these confusing and often contradictory experimental data. Since the thermotolerance can be expressed either under conditions in which HSPs synthesis is substantially impaired or when HSPs are overexpressed the conclusion is that an enhanced HSPs synthesis is neither a sufficient nor a necessary condition for the induction of the thermotolerance [23].

A different approach to this problem has been recently proposed by Van Wijk's group. According to these Authors [24], the thermotolerance can be divided in two different states : A and B. A corresponds to a transient, fast response that develops without any de novo protein synthesis. B is referred to a long-term response developing in coincidence with an increased rate of HSPs synthesis and, consequently, to a rise of HSPs intracellular level. A is mainly induced by mild hyperthermic treatments, while B occurs after a severe exposure to heat. These two different responses, however, do not exclude each other. On the contrary, in some instances the response occurring after a stressing treatment becomes a combination of both A and B states. If further experiments will support this model, all the data concerning the relationship between the HSP induction and the thermotolerance should be reconsidered and in some way reconciled.

From a theoretical point of view the possibility of either developing the thermotolerance in absence of HSPs synthesis or of inducing HSPs synthesis without developing any thermotolerance is very interesting. However, it must be pointed out that in practise the thermotolerance and the HSPs synthesis are very frequently coincident and that this fact may be of clinical relevance. As a matter of fact a comparison of the relative rates of HSPs synthesis before and after a hyperthermic treatment may be useful for evaluating its effectiveness. Moreover, as it has been suggested by Li, the intracellular level of the 70 HSP might be assumed as a "predictor" of the thermal sensitivity, monitoring the onset and the subsequent decrease of the thermotolerance [18] . On this basis, reliable protocols for fractionated hyperthermic treatments might be drawn up. However the in vivo measurements both of the rate of HSPs synthesis and of the HSPs

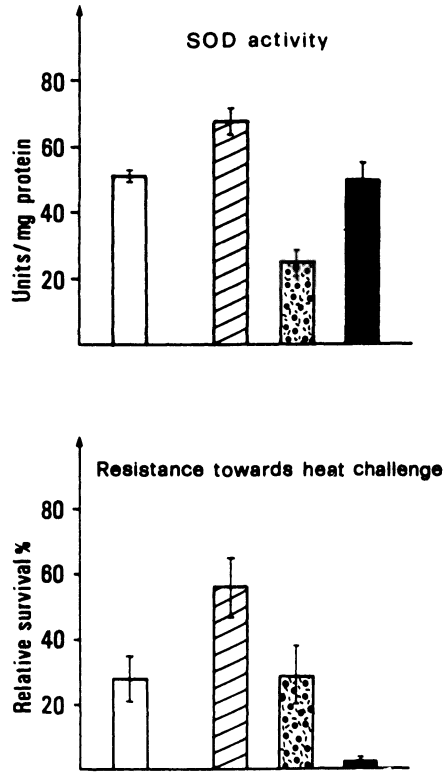


Fig. 5 Comparative evaluation of superoxide dismutase activity and of thermotolerance induction in M-14 human melanoma cells subjected to different stressing treatments.
Couples of culture flasks were treated as follows:

▨ - Exposure at 42 °C for 1 hr, followed by a two hrs recovery at 37 °C.

▩ - Exposure to 2,5 mM disulfiram, for 1 hr, followed by 2 hrs of recovery in drug-free medium.

■ - Exposure to 0,3 mM canavanine, for 18 hrs.

□ - No treatment, control cells.

After receiving the appropriate treatment, one flask of each experimental group was immediately processed for determining the SOD activity . The assay was performed by the indirect inhibitory test based on the reduction of nitrobluetetrazole, in the presence of a superoxide-generator system (Loven and al.[22] ). The other flask was further subjected to a hyperthermic challenge and the onset of thermotolerance was assayed by the colony-forming assay.

intracellular levels have been carried out only in experimental models with radioactive labelling, a procedure that is very dangerous and difficult in the clinical practice. A development of new methods allowing fast and harmless determination of such parameters would be of relevant medical interest.

