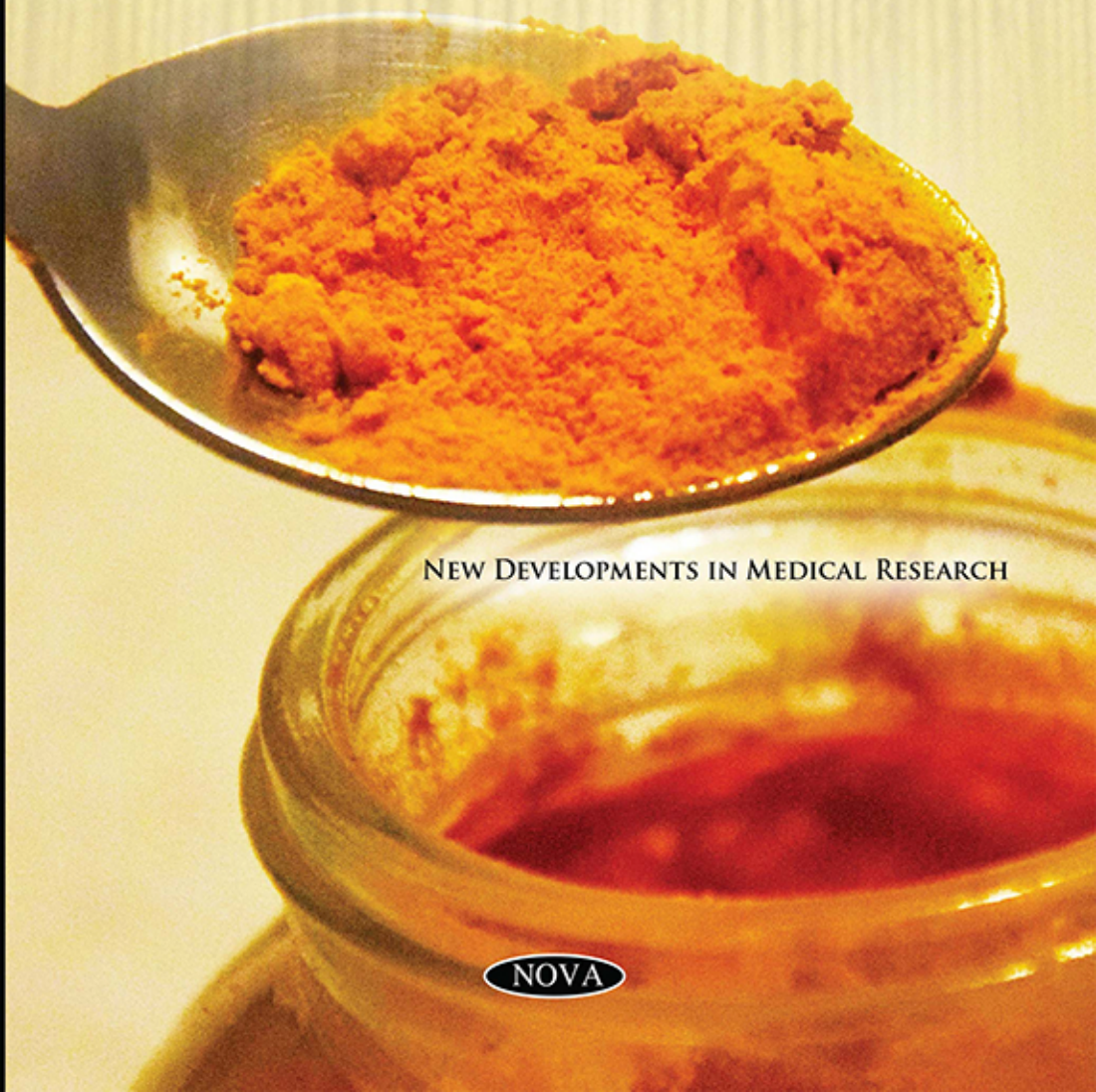
Valeria Martin
Editor

Curcumin

Clinical Uses, Health Effects and Potential Complications



New Developments in Medical Research

NOVA

NEW DEVELOPMENTS IN MEDICAL RESEARCH

CURCUMIN

CLINICAL USES, HEALTH EFFECTS AND POTENTIAL COMPLICATIONS

NEW DEVELOPMENTS IN MEDICAL RESEARCH

Additional books in this series can be found on Nova's website under the Series tab.

Additional e-books in this series can be found on Nova's website under the e-book tab.

NEW DEVELOPMENTS IN MEDICAL RESEARCH

CURCUMIN

CLINICAL USES, HEALTH EFFECTS AND POTENTIAL COMPLICATIONS

VALERIA MARTIN
EDITOR

nova publishers
New York

Library of Congress Cataloging-in-Publication Data

Library of Congress Control Number: 2015956836

ISBN: 978-1-63484-273-0 (eBook)

Published by Nova Science Publishers, Inc. † New York

CONTENTS

PREFACE

Curcumin is a natural product with polyphenolic structure. It is used in therapeutic remedies alone or in combination with other natural substances. Many researchers are investigating it because of its biological activities such as: anti-inflammatory, anti-cancer, anti-protozoal, anti-viral, anti-bacterial and has been found to be effective for treatment of Alzheimer, depression, headaches, fibromyalgia, leprosy, fever, menstrual problems, water retention, worms and kidney problems etc. It is an active ingredient in dietary spice, turmeric. It has reactive functional groups: a diketone moiety and two phenolic groups. Despite its unique biological activities, it suffers from some shortcomings which include: gastrointestinal problems, poor bioavailability due to its poor absorption, short half-life, poor solubility in aqueous solutions, rapid systemic elimination and antithrombotic activity which can interfere with blood clotting. The first chapter of this book reviews the different delivery systems used for incorporation of curcumin and its derivatives, release kinetics and up to date in vivo results. Chapter two discusses curcumin nano and microencapsulation and its implications on clinical uses. Chapter three studies the effect of curcumin in nasal epithelial cells. The last two chapters studies the epigenetic changes induced by curcumin and its congeners and the potential of utilizing these changes in the treatment of different diseases; and examines differential absorption of curcuminoids between free and liposomed curcumin formulations.

Chapter 1 - Curcumin exhibits anti-inflammatory, anti-diabetic, anti-cancer, anti-bacterial, anti-Alzheimer, antimicrobial and antimalarial activities etc. However, its poor water solubility and instability has resulted in its poor bioavailability. To enhance its bioavailability, it is incorporated into several drug delivery systems. This review will focus on the different delivery systems

used for incorporation of curcumin and its derivatives, release kinetics and up to date in vivo results.

Chapter 2 - Encapsulation techniques have been widely applied to a diverse array of bioactive substances in order to improve stability, bioavailability and hydrophilicity and also to avoid side effects caused by high dosages required by some drugs. In the case of curcumin, its hydrophobic character and low stability in alkaline conditions, thermal treatment, light, metallic ions, ascorbic acid and others turn it into a natural candidate for encapsulation. Researches over the last decades have demonstrated that curcumin encapsulation is feasible being carried out by techniques such as nanoprecipitation, micellization, emulsification and miniemulsification followed by solvent removal, crosslinking reaction. However, attention must be paid when choosing the encapsulant matrix because it directly affects encapsulation efficiency and also due to the existence of specific interactions with the chemical structure of curcumin. Some examples are synthetic and bio-based amorphous or semi-crystalline polymers, hydrogels, solid lipids micro or nano carriers, ionic complexation matrices and. In the case of nanosized capsules, colloidal stability is also an issue because agglomeration leads to a decrease in overall specific surface area and slow release rates. Regarding capsules size, although microscale often led to increase in bioavailability, specific clinical uses such as cancer treatment require nanosized capsules due to the phagocytosis mechanism. However, microcapsules remain as a low cost, easy to scale up methodology to encapsulate curcumin. Recent studies focus on characterizing the resulting material to assure curcumin is properly encapsulated and homogeneously distributed along the matrix. Also, gains in bioavailability and pharmacological effects must be extensively demonstrated as to compensate the increased complexity of the encapsulation techniques.

Chapter 3 - The airway epithelium, particularly the nasal epithelium, is the first line of defense against respiratory viral infections. Airway epithelial barriers are regulated predominantly by apical intercellular junctions, referred to as tight junctions. Respiratory syncytial virus (RSV) is a negative-stranded RNA virus of the genus *Pneumovirus*, family *Paramyxoviridae*; it is the major cause of bronchitis, asthma, and severe lower respiratory tract diseases in infants and young children. In human nasal epithelial cells (HNECs), the replication and budding of RSV, and subsequent epithelial responses including the release of proinflammatory cytokines and enhancement of tight junctions, are partially regulated by the nuclear factor-kappa B (NF-κB) pathway. On the other hand, the effects of curcumin are believed to be caused partially by the inhibition of NF-κB activity through a blockage of its entry into the nucleus.

Curcumin is also a potent inhibitor of proteasomes, cyclooxygenase (COX)-2, lipoxygenase, ornithine decarboxylase, c-Jun N-terminal kinase, and protein kinase C. The authors' data show that curcumin prevents the replication and budding of RSV, and subsequent epithelial responses, without causing cytotoxicity. Moreover, the increase of epithelial barrier function caused by infection with RSV was enhanced by curcumin. Curcumin has various pharmacologic effects as an inhibitor of NF-κB, eIF-2α dephosphorylation, the proteasome, and COX-2. RSV-infected HNECs treated with the eIF-2α dephosphorylation blocker salubrinal, the proteasome inhibitor MG132, or COX-2 inhibitors (such as curcumin) prevented the replication of RSV and subsequent epithelial responses. Treatment with salubrinal and MG132 also enhanced the upregulation of tight junction molecules induced by infection with RSV. These results suggest that curcumin can prevent the replication of RSV and subsequent epithelial responses without causing cytotoxicity, and may be an effective therapy for severe RSV-associated lower respiratory tract diseases in infants and young children.

Chapter 4 - The role of epigenetic changes in health and disease is well established. Recent discoveries of the role of epigenetic modifications in the development and progression of different diseases like cancer, diabetes, chronic kidney disease and neurodegeneration urged the parallel development of drugs that modulate these modifications. DNA methyltransferase inhibitors and histone deacetylase inhibitors were the first categories of drugs tested in clinical trials as epigenetic modifiers and FDA-approved for treatment of different tumors. Nutraceuticals like genistein, epigallocatechin-3-gallate and curcumin demonstrated activity as epigenetic modifiers and a long list of other nutraceuticals is waiting validation of their activity. Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa (turmeric), exhibits a wide spectrum of pharmacological activities. Curcumin demonstrated anti-inflammatory, antimicrobial, antiviral, antioxidant and antitumor activity in several studies. Curcumin is safe when administered at high doses; however, its low bioavailability due to poor absorption and rapid metabolism is a major drawback. Different formulation based approaches were adopted to overcome its low bioavailability like liposomal curcumin and curcumin nanoparticles. Additionally, several structural analogues were also synthesized to improve the solubility and bioavailability of curcumin. Curcumin and its congeners were shown to induce epigenetic changes in tumor cells. Curcumin modulated histone acetylation by inhibiting histone deacetylase (HDAC) and histone acetyltransferase (HAT) enzymes in tumor cells. Curcumin modulated microRNAs (miRNAs) expression in tumor cells

and demonstrated a controversial DNA hypomethylating effect. In this review, the epigenetic changes induced by curcumin and its congeners and the potential of utilizing these changes in the treatment of different diseases will be discussed.

Chapter 5 - Curcumin is a polyphenolic compound derived from turmeric, an old Indian dietary spice. Curcumin extracts possess diverse pharmacological effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities and have been very appreciated by traditional Asian medicine for a long time. In spite of this relevance, the pharmacological use of curcumin is limited due to poor water-solubility and low oral bioavailability. One of the main strategies to avoid this limitation is the encapsulation of curcumin extracts in lipid-based nano-delivery systems such as phospholipid complexes, micelles or liposomes that increase permeability by interacting with cell membrane components. In this work, the individual absorption of curcumin, demethoxycurcumin and bisdemethoxycurcumin was studied by comparing the behavior of free (water dispersion) and liposomed formulations using an *in situ* rat absorption model. The results show differences in the absorption rates for the curcuminoids in the water dispersion in correlation with their hydrophobicity, having the demethoxy derivatives a higher intestinal permeability compared to curcumin. In contrast, using the liposomed formulation no differences in the absorption were observed for the curcuminoids, probably revealing that the absorption is driven by the incorporation/fusion of the liposomes in the intestinal cell membrane. In spite of the lower absorption rate coefficient observed for the curcuminoids in the liposomed formulation compared to the free formulation, only the liposomed formulation led to significant quantities of curcumin metabolites in rat plasma, probably due to the highest concentration of curcuminoids achieved in the gut. These results show that, besides their biological activity, the preferential absorption of curcuminoids depending on their polarity has to be considered for therapeutical use of curcumin.

In: Curcumin
Editor: Valeria Martin
ISBN: 978-1-63484-272-3

Chapter 1

AN OVERVIEW OF NANO-BASED FORMULATIONS OF CURCUMIN AND ITS DERIVATIVES

B. A. Aderibigbe*[*], *T. Naki and A. Mugogodi
Department of Chemistry, University of Fort Hare, Alice Campus,
Eastern Cape, South Africa

ABSTRACT

Curcumin exhibits anti-inflammatory, anti-diabetic, anti-cancer, anti-bacterial, anti-Alzheimer, antimicrobial and antimalarial activities etc. However, its poor water solubility and instability has resulted in its poor bioavailability. To enhance its bioavailability, it is incorporated into several drug delivery systems. This review will focus on the different delivery systems used for incorporation of curcumin and its derivatives, release kinetics and up to date in vivo results.

INTRODUCTION

Curcumin is a natural product with polyphenolic structure [1]. It is used in therapeutic remedies alone or in combination with other natural substances [2]. Many researchers are investigating it because of its biological activities

[*] Corresponding author: email: blessingaderibigbe@gmail.com.

(Figure 1) such as: anti-inflammatory, anti-cancer, anti-protozoal, anti-viral, anti-bacterial and has been found to be effective for treatment of Alzheimer, depression, headaches, fibromyalgia, leprosy, fever, menstrual problems, water retention, worms, and kidney problems etc., [3]. It is an active ingredient in dietary spice, turmeric. It has reactive functional groups: a diketone moiety and two phenolic groups [4]. Despite its unique biological activities, it suffers from some shortcomings which include: gastrointestinal problems, poor bioavailability due to its poor absorption, short half-life, poor solubility in aqueous solutions, rapid systemic elimination and antithrombotic activity which can interfere with blood clotting, [5, 6]. And because of the aforementioned shortcomings, several researchers are investigating how to improve its bioavailability by incorporating it into several drug delivery systems.

The current trends in curcumin research is focused on the development of effective delivery systems that will increase its stability, hydrophilicity, bioavailability as well as targeted delivery with enhanced therapeutic effects.

In recent years, there has been an unprecedented growth in the applications of nanotechnology in drug delivery. Application of nanotechnologies in medicine involves disease diagnosis, targeted drug delivery and molecular imaging [7]. One unique advantage of the application of nanotechnology in drug delivery is targeted delivery whereby the drug is delivered at the target tissue/organ with improved therapeutic effects. Presently, different nanoformulation of drugs are being evaluated by various researchers such as: nanoemulsions, nanomicelles, nanoliposomes, nanoparticles, nanocarriers, nanotubes etc. Nanoformulations that are used in drug delivery exhibit several advantages such as: increases the surface area of the drug molecule, enhances water solubility, bioavailabilty and targeted delivery mechanism of the incorporated drug and can be used for combination therapy where two or more drugs are incorporated onto a single drug delivery system. They also increase the half-life and reduces toxic adverse effects of drugs, resulting in improved pharmacokinetic profile and therapeutic efficacy [8-11].

This review will be focused on the different delivery systems used for incorporation of curcumin and its derivatives, their release kinetics and up to date in vivo results.

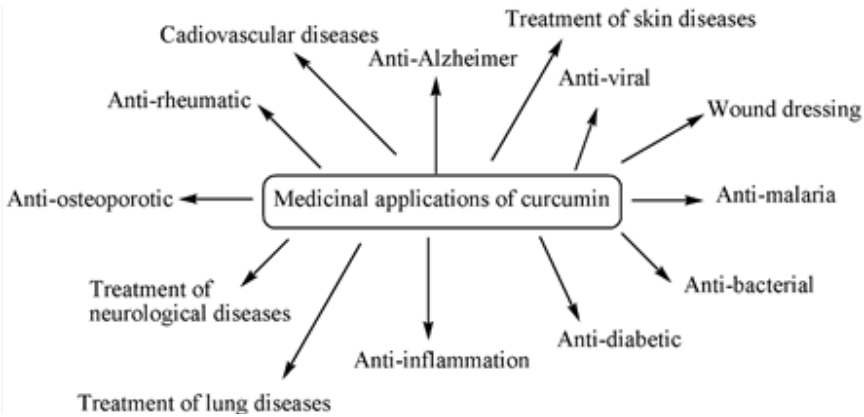


Figure 1. Biomedical applications of curcumin.

NANOLIPOSOMES

Nanoliposomes are made of bilayered phospholipid membranes with an aqueous interior [12]. They are nanosized vesicles used to deliver low molecular weight drugs. One outstanding feature of nanoliposomes is that they do not undergo rapid degradation and clearance by liver macrophages [12]. They are very useful for targeted drug delivery [13]. Their leaky vascular structure is unique in accumulating and releasing the drug in the tumor over an extended period of time [14]. Mourtas et al. 2014, formulated nanoliposomes of curcumin derivatives to target amyloid deposits in Alzheimer disease in the brain. The formulation exhibited high affinity for the amyloid deposits on post-mortem brains samples of Alzheimer diseased patients. The formulation was reported to be a potential therapeutic agent for treatment and diagnosis of Alzheimer disease [15]. Lazer et al., also designed nanoliposomes conjugated with curcumin which was exposed at the surface. They were reported to be potential formulation for diagnosis and targeted delivery system for treatment of Alzheimer disease [16]. Shin et al., reported chitosan-coated curcumin nanoliposomes prepared by ethanol injection method. Their physicochemical properties were compared with the properties of those prepared by dry thin film method. The encapsulation efficiency, mucoadhesive property of the chitosan-coated curcumin nanoliposomes prepared by ethanol injection method was found to be better than those prepared by dry thin film method. These observations suggested that the method of preparation of curcumin-

nanoliposomes can influence the adsorption of curcumin in the gastrointestinal tract [17]. Hasan et al., reported encapsulation of curcumin onto nanoliposomes to achieve an improved bioavailability. The liposomes that were composed of salmon's lecithin improved curcumin bioavailability when compared to those composed of rapeseed and soya lecithins. Liposomal encapsulation of curcumin enhanced the cellular effects of curcumin [18]. Taylor et al., investigated the effects of various types of nanoliposomes on the aggregation of the amyloid-$\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) peptide. In vitro studies showed that nanoliposomes with curcumin derivative inhibited the formation of fibrillar and/or oligomeric Aβ. Suggesting their potential application for treatment of Alzheimer disease [19]. Chen et al., encapsulated curcumin onto nanoliposomes and they exhibited enhanced physicochemical properties than the free curcumin [20].

Nanomicelles

Polymeric micelles are nanoscale assemblies of amphiphilic polymers that are composed of the core for drug encapsulation and the shell [21]. Encapsulation of hydrophobic compounds into polymeric micelles have been reported to enhance the solubility of the compound and is useful for intravenous applications [22]. They can prolong circulation time *in vivo* and enhance the cellular uptake. They can target tumors by enhanced permeability and retention effect, resulting in delivery of anticancer drugs with improved antitumor effects [23]. There are some research reports on the encapsulation of curcumin onto nanomicelles. Gao et al., reported the encapsulation of curcumin to biodegradable monomethoxy poly(ethylene glycol)poly(lactide) copolymer micelles [24]. The micelles encapsulated with curcumin released curcumin over an extended period of time. They exhibited cell growth inhibition and induction of cell apoptosis more than the free curcumin. In vivo analysis on colon cancer mouse model suggested that the micelle encapsulate with curcumin exhibited greater inhibitory effect on colon tumor growth than the free curcumin. These findings suggested that the incorporation of curcumin onto micelles has great potential in colon cancer therapy. Taurin et al., prepared styrene-co-maleic acid micelles encapsulating 5, 10 or 15% curcumin derivative by weight/weight ratio [25]. Cytotoxicity analysis was performed against triple negative breast cancer cell lines. The micelles encapsulated with curcumin derivative exhibited an enhanced cytotoxicity profile when compared to the free curcumin. Gülçür et al., designed sterically stabilized

micelles encapsulated with curcumin to target and hinder breast cancer [26]. In vitro cytotoxicity analysis of the micelles on MCF-7 breast cancer cells showed an enhanced anticancer activity than the free curcumin. The micelles were also able to target the resistant breast cancer cells with a higher efficacy than the free curcumin. Tripodo et al., evaluated nanomicelles, composed of inulin and vitamin E encapsulated with curcumin [27]. The release of curcumin from the micelles followed a controlled release profile and the nanomicelles were able to penetrate cellular membrane. These further confirmed that nanomicelles are potential nanocarriers for enhancing therapeutic properties of hydrophobic drugs such as curcumin. Kershawani et al., encapsulated curcumin derivative to nanomicelles prepared from hyaluronic conjugate of copoly(styrene maleic acid) [28]. The anticancer activity of curcumin loaded nanomicelles was evaluated against MiaPaCa-2 and AsPC-1 human pancreatic cancer cells and the cell killing was dose dependent. The result suggested that nanomicelles are potential drug delivery systems for treatment of pancreatic cancers. There is also a report on the formulation of curcumin in polyethylene glycol (PEG)-derivatized FTS-based nanomicellar system. The formulation exhibited enhanced cytotoxicity towards several cancer cell lines. Intravenous application of curcumin-loaded micelle resulted in an effective inhibition of tumor growth in a syngeneic mouse breast cancer model [29].

CARBON NANOTUBES

Carbon nanotubes are allotropes of carbons and they are currently investigated as drug delivery systems. They exhibit properties that make them useful for drug delivery such as: high surface area, ability to adsorb therapeutic molecules, rich electronic polyaromatic structure and outstanding stability [30]. They have been found to deliver drugs by penetrating into the cells while the drug incorporated onto it remains intact [31]. These findings have open doors to application of carbon nanotubes as drug delivery systems. Functionalized single-walled carbon nanotubes (SWCNTs) have been used for drug delivery of curcumin to splenic lymphocytes. The functionalized SWCNTs loaded with curcumin exhibited enhanced cell proliferation inhibition efficacy when compared to the free curcumin. Curcumin loaded onto functionalized SWCNTs exhibited good dispersion in water [32]. Functionalized SWCNTs loaded with curcumin-loaded was also evaluated for possible application in cancer therapy. It was found to inhibit PC-3 cell growth

suggesting that they are potential delivery system for cancer therapy [33]. In another research report prepared for U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland, SWCNT was loaded with curcumin. Its cytotoxic effect against selected cancer cell lines were evaluated. Its cytotoxic effect was prominent in metastatic breast cancer cell line MDA-231 but other cancer cell lines used i.e., non-metastatic cell lines T47D and MCF-7 for the study required higher concentration of curcumin encapsulation to the SWCNT [34].

NANOEMULSION

Nanoemulsions are colloidal particulate system with size that ranges from 10 to 1,000 nm [35]. They are useful for drug delivery. There are three types of nanoemulsion namely [35]: oil in water nanoemulsion, water in oil nanoemulsion and bi-continuous nanoemulsions. They offer several advantages such as [35-37]: enhances bioavailability of drugs, they are non-toxic, have large surface area for greater drug loading, solubilizes lipophilic drug and are useful for taste masking.

Ganta and Amiji, reported the preparation of flaxseed oil containing nanoemulsion formulation encapsulated with paclitaxel and curcumin [38]. In vitro analysis was performed on wild-type SKOV3 and drug resistant $SKOV3_{TR}$ human ovarian adenocarcinoma cells. The results showed that the encapsulated drugs were effectively delivered intracellular in both SKOV3 and $SKOV3_{TR}$ cells. Combination of paclitaxel and curcumin administered in the nanoemulsion formulations was reported to be very effective in improving the cytotoxicity in wild-type and resistant cells by promoting the apoptotic response. These findings suggested that nanoemulsion of curcumin in combination with paclitaxel has significant potential application for treatment of ovarian cancer. Ahmed et al., investigated the bioaccessibility of lipid-based formulations (i.e., oil-in-water, long, medium, and short chain triacylglycerols nanoemulsions) containing curcumin [39]. An *in vitro* study was performed on simulating small intestine conditions and the bioaccessibility of curcumin decreased in the order of medium chain triacylglycerols nanoemulsion > long chain triacylglycerols nanoemulsion > short chain triacylglycerols nanoemulsion. The nanoemulsions were physically stable than the conventional emulsion. Sari et al., reported encapsulation of curcumin onto medium chain triglyceride oil droplets of nanoemulsion prepared by ultrasonification [40]. In vitro release kinetics of curcumin from the

nanoemulsion indicated its stability in pepsin digestion. Donsi et al., fabricated nanoemulsions based on natural food ingredients (i.e., resveratrol and curcumin). Curcumin was encapsulated in a stable solid fat nanoemulsions using stearic acid as lipid phase. The encapsulation improved the dispersion of the bioactive agents and their bioavailability [41]. Rachmawati et al., developed tablet containing curcumin nanoemulsion for oral delivery. Tablet containing nanoemulsion had good physical characteristics and was found to maintain its particle size when dispersed in water [42]. Rachmawati et al., also reported the development of curcumin based nanoemulsions for transdermal delivery [43]. It was prepared by self-nanoemulsification method followed by *in vitro* study using a snake skin of Phyton reticulatus. The *in vitro* results suggested that the nanoemulsion is a potential drug delivery system for inflammatory pain management. Yu and Huang, developed organogel-based nanoemulsion for oral delivery of curcumin [44]. In vitro analysis of the nanoemulsion on Caco-2 cell showed that the digestion-diffusion was the mechanism of release of the nanoemulsion. In vivo studies on mice further confirmed the enhanced bioavailability of the curcumin when compared to the free curcumin.

There is a recent newspaper report on curcumin [45]. The nanodrug is a non-ionic polymer nanoemulsion containing curcumin. Clinical studies showed that the nanodrug was successful on a number of cancer patients. In vivo work showed that nanodrug is effective on cancer cells. The nanodrug is now in the second phase for treatment of people with resistant breast and gastrointestinal cancer. The nano drug is in different formulations such as oil, semi-solid and water-soluble forms. There are also other research reports on the nanoemulsion of curcumin with improved bioavailability [46, 47].

NANOSHELL

Nanoshells are drug delivery devices that are made up of inner core of silica and an outer metallic layer. They have been reported to concentrate in cancer lesion sites due to their sizes by enhanced permeation retention (EPR). They are presently being investigated for treatment of cancer by targeted delivery [48]. Zhu et al., prepared hollow structured superparamagnetic iron oxide nanoshells encapsulated with curcumin [49]. The nanoshell-based encapsulation of curcumin resulted in a stable aqueous dispersion of the curcumin. In vitro release study of curcumin from the nanoshell was faster than the free curcumin. These results suggested that the nanoshells are

promising intracellular carrier for hydrophobic anticancer drugs. Braden and Vishwanatha, invented biodegradable nanoshell [50] comprising poly-lactic-co-glycolic acid biocompatible polymer in contact with an amphiphilic polyol stabilizing agent; a spacer compound non-covalently sequestered in the nanoshell wall and a targeting agent bound to the non-covalently sequestered spacer compound resulting in the display of the targeting agent on the exterior surface of the nanoshell. The nanoshell was loaded with curcumin and its derivatives. These were found to be potential drug delivery systems for bioactive agent which can be used in combination with a conventional radioisotopes for controlled release of bioactive agents.

NANOPARTICLES

Nanoparticles offers several advantages when encapsulated with bioactive agents. Some of the advantages are [51, 52]: they enhance the solubility of the encapsulated bioactive agent, improve bioactive agents adhesion to biological surfaces resulting in rapid onset of therapeutic action with improved bioavailability, they have large surface area which can improve the loading and delivery of bioactive agents to targeted cells and tissues, they reduce the drug dose needed to achieve therapeutic benefit thereby lowering cost and side effects associated with the drug and their size can enhance active drug targeting.

Heo et al., developed cyclodextrin-conjugated gold nanoparticles which formed inclusion complexes with curcumin [53]. The formulation significantly hindered osteoclast formation of bone marrow-derived macrophages by suppression of the receptor activator of nuclear factor-κBligand-induced signalling pathway. In vivo analysis was performed on ovariectomy-induced osteoporosis model which suggested that the formulation can enhance bone density and prevent bone loss. Mulik et al., formulated apolipoprotein E3 mediated poly(butyl) cyanoacrylate nanoparticles containing curcumin with enhanced cell uptake for treatment of Alzheimer disease [54]. The nanoparticle containing curcumin was more photostable than the free curcumin. In vitro cell culture study showed enhanced therapeutic efficacy of nanoparticle against beta amyloid induced cytotoxicity in SH-SY5Y neuroblastoma cells than the free curcumin. Amano et al., prepared nanoparticles to suppress macrophages. The diameter of the nanoparticles containing curcumin ranged from 60 to 100 nm. They were useful for the selective suppression of macrophages in vivo in mice [55]. Krausz et al.,

synthesized curcumin nanoparticles which inhibited in vitro growth of methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa [56]. This inhibition was dose-dependent and it inhibited methicillin-resistant Staphylococcus aureus growth with enhanced wound healing in an in vivo murine wound model. Jambhrunkar et al., encapsulated curcumin in mesoporous silica nanoparticles [57]. The encapsulation resulted in enhanced solubility, cell cytotoxicity, drug release, and high cellular delivery efficiency of curcumin than the free crucumin. Bhawana et al., prepared nanoparticles of curcumin by wet-milling technique and the particle size distribution was in a range of 2-40 nm [58]. They were dispersible in water and the aqueous dispersion of nanocurcumin was much more effective against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Penicillium notatum, Aspergillus niger than the free curcumin. The nanocurcumin exhibited enhanced inhibitory effects on the Gram-positive bacteria than the Gram-negative bacteria. Furthermore, its antibacterial activity was much better than the antifungal activity. The mechanism of antibacterial action of curcumin nanoparticles was as a result of the penetrating effects of the nanoparticles through the cell wall onto the bacteria cell resulting in cell death. Anand et al., developed polymer-based nanoparticle from poly (lactide-co-glycolide) and polyethylene glycol (PEG)-5000, a stabilizer [59]. The cellular uptake of the nanoparticles was more efficient and more rapid than the free curcumin. They were also able to induce apoptosis of leukemic cells and suppressed proliferation of various tumor cell lines. Bisht et al., synthesized polymeric nanoparticle encapsulated formulation of curcumin by micellar aggregates of cross-linked and random copolymers of N-isopropylacrylamide, N-vinyl-2-pyrrolidone and poly (ethyleneglycol) monoacrylate [60]. The nanoparticles encapsulated with curcumin exhibited *in vitro* therapeutic efficacy against human pancreatic cancer cell lines. The mechanisms of action of the nanoparticles encapsulated with curcumin against the cancer cell lines was by induction of cellular apoptosis, blockade of nuclear factor kappa B (NFκB) activation and downregulation of steady state levels of multiple proinflammatory cytokines (IL-6, IL-8, and TNFα). These findings further confirmed the benefits of nanoformulation of curcumin. Lim et al. developed nanoparticle-encapsulated curcumin active against medulloblastoma and glioblastoma cells [61]. The curcumin nanoparticles inhibited malignant brain tumor growth through the modulation of cell proliferation, survival and stem cell phenotype. Ha et al., encapsulated curcumin nanoparticles onto copolymer [62]. They exhibited enhanced cellular uptake on cancer cell lines i.e., HT29 and HeLa. Nanoparticles laced with curcumin was reported to enhance

neurogenesis, opening doors to treatment of neurodegenerative disorders such as Alzheimer's disease [63]. Curcumin-encapsulated nanoparticles induced proliferation of the neural stem cells. There is also a report on solid lipid nanoparticles of curcumin for the treatment of oral mucosal infection [64]. There was an enhanced uptake of curcumin in the mucosal tissue. The nanoparticles exhibited high antimicrobial activity against *Staphylococcus aureus*, *Streptococcus mutans*, *Viridans streptococci*, *Escherichia coli*, *Lactobacillus acidophilus* and *Candida albicans* than the free curcumin. There are other research reports on the potential medical applications of nanoparticles of curcumin [65-71, 72].

