H. Feng · Y. Mao
J. H. Zhang *Editors*

Early Brain Injury or Cerebral Vasospasm

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Edited by
Hua Feng, Ying Mao, John H. Zhang

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Hua Feng
Department of Neurosurgery, Southwest Hospital, Third Military Medical University,
Gaotanyan 30, Chongqing 400038, China, fenghua8888@yahoo.com.cn

Ying Mao
Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai,
China, yingmao@vnet.citiz.net

John H. Zhang
Departments of Physiology and Neurosurgery, Loma Linda University School of Medicine,
Risley Hall, Room 223, 92354 Loma Linda, California, USA, jhzhang@llu.edu

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Preface

On October 9 11, 2009, the Tenth International Conference on Cerebral Vasospasm was held for the first time in Chongqing, China. Literally translated to mean "double happiness", Chongqing was the perfect venue to host members of the community from all over the world to witness and participate in such a historic event. Just like the city's meaning would have you to believe, the conference was a joyous time for both the Chinese neurosurgery researchers who organized this well established conference and the vasospasm researchers from other countries who were delighted to have a meeting on a tour boat called the Misty Star. For many, being on a ship was a new experience, while for others, it was a time to soak in the beauty of China. The conference catered to more than 90 researchers from various countries around the world, presenting over 90 articles ranging from clinical trials to molecular biology experiments. This was all done while enjoying a cruise down the largest river in China, the Yangtze River, and experiencing the greatness of China's historic hydroelectric dams, the Three Gorges and the Three Little Gorges.

The meeting focused on subarachnoid hemorrhage research with topics divided into two main subcategories early brain injury and delayed vasospasm. Since 1972 when the first conference on cerebral vasospasm took place, delayed vasospasm has been regarded as the single most important treatable cause of mortality and morbidity after subarachnoid hemorrhage. However, since the successfulness of steering patients out of vasospasm by an endothelin receptor antagonist failed to reduce mortality, more attention was placed on global cerebral injury, which was termed early brain injury. Since then, more than 20% of all published studies on subarachnoid hemorrhage in the last 3 years have been focused on early brain injury, with 45% targeting delayed vasospasm. As a result, the Tenth International Conference on Cerebral Vasospasm dedicated close to one third of all presentations to early brain injury.

The conference followed the Misty Star into the Chinese ghost city of Fengdu, which is located high atop a beautiful hill. Meeting attendees were able to take a gondola ride and dashed into the "Gates of Hell". Chinese people worship and respect the notion of death and for this reason, many believe that is why they built the "Gates of Hell" on a beautiful hill like Heaven on Earth. This seems to be a coincident with the research on subarachnoid hemorrhage; it was believed over 50 years ago that delayed vasospasm was the major cause of death in victims. Nevertheless, the failure of Clazosentan to reduce mortality led researchers to shy away from the theory of delayed cerebral vasospasm, and transformed subarachnoid hemorrhage research into the birth of early brain injury.

Towards the end of the conference, the Misty Star led the meeting participants to a vast clearing of water, before the greatest dam on earth, the Three Gorges Dam appeared before them. Crossing the greatest dam during the evening provided a magnificent view for the participants and set the mood for the researchers.

In closing, it is with great pleasure that we would like to present the Volume Two entitled "Clinical Management" a collection of 43 chapters showcasing the magnificent works

conducted by the conference participants. These chapters include studies on early brain injury, the pathophysiology of delayed cerebral vasospasm, the clinical manifestations of subarachnoid hemorrhage, and the latest strategies on treatments. Additionally, we are delighted to present two historic review articles conducted by our honored guest, Dr. Nicolas Dorsch and our distinguished keynote speaker, Dr. Ryszard Pluta. These chapters also include bench investigations conducted by researchers and scientists from all across Asia, North America, and European countries highlighting the achievements in subarachnoid hemorrhage since the Ninth International Conference on Cerebral Vasospasm in Istanbul, Turkey almost 3 years ago.

And finally, to our dear participating colleagues, we would like to thank YOU especially for your participation and support of the Tenth International Conference on Cerebral Vasospasm. We look forward to seeing you in Cincinnati, OH, USA at the 11th Conference in 2011.

Chongping, People's Republic of China — Hua Feng
Shanghai, People's Republic of China — Ying Mao
Loma Linda, CA, USA — John H. Zhang

Acknowledgement

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Part III: Therapeutical Studies

The Role of Apolipoprotein E in the Pathological Events Following Subarachnoid Hemorrhage: A Review

Zong-duo Guo, Xiao-chuan Sun, and John H. Zhang

Abstract Subarachnoid hemorrhage (SAH) strikes individuals with devastating neurological results. Traditional viewpoints do not explain all the differences that are usually found in clinical practice. The role of genetic predisposition in SAH has recently been investigated. Particular attention has been paid to the apolipoprotein E (apoE) genotype. APOE genotype is a major prognostic factor in patient outcome after spontaneous aneurysmal SAH. In patients with SAH, the expression of the apoE ε4 allele is associated with a higher risk of negative outcome and delayed ischemia. Evidence from experimental and clinical studies confirms that apoE plays an important role in the pathological events after SAH. This article reviews related research and surveys the links between the pathological events of SAH and apoE.

Keywords APOE · SAH · Clinical outcomes

Introduction

Subarachnoid hemorrhage (SAH) is a deadly stroke with a mortality rate that is reported to be as high as 50%. Among the remaining survivors, 50% are left severely disabled. The etiology of SAH is mainly ruptured intracranial aneurysm [11, 16]. Currently, outcome prediction of SAH relies on demographic, clinical, and radiological factors. However, accurate outcome prediction in aneurysmal SAH remains imprecise despite use of current clinical grading scales [2].

Apolipoprotein E (apoE), the major apolipoprotein in the central nervous system, has been shown to influence neurological diseases in a gene-specific manner. It is known that presence of the apoE4 allele is associated with poorer response to traumatic brain injury and ischemic stroke [19, 20], but the association between apoE genotype and outcome following SAH still needs further research. This article reviews the related research and investigates the association between apoE genotype and the pathological events after SAH.

APOE and Outcome After SAH

Recent research indicating that polymorphism of the human apoE gene (ε3/3, ε3/4, ε4/4, ε2/3, ε2/4, ε2/2) influences outcome after SAH suggests that apolipoprotein E (apoE indicates protein; APOE, gene) may play an important role in the process of recovery after SAH [17]. Kaushal et al. suggest a plausible role of the upstream regulatory region of APOE in the etiology of aneurysmal SAH [8].

Several clinical studies have demonstrated an association between the APOE4 allele and poor outcome after SAH [12, 21]. ApoE4 may act directly on the effect of brain ischemia, which accounts for the poorer outcome in SAH patients [15]. It is widely recognized that the products of hemolysis in the subarachnoid space after SAH may lead to widespread necrosis of the cortex [4]. It has been found that apolipoprotein E4 exerts its effect on the mechanism via products of hemolysis; this may also explain the divergent effects of APOE4 on the outcome of patients with hemorrhagic and ischemic stroke [15].

Research also found that the presence of the apoE4 isoform is associated with higher mortality and impaired functional outcome in a murine SAH model [7]. The apoE4 isoform was associated with a greater degree of cerebral edema and vasospasm in mice [7]. Experimental observations are consistent with several clinical studies that have identified an isoform-specific role for apoE in

X. c. Sun (✉) and Z. d. Guo
Department of Neurosurgery, First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, People's Republic of China
e mail: sunxch1445@gmail.com
J.H. Zhang
Department of Neurosurgery, Loma Linda University Medical Center, Loma Linda, CA 92354, USA

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modifying the incidence of delayed ischemic deficit and neurocognitive outcomes after SAH [15, 21].

ApoE and Early Brain Injury After SAH

Early brain injury (EBI) after SAH refers to the immediate injury to the brain as a whole, within the first 72 h of the ictus, secondary to an SAH [10]. It has been pointed out that EBI is the primary cause of mortality in SAH patients.

Much research has found that the APOE ε4 allele is a significant determinant to the prognosis after acute central nervous system injury with different etiologies [17]. Although these studies suggest a role for apoE in the pathological processes of acute brain injury, the mechanism underlying these observations remains uncertain [22, 23]. Kay et al. [9] found that decreased concentration of apoE in the CSF after SAH and the correlation of injury severity and clinical outcome provide new in vivo evidence that apoE is involved in the response of early brain injury. This finding confirms the influence of APOE genotype on outcome from acute brain damage [17].

ApoE and Cerebral Vasospasm After SAH

Vasospasm is the main cause of secondary brain injury in SAH. Recently, genetic susceptibility to the development of vasospasm and poor outcome after SAH has been associated with APOE polymorphisms. Although the mechanisms by which apoE influences brain recovery from acute insults remain imprecise, there is a growing body of literature reporting that apoE suppresses CNS inflammatory responses [3, 14, 16], which are believed to play a key role in the pathogenesis of vasospasm [1, 3]. Alternatively, apoE has been demonstrated to exert a direct neuroprotective effect against glutamate excitotoxicity [1], and it is plausible that apoE may be modifying ischemic outcome independent of any vascular effects.

Endothelin-1 is known to be one of the most potent vasoconstrictors in SAH, and was found to be released from cerebrospinal fluid leukocytes during the acute phase of SAH [5]. The animal study suggested that there is a synergistic relation between apolipoprotein E and endothelin-1 in vasoconstriction [18]. This synergistic effect was especially strong with apolipoprotein E4 compared with apolipoproteins E2 and E3. Therefore, the effect of apolipoprotein E4 on SAH patients may be due to its synergistic effect with endothelin-1 during the acute phase of hemorrhage in causing widespread and persistent vasospasm [15]. ApoE4 negatively affects cognitive morbidity and delayed ischemic neurologic deficit recovery. The presence of the APOE2 allele was not associated with functional outcomes even when considering presence of cerebral vasospasm [6]. The presence of an ε4 allele increases the risk of delayed ischemic neurologic deficit [12].

It has been demonstrated that a peptide derived from the apoE-binding region reduces luminal narrowing of the MCA caused by vasospasm, and improves functional outcomes following SAH. One mechanism that has been postulated to explain the role of apoE in modifying recovery after brain injury is an isoform-specific effect on glial activation and brain inflammation [13, 16]. Endogenous apoE modifies functional outcome, mortality and vasospasm in an isoform-specific fashion after SAH. Furthermore, an apoE derived therapeutic peptide improved functional outcome, decreased vasospasm and mortality after SAH.

Conclusion

Study of the genetic influence on the severity and outcome following SAH is still at an early stage. Increasing the knowledge of the nature of SAH and deepening the understanding of the relationship between SAH and genetics are required. Further insight into the role of apoE in the injured brain after SAH may help define the role of genetic influences in recovery from brain injury and result in the development of novel therapies that mimic that anti-inflammatory and neuroprotective properties of endogenous apoE.

Conflict of interest statement We declare that we have no conflict of interest.

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Mechanisms of Statin Treatment in Cerebral Vasospasm

Takashi Sugawara, Robert Ayer, Vikram Jadhav, Wanqiu Chen, Tamiji Tsubokawa, and John H. Zhang

Abstract 3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors, commonly known as statins, are widely used clinically for their lipid lowering properties. Recent experimental evidence shows that statins are also effective in ameliorating cerebral vasospasm, which occurs as sequelae of subarachnoid hemorrhage. This literature review focuses on the literature-based putative mechanisms involved in statin mediated attenuation of cerebral vasospasm, such as eNOS, vascular inflammation, apoptosis, especially the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway from our experimental study.

Keywords Statin · Cerebral vasospasm · Mechanism · PI3K · Akt · eNOS

Introduction

In addition to their cholesterol lowering effect, statins are well known to exhibit many pleiotropic actions. Statins improve the integrity of endothelial cells and preserve the endothelial function [8]. Statins are likely to protect against cerebral vasospasm by improving endothelial function [7], inhibiting Rho kinase [1], Endothelin-1 [6], Inflammation [12], NADPH oxidase [4], and Caveolin-1 [11] signaling pathway in endothelial cells and vascular smooth muscle. These statins' effects on each pathway have been shown mainly in the cardiovascular fields. To date, only five animal studies evaluated the effect of statins on cerebral vasospasm have been published (Table 1).

This statins' effect on cerebral vasospasm was shown by McGirt et al. in 2002 [9] for the first time. They showed that simvastatin pretreatment increased middle cerebral artery diameter and reduced neurological deficits with increasing eNOS protein simultaneously; however, simvastatin post-treatment caused a modest increase in middle cerebral artery diameter and reduced neurological deficits without increasing eNOS protein. They concluded that the mechanism may be attributable in part to eNOS upregulation. McGirt et al. also showed inflammation as the possible mechanism in 2006 [10]. They reported that basilar artery diameter was greater in simvastatin treated rabbits versus vehicle and simvastatin attenuated the increase in perivascular CD18-positive cells after SAH simultaneously. They concluded that subcutaneous administration of simvastatin after the onset of SAH attenuates perivascular granulocyte migration and ameliorates basilar artery vasospasm after experimental SAH in rabbits, and simvastatin may potentially serve as agents in the prevention of cerebral vasospasm after SAH.

Bulsara et al. evaluated amelioration of cerebral vasospasm during simultaneous upregulation of NO with simvastatin and immunosuppression with cyclosporin A in 2006 [2]. They showed that vasodilation greater than baseline is seen at day 10 in the simvastatin group, but the combination of simvastatin and cyclosporine does not ameliorate cerebral vasospasm in a canine model to a greater extent than simvastatin alone. They concluded that the results lead us to suggest that combined therapy with cyclosporine and simvastatin is not as efficacious in ameliorating vasospasm as simvastatin alone; interestingly, cyclosporin may limit the beneficial effect of simvastatin.

T. Sugawara
Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, USA
Department of Neurosurgery, National Hospital Organization Disaster Medical Center, Japan
R. Ayer, V. Jadhav, W. Chen, and T. Tsubokawa
Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, USA
J.H. Zhang (✉)
Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, USA
Department of Neurosurgery, Loma Linda University Medical Center, 11234 Anderson Street, Room 2562B, Loma Linda, CA 92354, USA
Department of Anesthesiology, Loma Linda University, Loma Linda, CA, USA
e mail: johnzhang3910@yahoo.com

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Gao Cheng et al. explored apoptosis inhibiting effects of atorvastatin and its potential apoptotic signal pathway in 2009 [3]. They reported that ameliorating cerebral vasospasm was obtained after prophylactic use of atorvastatin with marked reducing TUNEL positive cells both in basilar artery and in brain cortex by atorvastatin; apoptosis-related proteins P53, apoptosis-inducing factor and cytochrome c (in hippocampus and basal cortex) were up-regulated after SAH while they were not affected by atorvastatin, and up-regulation of caspase-3 and caspase-8 (in hippocampus and basal cortex) after SAH was decreased by atorvastatin treatment both in mRNA and in protein levels. They concluded that the neuroprotective effects of atorvastatin after SAH may be related to its inhibition of caspase dependent proapoptotic pathway based on their results.

We investigated the role of the PI3K/Akt pathway and endothelial nitric oxide synthase (eNOS) in the cerebral vasculature in statin-mediated attenuation of cerebral vasospasm using wortmannin, a pharmacologic irreversible PI3K inhibitor, and a rat endovascular perforation model of SAH.

Materials and Methods

Simvastatin was administered intraperitoneally in two dosages (1 mg/kg and 20 mg/kg) at 0.5, 24, and 48 h after SAH. Morphology, such as diameter, perimeter, and wall thickness, with histology of the ipsilateral intracranial carotid artery (ICA); proteins, such as Akt, eNOS, phosphorylated Akt (pAkt), and phosphorylated eNOS (peNOS), with western blot and fluorescence immunohistochemical staining; and neurological deficits with a modification of the scoring system reported by Garcia et al. [5], were assessed at 24 and 72 h after SAH.

Results

SAH significantly decreased ICA diameter and perimeter while increasing wall thickness at both 24 and 72 h. High dosages of simvastatin prevented the reduction of ICA diameter and perimeter following SAH, and both high and low dosages significantly reduced wall thickness at 24 and 72 h. The effects of simvastatin were reversed by wortmannin. High-dosage simvastatin increased pAkt and peNOS (phosphorylated forms) levels without increasing Akt and eNOS expression when compared with the SAH group. This treatment also improved neurological deficits at 24 and 72 h. Simvastatin did not induce changes in protein levels in the absence of SAH, as both vehicle and simvastatin treated shams at equal levels. This study elucidates the critical role of the PI3K activation leading to phosphorylation of Akt and

Table 1 Published animal studies evaluating the effect of statins on cerebral vasospasm

Authors and year	Statin used	Model	Sample size	Vasospasm criteria	Statin therapy	Statin effect	Mechanism
McGirt et al. (2002)	Simvastatin	Mice, endovascular perforation	34 statin, 36 nonstatin	MCA diameter at necropsy at 72 h	20 mg/kg s.c. (daily for 14 days pre-SAH)	Increased MCA diameter	eNOS upregulation
McGirt et al. (2006)	Simvastatin	Rabbit, injection	5 statin, 5 nonstatin	Cross-section of BA at 72 h after SAH	40 mg/kg s.c. (30 min, 24 h, 48 h postSAH)	Increased BA diameter	Attenuates perivascular granulocyte migration
Bulsara et al. (2006)	Simvastatin	Dog, double injection	4 statin, 5 nonstatin	BA diameter on angiography at 3, 7, 10 days after SAH	20 mg/kg orally (daily for 10 days postSAH)	Increased BA diameter	–
Sugawara et al. (2008)	Simvastatin	Rat, endovascular perforation	35 statin, 45 nonstatin	Cross-section of IC at 24 h and 72 h after SAH	1 mg/kg, 20 mg/kg i.p. (30 min, 24 h, 48 h postSAH)	Ameliorated cerebral vasospasm	Upregulated PI3K/Akt/eNOS pathway
Gao Cheng et al. (2009)	Atorvastatin	Rat, endovascular perforation	16 statin, 16 nonstatin	Cross-section of BA at 24 h after SAH	20 mg/kg orally (daily for 15 days preSAH)	Ameliorated cerebral vasospasm	Reduced the expression of cleaved caspase-3 and caspase-8

eNOS in simvastatin-mediated attenuation of cerebral vasospasm after SAH.

Conclusion

This study showed that PI3K activation, leading to phosphorylation of Akt and eNOS by statin, may be one of the important roles in simvastatin-mediated attenuation of cerebral vasospasm after SAH. Further investigation of proposed pathway is needed to clear the mechanisms of cerebral vasospasm after SAH.

Conflict of interest statement We declare that we have no conflict of interest.

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The Effect of Phosphodiesterase Inhibitor Tadalafil on Vasospasm Following Subarachnoid Hemorrhage in an Experimental Rabbit Model

Firat Narin, Burcak Bilginer, Ahmet Ilkay Isikay, Mehmet Bülent Onal, Figen Soylemezoglu, and Nejat Akalan

Abstract *Background*: Despite the years of study on it, cerebral vasospasm following subarachnoid hemorrhage is still an important cause of mortality and morbidity. The presented study was undertaken to show whether phosphodiesterase inhibitor tadalafil can attenuate the vasospasm process following subarachnoid bleeding.

Method: In this study, 20 male New Zealand White rabbits weighing 2,500 3,000 g were randomly assigned to four groups. Animals in group 1 were controls. In group 2, animals were given oral tadalafil at 12, 24 and 36 h and SAH was not induced. SAH induced animals in group 3 did not receive any medication. In group 4, animals received tadalafil at 12, 24 and 36 h after SAH induction. All animals were sacrificed via exsanguination at 48 h after induction of SAH. Brains and brainstems with overlying basilar arteries were removed and stored in fixative at +4°C overnight. Basilar arteries were sectioned from four separate zones, and four sections were obtained from each rabbit. Basilar artery luminal section areas were measured by using SPOT for Windows version 4.1. Statistical comparisons were performed using Kruskal Wallis and ANOVA tests.

Findings: The SAH induced group which had been treated with tadalafil had significantly greater basilar artery luminal area than the untreated group ($p < 0.05$). There was no significant difference between control group and non-SAH induced group in terms of luminal areas.

Conclusion: Tadalafil has a potentially preventive effect in treatment of cerebral vasospasm following subarachnoid bleeding.

F. Narin, B. Bilginer, A.I. Isikay (✉), M.B. Onal, and N. Akalan
Department of Neurosurgery, Hacettepe University School of Medicine, Ankara, Turkey
e mail: ilkayisikay@yahoo.com
F. Soylemezoglu
Department of Pathology, Hacettepe University School of Medicine, Ankara, Turkey

Keywords Tadalafil · Basilar artery · Cerebral vasospasm · Subarachnoid hemorrhage

Introduction

Despite developments in the past few decades, vasospasm still remains a challenge for those dedicated to the treatment of aneurysmal SAH. Since it was first described by Ecker and Riemenschneider [7] in 1951, post-SAH cerebral vasospasm is recognized as a detrimental clinical entity. Ongoing studies have failed to propose an effective solution for this devastating disease. Current treatment options, i.e. calcium channel antagonists (nimodipine, nicardipine), triple-H therapy and experimental therapies such as endothelin antagonists and measures to increase NO availability have made just a limited progress [12].

Since the recognition of the NO-cyclic nucleotide mono phosphate pathway as a pivotal element in this disease, phosphodiesterase inhibitors have also gained importance. The phosphodiesterase (PDE) enzyme superfamily is a group of enzymes that cleaves cAMP and cGMP. These cyclic nucleotides play important regulatory roles as second messengers in a wide variety of signal transduction pathways and in various tissues [2]. The multiple subtypes and varied tissue distribution make PDEs promising drug targets for a variety of diseases [3]. A cGMP-specific PDE, i.e. type 5 phosphodiesterase (PDE V), is abundant in lungs, platelets, and vascular smooth muscle. PDE V is highly specific for cGMP, both in its catalytic site, and in the two cGMP-binding allosteric sites [9]. NO regulates the relaxation of vascular smooth-muscle cells through activation of soluble guanylate cyclase. The activated cyclase converts guanosine triphosphate to cGMP in the cerebral arterial smooth muscle cells; The second messenger cGMP is in turn hydrolyzed by PDE which means the amount of cGMP in smooth-muscle cells is influenced by both NO and PDE [8].

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The amount of available cGMP may be increased by administration of PDE inhibitors. In fact PDE V inhibitors are widely prescribed agents for the treatment of erectile dysfunction and pulmonary hypertension [10]. Previously sildenafil citrate was reported to dilate vasospastic cerebral arteries in an experimental model of subarachnoid bleeding [1]. However, there is no literature data on tadalafil, the long acting type 5 PDE inhibitor, as to whether or not it attenuates cerebral vasospasm. The current study is designed to examine the effects of tadalafil on cerebral vasospasm after SAH.

Methods and Materials

Animal Model

The experimental protocols used in this study were approved by the Hacettepe University Animal Research Committee. Twenty male New Zealand White rabbits weighing 2,500 3,000 g were randomly assigned to 4 groups. Animals in group 1 (n = 5) were controls. In group 2 (n = 5) animals were treated with 6 mg/kg tadalafil at 12, 24, and 36 h, but SAH was not induced. In group 3 (n = 5) animals, only SAH was induced and no treatment was given. SAH induced animals in group 4 (n = 5) received tadalafil at 12, 24 and 36 h of SAH induction. Animals were placed in restrainer while drug was being given. All procedures were performed by two investigators working in tandem and not blinded to the treatment group during surgery and euthanasia. Vascular measurements were performed in a blinded fashion.

Induction of Experimental SAH

All animals were anesthetized by intramuscular injection of a mixture of ketamin (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg), paralyzed with pancuronium bromide (0.08 mg/kg), intubated and ventilated with a Harvard 683 model dual-phase ventilator (Harvard Apparatus Co.). A 23-gauge butterfly needle was inserted percutaneously into cisterna magna. After withdrawal of 1.0 ml CSF, 3 ml of non-heparinized blood from central ear artery was injected into subarachnoid space. The animals were then placed in head down position for 15 min to hold the blood in the basal cisterns. Arterial blood gases were analyzed during the surgical procedure and maintained within the physiological range. After recovering from anesthesia, the rabbits were observed for possible neurological deficits and then returned to the vivarium.

Perfusion-Fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 48 h after SAH induction. The animals were anesthetized, intubated, and ventilated as described above. The ear artery was catheterized for the monitoring of blood pressure and blood gas analysis. When satisfactory respiratory parameters were obtained, thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution (Hank's balanced sat solution [Sigma Chemical Co.], pH 7.4 at 37°C, 300 ml), the fixative was perfused (2% paraformaldehyde, 2.5% gluteraldehyde im 0.1 M phosphate buffer, pH 7.4 at 37°C, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were killed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Embedding, Morphometry, and Statistical Analysis

Basilar arteries were removed from the brain stems, and arterial segments from the proximal third of the artery were dissected for analysis. The arterial segments were washed several times with 0.1 mol/l phosphate-buffered solution (PBS, pH 7.4), fixed in 1% osmium tetroxide in PBS for 1 h at room temperature, and then washed again with PBS. Cross-sections were cut at a thickness of 0.5 μm. The sections were mounted onto glass slides and stained with H & E for light microscopic analysis. The vessels were measured using computer-assisted morphometry (SPOT for Windows Version 4.1). Automated measurements of the cross-sectional area of the arterial sections were taken by an investigator who was blinded to the identity of the group the animals belonged to. Four cross-sections of each vessel were selected randomly for measurement, calculating the average of these measurements. Statistical comparisons were performed using Kruskal Wallis and one way ANOVA tests. A p-value smaller than 0.05 was accepted as statistically significant.

Results

Physiologic parameters of animals, which are given in Table 1, did not differ significantly.

All 20 animals survived to the end of the study. Histological sections of the basilar artery luminal areas were analyzed by a computerized image-analysis system

Table 1 Summary of physiologic parameters of the groups

Group	n	Body weight (g)	pH	pCO_2	pO_2	MABP
1	5	2870 ± 41	7.43 ± 0.05	40.1 ± 1.12	107 ± 6.01	110 ± 4
2	5	2686 ± 52	7.43 ± 0.04	40.5 ± 1.11	112 ± 5.87	112 ± 4
3	5	2810 ± 38	7.45 ± 0.03	41.2 ± 1.07	112 ± 5.76	110 ± 5
4	5	2708 ± 48	7.43 ± 0.05	40.9 ± 1.08	106 ± 6.12	108 ± 3

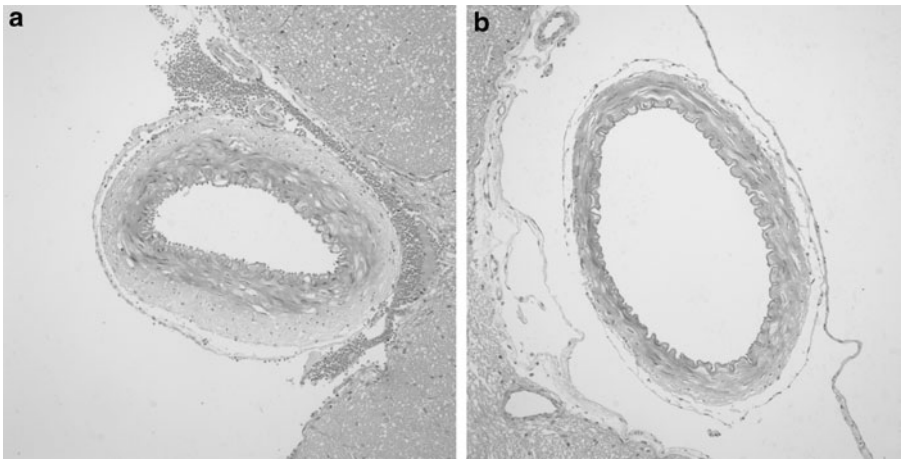

Fig. 1 (**a**) Basilar artery after SAH induction. Cross sectional appearance of the HE stained basilar artery under 20x magnification. (**b**) Basilar artery after SAH induction + tadalafil treatment. Cross sectional appearance of the HE stained basilar artery under 20x magnification

(Fig. 1). In the control group (no SAH, no treatment), mean basilar artery luminal area was 115,823.80 ± 18,048.15 μm^2. In group 2, in which SAH was not induced but tadalafil was given, mean luminal area was 117,294.00 ± 4,502.91 μm^2. In SAH-induced group mean luminal area was 10,491.00 ± 3,652.48 μm^2. Finally in the SAH-induced tadalafil treated group mean luminal area was measured to be 38,403.60 ± 3,242.70 μm^2 (Fig. 2).

Mean luminal area of group 3 is significantly smaller than the mean area of group 1 ($p < 0.05$). When control and tadalafil-only groups were compared, there was no significant difference in terms of luminal area. In contrast, mean luminal area of SAH-induced, tadalafil treated group was significantly greater than that of SAH-only group.

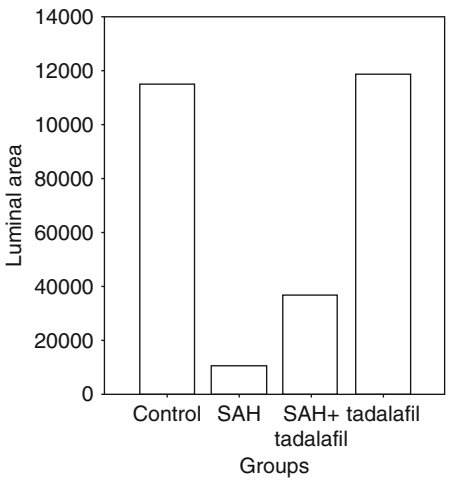

Fig. 2 Mean cross sectional areas in μm^2

Discussion

Subarachnoid bleeding and resultant cerebral vasospasm is one of the most important challenges to the neurosurgeon and neurocritical care provider. Even though the intracranial aneurysm is treated successfully by neurosurgery or interventional neuroradiology means, radiological cerebral vasospasm is inevitably the result in almost 70% of cases and approximately half of these suffer a clinically apparent condition known as delayed ischemic neurological deficit (DIND) [6]. The multifactorial nature of this disease is the reason for the failure of proposing an effective treatment, despite intense research going on for decades. A thorough understanding of the pathophysiologic events that take place in the disease process necessitates experimental models which should be feasible and also reliable. The rabbit model of experimental subarachnoid bleeding consequently is one of the most used models [11].

Cerebral vasospasm is reported to be maximal at 48 72 h after SAH induction [1, 3, 5]. Therefore, we sacrificed the animals at 48 h post-SAH assuming to demonstrate a significant vasospasm. We have demonstrated SAH-induced tadalafil-treated group had a significantly greater vessel lumen area than the SAH-only group. These results show that the PDE V inhibitor tadalafil is a potent cerebral vaso-

dilator in the treatment of cerebral vasospasm after experimental SAH in rabbits. This finding is consistent with the previous experimental and clinical researches on other PDE inhibitors [1, 3, 4].

In a similar study which was conducted to examine the effects of sildenafil citrate, another PDE inhibitor, the drug showed a significant vasodilatory effect on the drug-only group compared with control group [1]. In contrast, in our study we have failed to show a significant difference in terms of luminal area between control and tadalafil-only groups. Inoha et al. [8] showed that PDE V expression can only be identified immunohistochemically only in vasospastic arteries following SAH; they could not identify PDE V expression in control arteries. They also hypothesized that PDE V may function in the initiation of vasospasm. According to their findings, the limited activity of PDE V in control arteries, if any at all, may be responsible for a failure to cause vasodilation, since highly specific inhibitor tadalafil needs expression of the enzyme to show a differential effect.

We did not measure cGMP levels from basilar arteries to demonstrate that tadalafil has accomplished its expected biochemical activity, but increased basilar arterial lumen area in the tadalafil treated group suggests such an action. Further studies which may be conducted to give cGMP measurements from control and treated arteries will increase the knowledge pool on this issue.

The long acting type 5 phosphodiesterase enzyme inhibitor tadalafil is shown to effectively dilate vasospastic cerebral arteries following SAH. This action is achieved through oral administration of the drug at a dosage of 6 mg/kg, which is more than the dose that is used for erectile dysfunction and pulmonary hypertension; but this is common when experimental studies are considered. Based on these data, we can propose that tadalafil may well be a new candidate for clinical trials in the treatment of cerebral vasospasm.

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Effect of a Free Radical Scavenger, Edaravone, on Free Radical Reactions: Related Signal Transduction and Cerebral Vasospasm in the Rabbit Subarachnoid Hemorrhage Model

Akira Munakata, Hiroki Ohkuma, and Norihito Shimamura

Abstract *Objective*: It is hypothesized that free radical reactions evoked by oxyhemoglobin (oxyHb) cause cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH), even though the detailed mechanisms have not yet been fully established. The aims of this study were thus to investigate, through the use of the double-hemorrhage rabbit model, the possibility that free radical reactions play a role in cerebral vasospasm and to delineate the mechanism of signal transduction that causes cerebral vasospasm.

Methods: In the SAH group, SAH was simulated using the double-hemorrhage rabbit model. In the treatment group, edaravone (0.6 mg/kg), a potent free radical scavenger, was injected into the central ear vein twice a day. Four days after SAH, the basilar artery was excised. The degree of cerebral vasospasm was evaluated by measuring the diameter of each basilar artery, and the expression of Rho-kinase in the vascular wall was examined by western blotting.

Results: The diameter of the basilar artery in the edaravone-treated group was 0.64 ± 0.06 mm, which was statistically significantly larger than that in the nontreated SAH group (0.50 ± 0.03 mm; $p < 0.01$). The expression of Rho-kinase in the edaravone-treated group was statistically significantly reduced in comparison to that of the nontreated SAH group.