REFERENCES


1. S. Lindquist, The heat shock response.
   Annual Rev. Biochem. 55: 1151 (1986)
2. U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T 4.
   Nature (London) 227: 680 (1970)
3. P.H. O'Farrel, High resolution two dimensional electrophoresis of proteins.
   J. Biol. Chem. 250: 4007 (1975)
4. U. Ferrini, R.Falcioni, A. Delpino R. Cavaliere, G. Zupi and P.G. Natali, Heat shock proteins produced by two human melanoma cell lines: absence of correlation with thermosensitivity.
   Int. J. Cancer 34: 651 (1984)
5. G.C. Li and J.Y. Mak, Induction of heat shock protein synthesis in murine tumors during the development of thermotolerance.
   Cancer Res. 45: 3816 (1985)
6. G.C. Li and A. Laszlo, Thermotolerance in mammalian cells: a possible role for heat shock proteins.
   In "Changes in Eukaryotic Gene Expression in Response to Environmental Stress", B.G. Atkinson and D.B. Walden Ed., Academic Press, Orlando, (1985)
7. J.J. Heikkila, M. Kloc, J. Bury, G.A. Schultz and L.W. Browder, Acquisition of the heat-shock response and thermotolerance during early development of Xenopus laevis.
   Dev. Biol. 107: 483 (1985)
8. M. C. Roccheri, M.G. Di Bernardo and G. Giudice, Synthesis of heat shock proteins in developing sea urchins.
   Dev. Biol. 83: 173 (1981).
9. G. Graziosi, F. Micali, R. Marzani, F. De Cristini and A. Savioni, Variability of response of early Drosophila embryos to heat shock.
   J. Exp. Zool. 214: 141 (1980)
10. G.M. Hahn and G.C. Li, Thermotolerance and heat shock proteins in mammalian cells.
    Radiation Res. 92: 452 (1982)
11. J. Landry and P. Chrétien, Relationship between hyperthermia induced heat shock proteins and thermotolerance in Morris hepatoma cells.
    Can. J. Biochem. Cell Biol. 61: 428 (1983)
12. E.K. Boon-Niermeijer, J.E.M. Souren and R. Van Wijk, Thermotolerance induced by 2,4-dinitrophenol.
    Int. J. Hyperthermia 3: 133 (1987).
13. G.C. Li and A. Laszlo, Amino acid analogs while inducing heat shock proteins sensitize CHO cells to thermal damage.
    J. Cel. Physiol. 122: 91 (1985)

14. K. Watson, G. Dunlop and R. Cavicchioli, Mitochondrial and cytoplasmic protein syntheses are not required for heat shock acquisition of ethanol and thermotolerance in yeast.
FEBS Letters 172: 299 (1984)
15. A. Laszlo and G.C. Li, Heat resistant variants of Chinese fibroblasts altered in expression of heat shock protein.
Proc. Natl. Acad. Sci. USA 82: 8029 (1985)
16. R.L. Anderson, T.W. Tao, D.A. Betten and G.M. Hahn, Heat shock protein levels are not elevated in heat resistant B-16 melanoma cells.
Radiation Res. 105: 240 (1986)
17. R.A. Omar and K.W. Lanks, Heat shock protein synthesis and cell survival in clones of normal and simian virus 40-transformed mouse embryo cells.
Cancer Res. 44: 3976 (1984)
18. G.C. Li and J.Y. Mak, Re-induction of hsp 70 synthesis: an assay for thermotolerance.
Int. J. Hyperthermia 5: 389 (1989)
19. F. Mattei, A. Delpino, A.M. Mileo and U. Ferrini, Absence of acquired thermotolerance in murine tumors unable to increase the expression of heat shock proteins following stress stimuli.
Tumori 74: 513 (1988)
20. T. Yamamori and T. Yura, Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in Escherichia coli K-12.
Proc. Natl. Acad. Sci. USA 79: 860 (1982)
21. M.L. Cunnigham, J.G. Peak and M.J. Peak, Single-strand DNA breaks in rodent and human cells produced by superoxide anion or its reduction products.
Mutation Res. 184: 217 (1987)
22. D.P. Loven, D.B. Leeper and L.W. Oberlay, Superoxide dismutase levels in Chinese hamster ovary cells and ovarian carcinoma cells after hyperthermia or exposure to cycloheximide.
Cancer Res. 45: 3029 (1985)
23. S.W. Carper, J.J. Duffy and E.W. Gerner, Heat shock proteins in thermotolerance and other cellular processes.
Cancer Res. 47: 5249 (1987)
24. E.K Boon-Niermeijer, J.E.M. Souren, A.M. De Waal and R. Van Wijk, Thermotolerance induced by heat and ethanol.
Int. J. Hyperthermia 4: 211 (1988)

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