DENDRIMERS

Dendrimers are nanocarriers and they are composed of an interior core, several interior layers and exterior multivalent surface that has functionalities for possible drug anchoring [73]. Most dendrimers have molecular diameters of less than 10 nm and this unique property makes them useful as biomimics [74]. Their dendritic structure consists of a number of functional groups useful for encapsulation of drug molecules, targeting moieties and solubilizing groups [75]. The interior of dendrimers is also useful for encapsulation of drugs [76]. They have been used for encapsulation of several types of drugs [77-82]: anticancer drugs, antimicrobial, anti-malaria, antiviral, anti-inflammatory etc. Wang et al., prepared poly(amidoamine) dendrimer to encapsulate curcumin for drug delivery to cancer cells [83]. The dendrimer encapsulated with curcumin exhibited higher anti-proliferative activity against A549 cell lines than the free curcumin. The dendrimer also had a significant effect on the generation of intracellular reactive oxygen species, the mitochondrial membrane potential and cell apoptosis suggesting that encapsulation of curcumin onto dendrimers enhances its anticancer effects. Mollazade et al., investigated the anti-proliferative effect of PAMAM encapsulated with curcumin on T47D breast cancer cell line [84]. While PAMAM dendrimers encapsulated with curcumin decreased the IC50 for proliferation and also increased the inhibitory effect on telomerase activity. Abderrezak, et al., investigated the interaction of selected dendrimers with different compositions with hydrophilic and hydrophobic drugs (i.e., cisplatin, resveratrol, genistein and curcumin) [85]. Dendrimers encapsulated with curcumin was found to be very stable at physiological pH. Song et al., developed linear-dendrimer methoxy-poly (ethylene glycol)-b-poly (ε-

caprolactone) copolymer encapsulated with curcumin [86]. In vitro release cytotoxic activities against Hela and HT-29 cells showed enhanced anticancer activity of the curcumin. Debnath et al., prepared dendrimer-curcumin conjugate that exhibited enhanced water solubility and cytotoxic effects against SKBr3 and BT549 breast cancer cells [87]. Yallapu et al., evaluated the interaction of curcumin nanoformulations with cancer cells, serum proteins, and human red blood cells so as to determine their clinical application [88]. The significant binding capacity of dendrimer-curcumin to plasma protein was observed.

NANOGELS

Nanogels are nanosized particles that are formed by physically or chemically cross-linked polymer networks [89]. The exhibit good swelling ability and they have been proven to deliver drugs in controlled, sustained and targetable manner. They have unique feature such as [89]: their particle size and surface properties can be manipulated to minimise rapid clearance by phagocytic cells, resulting in passive and active drug targeting, controlled and sustained drug release to the target site; they improve the therapeutic efficacy and reduce side effects of drugs; they exhibit high drug loading capacity; they are non-immunogenic; they are biocompatible and biodegradable. Their size range is between 20–200 nm in diameter which makes them effective in hindering rapid renal clearance [89]. Madhusudana et al., developed interpenetrating polymeric network nanogels from gelatin by simple free radical emulsion polymerization [90]. The curcumin-encapsulated nanogels exhibited good bioavailability. The cytotoxicity test suggested that the nanogels is pH sensitive and that they are potential delivery systems for colorectal cancer drug delivery applications. Gonçalves et al., developed self-assembled nanogels obtained from dextrin for encapsulation of curcumin [91]. The *in vitro* release profile suggested that the nanogel are suitable carrier for controlled release of curcumin. The curcumin-loaded nanogel exhibited cytotoxic effects against HeLa cell lines. Wu et al., [92] developed multifunctional hybrid nanogels for intracellular delivery of curcumin. In vivo results proved that they are potential therapeutic agents for treatment of cancers and other diseases. Wei et al., also reported hyaluronic acid based nanogel-drug conjugates with improved anticancer activity [93]. They were found to be effective for efficient targeting and suppression of drug-resistant tumors.

POLYMER-DRUG CONJUGATES

The incorporation of bioactive agents to polymeric carrier usually result in enhanced therapeutic effects of the bioactive agents such as increased bioavailability, targeted drug delivery profile with reduced toxicity and overcome resistance build-up [94]. Polymer-drug conjugate is composed of five parts namely: the polymeric backbone, the drug, the spacer, the targeting group and the solubilising group [94, 95]. The mechanism of action of polymer-drug conjugates is influenced by its structural design such as [94, 96-98]: flexible backbone must be non-toxic, biocompatible and consist of solubilizing functionalities that will enhance its water solubility; molecular weight should be below the renal excretion to prevent quick excretion from the body; backbone should be biodegradable and susceptible to catabolic processes for elimination of the polymer after drug release process; the carriers should have reactive functionalities for polymer-drug conjugation which should be sufficiently remote from the main chain to permit enzyme approach and cleavage action that results in target drug release mechanism; there should be targeting moieties to enhance drug targeting capability; linkers introduced to the carrier should be stable in blood circulation ensuring that the polymeric prodrug is inert during transport. There are research reports on their applications, for treatment of diabetes, cancer, inflammation, viral infection, bacterial infection etc.

Tang et al., reported polymer-drug conjugates with high molecular weight encapsulated with curcumin [99]. The design of the backbone of the conjugates resulted in high drug loading efficiency, fixed drug loading content, enhanced stability of curcumin and water solubility. The polymer-drug conjugates of curcumin were cytotoxic to cancer cells (i.e., SKOV-3, OVCAR-3 ovarian cancers, and MCF-7). They exhibited enhanced cellular uptake resulting in hydrolytic cleavage and release of curcumin. The conjugates inhibited SKOV-3 cell cycle at G(0)/G(1) phase in vitro and induced cell apoptosis partially through the caspase-3 dependent pathway. Zhang et al., prepared polymer residue of hydrazone-containing pH responsive polymeric conjugate micelles that induce significant cytotoxicity in HeLa cell lines [100]. Yang et al., developed polymer-drug conjugate micelle to enhance the delivery of curcumin [101]. Multiple curcumin molecules were conjugated to poly(lactic acid) via tris(hydroxymethyl)aminomethane linker. The conjugates were found to be in a range of <100 nm. The drug-encapsulated conjugate micelles cellular uptake compartment of HepG2 cells was significant. Nkazi et al., demonstrated the polymer-drug co-conjugation of

curcumin and an analogue of ferrocene to a single polymeric carrier via hydrazone linker [102]. The conjugates were found to be potential delivery system for combination therapy. Manju and Sreenivasan, synthesized polyvinylpyrrolidone–curcumin conjugates [103]. The drug conjugates were self-assembled in aqueous solution to form nanosized micellar aggregates. The conjugates were also cationic. The conjugates exhibited higher cytotoxic effects than the free curcumin. In another research report by Aderibigbe et al., polyamidoamine conjugates containing curcumin and bisphosphonate were synthesized by aqueous phase Michael addition reaction [104]. The in vitro release studies of curcumin from the polyamidoamine was slower at pH 7.4 than at pH 5.8. The release profiles suggested that the conjugates are more stable at pH 7.4 and hence, are potential sustained drug delivery systems for combination therapy.

GRAPHENE OXIDE

Graphene oxide sheets are carbon based biomaterials and they are hydrophilic and they exhibit reasonable colloidal dispersibility under appropriate pH conditions [105]. The unique properties of graphene oxide that makes it to be preferred over other carbon-based materials are good aqueous dispersibility, low cost, absence of particles of toxic metal and colloidal stability [106, 107]. They also have high therapeutic loading capacity, high surface area, negative charge which establish electrostatic interactions with positively charged (i.e., highly hydrophilic) polymers and a variety of functional groups on its surface for possible surface bi-conjugation and because of these aforementioned properties, they are applied in biomedical field [106, 107]. In a research by Hatamie et al., curcumin-functionalized reduced graphene oxide sheets with concentrations greater than 70 μg/mL exhibited cytotoxic effects by inducing slight cell deaths, cell apoptosis and cell morphological changes. The results showed that functionalized reduced graphene oxide nanomaterials can be used effectively in drug delivery [108]. Some et al., incorporated curcumin onto graphene composites to produce biocomposites with anticancer activity [109]. The drug loading increased with increase in the number of oxygen-containing functional groups in graphene-derivatives. A synergistic effect of curcumin loaded graphene composites was very significant on cancer cell death of HCT 116 both in vitro and in vivo. The graphene quantum dot-curcumin composites contained the highest amount of curcumin nano-particles and exhibited the best anticancer activity compared to

the other composites and the free curcumin. This finding suggested that the quantum dot-curcumin composites are potential devices for synergistic chemotherapy with superficial bioprobes for tumor imaging.

CONCLUSION

Curcumin exhibit various medicinal properties such as anticancer, anti-inflammatory, anti-diabetic, anti-bacterial, anti-Alzheimer, antimicrobial and antimalarial activities etc. However, its application is hampered by its poor water solubility and bioavailability. As a results of these shortcomings, several researchers are currently focused on the development of effective delivery systems that will increase the hydrophilicity, stability, bioavailability and control the delivery of curcumin to target site. The application of nanotechnology to develop nanoformulations of curcumin have resulted in enhanced water solubility, bioavailability and targeted delivery mechanism and they have been found to be useful for combination therapy where other drugs are incorporated together with curcumin. This has also increased the half-life, reduced toxic adverse effects of curcumin resulting in improve pharmacokinetic profile and therapeutic efficacy. However, most of the studies were performed on animal models and cell lines. There is still a pressing need to understand the effects of curcumin nanoformulations on humans.

ACKNOWLEDGMENT

The financial assistance of Medical Research Council (Self-Initiated research), South Africa is hereby acknowledged. The views and opinions expressed are not those of the MRC but of the authors.

REFERENCES

[1] Srivastava, RM; Singh S; Dubey SK; Misra K; Khar A. Immunomodulatory and therapeutic activity of curcumin. *International Immunopharmacology*, 2010, 11, 331– 341.

[2] Araujo, CC; Leon, LL. Biological activities of Curcuma longa L. Mem. Inst. *Oswaldo Cruz*. 2001, 96, 723-728.

[3] Tumeric. https:// www.nlm.nih.gov/ medlineplus/ druginfo/ natural/ 662. html. Accessed 27th September 2015.

[4] Priyadarsini, KI. Chemical and structural features influencing the biological activity of curcumin. *Curr. Pharm. Des*., 2013, 19, 2093–2100.

[5] Neeraj, C; Sekhon, BS. Potential therapeutic effect of curcumin - an update. *J. Pharm. Educ. Res*., 2012, 3, 64-71.

[6] Anand, P; Kunnumakkara, AB; Newman, RA; Aggarwal, BB. Bioavailability of curcumin: problems and promises. *Mol. Pharm*., 2001, 4, 807–818.

[7] Sahoo, SK. Application of nanomedicine. *APBN*, 2005, 9, 1048-1050.

[8] Davis, ME; Chen, ZG; Shin, DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. *Nat. Rev. Drug Discov*., 2008, 7, 771-782.

[9] Farokhzad, OC; Langer, R. Impact of nanotechnology on drug delivery. *ACS Nano*, 2009, 3, 16-20.

[10] Shi, J; Votruba, AR; Farokhzad, OC; Langer, R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett*., 2010, 10, 3223-3230.

[11] Babu, PJ; Saranya, S; Mallepogu, V. Nanoformulations as Drug Delivery Vehicles for Cancer Treatment. Austin. *J. Nanomed. Nanotechnol*., 2015, 3, 1-3.

[12] Mukherjee, B; Dey, NS; Maji, R; Bhowmik, P; Das, PJ; Paul, P. Current Status and Future Scope for Nanomaterials in Drug Delivery. Book title: Application of nanotechnology in drug delivery, chapter 16, 525-544. Publisher: InTech, 2014.

[13] Kumar, A; Badde, S; Kamble, R; Pokharkar, VB. Development and characterization of liposomal drug delivery system for nimesulide. *Int. J. Pharm. Pharm. Sci*., 2010, 2, 87-89.

[14] Arab Tehrany, E; Kahn, CJ; Baravian, C; Maherani, B; Belhaj, N; Wang, X; Linder, M. Elaboration and characterization of nanoliposome made of soya, rapeseed and salmon lecithins: application to cell culture. Colloids Surf. *B Biointerfaces*., 2012, 95, 75-81.

[15] Mourtas, S; Lazar, AN; Markoutsa, E; Duyckaerts, C; Antimisiaris SG. Multifunctional nanoliposomes with curcumin-lipid derivative and brain targeting functionality with potential applications for Alzheimer disease. *Eur J Med Chem*., 2014, 80, 175-183.

[16] Lazar, AN; Mourtas, S; Youssef, I; Parizot, C; Dauphin, A; Delatour, B; Antimisiaris, SG; Duyckaerts, C. Curcumin-conjugated nanoliposomes

with high affinity for Aβ deposits: Possible applications to Alzheimer disease. *Nanomed. Nanotechnol. Biol. Med*., 2013, 9, 712–721.

[17] Shin, GH; Chung, SK; Kim, JT; Joung, HJ; Park, HJ. Preparation of Chitosan-Coated Nanoliposomes for Improving the Mucoadhesive Property of Curcumin Using the Ethanol Injection Method. *J. Agric. Food Chem*., 2013, 61, 11119–11126.

[18] Hasan, M; Belhaj, N; Benachour, H; Barberi-Heyob, M; Kahn, CJ; Jabbari, E; Linder, M; Arab-Tehrany, E. Liposome encapsulation of curcumin: Physico-chemical characterizations and effects on MCF7 cancer cell proliferation. *Int. J. Pharm*., 2014, 461, 519–528.

[19] Taylor, M; Moore, S; Mourtas, S; Niarakis, A; Re, F; Zona, C; La Ferla, B; Nicotra, F; Masserini, M., Antimisiaris, SG; Gregori, M; Allsop, D. Effect of curcumin-associated and lipid ligand-functionalized nanoliposomes on aggregation of the Alzheimer's Aβ peptide. *Nanomed. Nanotechnol. Biol. Med*., 2011, 7, 541–550.

[20] Chen, X; Zou, LQ; Niu, J; Liu, W; Peng, SF; Liu, CM. The Stability, Sustained Release and Cellular Antioxidant Activity of Curcumin Nanoliposomes. *Molecules*, 2015, 20, 14293-14311.

[21] MacKay, JA; Chen, M; McDaniel, JR; Liu, W; Simnick, AJ; Chilkoti, A. Self-assembling chimeric polypeptide-doxorubicin conjugate nanoparticles that abolish tumors after a single injection. *Nat.Mater*., 2009, 8, 993–999.

[22] Gong, C; Xie, Y; Wu, Q; Wang, Y; Deng, S; Xiong, D; Liu, L; Xiang, M; Qian, Z; Wei, Y. Improving anti-tumor activity with polymeric micelles entrapping paclitaxel in pulmonary carcinoma. *Nanoscale*, 2012, 4, 6004–6017.

[23] Gong, C; Wang, C; Wang, Y; Wu, Q; Zhang, D; Luo, F; Qian, Z. Efficient inhibition of colorectal peritoneal carcinomatosis by drug loaded micelles in thermosensitive hydrogel composites. *Nanoscale*, 2012, 4, 3095–3104.

[24] Gao, X; Zheng, F; Guo, G; Liu, XX; Fan, R; Qian, ZY; Huang, N; Wei, YQ. Improving the anti-colon cancer activity of curcumin with biodegradable nano-micelles. *J. Mater. Chem. B*, 2013, 1, 5778-5790.

[25] Taurin, S; Nehoff, H; Diong, J; Larsen, L; Rosengren, RJ; Greish, K. Curcumin-derivative nanomicelles for the treatment of triple negative breast cancer. *J. Drug Target*., 2013, 21, 675-683.

[26] Gülçür, E; Thaqi, M; Khaja, F; Kuzmis, A; Önyüksel, H. Curcumin in VIP-targeted sterically stabilized phospholipid nanomicelles: a novel

therapeutic approach for breast cancer and breast cancer stem cells. *Drug Deliv. Transl. Res*., 2013, 3, 562–574.

[27] Tripodo, G; Pasut, G; Trapani, A; Mero, A; Lasorsa, FA; Chlapanidas, T; Trapani, G; Mandracchia, D. Inulin-d-α-Tocopherol Succinate (INVITE) Nanomicelles as a Platform for Effective Intravenous Administration of Curcumin. *Biomacromol*., 2015, 16, 550–557.

[28] Kesharwani, P; Banerjee, S; Padhye, S; Sarkar, FH; Iyer, AK. Hyaluronic Acid Engineered Nanomicelles Loaded with 3, 4-Difluorobenzylidene Curcumin for Targeted Killing of CD44+ Stem-Like Pancreatic Cancer Cells. *Biomacromol*. 2015, 16, 3042–3053.

[29] Chen, Y; Zhang, X; Lu, J; Huang, Y; Li, J; Li, S. Targeted Delivery of Curcumin to Tumors *via* PEG-Derivatized FTS-Based Micellar System. *AAPS J*., 2014, 16, 600–608.

[30] He, H; Pham-Huy, LA; Dramou, P; Xiao, D; Zuo, P; Pham-Huy, C. Carbon Nanotubes: Applications in Pharmacy and Medicine. *BioMed Research International*, *Hindawi Publishing Corporation*, *Volume*, 2013, Article ID 578290, 12 pages, http:// dx.doi.org/ 10.1155/ 2013/ 578290

[31] Zhang, Y; Bai, Y; Yan, B. Functionalized carbon nanotubes for potential medicinal applications. *Drug Discov. Today*, 2010, 15, 428–435.

[32] Yuan, S; Zeng, L; Zhuang, Y; Hou, Q; Song, M. Functionalized single-walled carbon nanotubes for the improved solubilization and delivery of curcumin. Fuller. *Nanotub. Car. N*., 2015. DOI:10.1080/ 1536383X. 2015.1088007.

[33] Li, H; Zhang, N; Hao, Y; Wang, Y; Jia, S; Zhang, H; Zhang, Y; Zhang, Z. Formulation of curcumin *Drug Deliv*, 2014, 21, 379-387.

[34] Reeves, AE; Wickstrom, E; Vinogradov, SV. Curcumin-Combretastatin Nanocells as Breast Cancer Cytotoxic and Antiangiogenic Agent. http:// handle.dtic.mil/100.2/ADA494015. Accessed 30th September 2015.

[35] Jaiswal, M; Dudhe, R; Sharma, PK. Nanoemulsion: an advanced mode of drug delivery system, *Biotech*., 2015, 5, 123–127.

[36] Kim, CK; Cho, YJ; Gao, ZG. Preparation and evaluation of biphenyl dimethyl dicarboxylate microemulsions for oral delivery. *J. Control Release*, 2001, 70, 149–155.

[37] Wagner, JG; Gerrard, ES; Kaiser, DG. The effect of the dosage form on serum levels of indoxole. *Clin. Pharmacol. Ther*., 1996, 7, 610–619.

[38] Ganta, S; Amiji, M. Coadministration of Paclitaxel and Curcumin in Nanoemulsion Formulations to Overcome Multidrug Resistance in Tumor Cells. *Mol. Pharm*., 2009, 6, 928–939.

[39] Ahmed, K; Li, Y; McClements, DJ; Xia, H. Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chem.*, 2012, 132, 799–807.

[40] Sari, TP; Mann, B; Kumar, R; Singh, RRB; Sharma, R; Bhardwaj, M; Athira, S. Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloid.*, 2015, 43, 540-546.

[41] Donsì, F; Sessa, M; Mediouni, H; Mgaidi, A; Ferrari, G. Encapsulation of bioactive compounds in nanoemulsion- based delivery systems. *Procedia Food Sci*., 2011, 1, 1666-1671.

[42] Rachmawati, H; Yee, CW; Rahma, A. Formulation of Tablet Containing Curcumin Nanoemulsion *Int. J. Pharm. Pharm. Sci*., 2014, 6, 115-120.

[43] Rachmawati, H; Budiputra, DK; Suhandono, S; Anggadiredja, K. Curcumin Nanoemulsion for Transdermal Application: *Formulation and Evaluation Research and Development on Nanotechnology in Indonesia*, 2014, 1, 5-8.

[44] Yu, H; Huang, O. Improving the Oral Bioavailability of Curcumin Using Novel Organogel-Based Nanoemulsions. *J. Agric. Food Chem*., 2012, 60, 5373–5379.

[45] Iranian researchers. I ran daily April 27th 2015. http://www.iran-daily.com/News/116710.html. Accessed October 1, 2015.

[46] Ucisik, MH; Küpcü, S; Schuster, B; Sleytr, UB. Characterization of CurcuEmulsomes: nanoformulation for enhanced solubility and delivery of curcumin. *J. Nanobiotechnol*., 2013, 37, 13 pages.

[47] Onodera, T; Kuriyama, I; Andoh, T; Ichikawa, H; Sakamoto, Y; Lee-Hiraiwa, E; Mizushina, Y. Influence of particle size on the in vitro and in vivo anti-inflammatory and anti-allergic activities of a curcumin lipid nanoemulsion. *Int. J. Mol. Med*., 2015, 35, 1720-1728.

[48] Nanoshell. http:// nano.cancer. Accessed October 1, 2015.

[49] Zhu, XM; Yuan, J; Leung, KC; Lee, SF; Sham, KW; Cheng, CH; Au, DW; Teng, GJ; Ahuja, AT; Wang, YX. Hollow superparamagnetic iron oxide nanoshells as a hydrophobic anticancer drug carrier: intracelluar pH-dependent drug release and enhanced cytotoxicity. *Nanoscale*., 2012, 18, 5744-5754.

[50] Braden, ARC; Vishwanatha, JK. Formulation of active agent loaded activated PLGA nanoparticles for targeted cancer nano-therapeutics, US 9023395 B2, May 5, 2015.

[51] Arzt, E; Gumbsch, P. Small-scale materials and structures. In: *European White Book on Fundamental Research in Materials Science (Volume 5)*. Rühle M, Dosch H, Mittemeijer EJ, Van de Voorde MH (Eds). Max-

Planck-Institut für Metallforschung, Stuttgart, Germany, 176–180 (2001).

[52] Bamrungsap, S; Zhao, Z; Chen, T; Wang, L; Li, C; Fu, T; Tan, W. Nanotechnology in Therapeutics: A Focus on Nanoparticles as a Drug Delivery System. *Nanomed*., 2012, 7, 1253-1271.

[53] Heo, DN; Ko, WK; Moon HJ; Kim, HJ; Lee, SJ; Lee, JB; Bae, MS; Yi, J-K; Hwang, Y-S; Bang, JB; Kim, E-C; Do, SH; Kwon, IK. Inhibition of Osteoclast Differentiation by Gold Nanoparticles Functionalized with Cyclodextrin Curcumin Complexes. *ACS Nano*, 2014, 8, 12049–12062.

[54] Mulik, RS; Mönkkönen, J; Juvonen, RO; Mahadik, KR; Paradkar, AR. ApoE3 mediated poly(butyl) cyanoacrylate nanoparticles containing curcumin: study of enhanced activity of curcumin against beta amyloid induced cytotoxicity using in vitro cell culture model. *Mol. Pharm*., 2010 Jun 7, 815-825.

[55] Amano, C; Minematsu, H; Fujita, K; Iwashita, S; Adachi, M; Igarashi, K; Hinuma, S. Nanoparticles Containing Curcumin Useful for Suppressing Macrophages In Vivo in Mice. *PLoS One*., 2015 Sep 11, e0137207.

[56] Krausz, AE; Adler, BL; Cabral, V; Navati, M; Doerner, J; Charafeddine, RA; Chandra, D; Liang, H; Gunther, L; Clendaniel, A; Harper, S; Friedman, JM; Nosanchuk, JD; Friedman, AJ. Curcumin-encapsulated nanoparticles as innovative antimicrobial and wound healing agent. *Nanomedicine*., 2015 Jan, 11(1), 195-206.

[57] Jambhrunkar, S; Karmakar, S; Popat, A; Yu, M; Yu, C. Mesoporous silica nanoparticles enhance the cytotoxicity of curcumin. *RSC Adv*., 2014, 4, 709–712.

[58] Bhawana, Basniwal, RK; Buttar, HS; Jain, VK; Jain, N. Curcumin nanoparticles: preparation, characterization, and antimicrobial study. *J. Agric. Food Chem*., 2011, 59, 2056-2061.

[59] Anand, P; Nair, HB; Sung, B; Kunnumakkara, AB; Yadav, VR; Tekmal, RR; Aggarwal, BB. Design of curcumin-loaded PLGA nanoparticles formulation with enhanced cellular uptake, and increased bioactivity *in vitro* and superior bioavailability *in vivo*. *Biochem. Pharmacol*., 2010, 79, 330–338.

[60] Bisht, S; Feldmann, G; Soni, S; Ravi, R; Karikar, C; Maitra A; Maitra, A. Polymeric nanoparticle-encapsulated curcumin ("nanocurcumin"): a novel strategy for human cancer therapy. *J. Nanobiotechnol*., 2007, 5, 18 pages.

[61] Lim, KJ; Bisht, S; Bar, EE; Maitra, A; Charles, G. Eberhart. A polymeric nanoparticle formulation of curcumin inhibits growth, clonogenicity and stem-like fraction in malignant brain tumors. *Cancer Biol. Ther*., 2011, 11, 1-10.

[62] Ha, PT; Le, MH; Hoang, TMN; Le, TTH; Duong, T. Q; Tran, THH; Tran, DL; Nguyen, XP. Preparation and anti-cancer activity of polymer-encapsulated curcumin nanoparticles. *Adv. Nat. Sci. Nanosci. Nanotechnol*., 2012, 3, 7 pages.

[63] Tiwari, SK; Agarwal, S; Seth, B; Yadav, A; Nair, S; Bhatnagar, P; Karmakar, M; Kumari, M; Chauhan, LKS; Patel, DK; Srivastava, V; Singh, D; Gupta, SK; Tripathi, A; Chaturvedi, RK; Gupta, KC. Curcumin-Loaded Nanoparticles Potently Induce Adult Neurogenesis and Reverse Cognitive Deficits in Alzheimer's Disease Model *via* Canonical Wnt/β-Catenin Pathway. *ACS Nano*, 2014, 8, 76–103.

[64] Hazzah, HA; Farid, RM; Nasra, MMA; Hazzah, WA; El-Massik, MA; Abdallah, OY. Gelucire-Based Nanoparticles for Curcumin Targeting to Oral Mucosa: Preparation, Characterization, and Antimicrobial Activity Assessment. *J. Pharm. Sci*., 2015, DOI: 10.1002/jps.24590.

[65] Pandit, RS; Gaikwad, SC; Agarkar, GA; Gade, AK; Rai, M. Curcumin nanoparticles: physico-chemical fabrication and its in vitro efficacy against human pathogens. *3 Biotech*, 2015, DOI 10.1007/s13205-015-0302-9.

[66] Yen, FL; Tsai, MH; Yang, CM; Liang, CJ; Lin, CC; Chiang, YC; Lee, HC; Ko, HH; Lee, CW. Curcumin nanoparticles ameliorate ICAM-1 expression in TNF-alpha-treated lung epithelial cells through p47 (phox) and MAPKs/AP-1 pathways. *PLoS ONE*, 2013, 8, e63845-e63845.

[67] Udompornmongkol, P; Chiang, B-H. Curcumin-loaded polymeric nanoparticles for enhanced anti-colorectal cancer applications. *J. Biomaterial Appl*., 2015, 0885328215594479.

[68] Yallapu, MM; Maher, DH; Sundram, V; Bell, MC; Jaggi, M; Chauhan, SC. Curcumin induces chemo/radio-sensitization in ovarian cancer cells and curcumin nanoparticles inhibit ovarian cancer cell growth. *J. Ovarian Res*., 2010, 3, 11.

[69] Sun, M; Gao, Y; Guo, C; Cao, F; Song, Z; Xi, Y; Yu, A; Li, A; Zhai, G. Enhancement of transport of curcumin to brain in mice by poly(*n*-butylcyanoacrylate) nanoparticle. *J. Nanopart. Res*., 2010, 12, 3111-3122.

[70] Yin, H; Zhang, H; Liu, B. Superior anticancer efficacy of curcumin-loaded nanoparticles against lung cancer. *Acta Biochim Biophys Sin (Shanghai)*.. 2013, 45, 634-640.

[71] Hu, L; Kong, D; Hu, Q; Gao, N; Pang, S. Evaluation of High-Performance Curcumin Nanocrystals for Pulmonary Drug Delivery both *In Vitro* and *In Vivo*. *Nanoscale Res. Lett*., 2015, 10, 381 doi:10.1186/ s11671-015-1085-y.

[72] Yallapu, MM; Dobberpuhl, MR; Maher, DM; Jaggi, M; and ChandChauhan, S. Design of Curcumin loaded Cellulose Nanoparticles for Prostate Cancer. *Current Drug Metabolism*, 2012, 13, 120-128

[73] Noriega-Luna, B; Godínez, LA; Rodríguez, FJ; Rodríguez, A; Zaldívar-Lelo de Larrea, G; Sosa-Ferreyra, CF; Mercado-Curiel, RF; Manríquez, J; Bustos, E. Applications of dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection. *J. Nanomater*., 2014 (2014), Article ID 507273, 19 pages http://dx.doi.org/10.1155/2014/507273.

[74] Esfand, R; Tomalia, DA. Poly (amidoamine) (PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications. *Drug Discov. Today*, 2001, 6, 427–436.

[75] Kolhe, P; Khandare, J; Pillai, O; Kannan, S; Lieh-Lai, M; Kannan, RM. Preparation, cellular transport, and activity of polyamidoamine-based dendritic nanodevices with a high drug payload. *Biomaterials*, 2006, 27, 660–669.

[76] D'Emanuele, A; Attwood, D. Dendrimer-drug interactions. Adv. *Drug Deliv. Rev*., 2005, 57, 2147–2162.

[77] Cheng, Y; Wang, J; Rao, T; He, X; Xu, T. Pharmaceutical applications of dendrimers: promising nanocarriers for drug delivery. *Front. Biosci*., 2008, 13, 1447–1471.

[78] Ma, M; Cheng, Y; Xu, Z; Xu, P; Qu, H; Fang, Y; Xu, T; Wen, L. Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model drug. *Eur. J. Med. Chem*., 2007, 42, 93–98.

[79] Cheng, Y; Qu, H; Ma, M; Xu, Z; Xu, P; Fang, Y; Xu, T. Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: an in vitro study. *Eur. J. Med. Chem*., 2007, 42, 1032–1038.

[80] Tripathi, PK; Khopade, AJ; Nagaich, S; Shrivastava, S; Jain, S; Jain, NK. Dendrimer grafts for delivery of 5-flurouracil. *Pharmazie*, 2002, 57, 261–264.

[81] McCarthy, TD; Karellas, P; Henderson, SA; Giannis, M; O'Keefe, DF; Heery, G; Paul, J. R; Matthews, BR; Holan, G. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol. Pharm*., 2005, 2, 312–318.

[82] Chauhan, A; Diwan, P; Jain, N; Raghavan, K. Composition and complexes containing a macromolecular compound as potential anti-inflammatory agents. US Patent 20030180250 A1, 2003.

[83] Wang, L; Xu, X; Zhang, Y; Zhang, Y; Zhu, Y; Shi, J; Sun; Huang, Q. Encapsulation of curcumin within poly (amidoamine) dendrimers for delivery to cancer cells. *J. Mater. Sci. Mater. Med*., 2013, 24, 2137-2144.

[84] Mollazade, M; Nejati-Koshki, K; Akbarzadeh, A; Zarghami, N; Nasiri, M; Jahanban-Esfahlan, R; Alibakhshi, A. PAMAM Dendrimers Augment Inhibitory Effects of Curcumin on Cancer Cell Proliferation: Possible Inhibition of Telomerase. *Asian Pac. J. Cancer Prev*., 14, 2013, 6925-6928.

[85] Abderrezak, A; Bourassa, P; Mandeville, JS. Affiliation: Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada Sedaghat-Herati,Affiliation: Department of Chemistry, Missouri State University, Springfield, Missouri, United States of America ?R; Tajmir-Riahi, H-A. Dendrimers Bind Antioxidant Polyphenols and cisplatin DrugAffiliation: Département de Chimie-Biologie, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, Canada . *PLoS ONE*, 2012, 7, e33102.

[86] Song, Z; Zhu, W; Song, J; Wei, P; Yang, F; Liu, N; Feng, R. Linear-dendrimer type methoxy-poly (ethylene glycol)-b-poly (?-caprolactone) copolymer micelles for the delivery of curcumin. *Drug Deliv*., 2014, 22, 58-68.

[87] Debnath, S; Saloum, D; Dolai, S; Sun; Averick, S; Raja, K; Fata. Dendrimer-curcumin conjugate: a water soluble and effective cytotoxic agent against breast cancer cell lines. *Anticancer Agents Med. Chem*., 2013, 13, 1531–1539.

[88] Yallapu, MM; Ebeling, MC; Chauhan, N; Jaggi, M; Chauhan, SC. Interaction of curcumin nanoformulations with human plasma proteins and erythrocytes. *Int. J. Nanomed*., 2011, 6, 2779–2790.

[89] Sultana, F; Manirujjaman; Imran-Ul-Haque; Arafat, M; Sharmin, S. An Overview of Nanogel Drug Delivery System. *J. Appl. Pharm. Sci*., 2013, 3, S95-S105.

[90] Madhusudana, RK; Krishna Rao, KS; Ramanjaneyulu, G; Ha, CS. Curcumin encapsulated pH sensitive gelatin based interpenetrating polymeric network nanogels for anticancer drug delivery. *Int. J. Pharm*., 2015, 478, 788-795.

[91] Gonçalves, C; Pereira, P; Schellenberg, P; Coutinho, PJ; Gama, FM. Self-Assembled Dextrin Nanogel as Curcumin Delivery System. J. Biomater. *Nanobiotechnol*., 2012, 3, 178-184.

[92] Wu, W; Shen, J; Banerjee, P; Zhou, S. Water-dispersible multifunctional hybrid nanogels for combined curcumin and photothermal therapy. *Biomaterials*, 2011, 32, 598–609.

[93] Wei, X; Senanayake, TH; Warren, G; Vinogradov, S. V. Hyaluronic acid-based nanogel-drug conjugates with enhanced anticancer activity designed for the targeting of CD44-positive and drug-resistant tumors. *Bioconjug. Chem*., 2013, 24, 658–668.