Conclusion: Results from this study have indicated for the first time that free radical reactions mediated by oxyHb may play an important role in the pathogenesis of cerebral vasospasm through the expression of Rho-kinase.

Keywords Free radical scavenger · Free radicals · SAH · Vasospasm

Introduction

It is hypothesized that free radical reactions, such as lipid peroxide production in the arterial smooth muscle layer, evoked by oxyhemoglobin (oxyHb) released from a subarachnoid clot, cause cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) [9, 15, 22, 23, 27, 48]. In fact, previous studies using free radical scavengers in experimental SAH models showed amelioration of cerebral vasospasm as a result of scavenging free radicals [3, 11, 25, 52]. In addition, it has been revealed that intracellular signal transduction of vascular smooth muscle cells, such as the PKC [28, 29, 34, 41, 48, 50] and Rho/Rho-kinase [8, 18, 37, 38, 48] pathways, is activated during the development of cerebral vasospasm and, as a result, a sustained contraction of arterial smooth muscle cells occurs [18, 38, 48]. Even though it has been speculated that free radical reactions, evoked by oxyHb released from a subarachnoid clot, induce sustained contraction through an activated intracellular signal transduction, the detailed mechanisms of this free radical reaction-induced sustained contraction have not yet been clarified [48].

The aims of this study were to investigate, through the use of the double-hemorrhage rabbit model [4, 31, 44], the possibility that free radical reactions play a role in cerebral vasospasm and to delineate the mechanism of signal transduction that causes sustained contraction in cerebral vasospasm. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a potent free radical scavenger which is widely used in Japan for acute ischemic stroke [42, 51], was injected into veins to scavenge free radicals. The prevention of cerebral vasospasm by edaravone and associated changes in the regulation of Rho-kinase as a marker of the intracellular signal transduction were examined.

A. Munakata, H. Ohkuma, and N. Shimamura (✉)
Department of Neurosurgery, Hirosaki University School of Medicine, Hirosaki, Japan
e mail: shimab@cc.hirosaki u.ac.jp

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Materials and Methods

All experimental protocols were approved by the Hirosaki University Animal Research Committee. Thirty Japanese white rabbits weighing 2.5 to 3.0 kg were used. All animals were randomly assigned to three groups: in group 1 (sham, n = 10), the animals were given an intravenous injection of edaravone after sham surgery; in group 2 (SAH-edaravone, n = 10), SAH was produced and the animals were given an intravenous injection of edaravone; and in group 3 (SAH, n = 10), SAH was produced.

Production of SAH

In groups 2 and 3, SAH was produced according to the double-hemorrhage method.

The animals were anesthetized with an intravenous injection of pentobarbital (30 mg/kg) and an intramuscular injection of ketamine (20 mg/kg).

After anesthesia, under spontaneous breathing, a 23-gauge butterfly needle was percutaneously placed in the cisterna magna, and CSF (1.0 1.5 ml) was aspirated under aseptic technique before each injection of blood. The femoral artery was cannulated to obtain autologous arterial blood. Autologous nonheparinized arterial blood, 1.5 ml, was injected into the cisterna magna over 1 2 min. Animals were then placed in a 30° head-down, tilted position for 15 min to ensure that blood spread into the basal cistern. Forty-eight hours afterwards the second SAH was produced in the same manner as the first [4].

Intravenous Injection of Edaravone

In groups 1 and 2, edaravone (0.6 mg/kg) was injected into the central ear vein over 1 2 min, twice a day from day 0 to day 4 after sham or SAH surgery.

Histological Evaluation

Perfusion-fixation was performed on day 4 after SAH. Five animals of each group were deeply anesthetized using 100 mg/kg pentobarbital, the thorax was opened, and a cannula was immediately inserted into the ascending aorta via the left ventricle. Perfusion fixation was performed at 75 mmHg with 400 ml heparinized physiological saline (5,000 U/500 ml), followed by 500 ml of phosphate-buffered 4% paraformaldehyde (pH 7.4). Finally, the brain was carefully removed so as not to stretch and injure the basilar artery. The tissue was dehydrated in graded alcohol and embedded in paraffin. All 6-μm-thick sections were cut vertically, mounted on a glass slide, and stained with hematoxylin-eosin (HE).

Cerebral vasospasm was evaluated using the HE-stained sections. Vessel patency was quantified by measuring the basilar artery circumference with the National Institutes of Health image program (version 1.62). To correct for vessel deformation and off-transverse sections, the internal circumferences of five different sections of each vessel, separated by 200 μm, were measured and averaged. The luminal cross-sectional area of each vessel was estimated with the use of the calculated radius (r) value obtained from the measured circumference (r = measured circumference/2π; area of circle = πr^2) [30]. The mean diameter of the basilar arteries was calculated from the calculated radius (diameter = 2r).

Western Blotting

Another five animals of each group were killed by the intravenous injection of high dose pentobarbital (300 mg/kg) on day 4 after SAH. The basilar arteries were immediately removed and stored at -80°C until analysis.

Western blotting followed the standard technique. The primary antibody included goat anti-RockII (sc1851; Santa Cruz Biotechnology; Santa Cruz, CA). The membrane was incubated with the appropriate Cruz Marker compatible secondary antibody. Bands were detected with a chemiluminescence detection kit (ECL plus; Amersham Bioscience). Blot bands were quantified using the densitometry method (Scion image Beta 4.02), n = 5 for each group. The value of the sham is expressed as 100%, and other groups are expressed as a percentage of the sham group.

Statistical Analysis

Statistical analysis was performed with the use of JMP® (Version 5; SAS Institute Inc., Cary, NC, USA). Data are expressed as mean ± SEM. To compare two unpaired groups, the t-test was used. Differences were considered significant at the $p < 0.05$ level.

Results

Evaluation of Cerebral Vasospasm (Figs. 1 and 2)

In group 3 (SAH, n = 5), basilar artery diameter was statistically significantly reduced 4 days after SAH vs. group 1 (Sham, n = 5) (0.50 ± 0.03 mm vs. 0.73 ± 0.05 mm, $p < 0.01$). After SAH, basilar artery diameter was greater in group 2 (SAH + edaravone, n = 5) vs. group 3 (0.64 ± 0.06 mm vs. 050 ± 0.03 mm, $p < 0.01$). In addition, marked corrugation of the internal elastic lamina around the wall, with thickening of the vascular smooth muscle layer, was seen in group 3. In contrast, the corrugation was much less remarkable in group 2.

Expression of Rho-kinase (Fig. 3)

In group 3 (SAH, n = 5), expression of Rho-kinase was significantly increased in the vascular smooth muscle cells by Western blotting ($p < 0.01$; vs. groups 1 and 2)

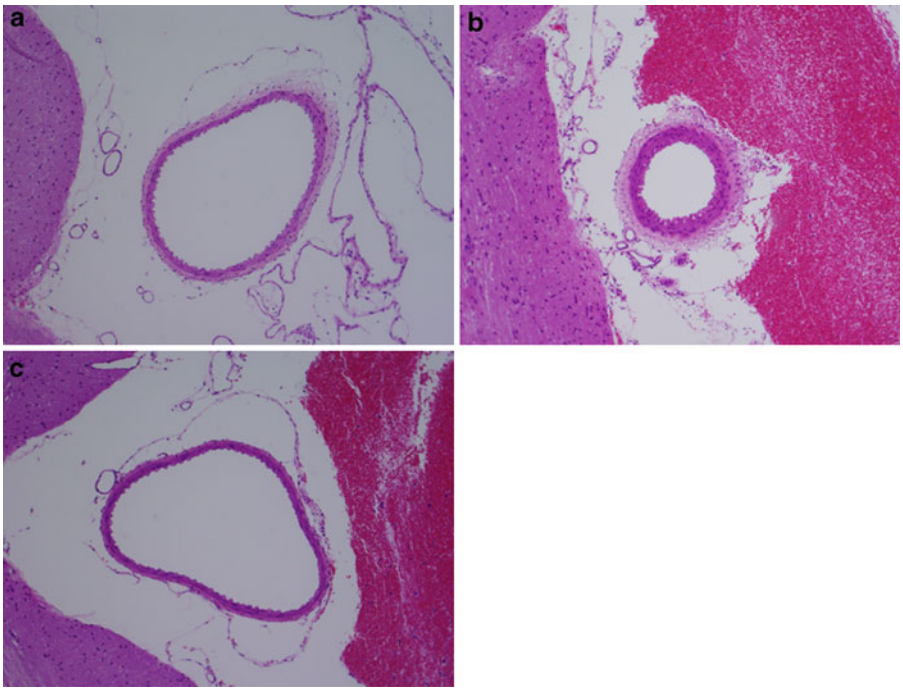

Fig. 1 (**a**) group 1 (Sham), (**b**) group 2 (SAH edaravone), (**c**) group 3 (SAH). Histopathological findings of the basilar artery were evaluated with HE staining. In group 3 (SAH), corrugation of the internal elastic lamina was found (**c**). In contrast, in group 2 (SAH edaravone), there was faint corrugation of the internal elastic lamina around the wall (**b**). Magnification × 100

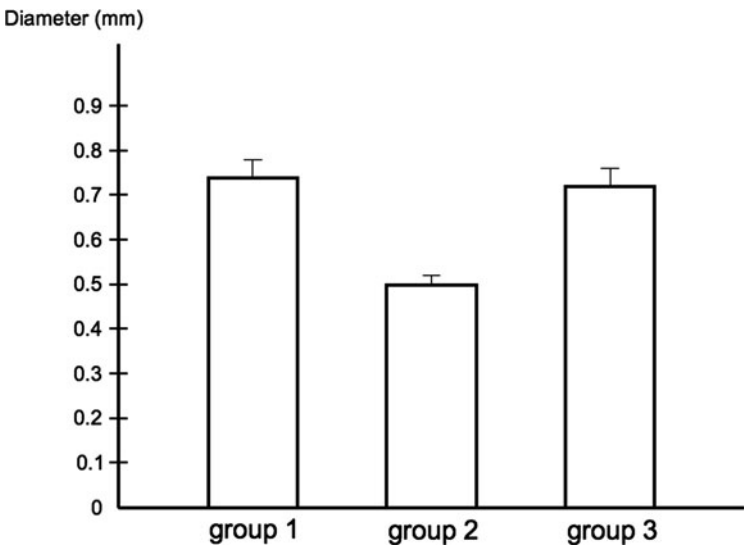

Fig. 2 (**a**) group 1 (Sham), (**b**) group 2 (SAH edaravone), (**c**) group 3 (SAH). In group 3, mean ± SEM basilar artery diameter was statistically significantly reduced 4 days after SAH vs. group 1 (0.50 ± 0.03 mm vs. 0.73 ± 0.05 mm, $p < 0.01$). Mean ± SEM basilar artery diameter was greater in group 2 (SAH + edaravone, n = 5) vs. group 3 (0.64 ± 0.06 mm vs. 050 ± 0.03 mm, $p < 0.01$)

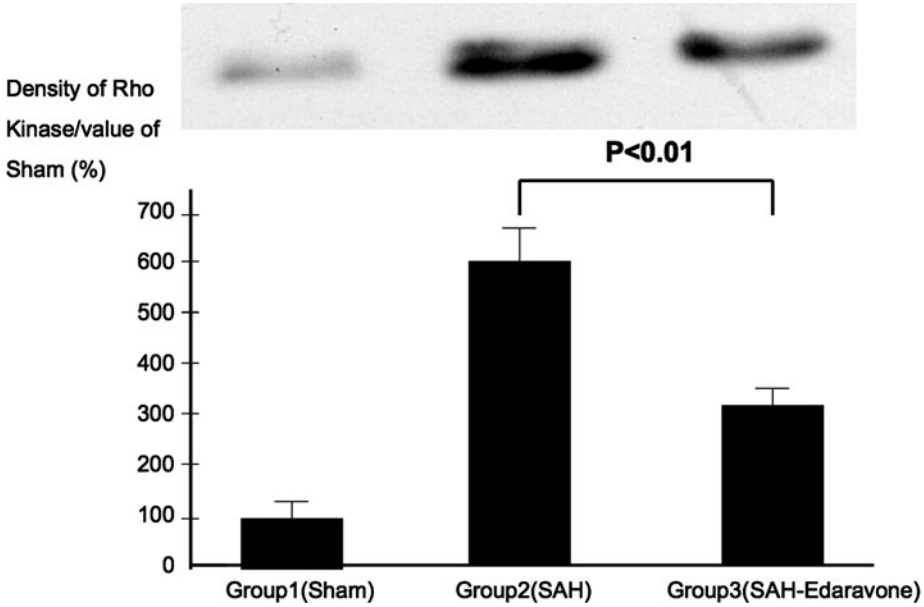

Fig. 3 (**a**) group 1 (Sham), (**b**) group 2 (SAH edaravone), (**c**) group 3 (SAH). In group 3, expression of Rho kinase was significantly increased in the vascular smooth muscle cells by Western blotting ($p < 0.01$; vs. groups 1 and 2)

Discussion

The transformation of oxyHb, released from a subarachnoid clot, into methemoglobin generates activated species of oxygen such as the superoxide anion, hydrogen peroxide and singlet oxygen [10, 17, 32, 33, 49]. Oxygen radicals can initiate peroxidative reactions in membrane polyunsaturated fatty acid, producing lipid peroxides [5, 7, 32, 49]. And it has been speculated that lipid peroxide production in vascular smooth muscle layer causes cerebral vasospasm after SAH [24, 25, 35, 36, 40, 46]. It has thus been considered that scavenging free radicals in the subarachnoid space will ameliorate cerebral vasospasm. In fact, previous studies using free radical scavengers in experimental SAH models showed statistically significant amelioration of cerebral vasospasm, even though the detailed mechanisms as to how free radicals induce sustained contraction of vascular smooth muscle have not yet been clarified [3, 11, 25, 52]. This study also revealed that using edaravone in an experimental rabbit SAH model also statistically significantly ameliorated lipid peroxide production in the vascular smooth muscle layer and cerebral vasospasm.

Several recent studies have revealed that the Rho/Rho-kinase pathway plays an important role in vascular diseases [13, 43, 45]. Sato et al. [37] showed that the Rho/Rho-kinase pathway is activated during the development of cerebral vasospasm after SAH. It has been considered that the Rho/Rho-kinase pathway is activated by some trimeric G-protein-coupled receptors, including lysophosphatidic acid, thrombin, and serotonin receptors, which are linked to the Rho/Rho-kinase pathway [39]. The α subunits of G_i, G_q, G_{12} and G_{13} activate Rho by regulating GDP/GTP exchange factors for Rho [6, 12, 16, 19]. Rho-kinase, which is activated by Rho [14, 21, 26] phosphorylates the myosin-binding subunit (MBS) of myosin phosphatase and, as a result, activity of myosin phosphatase is decreased [18]. In addition, the activated Rho-kinase phosphorylates myosin light chain (MLC) at the Ser19 residue, which is the site phosphorylated by Ca^{2+}/calmodulin-dependent MLC kinase [1]. So a sustained contraction of vascular smooth muscle was induced by Rho-kinase. But, the mechanism as to how SAH activates the Rho/Rho-kinase pathway has not yet been clarified.

Wickman et al. [48] have shown that the oxyHb-mediated sustained contraction of vascular smooth muscle is dependent on the Rho/Rho-kinase pathway and PKC by using the selective inhibitors of Rho-kinase, Y-27632, and HA-1077, even though the mechanism by which oxyHb activates the Rho/Rho-kinase pathway has not yet been clarified. OxyHb has been shown to exhibit a number of signaling processes such as free radical reactions, elevation of intracellular Ca^{2+}, activation of tyrosine kinases and mitogen-activated protein kinases [2, 20, 47]. Whether free radical reactions or other reactions mediated by oxyHb play a role in the activation of the Rho/Rho-kinase pathway has not yet been clarified. Since the Rho-kinase expression on western blotting was statistically significantly reduced due to ameliorate lipid peroxide production in the vascular smooth muscle layer by using edaravone, a potent free radical scavenger in experimental rabbit SAH model, it could be speculated that the free radical reaction mediated by oxyHb is concerned with the regulation of Rho/Rho-kinase pathway.

Conclusion

In conclusion, results from this study indicate that edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one), a potent free radical scavenger, may serve as an agent in the prevention of cerebral vasospasm in patients after SAH, since intravenous administration of edaravone after the onset of SAH statistically significantly ameliorated lipid peroxide production in the vascular smooth muscle layer and basilar artery vasospasm after experimental SAH in rabbits. In addition, results from this study also indicate, for the first time, that free radical reactions mediated by oxyHb might be playing an important role in the Rho/Rho-kinase pathway by expressing Rho-kinase.

Conflict of interest statement We declare that we have no conflict of interest.

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Comparison of Nimodipine Delivery Routes in Cerebral Vasospasm After Subarachnoid Hemorrhage: An Experimental Study in Rabbits

Mehmet Bulent Onal, Erdinc Civelek, Atilla Kircelli, Ilker Solmaz, Sahin Ugurel, Firat Narin, Ilkay Isikay, Burcak Bilginer, and Hakan Yakupoglu

Abstract *Background*: Nimodipine is the most widely preferred and administered calcium channel blocker in cerebral vasospasm prevention and treatment. There is no experimental or clinical study investigating the comparative effects of routine treatment modalities.

Method: 35 male New Zealand White rabbits were assigned randomly to one of seven groups: Control, only SAH, SAH/oral nimodipine, SAH/IV nimodipine, SAH/IT nimodipine, SAH/IA nimodipine, SAH/angiography.

Findings: Basilar artery vessel diameters are measured by angiography. Basilar artery vessel diameters and luminal sectional areas are measured in pathology slides. Basilar artery thicknesses were significantly higher in group 2 and 7 than the others ($p < 0.05$). Luminal sectional areas in group 5 and 6 were significantly higher than other groups ($p < 0.05$). We found no significant difference in group 1, 5 and 6 ($p > 0.05$). Basilar section areas in group 3 and 4 were significantly higher than group 2 but lower than group 1.

Conclusion: This is the first study to show the most effective drug delivery route in CVS after SAH. Nimodipine treatment in cerebral vasospasm is useful. This study showed that selective IA nimodipine treatment and IT nimodipine treatment must be preferred to IV and oral treatments of chronic vasospasm following SAH.

Keywords Subarachnoid hemorrhage · Vasospasm · Animal models · Drug delivery methods · Nimodipine

M.B. Onal, E. Civelek, A. Kircelli (✉), and I. Solmaz
Department of Neurosurgery, Gulhane Military Academy, Ugur Mumcunun Sokak, 78/2, 06700 Gaziosmanpasa, Ankara, Turkey
e mail: atillakircelli@gmail.com
F. Narin, I. Isikay, and B. Bilginer
Department of Neurosurgery, Hacettepe University School of Medicine, Ankara, Turkey
S. Ugurel
Department of Radiology, Gulhane Military Academy, Ankara, Turkey
H. Yakupoglu
Department of Neurosurgery, Medicana Hospital, Ankara, Turkey

Introduction

In spite of all promising therapeutic treatments, the successful treatment of delayed cerebral vasospasm (CVS) after subarachnoid hemorrhage (SAH) still remains as the major reason for poor clinical outcome and mortality [2, 16]. Calcium channel antagonists are cellular neuroprotective and vasodilalator agents. Their effect is to decrease the calcium influx. In addition to this, they also induce the development of the collateral circulation [9].

Nimodipine, the most widely administered calcium channel blocker, is used orally, intravenously, intra-arterial and also intrathecally in the treatment of vasospasm [3, 8, 11, 18]. Nimodipine has been shown to decrease the overall cerebral infarction after SAH by 34% and the incidence of poor outcome by 40% [13]. The purpose of the present study is to find out the efficiency differences between the drug delivery systems in chronic cerebral vasospasm treatment.

Materials and Methods

Animal Groups

The Hacettepe University Laboratory Animals Ethics Committee approved all protocols. Experiments were carried out in 35 male New Zealand White rabbits weighing 2,500 3,000 g. We preferred a rabbit model for this study because SAH induction and arterial angiography are easier to perform and demonstrate. All animals were starved for 8 h before the procedures and anesthetized with a mixture of ketamine (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg) administered intramuscularly. Additional doses were added at 20 30 min intervals when necessary.

Animals were assigned randomly to one of seven groups according to treatment protocols. All groups consisted of five rabbits. Animals in group 1 served as controls ($n = 5$),

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group 2: SAH only (n = 5), group 3 was treated with 0.7 mg/kg nimodipine orally two times at 24 and 48 h (h) after SAH induction (n = 5), group 4 was treated with 0.2 mg/kg nimodipine intravenously two times at 24 and 48 h after SAH induction (n = 5), group 5 was treated with 0.2 mg/kg nimodipine intrathecally two times at 24 and 48 h after SAH induction (n = 5), group 6 was treated with 0.2 mg/kg nimodipine by basilar artery angiography for selective intra-arterial treatment two times at 24 and 48 h after SAH induction (n = 5), and the ones in which angiography was performed two times at 24 and 48 h without SAH induction (n = 5) were assigned as group 7.

Nimodipine Solution

Nimodipine contains 24% ethanol, so to reduce the alcohol concentration without provoking crystallization, nimodipine was diluted by 20% saline.

SAH Formation

After shaving the dorsal parts of neck and head, under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. To enter the subarachnoid space, atlanto-occipital membrane was punctured in a head hyperflexion position. After withdrawal of 1 mL of CSF, equal volume of autologous fresh nonheparinized blood from the central ear artery was injected in 3 min into the subarachnoid space to induce SAH. The animals were then placed in a head-down position for 30 min to hold the blood in the basal cisterns.

Angiography Procedure

Under general anesthesia following preparation and draping of the right femoral regions of the rabbits under sterile conditions, an 18-gauge angiocath was introduced to the right femoral artery via surgical means (through cut-down) and fixated to the skin with surgical sutures. Then the rabbits were transferred to the angiography table and oblique lateral projection DSA (digital subtraction angiography) images of the basilar arteries of these six rabbits were obtained in an old-style CCD camera detector device, with fairly good quality. The technique of angiography was as follows: A bolus of ketalar-xyilazine mixture was given i.m for extended general anesthetic effect (without intubation) and the rabbit was laid supine on the table. After sterile preparation and draping, 18-gauge angiocath in the right femoral system of the rabbit was exchanged for a 3F 11 cm introducer sheath of a micropuncture set, through which 0.018 in. angled-tip microguidewire assisted catheterization of the left vertebral artery was achieved using a 1.8F/2.2F (distal/proximal) diameter microcatheter. 1 or 2 cc syringes (as necessary) were used for contrast media injection. Iobitridol 300 mg Iodine/mL (Xenetix 300) was the contrast media used for all rabbits. Approximately, 1 mL of contrast media was required per basilar artery run and one or two runs were obtained in each rabbit, as necessary. When the contrast media used for navigation of the microcatheter to the left vertebral artery is included, total contrast media use ranged between 4 and 8 mL (median 6 mL). Good quality images of the basilar artery were acquired through left vertebral artery injections in all rabbits but one, which had a dissection of the left vertebral artery during microcatheterization and right vertebral artery had to be used. Total blood volume loss during angiography procedure did not exceed 20 30 mL in all rabbits. Rabbits were sacrificed just after the procedure by perfusion-fixation method.

Every rabbit underwent angiography procedure in the fifth day of SAH induction to visualize and measure the diameter of the basilar artery. Animals in group 6 were also taken to this procedure in the 24th and 48th hours for selective IA nimodipine treatment.

Perfusion-Fixation

All animals subjected to experimental SAH study were euthanized by perfusion-fixation 5 days after SAH induction. After anesthetic injection thoracotomy, was performed, the left ventricle was cannulated, the right atrium opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution (Hanks' balanced salt solution [Sigma Chemical Co.], pH 7,4 at 37°C, 300 mL), the fixative was perfused (2% paraformaldehyde, 2, 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 37°C, 200 mL). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were killed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Neurological Parameters

All neurological evaluations were performed by an observer blinded to the study plan. The initial evaluation was completed between 6 and 12 h after the SAH; following assessments were completed on the third and fifth days. All of the scoring was performed 6 h after anesthesia. The neurological scale used for the assessments was based on previous study of Strong et al. with rabbits [7, 14, 18]. As a result, clinical

observations (spontaneous behavior, reaction to handling, posture, gait, limb hypertonia, righting reflexes, and feeding behavior) were each given a score: 0 (absent); 1 (mild); 2 (moderate); or 3 (severely impaired). Similarly, front and back reflexes were scored: 0 (normal); 1 (brisk); 2 (spreading); or 3 (clonus). Nystagmus was also observed: 0 (absent) or 1 (present). An overall score was calculated as the sum of the individual observations; a greater score reveals more significant neurological impairment, and a lower score reveals a lesser degree of neurological impairment.

Embedding, Morphometry, and Statistical Analysis

The basilar artery was embedded in paraffin and cut a thickness of 0.5 μm slices. The sections were mounted onto glass slides and stained with H and E for light microscopic analysis. Four sections from four separate zones of the basilar artery were obtained and luminal section areas were measured by using Image J computer program in the Department of Pathology.

The groups were compared with the analysis of variance (ANOVA) test using SPSS for Windows (version 11, 5). Following the one-way ANOVA test, a Kruskall Wallis test is performed to examine the differences between the groups. Statistical significance was accepted at $p < 0.05$.

Results

Measurement of the rabbits' physiological parameters revealed no significant differences in mean body weight, mean brain weight, mean blood pressure, and mean blood gas values among the seven groups. Gross pathological examination revealed a thick subarachnoid clot over the basal surface of the brain stem in each animal subjected to induction of SAH (Fig. 1).

Pathological Measurements

The findings of light microscopic examination of the basilar artery from group 1 7 are showed in Fig. 2. In group 2 vasospasm in the basilar artery was present. Macroscopically, a thickening of the arterial wall and narrowing of the vessel lumen were noted (Fig. 2b), when compared with the control group (Fig. 2a).

Measurements of cross sectional areas between the groups differed significantly (Table 1). The mean value of cross sectional areas in the basilar artery were 199,915 ± 18,000 μm^2 in the control group, 32,624 ± 2,100 μm^2 in the SAH only group, 100,386 ± 9,700 μm^2 in the SAH/Oral group, 102,509 ± 9,200 μm^2 in the SAH/iv group, 163,391 ± 11,000 μm^2 in the SAH/it group, 170,419 ± 13,000 μm^2 in the SAH/ia group and 69,281 ± 4,500 μm^2 in the SAH/Angio group.

The mean diameter of the basilar arteries in group 1 was 890.72 ± 70 μm, 367.50 ± 42 μm in the SAH only group, 661.29 ± 55 μm in the SAH/oral group, 587.65 ± 52 μm in the SAH/iv group, 756.93 ± 61 μm in the SAH/it group, 781.91 ± 63 μm in the SAH/ia group and 475.19 ± 47 μm in the SAH/angio group.

Compared with the control group (group 1), vasoconstriction and vessel wall thickness increase were significant in group 2. Measurements of cross sectional areas between the groups differed significantly. Median levels of cross sectional areas of basilar arteries in the SAH only group (Fig. 1b) were significantly lower than in the SAH/Oral, SAH/iv, SAH/İT, SAH/ia groups. SAH only and SAH/Angio groups did not show significant difference statistically.

Mortality and Neurological Parameters

One animal died right after injection of autologous blood into the atlanto-occipital cistern due to respiratory arrest. No

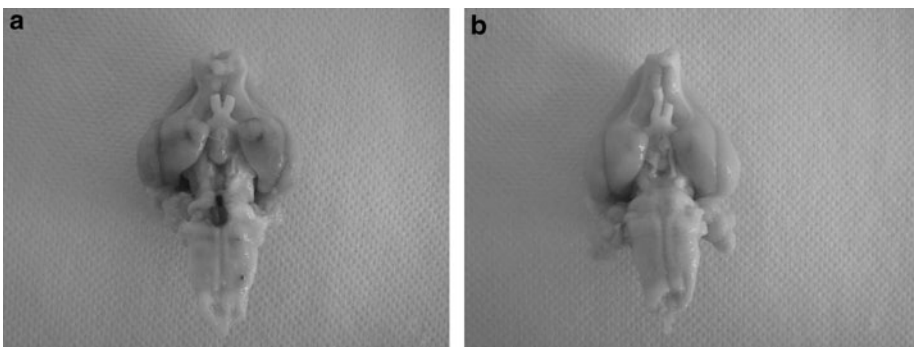

Fig. 1 Bottom surface of the rabbit brain totally removed from the brain stem. (**a**) After SAH induction, blood clot can be seen on the basilar artery surface easily. (**b**) Blood is suspended by perfusion from the control group basilar artery

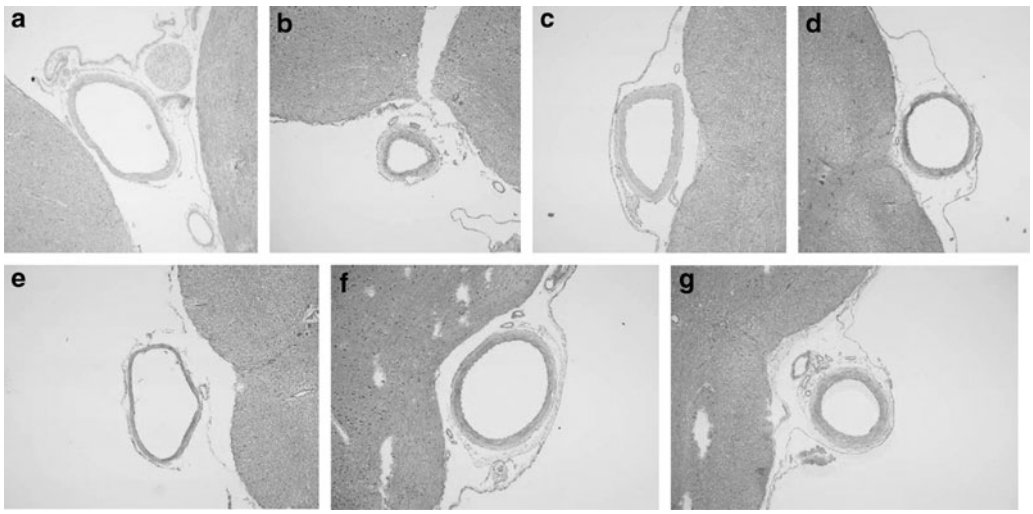

Fig. 2 Photomicrograph showing basilar artery luminal areas and wall thicknes in seven different groups. (**a**) Control, (**b**) SAH only, (**c**) SAH/ Oral nimodipine, (**d**) SAH/iv nimodipine, (**e**) SAH/it nimodipine, (**f**) SAH/ia nimodipine, (**g**) SAH/Angio

Table 1 Change in the basilar artery diameter, wall thickness and arterial luminal areas. This table shows a summary of the effects of nimodipine delivery methods and compares to each other. All values were derived from n 5 groups except the SAH/ Angiography group was derived from n 4. All values are expressed as mean ± standard deviation

Groups	Wall thickness	Perimeter of arterial lumen	Cross sectional areas
Control	26.7 ± 0.5 μm	890.72 ± 70 μm	199,915 ± 18,000 μm^2
SAH	31.2 ± 0.5 μm	367.50 ± 42 μm	32,624 ± 2,100 μm^2
SAH/Oral	25.6 ± 0.7 μm	661.29 ± 55 μm	100,386 ± 9,700 μm^2
SAH/IV	24.7 ± 0.6 μm	587.65 ± 52 μm	102,509 ± 9,200 μm^2
SAH/IT	22.6 ± 0.5 μm	756.93 ± 61 μm	163,391 ± 11,000 μm^2
SAH/IA	21.9 ± 0.5 μm	781.91 ± 63 μm	170,419 ± 13,000 μm^2
SAH/Angio	26.1 ± 0.4 μm	475.19 ± 47 μm	69,281 ± 4,500 μm^2

Table 2 Comparisons of angiographic measurements of different delivery methods are shown in the table. All values are expressed as mean ± standard deviation

Groups	Control	Only SAH	Treatment (fifth day)
SAH/oral nimodipine	0.643 ± 2.22	0.602 ± 1.98	0.697 ± 3.07
SAH/IV nimodipine	0.643 ± 2.22	0.602 ± 1.98	0.701 ± 2.69
SAH/IT nimodipine	0.643 ± 2.22	0.602 ± 1.98	0.747 ± 1.87
SAH/IA nimodipine	0.643 ± 2.22	0.602 ± 1.98	0.762 ± 2.82
SAH/angiography	0.643 ± 2.22	0.602 ± 1.98	0.699 ± 2.62

rabbits died in the control group. All other animals survived to complete the study. Clinical daily follow-up of the animals was free of problems until day 5 when they were sacrificed. Gross pathological examination showed no signs of infection. Clinical observation of the rabbits assessed by blinded veterinarians. A significant reduction of neurological scores and hypoactivity was observed in the SAH group and SAH/angiography group. There was no significant difference between only SAH group and SAH/angiography group ($p > 0.05$). Neurological scores in the treatment groups were significantly higher ($p < 0.05$) than only SAH and SAH/Angio groups at 36 h after SAH induction.