[94] Aderibigbe, BA. Polymeric Prodrugs Containing Metal-Based Anticancer Drugs. *J. Inorg. Organomet. Polym*., 2015, 25, 339–353.

[95] Ringsdorf, H. Structure and properties of pharmacologically active polymers, *J. Polym. Sci. Pol. Sym*., 1975, 51, 135–153.

[96] Pasut, G; Veronese, FM. Polymer–drug conjugation, recent achievements and general strategies. *Prog. Polym. Sci*., 2007, 32, 933–961.

[97] Rohini; Agrawal, N; Joseph, A; Mukerji, A. Polymeric Prodrugs: Recent Achievements and General Strategies, *J. Antivir. Antiretrovir*., 2013, S15, 12 pages.

[98] Jaracz, S; Chen, J; Kuznetsova, LV; Ojima, I. Recent advances in tumor-targeting anticancer drug conjugates. *Bioorg. Med. Chem*., 2005, 13, 5043–5054.

[99] Tang, H; Murphy, CJ; Zhang, B; Shen, Y; Van Kirk, EA; Murdoch, WJ; Radosz, M. Curcumin polymers as anticancer conjugates. *Biomaterials*., 2010, 31, 7139-7149.

[100] Zhang, Y; Gao, M; Chen, C; Wang, Z; Zhao, Y. Residue cytotoxicity of a hydrazone-linked polymer–drug conjugate: implication for acid responsive micellar drug delivery. *RSC Adv*., 2015, 5, 34800-34802.

[101] Yang, R; Zhang, S; Kong, D; Gao, X; Zhao, Y; Wang, Z. Biodegradable Polymer-Curcumin Conjugate Micelles Enhance the Loading and Delivery of Low-Potency Curcumin. *Pharm. Res*., 2012, 29, 3512–3525.

[102] Nkazi, BD; Neuse, EW; Aderibigbe, BA. Polymeric Co-Conjugates of Curcumin. *J. Inorg. Organomet. Polym*., 2012, 22, 886–891.

[103] Manju, S; Sreenivasan, K. Synthesis and characterization of a cytotoxic cationic polyvinylpyrrolidone–curcumin conjugate. *J. Pharm. Sci*., 2011, 100, 504–511.

[104] Aderibigbe, BA; Sadiku, ER; Ray, SS; Mbianda, XY; Fotsing, MC; Jayaramudu, J; Owonubi, SJ. Synthesis, characterization and the release kinetics of antiproliferative agents from polyamidoamine conjugates. *J. Microencapsul*., 2015, 32, 432–442.

[105] Shih, CJ; Lin, S; Sharma, R; Strano, MS; Blankschtein, D. Understanding the pH-dependent behavior of graphene oxide aqueous solutions: a comparative experimental and molecular dynamics simulation study. *Langmuir*, 2012, 28, 235.

[106] Bitounis, D; Ali-Boucetta, H; Hong, BH; Min, D-H; Kostarelos, K. Prospects and Challenges of Graphene in Biomedical Applications. *Adv. Mater*., 2013, 25, 2258–2268.

[107] Bao, H; Pan, Y; Li, L. Recent Advances in Graphene-Based Nanomaterials for Biomedical Applications. *Nano Life*, 2012, 2, 15 Pages.

[108] Hatamie, S; Akhavanb, O; Sadrnezhaad, SK; Ahadian, M; Shirolkar, MM; Wang, HQ. Curcumin-reduced graphene oxide sheets and their effects on human breast cancer cells Mater. *Sci. Eng*., 2015, 55, 482–489.

[109] Some, S; Gwon, A-R; Hwang, E; Bahn, GH; Yoon, Y; Kim, Y; Kim, SH; Bak, S; Yang, J; Jo, DG; Lee, H. Cancer Therapy Using Ultrahigh Hydrophobic Drug-Loaded Graphene Derivatives. *Scientific Reports*, 2014, 4, 9 pages.

In: Curcumin
Editor: Valeria Martin
ISBN: 978-1-63484-272-3

Chapter 2

CURCUMIN NANO AND MICROENCAPSULATION AND ITS IMPLICATIONS ON CLINICAL USES

Jéssica T. P. Silva[1], Anderson C. da Silva[1], Bruno A. da Rocha[2], Lívia Bracht[2], Ciomar A. B. Amado[2], Fernanda V. Leimann[1] and Odinei H. Gonçalves[1,*]

[1]Post-Graduation Program of Food Technology –
Federal University of Technology – Paraná, Campo Mourão, PR, Brazil
[2]Post-Graduation Program of Pharmacological Sciences,
Maringá State University, Maringá, PR, Brazil

ABSTRACT

Encapsulation techniques have been widely applied to a diverse array of bioactive substances in order to improve stability, bioavailability and hydrophilicity and also to avoid side effects caused by high dosages required by some drugs. In the case of curcumin, its hydrophobic character and low stability in alkaline conditions, thermal treatment, light, metallic ions, ascorbic acid and others turn it into a natural candidate for encapsulation. Researches over the last decades have demonstrated that curcumin encapsulation is feasible being carried out by techniques such

* CEP: 87301-899, Campo Mourão - PR, Brazil. Email: odinei@utfpr.edu.br.

as nanoprecipitation, micellization, emulsification and miniemulsification followed by solvent removal, crosslinking reaction. However, attention must be paid when choosing the encapsulant matrix because it directly affects encapsulation efficiency and also due to the existence of specific interactions with the chemical structure of curcumin. Some examples are synthetic and bio-based amorphous or semi-crystalline polymers, hydrogels, solid lipids micro or nano carriers, ionic complexation matrices and. In the case of nanosized capsules, colloidal stability is also an issue because agglomeration leads to a decrease in overall specific surface area and slow release rates. Regarding capsules size, although microscale often led to increase in bioavailability, specific clinical uses such as cancer treatment require nanosized capsules due to the phagocytosis mechanism. However, microcapsules remain as a low cost, easy to scale up methodology to encapsulate curcumin. Recent studies focus on characterizing the resulting material to assure curcumin is properly encapsulated and homogeneously distributed along the matrix. Also, gains in bioavailability and pharmacological effects must be extensively demonstrated as to compensate the increased complexity of the encapsulation techniques.

1. ENCAPSULATION TECHNIQUES

Encapsulation is well recognized as an efficient way to modify substances at micro or nanoscale levels. There is a range of different techniques available and the correct choice depends on a number of factors as encapsulated substance-matrix interactions, use of harm solvents, hydrophilicity of components and costs. In the case of curcumin, the most appropriate techniques involves its dissolution in an appropriate solvent then removing it in the presence of strong stirring or an antisolvent substance to generate micro or nano sized particles. The most prominent curcumin encapsulation procedures are described below.

Microemulsion

Microemulsion is used to improve water dispersibility of hydrophobic compounds. Other claimed advantages of microemulsions are easy of preparation, thermodynamic stability, high solubilizing capacity and formulation flexibility regarding stabilizer, lipid carrier and the encapsulated drug itself (Bergonzi, Hamdouch, Mazzacuva, Isacchi, & Bilia, 2014; Spernath

& Aserin, 2006). However, proper formulation design generally requires knowledge on phase equilibria (Lin, Lin, Chen, Yu, & Lee, 2009; Piao et al., 2010). Encapsulation efficiency is demonstrated to be dose-dependent (Bergonzi et al., 2014) although maximum loads are in general low which is a disadvantage of microemulsion encapsulation.

Microemulsion is an alternative to transdermal delivery of curcumin when no harm components are used such as alcohol-free formulations (Sintov, 2015). *In-vitro* skin penetration studies in rats were reported comparing four systems: W/O microemulsion, micellar system, a surfactant-oil mixture and a 1% CUR solution in propylene carbonate. Results indicated that the microemulsion nature played an important role in curcumin permeability since it was statistically higher for W/O microemulsion and all four systems were essentially composed by the same ingredients.

Curcumin microemulsion using terpene was successfully prepared by Liu, Cheng and Hung (C.-H. Liu, Chang, & Hung, 2011). They observed that skin permeation rates greatly increased when the limonene/water contents were appropriately adjusted. The optimal formulation was able to retain 29.88 μg of curcumin in porcine skin samples, which means that limonene microemulsion loaded with curcumin is a promising tool for the percutaneous delivery of curcumin.

Bergonzi and colleagues (2014) have used the microemulsion technique aiming to improve the solubility and oral uptake of curcumin. The encapsulation of curcumin in microemulsions did not affect these systems, thus, the microemulsions resulted stable, with low tendency to aggregate. All formulations provided considerable improvement in solubility of curcumin, whose the best formulation presented 14.57 mg/ml of solubilization capacity and a percentage of permeation through the artificial membrane of about 10% of curcumin after 6 h and about 70% after 24 h.

Emulsion and Microemulsion Polymerization

Emulsion polymerization is the most common method to prepare polymer lattices with high solid content and fast reaction rate. In microemulsion polymerization, the particles are generated in a thermodynamically stable microemulsion phase containing swollen micelles. This process is based on homogeneous or micellar nucleation. On the other hand, miniemulsion polymerization is known to progress via monomer droplet nucleation without mass transport. Generaly, miniemulsion polymerization is used for

encapsulation of hydrophobic functional molecules and its preparation requires a high-pressure shear process using an ultrasonicator or a high-presure homogenizer (Sasaki et al., 2015).

Duan and co-workers (2010) have prepared curcumin-loaded chitosan/poly(butyl cyanoacrylate) nanoparticles using the emulsion polymerization technique. They obtained particles with spherical shape, average size of 200 nm and 90.04% of encapsulation efficiency. In *in vitro* studies, curcumin nanoparticles have shown comparable therapeutic efficacy to free curcumin against hepatocellular cancer cells and proapoptotic effects. Regarding to in vivo studies, curcumin nanoparticles suppressed hepatocellular carcinoma growth and inhibited tumor angiogenesis.

In another study, Duan and co-workers (2012) have used the emulsion polymerization technique to co-encapsulate doxorubicin and curcumin in poly(butyl cyanoacrylate) nanoparticles. This time the authors obtained particles with 133 nm in diameter and entrapment efficiencies of 49.98% and 94.52% to doxorubicin and curcumin, respectively. According to them, the dual-agent loaded in nanoparticles system had the similar cytotoxicity to co-administration of two single-agent loaded in nanoparticles. Besides, the simultaneous administration of anticancer compound (doxorubicin) and chemosensitizer (curcumin) achieved the highest reversal efficacy.

Emulsification/Solvent Evaporation

In this method, the dissolution of encapsulant (usually a preformed polymer) and curcumin is carried out using a suitable non-polar solvent like chloroform or dichloromethane and then the solution is emulsified into nanometric droplets in water with the aid of high HLB surfactants. The emulsification step also requires a high throughput energy device (ultrasound probe or high shear homogenizer) or mechanical stirring. After this, the solvent is evaporated overnight or under reduced pressure leading to the precipitation of the curcumin-loaded polymer submicrometric particles (Asua, 2002). Mechanical stirring leads to micrometric particles while high energy dispersers result in nanometric particles (Leimann et al., 2013). This technique is well suitable to encapsulate curcumin due to its high hydrophobic behavior.

Tsai and colleagues (2011) have used emulsification/solvent evaporation technique to prepare Curcumin-loaded PLGA nanoparticles. The mean particle size was 158 nm with 46.6% of entrapment efficiency. The oral bioavailability of encapsulated curcumin was 22-fold higher than free curcumin. Supporting

the oral study, excretion results have shown that absorption of curcumin was significantly increased by nano-formulation, which indicates that PLGA nanoparticles could potentially be applied to increase bioavailability of other hydrophobic polyphenols.

Chereddy and colleagues (2013) also have produced curcumin-loaded PLGA nanoparticles. The authors found that PLGA nanoparticles were able to protected curcumin from light degradation, enhanced water solubility and sustained a release of curcumin over a period of 8 days. Histology studies confirmed that curcumin-loaded PLGA nanoparticles exhibited higher anti-inflammatory potential, re-epithelialization and granulation tissue formation. In conclusion, PLGA nanoparticles containing curcumin could be used to healing of wounds.

Yoon and co-workers (2015) have used the emulsification/solvent evaporation technique to prepare poly(D,L-latic acid)-glycerol containing curcumin. They obtained particles with mean diameter of approximately 200 nm. The *in vitro* anti-tumor efficacy of curcumin nanoparticles was comparable to that of a solution of curcumin, but *in vivo* studies have shown that curcumin nanoparticles allowed the prolonged circulation of the drug in the blood stream and improved anticancer activity after intravenous injection.

Nanoprecipitation

Nanoprecipitation (or solvent displacement) method consists in the dispersion of hydrophobic droplets in non-solvent. Generaly, the hydrophobic solute (polymer or lipid molecule) is dissolved into a polar organic solvent like acetone, ethanol our tetrahydrofuran. Then, this solution is dripped to a large amount of a non-solvent (generally water) of the solute, under stirring. The mixed binary solution becomes a non-solvent for the hydrophobic molecules and the system evolves phase separation, leading to the formation of particles of the hydrophobic solute. This method doesn't require precursor emulsion, differing from emulsion-based methods, which makes it a faster option (Lepeltier, Bourgaux, & Couvreur, 2014).

Shaikh et al., (2009a) have encapsulated curcumin in PLGA by the nanoprecipitation technique. The nanoparticles showed spherical shape with particle size of 264 nm. *In vivo* studies have shown that curcumin encapsulated obtained at least 9-fold increase in oral bioavailability when compared to curcumin administrated with piperine as absorption enhancer. Zein nanoparticles were successful prepared by Patel et al., (Patel, Hu, Tiwari, &

Velikov, 2010). The authors obtained spherical shape particles with average particle size around 100 – 150 nm. The UV irradiation study confirmed enhanced photostability of curcumin into the nanoparticles. Besides, the particles presented good colloidal stability at differents pHs. The nanoprecipitation technique was used by Yallapu et al., (2014) to evaluate the role of nanoencapsulated curcumin in prostate cancer. Their results have demonstrated that PLGA nanoparticles containing curcumin efficiently inhibit growth of prostate cancer cells *in vitro* and *in vivo*.

Supercritical Anti-Solvent Precipitation (SAS)

Supercritical anti-solvent precipitation uses CO_2 as the anti-solvent. It diffuses into the organic solvent, generating an expansion of the volume, a decrease in solvent density and a decrease in solvent power. The quick diffusion provides a uniform and high degree of supersaturation, which promotes the formation of nanoparticles whose size and morphology can be controlled. It is an attractive method for nanoparticle formation for bioactive and pharmaceutical compounds due the absence of residual solvent, mild operating temperatures and a narrow particle size distribution (Joye & McClements, 2013).

Anwar et al., (2015) have applied the SAS methodology to encapsulate curcumin. The authors have found that SAS was able to provide a significant increase in solubility and dissolution profile of curcumin. Nevertheless, rats' plasma analysis demonstrated that oral bioavailability of SAS nanoparticles containing curcumin have increased approximately 11.6-fold as compared to native curcumin.

Zabihi et al., (2014) evaluated the influence of process parameters on preparation of SAS nanocapsules containing curcumin. They have observed that the size and loading of the particles can be significantly enhanced by increasing ultrasound power, fluidizing potential in turn and high anti-solvent flow rate.

Solid Lipid Nanocarriers (SLN)

Solid lipid nanocarriers was successfully developed in the early 1990s as alternative materials to polymers for parenteral nutrition. The technique for obtaining it is identical to oil in water emulsion, but the lipid has to be in solid

shape. There are several techniques for SLN fabrication, including high pressure homogenization and emulsification-evaporation, but also numerous dispersion techniques such as ultrasonication and high speed stirring, as well as spray drying, microemulsion-based methods and various precipitation techniques (Bazylińska, Pucek, Sowa, Matczak-Jon, & Wilk, 2014). According to Gastaldi et al. (2014), the main advantages of SLN are good biocompatibility, relevant drug loading capability, absence of organic solvents, ease of scaling up and long time stability (around 3 yars).

Sun et al. (2013) have prepared curcumin-loaded SLN by high-pressure homogenization using different solid lipids. Most of the particles were observed to be distributed between 150 and 200 nm. Besides, in vitro release studies in 0.1 M phosphate buffer solution have demonstrated that 90% of free curcumin was rapidly degraded whiting 10 minutes. On the other hand, curcumin SLN remained stable, 60% of the curcumin could be detected after 12 hours, which prove that this encapsulation technique was able to improve the chemical stability of curcumin.

In order to enhance the oral bioavailability of curcumin, Kakkar et al., (2011) have produced NLS using a microemulsification technique. The particles produced had spherical shape with an average particle size of 134.6 nm and total drug content of 92.33%. No significant variation in particle size and curcumin content of curcumin SLN was observed after a period of 12 months at 5^{o}C. In vivo pharmacokinetics performed after oral administration demonstrated that curcumin SLN has improved the content of curcumin in plasma rats as compared to free curcumin.

Wang et al., (2013) have prepared SLN containing curcumin using stearic acid as solid lipid. The particles have shown size ranged from 20 to 80 nm. Besides, curcumin SLN enhanced the targeting of curcumin to lung and tumor, being able to increase the inhibition efficiency of curcumin from 19.5% to 69.3%, which indicates that curcumin SLN provide a novel method for medical application on lung cancer treatment.

Liposome Encapsulation

Liposome encapsulation was also evaluated in order to allow intravenous administration and to overcome the problem of poor bioavailability of free curcumin. Common liposome constituents are 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), l-α-dimyristoylphosphatidylglycerol (DMPG) and cholesterol. Curcumin-loaded liposome production usually involves the

following steps: dissolving curcumin and the lipids in a common solvent (tert-butanol chloroform, methanol, DMSO or a combination of them); and sterilization of such solution by filtration) (Chen et al., 2009; Li, Ahmed, & Mehta, 2007; Li, Braiteh, & Kurzrock, 2005). Due to the nature of the double wall vesicles, liposomal curcumin is less prone to degrade than free curcumin when in unfavorable conditions (pH 7.4, phosphate buffer solution). However, no significant difference was detected in plasma or whole blood media (Chen et al., 2009).

In a study conducted by Shi and co-workers (2012), the role of liposomal curcumin in radiation pneumonitis and lung carcinoma was avaliated. The combined treatment with liposomal curcumin and radiotherapy increased intratumoral apoptosis and significantly enhanced inhibition of tumou grouth. In this sense, the authors suggest that the systemic administration of liposomal curcumin is safe and need to be investigated for further clinical applications.

Chen and colleagues (Chen et al., 2009) have performed in vitro study of liposomal curcumin. They confirmed that liposomal curcumin presented higher stability than free curcumin in phosphate buffer solution, but liposomal and free curcumin had similar stability in human blood and plasma. Thus, the liposomal curcumin may be useful for intravenous administration to improve bioavailability and efficacy of curcumin.

Spray Drying

Spray drying encapsulation technique is typically used for the preparation of dry, stable food additives and flavor for food industry. Usually, modified starch, maltodextrin, gum and other substances are hydrated to be used as the wall material. For encapsulation, the core material is homogenized with the wall material and this mixture is fed into a spray dryer and atomized with a nozzle or spinning wheel. The water is evaporated due to the contact with the hot air and then the capsules are collected after they fall to the bottom of the drier. Typically, this technique provides spherical shape particles with a mean size range of 10 – 100 μm. The main advantages of spray drying encapsulation technique are continuous operation, economy, flexibility and production of particles with good quality (Z. Fang & Bhandari, 2010).

Paramera and co-workers (Paramera, Konteles, & Karathanos, 2011) have used the spray drying technique to encapsulate curcumin in modified starch. The authors obtained low value of encapsulation efficiency (around 56%). They claim that curcumin and modified starch are hydrogen bonded, thus the

low encapsulation capacity of modified starch is probably due to its low degree of substitution (<3%). Therefore, to obtain successful emulsification with hydrophobic components is necessary to substitute the starch. However, modified starch particles was able to drastically increase curcumin solubility in simulated gastric fluid.

Aiming to enhance the solubility of curcumin, Liu et al., (2016) have microencapsulated curcumin in whey protein isolate (WPI) using the spray drying technique. The complexation was possible due to hydrophobic interactions between curcumin and nonpolar region of WPI. The obtained microparticles displayed high curcumin retention rates (>95%) and increased solubility of 11,355-fold compared to the raw curcumin cristals. Besides, the microparticles dried at different inlet temperatures showed very similar radical scavenging activities, demonstrating that the drying temperature did not affect the antioxidant activity of curcumin.

Inclusion Encapsulation

Molecular inclusion is an encapsulation technique that generally uses cyclodextrins as the encapsulating material. Cyclodextrins are a group of natural cyclic oligosaccharides derived from starch. It presents a cylinder-shaped structure where the external part of the molecule is hydrophilic whereas the internal part is hydrophobic. This structure guarantees cyclodextrins a satisfactory medium for encapsulation of less polar molecules into the internal cavity through hydrophobic interactions (FANG; BHANDARI, 2010).

Jahed and colleagues (2014) have investigated the solubility enhancement of curcumin through complaxation by β-cyclodextrin. The results have shown that curcumin solubility increased linearly with increasing β-cyclodextrin concentration. Besides, Nuclear Magnetic Resonance spectroscopy revealed the hydrophobic interactions between the cavity of β-cyclodextrin and the aromatic rings of curcumin.

Mangolim and co-workers (2014) evaluated three different methodologies to complex curcumin with β-cyclodextrin: co-precipitation, freeze-drying and solvent evaporation. According to them, the co-precipitation method presented the best result of inclusion efficiency (74%). Besides, this formulation was able to increase the solubility of the curcumin 31-fold and keep it stable to pH variations and storage at -15 and 4oC. The authors have applied curcumin β-cyclodextrin complex in vanilla ice cream, which results

in a good sensorial acceptance. In this sense, the use of complex is viable in food industry.

Yallapu and contributors (YALLAPU; JAGGI; CHAUHAN, 2010) have complexed curcumin in β-cyclodextrin by using a solvent evaporation technique. Their formulation demonstrated greater potent therapeutic efficacy in prostate cancer cells compared to free curcumin, which means that it can be an effective curcumin formulation for prostate cancer therapy.

Lipid-core Nanocapsules (LNC)

Lipid-core nanocapsules (LNC) are a class of nanocapsules in which the oil core is formed by a dispersion of a liquid lipid and a solid lipid. A polymeric wall surrounds the oil core and the interface particle/water is stabilized with surfactant. The main advantages of this colloidal system are capacity to stabilize photolabile substances, control drug release, icrease cerebral biodistribution of different substances and improve effectiveness (CORADINI et al., 2014).

Zanotto-Filho and colleagues (2013) produced LNC containing curcuming by interfacial deposition of preformed polymer using PCL as polymeric wall. Curcumin LNC showed mean size of 196 nm and 100% of encapsulation efficiency. The encapsulated curcumin had important effects in rats bearing gliomas being able to decrease the tumor size and malignance and prolonged animal survival when compared to same dose of non-encapsulated curcumin. Those data suggest that encapsulation of curcumin in NLC is an option to improve its pharmacological efficacy in gliomas' treatment.

Friedrich and co-workers (2015) also have used the interfacial deposition of preformed polymer to obtain LNC containing curcumin and resveratrol. The proposal of this study was to investigate the effect of co-delivery by LNC upon topical application on excised human skin. The authors observed that curcumin allows the penetration of resveratrol (less lipophilic) across the major skin barrier into viable epidermis and dermis. This occurs because curcumin is able to interact with the lipid bilayers of the stratum corneum, facilitating the passage of resveratrol. Based on these results, curcumin LNC presents a great potential as carrier system for delivery of low water soluble compounds such as resveratrol and curcumin for local applications to human skin.

2. NANO AND MICROENCAPSULATION AS A STRATEGY TO ENHANCE CURCUMIN BIOAVAILABILITY

The pharmacokinetics and pharmacodynamics of curcumin have been widely investigated. Although it has been proven efficient in many cases (anti-inflammatory, anti-cancer, antioxidant, antimicrobial, etc) and safe (curcumin is safe even at doses as high as 12 g/Kg) (Lao et al., 2006), curcumin has not yet been approved as a therapeutic agent. The major problem for this is probably its poor oral bioavailability, since oral delivery is still the most important and frequently used application route for drug administration.

Curcumin poor bioavailability is a consequence of three main aspects: 1) poor absorption; 2) rapid metabolism and 3) rapid excretion (Goel, Kunnumakkara, & Aggarwal, 2008b). The first study on curcumin uptake, metabolism and excretion was from Wahlström & Blennow (1978) using Sprague-Dawley rats. They found that when curcumin was administrated orally in a dose of 1 g/kg, it was excreted in the faeces to about 75%, while negligible amounts of curcumin appeared in the urine. Measurements of blood plasma levels and biliary excretion showed that curcumin was poorly absorbed from the gut. In fact, other studies confirm the poor absorption of curcumin in rats and in humans. All of them relate negligible serum levels of curcumin (Ravindranath & Chandrasekhara 1980; Ravindranath & Chandrasekhara, 1981; Pan et al., 1999; Anand et al., 2007). When given orally at a dose of 2g/Kg to humans, it was observed extremely low or undetectable serum levels of curcumin (0,006 ± 0,005 μg/mL at 1 h) (Shoba et al., 1998).

Once it is absorbed, curcumin is rapidly and extensively metabolized. Metabolism of curcumin occurs in intestine and liver and includes sulfation, glucuronidation and reduction (Pan et al., 1999). In rats, the major curcumin metabolites are glucuronides and sulfate conjugates. Traces of dihydroferulic acid and ferulic acid are also found. In humans, gastrointestinal tract contributes substantially to the glucuronidation of curcuminoids, which may have important implications for their pharmacokinetic fate in vivo (Hoehle, Pfeiffer, & Metzler, 2007). The chemical structure of the major curcumin metabolites are shown in Figure 1.

Curcumin is also rapidly eliminated. Yang et al., (2007) showed that after curcumin (500 mg/kg, p.o.) administration, the maximum concentration (Cmax) and the time to reach maximum concentration (Tmax) were 0.06 ± 0.01 μg/ml and 41.7 ± 5.4 min, respectively. The elimination half-life, after

oral administration, was 28.1 ± 5.6 min. These authors claim that the oral bioavailability of curcumin was about 1%.

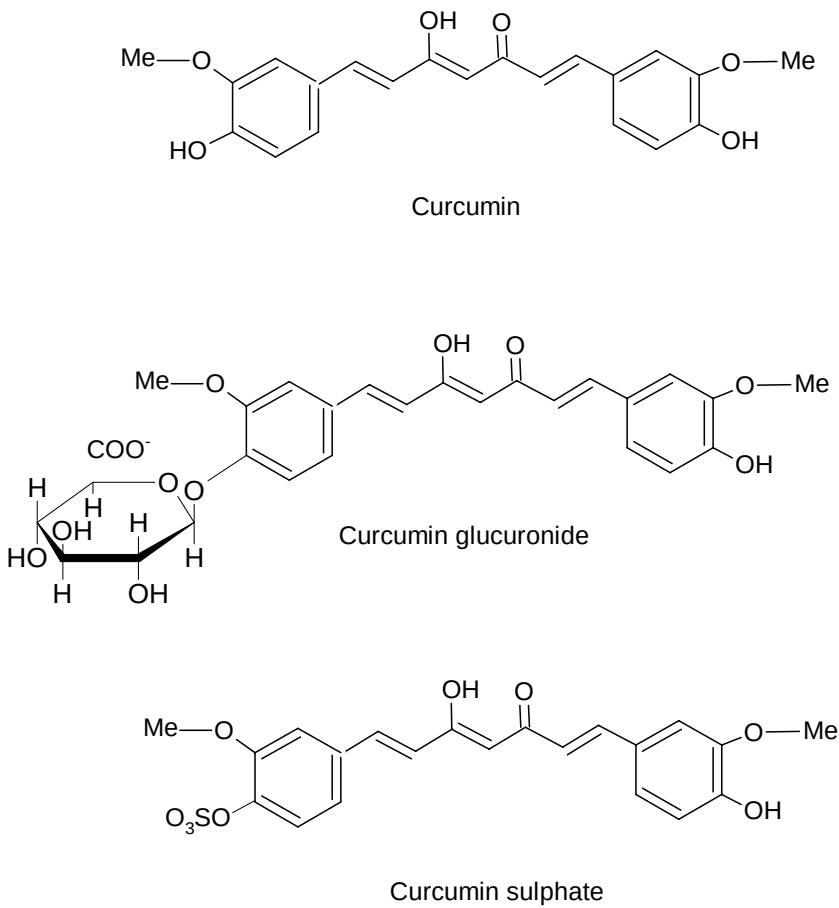


Figure 1. Structure of curcumin and its major metabolites following oral administration. Adaptation from Siviero et al., 2015.

Some of the possible ways to overcome curcumin poor bioavailability are based on encapsulation techniques. The encapsulation of curcumin with biomaterials such as chitosan, PLGA (poly(lactic-co-glycolic acid)) and PLLA (poly(l-lactic acid)) to increase the cellular delivery of curcumin has been studied by different authors. For example, it was already shown that PLGA nanoparticles (CUR-PLGA-NPs) improve the oral bioavailability of curcumin

in rats (Xie et al., 2011). After oral administration of CUR-PLGA-NPs the relative bioavailability was 5.6-fold higher compared with that of native curcumin. Similarly, curcumin loaded PLGA nanoparticles prepared by emulsion–diffusion–evaporation method presented oral bioavailability at least 9-fold higher when compared to curcumin administered with piperine, a known inhibitor of hepatic and intestinal glucuronidation (Shaikh, Ankola, Beniwal, Singh, & Kumar, 2009b).

Curcumin can also be nanoencapsulated using poly(l-lactic acid) (PLLA) using the miniemulsification-solvent evaporation technique (Silva-Buzanello et al., 2015). Curcumin-loaded PLLA nanoparticles (CUR-PLLA-NPs) showed greater biological effectiveness since dosages 8-fold smaller produced an inhibitory effect in the inflammatory process similar to that of free curcumin (Rocha et al., 2014). This result suggests higher bioavailability of CUR-PLLA-NPs, however, plasmatic concentrations of curcumin were not investigated.

Encapsulation of curcumin in the lauroyl sulphated chitosan (LSCS-CUR) also caused a great improvement on its bioavailability. In this system it was observed the attachment of LSCS-CUR particles to the gastrointestinal mucosa. As a result of this, the in vivo pharmacokinetics study showed that LSCS-CUR was better absorbed than free curcumin. Also, a single dose of LSCS-CUR particles sustained curcumin levels in the blood for 7 days (Shelma & Sharma, 2013).

Liposomes are excellent drug delivery systems since they can carry both hydrophilic and hydrophobic molecules. Liposomal encapsulation of curcumin can also enhance gastrointestinal absorption. Takahashi et al., (2009) produced liposome-encapsulated curcumin (LEC), prepared from commercially available lecithin grades. High bioavailability of curcumin was evident in the case of oral LEC since a faster rate and better absorption of curcumin were observed as compared to the other forms. These results indicated that curcumin enhanced the gastrointestinal absorption by liposome encapsulation. Other studies showed that liposomal encapsulated curcumin causes also an augmentation on cellular anti-cancer effect of the drug (Li et al., 2014; Hasan et al., 2014).

Micelles and phospholipid complexes can improve gastrointestinal absorption of drugs, resulting in improved bioavailability. Suresh & Srinivasan (2007) produced curcumin mixed micelles prepared with a mixture of phosphatidyl choline and sodium deoxycholate. Micellar curcumin was found to increase intestinal absorption using an in vitro model of everted rat intestinal sacs. It was observed that curcumin absorption in this model

increased from 48.7% to 56.1% when the same was present in micelles. Recently, lipopolysaccharide based nanocarriers were used to encapsulate curcumin and revealed 130-fold increase in oral bioavailability of curcumin. This system had also increased anti-cancer efficacy compared with pure curcumin (Chaurasia et al., 2015).

Microemulsion is also a useful technique to enhance water dispensability of hydrophobic molecules. Using this method, Bergonzi et al., (2014) have shown that an oil/water microemulsion system of curcumin improves its oral absorption. In this study the optimal formulation consisted of 3.3 g/100 g of vitamin E, 53.8 g/100 g of Tween 20, 6.6 g/100 g of ethanol and water (36.3 g/100 g). This formulation enhanced permeation in an in vitro model using parallel artificial membrane permeability assay (PAMPA).

Taking into account all the studies of encapsulation described here, it is plausible to say that the effect in improving oral bioavailability of curcumin may be associated with five main aspects: 1) improved water solubility, 2) higher release rate in the intestinal juice, 3) enhanced absorption, 4) increased residence time in the intestinal cavity and 5) diminished intestinal metabolism.

3. Effect of Nanoencapsulated Curcumin on Neurodegenerative Diseases

Alzheimer Disease

The success of improved healthcare over the past years is a direct consequence of demographic aging. Today, human life expectancy is longer, resulting in a huge proportion of elderly people. However, it was been well documented that the cases of dementias is increasing in senile but modern society (Villaflores, Chen, Chen, Yeh, & Wu, 2012).

Alzheimer's disease (AD) is a neurodegenerative disease, which has huge impact on society. This neuropathology has been found for more than 100 years but it is still incurable due to the incomplete understanding of its pathogenesis (FANG et al., 2014). Nowadays, millions of people are affected by AD. It is predicted that we are going to have big societal and economic impact if no efficient therapeutic and early-diagnosis approaches become available (Brambilla et al., 2011).