Angiographic Measurements

The basilar artery diameters of control, SAH and treatment groups are exposed on Table 2. Measurement studies were performed by two independent radiologist in a single blind fashion. Measurement of each vessel (basilar artery) on every angiogram was performed two times at four different levels, so the mean values were determined. The mean measures of the control and the SAH only groups are 0.643 ± 2.22 and 0.602 ± 1.98 mm, respectively. The other groups measures are as follows: Oral treated group 0.697 ± 3.07, IV group

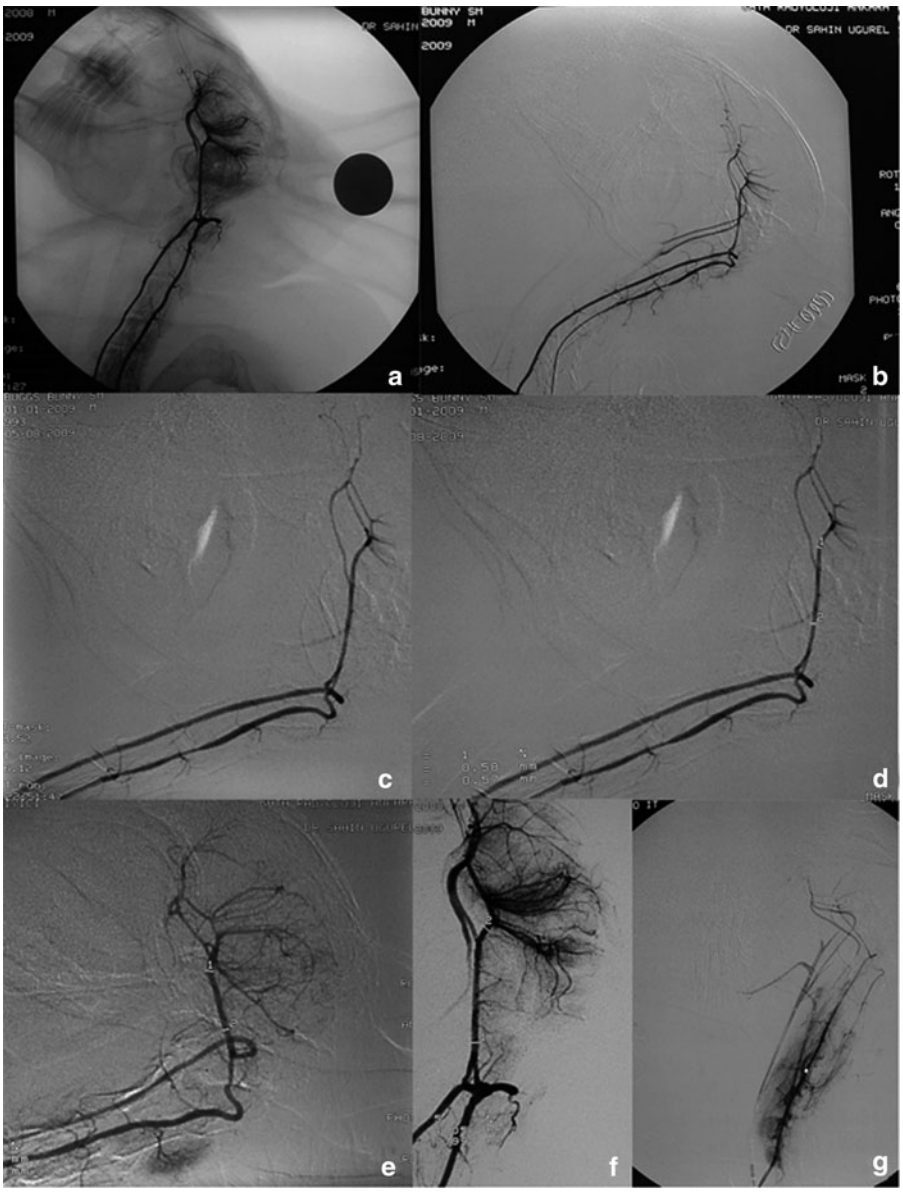

Fig. 3 Rabbit angiograms. (**a**) Control, (**b**) SAH only, (**c**) SAH/Oral nimodipine, (**d**) SAH/iv nimodipine, (**e**) SAH/it nimodipine, (**f**) SAH/ia nimodipine, (**g**) SAH/Angio

0.701 ± 2.69, IT group 0.747 ± 1.87, IA group 0.762 ± 2.82 and angio group was measured 0.699 ± 2.62 mm (Fig. 3).

Therefore the measures were harmonious with the pathological findings. IT and IA nimodipine treated groups have significant difference in the basilar artery calibres when compared with the others ($p < 0.05$). They did not have significant difference between each other ($p > 0.05$).

Discussion

Nimodipine is a calcium channel blocker, which is nowadays the only available efficient proved therapy for vasospasm after SAH to reduce the morbidity and mortality [15]. Calcium ions are in all phases of CVS. It is suggested that calcium channel blockers that obstructs the calcium influx have cellular neuroprotective and vasodilatory effects [10]. Calcium antagonists are recently used against CVS after subarachnoid hemorrhage clinically. Oral, intra-arterial, intravenous or intrathecal deliveries could be chosen for treatment but no comparative study can be found in the literature.

IV or oral application of nimodipine is currently recommended as the first line medication to prevent vasospasm [9]. Endovascular treatment has been suggested as an alternative option. IA nimodipine has been used by neurointerventional groups empirically [16].

Toyota et al. suggested a standard oral dose therapy but the pharmacokinetic studies show the oral administered nimodipine bioavailability among 2% and 28% in SAH patients [2, 17]. Intravenous, intra-arterial or intrathecal

administrations can replace the oral administration because of better bioavailability; but the alcohol existence in the intravenous nimodipine can cause local irritation and extreme phlebitis during infusion [15]. Some clinical studies report that oral or intravenous nimodipine treatment before CVS formation is ineffective angiographically, but these delivery methods could be effective in neuronal protection [1]. Böker et al. reported three selective IA nimodipine treatment cases of CVS and suggested that IA nimodipine was angiographically effective [5], Grotenhius et al. reported six cases treated with selective IA nimodipine treatment who had CVS and found the treatment ineffective [4]. Conti et al. found IA nimodipine injection more effective than its IT application in basilar artery but equally effective in vertebral artery [10]. Some authors introduce IT nimodipine treatment of CVS as an effective method. Directly given nimodipine treatment is lacking in dose dependent side effects [10]. Gioia et al. applied nimodipine sublingually, intravenously and intrathecally and suggested that only the IT group showed improvement [19]. Zabramski et al. also reported an increase in the vasospastic arteries treated with IT nimodipine [6]. IT application is catheter dependent and this treatment was disappointing until prolonged release implants were available. Surgical implantation requirement is a disadvantage but results are encouraging [5].

The intravenous or intraperipheral route of administration requires larger doses of drugs than intrathecal or selective intra-arterial routes, which could lead the adverse effects, reducing the therapeutic efficacy of the drug. The intrathecal and intra-arterial routes can overcome the inability of intravenous or peripherally administered drugs to allow distribution of the drugs through the entire neuraxis without the step of penetrating the blood brain barrier [12].

Conclusion

This is the first study to show the most effective drug delivery route in CVS after SAH. Our aim is to compare the drug delivery routes of effect proved agent nimodipine on cerebral vasospasm. SAH/Angiography group has no significant difference than only SAH group. Selective IA nimodipine and IT nimodipine treatments were found equally effective, but they were more effective than oral and intravenous treatments. Selective IA or IT treatments can be considered as more effective. After clinical experiments, IA and IT treatments of CVS after SAH can be preferred to oral or intravenous routes. Further studies are needed for this issue into clinical practice.

Conflict of interest statement We declare that we have no conflict of interest.

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Effect of Recombinant Osteopontin on Cerebral Vasospasm After Subarachnoid Hemorrhage in Rats

Hidenori Suzuki, Yu Hasegawa, Kenji Kanamaru, and John H. Zhang

Abstract *Background*: Osteopontin (OPN), a pleiotropic extracellular matrix glycoprotein, has been reported to have neuroprotective effects against early brain injury after subarachnoid hemorrhage (SAH). The aim of this study is to examine if osteopontin prevents cerebral vasospasm after SAH in rats.

Method: The endovascular perforation model of SAH was produced, and 62 rats were randomly assigned to sham + vehicle, SAH + vehicle, and SAH+ r-OPN (0.1 μg) groups. Cerebral vasospasm was evaluated by India ink angiography at 24 and 72 h after SAH, as well as neurobehavioral tests.

Findings: Significant vasospasm and neurological impairments occurred over the observed period after SAH. r-OPN significantly prevented vasospasm in the left middle cerebral artery at 24 h and improved neurological impairments at 48 h after SAH. In other time points studied, r-OPN had a tendency toward improving both vasospasm and neurological scores, but the difference was not significant.

Conclusions: This study shows that r-OPN has anti-vasospastic effects against cerebral vasospasm after SAH.

Keywords Osteopontin · Casting method · Cerebral vasospasm · Subarachnoid hemorrhage

Introduction

Osteopontin (OPN) is a secreted multifunctional extracellular matrix glycoprotein that has been linked to a variety of pathophysiological processes in the nervous system [1]. OPN has antioxidant, anti-inflammatory and antiapoptotic functions [1, 2], and therefore is believe to be neuroprotective [3]. Recently, we reported that recombinant OPN (r-OPN) prevented early brain injury and improved neurological outcome after subarachnoid hemorrhage (SAH) in rats. However, the effects of OPN on cerebral vasospasm have not been investigated. Considering pleiotropic effects of OPN, it would be worth examining if OPN has protective effects on vasospasm. Thus, in this study, we study whether r-OPN prevents cerebral vasospasm after SAH in rats.

Materials and Methods

All protocols were evaluated and approved by the Institutional Animal Care and Use Committee of Loma Linda University. The animals were cared for in accordance with the Guidelines of the Committee.

Experimental Model of SAH and Study Protocol

The endovascular perforation model of SAH was produced in male adult Sprague-Dawley rats (300 370 g, Harlan, Indianapolis, IN) as previously described [3]. Briefly, rats were anesthetized with 3% isoflurane in 60/40% medical air/oxygen in a small animal anesthesia induction box, followed by the injection of atropine (0.1 mg/kg s.c.). The animals were transorally intubated and the respiration was maintained with a small rodent respirator (Harvard Apparatus,

H. Suzuki and Y. Hasegawa
Department of Physiology, Loma Linda University School of Medicine, Risley Hall, Room 223, Loma Linda, CA, USA
e mail: johnzhang3910@yahoo.com
J.H. Zhang (✉)
Department of Physiology, Loma Linda University School of Medicine, Risley Hall, Room 223, Loma Linda, CA, USA
Department of Neurosurgery, Loma Linda University School of Medi cine, Loma Linda, CA, USA
K. Kanamaru
Department of Neurosurgery, Suzuka Kaisei Hospital, Suzuka, Japan

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Holliston, MA). Anesthesia was maintained with 2 3% isoflurane in 60/40% medical air/oxygen. After cannulation of the left femoral artery with a plastic catheter, catheters were connected with a three-way stopcock to a pressure transducer to measure blood pressure and to withdraw blood for blood gas measurements (GEM Premier 4000; Instrumentation Laboratory, Lexington, MA). Rectal temperature was kept at approximately 37°C with an electric heating pad. Rats were placed in the supine position and a midline skin incision was made on the neck. After exposing the left common carotid artery, external carotid artery (ECA) and internal carotid artery (ICA), the ECA was ligated, cut, and shaped into a 3-mm stump. A sharpened 4-0 monofilament nylon suture was advanced rostrally into the ICA from the ECA stump until resistance was felt (15 18 mm from the common carotid bifurcation) and then pushed 3 mm further to perforate the bifurcation of the anterior cerebral and middle cerebral arteries. Immediately after puncture, the suture was withdrawn into the ECA stump, and the ICA was reperfused to produce SAH. Sham-operated rats underwent identical procedures except that the suture was withdrawn once resistance was felt, without puncture. The incision was then closed, and rats were housed individually following their recovery from anesthesia. Animals had free access to food and water until euthanization.

Sixty-two rats were randomly assigned to sham + vehicle, SAH + vehicle, and SAH + r-OPN (0.1 μg) groups. Neurological scores were evaluated prior to and after the SAH production or sham-operation at each interval of 24 h until the sacrifice. Cerebral vasospasm was evaluated by India ink angiography at 24 and 72 h post-SAH, followed by the assessment of the severity of SAH.

Neurological Scoring

Neurological scores were evaluated with the scoring system reported by Sugawara et al. [4] in a blinded fashion. Briefly, the evaluation consists of six tests that can be scored 0 3 or 1 3. These six tests include: spontaneous activity; symmetry in the movement of all four limbs; forepaw outstretching; climbing; body proprioception; and response to whisker stimulation. The maximum score is 18 and the minimum score is 3. Higher scores indicate greater function.

India Ink angiography

The diameter of the left middle cerebral artery was measured by the cerebrovascular casting method, which has been used to assess vasospasm in rats and mice [5, 6]. Gelatin India ink solution, which was maintained at 50°C, was made by dissolving gelatin powder (7 g) in 100 mL of phosphate-buffered saline (PBS) and mixing with 100 mL of India ink (Design; Sanford Co, Bellwood, IL, USA) [5]. Rats were deeply anesthetized with isoflurane and cerebral vascular perfusion was performed as follows. The ascending aorta was cannulated through the left ventricle with a blunted 16-gauge needle attached to flexible plastic tubing to deliver infusion solutions by an automatic infusion pump (Syringe infusion pump 22; Harvard Apparatus, Holliston, MA). To establish a closed circuit for monitoring perfusion pressure, the tubing was connected to a three-way stopcock to a pressure transducer to measure blood pressure (Digi-Med; Micro-Med Inc, Louisville, Ky), a 60-mL syringe on the infusion pump, and the cannulated aorta. After an incision was made in the right atrium to allow for the outflow of perfusion solutions, 100 mL of PBS, 15 min of 10% formalin, and 10 min of 3.5% gelatin India ink solution were infused through the closed circuit. All perfusates were passed through a 0.2-μm pore size filter and delivered at 60 80 mmHg [5]. The rat was refrigerated for 24 h to allow gelatin solidification. The brains were harvested and high-resolution pictures of the base of the brain depicting the circle of Willis and basilar arteries were taken with a scale before and after the removal of a subarachnoid clot if needed. The brain was stored in 10% neutral buffered formalin.

An experienced person who was unaware of the treatment groups measured the diameter of the sphenoidal segment of the left middle cerebral arteries three times on the digitized images using Image J freeware (National Institutes of Health, MD) and determined a mean value. The smallest lumen diameter was recorded.

Severity of SAH

The severity of SAH was assessed in a blinded fashion using the high-resolution photographs as described previously [4]. In brief, the SAH grading system was as follows: the basal cistern was divided into six segments, and each segment was allotted a grade from 0 to 3 depending on the amount of subarachnoid blood clot in the segment; grade 0: no subarachnoid blood; grade 1: minimal subarachnoid blood; grade 2: moderate blood clot with recognizable arteries; grade 3: blood clot obliterating all arteries within the segment. The animals received a total score ranging from 0 to 18 after adding the scores from all six segments.

Intracerebroventricular Infusion

r-OPN was administered by an intracerebroventricular infusion as previously reported [3]. Rats were placed in a head

holder under 2 3% isoflurane anesthesia. The needle of a 10-μL Hamilton syringe (Microliter #701; Hamilton Company, Reno, NV) was inserted through a burr hole perforated on the skull into the left lateral ventricle using the following coordinates relative to bregma: 1.5 mm posterior; 1.0 mm lateral; 3.2 mm below the horizontal plane of bregma. Sterile PBS vehicle (1 μL), or mouse r-OPN (0.1 μg in 1 μL; EMD Chemicals, La Jolla, CA) was automatically infused at a rate of 0.5 μL/min for 2 min irrespective of the animal's body weight at 1 h before the SAH production or sham-operation. The needle was removed 10 min after finishing of an infusion, and the burr hole was quickly plugged with a bone wax.

Statistics

All values were expressed as mean ± SD. Comparisons were assessed using chi-square test or one-way ANOVA and Tukey-Kramer *post hoc* tests. $P < 0.05$ was considered statistically significant.

Results

None of sham-operated rats died. Physiological parameters, the severity of SAH (Fig. 1) and mortality were not significantly different between the vehicle- and r-OPN-treated SAH rats. In the vehicle-treated SAH rats, neurological scores were significantly worse compared with the sham-operated rats at all time points following SAH (Fig. 2). r-OPN significantly improved neurological score at 48 h post-SAH. Neurological scores in the r-OPN-treated SAH rats were not significantly different from that in the sham-operated rats over the observation period.

Significant vasospasm occurred in the left middle cerebral artery at 24 and 72 h post-SAH, which was significantly attenuated by r-OPN at 24 h post-SAH (Fig. 3). At 72 h post-SAH, r-OPN still had improving tendency of vasospasm, but the difference was not significant.

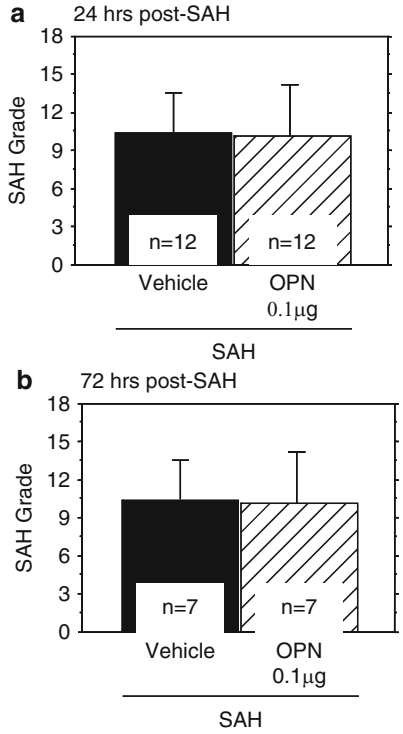

Fig. 1 SAH grading scores at 24 (**a**) and 72 (**b**) hours post SAH

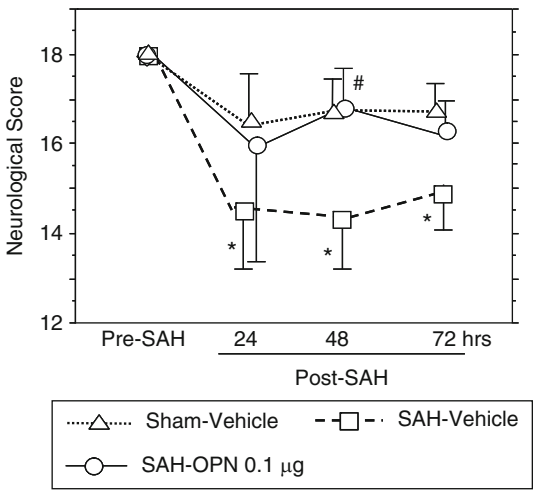

Fig. 2 Neurological scores after SAH. $^{*}P < 0.05$ versus sham operated rats; $^{\#}P < 0.05$ versus vehicle treated SAH rats. Pre SAH, n 16, 24, 22; 24 h post SAH, n 16, 19, 19; 48 and 72 h post SAH, n 6, 7, 7 in the sham operated rats, vehicle and r OPN treated SAH rats, respectively

Conclusion

This study showed that r-OPN prevented vasospasm in the left middle cerebral artery and improved neurological status. As OPN has not been reported to affect vessel diameter, the underlying mechanisms for OPN to prevent vasospasm should be studied.

OPN potentially affects some signaling pathways. For example, OPN inhibited interleukin (IL)-1β-induced inducible nitric oxide synthase (iNOS) expression and/or nitric oxide (NO) production in both in vitro and in vivo studies [7, 8]. As the signaling pathways that are closely linked with iNOS expression, the nuclear factor (NF)-κB transcription pathway, janus tyrosine kinase-signal transducers and activators of transcription (JAK/STAT) pathway, mitogen-activated

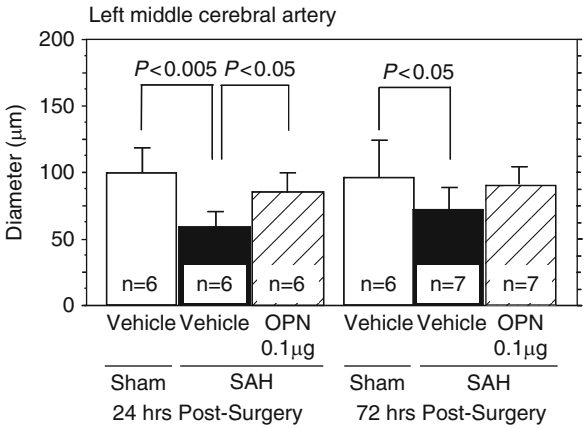

Fig. 3 Vasospasm in the left middle cerebral artery after SAH

protein kinases (MAPK) pathway are known [9]. A previous study showed that OPN provided protection against IL-1β-mediated cytotoxic effects by reducing IL-1β-induced NO production through the deactivation of NF-κB activity in isolated rat islets [7]. Also, a recent study showed that OPN downregulated iNOS expression by accelerating ubiquitination and degradation of Stat1 in a murine sepsis model [8]. Although no studies have reported if OPN can suppress MAPK activation, OPN may also suppress MAPK activation. In a rat SAH model, we recently reported that OPN inhibited NF-κB activity in the brain [3]. In addition, OPN was reported to inhibit IL-1β-induced protein kinase C (PKC) activation in cultured cells [10]. As IL-1β, iNOS, NF-κB, JAK/STAT, MAPK and PKC are all reported to be involved in the development of cerebral vasospasm, OPN may inhibit these signaling pathways and prevent vasospasm after SAH. OPN also has antioxidant, antiinflammatory and antiapoptotic effects [1, 2], which also potentially prevent vasospasm. In this regard, further investigations are needed.

Conflict of interest statement We declare that we have no conflict of interest.

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The Effect of Intracisternal Zn (II) Protoporphyrin IX on Vasospasm Process in the Experimental Subarachnoid Hemorrhage Model

Ilkay Isikay, Burcak Bilginer, Firat Narin, Figen Soylemezoglu, and Nejat Akalan

Abstract *Background*: Although there has been much work on it for years, cerebral vasospasm as a complication of subarachnoid bleeding is still an important cause of mortality and morbidity. The presented study was designed to examine the effects of heme oxygenase inhibitor, Zn (II) protoporphyrin IX, on vasospasm process.

Method: In this study 20 male New Zealand White rabbits weighing 2,500 to 3,000 g were randomly assigned to four groups. Animals in group 1 were controls. In group 2, animals were SAH induced only and no treatment given. SAH induced animals in group 3 received intracisternal Zn (II) protoporphyrin IX 0.5 mg/kg in DMSO solution, at 0 and 24 h of SAH induction. In group 4, animals received only intracisternal DMSO at 0 and 24 h after SAH induction. All animals were sacrificed via exsanguination at 72 h after induction of SAH. Brains and brainstems with overlying basilar arteries were removed and stored in fixative at $+4^{\circ}C$ overnight. Basilar arteries were sectioned from four seperate zones, and four sections were obtained from each rabbit. Basilar artery luminal section areas and vessel wall thicknesses were measured by using SPOT for Windows version 4.1. Statistical comparisons were performed using Mann-Whitney and ANOVA tests.

Findings: Basilar arterial wall thicknesses in group 3 were smaller than that of group 2 ($p < 0.05$). Luminal section areas in group 3 were significantly greater than luminal section areas of group 2 ($p < 0.05$).

Conclusion: ZnPP has a potentially beneficial effect on cerebral vasospasm after subarachnoid bleeding.

I. Isikay (✉), B. Bilginer, F. Narin, and N. Akalan
Department of Neurosurgery, Hacettepe University School of Medicine, Ankara, Turkey
e mail: ilkayisikay@yahoo.com
F. Soylemezoglu
Department of Pathology, Hacettepe University School of Medicine, Ankara, Turkey

Keywords Zn (II) protoporphyrin IX · Basilar artery · Cerebral vasospasm · Subarachnoid hemorrhage

Introduction

Cerebral vasospasm is the delayed narrowing of large capacity arteries at the base of the brain occurring after subarachnoid hemorrhage. This condition is often associated with diminished perfusion in the territory distal to the affected vessel [10]. If they do not succumb to death, almost half of patients suffering subarachnoid bleeding will end up with severe morbidity due to cerebral vasospasm [8]. Despite the years of research on the issue of vasospam, etiology and pathogenesis of this detrimental disease are not yet well understood [1, 4, 6, 11].

BOXes are recently recognised as possible contributors to and initiators of vasospasm. Although bilirubin itself does not produce vasospasm, concentrations in the CSF are closely related to the time course of vasospasm [2]. This knowledge led to a theory that compounds which are related to but different from bilirubin are associated with vasospasm. The theory was further supported by different in vitro and in vivo experiments [2, 3, 9, 12]. Following the hemolysis of blood in the subarachnoid space, heme, the prosthetic group of hemoglobin, is degraded into biliverdin, CO and a free iron by an enzyme called heme oxygenase. Biliverdin is further oxidized to bilirubin by the enzyme biliverdin reductase. Bilirubin, in the setting of highly oxidative post hemorrhagic environment, is the source of vasoactive compounds namely the 4-methyl-5-oxo-3-vinyl-(1,5-dihydropyrrol-2-ylidene) acetamide, 3-methyl-5-oxo-4-vinyl-(1,5-dihydropyrrol-2-ylidene)acetamide and 4-methyl-3-vinylmaleimide (MVM) [9]. Metalloporphyrins are competitive inhibitors of heme oxygenase enzyme [13].

Zinc (II) protoporphyrin IX (ZnPP) is a metalloporphyrin that readily inhibits heme oxygenase, which may in turn decrease BOX concentration through a reduction in bilirubin

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production [3]. Intraperitoneal infusion of ZnPP attenuates brain edema in animals after intracerebral hemorrhage; ZnPP also reduces ICH-induced caudate atrophy and ventricular enlargement [5]. Zinc protoporphyrin, but also zinc and protoporphyrin alone, contribute to brain-protective effects when administered early in a temporary focal ischemia model [14]. Although these experimental data imply a protective effect on central nervous system, contemporary literature lacks the knowledge whether intracisternal ZnPP may attenuate cerebral vasospasm or not. In this study our aim was to investigate effects of intracisternal ZnPP on cerebral vasospasm after SAH.

Methods and Materials

Animal Model

The experimental protocols used in this study were approved by the Hacettepe University Animal Research Committee. Twenty male New Zealand White rabbits weighing 2,500 3,000 g were randomly assigned to four groups. Animals in group 1 (n = 5) were controls. In group 2 (n = 5) animals were only SAH induced. SAH induced animals in group 3 (n = 5) received intracisternal Zn (II) protoporphyrin IX 0.5 mg/kg in DMSO solution, at 0 and 24 h of SAH induction. In group 4 (n = 5) animals received only intracisternal DMSO at 0 and 24 h after SAH induction. All procedures were performed by two investigators working in tandem and not blinded to the treatment group during surgery and euthanasia. Vascular measurements were performed in a blinded fashion.

Induction of Experimental SAH

All animals were anesthetized by intramuscular injection of a mixture of ketamine (Ketaset, 50 mg/kg) and xylazine (Rompun, 10 mg/kg), paralyzed with pancuronium bromide (0.08 mg/kg), intubated and ventilated with a Harvard 683 model dual-phase ventilator (Harvard Apparatus Co.). A 23-gauge butterfly needle was inserted percutaneously into cisterna magna. After withdrawal of 1.0 ml CSF, 3 ml of non-heparinized blood from central ear artery was injected into subarachnoid space. The animals were then placed in head down position for 15 min to hold the blood in the basal cisterns. Arterial blood gases were analyzed during the surgical procedure and maintained within the physiological range. After recovering from anesthesia, the rabbits were observed for possible neurological deficits and then returned to the vivarium. The same steps were repeated for ZnPP and vector injections.

Perfusion-Fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 72 h after SAH induction. The animals were anesthetized, intubated, and ventilated as described above. The ear artery was catheterized for the monitoring of blood pressure and blood gas analysis. When satisfactory respiratory parameters were obtained, thoracotomy was performed, the left ventricle cannulated, the right atrium opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution (Hank's balanced sat solution [Sigma Chemical Co.], pH 7.4 at 37°C, 300 ml), the fixative was perfused (2% paraformaldehyde, 2.5% gluteraldehyde im 0.1 M phosphate buffer, pH 7.4 at 37°C, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were killed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Embedding, Morphometry, and Statistical Analysis

Basilar arteries were removed from the brain stems, and arterial segments from the proximal third of the artery were dissected for analysis. The arterial segments were washed several times with 0.1 mol/l phosphate-buffered solution (PBS, pH 7.4), fixed in 1% osmium tetroxide in PBS for 1 h at room temperature, and then washed again with PBS. Cross-sections were cut at a thickness of 0.5 μm. The sections were mounted onto glass slides and stained with H and E for light microscopic analysis. The vessels were measured using computer-assisted morphometry (SPOT for Windows Version 4.1). Automated measurements of the cross-sectional area of the arterial sections were taken by an investigator who was blinded to the identity of the group the animals belong to. Four cross-sections of each vessel were selected randomly for measurement, calculating the average of these measurements. Statistical comparisons were performed using Mann-Whitney U and one way ANOVA tests. Statistical significance was accepted at $p < 0.05$.

Results

Mean body weight, mean brain weight, mean blood pressure and mean blood gas value measurements did not reveal significant differences between the four groups (Table 1).

Microscopic examination revealed a thick subarachnoid clot over the basal surface of the brain stem in each animal

Table 1 Summary of physiologic parameters of the groups

Group	n	Body weight (g)	pH	pCO_2	pO_2	MABP
1	5	2,846.8 ± 35.3	7.42 ± 0.03	41.1 ± 1.10	110 ± 5.98	105 ± 3
2	5	2,748.0 ± 12.4	7.44 ± 0.05	41.5 ± 1.10	107 ± 5.45	102 ± 3
3	5	2,760.0 ± 36.6	7.43 ± 0.03	41.3 ± 1.09	110 ± 6.00	107 ± 2
4	5	2,848.0 ± 40.6	7.43 ± 0.07	40.5 ± 1.04	106 ± 5.89	99 ± 3

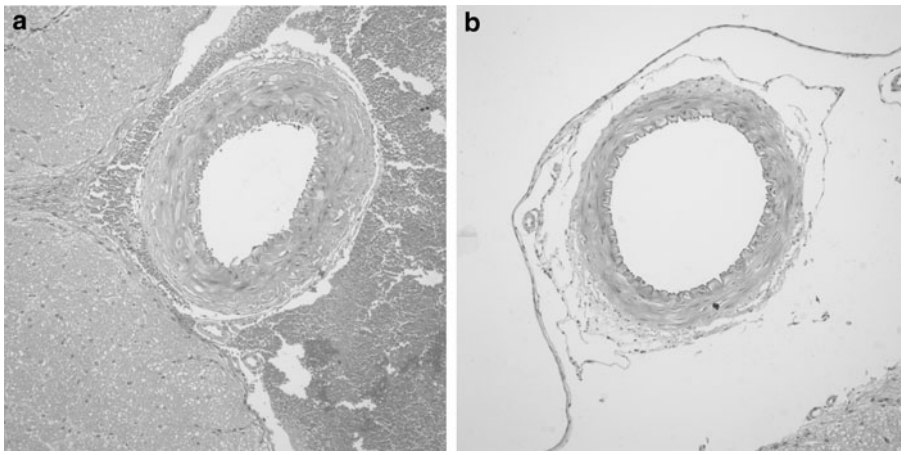

Fig. 1 (**a**) Basilar artery after SAH induction. Cross sectional appearance of the HE stained basilar artery under × 20 magnification. (**b**) Basilar artery after SAH induction + ZnPP treatment. Cross sectional appearance of the HE stained basilar artery under × 20 magnification

subjected to induction of SAH (Fig. 1). In the SAH-induced animals, arterial lumina were narrowed with folding and corrugation of elastic lamina. Tunica media was vacuolized and tunica adventitia was accumulated with red and inflammatory cells. Mean arterial luminal area in group 1 (control) was 96,148.6 ± 4,692.4 μm^2. SAH-only induced group 2 animals had a mean luminal area of 41,414.6 ± 3,181.3 μm^2. In the SAH-induced and ZnPP treated group (group 3), mean area was 68,267.0 ± 10,273.7 μm^2 (Fig. 2). Vessel wall thickness measurements were as follows: 43.8 ± 3.0 μm in group 1, 104.8 ± 7.8 μm in group 2, 59.8 ± 3.7 μm in group 3 and 103.0 ± 3.2 μm in group 4 (Fig. 3).

Compared with SAH-only and DMSO treated group, ZnPP treated group had a significantly greater mean luminal area ($p < 0.05$). Similarly mean arterial wall thickness was significantly small in ZnPP treated group.

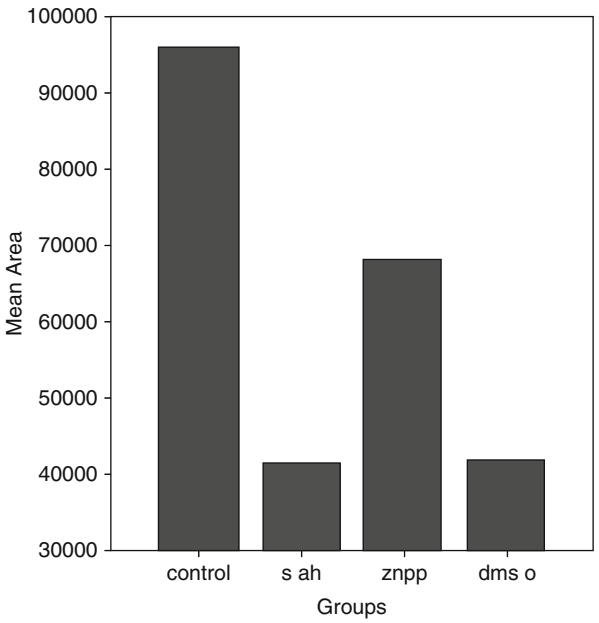

Fig. 2 Mean cross sectional areas in μm^2

Discussion

Cerebral vasospasm as a complication of subarachnoid bleeding is still an important cause of mortality and morbidity. The reason for the failure to propose an effective treatment is complexity and multifactorial nature of the disease. There is no single pharmacological agent or treatment protocol which is effective in inhibiting the multiple factors related to the disease process, since most of them cannot be used due to harmful side effects. Despite the clinical usage of treatments such as triple-H therapy and nimodipine to reduce the risk of vasospasm and cerebral infarction, SAH continues to have a high fatality rate and as a result a high prevalence of dependency and reduced quality of life among survivors.