The brain of an AD patient is characterized by a strong loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This results in atrophy of the affected regions, degeneration in the temporal lobe, parietal lobe, parts of frontal cortex, cingulated gyrus and mainly hippocampus (Andrieux & Couvreur, 2013). The exact sequence of synaptic dysfunction, neuronal loss and biochemical cascade of events are still unknown (Ray & Lahiri, 2009).

There are two characteristic events that cause AD: deposition of the amyloid beta (Aβ) peptide in the intercellular space; and formation of intraneuronal tangle owing to hyperphosphorylation of axonal Tau protein (Ray & Lahiri, 2009). Aβ presents high aggregability, which results in a very toxic aggregate. It is widely believed that the fundamental treatment for AD should dissociate Aβ aggregate (Endo, Nikaido, Nakadate, Ise, & Konno, 2014). However, there are evidences indicating that oxidative damage may contribute to AD pathogenesis before Aβ accumulation in the brain (Elmegeed, Ahmed, Hashash, Abd-Elhalim, El-Kady, 2015).

According to Fang et al. (2014), the mechanism of the anti-AD property of curcumin is due to its ability of increasing the brain clearance of Aβ. In addition, the antioxidant and the immune-stimulating properties of curcumin may help to control this disease.

Xiong et al. (2011) have examined the effects of curcumin on the generation of Aβ and the mechanism by which curcumin inhibits the production of Aβ. The results showed that curcumin treatment produced a concentration and time-dependent reduction in the levels of Aβ *in vitro*, meanwhile it was unclear which element of the amyloidogenic pathway was directly targeted.

In vivo studies conducted by Ishrat et al. (2009) aimed to test the effect of curcumin in cognitive deficit. Results have shown that the curcumin supplementation significantly influenced cognitive deficits in rats, besides increasing antioxidant and other substances in the hippocampus and cerebral cortex.

The therapeutic efficacy of curcumin is restricted due its rapid systemic elimination. To increase the retention time of this compound in the body, various techniques have been proposed. One of them is its encapsulation in biopolymers (Tsai, Chien, Lin, & Tsai, 2011). According to Taylor et al. (2011), such attachment could help to increase the bioavailability of curcumin and, possibly, increase its binding affinity for Aβ peptides which affects the aggregation process.

Facing this problem, many researchers have employed nanotechnology to overcome these limitations of pure curcumin and the effects of its nanoparticles formulations have been promising (Hoppe et al., 2013; Gendelman et al., 2015).

To design a new nanomedicine for AD it is necessary to consider the physiopathology of the disease and to adapt the nanocarriers to this new therapeutic field (Andrieux & Couvreur, 2013). The prerequisites for successful brain targeting by nanocarrier material to treat neurodegenerative disease are blood compatibility, blood stability and BBB epithelial surface adhesion (Sarvaiya & Agrawal, 2014).

Blood Brain Barrier (BBB) is a tightly protective reticulum surrounding the brain to restrict substances in the blood from entering the brain, such as harmful blood-borne substances and microorganisms. However, it poses an impassable obstacle when attempting to deliver drugs. Only small molecules with high lipid solubility and low molecular weight can cross this specialized barrier into the brain (SAHNI et al., 2011; TSAI et al., 2011). Andrieux e Couvreur (2013) claim that the strategy to cross the BBB seems to target specific transporters expressed at the surface of brain endothelial cells like transferrin or low-density lipoprotein (LDL) receptors.

Kakkar et al. (2013) evaluated the bioavailability of free curcumin and curcumin solid lipid nanoparticles (SLNs) in rats plasma and brain. Regarding to oral administration, the value of nanoencapsulated curcumin in rats plasma was 8.135 times greater than free curcumin. The results obtained by intravenous administration have demonstrated that the ratio of encapsulated curcumin in brain was 30 times higher than the value of plasma. This study gives us a proof of the protective effect by encapsulating the drug inside the lipid core, thus protecting the drug from physiological and enzymatic degradation, and elimination from the system.

Aiming to improve the antioxidant effects of two polyphenols, curcumin and resveratrol, Coradini et al. (2014) developed lipid-core nanocapsules (NLC) containing the combination of both polyphenols by precipitation of preformed polymer method, using poly(ε-caprolactone) (PCL). Co-encapsulation did not influence nanotechnological characteristics. Furthermore, *in vitro* tests have shown that the antioxidant activity of polyphenols was enhanced by nanoencapsulation and the nanocapsules exhibited controlled release profile, which means that this is a promising technique to improve the performance of medicines used to prevent and treat diseases associated with oxidative stress.

Hoppe et al. (2013) evaluated the effects of free and nanoencapsulated curcumin on cognitive impairments in rats. The nanoparticles were obtained by interfacial deposition of preformed polymer, using PCL as biodegradable polymer. The authors have observed that treatment with a low dose of nanoencapsulated curcumin (2.5 mg/Kg/day) significantly improved the cognitive damage induced by Aβ injection. Non-encapsulated curcumin just presented significant effects on cognitive damage at a high dose of 50 mg/Kg/day. This study suggests an improvement in the *in vivo* performance of the curcumin by its nanoencapsulation, showing a therapeutic alternative in the treatment of neurodegenerative diseases such as AD.

Taylor et al. (2011) developed and evaluated various formulations of nanoliposomes associated with curcumin on the aggregation of Aβ. All nanoliposomes with curcumin or curcumin derivative where able to inhibit the formation of oligomeric and/or fibrillar Aβ *in vitro*.

Curcumin-conjugated nanoliposomes with curcumin exposed at the surface were prepared by Lazar et al. (2013) in order to evaluate its action on Aβ. Authors found that nanoliposomes strongly labeled Aβ deposits in post-mortem brain tissue of AD patients and mice, indicating that curcumin-conjugated nanolipossomes could find application in the diagnosis and targeted drug delivery in AD.

Poly(latic-co-glycolic acid) (PLGA) nanoparticles with curcumin were prepared by the high-pressure emulsification-solvent evaporation technique by Tsai et al. (2011). In distribution studies, curcumin could be detected in rats liver, heart, spleen, lung, kidney and brain. The half-life of curcumin were significantly increased with curcumin encapsulated in nanoparticles from 2.32 to 19.9 in the cerebral cortex and from 7.56 to 16.7 in the hippocampus.

Mathew et al. (2012) have coupled PLGA nanoparticles with Tet-1 peptide, which has the affinity to neurons and possess retrograde transportation properties. Their results have shown that curcumin encapsulated on these nanoparticles are able to destroy amyloid aggregates, exhibit antioxidative property and are non-cytotoxic, proving it to be a potential therapeutic tool against Alzheimer disease.

In this regard, curcumin nanoscale systems are extremely promising to treat neurodegenerative disease like Alzheimer due its safe, effective and targeted-site-specific delivery.

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder, age associated, caused by a progressive loss of dopaminergic neurons of the *substantia nigra* (Jagatha, Mythri, Vali, & Bharath, 2008). The main symptoms are tremor, muscle rigidity, disturbances of balance and bradykinesia (Liu, Yu, Li, Ross, & Smith, 2011). It is the second most common neurodegenerative disease worldwide, affecting approximately 1% of people over age 60 and rising to over 4% by age 85 (Taylor, Main, & Crack, 2013).

The motor symptoms of PD are believed to be result of a loss of dopaminergic neurons, which the alpha-synuclein deposits, also called Lewy bodies, are the pathological hallmark (Shah & Duda, 2015).

Even though motor symptoms are the main criteria for clinical diagnosis, non-motor symptoms such as impaired olfaction, sleep disorders, constipation and neuropsychiatric manifestations may appear before and during PD onset and progression (Taylor et al., 2013).

The pathogenesis of neurodegenerative disorders such as Parkinson is multifactorial, including a complex combination of genetic and environmental factors (Ataie, Sabetkasaei, Haghparast, Moghaddam, & Kazeminejad, 2010). The leading mechanisms that implies in the pathogenesis of PD are oxidative stress, mitochondrial dysfunction, inflammation and abnormal protein aggregation and degradation (Shah & Duda, 2015).

Oxidative reactions are essential for cell metabolism. However, these reactions result in the formation of reactive oxygen species (ROS). Oxidative stress happens when the cells natural antioxidant capacity is overcome, resulting in oxidative injury (Shah & Duda, 2015). The most common species of ROS include molecules such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Taylor et al., 2013).

The dopaminergic neurons, located in the *substantia nigra*, are highly susceptible to oxidative damage due to the high oxygen consumption of this brain region. The synthesis and storage of dopamine can also increase the generation of ROS in the PD brain (J. M. Taylor et al., 2013).

Glutathione (GSH) is a tripeptide that plays the major role as antioxidant and redox modulator in the brain. GSH depletion is the earliest indicator of oxidative stress in presymptomatic PD patient. This tripepitide is synthesized from its constituent amino acids by enzymatic reaction involving the enzymes □-glutamyl cysteine ligase (□-GCL) and glutathione synthetase (GS) in the neuronal cytosol. Mitochondria supplies ATP for GSH synthesis and GSH decreases the load oxidative stress in the mitochondria. In PD, there is a

significant depletion of GSH associated with damage to the electrons transport chain components, which abruptly affects ATP synthesis, inhibiting GSH synthesis and consequently increasing oxidative stress (Vali et al., 2007).

Therefore, GSH depletion is an event that significantly contributes to the oxidative load in these neurons, increasing oxidative stress and decreasing mitochondrial function. In this way, there is a demand for molecules that restore brain GSH levels, protecting against oxidative damage (Jagatha et al., 2008).

According to Song et al. (2012), the discovery of neuroprotective compounds is one of the drug development strategies for PD. Recently, research aiming to discovery novel neuroprotective drug candidates has demonstrated that natural products, such as plant extracts and their bioactive compounds, can have a huge potential as neuroprotective candidates in PD treatment (More et al., 2013).

Natural antioxidants have been studied due to its action in reducing deleterious effects and neuronal death by oxidative stress. Nevertheless, not all antioxidants have been shown to cross the BBB and to have a neuroprotective effect in animal and human models. The limitation of these antioxidants seems to be their relatively short half-life, low bioavailability and lack of potent protection (Ataie et al., 2010).

Among the natural antioxidants, curcumin stands out for its ability to cross the BBB and bind redox metal ions (Sethi, Jyoti, Hussain, & Sharma, 2009). Curcumin owns free radical scavenging activity due to the various functional groups in its structure. The phenolic rings and diketone groups present on curcumin molecular structure serve as an electron trap, preventing formation of H_2O_2, OH^- and superoxide. It has been found to be ten times more active as an antioxidant than vitamin E. In this way, the most important biological activity of curcumin regarding to neuroprotection is its antioxidant function. (Mythri & Srinivas Bharath, 2012).

Many researchers have highlighted other effects of curcumin on PD. According to Jagatha et al. (2008), the antioxidant effect of curcumin can induct GHS synthesis *in vitro* and *in vivo* via increased expression of the □-GCL gene. Yang et al. (2014) have speculated that curcumin promotes synthesis and secretion of monoamine neurotransmitters in hippocampal tissue, improving neurological functions. Ebrahimi & Schluesener (2012) affirm that polyphenols such as curcumin are able to interact with cellular signaling pathways, which are involved in neurodegeneration, directly or indirectly.

To overcome the low bioavailability and stability of curcumin, nanoencapsulation techniques have been proposed. Moreover, the application of targeted delivery is a promising branch of nanotechnology, including benefits like noninvasive administration, elevated treatment outcome, improved compliance, optimized drug distribution and reduced systemic side effects (Gao, Pang, & Jiang, 2013).

The study conducted by Kakkar et al. (2013) aimed to confirm the delivery of curcumin loaded solid lipid nanoparticles to the brain. The presence of yellow fluorescent particles in the brain was an indicative of effective delivery of intact curcumin encapsulated across the BBB, indicating that the drug was protected by the encapsulation technique, preventing the drug from enzymatic and physiological degradation, and removal from the system. Furthermore, the authors observed that the high concentration of curcumin nanoparticles in the plasma and the brain was thanks to the use of non ionic surfactants (Tween 80 and lecithin), which enabled their permeation across the intestine due to high affinity between lipid particles and intestinal membrane.

Yoncheva et al. (2015) encapsulated curcumin in poly(acrylic acid-co-methyl methacrylate). The authors observed that encapsulated curcumin showed greater protective effects in rat brain synaptosomes and increased intracellular levels of GSH compared to free curcumin. These results indicated that poly(acrylic acid-co-methyl methacrylate) particles are a promising prototype for curcumin delivery applications in the treatment of neurodegenerative disorders.

Chitosan nanoparticles containing curcumin was prepared by Yadav et al. (2012) aiming to evaluate the effects of nanoencapsulated curcumin on arsenic toxicity in rats central nervous system. Results have shown that curcumin encapsulated in these nanoparticles enhanced its antioxidant and metal-chelating properties compared to free curcumin. Furthermore, the administration of chitosan nanoparticles containing curcumin was beneficial in recovery abnormalities induced by arsenic in brain.

Tiwari et al. (2014) encapsulated curcumin in PLGA nanoparticles using the emulsion solvent evaporation technique. They found that encapsulated curcumin potently induces proliferation of neural stem cells and neuronal differentiation *in vitro* and in the hippocampus of adult rats, comparing to uncoated curcumin, which suggests that curcumin nanoparticles enhance the brain self-repair mechanism.

In addition, Betbeder et al. (2015) evaluated the antioxidant and antinitrosant effect of PLGA nanoparticles containing curcumin. Results

indicated that the antioxidant and antinitrosant activities of encapsulated curcumin are light sensitive. Modifications over time and temperature may facilitate the accessibility of curcumin to ROS, either by facilitating penetration of ROS into the nanoparticle or by the redistribution of curcumin in the nanoparticle core toward the surface.

In this sense, nanodelivery systems represent a new direction for PD therapy, although the relatively early stages of their development.

4. CURCUMIN ENCAPSULATION AND CANCER TREATMENT

Cancer is one of the most serious diseases with incidence over 10 million cases per year (Rl, Kd, & Jemal, 2015). The treatment includes chemotherapy agents (anti-metabolites, DNA binders, alkylating agents and others), irradiation and surgical interventions (Baskaran, Madheswaran, Sundaramoorthy, Kim, & Yoo, 2014; Yallapu, Jaggi, & Chauhan, 2012). However, cancer treatment is not effective to reverse the disease process and showed numerous adverse effects. Additionally, the treatment is not safety being toxicity to normal human cells. Thus, the development of the chemotherapeutic substances that show anti-cancer selective activity without produce adverse effects is being the aim of many researchers.

Curcumin may be highlight among the numerous studies that seek new therapeutic strategies for cancer. The interest in curcumin as anti-cancer agent appeared when epidemiological studies showed that lower incidence and risk of various types of cancers in populations with curcumin consumption (Goel, Kunnumakkara, & Aggarwal, 2008a; Sinha, Anderson, McDonald, & Greenwald, 2003; Yallapu et al., 2012).

Curcumin is able to suppress key elements of cancer process pathology. Its act sequential or simultaneous on the genome (DNA), messengers (RNA) and enzymes (proteins) within cells. Additionally, curcumin can modulate of transcription factor activator protein-1 (AP-1), nuclear factor-kappaB (NF-kB), mitogen-activated protein kinase (MAPK), tumor protein 53 (p53), nuclear b-catenin and serine/threonine protein kinase (AKT) signaling pathways (Hatcher, Planalp, Cho, Torti, & Torti, 2008). Other studies have shown that curcumin is capable of facilitating the chemotherapeutic agents and radiation action on tumor cells (Yallapu et al., 2010). Curcumin is able also to inhibit the expression of epidermal growth and estrogen receptors (cancer-associated growth factors) (Kunnumakkara, Anand, & Aggarwal, 2008).

Another barrier of cancer treatment is the development of multidrug resistance resulting in an under response of the agents. The multidrug resistance occurs through overexpression of ATP-binding cassette transporters that act as drug-efflux pumps involved in the aggressive removal of drug molecules from the cells, which therefore reduces the intracellular levels of these therapeutic molecules (Szakacs, Paterson, Ludwig, Booth-Genthe, & Gottesman, 2006; Yallapu et al., 2012). Curcumin-based treatment is able to suppress multidrug resistance through downregulates P-glycoprotein (P-gp), antiapoptotic proteins (Bcl-2) and hypoxia-inducible factor (HIF-1a) (Ganta & Amiji, 2009).

However, despite the clear biological activity of curcumin in cancer treatment, the therapeutic use is impaired because its poor bioavailability. In this context, the development of numerous drugs carrier systems has showed important therapeutic applicability.

The development of curcumin-loaded polymeric nanoparticles showed significantly inhibition on the proliferation of two carcinoma cell lines *in vitro* (human hepatocellular – HuH-7 and mouse mammary – 4T1) in a dose- and time-dependent manner in comparison with curcumin. The IC50 of the free curcumin was significantly higher than curcumin-loaded nanoparticles. In this study, tumor growth in mice treated with curcumin-loaded polymeric nanoparticles PNPC-treated mice was significantly suppressed and/or almost completely stopped at the end of the treatment (Alizadeh et al., 2015).

Anticancer activity of curcumin-loaded liquid crystalline nanoparticles in comparison with free curcumin on the human colon cancer cell line (HCT116) was evaluated (Baskaran et al., 2014). Curcumin-loaded liquid crystalline nanoparticles were effective, and it exhibited markedly enhanced anticancer activity compared to free curcumin.

Additionally encapsulation of curcumin in poly (lactic-co-glycolic acid) (PLGA) nanospheres using solid/oil/water emulsion solvent evaporation method showed better efficiently on cancer therapy compared to free curcumin (Mukerjee & Vishwanatha, 2009). IC50 of curcumin-loaded PLGA nanoparticles was found to be between 20 μM to 22.5 μM while that of free curcumin to be between 32 μM to 34 μM at the cancer cell lines (prostate cancer). It was demonstrated that curcumin-loaded PLGA nanospheres were more effective in arresting cell growth as compared to that free curcumin and also that curcumin-loaded PLGA nanospheres were able to inhibit NF-kB function better to free curcumin.

Lung anticancer activity was also evaluated with curcumin treatment. Yin, Zhang, and Liu (2013) showed that curcumin-loaded nanoparticles prepared

from three kinds of amphiphilic methoxy poly(ethylene glycol) (mPEG)–polycaprolactone (PCL) block copolymers at equivalent dose, exhibited better cytotoxicity on lung carcinoma cell line (A549) than free curcumin. IC50 free curcumin at 24, 48 and 72 hours of incubation showed 81.1 ± 7.9; 66.6 ± 8.8 and 53.0 ± 6.8, respectively and the curcumin-loaded nanoparticles showed 67.9 ± 9.1; 51.3 ± 5.4 and 34.5 ± 4.3, respectively.

Another work involved lung adenocarcinoma cells (cell line A549), curcumin-loaded hydrogel nanoparticles showed an enhanced on apoptotic effect on human lung cancer compared with free curcumin. Authors associated this effect due to the greater aqueous dispersion of curcumin (Teong et al., 2015).

Thangavel et al. (2015) demonstrated that curcumin-loaded pH-sensitive redox nanoparticles (RNPN) showed prostate anticancer activity 2-fold higher that free curcumin. In this work, IC50 to free curcumin was 100 μmol/L *in vitro* and suppressed about 40% of tumor cell proliferation. However, curcumin® RNPn significantly enhanced cytotoxicity (IC50 50 ± 5.5 μmol/L) *in vitro*. The *in vivo* anticancer potential showed that free curcumin reduced tumor volume compared to control mice. In contrast, curcumin® RNPn exhibited a significant reduction in tumor volume.

The effect of curcumin nanoformulation on colorectal cancer cell lines (HT29) and human colon carcinoma cell lines (HCT116) was also investigated. Udompornmongkol and Chiang (2015) demonstrated that curcumin-loaded polymeric nanoparticles obtained via an emulsification solvent diffusion method exhibit decreased cell viability more potently as free curcumin at low dosages (5 and 10 mg/ml). Additionally, free curcumin showed IC50 of 6.945 mg/ml. In contrast, curcumin-loaded polymeric nanoparticles showed IC50 of 2.329 mg/ml. The authors conclude that curcumin nanoparticles imparted better anticancer activities because they were more easily uptaken and more readily induced cell apoptosis.

Curcumin also showed effect against metastatic cancer cells line and curcumin encapsulated in poly(lactic-co-glycolide) (PLGA) showed a greater anticancer activity compared to free curcumin (Yallapu, Gupta, Jaggi, & Chauhan, 2010). Free curcumin exhibited higher IC50 values for a cisplatin resistant/metastatic ovarian cancer cell line (A2780CP) and metastatic breast cancer cell line (MDA-MB-231) (15.2 and 16.4 μM, respectively). Whereas, curcumin nanoencapsulated showed IC50 lower than free curcumin (13.9 and 9.1 μM).

Magnetic nanoparticles of curcumin also showed effective on pancreatic cancer cells. *In vitro* analyses showed that both free curcumin and curcumin-

loaded magnetic nanoparticles had a very similar cytotoxicity on HPAF-II and Panc-1 human pancreatic cells line. However, in *in vivo* tests, animals treated with curcumin-loaded magnetic nanoparticles significantly inhibited tumor growth by 71.2% whereas the treatment with curcumin inhibited tumor growth by 35.9% (Yallapu et al., 2013).

Zanotto-filho et al. (2013) demonstrated that curcumin-loaded lipid-core nanocapsules were better effective than free curcumin on glioma cancer cell lines. *In vitro* tests showed that curcumin-loaded lipid-core nanocapsules was more cytotoxic than free curcumin in U251MG cells. *In vivo* tests, rats bearing C6 gliomas, after 14-day treatment, curcumin-loaded lipid-core nanocapsules at dose (1.5 mg/kg/day i.p. significantly decreased tumor size when compared the same dose of free curcumin (free curcumin only decreased significant on tumor size at a high dose of 50 mg/kg/day). Additionally, curcumin-loaded lipid-core nanocapsules decreased the presence of intratumoral hemorrhages, necrosis, and lymphocytic infiltration suggesting reduction of tumor aggressiveness.

In conclusion, curcumin presents an important biological activity against cancer demonstrating a wide action in different cancer types with a lower incidence of adverse effects. Additionally, its limitation (poor bioavalibity) can be suppressed by nanoformulation technology which makes this compound a key tool in the therapeutic anticancer.

5. INFLAMMATION TREATMENT

Inflammation is a complex response of organism defense involving vascular and cellular events. However, if the defense is not resolute, the inflammation process can be make progress to a serious framework resulting in a persistent damage (Nathan, 2002). The inflammatory response is related with many diseases (alzheimer, cancer, ischemia, rheumatoid arthritis, sepsis and other diseases) therefore the control of this response can be decrease diseases progression.

Actually, some drugs are used to control the inflammatory response such as non-steroidal and steroidal anti-inflammatory, the disease modifying antirheumatic drugs, biologic agents (infliximab, etanacerp) and others. However, this drugs many times not satisfactorily changes the inflammatory process. Furthermore, they may cause intense adverse effects such as gastrointestinal lesions, kidney failure, cardiovascular effects, and others, limited use in therapeutic (Batlouni, 2010).

In this context, in addition to demonstrating safety on its use, curcumin showed anti-inflamatory activity anti-inflamatory. Zhang et al. (1999) and Goel, Boland, and Chauhan (2001) showed that curcumin is able to inhibit the cyclooxygenase-2 enzyme expression resulting in prostaglandin synthesis suppression. Additionally, others studies demonstrated that curcumin down-regulation kinases proteins and inhibit NF-kB activation (Dorai, Gehani, 2000; Surh, 2002). Curcumin is able also to reduce inflammatory mediator concentration such as tumor necrosis factor (TNF), interleukine-1 (IL-1), interleukine-8 (IL-8) and the synthase nitric oxide (NOS) (Jobin et al., 1999; Singh & Aggarwal, 1995). Therefore, curcumin inhibits the metalloproteinase-3 production in chondrocytes on osteoporosis disease and induces apoptosis and inhibits the prostaglandin E_2 on synovial fibroblasts (Mathy-Hartert et al., 2009; Park et al., 2007).

Despite these important evidences of anti-inflammatory activity of curcumin, its poor bioavailability is still the major limitation of its use. In this context, the development of carrier systems of drugs may be critical key in its use in therapeutic.

Agrawal et al. (2015) showed that curcumin-loaded elastic vesicles is more effective than free curcumin in a model of acute topic inflammation and cotton pellet-induced chronic inflammation. This improved therapeutic efficacy, according to the authors, is due to the improvement in flux across murine skin.

Additionally, amorphous curcumin nanoparticles were developed by aqueous titration method and incorporate into carbopol gel showed improved of anti-inflammatory therapeutic efficacy when compared to curcumin crystalline gel and similar efficacy when compared to anti-inflammatory reference drug (diclofenac) in a carragenan-induced paw edema. Authors explained this effect by self-penetration enhancer could obtain high therapeutic level (Al-Rohaimi, 2015).

Treatment per oral of curcumin nanoparticles produced by miniemulsification/solvent evaporation showed enhanced at 8-fold of its efficacy anti-inflammatory compared to curcumin *in nature* using a carragennan-induced paw edema (Rocha et al., 2014). In another inflammatory model disease (complete Freud's adjuvant induced arthritis), curcumin loaded solid lipid nanoparticles by crystallization of the melted lipid droplets present in the microemulsions revealed improve of its efficacy anti-arthritic. In this study, curcumin loaded solid lipid nanoparticles compared to free curcumin showed enhance of analgesic, anti-oxidant, anti-inflammatory and immune-modulator activity. Additionally, solid lipid nanoparticles efficacy was similar

to naproxen activity (reference anti-inflammatory) (Arora, Kuhad, Kaur, & Chopra, 2015).

The ability of curcumin to inhibit pro-inflammatory cytokines was mentioned. Lee et al. (2015) measured curcumin nanoparticle by solvent and anti-solvent precipitation method with alterable surface charge (cationic, anionic or neutral), showing that cationic curcumin nanoparticle was more effective in inhibit LPS-induced inflammation and oxidative stress in alveolar macrophages.

Standardized novel solid lipid curcumin particle (commercially as Longvida®) showed stronger inhibition on nitrite and prostaglandin E2 production and interleukin 6 levels. Additionally, an important reduction on NF-kB activity took place (Nahar, Slitt, & Seeram, 2015).

Sun et al. (2010) showed that curcumin exosomes prepared by mixing curcumin with exosomes in PBS improved efficacy anti-inflammatory compared to free curcumin. In this work, curcumin exosomes significantly reduced pro-inflammatory cytokines (interleukin 6 and TNF). Additionally, in a model of LPS-induced septic shock, curcumin exosomes showed significant survival advantage and less IL-6 and TNF levels.

From the information available in the literature one may conclude that the improved anti-inflammatory efficacy of curcumin through drug carrier systems allow their use in the treatment of various diseases of inflammatory origin including.

CONCLUSION

Curcumin has been encapsulated in order to improve its bioavailability and hydrophilicity by a number of techniques. Literature confirms that the biological activity of curcumin increases when compared to pristine, *in natura* curcumin which compensates the gain in complexity that comes from the encapsulation procedure itself. Examples of successful uses of encapsulated curcumin are Alzheimer and Parkinson's disease, cancer treatment and also inflammation processes. Changes in pharmacokinetics as well in body response to encapsulated curcumin are responsible for the increase in the cellular delivery of curcumin. Regarding encapsulation, proper particles characterization (morphology and particles size, encapsulation efficiency and curcumin load) are required information in order to design curcumin-based treatments.

REFERENCES

Agrawal, R., Sandhu, S. K., Sharma, I., & Kaur, I. P. (2015). Development and Evaluation of Curcumin-loaded Elastic Vesicles as an Effective Topical Anti-inflammatory Formulation. *AAPS PharmSciTech*, *16*(2), 364–374. doi:10.1208/s12249-014-0232-6.

Alizadeh, A. M., Sadeghizadeh, M., Najafi, F., Ardestani, S. K., Erfani-Moghadam, V., Khaniki, M.,… Mohagheghi, M. A. (2015). Encapsulation of Curcumin in Diblock Copolymer Micelles for Cancer Therapy. *BioMed Research International*, *2015*, 14. doi:10.1155/2015/824746.

Al-Rohaimi, A. H. (2015). Comparative Anti-inflammatory Potential of Crystalline and Amorphous Nano Curcumin in Topical Drug Delivery. *Journal of Oleo Science*, *64*(1), 27–40. doi:10.5650/jos.ess14175.

Anand, P., Kunnumakkara, A. B., Newman, R. a, & Aggarwal, B. B. (2007). Bioavailability of curcumin: problems and promises. *Molecular Pharmaceutics*, *4*(6), 807–18. doi:10.1021/mp700113r.

Andrieux, K., & Couvreur, P. (2013). Nanomedicine as a promising approach for the treatment and diagnosis of brain diseases: The example of Alzheimer's disease. *Annales Pharmaceutiques Francaises*, *71*(4), 225–233.

Anwar, M., Ahmad, I., Warsi, M. H., Mohapatra, S., Ahmad, N., Akhter, S., … Ahmad, F. J. (2015). Experimental investigation and oral bioavailability enhancement of nano-sized curcumin by using supercritical anti-solvent process. *European Journal of Pharmaceutics and Biopharmaceutics*, *96*, 162–172. doi:10.1016/j.ejpb.2015.07.021.

Arora, R., Kuhad, A., Kaur, I. P., & Chopra, K. (2015). Curcumin loaded solid lipid nanoparticles ameliorate adjuvant-induced arthritis in rats. *European Journal of Pain*, *19*(7), 940–952. doi:10.1002/ejp.620.

Asua, J. M. (2002). Miniemulsion polymerization. *Progress in Polymer Science*, *27*(7), 1283–1346. doi:10.1016/S0079-6700(02)00010-2.

Ataie, A., Sabetkasaei, M., Haghparast, A., Moghaddam, A. H., & Kazeminejad, B. (2010). Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in the rat. *Pharmacology, Biochemistry, and Behavior*, *96*(4), 378–85. doi:10.1016/j.pbb.2010.06.009.

Baskaran, R., Madheswaran, T., Sundaramoorthy, P., Kim, H. M., & Yoo, B. K. (2014). Entrapment of curcumin into monoolein-based liquid crystalline nanoparticle dispersion for enhancement of stability and

anticancer activity. *International Journal of Nanomedicine*, *9*(1), 3119–3130. doi:10.2147/IJN.S61823.

Batlouni, M. (2010). Review Article Nonsteroidal Anti-inflammatory Drugs: Cardiovascular, Cerebrovascular and Renal Effects. *Arq Brass Cardiol*, *94*, 522–529.

Bazylińska, U., Pucek, A., Sowa, M., Matczak-Jon, E., & Wilk, K. a. (2014). Engineering of phosphatidylcholine-based solid lipid nanocarriers for flavonoids delivery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *460*, 483–493. doi:10.1016/j.colsurfa.2014.02.034.

Bergonzi, M. C., Hamdouch, R., Mazzacuva, F., Isacchi, B., & Bilia, a. R. (2014a). Optimization, characterization and in vitro evaluation of curcumin microemulsions. *LWT - Food Science and Technology*, *59*(1), 148–155. doi:10.1016/j.lwt.2014.06.009.

Bergonzi, M. C., Hamdouch, R., Mazzacuva, F., Isacchi, B., & Bilia, A. R. (2014b). Optimization, characterization and in vitro evaluation of curcumin microemulsions. *LWT - Food Science and Technology*, *59*(1), 148–155. doi:10.1016/j.lwt.2014.06.009.

Bergonzi, M. C., Hamdouch, R., Mazzacuva, F., Isacchi, B., & Bilia, A. R. (2014c). Optimization, characterization and in vitro evaluation of curcumin microemulsions. *LWT - Food Science and Technology*, *59*(1), 148–155. doi:10.1016/j.lwt.2014.06.009.

Betbeder, D., Lipka, E., Howsam, M., & Carpentier, R. (2015). Evolution of availability of curcumin inside poly-lactic-co-glycolic acid nanoparticles: impact on antioxidant and antinitrosant properties. *International Journal of Nanomedicine*, *10*, 5355–5366.

Brambilla, D., Le Droumaguet, B., Nicolas, J., Hashemi, S. H., Wu, L.-P., Moghimi, S. M., … Andrieux, K. (2011). Nanotechnologies for Alzheimer's disease: diagnosis, therapy, and safety issues. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(5), 521–540. doi:10.1016/j.nano.2011.03.008.

Chaurasia, S., Patel, R. R., Chaubey, P., Kumar, N., Khan, G., & Mishra, B. (2015). Lipopolysaccharide based oral nanocarriers for the improvement of bioavailability and anticancer efficacy of curcumin. *Carbohydrate Polymers*, *130*, 9–17. doi:10.1016/j.carbpol.2015.04.062.

Chen, C., Johnston, T. D., Jeon, H., Gedaly, R., McHugh, P. P., Burke, T. G., & Ranjan, D. (2009). An in vitro study of liposomal curcumin: Stability, toxicity and biological activity in human lymphocytes and Epstein-Barr virus-transformed human B-cells. *International Journal of Pharmaceutics*, *366*(1-2), 133–139. doi:10.1016/j.ijpharm.2008.09.009.

Chereddy, K. K., Coco, R., Memvanga, P. B., Ucakar, B., Rieux, A., Vandermeulen, G., & Préat, V. (2013). Combined effect of PLGA and curcumin on wound healing activity. *Journal of Controlled Release*, *171*, 208–215.