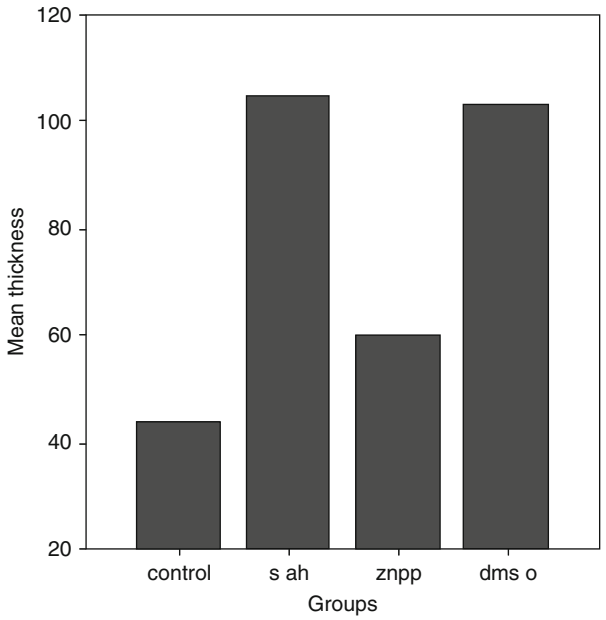

Fig. 3 Mean vessel wall thicknesses in μm

Triple H therapy comprises hypertension induction, together with hemodilution and hypervolemia. This therapy remains the single most effective treatment against vasospasm, but uncertain pathophysiological basis of this treatment and the possible complications (cerebral edema, cardiac arrhythmias, myocardial infarction) render it unreliable. Another group of medication, vasodilators, may be harmful by counteracting hypertension, which is believed to be beneficial in delayed cerebral vasospasm. However, cerebral arteriolar dilatation may cause shunting of perfusion and increase ischemia in affected brain tissue.

A great deal of experimental and clinical research has been conducted in an effort to explain pathophysiological events in the disease process. Recently, introduction of BOXes as a possible causative factor in this disease process rendered them a theoretical target for treatment options [3]. One of the two potential sources of BOXes is the oxidative milieu of the subarachnoid space, where oxidation of bilirubin is independent of HO enzyme system. The second is intracellular oxidation of bilirubin, which is formed through a number of reactions from heme the substrate for HO enzyme. Theoretically these two pathways are possible [3].

Very few literature data addresses the effect of ZnPP in central nervous system, and these are mainly concerned with ischemic processes. Kadoya et al. [7] showed that preischemic administration of ZnPP protects from reduction in cerebral infarct size, brain edema, sodium accumulation, and potassium loss after 2 h of middle cerebral artery occlusion followed by 22 h of reperfusion. These effects are not seen when ZnPP is administered after the occlusion. They hypothesized a mechanism of action that depend on antagonist effect over IL-1 and HO inhibition [7]. A similar study compared the effects of ZnPP, Zn and PP seperately on brain ischemia and showed that each component of ZnPP significantly reduces ischemia [14]. Although these studies imply a neuroprotection through inhibition of HO enzyme, there is no data concerning ZnPP when given intracisternally after subarachnoid hemorrhage.

Zinc protoporphyrin has a central zinc atom in its core, substituted for iron. This feature causes a competitive inhibition of HO enzyme, and finally a possible reduction in BOX concentration. We thought the possibility was worth investigating and conducted this study. Since ZnPP is known not to cross blood-brain barrier, intracisternal injection method is preferred. The effective systemic dose when given for central nervous system purposes was 50 mg/kg in literature, but we adjusted the dose to 0.5 mg/kg taking into account the limited volume of distribution in subarachnoid space.

Histopathological findings show a significant difference in terms of luminal cross sectional area and vessel wall thickness between SAH-only and ZnPP treated group. We can conclude that administration of intracisternal ZnPP may attenuate effects of vasospasm process. This is possibly due to a reduction in BOX concentration, since ZnPP reduces the amount of bilirubin, which is a substrate for oxidation reactions that finally lead to aforementioned vasoactive compounds.

It is clear that more research is needed to precisely define the causative background of these histopathological findings supported by quantitative measurements of BOXes in the CSF. Since this study is the first to investigate the effects of intracisternal ZnPP on vasospasm process, we think that it will shed light on further studies.

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Temporal Profile of the Effects of Intracisternal Injection of Magnesium Sulfate Solution on Vasodilation of Spastic Cerebral Arteries in the Canine SAH Model

Kentaro Mori, Masahiro Miyazaki, Yasukazu Hara, Yasuhisa Aiko, Takuji Yamamoto, Yasuaki Nakao, and Takanori Esaki

Abstract *Purpose*: The temporal profiles of the effects of intracisternal injection of magnesium sulfate ($MgSO_4$) on vasodilation and cerebrospinal fluid (CSF) magnesium ion (Mg^{2+}) concentration were investigated in the canine subarachnoid hemorrhage (SAH) model.

Method: Cerebral vasospasm was induced using the two-hemorrhage model in seven female beagles. On day 7, 0.5 ml/kg of 15 mmol/l $MgSO_4$ in Ringer solution was injected into the cerebellomedullary cistern. Angiography was performed on day 1 (before SAH), and before and 1, 3, and 6 h after the intracisternal injection on day 7. CSF Mg^{2+} was measured at the same time.

Results: The diameters of the basilar artery (BA), vertebral artery (VA), and superior cerebellar artery (SCA) before the intracisternal injection on day 7 were 0.59 $\pm$ 0.15, 0.41 $\pm$ 0.17, and 0.35 $\pm$ 0.17 mm, respectively, and were significantly decreased ($p < 0.01$) compared with the baseline diameters on day 1. The BA diameters at 1 h (0.74 $\pm$ 0.16 mm) and 3 h (0.73 $\pm$ 0.13 mm), the VA diameter at 1 h (0.64 $\pm$ 0.14 mm), and the SCA diameter at 3 h (0.54 $\pm$ 0.08 mm) after the injection were significantly increased ($p < 0.05$). The CSF Mg^{2+} concentration was significantly increased ($p < 0.01$) at 1 h (3.59 $\pm$ 0.76 mEq/l) and 3 h (2.00 $\pm$ 0.31 mEq/l) after the injection compared with the baseline value (1.35 $\pm$ 0.23 mEq/l).

Conclusions: The reversible effect of intracisternal $MgSO_4$ solution injection on the spastic artery depends on maintenance of the optimal CSF Mg^{2+} concentration.

Keywords Cerebrospinal fluid · Magnesium ion · Vasospasm · Subarachnoid hemorrhage

K. Mori (✉), T. Yamamoto, Y. Nakao, and T. Esaki
Department of Neurosurgery, Juntendo University Shizuoka Hospital, 1129 Nagaoka, Izunokuni, Shizuoka, 410 2295, Japan
e mail: kmori@med juntendo.jp
M. Miyazaki, Y. Hara, and Y. Aiko
Department of Radiology, Juntendo University Shizuoka Hospital, Izunokuni, Shizuoka, Japan

Introduction

Delayed cerebral vasospasm remains one of the major determinants of final neurological outcome even after advanced modern intravascular and open aneurysm procedures in patients with subarachnoid hemorrhage (SAH). Administration of calcium channel blockers is a promising method to prevent or ameliorate cerebral vasospasm based on the biological etiology [1]. Recent prospective studies of continuous intravenous administration of magnesium sulfate ($MgSO_4$) in patients with aneurysmal SAH have failed to show any vasodilatory effect [5, 7]. The blood brain barrier is relatively impermeable to serum magnesium ion (Mg^{2+}), so higher blood concentration of Mg^{2+} may not be reflected in increased cerebrospinal fluid (CSF) Mg^{2+} concentration. However, higher extracellular Mg^{2+} concentration in vitro causes vasodilation of spastic cerebral vessels [6]. Therefore, intracisternal administration of Mg^{2+} may reverse cerebral vasospasm caused by SAH. We previously found that intracisternal infusion of $MgSO_4$ solution improved reduced cerebral blood flow after experimental SAH in rats using a quantitative autoradiographic method [2], and that intracisternal injection of $MgSO_4$ solution dilated the spastic cerebral arteries in the canine experimental SAH model using angiography [3]. Recently, we reported the first clinical application of intracisternal infusion of $MgSO_4$ solution for the treatment of symptomatic cerebral vasospasm after aneurysmal SAH and demonstrated the vasodilatory effect on the spastic arteries [4]. However, optimal duration of the vasodilatory effect and optimal Mg^{2+} concentration in the CSF remain unclear, although the effect of CSF Mg^{2+} concentration on cat normal arterioles was dose dependent and caused dilation in the range of 2.4 9.6 mEq/l [6].

The present study investigated the temporal profile of the effects of intracisternal injection of $MgSO_4$ solution on vasodilation of spastic cerebral arteries and the CSF Mg^{2+} and calcium ion (Ca^{2+}) concentrations in the canine SAH model.

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Materials and Methods

Cerebral vasospasm was induced by experimental SAH using the two-hemorrhage canine model in seven female beagles weighing 10 11 kg. The animals were anesthetized with intravenous bolus injection of 20 mg/kg of pentobarbital and maintained by continuous intravenous infusion of 1 ml/kg/h of propofol on day 1. The animals were intubated using a 6 F tracheal tube and mechanically respirated with a magnetic resonance (MR)-compatible respirator. The $PaCO_2$ was maintained at 35 40 mmHg throughout the experimental procedures by monitoring of end-tidal CO_2. A 4 F double-lumen sheath was placed in the femoral artery for cerebral angiography. T1- and T2-weighted MR imaging was performed. Left vertebral digital subtraction angiography was performed to measure the baseline diameters of the vertebral artery (VA), basilar artery (BA), and superior cerebellar artery (SCA) after injection of 3 ml of iopamidol (Bayer). The cisterna magna was then punctured using a 20-gauge needle, 0.3 ml/kg CSF was removed by gravity flow, and 0.5 ml/kg autologous blood was injected into the cerebellomedullary cistern (first SAH). The CSF and serum concentrations of Mg^{2+} and Ca^{2+} were measured by ion-selective electrodes (Stat Profile® CCX; Nova Biomedical Corp., Waltham, MA, USA). The second SAH was similarly induced on day 3.

The animals were again anesthetized and intubated on day 7. A 4 F double-lumen sheath was placed in the femoral artery. MR imaging was performed. Left vertebral digital subtraction angiography was performed to assess the changes of the vertebrobasilar arteries after the experimental SAH. Immediately afterwards, the cisterna magna was punctured with a 20-gauge needle to remove 0.3 ml/kg of CSF and inject 0.5 ml/kg of 15 mmol/l $MgSO_4$ dissolved in Ringer solution into the cerebellomedullary cistern. Left vertebral angiography was repeated at 1, 3, and 6 h after the intracisternal injection of $MgSO_4$ solution to measure the diameters of the BA, VA, and SCA. MR imaging was also performed at 6 h. The CSF and serum concentrations of Mg^{2+} and Ca^{2+} were also measured at 0, 1, 3, and 6 h. The data are presented as means ± standard deviations. Values were compared using the paired t-test.

Results

Temporal Profile of Changes in CSF Mg^{2+} and Ca^{2+} Concentrations

Figure 1 shows the temporal profile of changes in CSF Mg^{2+} and Ca^{2+} concentrations. The Mg^{2+} concentration before

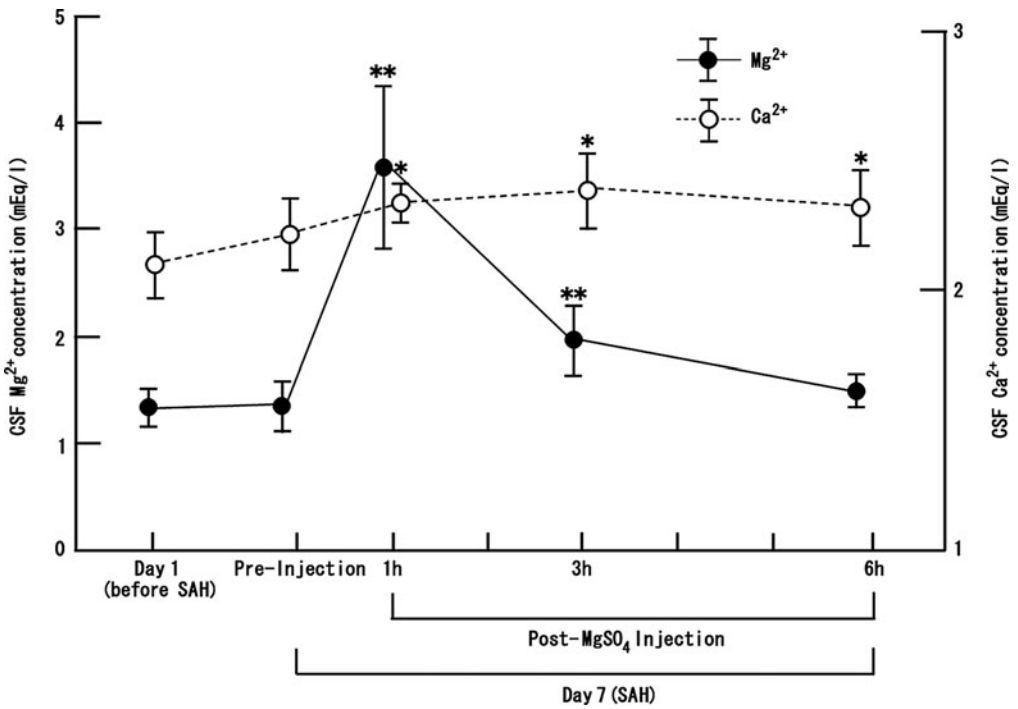

Fig. 1 Time profile of CSF Mg^{2+} and Ca^{2+} concentrations on day 1 (before SAH), and before, 1, 3, and 6 h after intracisternal injection of 0.5 ml/kg of 15 mmol/l $MgSO_4$ solution. *$p < 0.05$ paired t test (compared with CSF concentration before injection on day 7), **$p < 0.01$ paired t test (compared with CSF concentration before injection on day 7), *SAH* subarachnoid hemorrhage

injection on day 7 (1.35 ± 0.23 mEq/l) did not change compared with that of day 1 (1.34 ± 0.17 mEq/l). However, the Mg^{2+} concentrations at 1 and 3 h after the injection (3.59 ± 0.76 mEq/l and 2.00 ± 0.31 mEq/l, respectively) were significantly increased ($p < 0.01$) compared with before injection. The Mg^{2+} concentration returned to the baseline value at 6 h (1.51 ± 0.15 mEq/l). The Ca^{2+} concentration before injection on day 7 (2.21 ± 0.14 mEq/l) was slightly but significantly increased ($p < 0.05$) compared with day 1 (2.09 ± 0.13 mEq/l). The Ca^{2+} concentrations at 1, 3, and 6 h after the injection on day 7 (2.33 ± 0.08 mEq/l, 2.38 ± 0.14 mEq/l, and 2.32 ± 0.15 mEq/l, respectively) were significantly increased ($p < 0.05$) compared with before injection. The serum Mg^{2+} and Ca^{2+} concentrations did not change throughout the whole experimental period (data not shown).

Temporal Profile of Neuroimaging Changes

The mean Evans index was 19.5 ± 1.8% on day 1 and was significantly increased ($p < 0.01$) to 25.4 ± 2.8% on day 7 before intracisternal injection of $MgSO_4$. The mean Evans index after the injection (25.2 ± 2.0%) did not change compared to before injection. MR imaging did not show any pathological findings except for hydrocephalus after intracisternal injection of $MgSO_4$. Figure 2 shows the temporal changes of cerebral artery diameters. The BA, VA, and SCA diameters before SAH on day 1 were 1.17 ± 0.11, 0.92 ± 0.26, and 0.98 ± 0.15 mm, respectively. All cerebral arteries showed significant decreases ($p < 0.01$) in diameters (0.59 ± 0.15, 0.41 ± 0.17, and 0.35 ± 0.17 mm, respectively) before intracisternal injection of $MgSO_4$ on day 7 compared with day 1. The diameters of the BA at 1 h (0.74 ± 0.16 mm) and 3 h (0.73 ± 0.13 mm) after the injection were significantly increased ($p < 0.05$) as compared with before injection. The diameter of the VA at 1 h after injection (0.64 ± 0.14 mm) was significantly increased ($p < 0.05$) compared with before injection. The diameter of the SCA at 3 h after injection (0.54 ± 0.08 mm) was significantly increased ($p < 0.05$) compared with before injection. Figure 3 shows the representative angiograms.

Conclusion

The present study demonstrated that the diameter of spastic arteries significantly increased for up to 3 h after the intracisternal injection of 15 mmol/l $MgSO_4$ solution. The CSF Mg^{2+} concentration was also significantly increased at 1 h (3.59 ± 0.76 mEq/l) and 3 h (2.00 ± 0.31 mEq/l) after the intracisternal $MgSO_4$ solution injection. The CSF Mg^{2+} concentration returned to baseline value at 6 h. These observations suggest that the reversible effect of intracisternal $MgSO_4$ solution injection on the spastic arteries after experimental SAH depends on maintenance of the CSF Mg^{2+}

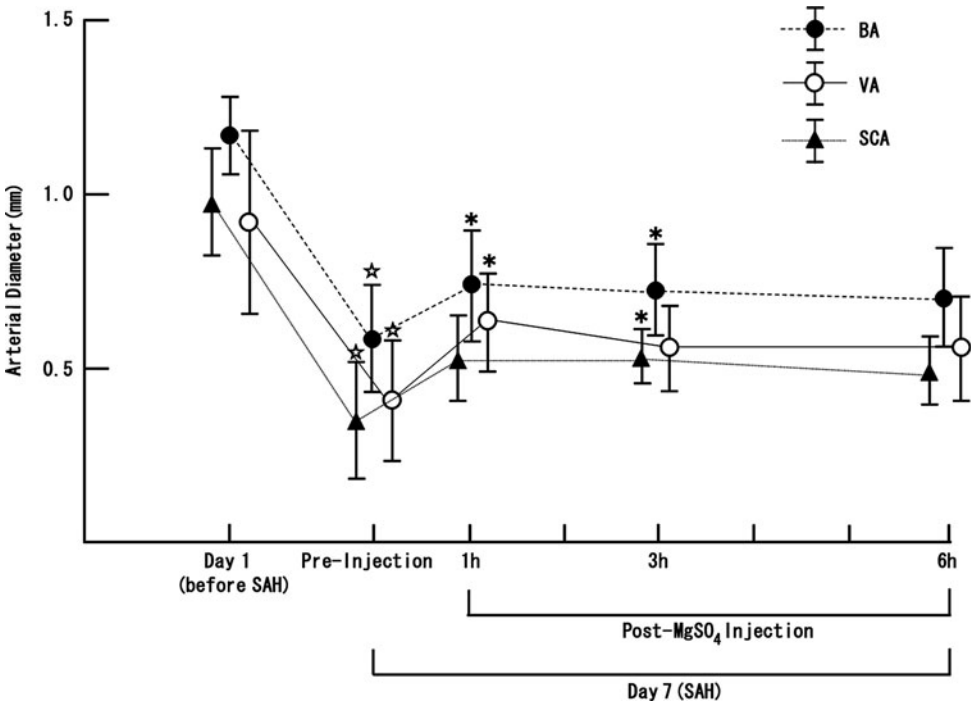

Fig. 2 Time profile of cerebral artery diameter on day 1 (before SAH), and before, 1, 3, and 6 h after intracisternal injection of 0.5 ml/kg of 15 mmol/l $MgSO_4$ solution. *SAH* subarachnoid hemorrhage, *BA* basilar artery, *VA* vertebral artery, *SCA* superior cerebellar artery, $p < 0.01$ paired t test (compared with artery diameter on day 1), *$p < 0.05$ paired t test (compared with artery diameter before injection on day 7)

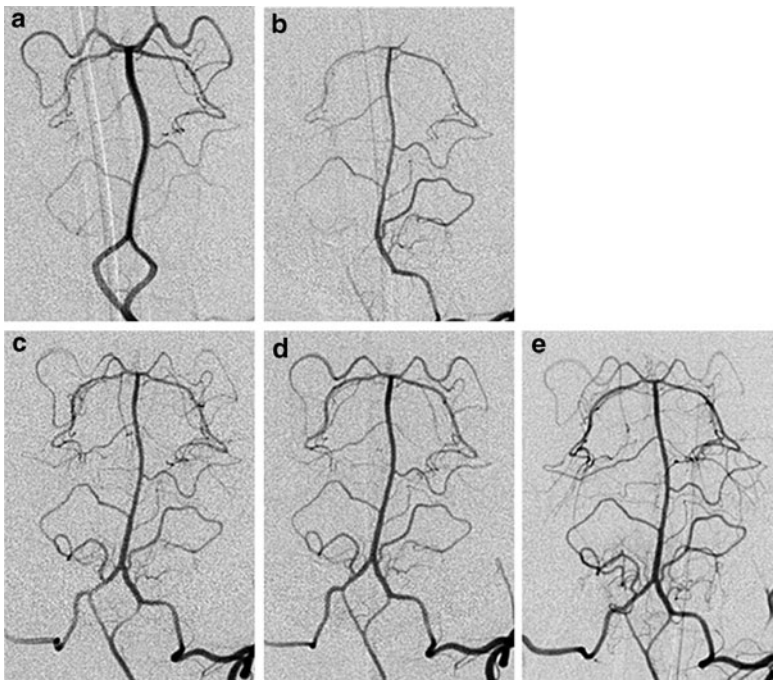

Fig. 3 Representative angiograms obtained on day 1 (**a**), before (**b**), and 1 h (**c**), 3 h (**d**), and 6 h (**e**) after intracisternal injection of $MgSO_4$ solution. The BA, VA, and SCA showed vasospasm on day 7 but were dilated at 1 and 3 h after the intracisternal injection

concentration at the optimal level. Our results also suggest that continuous intracisternal $MgSO_4$ solution infusion would be ideal in the clinical setting to constantly ameliorate cerebral vasospasm.

The present study also demonstrated a slight but significant increase of CSF Ca^{2+} concentration after the intracisternal injection of $MgSO_4$ solution, in agreement with our previous findings [3]. The increased CSF Ca^{2+} concentration is considered to result from the inhibition of extracellular Ca^{2+} influx due to the Ca^{2+} blocker effect of the increased extracellular Mg^{2+} concentration. Therefore, the mechanism of vasodilatory effect of intracisternal injection of $MgSO_4$ solution on spastic cerebral arteries after experimental SAH was mediated by the Ca^{2+} blocker effect of the increased extracellular Mg^{2+} concentration.

The present study indicates that intracisternal injection of $MgSO_4$ ameliorates cerebral vasospasm for at least 3 h in a canine model of SAH without any abnormal changes on MR imaging. Further study is needed to determine the optimal infusate concentration, duration of treatment, and the neurotoxicity of intracisternal injection of $MgSO_4$ solution for clinical application.

Conflict of interest statement We declare that we have no conflict of interest.

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Comparison of Intrathecal Cilostazol and Nimodipine Treatments in Subarachnoid Hemorrhage: An Experimental Study in Rabbits

Mehmet Bulent Onal, Burcak Bilginer, Firat Narin, M. İbrahim Ziyal, Figen Soylemezoglu, and Tuncalp Ozgen

Abstract *Objective* : Intrathecal administration of calcium channel antagonists has been proposed to reduce cerebral vasospasm (CVS) in animal subarachnoid hemorrhage (SAH) models. Also, delayed CVS treatment model with oral administration of cilostazol can be seen in the literature.

Methods : In this study, 25 male New Zealand white rabbits were randomly assigned to five groups: control, SAH only, SAH/nimodipine, SAH/cilostazol, SAH/vehicle. The animals' basilar arteries were sectioned from four separate zones and four sections were obtained from each rabbit. Basilar artery luminal section areas were measured by using SPOT for windows Version 4.1 computer program.

Results : Basilar artery luminal section areas in SAH/nimodipine and SAH/ cilostazol groups were significantly higher than SAH only group ($P < 0.05$).

Conclusion : Phosphodiesterase 3 inhibitor cilostazol has vasodilatory effects without affecting cerebral blood flow. Nimodipine is a calcium channel blocker and is still used in vasospasm therapy either oral or intravenously. This study demonstrates that prophylactic bolus intrathecal administration of either cilostazol or nimodipine equally prevents SAH-associated CVS in an animal model. We therefore propose that cilostazol is a candidate for clinical trials in the treatment of delayed vasospasm.

Keywords Subarachnoid hemorrhage · Vasospasm · Animal models · Cilostazol · Nimodipine · Intrathecal

M.B. Onal (✉), B. Bilginer, F. Narin, M.I. Ziyal, and T. Ozgen
Department of Neurosurgery, Hacettepe University School of Medicine, Ugur Mumcunun Sokak, 78/2, 06700, Gaziosmanpasa, Ankara, Turkey
e mail: bulentonal@hotmail.com
F. Soylemezoglu
Department of Pathology, Hacettepe University School of Medicine, Ankara, Turkey

Introduction

Subarachnoid hemorrhage (SAH) often results in death or severe disability. Delayed cerebral vasospasm and impaired vasodilatation are critical clinical complications that occur after SAH [20]. Cerebral vasospasm is an important complication of SAH. Symptomatic delayed cerebral vasospasm after SAH is one of the major causes of serious morbidity and death. It is characterized by the prolonged and reversible contraction of the cerebral arteries, which causes the ischemic process. Despite many clinical and experimental studies, the point at which we arrived has not been satisfactory yet [22]. Therefore, a more potent therapeutic modality for cerebral vasospasm is required to prevent delayed cerebral ischemia after SAH [19].

Symptomatic delayed cerebral vasospasm (DCV) develops in approximately 30 36% of patients after aneurismal subarachnoid hemorrhage [12, 16, 17]. An estimated two-thirds of patients undergoing angiography between days 4 and 14 after hemorrhage have some degree of vasospasm. Of these patients, approximately one-third develop symptoms related to vasospasm [2]. CVS begins most often 3 days after SAH and reaches a maximum on days 6 8 [16]

Nimodipine, the most widely administered calcium channel blocker, is used orally, intravenously, intra-arterially and also intrathecally in vasospasm therapy [7, 10, 17, 30]. Nimodipine has been shown to decrease the overall cerebral infarction after SAH by 34% and the incidence of poor outcome by 40% [21]. Intracellular calcium ion increase is known as one of the basic mechanisms in cerebral ischemia formation. The accumulation of the calcium ion depends on the decreased calcium release and also entry augmentation in the vascular smooth muscle cell [17].

The use of oral or intravenous nimodipine together with triple-H therapy including volume expansion, induced hypertension and hemodilution, to increase cerebral blood flow and also to decrease the blood viscosity are the mainstay of medical treatment for CVS [7, 18]. As the vasodilating effect of systemic calcium antagonists is limited by their

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hypotensive effect, topical applications such as intrathecal or intra-arterial administrations have become much popular; the search for an alternative drug has started [23].

Cilostazol is a phosphodiesterase type3 (PDE3) selective inhibitor, derivative of quinolinone, accumulates intracellular CAMP amount by blocking its hydrolysis on PDE3. Increased CAMP level functions on platelet aggregation inhibition, antitrombosis and vasodilatation in cerebral ischemia; it is therefore used in intermittent cladication treatment. It is useful in treatment of chronic peripheral arterial obstruction and used to prevent recurrent cerebral infarction [6, 14]. Cilostazol is an antiplatelet, vasodilatory, antimitogenic and cardiotonic drug that inhibits PDE3 potentially and selectively. Both vascular smooth muscle cells and platelets include PDE3, so antiplatelet and vasodilator effects of cilostazol are explanatory [4 6, 14]. Animal experimental models exposed that cilostazol also increases nitric oxide production. Cilostazol is shown to have lower hemorrhagic side effect when compared with other antiplatelet drugs.

The purpose of the present study is to investigate the effects of intrathecal cilostazol bolus infusion and to compare this with IT nimodipine bolus infusions in the treatment of experimentally induced cerebral vasospasm in a rabbit model.

Methods and Materials

All protocols were approved by the Hacettepe University Laboratory Animals Ethics Committee. Our study was carried out in 25 male adult New Zealand White rabbits of weighting 2,000 2,500 g. All animals were left hungry for 6 h before the experiment and anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) administered intramuscularly. Additional doses of the anesthetic mixture were given at almost 30 min intervals if necessary. All animals breathed spontaneously during the procedures. Arterial PO_2 and PCO_2 blood samples were taken from ear arteries of each animal respectively during the procedures, so that animals with poor blood gas levels just as PO_2 less than 70 mmHg and PCO_2 higher than 40 mmHg were excluded from the study.

The solution of cilostazol is prepared according to the report of Clark et al. Cilostazol tablet is dissolved in dimethylsulphoxide as 30 mg/ml stock solution and diluted 10 mg/ml with phosphate-buffered buffered saline to form 10 mg/ml solution of cilostazol.

Induction of SAH

Several methods have been used to induce experimental chronic vasospasm in animals. The method we chose is to inject fresh blood into the cisterna magna, in which this model was reported 31 55% successful in obtaining vasospasm in the basilar artery [3].

An occipitocervical small midline incision was performed and the suboccipital muscles were dissected to expose the atlanto-occipital membrane. The cisterna magna was punctured with a 27-gauge needle, and 0.4 ml of cerebral spinal fluid was gently aspirated. Freshly drawn autologous blood (0.4 ml) taken from the ear artery was then injected slowly into the cisterna magna. All animals were kept in the 45° upside down position for 45 min immediately after the injection of blood had been administered to obtain the blood spread out to all basal cisterns. The wound was then closed.

Experimental Protocol

25 animals were assigned randomly to one of five groups of 5 at each: Group 1 (control group, n = 5) was a sham surgery group in which SAH was not induced. In this group, after anesthesia induction the cisterna magna was tapped by a 27-gauge needle and 0.4 ml of physiological saline (0.9% NaCl) was slowly injected into the cisterna magna after aspiration of the same amount of the cerebrospinal fluid. In group 2 (SAH group, n = 5), the SAH protocol was used to induce cerebral vasospasm as described before. Group 3 (SAH + NMD) was treated with an 0.05 mg/kg nimodipine intrathecally for six times at 6, 12, 18, 24, 30 and 36 h after SAH induced. Group 4 (SAH + CLZ) was treated with an intrathecal 10 mg/kg cilostazol three times at 12, 24 and 36 h after SAH induction. Group 5 (SAH + VHC) was served as a vehicle group and DMSO solution injected intrathecally three times at 12, 24 and 36 h after SAH induction.

At the end of the experimental procedure, after 72 h, the rabbits in all groups were sacrificed under general anesthesia. It is reported that cerebral vasospasm reaches its maximum level during the third day of SAH, so we ended the experiment on the third day [29].

After transcardiac perfusion fixation method with 1,000 ml ringer lactate and then 100 ml of % 4 parafomaldehyde, the brain and brainstem were totally removed and placed in % 4 paraformaldehyde solutions. Basilar arteries were separated from the brain stems, and arterial segments from the proximal third of the artery were dissected for analysis. The arterial segments were washed several times with 0.1 mol = l phosphate-buffered solution (PBS, pH 7.4), fixed in 1% osmium tetroxide in PBS for 1 h at room temperature, and then washed again with PBS. The basilar artery embedded in paraffin and cut a thickness of 0.5 μm slices. The sections were mounted onto glass slides and

stained with H and E for light microscopic analysis. Four sections from four separate zones of the basilar artery were obtained and luminal section areas were measured by using SPOT for Windows Version 4.1 computer program in the Department of Pathology.

Statistical Analysis

The groups were compared with the analysis of variance (ANOVA) test using SPSS for Windows (version 11.5). Following the one-way ANOVA test, a Kruskall Wallis test is performed to examine the differences between the groups. Statistical significance was accepted at $p < 0.05$.

Results

Measurement of the rabbits' physiological parameters revealed no significant differences in mean body weight, mean brain weight, mean blood pressure, and mean blood gas values among the four groups (Fig. 1). Histopathological examination revealed a thick subarachnoid clot over the basal surface of the brain stem in each animal subjected to induction of SAH. Significant narrowing of the diameter of spastic arteries with folding and corrugation of lamina elastica, vacuolization of the tunica media and accumulation of red and inflammatory cells around the outer adventitia were seen in rabbits with SAH, as compared to the control group.

The mean cross sectional areas were measured 115,823.80 ± 18,048.147 μm^2 in group 1; 10,491.0000 ± 3,652.47649 μm^2 in group 2; 45,452.6000 ± 16,934.013 μm^2 in group 3; 33,581.0000 ± 1,235.63445 μm^2 in group 4, and 10,316.6000 ± 759.72186 μm^2 in group 5 (Table 1).