Coradini, K., Lima, F. O., Oliveira, C. M., Chaves, P. S., Athayde, M. L., Carvalho, L. M., & Beck, R. C. R. (2014). Co-encapsulation of resveratrol and curcumin in lipid-core nanocapsules improves their in vitro antioxidant effects. *European Journal of Pharmaceutics and Biopharmaceutics*, *88*(1), 178–185. doi:10.1016/j.ejpb.2014.04.009.

Dorai T, Gehani N, K. A. (2000). Therapeutic potential of curcumin in human prostate cancer. II. Curcumin inhibits tyrosine kinase activity of epidermal growth factor receptor and depletes the protein. *Molecular Urology*, *4*(1), 1–6.

Duan, J., Mansour, H. M., Zhang, Y., Deng, X., Chen, Y., Wang, J., … Zhao, J. (2012). Reversion of multidrug resistance by co-encapsulation of doxorubicin and curcumin in chitosan/poly(butyl cyanoacrylate) nanoparticles. *International Journal of Pharmaceutics*, *426*(1-2), 193–201. doi:10.1016/j.ijpharm.2012.01.020.

Duan, J., Zhang, Y., Han, S., Chen, Y., Li, B., Liao, M., … Huang, B. (2010). Synthesis and in vitro/in vivo anti-cancer evaluation of curcumin-loaded chitosan/poly(butyl cyanoacrylate) nanoparticles. *International Journal of Pharmaceutics*, *400*(1-2), 211–220. doi:10.1016/j.ijpharm.2010.08.033.

Ebrahimi, A., & Schluesener, H. (2012). Natural polyphenols against neurodegenerative disorders: Potentials and pitfalls. *Ageing Research Reviews*, *11*(2), 329–345. doi:10.1016/j.arr.2012.01.006.

Elmegeed, G. A., H.H., A., M.a., H., M.M., A.-E., & D.S., E.-K. (2015). Synthesis of novel steroidal curcumin derivatives as anti-Alzheimer's disease candidates: Evidences-based on in vivo study. *Steroids*, *101*, 78–89. doi:10.1016/j.steroids.2015.06.003.

Endo, H., Nikaido, Y., Nakadate, M., Ise, S., & Konno, H. (2014). Structure activity relationship study of curcumin analogues toward the amyloid-beta aggregation inhibitor. *Bioorganic & Medicinal Chemistry Letters*, *24*(24), 5621–6. doi:10.1016/j.bmcl.2014.10.076.

Fang, L., Gou, S., Liu, X., Cao, F., & Cheng, L. (2014). Bioorganic & Medicinal Chemistry Letters Design, synthesis and anti-Alzheimer properties of dimethylaminomethyl-substituted curcumin derivatives, *24*, 40–43.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols - A review. *Trends in Food Science and Technology*, *21*(10), 510–523. doi:10.1016/j.tifs.2010.08.003.

Friedrich, R. B., Kann, B., Coradini, K., Offerhaus, H. L., Beck, R. C. R., & Windbergs, M. (2015). Skin penetration behavior of lipid-core nanocapsules for simultaneous delivery of resveratrol and curcumin. *European Journal of Pharmaceutical Sciences,* 78, 204–213. doi:10.1016/j.ejps.2015.07.018.

Ganta, S., & Amiji, M. (2009). Coadministration of paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells. *Molecular Pharmaceutics*, *6*(3), 928–939. doi:10.1021/mp800240j.

Gao, H., Pang, Z., & Jiang, X. (2013). Targeted Delivery of Nano-Therapeutics for Major Disorders of the Central Nervous System. *Pharmaceutical Research*, *30*, 2485–2498. doi:10.1007/s11095-013-1122-4.

Gastaldi, L., Battaglia, L., Peira, E., Chirio, D., Muntoni, E., Solazzi, I., … Dosio, F. (2014). Solid lipid nanoparticles as vehicles of drugs to the brain: current state of the art. *European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft Für Pharmazeutische Verfahrenstechnik e.V*, *87*(3), 433–44. doi:10.1016/j.ejpb.2014.05.004.

Gendelman, H. E., Anantharam, V., Bronich, T., Ghaisas, S., Jin, H., Kanthasamy, A. G., … Mallapragada, S. K. (2015). Nanoneuromedicines for degenerative, inflammatory, and infectious nervous system diseases. *Nanomedicine: Nanotechnology, Biology and Medicine*, *11*(3), 751–767. doi:10.1016/j.nano.2014.12.014.

Goel, A., Boland, C. R., & Chauhan, D. P. (2001). Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer Letters*, *172*(2), 111–118. doi:10.1016/S0304-3835(01)00655-3.

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008a). Curcumin as "Curecumin:" From kitchen to clinic. *Biochemical Pharmacology*, *75*(4), 787–809. doi:10.1016/j.bcp.2007.08.016.

Goel, A., Kunnumakkara, A. B., & Aggarwal, B. B. (2008b). Curcumin as "Curecumin:" from kitchen to clinic. *Biochemical Pharmacology*, *75*(4), 787–809. doi:10.1016/j.bcp.2007.08.016.

Hasan, M., Belhaj, N., Benachour, H., Barberi-Heyob, M., Kahn, C. J. F., Jabbari, E., … Arab-Tehrany, E. (2014). Liposome encapsulation of curcumin: physico-chemical characterizations and effects on MCF7 cancer

cell proliferation. *International Journal of Pharmaceutics*, *461*(1-2), 519–28. doi:10.1016/j.ijpharm.2013.12.007.

Hatcher, H., Planalp, R., Cho, J., Torti, F. M., & Torti, S. V. (2008). Curcumin: From ancient medicine to current clinical trials. *Cellular and Molecular Life Sciences*, *65*(11), 1631–1652. doi:10.1007/s00018-008-7452-4.

Hoehle, S. I., Pfeiffer, E., & Metzler, M. (2007). Glucuronidation of curcuminoids by human microsomal and recombinant UDP-glucuronosyltransferases. *Molecular Nutrition & Food Research*, *51*(8), 932–938. doi:10.1002/mnfr.200600283.

Hoppe, J. B., Coradini, K., Frozza, R. L., Oliveira, C. M., Meneghetti, A. B., Bernardi, A., … Salbego, C. G. (2013). Free and nanoencapsulated curcumin suppress β-amyloid-induced cognitive impairments in rats: involvement of BDNF and Akt/GSK-3β signaling pathway. *Neurobiology of Learning and Memory*, *106*, 134–44. doi:10.1016/j.nlm.2013.08.001.

Ishrat, T., Hoda, M. N., Khan, M. B., Yousuf, S., Ahmad, M., Khan, M. M., … Islam, F. (2009). Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT)☆. *European Neuropsychopharmacology*, *19*(9), 636–647. doi:10.1016/j.euroneuro.2009.02.002.

Jagatha, B., Mythri, R. B., Vali, S., & Bharath, M. M. S. (2008). Curcumin treatment alleviates the effects of glutathione depletion in vitro and in vivo: Therapeutic implications for Parkinson's disease explained via in silico studies. *Free Radical Biology and Medicine*, *44*(5), 907–917. doi:10.1016/j.freeradbiomed.2007.11.011.

Jahed, V., Zarrabi, A., Bordbar, A., & Hafezi, M. S. (2014). NMR (1H, ROESY) spectroscopic and molecular modelling investigations of supramolecular complex of β-cyclodextrin and curcumin. Food Chemistry, 165, 241–246. doi:10.1016/j.foodchem.2014.05.094.

Jobin, C., Bradham, C. A., Russo, M. P., Juma, B., Narula, A. S., Brenner, D. A., & Sartor, R. B. (1999). Curcumin Blocks Cytokine-Mediated NF-kB Activation and Proinflammatory Gene Expression by Inhibiting Inhibitory Factor I-kB Kinase Activity. *The Journal of Immunology*, *163*, 3474–3483.

Joye, I. J., & McClements, D. J. (2013). Production of nanoparticles by anti-solvent precipitation for use in food systems. *Trends in Food Science and Technology*, *34*(2), 109–123. doi:10.1016/j.tifs.2013.10.002.

Kakkar, V., Mishra, A. K., Chuttani, K., & Kaur, I. P. (2013). Proof of concept studies to confirm the delivery of curcumin loaded solid lipid

nanoparticles (C-SLNs) to brain. *International Journal of Pharmaceutics*, *448*(2), 354–9. doi:10.1016/j.ijpharm.2013.03.046.

Kakkar, V., Singh, S., Singla, D., & Kaur, I. P. (2011). Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin. *Molecular Nutrition & Food Research*, *55*(3), 495–503. doi:10.1002/mnfr.2010 00310.

Kunnumakkara, A. B., Anand, P., & Aggarwal, B. B. (2008). Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Letters*, *269*(2), 199–225. doi:10.1016/j.canlet.2008.03.009.

Lao, C. D., Ruffin, M. T., Normolle, D., Heath, D. D., Murray, S. I., Bailey, J. M., … Brenner, D. E. (2006). Dose escalation of a curcuminoid formulation. *BMC Complementary and Alternative Medicine*, *6*, 10. doi:10.1186/1472-6882-6-10.

Lazar, A. N., Mourtas, S., Youssef, I., Parizot, C., Dauphin, A., Delatour, B., … Duyckaerts, C. (2013). Curcumin-conjugated nanoliposomes with high affinity for Aβ deposits: possible applications to Alzheimer disease. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *9*(5), 712–21. doi:10.1016/j.nano.2012.11.004.

Lee, W.-H., Loo, C.-Y., Young, P. M., Rohanizadeh, R., & Traini, D. (2015). Curcumin Nanoparticles Attenuate Production of Pro-inflammatory Markers in Lipopolysaccharide-Induced Macrophages. *Pharmaceutical Research*. doi:10.1007/s11095-015-1789-9.

Leimann, F. V., Biz, M. H., Musyanovych, A., Sayer, C., Landfester, K., & Hermes de Araújo, P. H. (2013). Hydrolysis of poly(hydroxybutyrate- co -hydroxyvalerate) nanoparticles. *Journal of Applied Polymer Science*, *128*(5), 3093–3098. doi:10.1002/app.38506.

Lepeltier, E., Bourgaux, C., & Couvreur, P. (2014). Nanoprecipitation and the "Ouzo effect:" Application to drug delivery devices. *Advanced Drug Delivery Reviews*, *71*, 86–97. doi:10.1016/j.addr.2013.12.009.

Li, L., Ahmed, B., & Mehta, K. (2007). Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. *Molecular Cancer Therapeutics*, *6*(April), 1276–1283. doi:10.1158/1535-7163.MCT-06-0556.

Li, L., Braiteh, F. S., & Kurzrock, R. (2005). Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. *Cancer*, *104*(6), 1322–31. doi:10.1002/cncr.21300.

Li, L., Braiteh, F. S., Kurzrock, R., Hasan, M., Belhaj, N., Benachour, H., … Arab-Tehrany, E. (2014). Liposome-encapsulated curcumin: in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. *International Journal of Pharmaceutics*, *104*(1-2), 519–28. doi:10.1016/j.ijpharm.2013.12.007.

Lin, C.-C., Lin, H.-Y., Chen, H.-C., Yu, M.-W., & Lee, M.-H. (2009). Stability and characterisation of phospholipid-based curcumin-encapsulated microemulsions. *Food Chemistry*, *116*(4), 923–928. doi:10.1016/j.foodchem.2009.03.052.

Liu, C.-H., Chang, F.-Y., & Hung, D.-K. (2011). Terpene microemulsions for transdermal curcumin delivery: Effects of terpenes and cosurfactants. *Colloids and Surfaces B: Biointerfaces*, *82*(1), 63–70. doi:10.1016/j.colsurfb.2010.08.018.

Liu, W., Chen, X. D., Cheng, Z., & Selomulya, C. (2016). On enhancing the solubility of curcumin by microencapsulation in whey protein isolate via spray drying. *Journal of Food Engineering*, *169*, 189–195. doi:10.1016/j.jfoodeng.2015.08.034.

Liu, Z., Yu, Y., Li, X., Ross, C. a., & Smith, W. W. (2011). Curcumin protects against A53T alpha-synuclein-induced toxicity in a PC12 inducible cell model for Parkinsonism. *Pharmacological Research*, *63*(5), 439–444. doi:10.1016/j.phrs.2011.01.004.

Mangolim, C. S., Moriwaki, C., Nogueira, A. C., Sato, F., Baesso, M. L., Neto, A. M., & Matioli, G. (2014). Curcumin–β-cyclodextrin inclusion complex: Stability, solubility, characterisation by FT-IR, FT-Raman, X-ray diffraction and photoacoustic spectroscopy, and food application. Food Chemistry, 153, 361–370. doi:10.1016/j.foodchem.2013.12.067.

Mathew, A., Fukuda, T., Nagaoka, Y., Hasumura, T., Morimoto, H., Yoshida, Y., … Kumar, D. S. (2012). Curcumin loaded-PLGA nanoparticles conjugated with Tet-1 peptide for potential use in Alzheimer's disease. *PloS One*, *7*(3), 1–10. doi:10.1371/journal.pone.0032616.

Mathy-Hartert, M., Jacquemond-Collet, I., Priem, F., Sanchez, C., Lambert, C., & Henrotin, Y. (2009). Curcumin inhibits pro-inflammatory mediators and metalloproteinase-3 production by chondrocytes. *Inflammation Research*, *58*(12), 899–908. doi:10.1007/s00011-009-0063-1.

More, S. V., Kumar, H., Kang, S. M., Song, S., Lee, K., & Choi, D. (2013). Advances in Neuroprotective Ingredients of Medicinal Herbs by Using Cellular and Animal Models of Parkinson ' s Disease. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 1–15.

Mukerjee, A., & Vishwanatha, J. K. (2009). Formulation, characterization and evaluation of curcumin-loaded PLGA nanospheres for cancer therapy. *Anticancer Research*, *29*(10), 3867–75. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19846921.

Mythri, R. B., & Srinivas Bharath, M. M. (2012). Curcumin: A Potential Neuroprotective Agent in Parkinson's Disease. *Current Pharmaceutical Design*, *18*(1), 91–99. doi:10.2174/138161212798918995.

Nahar, P. P., Slitt, A. L., & Seeram, N. P. (2015). Anti-Inflammatory Effects of Novel Standardized Solid Lipid Curcumin Formulations. *Journal of Medicinal Food*, *18*(7), 786–792. doi:10.1089/jmf.2014.0053.

Nathan, C. (2002). Points of control in inflammation. *Nature*, *420*(6917), 846–852.

Pan, M. H., Huang, T. M., & Lin, J. K. (1999). Biotransformation of curcumin through reduction and glucuronidation in mice. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *27*(4), 486–94.

Paramera, E. I., Konteles, S. J., & Karathanos, V. T. (2011). Stability and release properties of curcumin encapsulated in Saccharomyces cerevisiae, β-cyclodextrin and modified starch. *Food Chemistry*, *125*(3), 913–922. doi:10.1016/j.foodchem.2010.09.071.

Park, C., Moon, D.-O., Choi, I.-W., Choi, B. T., Nam, T.-J., Rhu, C.-H., … Choi, Y. H. (2007). Curcumin induces apoptosis and inhibits prostaglandin E(2) production in synovial fibroblasts of patients with rheumatoid arthritis. *International Journal of Molecular Medicine*, *20*(3), 365–372.

Patel, A., Hu, Y., Tiwari, J. K., & Velikov, K. P. (2010). Synthesis and characterisation of zein–curcumin colloidal particles. *Soft Matter*, *6*(24), 6192. doi:10.1039/c0sm00800a.

Piao, H.-M., Balakrishnan, P., Cho, H.-J., Kim, H., Kim, Y.-S., Chung, S.-J., … Kim, D.-D. (2010). Preparation and evaluation of fexofenadine microemulsions for intranasal delivery. *International Journal of Pharmaceutics*, *395*(1-2), 309–316. doi:10.1016/j.ijpharm.2010.05.041.

Ravindranath, V., & Chandrasekhara, N. Metabolism of curcumin--studies with [3H]curcumin. *Toxicology*, *22*(4), 337–44.

Ravindranath, V., & Chandrasekhara, N. (1980). Absorption and tissue distribution of curcumin in rats. *Toxicology*, *16*(3), 259–265. doi:10.1016/0300-483X(80)90122-5.

Ray, B., & Lahiri, D. K. (2009). Neuroinflammation in Alzheimer's disease: different molecular targets and potential therapeutic agents including

curcumin. *Current Opinion in Pharmacology*, *9*(4), 434–444. doi:10.1016/j.coph.2009.06.012.

Rl, S., Kd, M., & Jemal, A. (2015). Cancer statistics, 2015 . *CA Cancer J Clin*, *65*(1), 21254. doi:10.3322/caac.21254.

Rocha, B. A., Gonçalves, O. H., Leimann, F. V, Rebecca, E. S. W., Silva-buzanello, R. A., Filho, L. C., … Bersani-amado, C. A. (2014). Curcumin encapsulated in poly-L-lactic acid improves its anti-inflammatory efficacy in vivo, *2*(December), 62–73.

Rocha, B., Gonçalves, O., Leimann, F., Rebecca, E., Silva-Buzanello, R., Filho, L., … Bersani-Amado, C. (2014). Curcumin encapsulated in poly-L-lactic acid improves its anti-inflammatory efficacy in vivo. *Advancement in Medicinal Plant Research*, *2*(4), 62–73.

Sahni, J. K., Doggui, S., Ali, J., Baboota, S., Dao, L., & Ramassamy, C. (2011). Neurotherapeutic applications of nanoparticles in Alzheimer's disease. *Journal of Controlled Release*, *152*(2), 208–231. doi:10.1016/j.jconrel.2010.11.033.

Sarvaiya, J., & Agrawal, Y. K. (2014). Chitosan as a suitable nanocarrier material for anti-Alzheimer drug delivery. *International Journal of Biological Macromolecules*, *72C*, 454–465. doi:10.1016/j.ijbiomac.2014.08.052.

Sasaki, Y., Konishi, N., Kasuya, M., Kohri, M., Taniguchi, T., & Kishikawa, K. (2015). Preparation of size-controlled polymer particles by polymerization of O/W emulsion monomer droplets obtained through phase inversion temperature emulsification using amphiphilic comb-like block polymers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *482*, 68–78. doi:10.1016/j.colsurfa.2015.04.019.

Sethi, P., Jyoti, A., Hussain, E., & Sharma, D. (2009). Curcumin attenuates aluminium-induced functional neurotoxicity in rats. *Pharmacology Biochemistry and Behavior*, *93*(1), 31–39. doi:10.1016/j.pbb.2009.04.005.

Shah, S. P., & Duda, J. E. (2015). Dietary modifications in Parkinson ' s disease: A neuroprotective intervention? *Medical Hypotheses*, 10–13.

Shaikh, J., Ankola, D. D., Beniwal, V., Singh, D., & Kumar, M. N. V. R. (2009a). Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *European Journal of Pharmaceutical Sciences*, *37*(3-4), 223–230. doi:10.1016/j.ejps.2009.02.019.

Shaikh, J., Ankola, D. D., Beniwal, V., Singh, D., & Kumar, M. N. V. R. (2009b). Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with

piperine as absorption enhancer. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences*, *37*(3-4), 223–30. doi:10.1016/j.ejps.2009.02.019.

Shelma, R., & Sharma, C. P. (2013). In vitro and in vivo evaluation of curcumin loaded lauroyl sulphated chitosan for enhancing oral bioavailability. *Carbohydrate Polymers*, *95*(1), 441–8. doi:10.1016/j.carbpol.2013.02.029.

Shi, H., Gao, X., Li, D., Zhang, Q., Wang, Y., Zheng, Y., … Chen, L. (2012). A systemic administration of liposomal curcumin inhibits radiation pneumonitis and sensitizes lung carcinoma to radiation. *International Journal of Nanomedicine*, *7*, 2601–2611.

Shoba, G., Joy, D., Joseph, T., Majeed, M., Rajendran, R., & Srinivas, P. S. (1998). Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Medica*, *64*(4), 353–6. doi:10.1055/s-2006-957450.

Silva-Buzanello, R. A. da, Ferro, A. C., Bona, E., Cardozo-Filho, L., Araújo, P. H. H. de, Leimann, F. V., & Gonçalves, O. H. (2015). Validation of an Ultraviolet-visible (UV-Vis) technique for the quantitative determination of curcumin in poly(L-lactic acid) nanoparticles. *Food Chemistry*, *172*, 99–104. doi:10.1016/j.foodchem.2014.09.016.

Singh, S., & Aggarwal, B. B. (1995). Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) [corrected]. *The Journal of Biological Chemistry*, *270*(42), 24995–25000. doi:10.1074/jbc.270.50.30235.

Sinha, R., Anderson, D. E., McDonald, S. S., & Greenwald, P. (2003). Cancer risk and diet in India. *Journal of Postgraduate Medicine*, *49*(3), 222–228.

Sintov, A. C. (2015). Transdermal delivery of curcumin via microemulsion. *International Journal of Pharmaceutics*, *481*(1-2), 97–103. doi:10.1016/j.ijpharm.2015.02.005.

Siviero, A., Gallo, E., Maggini, V., Gori, L., Mugelli, A., Firenzuoli, F., & Vannacci, A. (2015). Title: Curcumin, a golden spice with a low bioavailability. *Perspectives in Medicine*. doi:10.1016/j.hermed.2015.03.001.

Song, J. X., Sze, S. C. W., Ng, T. B., Lee, C. K. F., Leung, G. P. H., Shaw, P. C., … Zhang, Y. B. (2012). Anti-Parkinsonian drug discovery from herbal medicines: What have we got from neurotoxic models? *Journal of Ethnopharmacology*, *139*(3), 698–711. doi:10.1016/j.jep.2011.12.030.

Spernath, A., & Aserin, A. (2006). Microemulsions as carriers for drugs and nutraceuticals. *Advances in Colloid and Interface Science*, *128-130*(2006), 47–64. doi:10.1016/j.cis.2006.11.016.

Sun, D., Zhuang, X., Xiang, X., Liu, Y., Zhang, S., Liu, C., … Zhang, H.-G. (2010). A Novel Nanoparticle Drug Delivery System: The Anti-inflammatory Activity of Curcumin Is Enhanced When Encapsulated in Exosomes. *Molecular Therapy*, *18*(9), 1606–1614. doi:10.1038/mt.2010.105.

Sun, J., Bi, C., Chan, H. M., Sun, S., Zhang, Q., & Zheng, Y. (2013). Curcumin-loaded solid lipid nanoparticles have prolonged in vitro antitumour activity, cellular uptake and improved in vivo bioavailability. *Colloids and Surfaces B: Biointerfaces*, *111*, 367–375. doi:10.1016/j.colsurfb.2013.06.032.

Suresh, D., & Srinivasan, K. (2007). Studies on the in vitro absorption of spice principles – Curcumin, capsaicin and piperine in rat intestines. *Food and Chemical Toxicology*, *45*(8), 1437–1442. doi:10.1016/j.fct.2007.02.002.

Surh, Y. J. (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. *Food and Chemical Toxicology*, *40*(8), 1091–1097. doi:10.1016/S0278-6915(02)00037-6.

Szakacs, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C., & Gottesman, M. M. (2006). Targeting multidrug resistance in cancer. *Nat Rev Drug Discov*, *5*(3), 219–234.

Takahashi, M., Uechi, S., Takara, K., Asikin, Y., & Wada, K. (2009). Evaluation of an oral carrier system in rats: bioavailability and antioxidant properties of liposome-encapsulated curcumin. *Journal of Agricultural and Food Chemistry*, *57*(19), 9141–6. doi:10.1021/jf9013923.

Taylor, J. M., Main, B. S., & Crack, P. J. (2013). Neurochemistry International Neuroinflammation and oxidative stress: Co-conspirators in the pathology of Parkinson' s disease. *Neurochemistry International*, *62*(5), 803–819. doi:10.1016/j.neuint.2012.12.016.

Taylor, M., Moore, S., Mourtas, S., Niarakis, A., Re, F., Zona, C., … Allsop, D. (2011). Effect of curcumin-associated and lipid ligand-functionalized nanoliposomes on aggregation of the Alzheimer's Aβ peptide. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(5), 541–550. doi:10.1016/j.nano.2011.06.015.

Teong, B., Lin, C.-Y., Chang, S.-J., Niu, G. C.-C., Yao, C.-H., Chen, I.-F., & Kuo, S.-M. (2015). Enhanced anti-cancer activity by curcumin-loaded hydrogel nanoparticle derived aggregates on A549 lung adenocarcinoma

cells. *Journal of Materials Science: Materials in Medicine*, *26*(1), 49. doi:10.1007/s10856-014-5357-3.

Thangavel, S., Yoshitomi, T., Sakharkar, M. K., & Nagasaki, Y. (2015). Redox nanoparticles inhibit curcumin oxidative degradation and enhance its therapeutic effect on prostate cancer. *Journal of Controlled Release: Official Journal of the Controlled Release Society*, *209*, 110–119. doi:10.1016/j.jconrel.2015.04.025.

Tiwari, S. K., Agarwal, S., Seth, B., Yadav, a, Nair, S., Bhatnagar, P., … Gupta, K. C. (2014). Curcumin-loaded nanoparticles potently induce adult neurogenesis and reverse cognitive deficits in Alzheimer's disease model via canonical Wnt/beta-catenin pathway. *ACS Nano*, *8*(1), 76–103. doi:10.1021/nn405077y.

Tsai, Y., Jan, W., Chien, C., Lee, W., Lin, L., & Tsai, T. (2011). Optimised nano-formulation on the bioavailability of hydrophobic polyphenol, curcumin, in freely-moving rats, *127*, 918–925. doi:10.1016/j.foodchem. 2011.01.059.

Tsai, Y.-M., Chien, C.-F., Lin, L.-C., & Tsai, T.-H. (2011). Curcumin and its nano-formulation: the kinetics of tissue distribution and blood-brain barrier penetration. *International Journal of Pharmaceutics*, *416*(1), 331–8. doi:10.1016/j.ijpharm.2011.06.030.

Udompornmongkol, P., & Chiang, B.-H. (2015). Curcumin-loaded polymeric nanoparticles for enhanced anti-colorectal cancer applications. *Journal of Biomaterials Applications*, *0*(1), 1–10. doi:10.1177/0885328215594479.

Vali, S., Mythri, R. B., Jagatha, B., Padiadpu, J., Ramanujan, K. S., Andersen, J. K., … Bharath, M. M. S. (2007). Integrating glutathione metabolism and mitochondrial dysfunction with implications for Parkinson's disease: A dynamic model. *Neuroscience*, *149*(4), 917–930. doi:10.1016/j. neuroscience.2007.08.028.

Villaflores, O. B., Chen, Y. J., Chen, C. P., Yeh, J. M., & Wu, T. Y. (2012). Effects of curcumin and demethoxycurcumin on amyloid-β precursor and tau proteins through the internal ribosome entry sites: A potential therapeutic for Alzheimer's disease. *Taiwanese Journal of Obstetrics and Gynecology*, *51*(4), 554–564. doi:10.1016/j.tjog.2012.09.010.

Wahlström, B., & Blennow, G. (1978). A study on the fate of curcumin in the rat. *Acta Pharmacologica et Toxicologica*, *43*(2), 86–92.

Wang, P., Zhang, L., Peng, H., Li, Y., Xiong, J., & Xu, Z. (2013). The formulation and delivery of curcumin with solid lipid nanoparticles for the treatment of on non-small cell lung cancer both in vitro and in vivo.

Materials Science & Engineering. C, Materials for Biological Applications, *33*(8), 4802–8. doi:10.1016/j.msec.2013.07.047.

Xie, X., Tao, Q., Zou, Y., Zhang, F., Guo, M., Wang, Y., … Yu, S. (2011). PLGA nanoparticles improve the oral bioavailability of curcumin in rats: characterizations and mechanisms. *Journal of Agricultural and Food Chemistry*, *59*(17), 9280–9. doi:10.1021/jf202135j.

Xiong, Z., Hongmei, Z., Lu, S., & Yu, L. (2011). Curcumin mediates presenilin-1 activity to reduce b-amyloid production in a model of Alzheimer ' s disease. *Pharmacological Reports*, *63*, 1101–1108.

Yadav, A., Lomash, V., Samim, M., & Flora, S. J. S. (2012). Curcumin encapsulated in chitosan nanoparticles: a novel strategy for the treatment of arsenic toxicity. *Chemico-Biological Interactions*, *199*(1), 49–61. doi:10.1016/j.cbi.2012.05.011.

Yallapu, M. M., Ebeling, M. C., Khan, S., Sundram, V., Chauhan, N., Gupta, B. K., … Chauhan, S. C. (2013). Novel Curcumin-Loaded Magnetic Nanoparticles for Pancreatic Cancer Treatment. *Molecular Cancer Therapeutics*, *12*(8), 1471–1480. doi:10.1158/1535-7163.MCT-12-1227.

Yallapu, M. M., Gupta, B. K., Jaggi, M., & Chauhan, S. C. (2010). Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. *Journal of Colloid and Interface Science*, *351*(1), 19–29. doi:10.1016/j.jcis.2010.05.022.

Yallapu, M. M., Jaggi, M., & Chauhan, S. C. (2012). Curcumin nanoformulations: a future nanomedicine for cancer. *Drug Discovery Today*, *17*(1-2), 71–80. doi:10.1016/j.drudis.2011.09.009.

Yallapu, M. M., Khan, S., Maher, D. M., Ebeling, M. C., Sundram, V., Chauhan, N., … Chauhan, S. C. (2014). Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer. *Biomaterials*, *35*(30), 8635–8648. doi:10.1016/j.biomaterials.2014.06.040.

Yallapu, M. M., Maher, D. M., Sundram, V., Bell, M. C., Jaggi, M., & Chauhan, S. C. (2010). Curcumin induces chemo/radio-sensitization in ovarian cancer cells and curcumin nanoparticles inhibit ovarian cancer cell growth. *Journal of Ovarian Research*, *3*, 11. doi:10.1186/1757-2215-3-11.

Yang, J., Song, S., Li, J., & Liang, T. (2014). Neuroprotective effect of curcumin on hippocampal injury in 6-OHDA-induced Parkinson's disease rat. *Pathology, Research and Practice*, *210*(6), 357–62. doi:10.1016/j.prp.2014.02.005.

Yang, K.-Y., Lin, L.-C., Tseng, T.-Y., Wang, S.-C., & Tsai, T.-H. (2007). Oral bioavailability of curcumin in rat and the herbal analysis from Curcuma longa by LC-MS/MS. *Journal of Chromatography. B, Analytical*

Technologies in the Biomedical and Life Sciences, *853*(1-2), 183–9. doi:10.1016/j.jchromb.2007.03.010.

Yin, H., Zhang, H., & Liu, B. (2013). Superior anticancer efficacy of curcumin-loaded nanoparticles against lung cancer. *Acta Biochimica et Biophysica Sinica*, *45*(8), 634–640. doi:10.1093/abbs/gmt063.

Yoncheva, K., Kondeva-Burdina, M., Tzankova, V., Petrov, P., Laouani, M., & Halacheva, S. S. (2015). Curcumin delivery from poly(acrylic acid-co-methyl methacrylate) hollow microparticles prevents dopamine-induced toxicity in rat brain synaptosomes. *International Journal of Pharmaceutics*, *486*(1-2), 259–267. doi:10.1016/j.ijpharm.2015.03.061.

Yoon, I.-S., Park, J.-H., Kang, H. J., Choe, J. H., Goh, M. S., Kim, D.-D., & Cho, H.-J. (2015). Poly(d,l-lactic acid)-glycerol-based nanoparticles for curcumin delivery. *International Journal of Pharmaceutics*, *488*(1-2), 70–7. doi:10.1016/j.ijpharm.2015.04.046.

Zabihi, F., Xin, N., Li, S., Jia, J., Cheng, T., & Zhao, Y. (2014). Polymeric coating of fluidizing nano-curcumin via anti-solvent supercritical method for sustained release. *Journal of Supercritical Fluids*, *89*, 99–105. doi:10.1016/j.supflu.2014.02.021.

Zanotto-filho, A., Coradini, K., Braganhol, E., Schröder, R., Melo, C., Oliveira, D., … Moreira, F. (2013). European Journal of Pharmaceutics and Biopharmaceutics Curcumin-loaded lipid-core nanocapsules as a strategy to improve pharmacological efficacy of curcumin in glioma treatment, *83*, 156–167.

Zhang, F., Altorki, N. K., Mestre, J. R., Subbaramaiah, K., & Dannenberg, A. J. (1999). Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. *Carcinogenesis*, *20*(3), 445–51. doi:10.1093/carcin/20.3.445.

In: Curcumin
Editor: Valeria Martin
ISBN: 978-1-63484-272-3

Chapter 3

THE EFFECTS OF CURCUMIN IN HUMAN NASAL EPITHELIAL CELLS

Kenichi Takano[1,*], Kazufumi Obata[1], Tetsuo Himi[1] and Takashi Kojima[2]

[1]Department of Otolaryngology,
Sapporo Medical University School of Medicine, Sapporo, Japan
[2]Department of Cell Science, Research Institute for Frontier Medicine,
Sapporo Medical University School of Medicine, Sapporo, Japan

ABSTRACT

The airway epithelium, particularly the nasal epithelium, is the first line of defense against respiratory viral infections. Airway epithelial barriers are regulated predominantly by apical intercellular junctions, referred to as tight junctions. Respiratory syncytial virus (RSV) is a negative-stranded RNA virus of the genus *Pneumovirus*, family *Paramyxoviridae*; it is the major cause of bronchitis, asthma, and severe lower respiratory tract diseases in infants and young children. In human nasal epithelial cells (HNECs), the replication and budding of RSV, and subsequent epithelial responses including the release of proinflammatory cytokines and enhancement of tight junctions, are partially regulated by the nuclear factor-kappa B (NF-κB) pathway. On the other hand, the

* Corresponding author: Kenichi Takano, MD, PhD, Department of Otolaryngology, Sapporo Medical University School of Medicine, S1W16, Chuo-ku, Sapporo 060-8543, Japan, Tel +81-11-688-9655, FAX +81-11-615-5405, E-mail: kent@sapmed.ac.jp.

effects of curcumin are believed to be caused partially by the inhibition of NF-κB activity through a blockage of its entry into the nucleus. Curcumin is also a potent inhibitor of proteasomes, cyclooxygenase (COX)-2, lipoxygenase, ornithine decarboxylase, c-Jun N-terminal kinase, and protein kinase C. Our data show that curcumin prevents the replication and budding of RSV, and subsequent epithelial responses, without causing cytotoxicity. Moreover, the increase of epithelial barrier function caused by infection with RSV was enhanced by curcumin. Curcumin has various pharmacologic effects as an inhibitor of NF-κB, eIF-2α dephosphorylation, the proteasome, and COX-2. RSV-infected HNECs treated with the eIF-2α dephosphorylation blocker salubrinal, the proteasome inhibitor MG132, or COX-2 inhibitors (such as curcumin) prevented the replication of RSV and subsequent epithelial responses. Treatment with salubrinal and MG132 also enhanced the upregulation of tight junction molecules induced by infection with RSV. These results suggest that curcumin can prevent the replication of RSV and subsequent epithelial responses without causing cytotoxicity, and may be an effective therapy for severe RSV-associated lower respiratory tract diseases in infants and young children.

INTRODUCTION

The upper respiratory tract epithelium, particularly the nasal epithelium, consists of pseudostratified ciliated columnar epithelial cells, including membranous or microfold cells (M cells), that are specialized for antigen uptake and form a continuous barrier against a wide variety of exogenous antigens [1-6]. The respiratory tract epithelium also contains dendritic cells that take up transported antigens via M cells and present the antigens to CD4+ T cells while maintaining the integrity of the airway epithelial barrier [7-9]. The epithelium plays a crucial role as an interface of adaptive and innate responses, via tight junctions (TJs), to prevent invasion of inhaled environmental agents such as allergens and pathogens. Dynamic changes in TJs have been identified in human nasal mucosa affected by allergic rhinitis or viral infection.

The airway epithelium of the human upper respiratory mucosa acts as the first physical barrier that protects against inhaled substances and pathogens [3, 10, 11]. The epithelium is a highly regulated and impermeable barrier formed exclusively by tight junctions (TJs) [3, 10, 11]. TJs, which are the most apically located of the intercellular junctional complexes, inhibit solute and water flow through the paracellular space (termed the barrier function) [12,

13]. In addition, they separate the apical and basolateral cell surface domains to establish cell polarity (termed the fence function) [14, 15]. TJs participate in signal transduction mechanisms that regulate epithelial cell proliferation, gene expression, differentiation, and morphogenesis [16].

TJs are formed by integral membrane proteins (i.e., claudins, occludin, and junction adhesion molecules (JAMs)), as well as by several peripheral membrane proteins. These peripheral membrane proteins include scaffold PDZ expression proteins (i.e., zonula occludens (ZO)-1, -2, -3, and multi-PDZ domain protein-1) and membrane-associated guanylate kinase with inverted orientation (MAGI)-1, -2, -3. They also include cell polarity molecules such as ASIP/PAR-3, PAR-6, PALS-1, and PALS-1-associated TJ, and the non-PDZ-expressing proteins cingulin, symplekin, ZONAB, GEF-H1, aPKC, PP2A, Rab3b, Rab13, PTEN, and 7 H6 [17-19]. ZO-1, -2, and -3 proteins are members of the membrane-associated guanylate kinase family and display a characteristic multi-domain structure comprised of SH3, guanylate kinase-like, and multiple PDZ (PSD95–Dlg–ZO1) domains [20]. ZO-1 and -2 are also closely associated with the polymerization of claudins [21]. Tricellulin was first identified at contacts with three epithelial cells and has a barrier function [22]. More recently, lipolysis-stimulated lipoprotein receptor was identified as a tricellular TJ-associated membrane protein that recruits tricellulin to tricellular TJs [23].

The claudin family consists of at least 27 members, is solely responsible for forming TJ strands, and has four transmembrane domains and two extracellular loops [18, 24]. The first extracellular loop is the co-receptor of the hepatitis C virus [25] and influences paracellular charge selectivity [26]. The second extracellular loop is the receptor of *Clostridium perfringens* enterotoxin (CPE) [27]. Because claudin-4 is also a high-affinity receptor of CPE [28], full-length CPE with a direct cytotoxic effect, and the C-terminal receptor binding domain of CPE (C-CPE) without a cytotoxic effect, are employed for selective treatment and drug delivery against claudin-4-expressing cells [29, 30].

Occludin, the first discovered integral membrane protein of TJs, is expressed ubiquitously in the most apically located basolateral membranes and is the most reliable immunohistochemical marker for TJs [18, 31]. Overexpression of occludin increases the membrane barrier function, as indicated by an increase in transepithelial electrical resistance in mammalian epithelial cells [32, 33]. However, TJ strands can be formed without occludin in some cell types, including occludin-deficient embryonic stem cells [34, 35]. Moreover, an occludin-deficient mouse model does not display perturbations

of epithelial barrier function. However, a complex pathophysiological phenotype is observed in occludin-deficient mice with growth retardation, chronic inflammation and hyperplasia of the gastric epithelium, calcification in the brain, testicular atrophy, loss of cytoplasmic granules in striated duct cells of the salivary gland, and thinning of the compact bone [36]. Compared with claudins, occludin has a relatively long cytoplasmic C terminus containing several phosphorylation sites and a coiled-coil domain that may interact with compounds including PKC-z, c-Yes, connexin26, and the regulatory subunit of phosphatidylinositol 3-kinase [37], as well as occludin itself, and ZO-1 and -3 [17, 18]. Thus, research has suggested several roles of occludin in signal transduction and apoptosis [38, 39]. Furthermore, occludin is required for hepatitis C virus infection, similar to claudins [40].

JAMs (JAM-A, -B, -C, and -4) are immunoglobulin superfamily proteins expressed at cell junctions in epithelial and endothelial cells, as well as on the surfaces of leukocytes, platelets, and erythrocytes [41]. JAMs are important for a variety of cellular processes including TJ assembly, leukocyte transmigration, platelet activation, angiogenesis, and adenovirus binding. Current evidence indicates that JAM-A dimerization is necessary for functional regulation of the cellular barrier [42].

An indispensable role of TJs in pathogen infection has been demonstrated widely because their disruption increases paracellular permeability and polarity defects that facilitate viral or bacterial entry and spread. In addition, some TJ proteins function as receptors for viruses. Thus, extracellular stimuli, pathogenic bacteria, and viruses target and affect the function of TJs, leading to disease [19].

Respiratory syncytial virus (RSV) is a negative-stranded RNA virus in the genus *Pneumovirus,* family *Paramyxoviridae*, and is the major cause of bronchitis, asthma, and severe lower respiratory tract disease in infants and young children [43]. There is no effective vaccine against RSV, and the use of passive RSV-specific antibodies is limited to high-risk patients [44]. The RSV envelope contains three transmembrane surface proteins: fusion F glycoprotein, attachment G glycoprotein, and small hydrophobic protein (SH protein) [45, 46]. Nucleolin is a functional cellular receptor for RSV [47], and the fusion envelope glycoprotein of RSV binds specifically to nucleolin at the apical cell surface for entry into the host cell. RSV also contains the M2-1 protein that induces transcriptional processivity and is an anti-termination factor [48]. Furthermore, the M2-1 protein induces the production of cytokines and chemokines via activation of nuclear factor kappa B (NF-κB) [49]. RSV also induces and activates protein kinase R (PKR), a cellular kinase relevant to

limiting viral replication by phosphorylating and activating eukaryotic translation initiation factor 2 (eIF-2α) [50-52].

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl) -1, 6-heptadiene-3, 5-dione] is a major phenolic compound from the rhizome of *Curcuma longa.* Curcumin has various biological effects including antiviral, anti-inflammatory, antioxidant, and anticancer activities [53–55]. The effects of curcumin are, in part, caused by inhibiting NF-κB by preventing its entry into the nucleus [56]. Curcumin is also a potent inhibitor of the proteasome, cyclooxygenase (COX)-2, lipooxygenase, ornithine decarboxylase, c-Jun N-terminal kinase, and protein kinase C [57-59]. Curcumin modulates eukaryotic initiation factors (eIFs), which play important roles in translation initiation, and cell growth and proliferation [54]. Curcumin also strengthens the epithelial barrier by enhancing tight and adherens junctions [60, 61].

In this chapter, we review the possibility of a novel effect of curcumin on human nasal epithelial cells (HNECs). Specifically, curcumin may prevent the replication of RSV and the epithelial responses to it, without cytotoxicity, and may be effective for treating severe lower respiratory tract disease in infants and young children caused by RSV infection.

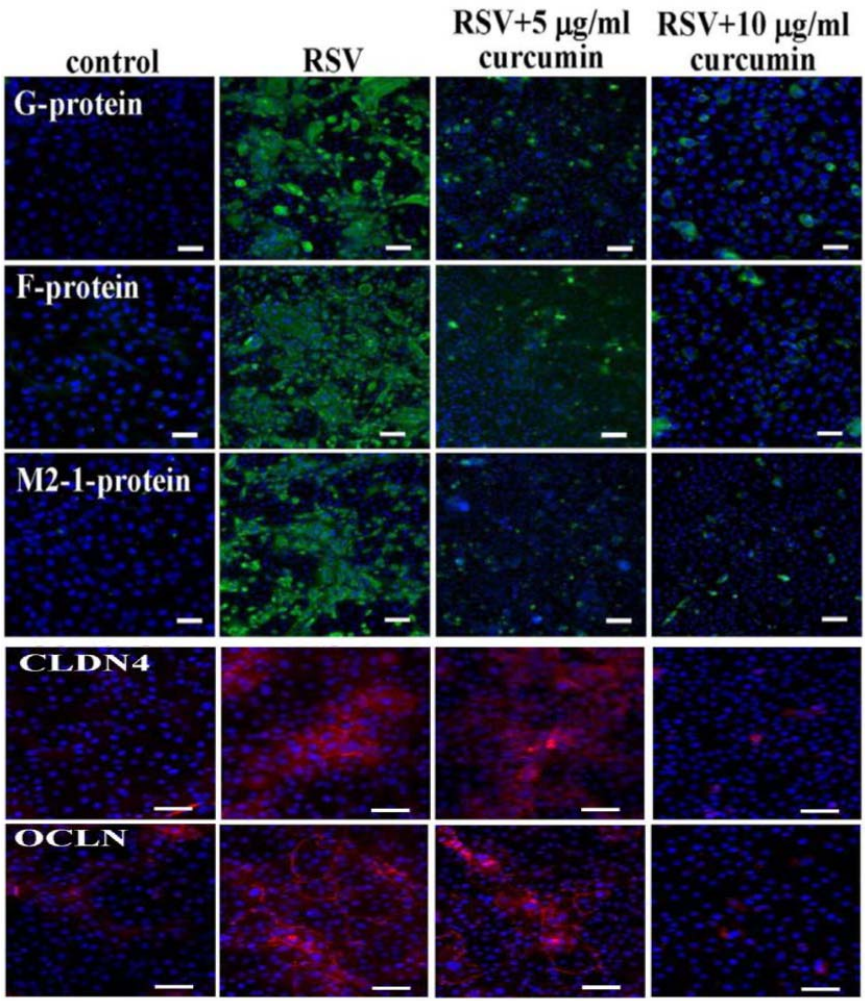
CURCUMIN PREVENTS THE REPLICATION AND BUDDING OF RSV

RSV is believed to replicate in the airway mucosa where it may produce uncomplicated upper respiratory infection or spread distally to the lower airways producing more severe lower respiratory tract infection. To investigate the detailed mechanisms of replication and budding of RSV in HNECs, and the epithelial cell responses including effects on TJs, we established an RSV-infection model using hTERT-transfected HNECs [62]. The replication and budding of RSV, and the epithelial cell responses in HNECs, were regulated by the PKCδ/HIF-1α/NF-κB pathway [62]. In addition, the PKCδ/HIF-1α/NF-κB pathway, through TGF-β in an autocrine manner, may be important in epithelial cell responses at a late stage after RSV infection. The control of this pathway in HNECs may be useful, not only for preventing the replication and budding of RSV, but also in therapy for RSV-induced respiratory disease. For example, we found that humulone, the main constituent of hop bitter acids, prevented the expression of RSV/G protein, the formation of virus filaments, and the release of IL-8 and RANTES (regulated on activation, normal T-cell

expressed and secreted), in a dose-dependent manner in RSV-infected HNECs. The effects of humulone in RSV-infected HNECs may be regulated via distinct signaling pathways including NF-κB [63].

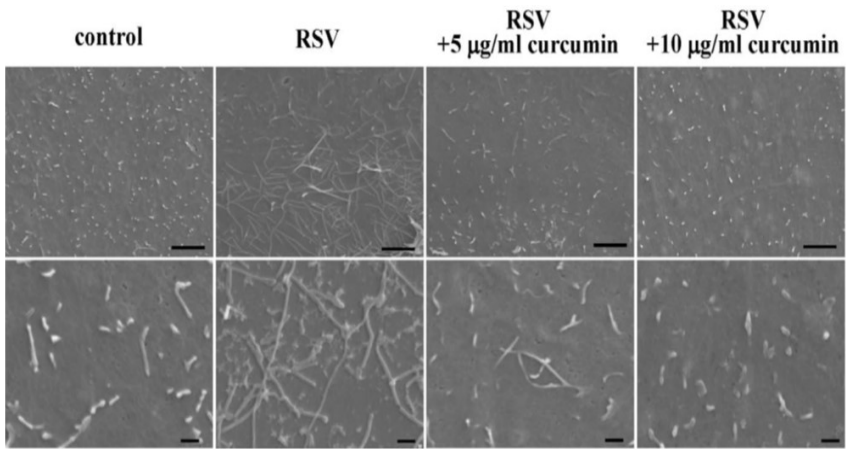
The NF-κB inhibitor, IMD0354, prevents replication of RSV in an established RSV-infection model using HNECs [62]. Curcumin did not affect the viability of HNECs even at high concentrations. When HNECs were pretreated with 10 mg/mL IMD0354 and 10 mg/mL curcumin 24 h before infection with RSV, the production of RSV/G- and M2-1-proteins was prevented. This indicated that the replication of RSV was inhibited and there was a decrease of phospho-NF-κB, and an increase of claudin-4 and occluding. In HNECs infected with RSV without curcumin, the TJ molecules claudin-1, -3, -4, -9, and -12, occludin, cingulin, and MAGI-1, the pattern recognition receptor retinoic acid-inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), proinflammatory cytokines IL-28A, IL-23A, and IL-11, and COX-1 and COX-3 were upregulated compared to the control that was not infected with RSV. In contrast, in HNECs infected with RSV and treated with curcumin, claudin-1, -4, and -12, occludin, and cingulin levels were upregulated while MAGI-1, RIG-I, MDA5, IL-28A, IL-23A, IL-11, COX-1, COX-2, and COX-3 levels were downregulated compared to those in the cells with RSV infection only [64].

When HNECs were pretreated with 5 mg/mL curcumin for 30 min before infection with RSV, at a multiplicity of infection of 1 for 24 h, expression of G and M2-1 proteins was inhibited. The upregulation of claudin-4 and occludin after infection with RSV was enhanced by 1 and 5 mg/mL and prevented by 10 mg/mL curcumin [64]. As shown Figure 1, the expression of G, F and M2-1 proteins after infection with RSV was markedly inhibited by curcumin, whereas upregulation of claudin-4 and occludin at the membrane was inhibited. In agreement with these observations, upregulation of transepithelial electrical resistance values, which reflect the epithelial barrier function, after infection with RSV was enhanced by curcumin. Scanning electron microscopy showed that the formation of virus filaments and small membranous structures at the surfaces of HNECs after infection with RSV were also inhibited by curcumin (Figure 2). However, curcumin did not affect the replication of RSV in A549 human lung adenocarcinoma cells [64]. Thus, curcumin appears to be more effective in preventing replication of RSV in HNECs than in lung epithelial cells.



The expression of G, F and M2-1 proteins after infection with RSV was markedly inhibited by curcumin. The upregulation of claudin-4 and occludin at the membranes after infection with RSV was inhibited by curcumin. CLDN: claudin, OCLN: occludin. Bars: 40 mm. (This figure was quoted reference 64.)

Figure 1. Immunostaining for G, F, M2-1, claudin-4, and occludin proteins in human nasal epithelial cells (HNECs) pretreated with curcumin before infection with respiratory syncytial virus (RSV).



The formation of virus filaments and small membranous structures at the surfaces of HNECs after infection with RSV were inhibited by curcumin. Bars: 1 μm. (This figure was quoted reference 64.)

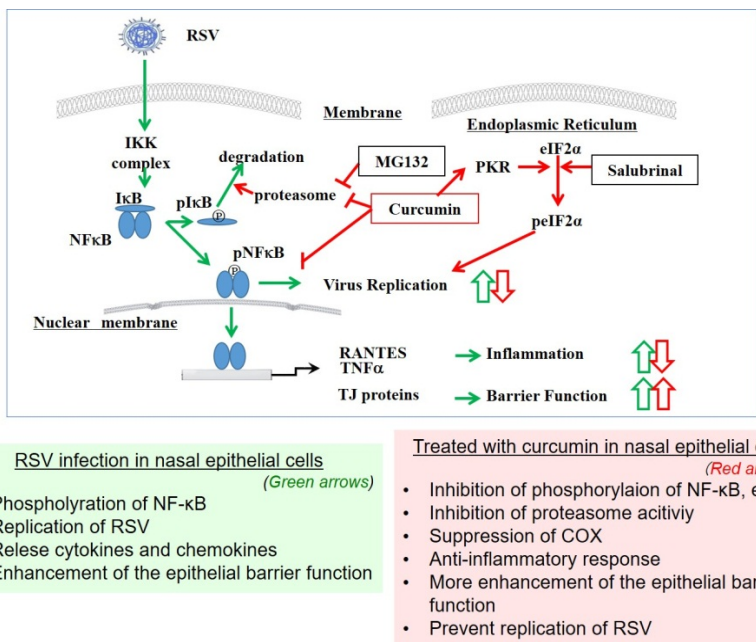
Figure 2. Scanning electron microscopic image in human nasal epithelial cells (HNECs) pretreated with curcumin before infection with respiratory syncytial virus (RSV).

CURCUMIN AFFECTS PHOSPHORYLATION OF NF-κB AND eIF2α, AND EXPRESSION OF PKR

The replication of RSV is closely associated not only with the activation of NF-κB, but also with the phosphorylation of eIF2α and the expression of PKR in infected cells [50]. RSV induces and activates PKR, which then activates eIF2α [50–52]. In HNECs infected with RSV, the expression of PKR and the phosphorylation of eIF2α were enhanced by curcumin, whereas the phosphorylation of NF-κB was decreased [64]. Salubrinal, an eIF-2α dephosphorylation inhibitor, prevented the expression of G and M2-1 proteins after infection with RSV, and upregulated the phosphorylation of NF-κB and eIF-2α. The upregulation of TNFα and RANTES after infection with RSV was also significantly inhibited by salubrinal while the upregulation of claudin-4, occludin, and PKR was not affected by any dose of this compound. Salubrinal can inhibit Epstein-Barr virus replication [65, 66]. In HNECs, curcumin can prevent the replication of RSV and the release of TNFα and RANTES via

phosphorylation of NF-κB, as well as PKR/phosphorylation of eIF2α, like salubrinal.

Curcumin is a potent proteasome inhibitor [59]. The proteasome inhibitor MG132, which blocks activation of NF-κB by preventing proteasome-mediated degradation of IκB, inhibits replication of RSV in Vero cells [67]. In HNECs infected with RSV, MG132 also inhibited the replication of RSV, as well as the release of TNFα and RANTES, and the expression of RIG-I and MDA5. Hence, curcumin may prevent the replication of RSV, release of TNFα and RANTES, and expression of RIG-I and MDA5 by inhibiting the proteasome.



Green arrows indicate signal transduction with RSV. Red arrows indicate treatment with curcumin. (This figure was quoted reference 64.)

Figure 3. Overview of signal transduction events in human nasal epithelial cells (HNECs) infected with respiratory syncytial virus (RSV), with and without curcumin treatment.

CURCUMIN IS A POTENT INHIBITOR OF COX-2

Infection with RSV induces the expression of COX-2 but not COX-1 [68]. Thus, COX-2 is a potential therapeutic target in RSV-induced diseases in the human lung [69, 70]. GeneChip analyses showed that COX-1 and COX-3 were upregulated by infection with RSV, and curcumin downregulated the expression of COX-1, COX-2, and COX-3 after such an infection [64]. When HNECs were pretreated with inhibitors of COX-1 or COX-2 before infection with RSV, the expression of G and M2-1 protein, and the upregulation of phospho-NF-κB after infection with RSV, were inhibited by the COX-2 but not COX-1 inhibitors [64]. The upregulation of RANTES after infection with RSV was inhibited by both COX-1 and COX-2 inhibitors while the upregulation of TNFα was inhibited only by COX-2 inhibitors. Inhibitors of COX-1 and COX-2 did not affect the upregulation of claudin-4 and occludin proteins after infection with RSV. Hence, curcumin may be a potent inhibitor of COX-2 in HNECs, and effects on this enzyme may play a crucial role in preventing the epithelial inflammatory responses to RSV infection.

CONCLUSION

Curcumin was not cytotoxic even at high doses in normal HNECs, and completely prevented the replication and budding of RSV. Curcumin also prevented the epithelial responses to RSV in HNECs and strengthened the epithelial barrier of HNECs via its pharmacologic effects (Figure 3). These results indicate that curcumin has the potential to inhibit RSV in upper airway HNECs and may be effective for the prevention of severe lower respiratory tract disease in infants and young children.

REFERENCES

[1] Takano, K; Kojima T; Ogasawara, N; Go, M; Kikuchi, S; Ninomiya, T; Kurose, M; Koizumi, J; Kamekura, R; Murata, M; Tanaka, S; Chiba, H; Himi, T; Sawada, N. Expression of tight junction proteins in epithelium including Ck20-positive M-like cells of human adenoids in vivo and in vitro. *J Mol Histol*, 2008, 39, 265-273.

[2] Takano K; Kojima T; Sawada N; Himi T. Role of tight junctions in signal transduction: an update. *EXCLI J*, 2014, 13, 1145-1162.

[3] Holgate, ST. The airway epithelium is central to the pathogenesis of asthma. *Allergol Int*, 2008, 57, 1-10.

[4] Fujimura, Y. Evidence of M cells as portals of entry for antigens in the nasopharyngeal lymphoid tissue of humans. *Virchows Arch*., 2000, 436, 560-566.

[5] Kim, DY; Sato, A; Fukuyama, S; Sagara, H; Nagatake, T; Kong, IG; Goda, K; Nochi, T; Kunisawa, J; Sato, S; Yokota, Y; Lee, CH; Kiyono H. The airway antigen sampling system: respiratory M cells as an alternative gateway for inhaled antigens. *J Immunol*, 2011, 186, 4253-4262.

[6] Nawijn, MC; Hackett, TL; Postma, DS; van Oosterhout, AJ; Heijink, IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol*, 2011, 32, 248-255.

[7] Steinman, RM; Pack, M; Inaba, K. Dendritic cells in the T-cell areas of lymphoid organs. *Immunol Rev*, 1997, 156, 25-37.

[8] Yamanaka, T; Helgeland, L; Farstad, IN; Fukushima, H; Midtvedt, T; Brandtzaeg, P. Microbial colonization drives lymphocyte accumulation and differentiation in the follicle-associated epithelium of Peyer's patches. *J Immunol*, 2003, 170, 816-822.

[9] Hammad, H; Lambrecht, BN. Dendritic cells and airway epithelial cells at the interface between innate and adaptive immune responses. *Allergy*, 2011, 66, 579-587.

[10] Schleimer, RP: Kato, A; Kern, R; Kuperman, D; Avila, PC. Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol*, 2007, 120, 1279-1284.

[11] Kojima, T; Go, M; Takano, K; Kurose, M; Ohkuni, T; Koizumi, J; Kamekura, R; Ogasawara, N; Masaki, T; Fuchimoto, J; Obata, K; Hirakawa, S; Nomura, K; Keira, T; Miyata, R; Fujii, N; Tsutsumi, H; Himi, T; Sawada, N. Regulation of tight junctions in upper airway epithelium. *Biomed Res Int*., 2013, 947072. doi: 10.1155/2013/947072.

[12] Gumbiner, BM. Breaking through the tight junction barrier. *J Cell Biol*, 1993, 123, 1631-1633.

[13] Schneeberger, EE; Lynch, RD. Structure, function, and regulation of cellular tight junctions. *Am J Physiol Lung Cell Mol Physiol*, 1992, 262, L647-L661.

[14] van Meer, G; Simons, K. The function of tight junctions in maintaining differences in lipid composition between the apical and the basolateral cell surface domains of MDCK cells. *EMBO J*, 1986, 5, 1455-1464.

[15] Cereijido, M; Valdes, J; Shoshani, L; Contreras, RG. Role of tight junctions in establishing and maintaining cell polarity. *Annu Rev Physiol*, 1998, 60, 161-177.

[16] Balda, MS; Matter, K. Tight junctions and the regulation of gene expression. *Biochim Biophys Acta*, 2009, 1788, 761-767.

[17] Schneeberger, EE; Lynch, RD. The tight junction: A multifunctional complex. *Am J Physiol Cell Physiol*, 2004, 286, 1213-1228.

[18] Tsukita, S; Furuse, M; Itoh, M. Multifunctional strands in tight junctions. *Nat Rev Mol Cell Biol*, 2001, 2, 285-293.

[19] Sawada, N. Tight junction-related human diseases. *Pathol Int*, 2013, 63, 1-12.

[20] Anderson, JM. Cell signalling: MAGUK magic. *Curr Biol*, 1996, 6, 382-384.

[21] Umeda, K; Ikenouchi, J; Katahira-Tayama, S; Furuse, K; Sasaki, H; Nakayama, M; Matsui, T; Tsukita, S; Furuse, M; Tsukita, S. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight junction strand formation. *Cell*, 2006, 126, 741-754.

[22] Ikenouchi, J; Furuse, M; Furuse, K; Sasaki, JH; Tsukita, S; Tsukita, S. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol*, 2005, 171, 939-945.

[23] Masuda, S; Oda, Y; Sasaki, H; Ikenouchi, J; Higashi, T; Akashi, M; Nishi, E; Furuse, M. LSR defines cell corners for tricellular tight junction formation in epithelial cells. *J Cell Sci*, 2011, 124, 548-555.

[24] Mineta, K; Yamamoto, Y; Yamazaki, Y; Tanaka, H; Tada, Y; Saito, K; Tamura, A; Igarashi, M; Endo, T; Takeuchi, K; Tsukita, S. Predicted expansion of the claudin multigene family. *FEBS Lett*, 2011, 585, 606-612.

[25] Meredith, LW; Wilson, GK; Fletcher, NF; McKeating, JA. Hepatitis C virus entry: beyond receptors. *Rev Med Virol*, 2012, 22, 182-193.

[26] Krug, SM; Günzel, D; Conrad, MP; Lee, IF; Amasheh, S; Fromm, M; Yu, AS. Charge-selective claudin channels. *Ann N Y Acad Sci*, 2012, 1257, 20-28.

[27] Fujita, K; Katahira, J; Horiguchi, Y; Sonoda, N; Furuse, M; Tsukita, S. Clostridium perfringens enterotoxin binds to the second extracellular loop of claudin-3, a tight junction integral membrane protein. *FEBS Lett*, 2000, 476, 258-261.

[28] Katahira, J; Sugiyama, H; Inoue, N; Horiguchi, Y; Matsuda, M; Sugimoto, N. Clostridium perfringens enterotoxin utilizes two structurally related membrane proteins as functional receptors in vivo. *J Biol Chem*, 1997, 272, 26652-26658.

[29] Michl, P; Buchholz, M; Rolke, M; Kunsch, S; Löhr, M; McClane, B; Tsukita, S; Leder, G; Adler, G; Gress, TM. Claudin-4: a new target for pancreatic cancer treatment using Clostridium perfringens enterotoxin. *Gastroenterology*, 2001, 121, 678-684.

[30] Saeki, R; Kondoh, M; Kakutani, H; Tsunoda, S; Mochizuki, Y; Hamakubo, T; Tsutsumi, Y; Horiguchi, Y; Yagi, K. A novel tumor-targeted therapy using a claudin-4-targeting molecule. *Mol Pharmacol*, 2009, 76, 918-926.

[31] Furuse, M; Hirase, T; Itoh, M; Nagafuchi, A; Yonemura, S; Tsukita, S; Tsukita, S. Occludin: a novel integral membrane protein localizing at tight junctions. *J Cell Biol*, 1993, 123, 1777-1788.

[32] Balda, MS; Whitney, JA; Flores, C; Gonzalez, S; Cereijido, M; Matter, K. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol*, 1996, 134, 1031-1049.

[33] McCarthy, KM; Skare, IB; Stankewich, MC; Furuse, M; Tsukita, S; Rogers, RA; Lynch, RD; Schneeberger, EE. Occludin is a functional component of the tight junction. *J Cell Sci*, 1996, 109, 2287-2298.

[34] Hirase, T; Staddon, JM; Saitou, M; Ando-Akatsuka, Y; Itoh, M; Furuse, M; Fujimoto, K; Tsukita, S; Rubin, LL. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J Cell Sci*, 1997, 110, 1603-1613.

[35] Saitou, M; Fujimoto, K; Doi, Y; Itoh, M; Fujimoto, T; Furuse, M; Takano, H; Noda, T; Tsukita, S. Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. *J Cell Biol*, 1998, 141, 397-408.

[36] Saitou, M; Furuse, M; Sasaki, H; Schulzke, JD; Fromm, M; Takano, H; Noda, T; Tsukita, S. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol Biol Cell*, 2000, 11, 4131-4142.

[37] Cummins, BM. Occludin: One protein, many forms. *Mol Cell Biol*, 2012, 32, 242-250.

[38] Murata, M; Kojima, T; Yamamoto, T; Go, M; Takano, K; Osanai, M; Chiba, H; Sawada, N. Down-regulation of survival signaling through

MAPK and Akt in occludin-deficient mouse hepatocytes in vitro. *Exp Cell Res*, 2005, 310, 140-151.

[39] Osanai, M; Murata, M; Nishikiori, N; Chiba, H; Kojima, T; Sawada, N. Epigenetic silencing of occludin promotes tumorigenic and metastatic properties of cancer cells via modulations of unique sets of apoptosis-associated genes. *Cancer Res*, 2006, 66, 9125-9133.

[40] Zeisel, MB; Fofana, I; Fafi-Kremer, S; Baumert, TF. Hepatitis C virus entry into hepatocytes: Molecular mechanisms and targets for antiviral therapies. *J Hepatol*, 2011, 54, 566-576.

[41] Martin-Padura, I; Lostaglio, S; Schneemann, M; Williams, L; Romano, M; Fruscella, P; Panzeri, C; Stoppacciaro, A; Ruco, L; Villa, A; Simmons, D; Dejana, E. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J Cell Biol*, 1998, 142, 117-127.

[42] Monteiro, AC; Parkos, CA. Intracellular mediators of JAM-A-dependent epithelial barrier function. *Ann NY Acad Sci*, 2012, 1257, 115-124.

[43] Bitko, V; Velazquez, A; Yang, L; Yang, YC; Barik, S. Transcriptional induction of multiple cytokines by human respiratory syncytial virus requires activation of NF-κB and is inhibited by sodium salicylate and aspirin. *Virology*, 1997, 232, 369-378.

[44] Johnson, S; Oliver, C; Prince, GA; Hemming, VG; Pfarr, DS; Oliver, C; Prince, GA; Hemming, VG; Pfarr, DS; Wang, SC; Dormitzer, M; O'Grady, J; Koenig, S; Tamura, JK; Woods, R; Bansal, G; Couchenour, D; Tsao, E; Hall, WC; Young, JF. Development of a humanized monoclonal antibody (MEDI-493) with potent in vitro and in vivo activity against respiratory syncytial virus. *J Infect Dis*, 1997, 176, 1215-1224.

[45] Levine, S; Klaiber-Franco, R; Paradiso, PR. Demonstration that glycoprotein G is the attachment protein of respiratory syncytial virus. *J Gen Virol*, 1987, 68, 2521-2524.

[46] Collins, PL; Mottet, G. Post-translational processing and oligomerization of the fusion glycoprotein of human respiratory syncytial virus. *J Gen Virol*, 1991, 72, 3095-3101.

[47] Tayyari, F; Marchant, D; Moraes, TJ; Duan, W; Mastrangelo, P; Hegele, RG. Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. *Nat Med*, 2011, 17, 1132-1135.