Compared with the sham group (group 1), vasoconstriction of vessel was significant in group 2 and group 5 ($p < 0.05$). Measurements of cross sectional areas between the groups differed significantly. Median levels of cross-sectional areas of basilar arteries in the SAH only group (group 2) were significantly lower than in the SAH+ cilostazol and SAH+ nimodipine groups. Graph shows the mean cross sectional areas of vessels (Fig. 2).

Discussion

Cerebral vasospasm is one of the major causes of morbidity and mortality after aneurysmal subarachnoid hemorrhage (SAH). Various kinds of treatment have been investigated and tried, but no satisfactory therapy has emerged for the prevention or treatment of vasospasm [1].

It is reported that the angiographic vasospasm after subarachnoid hemorrhage (SAH) occurs in 67.3% cases, but delayed ischemic deficit or symptomatic vasospasm in 32.6% [9]. The definite causes of delayed cerebral vasospasm (DCV) are not understood yet, although it is known that DCV and subsequent brain ischemia are critical complications, are seen after subarachnoid hemorrhage (SAH) [14]. Management with fluid loading or induced hypertension and with calcium antagonists has been reported widely for both prevention and treatment, and can reduce the incidence and improve the outcome of vasospasm. Although many more forms of useful treatments like transluminal angioplasty or different vasodilator agents are reported in the literature, large numbers of patients are still being reported in whom no specific treatment is used [9].

Calcium entry blocking agents are recently too popular for both prophylactic and postinsult treatment measures for intracerebral hemorrhage, focal ischemia, global ischemia and vasospasm following subarachnoid hemorrhage [27]. However the literature shows that, the effectiveness of these drugs on prevention or reverse in cerebral vascular constriction depends upon administration route, time of administration and dose of the drug [27].

Nimodipine is a calcium channel blocker, and it has various effects on cerebral circulation. Cerebrovascular smooth muscle is more sensitive than systemic arterial smooth muscle to changes in extracellular calcium concentration and to calcium antagonists such as nimodipine. Nimodipine does not reduce the incidence of severity of cerebral vasospasm detected by angiography in humans or primates, but it reduces the size of cerebral infarcts in rats when given before but not after occlusion of a cerebral artery [21]. Calcium antagonists reduce the influx of calcium into the cell by blocking the L-type calcium channels. The usage of these drugs for prevention of delayed ischemia is based on the principle that they can block the calcium influx into the vascular smooth muscle cell, therefore decreasing the vasospasm rate [17].

The neuroprotective properties of these drugs is also known, however it's a conflict that they can cause hypotension. Lack of prevention of CVS and systemic side effects like this with oral or intravenous administration lead the experiments with topical application [17]. Gioia et al. reported that, intrathecal administration of nimodipine produced a transient drop in blood pressure. In contrast, nimodipine given sublingually or intravenously produced a persistent hypotensive effect without affecting the vasospasm. They found that intrathecal nimodipine reverse the blood induced vasospasm, and suggested intrathecal administration of nimodipine can be an effective treatment of CVS [11, 31].

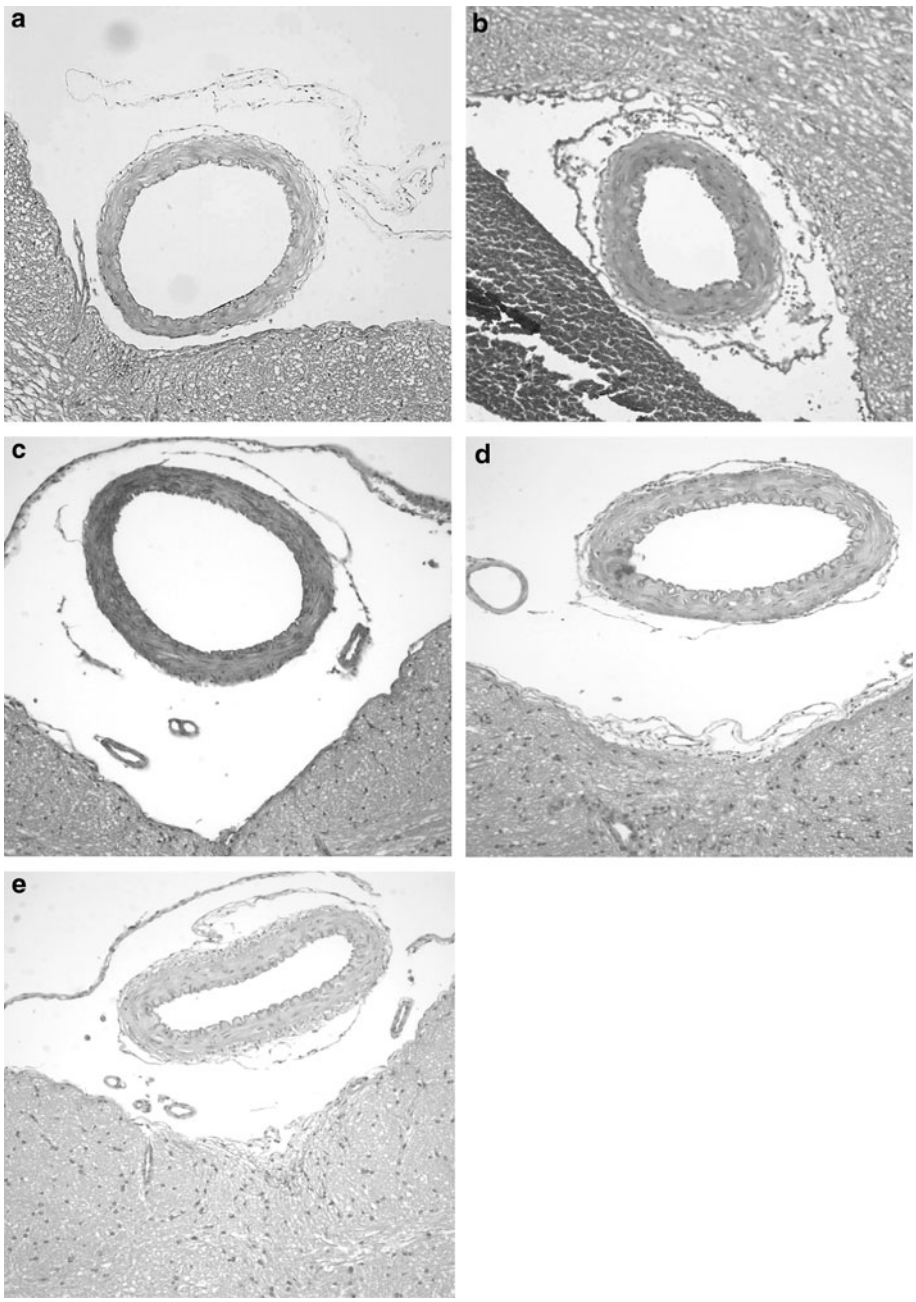

Fig. 1 Photomicrograph showing basilar artery luminal areas and wall thickness in five different groups: (a) Normal basilar artery cross sectional area at × 20 magnification, (b) Basilar artery after SAH induction, (c) Basilar artery after SAH + Cilostazol treatment, (d) Basilar artery after SAH + Nimodipine treatment, (e) Basilar artery after SAH + vehicle treatment. At ×20 magnification normal basilar artery mean cross sectional area is 115,823.80 ± 18,048.147 μm^2. Cross sectional area of the basilar artery after SAH is found 10,491.0000 ± 3,652.47649 μm^2. The mean value of cross sectional area of the basilar artery after SAH + cilostazol treatment is measured 45,452.6000 ± 16,934.013 μm^2. The mean value of cross sectional area of the basilar artery after SAH + Nimodipine treatment is 33,581.0000 ± 1,235.63445 μm^2 and the mean value of cross sectional area of the basilar artery after SAH+ vehicle treatment is 10,316.6000 ± 759.72186 μm^2

Table 1 Physiological parameters of the groups. Measurement of the rabbits' physiological parameters revealed no significant differences

Group	n	Body weight (g)	pH	pCO_2	pO_2	MABP
1	5	2356 ± 42.6	7.43 ± 0.06	41.2 ± 1.1	112 ± 5.56	101 ± 2.27
2	5	2245 ± 37.9	7.42 ± 0.03	40.7 ± 0.9	109 ± 4.87	103 ± 1.89
3	5	2390 ± 47.4	7.43 ± 0.05	41.1 ± 1.2	108 ± 5.02	99 ± 2.51
4	5	2287 ± 50.1	7.44 ± 0.03	40.3 ± 0.7	111 ± 6.22	103 ± 2.98
5	5	2326 ± 25.7	7.43 ± 0.05	41.4 ± 1.1	107 ± 7.54	101 ± 2.78

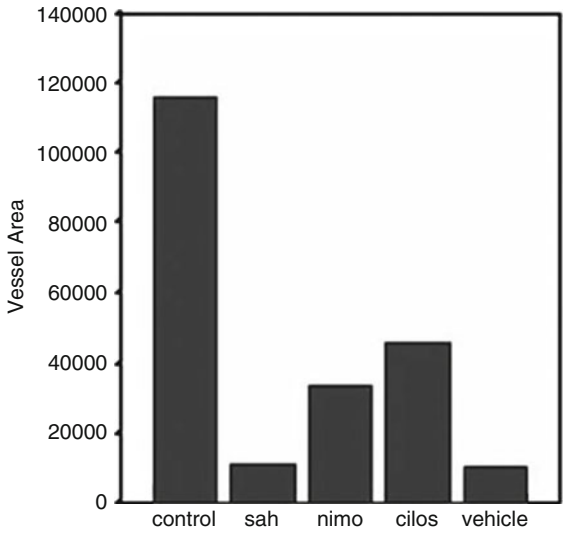

Fig. 2 Graph shows the mean cross sectional areas of vessels

Cilostazol is a selective phosphodiesterase 3 inhibitor, which is a CAMP-mediated antiplatelet and vasodilator agent. It is reported that, cilostazol prevents the thrombus formation and the blood flow reduction in rat carotid arterial thrombosis model, and also reduces the infarct size after transient focal cerebral ischemia in rats [14]. We suggest that these influences may show beneficial effects to improve the disturbance of microcirculation after SAH. Cilostazol was recently approved by the Food and Drug Administration for treatment of intermittent claudication (Dawson et al.). Gotoh et al. reported that cilostazol treatment achieves a considerable risk reduction, which is 41.7%, in patients with recurrent cerebral infarction. Elevation of cyclic-AMP levels was demonstrated to suppress the generation of superoxide anion and hydrogen peroxide in alveolar macrophages. It is also reported that cilostazol effectively scavanges hydroxyl and peroxyl radicals and reduces intracellular hydrogen peroxide [1, 8, 15, 25, 26]. Choi et al. reported that cilostazol decrease of infarct size was associated with decreased oligonucleosomal DNA fragmentation, increased Bcl-2, decreased Bax protein, and reduced cytochrome c release from mitochondria, so it is a potent neuroprotective agent. They also showed that cilostazol significantly reduces cerebral infarct volume [6]. Today, cilostazol is suggested as a new candidate for future clinical trials of delayed cerebral vasospasm [5].

Experimental work has shown that vasodilator drugs can reverse established angiographically identified vasospasm when administered by the intrathecal route, despite being ineffective when administered by the intravenous or intra-arterial route [11, 24, 28]. In a chronic vasospasm study using dogs, prolonged oral administration of nimodipine did not significantly reduce the severity of arterial narrowing. However, in the same animal model, angiographic evaluation 20 30 min after the subarachnoid administration of nimodipine revealed partial resolution of chronic spasm in four of the six dogs. Similar results with topically or intrathecally administered calcium antagonists have been reported by others [27, 31].

The intravenous or intraperipheral route of administration requires larger doses of drugs than intrathecal route, which could lead the adverse effects, reducing the therapeutic efficacy of the drug. The intrathecal route can overcome the inability of intravenous or peripherally administered drugs to allow distribution of the drugs through the entire neuraxis without the step of penetrating the blood-brain barrier [13]. Intrathecal vasodilatory therapy for the treatment of cerebral vasospasm could minimize the risk of systemic adverse effects such as hypotension [24]. Therefore, we decided to choose the intrathecal administration for cerebral vasospasm prevention.

Conclusion

Our aim is to compare the intrathecal route with the other administration routes of effect-proven agent nimodipine on cerebral vasospasm, and also compare it with a promising new agent on delayed cerebral vasospasm called cilostazol. In this manner, the best agent, and the best administration route can be determined by time and new reports. We found no statistically significant differences between intrathecal nimodipine and cilostazol treatment groups ($p < 0.05$), and also between only SAH and vehicle groups ($p < 0.05$). The differences between the sham group and the cilostazol and nimodipine treated groups were statistically significant ($p < 0.05$). We decide that because of the adverse effects with the oral or intravenous route, intrathecal route of administration can reach the therapeutic concentration faster and with less dosage in the cerebrospinal fluid. We all agree that more data is needed to decide the optimal concentration, timing, duration of the infusion and the optimal drug selection. However, in our study, findings indicate that intracisternal infusion of cilostazol is promising for vasodilation prevention to help cerebral vasospasm patients. We suggest that cilostazol will take the place of calcium antagonists in the near future in delayed cerebral vasospasm treatment.

Conflict of interest statement We declare that we have no conflict of interest.

Acknowledgement This study was founded by Turkish Neurosurgery Society.

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Blocking Cerebral Lymphatic Drainage Deteriorates Cerebral Oxidative Injury in Rats with Subarachnoid Hemorrhage

Bao-liang Sun, Fang-min Xie, Ming-feng Yang, Ming-zhi Cao, Hui Yuan, Hai-tao Wang, Jing-ru Wang, and Li Jia

Abstract Substances and fluid in the brain and subarachnoid spaces may be drained into extracranial lymphatics. This study aimed to investigate the possible role of cerebral lymphatic drainage in the process of cerebral injury following subarachnoid hemorrhage (SAH). Wistar rats were divided into non-SAH, SAH, and SAH plus cervical lymphatic blockage (SAH + CLB) groups. Autologous arterial hemolysate was injected into rats' cisterna magna to induce SAH. At time of 24 and 72 h after SAH, the rats were sacrificed for serum lactate dehydrogenase (LDH) activity, brain tissue superoxide dismutase (SOD) activity, and brain tissue malonaldehyde (MDA) content detection. It was found that serum LDH activity increased in rats of SAH group comparing with non-SAH group. SAH also resulted in decreased brain tissue SOD activity and increased brain tissue MDA content. In rats of SAH + CLB group, the increase of serum LDH activity was to a lager extent. Meanwhile, brain tissue SOD activity decreased and MDA content increased to a lager extent, as compared with SAH group. It was concluded that blockage of cerebral lymphatic drainage deteriorates cerebral oxidative injury after SAH, indicating cerebral lymphatic drainage may exert intrinsic protective effects against cerebral injury following SAH.

B. l. Sun (✉), M. f. Yang, H. Yuan, H. t Wang, and J. r. Wang
Key Lab of Cerebral Microcirculation in Universities of Shandong, Department of Neurology, Affiliated Hospital of Taishan Medical College, Taian, Shandong, 271000, China
e mail: blsun88@163.com
F. m. Xie
Department of Neurosurgery, Affiliated Hospital of Taishan Medical College, Taian, Shandong, 271000, China
M. z. Cao
Department of Neurosurgery, Shanxian Central Hospital, Heze, Shandong, 274300, China
L. Jia
Key Lab of Cerebral Microcirculation in Universities of Shandong, Department of Neurology, Affiliated Hospital of Taishan Medical College, Taian, Shandong, 271000, China
Clinical College, Jining Medical College, Jining, Shandong, 272013, China

Keywords Subarachnoid hemorrhage · Cerebral lymphatic drainage · Cerebral oxidative injury · Superoxide dismutase · Malonaldehyde · Lactate dehydrogenase

Introduction

Subarachnoid hemorrhage (SAH) is a potentially life threatening condition. Previous studies have indicated that cerebral injury due to cerebral vasospasm, disturbances of microcirculation, increased intracranial pressure, and oxidative stress, are responsible for the high incidence of morbidity and mortality from this disease [5, 8, 11, 14]. Oxyhemoglobin released from the blood in the subarachnoid spaces is regarded as the primary cause of cerebral vasospasm and secondary cerebral injury after the onset of SAH [10, 12]. Endothelin-1 and other macromolecules also play important roles in the process of cerebral vasospasm [6, 7]. Attention should be paid to the elimination of those macromolecular substances from the brain and/or subarachnoid spaces. It was demonstrated that both cerebrospinal fluid and interstitial fluid of the brain may be drained in part to extracranial lymphatics [9, 16]. Cerebral lymphatic drainage pathway functions to maintain the homeostasis of the central nervous system [16]. In this experiment, the cerebral lymphatic drainage pathway was blocked to investigate the role of this pathway in cerebral oxidative injury in rats with experimental SAH.

Materials and Methods

Animal Preparations

This study was approved by the Institutional Review Board of Taishan Medical College. Wistar rats of both sexes (Experimental Animal Center of Shandong University)

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weighing 330 380 g were housed in groups of five per cage at a constant temperature (24 ± 1°C) and humidity (60 ± 5%), on a 12-h light-dark cycle. The rats were given free access to food and water before and after experiments. Animals were randomly divided into non-SAH, SAH, and SAH + CLB groups.

CLB rat models were replicated 24 h before the induction of SAH using the method described previously by us [13]. Briefly, making the skin incision along the midline of the neck, the bilateral superficial lymphatic nodes were exposed carefully and were removed after ligating their afferent and efferent vessels with a suture. The trachea was exposed after splitting of the subcutaneous tissue and muscles. The deep cervical lymph nodes could be viewed lateral to thyroid cartilage and posterolateral to common carotid artery and vagus nerve. The afferent and efferent lymph vessels of the deep cervical lymph nodes were obstructed. Then, the lymphatic nodes were removed. Submaxillary lymph nodes were also extirpated after obstructing their afferent and efferent lymph vessels. Rat SAH models were replicated by a modified cisterna magna injection with autologus arterial hemolysate. Rats were anesthetized by intraperitoneal use of chloral hydrate and were kept warm with a heating lamp. The left femoral artery was cannulated and 0.4 ml blood was drawn into a heparinized microinjector (310, Stoelting Co. Ltd, USA). The blood was frozen at −80°C for 15 min. Then, the frozen blood was melted at 37°C and the autologus arterial hemolysate was obtained. The rat's head was fixed in a stereotaxic frame (51600, Stoelting Co. Ltd, USA) to maintain a head down position of 30°. An incision was made in the midline and the skin, muscles were carefully separated using an operating microscope to expose the atlanto-occipital membrane. The needle was lowered into cisterna magna under direct vision. To produce SAH, 0.3 ml arterial hemolysate was injected into the cistern very slowly over 20 min with a constant rate. The non-SAH rats were manipulated in the same way, except that same volume of saline was injected into the cistern.

Arterial Blood Gas and Blood Pressure Monitoring

At different time, arterial blood samples were collected from the left femoral artery via the three-way stopcock. The blood samples were used for detection of blood gases, in which arterial pH, partial pressure of oxygen (PaO_2), and partial pressure of carbon dioxide ($PaCO_2$) were involved. After separation of the skin and muscles, the right femoral artery was exposed and cannulated with a cannula connected to a three-way stopcock. The cannula was advanced distally into the abdominal aorta. The pressure module of a Biopac system (MP150, USA) was connected to the cannula. The mean arterial blood pressure (MABP) was monitored and calculated automatically by the software AcqKnowledge version 3.7.2.

Serum Lactate Dehydrogenase Activity Detection

3.5 ml of blood was drawn from abdominal aorta using a syringe without any anticoagulant before the rat was sacrificed for harvesting the brain. The blood was centrifuged in a refrigerated high-speed centrifuge (3 k30, Sigma, USA) for 15 min at 4°C, 3,500 rpm and the supernatant liquid was stored in a −80°C refrigerator. The supernatant liquid was centrifuged for 10 min at 4°C, 3,500 rpm again and the serum was achieved. A colorimetric method was applied to determine serum lactate dehydrogenase (LDH) activity. LDH kit was provided by Jiancheng Biotechnologic Company, Nanjing, China. The detection was made according to the manufacturer's protocol.

Detection of Brain Tissue Superoxide Dismutase Activity and Malonaldehyde Content

Rats were sacrificed after anesthesia with chloral hydrate. The brain was harvested and stored at −80°C. Left cortex was obtained and weighed. The cortex was homogenized with cold physiological saline in iced bath. The tissue homogenate was then centrifuged in a refrigerated high-speed centrifuge for 15 min at 4°C, 4,000 rpm. The supernatant liquid was used for detection. Lowry method was employed to quantitatively detect protein concentration. The superoxide dismutase (SOD) activity and malonaldehyde (MDA) content were determined by a xanthine oxidase method and a sulfurbarbital acid method, respectively. Both SOD and MDA kits were obtained from Jiancheng Institute of Biologic Engineering, Nanjing, China.

Statistical Analysis

Data were expressed as mean ± standard deviation. Comparisons were made with the use of one-way analysis of variance (ANOVA). The level of significance for all tests was $P < 0.05$.

Results

Behavior Observation and SAH Models Verification

Rats in the SAH group showed dysphoria and quiver after cisternal injection, accompanied by increased respiration rate and heart rate. After recovering from anesthesia, the rat showed dispirited and reduced movement. No paralysis due to SAH occurred. Above abnormalities were severe in rats of SAH + CLB group than those in rats of SAH group. The changes of behavior were to a less extent in rats of non-SAH group. Rats were sacrificed and anatomic examinations were performed in the end of the experiment. Extensive arterial hemolysate was found in the subarachnoid spaces, especially in the basilar region in rats of SAH and SAH + CLB groups.

Arterial Blood Gas and Blood Pressure

After cisternal injection of autologus arterial hemolysate or saline, the MABP increased 30 min after induction of SAH ($P < 0.05$, $P < 0.01$). There was no significant difference among non-SAH, SAH and SAH + CLB groups. MABP returned to normal level 1 h after cisternal injection (Table 1).

Serum Lactate Dehydrogenase Activity

Comparing with non-SAH group, serum LDH activities in rats of SAH group increased by 52.60% and 89.89% at time of 24 and 72 h after induction of SAH ($P < 0.01$). Serum

Table 1 Arterial blood gas analysis ($x \pm s$, n 6)

Group	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	MABP (mmHg)
Non SAH				
Baseline	7.38 ± 0.02	125.6 ± 7.6	33.2 ± 4.5	101.7 ± 7.9
0.5 h	7.40 ± 0.03	124.5 ± 8.1	32.8 ± 5.1	121.3 ± 6.7*
1 h	7.39 ± 0.02	124.8 ± 5.8	33.4 ± 3.5	103.7 ± 7.8
6 h	7.39 ± 0.01	123.9 ± 4.7	33.2 ± 4.8	102.5 ± 5.8
SAH				
Baseline	7.39 ± 0.02	124.8 ± 6.1	33.5 ± 5.6	102.3 ± 8.5
0.5 h	7.38 ± 0.03	123.7 ± 6.6	34.3 ± 6.1	132.1 ± 8.4**
1 h	7.38 ± 0.02	123.9 ± 5.4	33.3 ± 3.9	100.1 ± 8.3
6 h	7.40 ± 0.03	122.8 ± 4.1	32.7 ± 4.7	102.1 ± 9.1
SAH + CLB				
Baseline	7.38 ± 0.02	123.8 ± 9.2	32.8 ± 6.3	103.2 ± 5.7
0.5 h	7.40 ± 0.02	122.7 ± 7.3	34.3 ± 7.8	133.4 ± 7.8**
1 h	7.41 ± 0.03	124.3 ± 4.6	35.4 ± 5.6	105.9 ± 7.7
6 h	7.39 ± 0.03	122.3 ± 7.2	32.7 ± 4.6	104.2 ± 8.6

*$P < 0.05$, **$P < 0.01$ vs. baseline

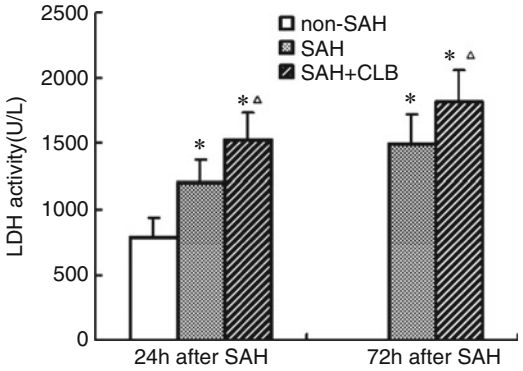

Fig. 1 Serum lactate dehydrogenase activities after SAH ($x \pm s$, n 6). *$P < 0.01$ vs. non SAH; $^{\Delta}P < 0.05$ vs. SAH

LDH activity in rats of SAH + CLB group increased by 27.38% and 21.63% at time of 24 and 72 h after induction of SAH, as compared with those in rats of SAH group ($P < 0.05$) (Fig. 1).

Brain Tissue Superoxide Dismutase Activity and Malonaldehyde Content

After induction of SAH, the brain tissue SOD activities in SAH rats significantly decreased at times of 24 and 72 h, which were 60.52% and 54.46% of non-SAH rats at time of 24 and 72 h, respectively. Brain tissue SOD activities in SAH + CLB group decreased more significantly, which were 51.52% and 41.01% of non-SAH group ($P < 0.01$) and reduced by 14.88% and 29.42% comparing with SAH group. The SOD activity at the time of 72 h after SAH induction in SAH + CLB group was lower than that of SAH group statistically ($P < 0.01$).

Seventy two and twenty four hours after induction of SAH, brain tissue MDA contents in rats of SAH group increased markedly, which were 1.76 and 1.96 times of rats in non-SAH group ($P < 0.01$). An additional enhancement of brain tissue MDA contents in rats of SAH + CLB group was found. MDA contents were 24.46% and 21.88% higher than those in rats of SAH group at time of 24 and 72 h, respectively ($P < 0.05$) (Fig. 2).

Discussion

The central nervous system does not possess defined lymphatic channels that are comparable with lymphatic vessels in organs elsewhere in the body. Nonetheless, studies suggest a link between cerebrospinal fluid and extracranial lymph [4]. Radiolabeled proteins injected into the cerebrospinal fluid of

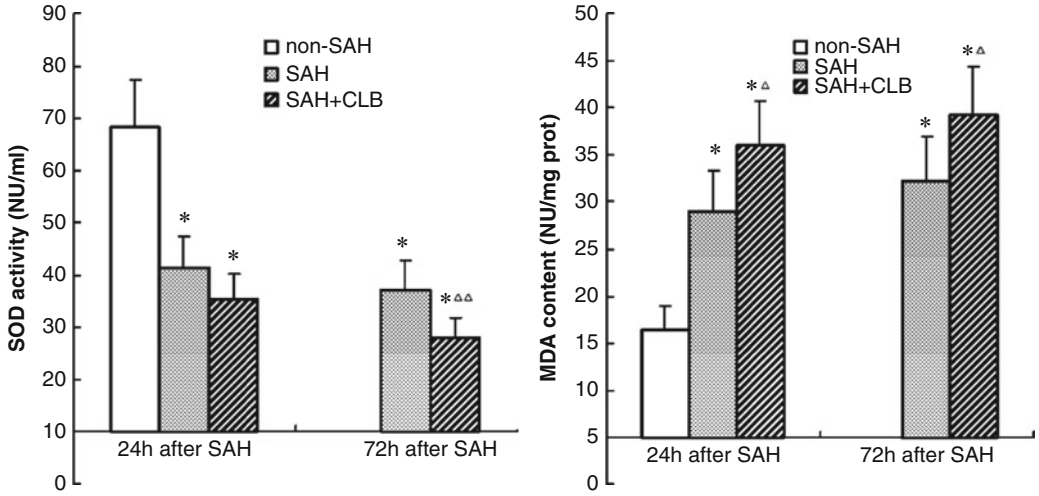

Fig. 2 Brain tissue superoxide dismutase (SOD) activities (*left panel*) and malonaldehyde (MDA) contents (*right panel*) after SAH ($x \pm s$, n 6). $^{*}P < 0.01$ vs. non SAH; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. SAH

rabbits or cats or into brain parenchyma of rabbits were recovered in cervical lymph [16]. Although the exact connections between the interstitial fluid and extracranial lymphatics are still being debated, two routes for cerebral lymphatic drainage, namely, perineurolymphatics and prelymphatics, were suggested [15, 16]. Substances, especially large molecules in the brain and cerebrospinal fluid, are mainly drained to cervical lymphatic tubes and lymph nodes [13, 16]. When cerebral lymphatic drainage is blocked, there appear various abnormalities in the morphology and function of the brain in different animals [3, 16].

Serum LDH level is regarded as a biochemical marker of neuronal injury [17]. Decreased SOD activity and increased MDA content in brain tissues indicate oxidative stress in cerebral ischemia and SAH [1, 2]. It was found in this experiment that SAH led to increased serum LDH activity and increased brain tissue MDA content, as well as decreased brain tissue SOD activity, indicating the occurrence of SAH-related cerebral oxidative injury. The results also showed that obstruction of cerebral lymphatic drainage deteriorates cerebral oxidative injury following experimental SAH. After onset of SAH, large amounts of blood and blood products, including hemoglobin and oxyhemoglobin, are present in the subarachnoid spaces. The severe outcome of SAH is mainly regarded as the result of secondary cerebral injury caused by SAH-induced cerebral ischemia, the impact to the brain during bleeding, etc. [8, 11, 14]. SAH-induced cerebral ischemia may lead to oxidative neuronal damage. As a result, a great amount of metabolites, including proteins and other large molecules (e.g., neurotoxic and vasoconstricting substances), accumulate in the interstitial spaces of the brain, which, in turn, induce brain edema and aggravate cerebral ischemia. An additional amount of large molecules accumulate in the interstitial space after obstruction of cerebral lymphatic drainage [13], and, therefore, cerebral ischemia and oxidative injury are exacerbated. It is reasonable to speculate from the results that cerebral lymphatic drainage may exert intrinsic protective effects against cerebral injury following SAH.

Conflict of interest statement We declare that we have no conflict of interest.

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Comparison of Intrathecal Dotarizine and Nimodipine Treatments in Cerebral Vasospasm After Subarachnoid Hemorrhage: An Experimental Study in Rabbits

Mehmet Bulent Onal, Ilker Solmaz, Erdinc Civelek, Atilla Kircelli, Ozkan Tehli, Yusuf Izci, Ersin Erdogan, and Engin Gonul

Abstract *Background*: Cerebral vasospasm (CVS) is one of the most considerable complications of subarachnoid hemorrhage (SAH). The aim of this study was to assess and to compare the ability of intrathecal dotarizine and nimodipine to prevent and treat vasospasm in a rabbit model of subarachnoid hemorrhage.

Method: Thirty male New Zealand white rabbits weighing 2,500 3,000 g were allocated into five groups randomly. The treatment groups were as follows: Control, only SAH, SAH/Dotarizine, SAH/Nimodipine, SAH/Vehicle. Forty-eight hours after SAH injection, all animals underwent femoral artery catheterization procedure by open surgery under anesthesia and angiography performed for each animal in the fifth day just before sacrifice.

Findings: Basilar artery vessel diameters are measured by angiography. Basilar artery vessel diameters and luminal sectional areas are measured in pathology slides. There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($p < 0.05$).

Conclusions: These findings demonstrate that calcium channel blocker dotarizine has marked vasodilatory effect in an experimental model of SAH in rabbits. Nimodipine is an effect-proven agent in CVS, but dotarizine may take place of it.

Keywords Subarachnoid hemorrhage · Cerebral vasospasm · Animal models · Nimodipine · Dotarizine · Intrathecal

M.B. Onal (✉), I. Solmaz, E. Civelek, A. Kircelli, Y. Izci, E. Erdogan, and E. Gonul
Department of Neurosurgery, Gulhane Military Academy, Ugur Mumcunun Sokak, 78/2, 06700 Gaziosmanpasa, Ankara, Turkey
e mail: bulentonal@hotmail.com
O. Tehli
Erzurum Military Hospital, Erzurum, Turkey

Introduction

Cerebral vasospasm (CVS), causing high rates of morbidity and mortality, is still counted as one of the most important complications after subarachnoid hemorrhage (SAH). Despite all the studies, the point we came today is not satisfactory yet [3]. Arterial vasospasm following SAH is not yet entirely understood pathophysiologically. The literature suggests that the vascular smooth muscle cell constriction can be hindered by calcium channel blockers so that the deficits after delayed ischemia can be reduced. Therefore nimodipine is currently recommended as the first line medication together with hypervolemia, hypertension, hemodilution in clinical practice for the medical treatment of CVS [2, 7].

Dotarizine is an antimigraine and antivertigo drug which belongs to diphenylbutyl- piperazines [6]. This novel piperazine derivative is structurally related to flunarizine. Dotarizine denotes strong antiserotonergic properties and has no tendency to accumulate in the tissue. In vivo experimental studies of dotarizine revealed that dotarizine produce a slight peripheral vasodilatation but a definite cerebrovascular dilatation. Tejerina et al. demonstrated more selective influence of dotarizine on rabbit basilar artery rather than on the aorta [11, 13]. In rabbit aortic smooth muscle, dotarizine inhibits calcium uptake and vessel contractility. Kuridze et al. [6] mentioned that dotarizine may be useful in the treatment of cerebrovascular disorders where vasoconstriction plays a significant role. Villaroya et al. suggests that dotarizine presents superiority over flunarizine in their comparative calcium antagonist study. They concluded that dotarizine is safer clinically because it does not accumulate in the tissues so less adverse reactions can be seen [13].

The purpose of the present study is to investigate the effects of intrathecal (IT) dotarizine bolus infusion and to compare this with IT nimodipine bolus infusions in the

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treatment of experimentally induced cerebral vasospasm in a rabbit model.

Methods and Materials

Animal Model

The protocol of this study was reviewed and approved by Gulhane Military Academy. A total of 30 adult male New Zealand White rabbits weighing 2.5 3.0 kg were randomly assigned to five experimental groups. The groups did not differ significantly in physiological parameters (body weight, $PaCO_2$, mean arterial blood pressure, sex). Animals were anesthetized with ketamine (Ketalar, 50 mg/kg) and xylazine (Xylazin, 10 mg/kg) administered intramuscularly. Additional doses were added at 20 30 min intervals when necessary.

All groups were consisted of six rabbits. Animals in group 1 served as controls (n = 6), group 2 was named the SAH only group (n = 6), group 3 was treated with 50 mg/kg intrathecal dotarizine two times at 24 and 48 hours (h) after SAH induction (n = 6), group 4 was the nimodipine treatment group in which the animals were given 0.2 mg/kg nimodipine two times at 24 and 48 h after SAH induction (n = 6) and group 5 was the vehicle group in which the animals was given 0.5 ml vehicle two times at 24 and 48 h after SAH induction (n = 6).

An injection of 0.3 ml autologous blood from ear artery into the subarachnoid space was made to induce SAH.

Every animal underwent angiography procedure to visualize both vertebral and basilar arteries in the 5th day after SAH formation just before sacrificed. After 5th day angiography, all animals subjected to experimental SAH study were euthanized by perfusion-fixation under general anesthesia.

Dotarizine Solution and Vehicle

Dotarizine was dissolved in dimethylsulphoxide (DMSO) and diluted in saline solutions to the desired concentrations. A15 mg/ml dotarizine concentration was produced and added to group 3 in doses of 50 mg/kg at 24 and 48 h after SAH induction (n = 6). DMSO was approved as the vehicle and was diluted in 10 ml of distilled water and 0.5 ml/kg were given to group 5 with intrathecal infusions at 24 and 48 h after SAH induction (n = 6).

SAH Formation

After shaving the dorsal parts of neck and head, under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. To enter the subarachnoid space, atlanto-occipital membrane was punctured in a head hyperflexion position. After withdrawal of 0.3 ml of CSF, equal volume of autologous fresh nonheparinized blood from the central ear artery was injected in 3 min into the subarachnoid space to induce SAH. The animals were then placed at a 65° angle head-down position for 15 min to allow blood dissemination throughout the subarachnoid space.

Angiography Procedure

Under general anesthesia following preparation and draping of the right femoral regions of the rabbits under sterile conditions, an 18-gauge angiocath was introduced to the right femoral artery via surgical means (through cut-down) and fixated to the skin with surgical sutures. Then the rabbits were transferred to the angiography table and oblique lateral projection DSA (digital substraction angiography) images of the basilar arteries of these rabbits were obtained in an old-style CCD camera detector device, with fairly good quality. The technique of angiography was as follows:

A bolus of ketalar-xyilazine mixture was given i.m for extended general anesthetic effect (without intubation) and the rabbit was laid supine on the table. After sterile preparation and draping, 18-gauge angiocath in the right femoral system of the rabbit was exchanged for a 3F 11 cm introducer sheath of a micro-puncture set, through which 0.018 in. angled-tip micro-guide wire assisted catheterization of the left vertebral artery was achieved using a 1.8F/2.2F (distal/proximal) diameter microcatheter. 1 or 2 cc syringes (as necessary) were used for contrast media injection. Iobitridol 300 mg Iodine/ml (Xenetix 300) was the contrast media used for all rabbits. Approximately 1 ml of contrast media was required per basilar artery run and one or two runs were obtained in each rabbit, as necessary. When the contrast media used for navigation of the microcatheter to the left vertebral artery is included, total contrast media use ranged between 4 and 8 ml (median 6 ml). Good quality images of the basilar artery were acquired through left vertebral artery injections in all rabbits but one, which had a dissection of the left vertebral artery during microcatheterization and the right vertebral artery had to be used. Total blood volume loss during angiography procedure did not exceed 20 30 ml in all rabbits. Rabbits were sacrificed just after the procedure by perfusion-fixation method. Every rabbit

underwent angiography procedure in the 5th day of SAH induction to visualize and measure the diameter of the basilar artery.

Perfusion-Fixation

All animals subjected to experimental SAH study were euthanized by perfusion-fixation 5 days after SAH induction. After anesthetic injection thoracotomy, was performed, the left ventricle was cannulated, the right atrium opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution (Hanks' balanced salt solution [Sigma Chemical Co.], pH 7,4 at 37°C, 300 ml), the fixative was perfused (2% paraformaldehyde, 2, 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 37°C, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were killed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Embedding, Morphometry, and Statistical Analysis

The basilar artery was embedded in paraffin and cut a thickness of 0.5 μm slices. The sections were mounted onto glass slides and stained with H & E for light microscopic analysis. Four sections from four separate zones of the basilar artery were obtained and luminal section areas were measured by using Image J computer program in the Department of Pathology.

The groups were compared with the analysis of variance (ANOVA) test using SPSS for Windows (version 11, 5). Following the one-way ANOVA test, a Kruskall Wallis test is performed to examine the differences between the groups. Statistical significance was accepted at $p < 0.05$.

Results

Mortality, Morbidity, and Neurological Parameters

Physiological parameters of animals, which are given in Table 1, did not differ significantly.

One animal belonging to the SAH only group died immediately after SAH formation as a result of respiratory arrest. One animal of the vehicle group died of blood loss during angiography. All other animals were in good condition during all the procedures. A significant reduction of neurological scores and hypo-activity was observed in the SAH group and SAH/vehicle group. Clinical daily follow up of the animals was uneventful until the 5th day when they were sacrificed.

Pathological Measurements

Changes in the basilar artery diameter, wall thickness and arterial luminal areas are shown in Table 2. Histological sections of the basilar artery luminal areas were analyzed by a computerized image-analysis system (Fig. 1). In the control group (no SAH, no treatment) mean basilar artery luminal area was 152,675 ± 3,400 μm^2. In group 2 in which SAH was induced, mean luminal area was 26,932 ± 6,210 μm^2. In SAH-induced and dotarizine treated group mean luminal area was 118,691 ± 4,317 μm^2. In SAH- induced and nimodipine treated group mean luminal area was 123,432 ± 8,294 μm^2. Finally in the vehicle group mean luminal area was measured to be 41,418 ± 4,416 μm^2.

Mean luminal area of groups 3 and 4 are significantly smaller than the mean area of group 1 ($p < 0.05$). When treatment groups (groups 3 and 4) were compared, there was no significant difference in terms of luminal area ($p > 0.05$). In contrast, mean luminal area of SAH-induced and treated groups were significantly greater than that of SAH-only group.

Table 1 Summary of physiologic parameters of the groups. Measurement of the rabbits' physiological parameters revealed no significant differences between days 0 and 5

Days	Group	n	Body weight (g)	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	MABP (mmHg)
0	Control	6	2,634.5 ± 25.3	7.44 ± 0.03	51.3 ± 1.04	63.2 ± 5.76	73.4 ± 5.76
5			2,634.5 ± 25.3	7.43 ± 0.04	52.4 ± 1.10	64.3 ± 5.17	74.1 ± 5.17
0	Only SAH	6	2,812.0 ± 32.4	7.43 ± 0.05	49.1 ± 1.09	68.6 ± 7.37	71.6 ± 3.17
5			2,812.0 ± 32.4	7.42 ± 0.07	48.7 ± 1.03	61.3 ± 4.17	71.5 ± 2.36
0	SAH/ IT Dot	6	2,778.2 ± 26.6	7.42 ± 0.03	52.4 ± 1.10	66.1 ± 6.14	63.4 ± 3.64
5			2,778.2 ± 26.6	7.42 ± 0.05	51.2 ± 1.20	67.4 ± 5.26	68.2 ± 1.86
0	SAH/ IT Nim	6	2,648.4 ± 30.6	7.43 ± 0.07	52.9 ± 1.07	68.5 ± 5.76	69.4 ± 4.75
5			2,648.4 ± 30.6	7.42 ± 0.05	51.3 ± 1.04	67.8 ± 5.47	72.6 ± 3.17
0	SAH/ Vehicle	6	2,734.7 ± 31.6	7.42 ± 0.06	55.9 ± 1.03	69.3 ± 5.16	70.1 ± 6.13
5			2,734.7 ± 31.6	7.41 ± 0.03	54.4 ± 1.10	70.4 ± 5.42	72.4 ± 3.11

Compared with the control group (group 1), vasoconstriction of vessel was significant in groups 2 and 5. Measurements of cross sectional areas between the groups differed significantly ($p < 0.05$). Median levels of cross sectional areas of basilar arteries in the SAH only group (group 2) were significantly lower than in the SAH+ dotarizine and SAH+ nimodipine groups. The differences between the control group and the dotarizine and nimodipine treated groups were statistically significant ($p < 0.05$). There were no statistically significant differences between intrathecal dotarizine and cilostazol treatment groups ($p > 0.05$), and also between only SAH and vehicle groups ($p > 0.05$).

Table 2 Changes in the basilar artery diameter, wall thickness and arterial luminal areas. This table shows a summary of the effects of nimodipine and dotarizine treatments compares them with the other groups. All values were derived from n = 6 groups except the SAHonly and SAH/ Vehicle groups were derived from n = 4. All values are expressed as mean ± standard deviation

Groups	Wall thickness (μm)	Perimeter of arterial lumen (μm)	Cross sectional areas (μm^2)
Control	21.3 ± 0.2	715 ± 31	152,675 ± 3,400
SAH only	27.6 ± 0.4	267 ± 29	26,932 ± 6,210
SAH/Dotarizine	18.3 ± 0.2	503 ± 37	118,691 ± 4,317
SAH/Nimodipine	17.7 ± 0.1	597 ± 51	123,432 ± 8,294
SAH/Vehicle	26.9 ± 0.3	289 ± 44	41,418 ± 4,416

Angiographic Measurements

The angiographic basilar artery measurements of five groups are exposed on Table 3. Measurement studies were performed by two independent radiologist in a single blind fashion. Measurement of each vessel (basilar artery) on every angiogram was performed two times at four different levels, so the mean values were determined (Fig. 2). The mean measures of the control and the SAH only groups are 0.672 and 0.539 mm, respectively. IT dotarizine and IT nimodipine treated SAH induced groups basilar artery measures were 0.832 and 0.869 mm in order. Treatment groups have no significant difference statistically ($p > 0.05$). Basilar artery angiographic measures of SAH/ Vehicle group was found 0.547 mm in which no significant difference was found between only SAH group ($p > 0.05$).

Table 3 Angiographic measurements of five groups compared to each other are shown on the table

Groups	Control (mm)	only SAH (mm)	Treatment (5th day)
SAH/Dotarizine	0.539 ± 0.03	0.672 ± 0.01	0.832 ± 0.03
SAH/ Nimodipine	0.539 ± 0.03	0.672 ± 0.01	0.869 ± 0.01
SAH/Vehicle	0.539 ± 0.03	0.672 ± 0.01	0.547 ± 0.02

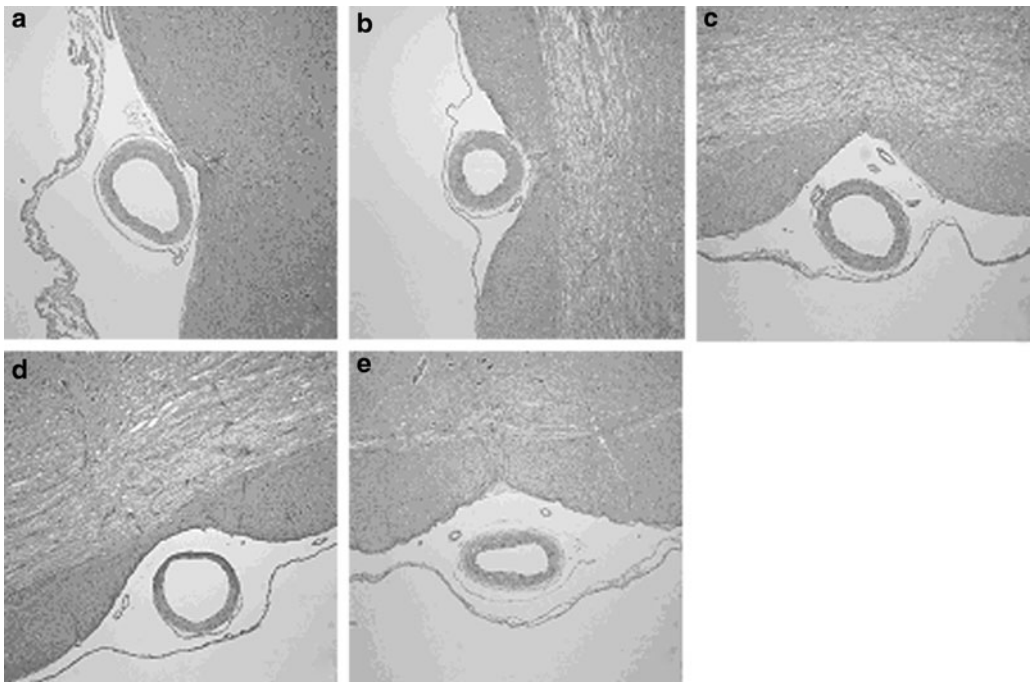

Fig. 1 Photomicrograph showing basilar artery luminal areas and wall thickness in five different groups. (**a**) Control, (**b**) SAH only, (**c**) SAH/ Dotarizine, (**d**) SAH/Nimodipine, (**e**) SAH/Vehicle

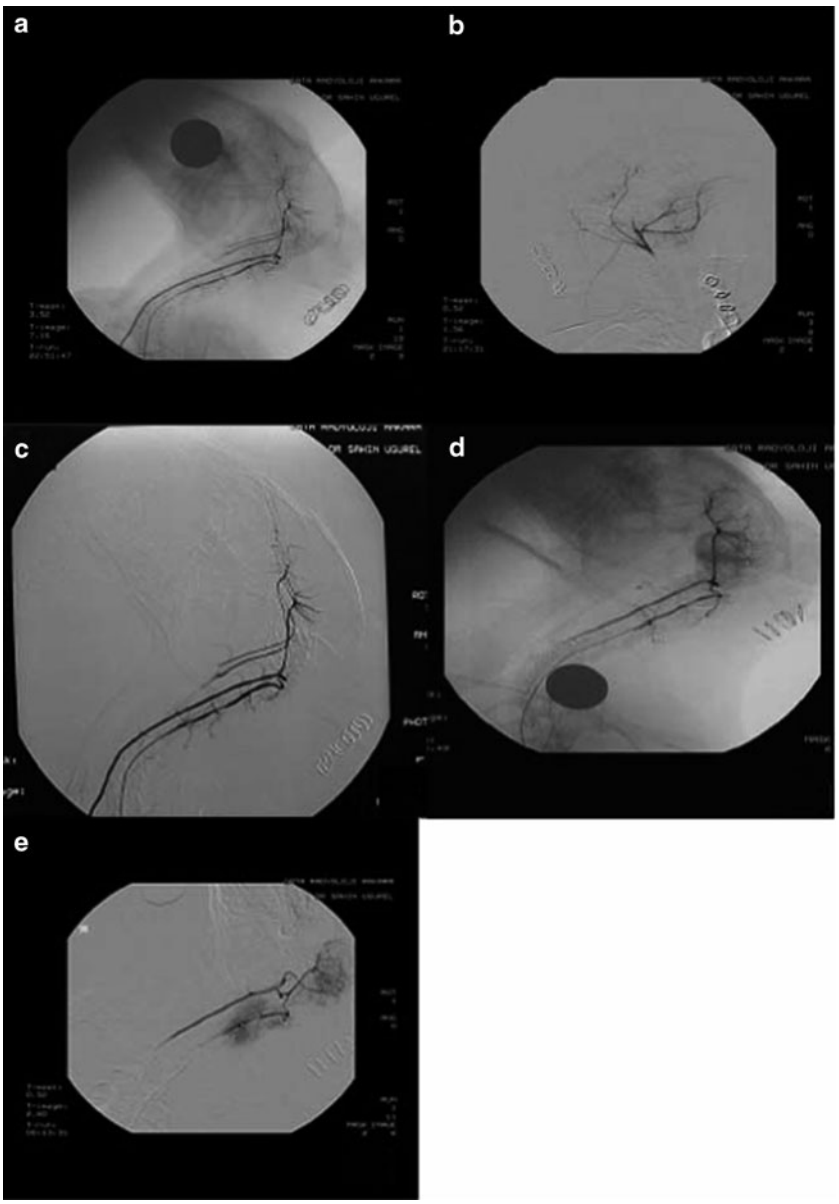

Fig. 2 Rabbit angiograms. (**a**) Control, (**b**) Only SAH, (**c**) SAH/ Dot, (**d**) SAH/Nimo, (**e**) SAH/Vehicle

Discussion

Cerebral vasospasm following aneurysmal vasospasm has been the subject of intensive research. However the underlying pathophysiological mechanisms remain obscure [8]. The combination therapy of hypervolemia, hemodilution, and hypertension (triple-H therapy) represents the mainstay of treatment for CVS [5, 9].

Dotarizine is a novel piperazine derivate that is currently a promising drug because of its antimigraine and antivertigo effects. CVS is responsible from the major ratio or morbidity and mortality after SAH. Nowadays calcium antagonists are popular in CVS treatment. Dotarizine, a calcium antagonist, inhibits calcium entry through calcium channels. It is much more selective for the basilar artery [8, 11, 13]. Villarroya et al. suggest that dotarizine has the advantage of reversibility of its calcium antagonistic effects. Dotarizine is a safer drug as it does not accumulate in tissue cells so less adverse reactions can be seen [13].

The mechanism of action of calcium antagonists in SAH is attributed mainly to both a neuroprotective cellular effect and a vasodilator effect. Calcium antagonists reduce the risk of poor outcome and secondary ischemia after aneurysmal SAH [2]. Nimodipine is a dihydropyridine calcium channel antagonist that has been shown to decrease the incidence of overall cerebral infarction after SAH by 34% and the incidence of poor outcomes by 40% [4, 5, 9]. It is the most widely preferred calcium channel blocker in CVS treatment.

The use of oral or intravenous nimodipine together with triple-H therapy including volume expansion, induced hypertension and hemodilution to increase cerebral blood flow and also to decrease the blood viscosity are the mainstay of medical treatment for CVS [1]. The intravenous use of nimodipine has been associated with possible induction of systemic hypotension, requiring dose adjustments to keep mean arterial pressure higher than 90 mmHg [12]. Villarroya et al. suggest that dotarizine has the advantage of reversi bility of its calcium antagonistic effects. Dotarizine is a safer drug as it does not accumulate in tissue cells so less adverse reactions can be seen [13].

The intravenous or intraperipheral route of administration requires larger doses of drugs than intrathecal or selective intra-arterial routes, which could lead to the adverse effects, reducing the therapeutic efficacy of the drug [2, 10]. It is suggested that the results for poor outcome in CVS treatments depend largely on a single large trial of oral nimodipine. That is why we decided to choose the intrathecal route in our study. In our opinion, because of its less adverse effects as a calcium channel blocker, intrathecal dotarizine may be a potential alternative to nimodipine for the treatment and prevention of vasospasm.

We found no statistically significant differences between intrathecal nimodipine and dotarizine treatment groups ($p > 0.05$). Nimodipine is an effect-proven agent in CVS, but dotarizine may take in place of it. We suggest that this drug could be used as possible anti-vasospastic agent in patients without major adverse effects. Further clinical experiments should be done.

Conflict of interest statement We declared that we have no conflict of interest.

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Changes of Blood–Brain Barrier Permeability Following Intracerebral Hemorrhage and the Therapeutic Effect of Minocycline in Rats

Wei Shi, Zizhang Wang, Jingnan Pu, Ruizhi Wang, Zhengyu Guo, Chongxiao Liu, Jianjun Sun, Ligui Gao, and Ren Zhou

Abstract *Objective*: To investigate the changes of blood brain barrier (BBB) permeability and expressions of VEGF, NGF and HPS70 in brain at different time points following intracerebral hemorrhage (ICH) in rats, and observe therapeutic effect of minocycline (MC).

Methods: Rat ICH model was induced with Type IV collagenase. Early MC treatment was administrated via intraperitoneal injection. BBB permeability was evaluated by Evans blue (EB) amount exuded out of cerebral vessels. VEGF, NGF, and HPS70 expressions were determined with immunohistochemical staining.

Results: EB exudation amount in MC treatment group was less than the ICH group ($P < 0.05$). The former showed a transient EB exudation peak only 1 h after modeling and then gradually decreased, while the latter showed two EB exudation peaks 1 and 4 days after modeling, respectively. The number of VEGF-positive cells in MC treatment group was less than the ICH group ($P < 0.05$), whereas the number of NGF- and HSP70-positive cells were more than the ICH group ($P < 0.05$). All three were mainly expressed in neurons and gitter cells, but there were only few expressions in the control group.

Conclusion: After ICH, the BBB permeability was destroyed, with neuron function affected. In the early stage, VEGF increased BBB permeability, while NGF and HSP70 showed protective effects on nerve cells. Early intraperitoneal injection with MC could reduce the damage of BBB and increase the protective effect on nerve cells, the mechanism of which may be achieved by reducing VEGF expression and enhancing NGF and HSP70 expressions.

Keywords Blood brain barrier · Intracerebral hemorrhage · VEGF · NGF · HPS70

Abbreviations

BBB	Blood brain barrier
ICH	Intracerebral hemorrhage
IP	Ischemic penumbra
MC	Minocycline
VEGF	Vascular endothelial cell growth factor
VEC	Vascular endothelial cell

Introduction

Intracerebral hemorrhage (ICH) is a common and frequently encountered disease which is harmful to human health, with high incidence (81/110,000 per year) and case fatality (43 45%). The secondary brain injury following ICH is one of the important reasons of high disability and mortality in ICH patients [1, 2]. Although molecular biology research, neuroimaging and neurological rehabilitation of ICH have made great progress in recent years, the therapeutic effect and prognosis were not improved fundamentally. Therefore, the study of brain injury following ICH is still the focus of the field of neuroscience. In our study, based on the ICH model of rats, the expressions of VEGF, NGF and HPS70 in brain at different time points following ICH in rats and the changes of BBB permeability surrounding the hematoma were observed. The expression changes of VEGF, NGF and HPS70 in brain at different time points following ICH in rats, the changes of BBB permeability and the therapeutic effect of minocycline (MC) as well as its mechanism were investigated, in order to provide a new way for the early prevention and treatment of secondary brain injury following ICH.

W. Shi (✉), Z. Wang, J. Pu, R. Wang, Z. Guo, C. Liu, J. Sun, L. Gao, and R. Zhou
Department of Neurosurgery, Second Affiliated Hospital of Xi'an Jiaotong University, 157 Xiwu Road, Xi'an 710004, P.R China
e mail: sweins@21cn.com

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Materials and Methods

Experimental Animals

Totally 78 healthy male adult SD rats, weighting 200 250 g, were purchased from Experimental Animal Center, Xi'an Jiaotong University. The rats were given standard foodstuff and free access to water in a quiet environment, with constant temperature of 20 25°C.

Animal Grouping

The 78 rats were randomly divided into three groups: (1, 2) ICH group and MC treatment groups: These two groups were further divided into six subgroups including 1, 2, 4, 5, 7, and 14 days according to the different time points following ICH, with six rats in each subgroup. (3) Control group: Six rats were included in the control group, with 4 days after modeling as the general control point.

Establishment of Rat ICH Model

Rat ICH model was induced with Type IV collagenase. After 12 h fasting and 4 h water deprivation preoperatively, intraperitoneal anesthesia was performed with 2% chloral hydrate (350 mg/kg), with additional anesthesia if necessary to ensure the spontaneously breathing during operation. The rat head was fixed on the stereotaxic apparatus at the prone position. The scalp was cut longitudinally. The bregma was adopted as the starting point, and cut toward right for 3 mm, then backward for 1 mm, and then the point of 5 mm depth was for the injection point (caudate nucleus). The skull was opened with the drill. After needling, 1 μl of the mixture of type IV collagenase, heparin and normal saline (0.2 U type IV collagenase and 2 U heparin per 1 μl mixture) was slowly injected. After 10 min, the needle was retracted slowly. The skull hill was blocked with bone wax, and then the scalp was sutured. During the operation, the breathing of the rats was paid attention to, and the respiratory tract was kept unobstructed. The body temperature of the rats was controlled at 36.5 37.5°C. After operation, the rats were placed in an air-conditioned animal room. For the MC treatment group, 6 h after the model was established according to the above method, 45 mg/kg of MC (initial dose) was administrated via intraperitoneal injection. Thereafter, 22.5 mg/kg of MC was administrated per 12 h for continuous 7 days [3]. In the control group, only normal saline was administrated.

Determination of BBB Permeability

Two percent Evan's blue (EB) 3 mg/kg was injected via vena caudalis of rats in all three groups 2 h before sacrifice. Before brain sampling, normal saline was poured into the left ventricle until the clear liquid was outpoured from the right atrial appendage. About 3 mm thickness of brain tissue in the injury cross section was harvested. The section was divided into four parts including cortex and subcortex in the ICH side, and cortex and subcortex in the healthy side. These four parts were put into a homogenizer for homogenate, and the homogenate liquid was 2 ml of 50% trichloroacetic acid (TCA). After fully homogenized, the brain tissue homogenate liquid was centrifuged at 15,000 rpm for 20 min. The absorbance was detected at 635 nm which was the maximum absorption spectra of EB. The EB content was obtained according to the EB standard curve. The result was represented as μg/g brain tissue.

Sampling and Preparation

After modeling, excessive 2% chloral hydrate was adopted for anesthesia at each corresponding time point. Thoracotomy was performed quickly and the heart was exposed immediately. Then aortic cannulation was performed via the left ventricle. A microstomia was made in the right atrial appendage, and 100 ml of 4% paraformaldehyde was poured rapidly. Then the pour was slowed down for 30 min for fixation until the animal limbs stiffened. The animal was sacrificed by decapitation and the brain was obtained. About 5 mm thickness of coronal slices were made with the microsyringe needle tract as the center. The samples were dehydrated for 2 3 days in 4% paraformaldehyde/30% sucrose/0.1 M PBS solution, respectively, until the samples sank to the bottom, suggesting complete dehydration. Then the serial sections were made in the constant freezing microtome for the immunohistochemistry, with the thickness of 20 μm.

Expressions of VEGF, NGF and HPS70 Detected by Immunohistochemistry

Firstly, frozen sections were taken out from the cryogenic refrigerator, and then washed three times with PBS, each for 3 min. The sections were incubated with 50 μl peroxidase for 10 min at room temperature, and then washed three times with PBS, each for 3 min. Then the sections were incubated with 50 μl 5% BSA for 60 min at room temperature. The redundant liquid was removed, and then the sections were

incubated with 50 μl of VEGF (NGF, HPS70) primary antibody at 4°C overnight. Then the sections were washed three times with PBS, each for 5 min. The redundant PBS was removed, and then the sections were incubated with biotin-labeled secondary antibody for 90 min at room temperature, and then washed three times with PBS, each for 3 min. The sections were incubated with SABC for 20 min at room temperature, and then washed with PBS three times with PBS, each for 3 min. The PBS was removed, and then the sections were incubated with 100 μl fresh DAB solution. The sections were observed under microscope for 10 min, followed by dehydration, clearing and sealing with neutral gum.

Statistical Analyses

All three groups of animal sections were observed under microscope (×400). Five fields of vision surrounding the hematoma cavity of the injured side were randomly selected. The number of VEGF-, NGF- and HPS70-positive cells was counted, and the average served as the results. All data were expressed by $x \pm s$. SPSS 13.0 software was adopted for the statistical analyses. Double-factor ANOVA was adopted for the comparison of each group. $P < 0.05$ was considered statistically significant.

Results

Changes of EB Content in Rat Brain Tissue Following ICH

EB exudation could be observed 1 day after modeling in the ICH group and the MC treatment group, and two peaks of EB exudation appeared 1 and 4 days after modeling, respectively. The MC treatment group showed a transient peak of EB exudation only 1 h after modeling and then gradually decreased. The control group only showed a small quantity of EB content, without exudation fluctuation. There was statistical significance in the EB exudation in the brain tissue at each time point in the three groups ($P < 0.05$) (see Table 1).

VEGF Expression in Brain Tissue Detected by Immunohistochemistry

In the early stage of ICH, VEGF was mainly expressed in vascular endothelial cells (VEC), neurons in perihematoma and cortex and the cytoplasm of gitter cells. Positive cells showed brown, and appeared after 1 day following ICH (Fig. 1a, b). The number of VEGF-positive cells was gradually increased with the time extension following ICH. It was significantly increased after 4 days, and mainly expressed in ischemic penumbra VEC after 7 days. Many microvascular and blood vessel-like structure could be observed (Fig. 1c, d), and a large quantity of neovascularization could be observed after 14 days (Fig. 1e, f). Simultaneously, the number of VEGF-positive cells in the MC treatment group was less than the ICH group, whereas there were only few VEGF-positive cells in the control group. There were statistical significances in the number of VEGF-positive cells among these three groups at different time points ($P < 0.05$) (see Table 2).

NGF and HSP70 Expressions in Brain Tissue Detected by Immunohistochemistry

NGF-positive cells in the brain tissue surrounding the hematoma showed widely distributed small brown particles in the cytoplasm. The nuclei were also partly colored. It was mainly expressed in neurons and gitter cells. HSP70 was mainly expressed in the neurons and gitter cells in the ischemic penumbra surrounding the hemorrhagic focus. The positive cells were that cells showed brown cytoplasm or nuclei. Normal brain tissue did not express HSP70. The former could be observed only 1 h following ICH in the ICH group and the MC treatment group. Subsequently, the number of NGF-positive cells was gradually increased with the

Table 1 Changes of EB exudation in rat brain tissue following ICH ($x \pm s, n = 6$)

Groups/time	1 day	2 days	4 days	5 days	7 days	14 days
ICH group	251.2 ± 8.3*, **	229.8 ± 7.9*, **	269.6 ± 2.4*, **	190.1 ± 11.7*, **	120.7 ± 12.1*, **	70.6 ± 4.1*, **
MC treatment group	211.8 ± 11.1*	191.3 ± 2.1*	211.8 ± 9.9*	133.6 ± 6.6*	71.8 ± 6.7*	31.1 ± 5.9*
Control group			15.4 ± 1.3			

Note: Comparison between the ICH group as well as the MC treatment group and the control group at each time point. *$P < 0.05$
Comparison between the ICH group and the MC treatment group at each time point. **$P < 0.05$

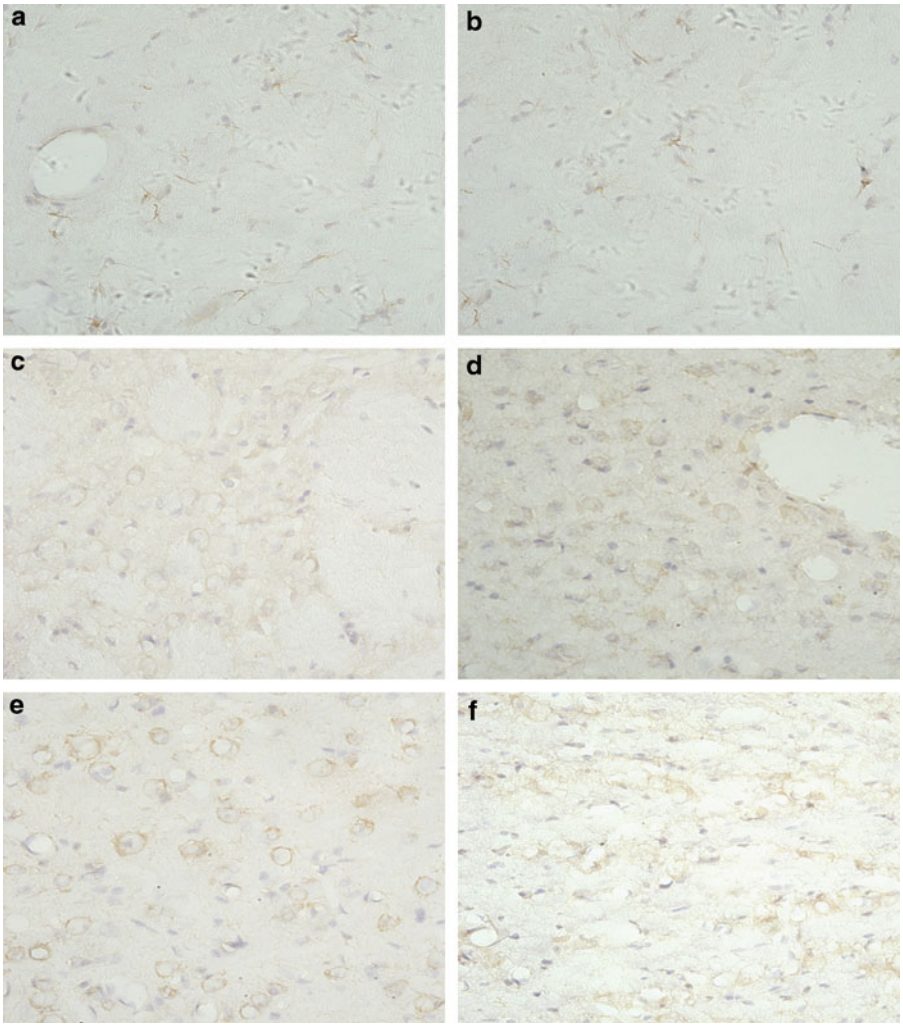

Fig. 1 (**a**) VEGF positive cells surrounding the hemorrhagic focus 1 day after ICH in the ICH group. VEGF was mainly expressed in neurons ×400; (**b**) VEGF positive cells surrounding the hemorrhagic focus 1 day after ICH in the MC treatment group. VEGF was mainly expressed in neurons. The difference was not significant between the MC treatment group and the ICH group. ×400; (**c**) VEGF positive cells 7 days after ICH in IP region in the ICH group. VEGF was mainly expressed in VEC ×400; (**d**) VEGF positive cells 7 days after ICH in IP region in the ICH group. VEGF was mainly expressed in VEC, and the expression amount in the MC treatment group was less than the ICH group ×400; (**e**) VEGF positive cells 14 days after ICH in IP region in the ICH group. VEGF was mainly expressed in VEC ×400; (**f**) VEGF positive cells 14 days after ICH in IP region in the MC treatment group. VEGF was mainly expressed in VEC, and the expression amount in the MC treatment group was less than the ICH group ×400

Table 2 Cell counting for VEGF positive cells at different time points following ICH ($x \pm s, n \quad 6$)

Group/time	1 day	2 days	4 days	5 days	7 days	14 days
ICH group	25.3 ± 0.9*, **	36.4 ± 1.1*, **	57.4 ± 4.6*, **	82.7 ± 5.0*, **	97.4 ± 4.2*, **	107.9 ± 2.4*, **
MC treatment group	19.9 ± 1.1*	31.4 ± 1.2*	42.8 ± 2.1*	47.5 ± 5.8*	72.6 ± 5.1*	83.6 ± 5.2*
Control group			6.5 ± 0.9			

Note: Comparison between the ICH group as well as the MC treatment group and the control group at each time point. *$P < 0.05$
Comparison between the ICH group and the MC treatment group at each time point. **$P < 0.05$

extension of time, and significantly increased after 4 days (Fig. 2a, b), and then gradually decreased after 7 days. However, HSP70 expression peaked between 24 and 48 h following ICH in the ICH group and the MC treatment group (Fig. 2c, d). Subsequently, the number of HSP70 positive cells was gradually reduced with the time extension. Simultaneously, the number of NGF- and HSP70-positive cells in MC treatment group was more than the ICH group. However, there were only few NGF- and HSP70-positive cells in the control group. There were statistical significances in the number of NGF- and HSP70-positive cells at each time point in these three groups ($P < 0.05$) (see Tables 3 and 4).