[48] Sutherland, KA; Collins, PL; Peeples, ME. Synergistic effects of gene-end signal mutations and the M2-1 protein on transcription termination by respiratory syncytial virus. *Virology*, 2001, 288, 295-307.

[49] Reimers, K; Buchholz, K; Werchau, H. Respiratory syncytial virus M2-1 protein induces the activation of nuclear factor kappa B. *Virology*, 2005, 331, 260-268.

[50] Groskreutz, DJ; Babor, EC; Monick, MM; Varga, SM; Hunninghake, GW. Respiratory syncytial virus limits a subunit of eukaryotic translation initiation factor 2 (eIF2α) phosphorylation to maintain translation and viral replication. *J Biol Chem*, 2010, 285, 24023-24031.

[51] Groskreutz, DJ; Monick, MM; Powers, LS; Yarovinsky, TO; Look, DC; Hunninghake, GW. Respiratory syncytial virus induces TLR3 protein and protein kinase R, leading to increased double-stranded RNA responsiveness in airway epithelial cells. *J Immunol*, 2006, 176, 1733-1740.

[52] Lindquist, ME; Mainou, BA; Dermody, TS; Crowe, JE. Activation of protein kinase R is required for induction of stress granules by respiratory syncytial virus but dispensable for viral replication. *Virology*, 2011, 413, 103-110.

[53] Rafiee, P; Nelson, VM; Manley, S; Wellner, M; Floer, M; Binion, DG; Shaker, R. Effect of curcumin on acidic pH-induced expression of IL-6 and IL-8 in human esophageal epithelial cells (HET-1A): role of PKC, MAPKs, and NF-κB. *Am J Physiol Gastrointest Liver Physiol*, 2009, 296, G388-G398.

[54] Chen, L; Tian, G; Shao, C; Cobos, E; Gao, W. Curcumin modulates eukaryotic initiation factors in human lung adenocarcinoma epithelial cells. *Mol Biol Rep*, 2010, 37, 3105-3110.

[55] Wilken, R; Veena, MS; Wang, MB; Srivatsan, ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer*, 2011, 10, 12.

[56] Singh, S; Aggarwal, BB. Activation of transcription factor NF-κB is suppressed by curcumin (diferuloylmethane). *J Biol Chem*, 1995, 270, 24995-25000.

[57] Plummer, SM; Holloway, KA; Manson, MM; Munks, RJ; Kaptein, A; Farrow, S; Howells, L. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-κB activation via the NIK/IKK signalling complex. *Oncogene*, 1999, 18, 6013-6020.

[58] Milacic, V; Banerjee, S; Landis-Piwowar, KR; Sarkar, FH; Majumdar, AP; Dou, QP. Curcumin inhibits the proteasome activity in human colon cancer cells in vitro and in vivo. *Cancer Res*, 2008, 68, 7283-7292.

[59] Zhou, H; Beevers, CS; Huang, S. The targets of curcumin. *Curr Drug Targets*, 2011, 12, 332-347.

[60] Al-Sadi, RM; Ma, TY. IL-1β causes an increase in intestinal epithelial tight junction permeability. *J Immunol*, 2007, 178, 4641-4649.

[61] Wong, TS; Chan, WS; Li, CH; Liu, RW; Tang, WW; Tsao, SW; Tsang, RK; Ho, WK; Wei, WI; Chan, JY. Curcumin alters the migratory phenotype of nasopharyngeal carcinoma cells through upregulation of E-cadherin. *Anticancer Res*, 2010, 30, 2851-2856.

[62] Masaki, T; Kojima, T; Okabayashi, T; Ogasawara, N; Ohkuni, T; Obata, K; Takasawa, A; Murata, M; Tanaka, S; Hirakawa, S; Fuchimoto, J; Ninomiya, T; Fujii, N; Tsutsumi, H; Himi, T; Sawada, N. A nuclear factor-κB signaling pathway via protein kinase C δ regulates replication of respiratory syncytial virus in polarized normal human nasal epithelial cells. *Mol Biol Cell*, 2011, 22, 2144-2156.

[63] Fuchimoto, J; Kojima, T; Okabayashi, T; Masaki, T; Ogasawara, N; Obata, K; Nomura, K; Hirakawa, S; Kobayashi, N; Shigyo, T; Yokota, S; Fujii, N; Tsutsumi, H; Himi, T; Sawada, N. Humulone suppresses replication of respiratory syncytial virus and release of IL-8 and RANTES in normal human nasal epithelial cells. *Med Mol Morphol*, 2013, 46, 203-209.

[64] Obata, K; Kojima, T; Masaki, T; Okabayashi, T; Yokota, S; Hirakawa, S; Nomura, K; Takasawa, A; Murata, M; Tanaka, S; Fuchimoto, J; Fujii, N; Tsutsumi, H; Himi, T; Sawada, N. Curcumin prevents replication of respiratory syncytial virus and the epithelial responses to it in human nasal epithelial cells. *PLoS One*, 2013, 18, e70225.

[65] Boyce, M; Bryant, KF; Jousse, C; Long, K; Harding, HP; Scheuner, D; Kaufman, RJ; Ma, D; Coen, DM; Ron, D; Yuan, J. A selective inhibitor of eIF2α dephosphorylation protects cells from ER stress. *Science*, 2005, 307, 935–939.

[66] Taylor, GM; Raghuwanshi, SK; Rowe, DT; Wadowsky, RM; Rosendorff, A. Endoplasmic reticulum stress causes EBV lytic replication. *Blood*, 2011, 118, 5528–5539.

[67] Lupfer, C; Pastey, MK. Decreased replication of human respiratory syncytial virus treated with the proteasome inhibitor MG132. *Virus Res*, 2010, 149, 36–41.

[68] Radi, ZA; Meyerholz, DK; Ackermann, MR. Pulmonary cyclooxygenase-1 (COX-1) and COX-2 cellular expression and distribution after respiratory syncytial virus and parainfluenza virus infection. *Viral Immunol*, 2010, 23, 43–48.

[69] Liu, T; Zaman, W; Kaphalia, BS; Ansari, GA; Garofalo, RP; Casola, A. RSVinduced prostaglandin E2 production occurs via cPLA2 activation: role in viral replication. *Virology*, 2005, 343, 12–24.

[70] Richardson, JY; Ottolini, MG; Pletneva, L; Boukhvalova, M; Zhang, S; Vogel, SN; Prince, GA; Blanco, JC. Respiratory syncytial virus (RSV) infection induces cyclooxygenase 2: a potential target for RSV therapy. *J Immunol*, 2005, 174, 4356–4364.

In: Curcumin
Editor: Valeria Martin
ISBN: 978-1-63484-272-3

Chapter 4

EPIGENETIC MODIFICATIONS INDUCED BY CURCUMIN AND ITS CONGENERS

Tamer E. Fandy*
Department of Pharmaceutical Sciences, Albany College of Pharmacy (Vermont campus), Colchester, VT

ABSTRACT

The role of epigenetic changes in health and disease is well established. Recent discoveries of the role of epigenetic modifications in the development and progression of different diseases like cancer, diabetes, chronic kidney disease and neurodegeneration urged the parallel development of drugs that modulate these modifications. DNA methyltransferase inhibitors and histone deacetylase inhibitors were the first categories of drugs tested in clinical trials as epigenetic modifiers and FDA-approved for treatment of different tumors. Nutraceuticals like genistein, epigallocatechin-3-gallate and curcumin demonstrated activity as epigenetic modifiers and a long list of other nutraceuticals is waiting validation of their activity. Curcumin, a hydrophobic polyphenol derived from the rhizome of the herb Curcuma longa (turmeric), exhibits a wide spectrum of pharmacological activities. Curcumin demonstrated anti-inflammatory, antimicrobial, antiviral, antioxidant and antitumor activity in several studies. Curcumin is safe when administered at high doses; however, its low bioavailability due to poor absorption and rapid metabolism is a major drawback. Different formulation based approaches

* Corresponding author: Tamer.fandy@acphs.edu.

were adopted to overcome its low bioavailability like liposomal curcumin and curcumin nanoparticles. Additionally, several structural analogues were also synthesized to improve the solubility and bioavailability of curcumin. Curcumin and its congeners were shown to induce epigenetic changes in tumor cells. Curcumin modulated histone acetylation by inhibiting histone deacetylase (HDAC) and histone acetyltransferase (HAT) enzymes in tumor cells. Curcumin modulated microRNAs (miRNAs) expression in tumor cells and demonstrated a controversial DNA hypomethylating effect. In this review, the epigenetic changes induced by curcumin and its congeners and the potential of utilizing these changes in the treatment of different diseases will be discussed.

PHYSICAL PROPERTIES AND STRUCTURAL ACTIVITY RELATIONSHIP OF CURCUMIN

Organic solvent extraction of the ground rhizomes of Curcuma Longa (turmeric) followed by crystallization is generally defined as curcumin. However, the extract is actually composed of a mixture of three polyphenolic constituents; the main constituent is 1,7-bis-(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione (also known as curcumin or diferuloylmethane) in addition to the desmethoxy (demethoxycurcumin) and bis-desmethoxy (bis-demethoxycurcumin) derivatives in varying proportions (Figure 1). Curcumin is an oil soluble pigment, practically insoluble in water at acidic and neutral pH and soluble in alkaline pH. Curcumin extract has a long history of use as a spice and coloring agent. Curcumin is relatively stable at acidic pH, but rapidly decomposes at alkaline pH into ferulic acid and ferulolymethane, which further degrades into vanillin and acetone [1, 2]. Curcumin exhibits keto-enol tautomerism and the enol form was shown to be the dominant form in aqueous solutions.

Curcumin structure possesses distinct chemical properties that facilitate its interaction with a variety of biomolecular targets including proteins, lipids and nucleic acids. For instance, hydrogen bonding interaction through its β-dicarbonyl moiety, phenolic hydroxyl groups and methoxy groups. The conformational flexibility imparted by rotamerization around multiple carbon-carbon single bonds and behavior as a Michael reaction acceptor also contributes to its bioreactivity. Additionally, the ability of curcumin to chelate metal cations facilitates its interaction with metalloproteins like glyoxalase I through chelation to zinc atom and thioredoxin reductase through chelation to

selenium atom [3, 4]. The high partition coefficient (log P) of curcumin (around 2.5) also promotes its intracellular targeting of bioreactive molecules.

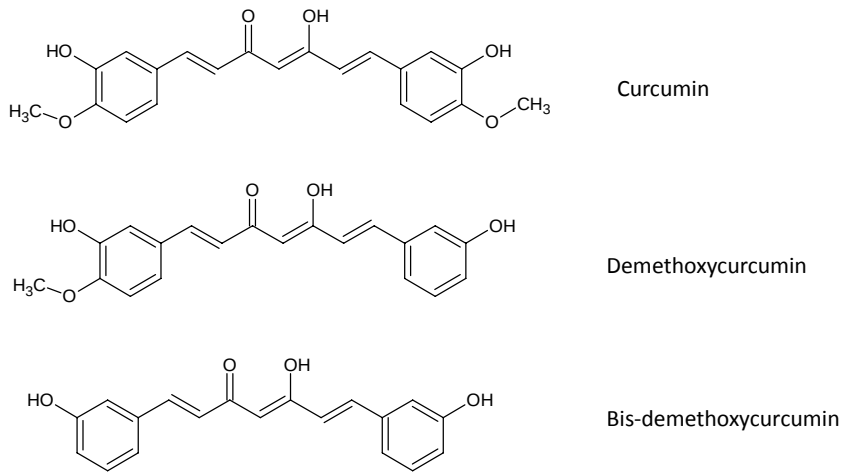


Figure 1. Chemical structure of the three main constituents in the curcumin extract.

THE PHARMACODYNAMICS OF CURCUMIN

Curcumin has diverse molecular targets and exerts its pharmacological activity via direct interaction with these targets. Curcumin has been found to physically bind to a myriad of proteins including transcriptional factors, growth factors/hormones and their receptors, cytokines and enzymes [5]. Moreover, curcumin regulates the expression of genes that are critically involved in the regulation of cellular signaling pathways, like NF-κB, Akt, MAPK, and other pathways through epigenetic mechanisms [6]. Curcumin was also shown to bind DNA in a pH and sodium concentration-dependent manner [7]. Binding to RNA was also reported, albeit with a lesser affinity [8].

Curcumin was reported to induce DNA damage in cancer cells through two different mechanisms. The first involved DNA double strand breaks as evidenced by accumulation of the phosphorylated H2AX [9]. The second mechanism involved the inhibition of DNA repair proteins like BRCA1 in breast cancer cells [10]. Curcumin also induced apoptosis and cell cycle arrest in different types of tumors. In glioblastoma and medulloblastoma cells, curcumin induced apoptosis was associated with upregulation of the pro-

apoptotic Bax and downregulation of the anti-apoptotic survivin and Bcl-2 [11]. Furthermore, curcumin incorporated into solid lipid nanoparticles induced apoptosis in leukemia, lung and prostate cancer cells by activating the intrinsic pathway of apoptosis [12].

Previous studies showed that curcumin has a very powerful antioxidant effect. Its antioxidant effect was eight times more powerful than vitamin E and was significantly more effective in preventing lipid peroxide formation than the synthetic antioxidant BHT [13]. The sites of activity responsible for its antioxidant effects are controversial, with claims that the antioxidant activity is mainly due to the hydroxyl moiety. Other reports claim the involvement of double bonds and the carbonyl groups, separately or together, with the para-hydroxyl groups [14, 15]. The antioxidant effect of curcumin contributes to its hepatoprotective effect and its activity in chronic liver diseases [16]. On the other hand, curcumin demonstrated a pro-oxidant role in the presence of copper by generating reactive oxygen species at a high free copper level in a reducing environment resulting in DNA damage, inhibition of vital signaling pathways in cancer cells and apoptosis [17].

Curcumin is a pleiotropic molecule that targets numerous inflammatory molecules. In vitro and in vivo studies demonstrated that curcumin is a potential therapeutic agent in many chronic diseases such as inflammatory bowel disease, arthritis, pancreatitis and cancer. The anti-inflammatory mechanism of curcumin involves the Nrf2-keap1 pathway and alleviation of oxidative stress. Curcumin reduced inflammation by decreasing NF-κB activation as well as suppressing mRNA induction of iNOS, TNF-α, and IL-6 in the pancreas [18]. The anti-inflammatory properties of curcumin have therapeutic potential in treating Parkinson's disease and major depressive disorder [19, 20].

The antimicrobial activity of curcumin against different bacteria, viruses, fungi, and parasites have been reported. The anti-tubercular activity of synthetic curcumin analogs was also reported [21]. The promising results for the antimicrobial activity of curcumin made it a good candidate for combination with established antimicrobial agents for a synergistic effect [22].

Curcumin and Epigenetic Therapy

Despite its poor bioavailability and low plasma concentration, curcumin demonstrated efficacy as antitumor agent in pancreatic and colorectal cancer [23]. This implies that the mechanism of action is not dependent on

cytotoxicity and rather dependent on other mechanisms that do not require high plasma concentration like epigenetic mechanisms. Epigenetics is defined as a heritable alteration in gene expression that takes place during development and cell proliferation, without any change in gene sequence. Epigenetic mechanisms include changes in DNA methylation, histone modifications, and altered microRNA (miRNAs) expression [24-26]. Epigenetic modifiers are agents that modulate the epigenetic landscape and consequently gene expression. Although epigenetic modifiers were developed originally for the treatment of cancer, the utility of these agents in other chronic diseases started to gain recognition [27-29]. Curcumin is among other several dietary agents like epigallocatechin-3-gallate (EGCG) and genistein that are considered epigenetic modifiers [30]. Curcumin induces epigenetic changes by different mechanisms including binding to miRNA and/or binding to enzymes that catalyze DNA methylation or histone modifications.

Curcumin and Histone Modifications

Histone tails and globular domain undergo a wide range of post-translational modifications. The dynamic process of histone tails acetylation is by far the most widely studied modification. Histone acetylation is catalyzed by histone acetyltransferase (HAT) enzymes and is associated with gene expression activation [31]. On the other hand, histone deacetylation is catalyzed by the histone deacetylase (HDAC) enzymes and is associated with gene inactivation [32]. Histone methylation, phosphorylation, ubiquitination, sumoylation and ADP-ribosylation represent other histone modifications and their clinical implications and role in chronic diseases is currently recognized [33].

Aberrant activity of HAT and HDAC were linked to the pathogenesis of cancer. Consequently, drugs that modulate the activity of these enzymes may have an anti-tumor effect. The activity of curcumin as an HDAC inhibitor was compared to several carboxylic acid derivatives to understand the structural requirements for HDAC inhibition. Curcumin was shown to be a more potent inhibitor of HDAC than sodium butyrate and valproic acid, a well-known HDAC inhibitors [34]. Other reports demonstrated that curcumin decreased the expression of class I HDAC enzymes isoforms with consequent increase in histone acetylation [35]. Curcumin inhibition of HDAC 8 isoform activity and expression increased the expression of the suppressors of cytokine signaling SOCS-1 and SOCS-3 and inhibited the clonogenic activity of hematopoietic

progenitor cells from patients with myeloproliferative neoplasms [36]. Other reports demonstrated increase in expression of other HDAC isoforms upon curcumin treatment, indicating that the effect of curcumin on HDAC expression is variable and probably cell line specific [37].

Curcumin was also reported to inhibit the activity of certain HAT isoforms. For instance, curcumin demonstrated in vitro and in vivo specific inhibition of the p300/CBP HAT activity but not of p300/CBP-associated factor [38]. Curcumin also inhibited the acetylation of non-histone proteins like p53 and HIV-Tat protein with consequent inhibition of viral proliferation, indicating that it may serve as a lead compound in combinatorial HIV therapeutics. Furthermore, the dicarbonyl moiety of the curcumin structure was shown to be required for its HAT inhibitory activity by acting as a Michael reaction acceptor, which is substantiated by the lack of p300 inhibition by the curcumin analogue tetrahydrocurcumin [39]. Curcumin was also shown to be a potent NF-κB inhibitor [40] and the mechanism of inhibition is believed to be mediated through inhibition of p300 HAT activity with consequent inhibition of NF-κB acetylation and decreased binding [41]. Recently, the p300 HAT inhibitory activity of curcumin was linked to its anti-nociceptive role in neuropathic pain in a rat model [42]. The mechanism involved decreased acetylation of the promoter region of the pro-nociceptive proteins BDNF and Cox-2 after curcumin treatment in a dose-dependent manner. Moreover, curcumin inhibition of HAT was also linked to DNA damage response (DDR) through the suppression of three major DDR pathways: non-homologous end joining (NHEJ), homologous recombination (HR) and the DNA damage checkpoint [43]. The effect of curcumin on histone modifications other than acetylation is not well defined and requires further investigation.

Curcumin and miRNAs Expression

miRNAs are small, noncoding regulatory RNAs ranging in size from 17 to 25 nucleotides. miRNAs repress gene expression post transcriptionally by recognizing complementary target sites in the 3'-untranslated regions of target mRNA [44]. miRNAs play essential roles in vital cellular functions like cell cycling, apoptosis and cellular differentiation. Additionally, they play an important role in tumor development, invasion, metastasis, and angiogenesis [45]. Modulating the expression of miRNAs is a rationale strategy for the treatment of cancer. Curcumin upregulated miRNA-22 and downregulated

miRNA-199a* expression in human pancreatic tumor cells [46]. Upregulation of miRNA-22 expression suppressed the expression of its target genes SP1 and estrogen receptor 1 (ESR1), while inhibiting miRNA-22 with antisense oligonucleotides enhanced SP1 and ESR1 expression. The combination of gemcitabine with curcumin or its analogue CDF was reported and demonstrated better results than single agents [47]. CDF upregulated miR-200 expression and downregulated the expression of miR-21 (a signature of tumor aggressiveness) with consequent induction of its target, the tumor suppressor PTEN. Also, in chronic myelogenous leukemia (CML) cells, curcumin increased the selective packaging of miR-21 in exosomes, which are nanosize vesicles released from cancer cells loaded with microRNAs and can influence gene expression in target cells [48]. Furthermore, the addition of curcumin to CML cells downregulated Bcr-Abl expression through the cellular increase of miR-196b, further contributing to the antileukemic effect of curcumin.

Low dose curcumin demonstrated anti-proliferative effect in prostate cancer cells. The effect was mediated through upregulating the expression of the cell cycle inhibitor CDKN1A without induction of its transcription [49]. miR-208 targets the 3'UTR of the CDKN1A and curcumin inhibited its expression suggesting a role for miRNAs in upregulation of CDKN1A. Indeed, overexpression of miR-208 inhibited upregulation of CDKN1A and the anti-proliferative effect of curcumin. In colorectal cells, curcumin induced upregulation of tumor-suppressive miR-34a and downregulation of miR-27a. Furthermore, curcumin treatment in a mouse xenograft model suppressed tumor growth and was associated with alterations in the expression of miR-34a and miR-27a, consistent with the in vitro findings [50]. The impact of oral curcumin administration as a chemopreventive agent on the miRNA signature of engrafted melanoma was recently investigated in a murine model [51]. mmu-miR-205-5p was significantly upregulated after curcumin treatment and two of its target genes (Bcl-2 and PCNA) were downregulated. Curcumin-induced miRNA expression changes was also shown to suppress liver fibrosis [52]. Curcumin upregulated the expression of miR-29b, which targets the DNA methyltransferase 3b (DNMT3b) enzyme promoting a hypomethylating effect in the promoter region of the PTEN tumor suppressor.

Curcumin downregulated the expression of WT1 in leukemic cells. The mechanism involves the expression upregulation of miR-15a/16-1. Furthermore, anti-miR-15a/16-1 oligonucleotides partly reversed WT1 downregulation induced by curcumin in leukemic cells [53]. Furthermore, curcumin directly induced the tumor-suppressive miRNA, miR-203 in bladder cancer. miR-203 is frequently downregulated in bladder cancer due to

promoter DNA methylation. Curcumin induced hypomethylation of the miR-203 promoter with subsequent upregulation of miR-203 expression and downregulation of its target genes Akt2 and Src. Collectively, these changes culminated in decreased proliferation and increased apoptosis of bladder cancer cells [54].

Curcumin and DNA Methylation

Reversal of promoter DNA methylation is a rational strategy to re-express silenced tumor suppressor genes (TSG). Currently, both Vidaza® and Dacogen® are FDA-approved DNA hypomethylating agents for the treatment of myelodysplastic syndrome (MDS). These agents were approved based on their DNA hypomethylating activity and clinical efficacy in MDS patients. Nonetheless, different studies did not support reversal of DNA methylation as the mechanism of action of these agents in MDS or leukemia [55, 56]. Recently, molecular docking studies suggested a possible covalent interaction between curcumin and the catalytic pocket of DNMT1 [57]. Global DNA methylation analysis demonstrated a comparable methylation reversal to the potent DNA hypomethylating agent decitabine after treatment of leukemia cells with a commercial curcumin mixture (consists of curcumin, demethoxycurcumin and bisdemethoxycurcumin). Similarly, in prostate cancer cells, curcumin mixture demonstrated significant reversal of Nrf2 gene promoter methylation [58]. Furthermore, pure analytical grade curcumin induced DNA methylation reversal and the expression of Neurog1 in LNCaP prostate cancer cells [59]. Another interesting study demonstrated that the activity of curcumin as a hypomethylating agent is dependent on the density of methylation at the CpG sites and that curcumin selectively demethylated partially-methylated loci and not fully-methylated CpG sites [60].

On the contrary, several studies demonstrated the lack of hypomethylating activity after curcumin treatment. Curcumin demonstrated no significant global DNA hypomethylating activity in both leukemia and colorectal cancer cells even after 6 days of treatment [60, 61]. In order to resolve the reported conflict about the hypomethylating activity of curcumin, we designed a study that investigated the global and gene-specific hypomethylating activity of pure curcumin using both low clinically relevant concentrations and high cytotoxic curcumin concentrations [62]. The study also compared the hypomethylating activity of curcumin and its structural analogue dimethoxycurcumin (DMC), which is more metabolically stable than curcumin and could overcome the

poor bioavailability drawback associated with curcumin [63]. The hypomethylating activity of both compounds was tested using the gold standard in DNA methylation analysis, DNA pyrosequencing. Both compounds did not show any significant hypomethylating activity using LINE-1 assay, a surrogate marker for global DNA methylation. Moreover, analysis of 7 CpG sites in the promoter region of the TSG p15 and 5 CpG sites in the promoter region of the TSG CDH-1 revealed that both compounds lack any hypomethylating activity even when used at very high concentrations. On the other hand, both decitabine and 5-azacytidine, a well-known hypomethylating agents, demonstrated dose-dependent global and gene-specific DNA hypomethylating activity.

Surprisingly, DMC induced the expression of promoter-methylated genes without reversing DNA methylation [62]. Indeed, previous reports demonstrated induction of gene expression despite promoter methylation. Class III HDAC SIRT1 was shown to localize to promoter methylated silenced tumor suppressor genes and inhibition of SIRT1 re-expressed the silenced genes despite retention of promoter methylation [64]. Another report demonstrated that treatment with different HDAC inhibitors was able to induce the expression of promoter methylated genes without reversing DNA methylation in colorectal cancer cells [65]. Moreover, the expression of the estrogen receptor alpha gene was induced by treatment with the HDAC inhibitor trichostatin A (TSA) without promoter DNA methylation reversal [66]. Consequently, the effect of DMC on histone modifications that can induce chromatin remodeling and gene expression was investigated [62]. DMC increased significantly the H3K36me3 mark near the promoter region of densely methylated genes and that was associated with induction of gene expression. Taken together, curcumin and its structural analogues are not potent hypomethylating agents; however, they can induce the expression of promoter-methylated genes.

CURCUMIN AND COMBINATION THERAPY

The combination of curcumin with other anticancer agents is an attractive approach based on its safety profile and multiple targets. The effects of curcumin and/or cisplatin treatment have been evaluated in head and neck squamous cell carcinoma as well as in a rat model of cisplatin-induced ototoxicity [67]. Curcumin chemosensitized cancer cells to cisplatin therapy in vitro and protected it from its ototoxicity in vivo. Co-treatment of curcumin

and Poly(ADP-ribose) polymerase (PARP) inhibitor might be useful for aggressive breast cancer treatment based on reducing homologous recombination (HR) and apoptosis induction [68]. Curcumin enhanced the efficacy of the chemotherapeutic agent cytarabine and imatinib mesylate through the inhibition of heat shock proteins [69]. Curcumin showed a synergistic anti-proliferative effect when combined with cytarabine in primary AML leukemia cells and repressed the expression of the multidrug resistance genes (MDR) [70].

CONCLUSION AND FUTURE DIRECTIONS

An ideal chemopreventive and chemotherapeutic agent should affect multiple molecular targets in cancer cells with minimal toxicity in normal healthy cells. Curcumin inhibits multiple vital pathways in cancer cells and demonstrated cytoprotective effect in normal cells based on its antioxidant effect. The poor bioavailability of curcumin is a major hurdle in its successful use as an antitumor agent and the development of modified synthetic analogues would improve its pharmacokinetic profile. The combination of curcumin analogues based on their effect on histone acetylation with other epigenetic modifiers like DNA hypomethylating agents may provide a new therapeutic modality in different types of tumors. The impact of curcumin or its analogues on other histone modifications like methylation, phosphorylation, ubiquitination and sumoylation are currently not well-defined and requires investigation.

ACKNOWLEDGMENTS

I would like to thank Ms. Engy Youssef for her help and support in the preparation of the manuscript.

REFERENCES

[1] Tonnesen, HH. Solubility, chemical and photochemical stability of curcumin in surfactant solutions. Studies of curcumin and curcuminoids, XXVIII. *Pharmazie*., 2002, 57, 820-4.

[2] Tonnesen, HH; Masson, M; Loftsson, T. Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. *Int J Pharm*., 2002, 244, 127-35.

[3] Singh, DV; Misra, K. Curcuminoids as inhibitors of thioredoxin reductase: a receptor based pharmacophore study with distance mapping of the active site. *Bioinformation*., 2009, 4, 187-92.

[4] Liu, M; Yuan, M; Luo, M; Bu, X; Luo, HB; Hu, X. Binding of curcumin with glyoxalase I: Molecular docking, molecular dynamics simulations, and kinetics analysis. *Biophys Chem*., 2010, 147, 28-34.

[5] Anand, P; Thomas, SG; Kunnumakkara, AB; Sundaram, C; Harikumar, KB; Sung, B; et al. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem Pharmacol*., 2008, 76, 1590-611.

[6] Mukhopadhyay, A; Bueso-Ramos, C; Chatterjee, D; Pantazis, P; Aggarwal, BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene*., 2001, 20, 7597-609.

[7] Zsila, F; Bikadi, Z; Simonyi, M. Circular dichroism spectroscopic studies reveal pH dependent binding of curcumin in the minor groove of natural and synthetic nucleic acids. *Org Biomol Chem*., 2004, 2, 2902-10.

[8] Nafisi, S; Adelzadeh, M; Norouzi, Z; Sarbolouki, MN. Curcumin binding to DNA and RNA. *DNA Cell Biol*., 2009, 28, 201-8.

[9] Jiang, Z; Jin, S; Yalowich, JC; Brown, KD; Rajasekaran, B. The mismatch repair system modulates curcumin sensitivity through induction of DNA strand breaks and activation of G2-M checkpoint. *Mol Cancer Ther*., 2010, 9, 558-68.

[10] Rowe, DL; Ozbay, T; O'Regan, RM; Nahta, R. Modulation of the BRCA1 Protein and Induction of Apoptosis in Triple Negative Breast Cancer Cell Lines by the Polyphenolic Compound Curcumin. *Breast Cancer (Auckl)*., 2009, 3, 61-75.

[11] Khaw, AK; Hande, MP; Kalthur, G. Curcumin inhibits telomerase and induces telomere shortening and apoptosis in brain tumour cells. *J Cell Biochem*., 2013, 114, 1257-70.

[12] Vandita, K; Shashi, B; Santosh, KG; Pal, KI. Enhanced apoptotic effect of curcumin loaded solid lipid nanoparticles. *Mol Pharm*., 2012, 9, 3411-21.

[13] Reddy, AC; Lokesh, BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem*., 1992, 111, 117-24.

[14] Sun, YM; Zhang, HY; Chen, DZ; Liu, CB. Theoretical elucidation on the antioxidant mechanism of curcumin: a DFT study. *Org Lett*., 2002, 4, 2909-11.

[15] Sugiyama, Y; Kawakishi, S; Osawa, T. Involvement of the beta-diketone moiety in the antioxidative mechanism of tetrahydrocurcumin. *Biochem Pharmacol*., 1996, 52, 519-25.

[16] Vera-Ramirez, L; Perez-Lopez, P; Varela-Lopez, A; Ramirez-Tortosa, M; Battino, M; Quiles, JL. Curcumin and liver disease. *Biofactors*., 2013, 39, 88-100.

[17] Leung, MH; Harada, T; Kee, TW. Delivery of curcumin and medicinal effects of the copper(II)-curcumin complexes. *Curr Pharm Des*., 2013, 19, 2070-83.

[18] Gulcubuk, A; Haktanir, D; Cakiris, A; Ustek, D; Guzel, O; Erturk, M; et al. Effects of curcumin on proinflammatory cytokines and tissue injury in the early and late phases of experimental acute pancreatitis. *Pancreatology*., 2013, 13, 347-54.

[19] Xu, Y; Ku, BS; Yao, HY; Lin, YH; Ma, X; Zhang, YH; et al. Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. *Pharmacol Biochem Behav*., 2005, 82, 200-6.

[20] Xu, Y; Ku, BS; Yao, HY; Lin, YH; Ma, X; Zhang, YH; et al. The effects of curcumin on depressive-like behaviors in mice. *Eur J Pharmacol*., 2005, 518, 40-6.

[21] Bukhari, SN; Franzblau, SG; Jantan, I; Jasamai, M. Current prospects of synthetic curcumin analogs and chalcone derivatives against mycobacterium tuberculosis. *Med Chem*., 2013, 9, 897-903.

[22] Varaprasad, K; Vimala, K; Ravindra, S; Narayana Reddy, N; Venkata Subba Reddy, G; Mohana Raju, K. Fabrication of silver nanocomposite films impregnated with curcumin for superior antibacterial applications. *J Mater Sci Mater Med*., 2011, 22, 1863-72.

[23] Dhillon, N; Aggarwal, BB; Newman, RA; Wolff, RA; Kunnumakkara, AB; Abbruzzese, JL; et al. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res*., 2008, 14, 4491-9.

[24] Jones, PA; Baylin, SB. The epigenomics of cancer. *Cell*., 2007, 128, 683-92.

[25] Jones, PA; Baylin, SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet*., 2002, 3, 415-28.

[26] Baylin, SB; Jones, PA. A decade of exploring the cancer epigenome - biological and translational implications. *Nat Rev Cancer*., 2011, 11, 726-34.

[27] Egger, G; Liang, G; Aparicio, A; Jones, PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature*., 2004, 429, 457-63.

[28] Liakopoulos, V; Georgianos, PI; Eleftheriadis, T; Sarafidis, PA. Epigenetic mechanisms and kidney diseases. *Curr Med Chem*., 2011, 18, 1733-9.

[29] Ling, C; Groop, L. Epigenetics: a molecular link between environmental factors and Type 2 diabetes. *Diabetes*. 2009, 58, 2718-25.