Discussion

Currently, it is considered that the mechanism of brain injury following ICH is mainly divided into two categories: One is primary brain injury, the main pathological change of which is the mass effect of hematoma [4, 5], resulting in the acute expansive destruction of brain tissue, brain tissue displacement due to the mechanical pressure, loss of nerve function due to local microvessel ischemic contracture, obstruction and necrosis [6], thereby leading to the corresponding brain injury. The other is secondary brain injury, which was mainly caused by the release of hematoma ingredients and their

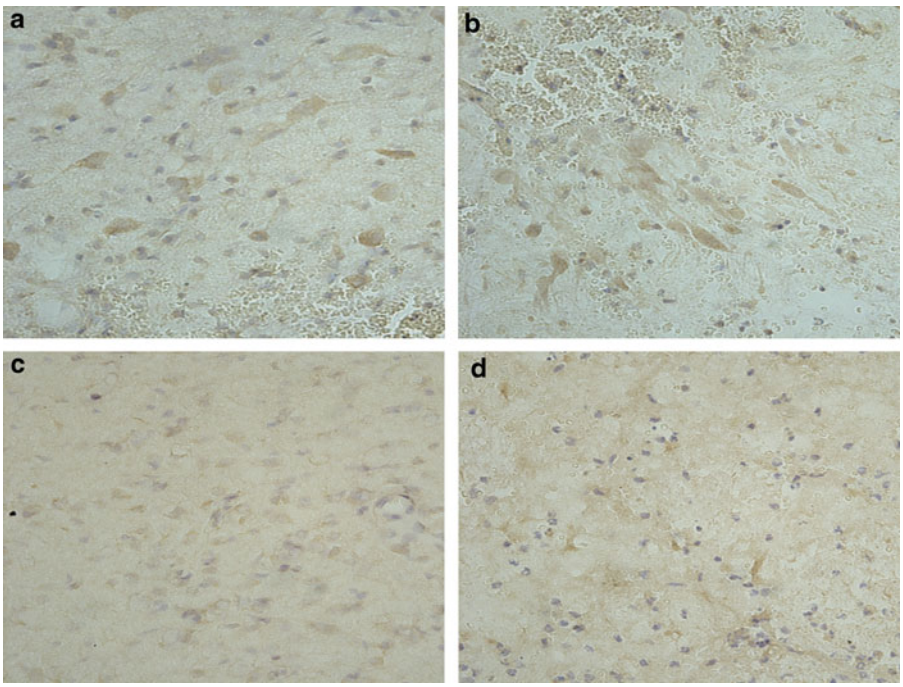

Fig. 2 (**a**) NGF positive cells surrounding the hemorrhagic focus 4 days after ICH in the ICH group ×400; (**b**) NGF positive cells surrounding the hemorrhagic focus 4 days after ICH in the MC treatment group ×400; (**c**) HSP70 positive cells surrounding the hemorrhagic focus 2 days after ICH in the ICH group ×400; (**d**) HSP70 positive cells surrounding the hemorrhagic focus 2 days after ICH in the MC treatment group ×400

Table 3 Cell counting for NGF positive cells at different time points following ICH ($x \pm s, n \quad 6$)

Group/time	1 day	2 days	4 days	5 days	7 days	14 days
ICH group	229.7 ± 2.0*, **	232.1 ± 3.2*, **	233.8 ± 1.4*, **	241.9 ± 2.6*, **	247.4 ± 3.3*, **	240.8 ± 2.6*, **
MC treatment group	265.1 ± 2.6*	267.2 ± 2.2*	272.7 ± 7.9*	282.0 ± 2.1*	285.5 ± 2.1*	281.5 ± 2.0*
Control group			68.8 ± 5.8			

Note: Comparison between the ICH group as well as the MC treatment group and the control group at each time point. *P < 0.05
Comparison between the ICH group and the MC treatment group at each time point. **P < 0.05

Table 4 Cell counting for HSP70 positive cells at different time points following ICH ($x \pm s, n \quad 6$)

Group/time	1 day	2 days	4 days	5 days	7 days	14 days
ICH group	51.1 ± 1.9*, **	56.8 ± 0.4*, **	34.1 ± 1.1*, **	22.5 ± 0.9*, **	5.7 ± 0.4*, **	1.9 ± 0.2*, **
MC treatment group	80.8 ± 1.5*	89.8 ± 0.9*	53.9 ± 0.7*	35.8 ± 1.0*	7.6 ± 0.3*	2.8 ± 0.3*
Control group			0.4 ± 0.1			

Note: Comparison between the ICH group as well as the MC treatment group and the control group at each time point. *P < 0.05
Comparison between the ICH group and the MC treatment group at each time point. **P < 0.05

cleavage products (such as the thrombin and hemoglobin), the chemotaxis of the inflammatory cells and the secretion of inflammatory factors [7], the apoptosis of neurons and VEC, brain edema and brain swelling due to the destruction of BBB permeability [8, 9], resulting in the death of the patients caused by cerebral hernia. Studies for the secondary brain injury following ICH showed that the inflammatory reaction [10, 11], the release of inflammatory factors and the leakage of new vessels occurred during the early stage following ICH, thus resulting in the destruction of BBB permeability accompanied by vasogenic brain edema [7]. Simultaneously, some active substances in the blood can destroy VEC, causing the increase of a great quantity of the surrounding complement forming membrane attack complex and free radicals that can destroy VEC. The instruction of the new vessels is imperfect. All of these factors can lead to the increase of BBB permeability and induce the overexpression of inflammatory cytokines by gradual accumulation, eventually leading to DNA damage, apoptosis and necrosis of nerve cells. Although MC is the second-generation semi-synthetic derivate of tetracyclines and it has been already successfully used in clinical practice for many years [12, 13]. Lately it has been discovered that MC has a good inhibitory effect on inflammatory reaction, which is entirely different from its anti-infection effect. Moreover, it has high lipophilicity and tissue penetration, and easily penetrates BBB to the central nervous system. Recently, it has been confirmed in animal models of central nervous system that MC has neuroprotective effects such as

anti-inflammatory effect, anti-apoptotic effect and antioxidative effect [14 22]. Our results showed that EB exudation could be observed only 1 h after modeling in the ICH group and the MC group, and two EB exudation peaks were observed 1 and 4 days after modeling in the ICH group; while the MC group showed a transient peak of EB exudation only 1 h after modeling and then gradually decreased, and only a very small amount of EB exudation was observed in the control group. There was statistical significance in the EB exudation amount in the brain tissue at each time point in these three groups ($P < 0.05$), suggesting that there existed two BBB openings following ICH. The first BBB opening was due to the primary brain injury following ICH, and the first BBB opening was the initiating agent of the second BBB opening and also the main reason for secondary brain injury. Accordingly, MC intervention treatment via intraperitoneal injection during the early stage of BBB opening following ICH was performed in our study, which could inhibit the inflammatory reaction and the release of inflammatory factors in time during the early stage following ICH, thereby reducing the damage to the BBB and enhancing nerve cell protective action to block the second opening of BBB and effectively prevent and cure the secondary brain injury after ICH in the early stage. Therefore, the early MC intervention treatment following ICH is an effective measure for the prevention and cure of the secondary brain injury after ICH.

VEGF is a highly specific vascular endothelial growth factor which was discovered in recent years. It specifically promotes the endothelial cells proliferation, involves in blood vessel growth and increases vasopermeability, playing an important role in a variety of physiological and pathological process. NGF was the first discovered neurotrophic factor. It is a cell regulatory factor with dual biological functions including neurons' nutrition and promoting neurite growth. HSP70, also known as stress protein, is a group of highly conserved peptides in the structure, involved in cell injury and repair. It is generated under stress conditions (such as nutritional deficiencies, ischemic hypoxia, etc.) and expressed in ischemic penumbra following ICH with neuroprotective effect. It is the marker of reversible damage of nerve cells. Therefore, it is considered a sensitive and reliable marker for ICH [23]. Our results showed that VEGF, NGF and HSP70 expressions appeared only 1 h following ICH in the ICH group and the MC treatment group. It was mainly expressed in neurons and gitter cells. Subsequently, the number of the VEGF- and NGF-positive cells was gradually increased with the extension of time. VEGF was significantly increased after 4 days and highly expressed in the vascular endothelial cells surrounding the hematoma between 7 and 14 days; while NGF expression was significantly increased after 4 days, and peaked after 7 days, and then gradually decreased. However, HSP70 expression peaked between 24 and 48 h, and the number of HSP70 positive cells was gradually reduced with the time extension. Simultaneously, the number of VEGF-positive cells in MC treatment group was less than the ICH group, whereas the number of NGF- and HSP70-positive cells was more than the ICH group. However, there were only few expressions of VEGF, NGF and HSP70 in the control group. There was statistical significance in the number of VEGF-, NGF- and HSP70-positive cells at each time point in these three groups ($P < 0.05$), suggesting that there was a certain time and spatial distribution regularity in the VEGF-, NGF- and HSP70-positive cells following ICH. Therefore, the early MC intervention treatment following ICH via intraperitoneal injection was adopted in our study, which decreased VEGF expression and increased NGF and HSP70 expressions, thus protecting neurons in the early stage following ICH and promoting the recovery of neurological function, so it had an important value for effectively preventing and curing the secondary brain injury. The mechanism of this effect may be due to the inhibitory effect of MC early application on VEGF following ICH [24], thus inhibiting inflammatory reaction and the expressions of inflammatory factors following ICH. However, NGF may regulate the expression of protective NGF high-affinity receptor TrkA during the cerebral ischemia reperfusion injury by NGF and activate the downstream PI-3K/Akt pathway, thus inhibiting the excessive release of aminoglutaminic acid and reducing the brain injury due to Ca^{2+} overload, thus playing the protective role for the brain. However, the neuroprotective effect of HSP70 was probably related to the cerebral ischemia due to hematoma oppression, resulting in increased HSP70 expression and the inhibition of cell apoptosis. However, our experiment was the initial study about VEGF, NGF and HSP70 expressions following ICH and the relationship between MC intervention treatment and ICH, many pathophysiological mechanisms of which were still unclear. Therefore, it still needs further in-depth study. The corresponding agents or antagonists should be prepared after assessing the advantages and disadvantages, in order to provide a more extensive prospect for ICH treatment.

Conflict of interest statement We declare that we have no conflict of interest.

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Comparison of Intrathecal Flunarizine and Nimodipine Treatments in Cerebral Vasospasm After Experimental Subarachnoid Hemorrhage in Rabbits

Erdinc Civelek, Ilker Solmaz, Mehmet Bulent Onal, Atilla Kircelli, Caglar Temiz, Halil Ibrahim Secer, Yusuf Izci, and Engin Gonul

Abstract *Background*: The aim of this study was to assess and to compare the ability of intrathecal flunarizine and nimodipine to prevent vasospasm in a rabbit model of subarachnoid hemorrhage (SAH).

Method: Forty male New Zealand white rabbits were allocated into 5 groups randomly. The treatment groups were as follows: (1) control (no SAH [n = 8]), (2) SAH only (n = 8), (3) SAH plus vehicle (n = 8), (4) SAH plus nimodipine (n = 8), and (5) SAH plus flunarizine (n = 8). Before sacrifice, all animals underwent femoral artery catheterization procedure by open surgery under anesthesia and angiography performed for each animal.

Findings: There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($p < 0.05$). Basilar artery vessel diameter and luminal section areas in group 4 were significantly higher than in group 2 ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 5 were significantly higher than in group 2 ($p < 0.05$).Basilar artery vessel diameter and basilar artery luminal section areas in group 5 were significantly higher than in group 4 ($p < 0.05$).

Conclusions: These findings demonstrate that flunarizine has marked vasodilatatory effect in an experimental model of SAH in rabbits.

E. Civelek (✉), I. Solmaz, M.B. Onal, A. Kircelli, C. Temiz, H.I. Secer, Y. Izci, and E. Gonul
Department of Neurosurgery, Gulhane Military Academy, Ugur Mum cunun Sokak, 78/2, 06700, Gaziosmanpasa, Ankara, Turkey
e mail: civsurgeon@yahoo.com

Keywords Flunarizine · Nimodipine · Bsilar artery · Cerebral vasospasm · Subarachnoid hemorrhage

Introduction

Delayed vasospasm after SAH is a major component of brain damage after SAH. It is characterized by the prolonged and reversible contraction of the cerebral arteries that adds to ischemic process [7]. Studies on the prevention and reversal of cerebral vasospasm are focused on various therapeutic agents and the different pathways thought to be involved in vasoconstriction. Although many approaches to prevent vasospasm have been proposed, including hypertensive hypervolemic hemodilution therapy (triple H) [11], cisternal drainage with or without intrathecal administration of plasminogen activators [8], and calcium antagonists, a conclusive method has not yet been determined.

Flunarizine (1-Cinnamyl-4-(bis (p-fluorophenyl) methyl) piperazine dihydrochloride) is a selective Ca^{++}-antagonist with weaker H1-histaminergic antagonistic properties. Flunarizine reduces vasospasm caused by an exaggerated Ca^{++} influx in depolarized arteries and by vasoactive substances released from aggregating platelets. This drug also antagonizes the mutual amplification of the vasoconstrictor action of the platelet products. Flunarizine is particularly effective in the cerebral blood vessels, also when they are hyperreactive because of hypoxia. The compound does not interfere with normal arteriolar autoregulation or heart function. This compound had no vasodilating properties in the pulmonary circulation. Thus flunarizine may be particularly effective in preventing vasospasm without lowering blood pressure or inducing a steal phenomenon [16].

In this study, our aim was to examine the effects of intrathecal flunarizine and to compare the efficiency of intrathecal nimodipine and intrathecal flunarizine.

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Methods and Materials

Animal Model

The Gulhane Military Medical Academy Laboratory Animals Ethics Committee approved all protocols. Experiments were carried out in 40 male New Zealand White rabbits weighing 2,500 3,000 g. SAH induction and arterial angiography are easier to perform and demonstrate in rabbits, so the rabbit model was preferred for this study. All animals were starved for 8 hours before the procedures anesthetized with a mixture of ketamine (Ketaset, 50 mg = kg) and xylazine (Rompun, 10 mg = kg) administered intramuscularly. Additional doses were added at 20 30 min intervals when necessary. Animals were assigned randomly to one of five groups according to treatment protocols. All groups consisted of eight rabbits. Animals in group 1 served as control (n = 8) and group 2 as SAH only (n = 8). The vehicle (dimethylsulphoxide(DMSO, Merck)) was diluted in 10 ml of distilled water and 0.5 ml/kg were given to group 3 in 30 min intrathecal infusions at 1, 5 and 7 days after SAH induction (n = 8). Nimodipine (Nimotop®) were administered to group 4 in a dosage of 12 μg/kg in 30 min intrathecal infusions at 1, 5 and 7 days after SAH induction (n = 8). Flunarizine (Sibelium®) was dissolved in dimethylsulphoxide (DMSO, Merck) at 10^{-2} M and diluted in saline solutions in a concentration of 2 mg/ml and administered to group 5 in 30 min intrathecal infusions in doses of 0.5 mg/kg at 1, 5 and 7 days after SAH induction (n = 8).

SAH Formation

After shaving the dorsal parts of neck and head, under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. To enter the subarachnoid space easily, atlanto-occipital membrane was punctured in a head hyperflexion position. After withdrawal of 1 ml of CSF, equal volume of autologous fresh nonheparinized blood from the central ear artery was injected in 3 min into the subarachnoid space to induce SAH. The animals were then placed in a head-down position for 30 min to hold the blood in the basal cisterns. Every rabbit underwent angiography procedure in the seventh day of SAH induction to visualize and measure the diameter of the basilar artery.

Perfusion-Fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 7 days after SAH induction. After induction of anesthesia, thoracotomy was performed. Left ventricle was cannulated while the right atrium was opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution (Hanks' balanced salt solution [Sigma Chemical Co.], pH 7,4 at 37°C, 300 ml), the fixative was perfused (2% paraformaldehyde, 2, 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 37°C, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were sacrificed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Neurological Parameters

All neurological evaluations were performed by an observer blinded to the study plan. The initial evaluation was completed between 6 and 12 h after the SAH; following assessments were completed on the third and fifth days. All of the scoring was performed 6 h after anesthesia. The neurological scale used for the assessments was based on previous study of Strong et al. with rabbits [14]. As a result, clinical observations (spontaneous behavior, reaction to handling, posture, gait, limb hypertonia, righting reflexes, and feeding behavior) were each given a score: 0 (absent); 1 (mild); 2 (moderate); or 3 (severely impaired). Similarly, front and back reflexes were scored: 0 (normal); 1 (brisk); 2 (spreading); or 3 (clonus). Nystagmus was also observed: 0 (absent) or 1 (present). An overall score was calculated as the sum of the individual observations; a greater score reveals more significant neurological impairment, and a lower score reveals a lesser degree of neurological impairment.

Embedding, Morphometry, and Statistical Analysis

The basilar artery was embedded in paraffin and cut a thickness of 0.5 μm slices. The sections were mounted onto glass slides and stained with H and E for light microscopic analysis. Four sections from four separate zones of the basilar artery were obtained and luminal section areas were measured by using Image J computer program in the Department of Pathology.

The groups were compared with the analysis of variance (ANOVA) test using SPSS for Windows (version 11, 5). Following the one-way ANOVA test, a Kruskall Wallis test is performed to examine the differences between the groups. Statistical significance was accepted at $p < 0.05$.

Results

Physiological parameters of the rabbits revealed no significant differences in mean body weight, mean brain weight, mean blood pressure, and mean blood gas values among the five groups. Gross pathological examination revealed a thick subarachnoid clot over the basal surface of the brain stem in each animal subjected to induction of SAH.

No rabbits died in the groups. Clinical daily follow-up of the animals was free of problems until the day 5 when they were sacrificed. Gross pathological examination showed no signs of infection. Clinical observation of the rabbits assessed by blinded veterinarians. A reduction of neurological scores and hypoactivity was observed in the SAH group and SAH plus vehicle group. Neurological scores in the nimodipine and flunarizine treatment groups were significantly higher ($p > 0.05$) than only SAH and SAH plus vehicle groups.

The mean wall thickness of the basilar arteries in group 1 (control group) was 23.1 ± 0.3 μm, whereas in group 2 (SAH only) it was 30.2 ± 0.5 μm; in the SAH + vehicle group: 24.6 ± 0.3 μm; in the SAH + Nimodipine group: 23.5 ± 0.2 μm and in the SAH + Flunarizine group: 21.1 ± 0.2 μm (Table 1). The mean diameter of arterial lumen in group 1 (control group) was 830.52 ± 60 μm, whereas in group 2 (SAH only) it was 358.50 ± 32 μm; in the SAH + vehicle group: 760.19 ± 25 μm; in the SAH + Nimodipine group: 563.15 ± 12 μm and in the SAH + Flunarizine group: 768.13 ± 41 μm (Table 1). The mean cross-sectional areas of basal arteries in group 1 (control group) was 173,315 ± 13,000 μm^2, whereas in group 2 (SAH only) it was 31,224 ± 2,000 μm^2; in the SAH + vehicle group: 100,261 ± 9,100 μm^2; in the SAH + Nimodipine group: 101,483 ± 8,900 μm^2 and in the SAH + Flunarizine group: 161,216 ± 12,000 μm^2 (Table 1).

The pictures of light microscopic examination of the basilar artery from group 1 5 are shown in Fig. 1. The pictures of angiographic examination of the basilar artery from group 1 5 are shown in Fig. 2.

There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 4 were significantly higher than in group 2 ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 5 were significantly higher than in group 2 ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 5 were significantly higher than in group 4 ($p < 0.05$).

Discussion

Cerebral vasospasm and the resulting cerebral ischemia after SAH are still the major determinants of morbidity and mortality in patients affected by cerebral aneurysm. Main reason is that delayed cerebral vasospasm is an effect of multiple factors initiated by ruptured aneurysm. Cerebral vasospasm is due to the presence of blood clot and its metabolism that causes releasing of some vasoactive substances. There is no single pharmacological agent or treatment protocol which is effective in inhibiting the multiple factors.

Vasodilators used for the treatment of delayed cerebral vasospasm may cause some unwanted effects on the brain. They may cause hypotension that is believed to be harmful in delayed cerebral vasospasm. If any of these drugs increases the cerebral blood flow by dilating cerebral arterioles, this may cause steal phenomena, which means the shunting of perfusion to normal areas of the brain. In our study, it has been shown that flunarizine dilates the basilar artery without adversely affecting the cerebral blood flow and blood pressure. And it seems that intrathecal flunarizine is more effective than intrathecal nimodipine in delayed cerebral vasospasm.

Fujita et al. administered flunarizine orally and concluded that flunarizine significantly inhibits the occurrence of severe neurological deficit due to delayed vasospasm in poor-grade aneurysm patients [3]. Zumkeller et al. concluded that flunarizine is a potential alternative of nimodipine in treatment of cerebral vasospasm as well as for cerebral blood flow improvement [17]. Kuba et al. concluded that flunarizine has a more prolonged and greater pharmacological activity on the responses of the

Table 1 Changes in the basilar artery diameter, wall thickness and arterial luminal areas. All values were derived from n = 8 groups and all values are expressed as mean ± standard deviation

Groups	Wall thickness (μm)	Diameter of arterial lumen (μm)	Cross sectional areas (μm^2)
Control	23.1 ± 0.3	830.52 ± 60	173,315 ± 13,000
SAH only	30.2 ± 0.5	358.50 ± 32	31,224 ± 2,000
SAH plus vehicle	24.6 ± 0.3	760.19 ± 25	100,261 ± 9,100
SAH plus nimodipine	23.5 ± 0.2	563.15 ± 12	101,483 ± 8,900
SAH plus flunarizine	21.1 ± 0.2	768.13 ± 41	161,216 ± 12,000

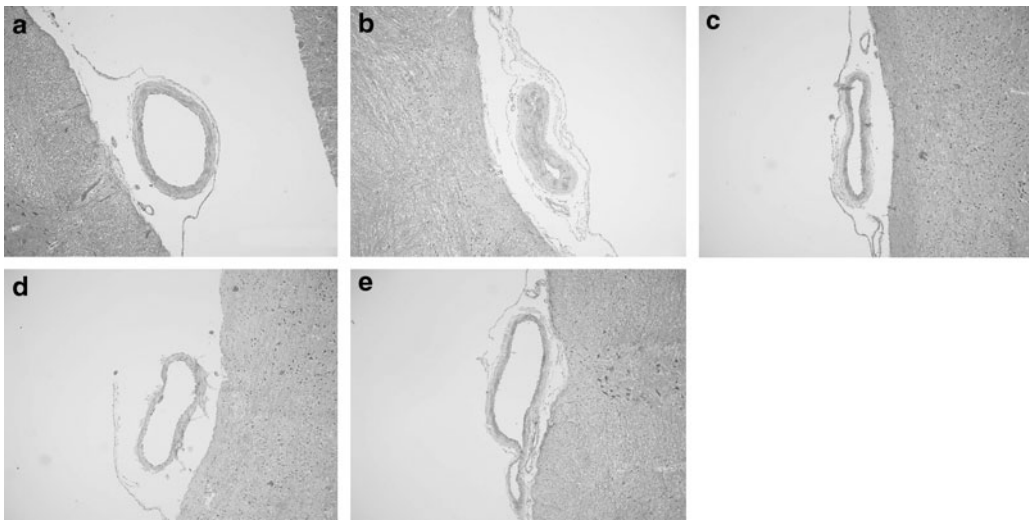

Fig. 1 The pictures of light microscopic examination of the basilar artery from group 1 5

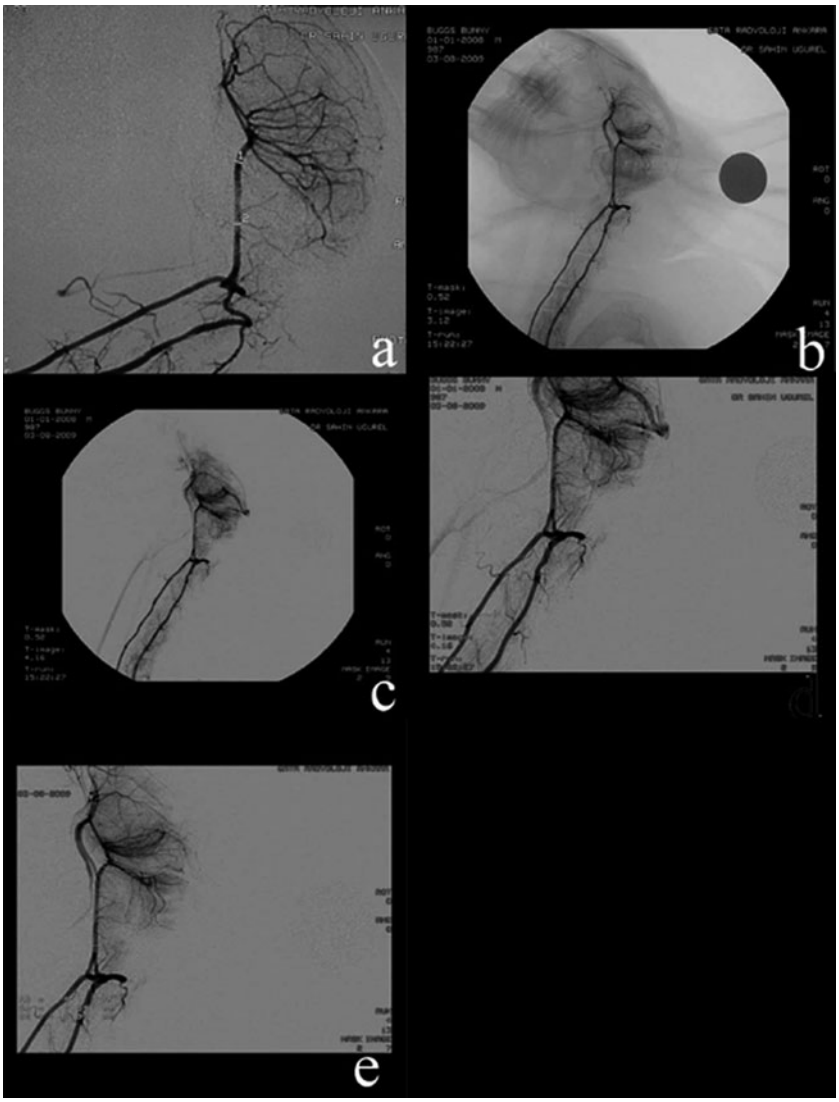

Fig. 2 The pictures of angiographic examination of the basilar artery from group 1 5

cerebral circulation than equal doses of cinnarizine or papaverine [6].

In animal experiments the vasodilatory effect of flunarizine was proven in the basillary artery in rabbits [15]. Also in animal experiments the positive effect of flunarizine on cerebral blood flow and the reduction of extracellular potassium and calcium were proven after induced global ischemia [12, 1]. Thus flunarizine prevents pathological cell alterations in the brain when administered before oxygen supply is reduced [13]. Even if flunarizine is given after ischemia, neuronal necrosis is significantly reduced in rats [2]. It is quite certain that cerebral blood flow is not negatively affected but rather, in contrast to nimodipine, is evidently higher. Based on the experimental data flunarizine is expected to protect ischemic brain regions [10, 9].

The arterial blood pressure does not decrease after flunarizine administration, because it does not affect the circulatory system. Normally nimodipine administration results in a decrease in blood pressure and, furthermore, lowers the overall perfusion status in other organs (e.g. the kidneys). A pilot-study in 55 stroke patients who were given 25 mg flunarizine twice a day showed positive results in time-course, restoration of neurological status, and duration of disease [4].

The intravenous or intraperipheral route of administration requires larger doses of drugs than the intrathecal route, which could lead to adverse effects, reducing the therapeutic efficacy of the drug. The intrathecal route can overcome the inability of intravenously or peripherally administered drugs to allow distribution of the drugs through the entire neuraxis without penetrating the blood brain barrier. However, the morbidity associated with long-term intrathecal drug infusion through catheters, which are required for continuous drug administration, has precluded the application of this procedure in the clinical setting. The catheter can cause adverse reactions, such as chronic inflammation and dural fibrosis, or result in spinal compression or infection [5]. Another limitation is that a second intervention may be required to remove the catheter. Furthermore, the distribution of the drug in the subarachnoid space is not localized, and the agents may be redistributed to the systemic and cranial circulation without reaching the target. To overcome the problems described above, a liposomal drug delivery system for intrathecal application that can maintain effective concentrations of the drug over a period of time may be developed [5].

In our opinion, because of its effect as a calcium channel blocker, intrathecal flunarizine is a potential alternative for the treatment and prevention of vasospasm. In a search for more effective agents in the treatment of cerebral vasospasm a promising new drug which influences cerebrovascular reactivity appears to be flunarizine, a novel piperazine derivative. Flunarizine reduces the severity of narrowing of in basilar artery and produces no obvious adverse effect in the rabbit SAH model.

Conflict of interest statement We declare that we have no conflict of interest.

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Treatment with Ginsenoside Rb1, A Component of *Panax Ginseng*, Provides Neuroprotection in Rats Subjected to Subarachnoid Hemorrhage-Induced Brain Injury

Yingbo Li, Jiping Tang, Nikan H. Khatibi, Mei Zhu, Di Chen, Liu Tu, Li Chen, and Shali Wang

Abstract *Objective*: Recent trials have shown Ginsenoside Rb1 (GRb1), an active component of a well known Chinese medicine *Panax Ginseng*, plays a significant role in improving the complications seen after an ischemic brain event. In the present study, we investigated the use of GRb1 as a treatment modality to reduce brain edema, reduce arterial vasospasm, and improve neurobehavioral function after subarachnoid hemorrhage-induced brain injury (SAH) in rats.

Method: Male Sprague-Dawley rats weighing between 250 and 300 g were randomly assigned to three groups: (1) Sham group (n = 10), (2) Vehicle group (SAH + no treatment; n = 12); (3) Treatment group (SAH + GRb1 treatment at 20 mg/kg; n = 11). Subarachnoid hemorrhage was induced using the modified double hemorrhage model followed by treatment administration intravenously. Post-operative assessment included neurobehavioral testing using the spontaneous activity scoring system, brain water content, and histological examination of the basilar artery.

Results: Post-operative findings indicated treatment with GRb1 had significantly reduced brain edema and improved neurobehavioral functioning. In addition, histological examination revealed a significant reduction in basilar artery vasospasm and lumen thickness with treatment.

Conclusion: The results of the study suggest that GRb1 treatment reduces brain edema, improves neurobehavioral function, and blocks vasculature thickening and spasm after SAH in rats. Given the novelty of the study, further research will be needed to confirm the benefits of treatment and mechanisms behind neuroprotection.