[30] Fandy, TE. Development of DNA methyltransferase inhibitors for the treatment of neoplastic diseases. *Curr Med Chem*., 2009, 16, 2075-85.

[31] Zhang, K; Dent, SY. Histone modifying enzymes and cancer: going beyond histones. *J Cell Biochem*., 2005, 96, 1137-48.

[32] West, AC; Johnstone, RW. New and emerging HDAC inhibitors for cancer treatment. *J Clin Invest*., 2014, 124, 30-9.

[33] Bannister, AJ; Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res*., 2011, 21, 381-95.

[34] Bora-Tatar, G; Dayangac-Erden, D; Demir, AS; Dalkara, S; Yelekci, K; Erdem-Yurter, H. Molecular modifications on carboxylic acid derivatives as potent histone deacetylase inhibitors: Activity and docking studies. *Bioorg Med Chem*., 2009, 17, 5219-28.

[35] Liu, HL; Chen, Y; Cui, GH; Zhou, JF. Curcumin, a potent anti-tumor reagent, is a novel histone deacetylase inhibitor regulating B-NHL cell line Raji proliferation. *Acta Pharmacol Sin*., 2005, 26, 603-9.

[36] Chen, CQ; Yu, K; Yan, QX; Xing, CY; Chen, Y; Yan, Z; et al. Pure curcumin increases the expression of SOCS1 and SOCS3 in myeloproliferative neoplasms through suppressing class I histone deacetylases. *Carcinogenesis*., 2013, 34, 1442-9.

[37] Meja, KK; Rajendrasozhan, S; Adenuga, D; Biswas, SK; Sundar, IK; Spooner, G; et al. Curcumin restores corticosteroid function in monocytes exposed to oxidants by maintaining HDAC2. *Am J Respir Cell Mol Biol*., 2008, 39, 312-23.

[38] Balasubramanyam, K; Varier, RA; Altaf, M; Swaminathan, V; Siddappa, NB; Ranga, U; et al. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J Biol Chem*., 2004, 279, 51163-71.

[39] Marcu, MG; Jung, YJ; Lee, S; Chung, EJ; Lee, MJ; Trepel, J; et al. Curcumin is an inhibitor of p300 histone acetylatransferase. *Med Chem*., 2006, 2, 169-74.

[40] Singh, S; Aggarwal, BB. Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane) (corrected). *J Biol Chem*., 1995, 270, 24995-5000.

[41] Yun, JM; Jialal, I; Devaraj, S. Epigenetic regulation of high glucose-induced proinflammatory cytokine production in monocytes by curcumin. *J Nutr Biochem*., 2011, 22, 450-8.

[42] Zhu, X; Li, Q; Chang, R; Yang, D; Song, Z; Guo, Q; et al. Curcumin alleviates neuropathic pain by inhibiting p300/CBP histone acetyltransferase activity-regulated expression of BDNF and cox-2 in a rat model. *PLoS One*., 2014, 9, e91303.

[43] Ogiwara, H; Ui, A; Shiotani, B; Zou, L; Yasui, A; Kohno, T. Curcumin suppresses multiple DNA damage response pathways and has potency as a sensitizer to PARP inhibitor. *Carcinogenesis*., 2013, 34, 2486-97.

[44] Croce, CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet*., 2009, 10, 704-14.

[45] Nana-Sinkam, SP; Croce, CM. MicroRNA regulation of tumorigenesis, cancer progression and interpatient heterogeneity: towards clinical use. *Genome Biol*., 2014, 15, 445.

[46] Sun, M; Estrov, Z; Ji, Y; Coombes, KR; Harris, DH; Kurzrock, R. Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. *Mol Cancer Ther*., 2008, 7, 464-73.

[47] Ali, S; Ahmad, A; Banerjee, S; Padhye, S; Dominiak, K; Schaffert, JM; et al. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR-200 and miR-21 expression by curcumin or its analogue CDF. *Cancer Res*., 2010, 70, 3606-17.

[48] Taverna, S; Giallombardo, M; Pucci, M; Flugy, A; Manno, M; Raccosta, S; et al. Curcumin inhibits in vitro and in vivo chronic myelogenous leukemia cells growth: a possible role for exosomal disposal of miR-21. *Oncotarget*., 2015, 6, 21918-33.

[49] Guo, H; Xu, Y; Fu, Q. Curcumin inhibits growth of prostate carcinoma via miR-208-mediated CDKN1A activation. *Tumour Biol*., 2015.

[50] Toden, S; Okugawa, Y; Buhrmann, C; Nattamai, D; Anguiano, E; Baldwin, N; et al. Novel Evidence for Curcumin and Boswellic Acid-Induced Chemoprevention through Regulation of miR-34a and miR-27a in Colorectal Cancer. *Cancer Prev Res (Phila)*., 2015, 8, 431-43.

[51] Dahmke, IN; Backes, C; Rudzitis-Auth, J; Laschke, MW; Leidinger, P; Menger, MD; et al. Curcumin intake affects miRNA signature in murine melanoma with mmu-miR-205-5p most significantly altered. *PLoS One*., 2013, 8, e81122.

[52] Zheng, J; Wu, C; Lin, Z; Guo, Y; Shi, L; Dong, P; et al. Curcumin up-regulates phosphatase and tensin homologue deleted on chromosome 10 through microRNA-mediated control of DNA methylation--a novel mechanism suppressing liver fibrosis. *Febs J*., 2014, 281, 88-103.

[53] Gao, SM; Yang, JJ; Chen, CQ; Chen, JJ; Ye, LP; Wang, LY; et al. Pure curcumin decreases the expression of WT1 by upregulation of miR-15a and miR-16-1 in leukemic cells. *J Exp Clin Cancer Res*., 2012, 31, 27.

[54] Saini, S; Arora, S; Majid, S; Shahryari, V; Chen, Y; Deng, G; et al. Curcumin modulates microRNA-203-mediated regulation of the Src-Akt axis in bladder cancer. *Cancer Prev Res (Phila)*., 2011, 4, 1698-709.

[55] Fandy, TE; Herman, JG; Kerns, P; Jiemjit, A; Sugar, EA; Choi, SH; et al. Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. *Blood*., 2009, 114, 2764-73.

[56] Raj, K; John, A; Ho, A; Chronis, C; Khan, S; Samuel, J; et al. CDKN2B methylation status and isolated chromosome 7 abnormalities predict responses to treatment with 5-azacytidine. *Leukemia*., 2007, 21, 1937-44.

[57] Liu, Z; Xie, Z; Jones, W; Pavlovicz, RE; Liu, S; Yu, J; et al. Curcumin is a potent DNA hypomethylation agent. *Bioorg Med Chem Lett*., 2009, 19, 706-9.

[58] Khor, TO; Huang, Y; Wu, TY; Shu, L; Lee, J; Kong, AN. Pharmacodynamics of curcumin as DNA hypomethylation agent in restoring the expression of Nrf2 via promoter CpGs demethylation. *Biochem Pharmacol*., 2011, 82, 1073-8.

[59] Shu, L; Khor, TO; Lee, JH; Boyanapalli, SS; Huang, Y; Wu, TY; et al. Epigenetic CpG demethylation of the promoter and reactivation of the expression of Neurog1 by curcumin in prostate LNCaP cells. *Aaps J*., 2011, 13, 606-14.

[60] Link, A; Balaguer, F; Shen, Y; Lozano, JJ; Leung, HC; Boland, CR; et al. Curcumin modulates DNA methylation in colorectal cancer cells. *PLoS One*., 2013, 8, e57709.

[61] Medina-Franco, JL; Lopez-Vallejo, F; Kuck, D; Lyko, F. Natural products as DNA methyltransferase inhibitors: a computer-aided discovery approach. *Mol Divers*., 2011, 15, 293-304.

[62] Hassan, HE; Carlson, S; Abdallah, I; Buttolph, T; Glass, KC; Fandy, TE. Curcumin and dimethoxycurcumin induced epigenetic changes in leukemia cells. *Pharm Res*., 2015, 32, 863-75.

[63] Tamvakopoulos, C; Dimas, K; Sofianos, ZD; Hatziantoniou, S; Han, Z; Liu, ZL; et al. Metabolism and anticancer activity of the curcumin analogue, dimethoxycurcumin. *Clin Cancer Res*., 2007, 13, 1269-77.

[64] Pruitt, K; Zinn, RL; Ohm, JE; McGarvey, KM; Kang, SH; Watkins, DN; et al. Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet*., 2006, 2, e40.

[65] Raynal, NJ; Si, J; Taby, RF; Gharibyan, V; Ahmed, S; Jelinek, J; et al. DNA methylation does not stably lock gene expression but instead serves as a molecular mark for gene silencing memory. *Cancer Res*., 2012, 72, 1170-81.

[66] Yang, X; Ferguson, AT; Nass, SJ; Phillips, DL; Butash, KA; Wang, SM; et al. Transcriptional activation of estrogen receptor alpha in human breast cancer cells by histone deacetylase inhibition. *Cancer Res*., 2000, 60, 6890-4.

[67] Fetoni, AR; Paciello, F; Mezzogori, D; Rolesi, R; Eramo, SL; Paludetti, G; et al. Molecular targets for anticancer redox chemotherapy and cisplatin-induced ototoxicity: the role of curcumin on pSTAT3 and Nrf-2 signalling. *Br J Cancer*., 2015.

[68] Choi, YE; Park, E. Curcumin enhances poly(ADP-ribose) polymerase inhibitor sensitivity to chemotherapy in breast cancer cells. *J Nutr Biochem*., 2015.

[69] Sarkar, R; Mukherjee, A; Mukherjee, S; Biswas, R; Biswas, J; Roy, M. Curcumin augments the efficacy of antitumor drugs used in leukemia by modulation of heat shock proteins via HDAC6. *J Environ Pathol Toxicol Oncol*., 2014, 33, 247-63.

[70] Shah, K; Mirza, S; Desai, U; Jain, N; Rawal, R. Synergism of Curcumin and Cytarabine in the down Regulation of Multi Drugresistance Genes in Acute Myeloid Leukemia. *Anticancer Agents Med Chem*., 2015.

In: Curcumin
Editor: Valeria Martin
ISBN: 978-1-63484-272-3

Chapter 5

DIFFERENTIAL ABSORPTION OF CURCUMINOIDS BETWEEN FREE AND LIPOSOMED CURCUMIN FORMULATIONS

Enrique Barrajón Catalán[1,2,*,†], Lorena Funes[1,#], María Herranz-López[1], Isabel González-Álvarez[2], Marival Bermejo[2] and Vicente Micol[1,3]

[1]Instituto de Biología Molecular y Celular (IBMC).
Universidad Miguel Hernández, Alicante, Spain
[2]Area de Farmacia y Tecnología Farmacéutica. Facultad de Farmacia.
Universidad Miguel Hernández, Alicante, Spain
[3]CIBER (Fisiopatología de la Obesidad y la Nutrición,
CIBERobn, Instituto de Salud Carlos III)

ABSTRACT

Curcumin is a polyphenolic compound derived from turmeric, an old Indian dietary spice. Curcumin extracts possess diverse pharmacological effects including anti-inflammatory, antioxidant, antiproliferative and antiangiogenic activities and have been very appreciated by traditional

[*] Both authors contributed evenly.
[†] Corresponding author: e.barrajon@umh.es.

Asian medicine for a long time. In spite of this relevance, the pharmacological use of curcumin is limited due to poor water-solubility and low oral bioavailability. One of the main strategies to avoid this limitation is the encapsulation of curcumin extracts in lipid-based nano-delivery systems such as phospholipid complexes, micelles or liposomes that increase permeability by interacting with cell membrane components. In this work, the individual absorption of curcumin, demethoxycurcumin and bisdemethoxycurcumin was studied by comparing the behavior of free (water dispersion) and liposomed formulations using an *in situ* rat absorption model. The results show differences in the absorption rates for the curcuminoids in the water dispersion in correlation with their hydrophobicity, having the demethoxy derivatives a higher intestinal permeability compared to curcumin. In contrast, using the liposomed formulation no differences in the absorption were observed for the curcuminoids, probably revealing that the absorption is driven by the incorporation/fusion of the liposomes in the intestinal cell membrane. In spite of the lower absorption rate coefficient observed for the curcuminoids in the liposomed formulation compared to the free formulation, only the liposomed formulation led to significant quantities of curcumin metabolites in rat plasma, probably due to the highest concentration of curcuminoids achieved in the gut. These results show that, besides their biological activity, the preferential absorption of curcuminoids depending on their polarity has to be considered for therapeutical use of curcumin.

INTRODUCTION

Curcumin is the yellow spice derived from the rhizome of *Curcuma longa* L. This plant is native of India and Southeast Asia and it has been used in cooking and to treat a broad range of disorders in Ayurvedic medicine for at least 4000 years [1]. Dried rhizomes of *C. longa* are milled obtaining turmeric, which contains not only curmumin and other derivatives, but also a mixture of volatile and nonvolatile oils, proteins, fat, minerals and carbohydrates. Curcumin extracts are obtained from turmeric with different purities depending on the commercial presentations, containing a mixture of different curcuminoids, mainly curcumin (CUR), demethoxycurcumin (DCUR) and bisdemethoxycurcumin (BDCUR) (Figure 1).

In addition to its ethnobotanical traditional use, curcumin has demonstrated diverse pharmacological effects including anti-inflammatory, antioxidant, antiproliferative, antitumoral and antiangiogenic activities [2, 3]. Various clinical trials have used curcumin, especially as anticancer agent [4,

5], however, poor bioavailability and rapid metabolism have emerged as drawbacks that limit its clinical use. The main limitation is the poor absorption of curcuminoids, which is mainly due to the water insoluble character of curcumin. In this sense, most of the curcumin orally administered is not absorbed but found in feces, both in human and rat models [2, 6]. In addition to its low absorption, a rapid metabolism of absorbed curcuminoids is also occurring. Curcuminoids suffer an important first-pass metabolism which converts them to sulphate or glucuronide derivatives in a high percentage when administered orally, or reduced forms as di, tetra or hexahydrocurcumin when administered i.v. or i.p. [2, 6]. Although there are contradictory data about the activity of curcuminoids' metabolites when compared with non-metabolized compounds, the most accepted theory is that most of them are less active when metabolized [2], which diminishes even more the potential biological effects of curcumin extracts and new approaches for formulation are necessary.

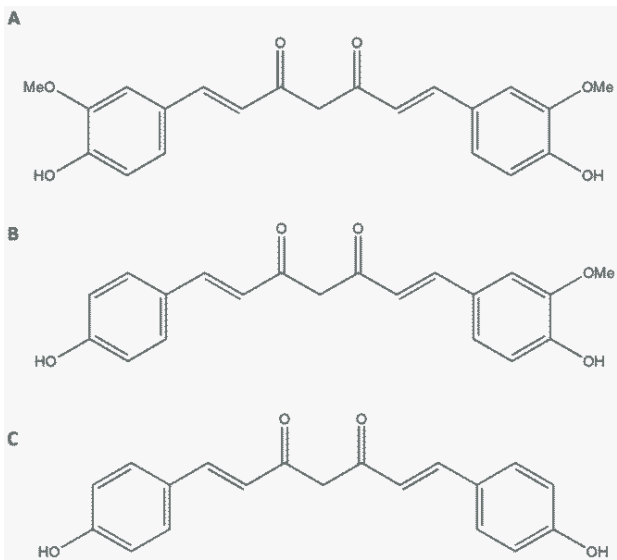


Figure 1. Structures of the main curcuminoids present in the curcumin extract: curcumin (A), demethoxycurcumin (B) and bisdemethoxycurcumin (C).

To overcome the low absorption, different approximations have been used to improve curcumin bioavailability such as liposomes, micelles, phospholipid complexes. nanoparticles, cyclodextrins, emulsions and others, reviewed in [2, 7-10]. On the other hand, metabolism inhibitors such as piperine, an inhibitor

of hepatic and intestinal glucuronidation, has been combined with curcumin in different studies to increase curcumin plasma concentration [2, 11]. Both strategies have succeeded and have increased notably the bioavailability of curcuminoids.

Some preclinical studies have shown that extracts bearing different curcuminoids profile do not bring different biological activity [2], but other studies revealed significant differences when the different curcuminoids were analyzed in terms of antioxidant [12], antimetastasic [13] and antinflammatory [14] activities. In this regard, although there are numerous studies on curcumin bioavailability and absorption [15-18], none of them has been focused on characterizing the differential absorption between the three main curcuminoids present in curcumin extracts by comparing free and liposomed formulations containing curcumin.

In this work the intestinal absorption coefficients of the main curcuminoids derived from a curcumin commercial extract have been studied using the *in situ* Doluisio's absorption method in rats. The absorption rate constants have been obtained for two different formulations, free curcumin and liposomed curcumin extracts and compared in order to obtain data from individual compounds in each formulation (free or liposomed). Finally, blood samples were analyzed and plasmatic curcuminoids were determined in all cases. This study was aimed to determine which of the curcuminoids was preferentially absorbed in each formulation, which may contribute to the design of more effective curcumin formulations for therapeutical use.

RESULTS AND DISCUSSION

In this study, the absorption of the main curcuminoids contained in a commercial curcumin extract either in their free form or vehiculized into unilamellar phospholipid vesicles was evaluated using an *in situ* rat absorption model. Liposomed curcumin formulation was prepared by formation of large unilamelar vesicles (LUVs) using soy phosphatidylcholine through lipid film hydration followed by 200 nm membrane extrusion [19], see methods section for further information. The encapsulation into liposomes led to two significant effects on the perfusion solution to be used in the *in situ* rat absorption assay. First, the concentration of the individual curcuminoids and that of total curcuminoids were increased up to 3.6 fold compared to free curcumin formulation (Table 1), most probably due to the higher solubility of curcuminoids into the liposomes lipophilic environment. This would facilitate

the preparation of formulations containing a higher concentration of curcuminoids, probably leading to higher absorption and therefore improved efficacy.

Secondly, the individual curcuminoids were encapsulated in a different extent into the phospholipid vesicles indicating a preferential partition depending on their structure. In the perfusion sample containing free curcumin extract, the concentration of BDCUR was almost four fold compared to those of CUR or DCUR (16:21:73 CU/DCUR/BDCUR ratio in percentage). This was in agreement with the highest polarity of the BDCUR, which contains two free hydroxyl groups, compared to the other curcuminoids. In contrast, the most lipophilic curcuminoid CUR exhibited a higher concentration in the perfusion sample containing the liposomed formulation (51:17:32 CU/DCUR/BDCUR ratio in percentage), which correlates with its higher potential to partition into phospholipid vesicles (Table 1).

Once the differences between the two formulations were established on the basis of curcuminoids polarity and lipid affinity, free and liposomed curcumin extracts were challenged in an *in situ* Doluisio's absorption assay in order to determine which of the curcuminoids was preferentially absorbed and to determine the differences between the absorption profiles of these two formulations.

Doluisio's assay was performed as described in methods section. Samples of intestinal lumen were taken at different times and curcuminoids' concentrations were measured by HPLC. Individual curcuminoid and total curcuminoids concentration were obtained for every time point and absorption rate coefficients, k_{app}, were calculated both for individual and total curcuminoids. As shown in Figure 2a (Table 1), when free curcumin extract was utilized, significant differences were observed between the three main curcuminoids. The loss of the methoxyl groups increased significantly ($p < 0.001$) the k_{app}, indicating that in the free extract, the less hydrophobic curcuminoids, i.e., DCUR and BDCUR, were preferably absorbed suggesting that these compounds still maintain certain level of hydrophobicity to be absorbed through a passive diffusion mechanism. No significant differences ($p > 0.05$) were obtained between DCUR and BDCUR, suggesting that the loss of a single methoxyl group from CUR was sufficient to increase the absorption of the compound. As the most abundant compound in the perfusion sample corresponding to the free formulation was BDCUR, the k_{app} of the whole extract was very close to that one of BDCUR value.

On the contrary, the perfusion sample containing the liposomed extract exhibited an absorption profile completely different to that one observed for

the free formulation. All the curcuminoids showed similar k_{app} values (Figure 2b, Table 1) regardless of their structure, suggesting that hydrophobicity did not influence the absorption when compounds were vehiculized into liposomes. No significant differences in k_{app} values were found when total curcuminoids absorption was compared to those of the individual compounds ($p > 0.05$). This results reveal that curcumin absorption in the liposomed formulation is likely mediated by the interaction of liposomes with parietal cell membrane in rat's gut and it's not influenced by the polarity of the compounds as occurred in free curcumin formulation.

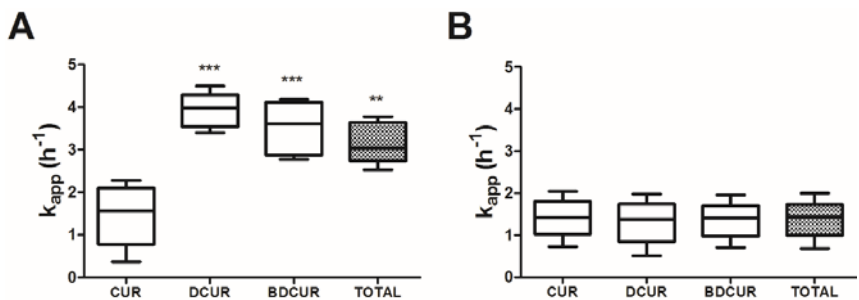


Figure 2. Absorption rate coefficients box plots for all the individual and total curcuminoids in free (A) or liposomed (B) formulations. Differences between boxes are related to CUR (*** $p < 0.001$, ** $p < 0.01$).

Table 1. Initial concentration (C_0) of curcuminoids in the perfusion samples and k_{app} values for both free and liposomed curcumin formulations. Both values are detailed for every single curcuminoid and for the total (sum of the three main curcuminoids) mixture

	Free curcumin				Liposomed curcumin			
	CUR	DCUR	BDCUR	TOTAL	CUR	DCUR	BDCUR	TOTAL
Conc (C_0) (mg/L)	11.70 ±0.60	14.80 ±4.60	44.40 ±1.60	71.00 ±5.80	133.50 ±5.10	44.30 ±1.80	82.30 ±4.60	260.10 ±11.20
k_{app} (h^{-1})	1.46 ±0.74	3.92 ±0.42	3.52 ±0.63	3.16 ±0.49	1.42 ±0.48	1.31 ±0.54	1.35 ±0.45	1.38 ±0.47

Finally, after the concentration of curcuminoids in the formulations and absorption behavior were studied, the presence of curcuminoids in rat plasma samples after the assay was explored. For this purpose, blood samples were

obtained after the Doluisio's assay for all the animals. Total blood samples were processed as described in methods section to eliminate protein and lipid interferences and analyzed by HPLC. Curcuminoid compounds were only observed in blood plasma samples derived from the animals treated with the liposomed formulation, indicating that although k_{app} was apparently higher for the curcuminoids in the free formulation, the higher concentration of curcuminoids achieved in the gut by the liposomed formulation led to an increased net absorption according to Fick's Law. Quantification of total plasma curcuminoids using HPLC coupled to fluorescence detection revealed a maximum concentration of 21.05 ± 2.22 ng/mL in rat samples taken 30 min after being treated with the liposomed formulation, which is agreement to data previously reported [15]. In conclusion, our results show that a higher concentration of curcuminoids, especially CUR, could be achieved in the gut when curcumin extract was vehiculized into liposomes compared to aqueous buffer. Moreover, a differential absorption of the main curcuminoids of the curcumin extract was observed when these compounds were in an aqueous buffer, leading to a higher absorption for the less hydrophobic curcuminoids, i.e., demethoxy and bisdemethoxy curcumin. In contrast, no differences in absorption were observed when all the curcuminoids where vehiculized into phospholipid vesicles indicating that the absorption path takes place through the interaction of liposomes with parietal cell membrane in rat's gut and is not influenced by the polarity of the compounds. Between the two formulations utilized, only the liposomed formulation of curcumin led to significant quantities of curcumin metabolites in rat plasma corroborating previously reported results. Based on the absorption studies and considering that some studies have pointed out that curcumin is more active that the dimethoxy derivatives, a liposomed formulation would be the choice for a therapeutical application. Our results demonstrate that future investigations on the design of curcumin extract formulations for therapy should consider not only the biological activity of the curcuminoids, but also the preferential absorption of these compounds depending on their polarity.

METHODS

Curcumin Liposomes Preparation

Appropriate amounts of soy phosphatidylcholine (Lipoid, Germany) were dissolved in chloroform/methanol 1:1 (Sigma-Aldrich, Spain) and mixed with

the curcumin extract. Curcumin extract was kindly provided by Monteloeder S.L (95% curcuminoids). The organic solvent was evaporated from the lipid solution using a N_2 stream and dried by vacuum for 3 h. The lipid film was hydrated by vortexing at 37ºC using isotonic saline matrix adjusted to pH 7.0 with 1% Sörensen phosphate buffer (v/v). Large unilamellar vesicles were obtained by at least 25x extrusion cycles through 100 nm polycarbonate membranes (Whatman, Maidstone, Kent, UK) using an extruder device (Avestin, Otawa, Canada). Unencapsulated and precipitated material was discarded by centrifugation. The final liposomal formulation showed a curcumid lipid ratio of 18.95 ± 2.60 µg of total curcuminoids/mg of lipid.

Absorption Experiments and Pharmacokinetic Calculations

The absorption experiments were performed using an *in situ* loop technique previously described by Doluisio [20]. Data were previously corrected for water reabsorption, k_{app} was estimated using non-linear regression with Excel and Solver tool.

The study protocol was approved by the University Miguel Hernández Ethical Committee and followed the guidelines described in the EC Directive 86/609, the Council of the Europe Convention ETS 123 and Spanish national laws governing the use of animals in research (Real Decreto 53/2013). Five male Wistar rats weighing 280-320 g were used for each formulation. After eight hours of fasting with access to water, rats were anesthetized with thiopental sodium (30 mg/kg intra-peritoneal). A midline abdominal incision was made. The intestinal segment was manipulated carefully in order to minimize any intestinal blood supply disturbances. The bile duct was tied to avoid drug enterohepatic circulation and limit the presence of bile salts in the lumen. Studies employed the entire small intestine (length around 100 cm). The proximal ligatures of the duodenal and ileal regions were place approximately 1 cm from the pylorus and 2 cm above the ileocecal junction. A catheter was tight up at both intestinal ends and connected to a glass syringe by the use of a stopcock type valve. Under this set up, the intestinal segment is an isolated compartment and the drug solution can be perfused and tested.

The drug test solutions were prepared in an isotonic saline matrix adjusted to pH 7.0 with 1% Sörensen phosphate buffer (v/v). Drug solutions were pre-warmed at 37ºC in advance. The drug solution was perfused into the loop and then the entire intestine was restored into the abdominal cavity. The body temperature was maintained during anesthesia by heating with a lamp. Samples were withdrawn every 5 min for 30 min. To correct for variation in drug luminal concentration over time due to water re-absorption, the

remaining fluid at 30 min was recovered and volume measured. Rats were sacrificed humanely at the end of experimentation. In order to separate solid components (e.g., mucus, intestinal contents) from drug solution, samples were centrifuged 5 min at 5000 r.p.m (1530 g)., and then quantified by HPLC.

Data were analyzed in terms of an apparent first order absorption rate constant. The apparent first-order rate constants were obtained from the expression:

$$C = C_0 \cdot e^{-kapp \cdot t}$$

where the C is the drug concentration remaining in the lumen, k_{app} is the apparent absorption rate constant, and C_0 is initial drug concentration.

HPLC Measurements

Curcuminoids were identified and quantified by HPLC using an Agilent LC 1100 series (Agilent Technologies, Inc., Palo Alto, CA, USA) controlled by the Chemstation software and equipped with a pump, autosampler, column oven and fluorescence and UV–diode array detector. All solvents used were of HPLC grade (Sigma–Aldrich, Europe). An isocratic method consisting of methanol, isopropyl alcohol, water and acetic acid (20:4:27:48:5 v/v) was used. The flow rate was 0.5 mL/min. The isocratic elution was monitored with fluorescence detector at a wavelength of excitation of 420 nm and emission of 540 nm. A Merck LiChrospher 100 RP18, 5 μm, 250 x 4mm (i.d.) column was used for analytical purposes. Quantitation of curcuminoids content was performed using a curcumin commercial standard (Sigma-Aldrich, Europe). Calibration graphs for the quantitative evaluation of the compounds were performed by means of a six-point regression curve ($R^2 > 0.999$).

Plasma Recovery of Curcuminoids

Plasma samples were mixed with equal volume of 0.1 M phosphate buffer, pH 12 and incubated at 37ºC and 500 rpm for 1 hour. Then, curcuminoids were recovered by a liquid extraction with ethyl acetate:isopropanol (9:1). Solvents were evaporated with a N_2 stream and the final pellets were resuspended in HPLC mobile phase before analysis.

ACKNOWLEDGMENTS

This work was supported by AGL2011-29857-C03-03 and AGL2015-67995-C3-1-R grants (Spanish Ministry of Science and Innovation), PROMETEO/2012/007 and ACOMP/2013/093 grants from Generalitat Valenciana (GV), and CIBER (CB12/03/30038, Fisiopatología de la Obesidad y la Nutrición, CIBERobn, Instituto de Salud Carlos III). MH is a recipient of a VALi+D fellowship from GV (ACIF/2010/162). We thank Monteloeder, SL (Alicante, Spain) for the curcumin extract.

REFERENCES

[1] Catania, A., Enhancement of efficacy and selectivity of chemopreventive compounds in human breast cancer cells by using Immunoliposomes, in Facoltà di Farmacia2011, *Università degli Studi di Catania: Catania* (Italy). p. 168.

[2] Anand, P., et al., Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics*, 2007. 4(6): p. 807-818.

[3] Kunnumakkara, A.B., P. Anand, and B.B. Aggarwal, Curcumin inhibits proliferation, invasion, angiogenesis and metastasis of different cancers through interaction with multiple cell signaling proteins. *Cancer Letters,* 2008. 269(2): p. 199-225.

[4] Aggarwal, B.B., A. Kumar, and A.C. Bharti, Anticancer potential of curcumin: Preclinical and clinical studies. *Anticancer Research*, 2003. 23(1 A): p. 363-398.

[5] Hsu, C.H. and A.L. Cheng, *Clinical studies with curcumin*, 2007. p. 471-480.

[6] Sharma, R.A., W.P. Steward, and A.J. Gescher, Pharmacokinetics and pharmacodynamics of curcumin. *Advances in Experimental Medicine and Biology,* 2007. 595: p. 453-470.

[7] Wang, S., et al., Nanotechnologies for curcumin: An ancient puzzler meets modern solutions. *Journal of Nanomaterials*, 2011. 2011.

[8] Yallapu, M.M., M. Jaggi, and S.C. Chauhan, Curcumin nanoformulations: A future nanomedicine for cancer. *Drug Discovery Today,* 2012. 17(1-2): p. 71-80.

[9] Douglass, B.J. and D.L. Clouatre, Beyond Yellow Curry: Assessing Commercial Curcumin Absorption Technologies. *Journal of the American College of Nutrition*, 2015. 34(4): p. 347-358.

[10] Jäger, R., et al., Comparative absorption of curcumin formulations. *Nutrition Journal*, 2014. 13(1).

[11] Shaikh, J., et al., Nanoparticle encapsulation improves oral bioavailability of curcumin by at least 9-fold when compared to curcumin administered with piperine as absorption enhancer. *European Journal of Pharmaceutical Sciences*, 2009. 37(3-4): p. 223-230.

[12] Ahmad, N., et al., A comparative study of PNIPAM nanoparticles of curcumin, demethoxycurcumin, and bisdemethoxycurcumin and their effects on oxidative stress markers in experimental stroke. *Protoplasma,* 2013. 250(6): p. 1327-38.

[13] Yodkeeree, S., et al., Curcumin, demethoxycurcumin and bisdemethoxycurcumin differentially inhibit cancer cell invasion through the down-regulation of MMPs and uPA. *J Nutr Biochem*, 2009. 20(2): p. 87-95.

[14] Sandur, S.K., et al., Curcumin, demethoxycurcumin, bisdemethoxycurcumin, tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and anti-proliferative responses through a ROS-independent mechanism. *Carcinogenesis,* 2007. 28(8): p. 1765-73.

[15] Sun, M., et al., Evaluation of an oral carrier system in rats: Bioavailability and gastrointestinal absorption properties of curcumin encapsulated PBCA nanoparticles. *Journal of Nanoparticle Research,* 2012. 14(2).

[16] Cui, J., et al., Enhancement of oral absorption of curcumin by self-microemulsifying drug delivery systems. *International Journal of Pharmaceutics,* 2009. 371(1-2): p. 148-155.

[17] Fong, Y.K., et al., In vitro and in situ evaluation of herb-drug interactions during intestinal metabolism and absorption of Baicalein. *Journal of Ethnopharmacology*, 2012. 141(2): p. 742-753.

[18] Yu, H. and Q. Huang, Investigation of the absorption mechanism of solubilized curcumin using caco-2 cell monolayers. *Journal of Agricultural and Food Chemistry*, 2011. 59(17): p. 9120-9126.

[19] Olson, F., C.A. Hunt, and F.C. Szoka, Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochimica et Biophysica Acta*, 1979. 557(1): p. 9-23.

[20] Doluisio, J.T., et al., Drug absorption. I. An in situ rat gut technique yielding realistic absorption rates. *Journal of Pharmaceutical Sciences,* 1969. 58(10): p. 1196-1200.

INDEX

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V