Keywords *Panax Ginseng* · Chinese medicine · Ginsenoside Rb1 (GRb1) · Subarachnoid hemorrhage (SAH)

Introduction

Subarachnoid hemorrhage (SAH) is a devastating event responsible for thousands of deaths each year. It accounts for 5% of all stroke types with close to 12% of patients dying before reaching medical attention [1]. Of those that survive, roughly 33% develop major neurologic deficits [2]. Despite the advances made in preventing and treating patients afflicted with SAH, morbidity and mortality rates have not changed [1]. This is partly a result of elevated intracranial pressure (ICP) from the initial bleed preventing adequate cerebral blood flow [3]. As a result, today much focus is spent on reducing SAH complications that can lead to elevated ICP including vasospasm, re-rupture of the aneurysm, and cerebral edema.

Panax Ginseng is a well known traditional Chinese medicine used for thousands of years in clinics throughout China [4]. According to the "Chinese Pharmacopoeia," ginsenoside Rb1 (GRb1) is a major component of *Panax Ginseng*, and has many protective effects on the body. Just recently, studies in cerebral ischemic models have demonstrated the use of GRb1 intraperitoneal injections in attenuating the damaging effects of ischemia in rat brains [5]. However the potential use of GRb1 in SAH has not been tested. In the present study, we hypothesize that GRb1 treatment will improve outcomes in SAH induced brain injury in rats by

Y. Li, D. Chen, L. Tu, L. Chen, and S. Wang (✉)
Institute of Neuroscience, Chongqing Medical University, Chongqing, 400016, China
e mail: ypshali@gmail.com
J. Tang
Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA, 92354, USA
N.H. Khatibi
Department of Anesthesiology, Loma Linda University, Loma Linda, CA, 92354, USA
M. Zhu
Institute of Neuroscience, Chongqing Medical University, Chongqing, 400016, China
Department of Physiology, Luzhou Medical College, Luzhou, Sichuan, 646000, China

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decreasing brain edema, improving neurobehavioral function, and decreasing basilar artery thickness.

Materials and Methods

All animal research was conducted in accordance with protocols approved by Chongqing Medical University, Institutional Animal Care and Use Committee (IACUC). Male Sprague-Dawley rats weighing between 250 and 300 g (Animal care center of Chongqing Medical University, Chongqing, China) were housed in light and temperature controlled environment with food and water ad libitum for the duration of this study.

Ginsenosides Rb1 was purchased from *National Institute for the Control of Pharmaceutical and Biological Products* (Beijing, China). All generic reagents were commercially obtained.

Randomized Grouping and SAH Model

Male Sprague-Dawley rats were randomly assigned into three groups: (1) Sham group (n = 10), (2) Vehicle group (SAH + vehicle treatment; n = 12); (3) Treatment group (SAH + GRb1 treatment at 20 mg/kg; n = 11).

A modified double hemorrhage SAH model was adapted as previously reported [6]. Briefly, rats were anesthetized with chloral hydrate administered intraperitoneally at a dose of 300 mg/kg. Throughout the duration of the surgery, rats were allowed to breathe spontaneously with body temperatures maintained at approximately at 37 ± 0.5°C using a homoeothermic operating table (DWV-IIHW, Cheng Yi, China). After positioning the head of the rat in the stereotactic frame, a parietal-occipital incision was made separating the muscle layer and exposing the atlanto-occipital membrane. Afterwards, the cisterna magna was punctured using a 27-gauge needle, allowing aspiration of 0.1 ml of cerebral spinal fluid. Non-heparinized autologous blood (0.1 ml/100 g) from the caudal artery was injected aseptically into the cisterna magna over 2 min. To permit blood distribution around the basal arteries, the rats were inclined at a 30° angle for 30 min with the head in the downward position. The needle was left in place for additional 30 min after injection to prevent the possible leakage of blood. After removal of the needle, the skull hole was closed with bone wax. The skin incision was sutured closed and the rats were allowed to recover. Once anesthesia wore off, the rats were returned to their cages, with the room temperature maintained between 24°C and 26°C. The procedure was repeated 24 h later where 0.2 ml of autologous blood was injected. GRb1 was administrated via vena caudalis at a dose of 20 mg/kg starting 30 min after the first SAH followed by the administration of the same dose per day for additional 7 days.

Sham surgery was performed in the same manner, however, saline solution (0.3/0.2 ml) was substituted for autologous blood. Rats that died during surgery were excluded from the experiment.

Calculating Mortality

Mortality was calculated 120 h after the second SAH. Rats that died during the surgery were excluded from the study.

Spontaneous Activity Scores

Testing for spontaneous activity was performed at 6 and 24 h after the first SAH and again at 6, 24, 48, 72, 96 and 120 h after the second SAH. Briefly, spontaneous activity was assessed by the rat's ability to approach all four walls of the cage. The neurological condition was graded as follows: Grade 1-no deficit, i.e., the rat moved around, explored the environment actively and approached at least three walls of the cage without motor deficits; Grade 2-slightly affected, i.e., the rat moved about in the cage with a delay but did not approach all sides and hesitated to move, although it eventually reached at least one upper rim of the cage; Grade 3-moderately affected, i.e., the rat did not rise up at all and barely moved in the cage without abnormality; Grade 4-severely affected, i.e., the rat did not move at all, and showed tetra- or paraplegia.

Brain Water Content

Brain water content was measured as previously described [7]. Briefly, rats were sacrificed at 24 and 120 h post SAH and brains were immediately removed and divided into cerebrum and cerebellum. The cerebellum was used as an internal control for brain water content. Tissue samples were then weighed on an electronic analytical balance (APX-60, Denver Instrument; Arvada, CO) to the nearest 0.1 mg to obtain the wet weight (WW). The tissue was then dried at 105°C for 24 h to determine the dry weight (DW). The percent brain water content was calculated as [(WW DW)/WW] × 100.

Histological Examination

In order to measure the morphology of the basilar artery, both light microscopy (LM) and transmission electron microscopy (TEM) were employed. Roughly 120 h after the second SAH, rats were anesthetized and perfused with cold physiological saline solution. This was followed immediately with 4% paraformaldehyde and 2.5% glutaraldehyde perfusion in a 0.1 M phosphate buffer solution [8]. The whole brain was then removed and fixated in solution overnight at 4°C.

For the TEM studies, the basilar artery was dissected out from the brain and carefully washed in 0.1 M PBS solution. It was then placed in a 1% osmium tetroxide solution and dehydrated in graded ethanol, embedded in an epoxy resin, and examined by a TOSHIBA 7005 TEM.

Basilar artery thickness and lumen cross-sectional area was determined using the image pro-plus imaging analysis system. Four measurements were taken by an independent blinded observer and the values were averaged.

Statistical Analysis

Results are presented as mean ± standard error of the mean. All data was statistically analyzed with one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls (SNK) and Least-significant Difference (LSD) for multiple comparisons. A p-value less than 0.05 was considered statistically significant. All data analysis was conducted using the SPSS 10.0 Statistical Software.

Results

Mortality

There was a statistically significant improvement in mortality rate between the vehicle and treatment groups 120 h after the second SAH (Sham group, 10%; Vehicle group, 25%; and Treatment group, 18.18%).

Spontaneous Activity Scores

The results from the spontaneous activity testing found a statistically significant difference between the vehicle and treatment groups 96 h after the second SAH. This significance did not translate to other time points (Fig. 1).

Brain Water Content

Twenty-four hours after the second SAH, there was a significant reduction in brain edema in the cerebrum of the treatment group when compared with that of the vehicle group (Fig. 2). However, the difference was not found at 120 h after SAH.

Histological Examination

Histological examination of the vehicle group basilar artery revealed severe vasospasm with decrease lumen size and

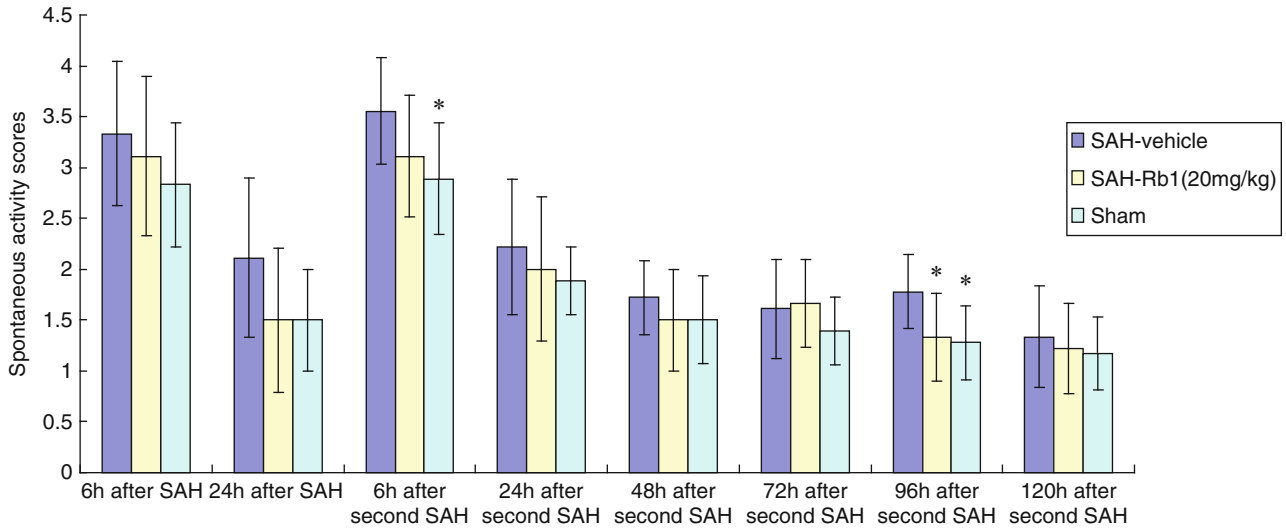

Fig. 1 Ginsenosides Rb1 (20 mg/kg) improved spontaneous activity scores significantly at 96 h after SAH (*$p < 0.05$; compared to vehicle)

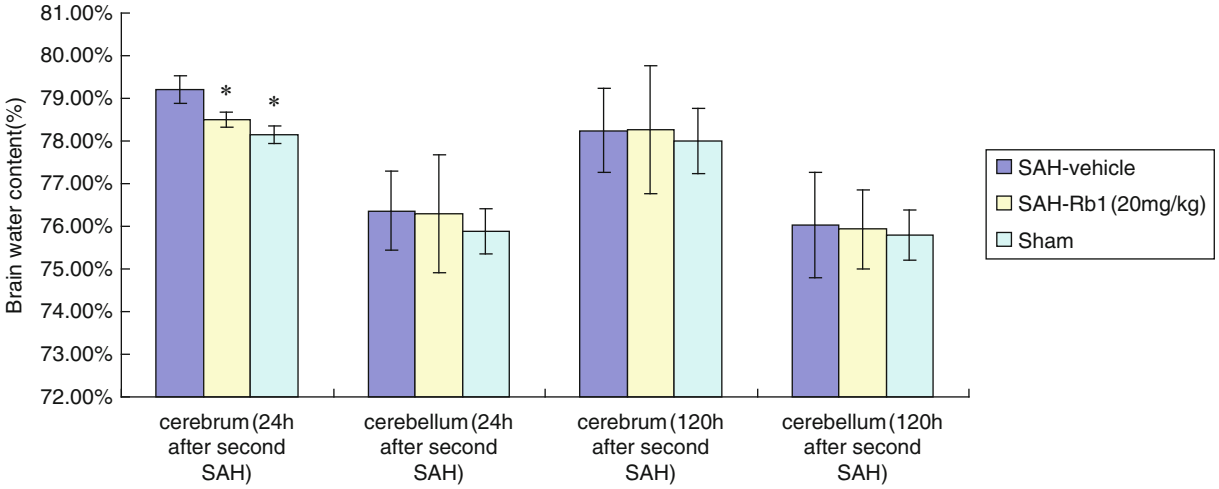

Fig. 2 Ginsenosides Rb1 (20 mg/kg) reduced brain water content at 24 h after SAH (*$p < 0.05$; compared to vehicle)

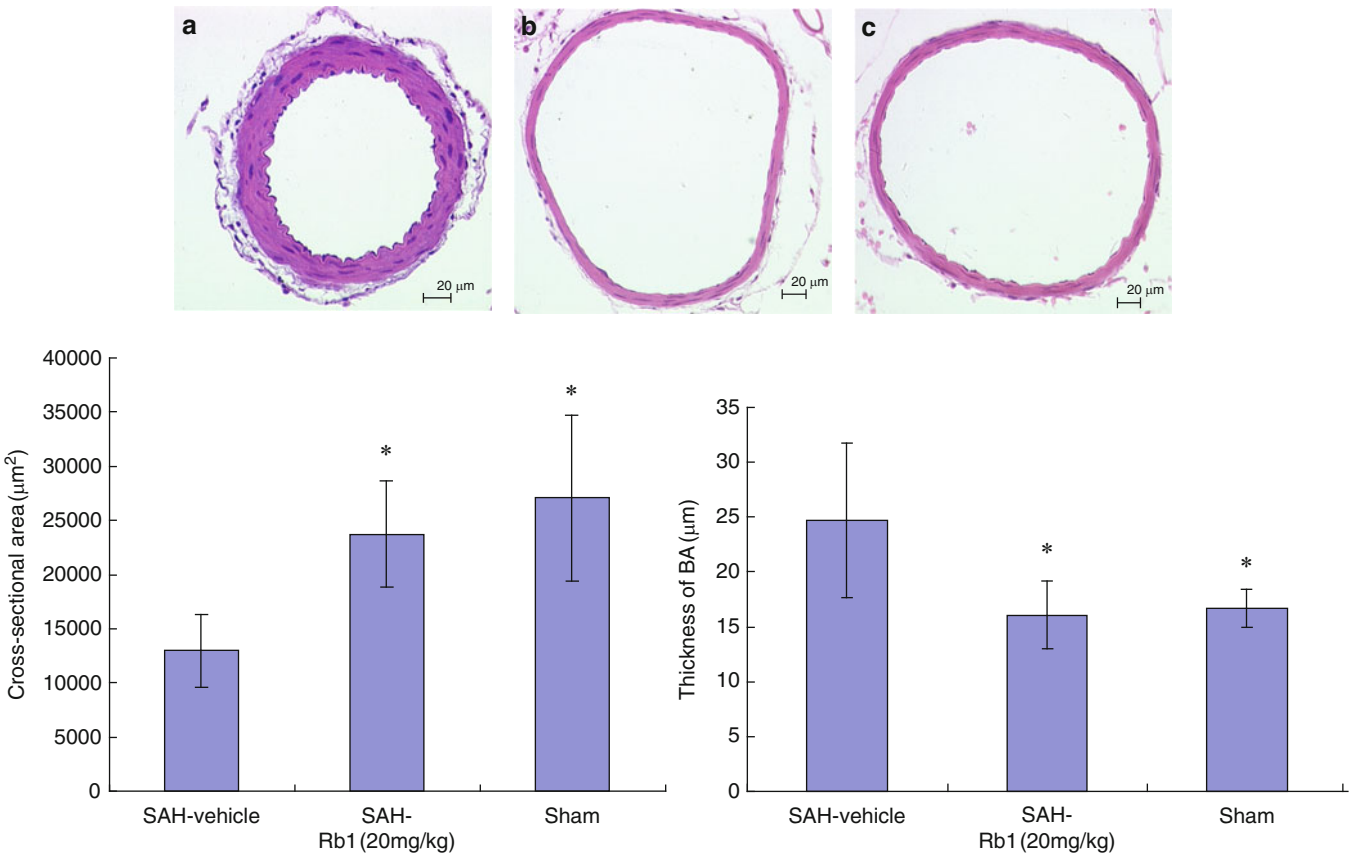

Fig. 3 In the vehicle group, the vessel lumen decreased in size while the wall thickness increased with internal elastic lamina corrugated severely. The mean cross sectional area was significantly larger while the basilar artery wall thickness was significantly lower in the treatment group (*$p < 0.05$; compared to vehicle). (**a**) Vehicle group, (**b**) Treatment group, (**c**) Sham group

a thickened vessel wall. In the treatment group, changes were milder, with a significant reduction in vasospasm and vessel thickness (Fig. 3). The mean cross-sectional area of the basilar artery was significantly larger than that of the vehicle group.

Conclusion

The aim of this study was to determine whether GRb1 treatment could be used to reduce the increased brain edema and neurobehavioral deterioration in rats subjected to subarachnoid hemorrhage-induced brain injury. Previous works on GRb1 have looked at its beneficial effects in brain ischemic models, but no studies to this date that we are aware of have looked at this treatment in SAH.

The present study established three significant points. First, GRb1 reduced brain edema in cerebrum compared to vehicle. Second, neurobehavioral testing using the spontaneous activity scoring system revealed a significant improvement in post operative function with treatment. And finally, histological examination under a TEM revealed a significant reduction in basilar artery vasospasm and lumen thickness with GRb1 treatment. This is the first study of its kind linking GRb1 treatment to a better outcome in SAH induced rats.

Current data suggests that the voltage-depended calcium ion channel plays a key role in preventing vasospasm, one of the major consequences of SAH. Inhibition of the voltage-depended calcium ion channel and/or alteration in intracellular calcium levels can mitigate contraction of endothelial cells leading to vasospasm [9]. Using patch-clamp technology, it was discovered that GRb1 could block Ca^{2+} channel currents in cardiac muscle cell [10]. This could possibly explain the improvements that were seen with GRb1 treatment in our experiments. Other studies have shown GRb1 to increase the levels of nitric oxide via PI3K/Akt regulation [11].

Given the current findings, the possibility of using GRb1 as a therapeutic option in SAH is supported. Given the novelty of the study, this study provides a basis for further researching looking at the role of GRb1 in subarachnoid hemorrhage-induced brain injury.

Conflict of interest statement We declare that we have no conflict of interest.

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The Effects of Intrathecal Nicergoline and Nimodipine in Cerebral Vasospasm: An Experimental Study in Rabbits

Ilker Solmaz, Mehmet Bulent Onal, Erdinc Civelek, Atilla Kircelli, Onder Ongoru, Sahin Ugurel, Ersin Erdogan, and Engin Gonul

Abstract *Background*: The aim of this study was to assess and to compare the ability of intrathecal nicergoline and nimodipine in prevention of cerebral vasospasm in a rabbit model of subarachnoid hemorrhage (SAH).

Method: Twenty male New Zealand white rabbits were allocated into four groups randomly. Subarachnoid hemorrhage was induced by injecting autologous blood into the cisterna magna. The treatment groups were as follows: (1) control [no SAH (n = 5)], (2) SAH only (n = 5), (3) SAH plus nimodipine (n = 5), and (4) SAH plus nicergoline (n = 5).

Findings: There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($p < 0.05$). Basilar artery vessel diameter and luminal section areas in group 3 were significantly higher than in group 2 ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 4 were significantly higher than in group 2 ($p < 0.05$). Therewas no significant difference between basilar artery vessel diameter and basilar artery luminal section areas in group 3 and group 4.

Conclusions: These findings demonstrate that intrathecal nicergoline has a vasodilatatory effect in an experimental model of SAH in rabbits but not more than that of nimodipine.

Keywords Nicergoline · Nimodipine · Basilar artery · Cerebral vasospasm · Subarachnoid hemorrhage

I. Solmaz, M.B. Onal, E. Civelek (✉), A. Kircelli, E. Erdogan, and E. Gonul
Department of Neurosurgery, Gulhane Military Academy, Ugur Mumcunun Sokak, 78/2, 06700 Gaziosmanpasa, Ankara, Turkey
e mail: civsurgeon@yahoo.com
O. Ongoru
Department of Pathology, Gulhane Military Academy, Ankara, Turkey
S. Ugurel
Department of Radiology, Gulhane Military Academy, Ankara, Turkey

Introduction

Cerebral vasospasm is a slowly developing, sustained constriction of cerebral vessels. The etiology and pathogenesis of symptomatic cerebral vasospasm are still not well understood. Although many approaches to prevent vasospasm have been proposed, including hypertensive hypervolemic hemodilution therapy (triple H) [8], cisternal drainage with or without intrathecal administration of plasminogen activators [6], and calcium antagonists, a conclusive method has not yet been determined.

Nicergoline is an ergot alkaloid derivative which has alpha-adrenergic receptor blocking and calcium antagonistic properties. It increases cerebral blood flow, improves hemodynamics and glucose metabolism in aged rats with cerebral ischemia [9], inhibits acetylcholinesterase activity in the rat brain and corrects reduced choline acetyltransferase and muscarinic cholinergic receptor activities in the aged rat brain [7]. Nicergoline can produce an appreciable short-term increase of cerebral blood flow in patients with established cerebrovascular disease. It may show protective effects against the anoxic brain damages due to its ameliorating action on cerebral energy metabolism, mainly contributed by an activation of cerebral cytochrome oxidase, without relation to its alpha-blocking action [4].

Blasco et al. concluded that the efficacy of the alpha-blocker nicergoline in the protection of retinal and cochlear tissue from ischemia could depend on its capacity to improve the blood flow, as observed in cerebral and peripheral circulation [3]. Iwata et al. concluded that nicergoline may induce the up-regulation of intracellular antioxidant defenses and protect the neuronal cells against oxidative stress [5]. Nicergoline is also an antioxidant that inhibits not only lipid peroxidation but also free radical generation from neutrophils [10]. In this study, our aim was to examine the effects of intrathecal nicergoline and to compare the efficiency of intrathecal nimodipine and intrathecal nicergoline.

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Methods and Materials

Animal Model

The Gulhane Military Medical Academy Laboratory Animals Ethics Committee approved all protocols. Experiments were carried out in 20 male New Zealand White rabbits weighing 2,500 3,000 g. All animals were starved for 8 h before the procedures and anesthetized with a mixture of ketamine (Ketaset, 50 mg = kg) and xylazine (Rompun, 10 mg = kg) administered intramuscularly. Additional doses were added at 20 30 min intervals when necessary. Animals were assigned randomly to one of four groups according to treatment protocols. All groups were consisted of five rabbits. Animals in group 1 served as control (n = 5) and group 2 as SAH only (n = 5). Nimodipine (Nimotop®) were administered to group 3 in a dosage of 12 μg/kg in 30 min intrathecal infusions at 1, 3 and 5 days after SAH induction (n = 5). Nicergoline (Sermion®) was administered to group 4 in 30 min intrathecal infusions in doses of 32 μg/kg at 1, 3 and 5 days after SAH induction (n = 5).

SAH Formation

After shaving the dorsal parts of neck and head, under sterile conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. To enter the subarachnoid space easily, atlanto-occipital membrane was punctured in a head hyperflexion position. After withdrawal of 1 ml of CSF, equal volume of autologous fresh nonheparinized blood from the central ear artery was injected in 3 min into the subarachnoid space to induce SAH. The animals were then placed in a head-down position for 30 min to hold the blood in the basal cisterns. Every rabbit underwent angiography procedure in the fifth day of SAH induction to visualize and measure the diameter of the basilar artery.

Perfusion-fixation

All animals subjected to experimental SAH were euthanized by perfusion-fixation 7 days after SAH induction. After induction of anesthesia, thoracotomy was performed. Left ventricle was cannulated while the right atrium was opened widely, and the abdominal aorta was clamped. After perfusion of a flushing solution [Hanks' balanced salt solution (Sigma Chemical Co.), pH 7.4 at 37°C, 300 ml], the fixative was perfused (2% paraformaldehyde, 2, 5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at 37°C, 200 ml). Perfusion was performed at a standard height of 100 cm from the chest. Animals in the control group were sacrificed using the same procedure. Brains were then removed and stored in fixative at 4°C overnight.

Neurological Parameters

All neurological evaluations were performed by an observer blinded to the study plan. The initial evaluation was completed between 6 and 12 h after the SAH; following assessments were completed on the third and fifth days. All of the scoring was performed 6 h after anesthesia. The neurological scale used for the assessments was based on a previous study by Strong et al. with rabbits. As a result, clinical observations (spontaneous behavior, reaction to handling, posture, gait, limb hypertonia, righting reflexes, and feeding behavior) were each given a score: 0 (absent); 1 (mild); 2 (moderate); or 3 (severely impaired). Similarly, front and back reflexes were scored: 0 (normal); 1 (brisk); 2 (spreading); or 3 (clonus). Nystagmus was also observed: 0 (absent) or 1 (present). An overall score was calculated as the sum of the individual observations; a greater score reveals more significant neurological impairment, and a lower score reveals a lesser degree of neurological impairment.

Embedding, Morphometry, and Statistical Analysis

The basilar artery was embedded in paraffin and cut a thickness of 0.5 μm slices. The sections were mounted onto glass slides and stained with H and E for light microscopic analysis. Four sections from four separate zones of the basilar artery were obtained and luminal section areas were measured by using Image J computer program in the Department of Pathology. The groups were compared with the analysis of variance (ANOVA) test using SPSS for Windows (version 11, 5). Following the one-way ANOVA test, a Kruskall Wallis test is performed to examine the differences between the groups. Statistical significance was accepted at $p < 0.05$.

Results

No rabbits died in the groups. The animals were free of problems until the fifth day when they were sacrificed. Gross pathological examination showed no signs of infection.

Clinical observation of the rabbits assessed by blinded veterinarians. A reduction of neurological scores and hypoactivity was observed in the SAH group. Neurological scores in the nimodipine and nicergoline treatment groups were significantly higher ($p > 0.05$) than only SAH group.

Physiological parameters of the rabbits were followed and showed no significant differences in mean body weight, mean brain weight, mean blood pressure, and mean blood gas values among the four groups. Gross pathological examination revealed a subarachnoid clot over the basal surface of the brain stem in each animal with SAH.

The mean wall thickness of the basilar arteries in group 1 (control group) was 22.1 ± 0.1 μm, whereas in group 2 (SAH only) it was 32.3 ± 0.4 μm; in the SAH + Nimodipine group 24.7 ± 0.2 μm and in the SAH + Nicergoline group: 25.1 ± 0.2 μm (Table 1). The mean diameter of arterial lumen in group 1 (control group) was 826.46 ± 30 μm, whereas in group 2 (SAH only) it was 343.50 ± 22 μm; in the SAH + Nimodipine group: 555.65 ± 33 μm and in the SAH + Nicergoline group: 586.13 ± 41 μm (Table 1). The mean cross-sectional areas of basal arteries in group 1 (control group) was 1,683,205 ± 11,000 μm^2, whereas in group 2 (SAH only) it was 36,112 ± 3,000 μm^2; in the SAH + Nimodipine group: 100,513 ± 7,600 μm^2 and in the SAH + Nicergoline group: 111,315 ± 9,000 μm^2 (Table 1).

The pictures of light microscopic examination of the basilar artery from group 1 4 are shown in Fig. 1. The pictures of angiographic examination of the basilar artery from group 1 5 are shown in Fig. 2.

There was a statistically significant difference between the mean basilar artery cross-sectional areas and the mean arterial wall thickness measurements of the control and SAH-only groups ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 3 were significantly higher than in group 2 ($p < 0.05$). Basilar artery vessel diameter and basilar artery luminal section areas in group 4 were significantly higher than in group 2 ($p < 0.05$). There was no significant difference between basilar artery vessel diameter and basilar artery luminal section areas in group 3 and group 4.

Table 1 Changes in the basilar artery diameter, wall thickness and arterial luminal areas. All values were derived from n = 5 groups and all values are expressed as mean ± standard deviation

Groups	Wall thickness	Diameter of arterial lumen	Cross sectional areas
Control	22.1 ± 0.1 μm	826.46 ± 30 μm	1,683,205 ± 11,000 μm^2
SAH only	32.3 ± 0.4 μm	343.50 ± 22 μm	36,112 ± 3,000 μm^2
SAH plus nimodipine	24.7 ± 0.2 μm	555.65 ± 33 μm	100,513 ± 7600 μm^2
SAH plus nicergoline	25.1 ± 0.2 μm	586.13 ± 41 μm	111,315 ± 9,000 μm^2

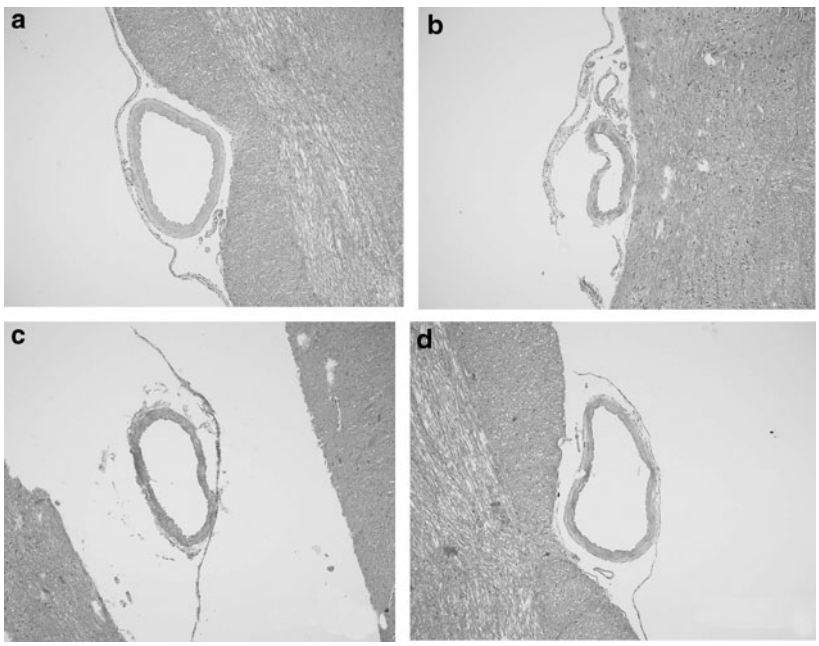

Fig. 1 The pictures of light microscopic examination of the basilar artery from group 1 4

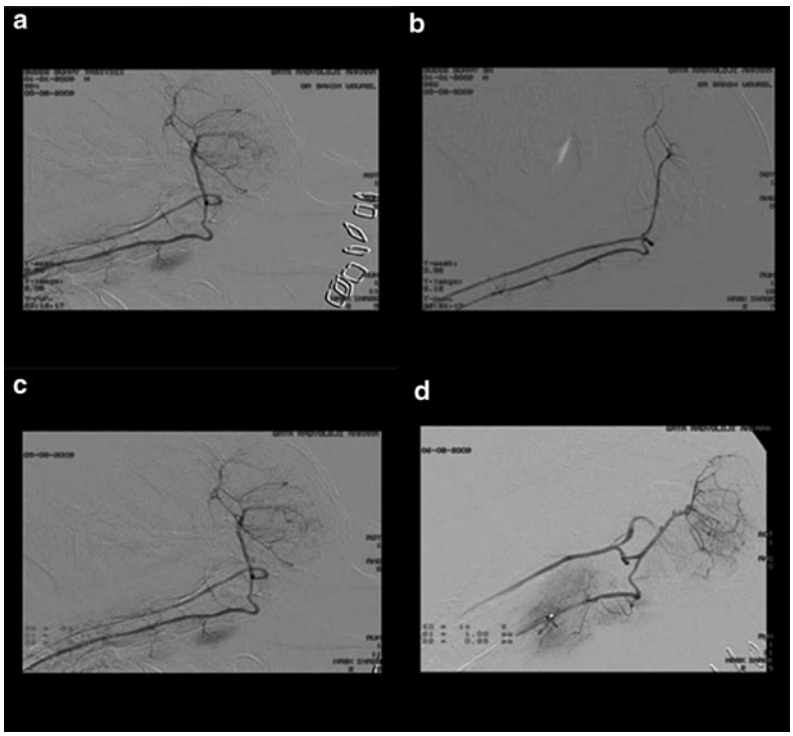

Fig. 2 The pictures of angiographic examination of the basilar artery from group 1 5

Discussion

Cerebral vasospasm is due to the presence of blood clot and its metabolism that causes releasing of some vasoactive substances. There is no single pharmacological agent or treatment protocol which is effective in inhibiting the multiple factors. The ergot alkaloid derivative nicergoline became clinically available about 40 years ago in the 1970s. Nicergoline has a broad spectrum of action: (a) as an alpha (1)-adrenoceptor antagonist, it induces vasodilation and increases arterial blood flow; (b) it enhances cholinergic and catecholaminergic neurotransmitter functions; (c) it inhibits platelet aggregation; (d) it promotes metabolic activity, resulting in increased utilization of oxygen and glucose; and (e) it has neurotrophic and antioxidant properties. Acting on several basic pathophysiological mechanisms, nicergoline has therapeutic potential in a number of disorders. These include mild to moderate dementia, Alzheimer's disease, rehabilitation therapy of patients with chronic ischemic stroke and balance disorders. This drug is commonly used for treating chronic cerebral infarction; it may also have a protective effect on progression of Parkinson's disease or Alzheimer's disease [11]. It improves metabolic and hemodynamic conditions of cerebral tissue [1] and exerts an antiplatelet aggregation action [2]. Nicergoline is used in the treatment of cerebral transient ischemia in the attempt to increase cerebral blood flow to areas where the perfusion is compromised by acute or chronic arterial obstruction or vasospasm [2].

There is no any other study of this drug related to cerebral vasospasm in the literature. Our study showed that nicergoline has similar antivasospastic effects of nimodipine on cerebral vasculature without any serious side effects. Vasodilators used for the treatment of delayed cerebral vasospasm may cause some unwanted effects on the brain. They may cause hypotension that is believed to be harmful in delayed cerebral vasospasm. Nicergoline has no such hypotensive effect. In our opinion, intrathecal nicergoline may be a potential alternative for the treatment and prevention of vasospasm.

Nicergoline reduces the severity of narrowing of in basilar artery and produces no obvious adverse effect in the rabbit SAH model. This drug may be considered as an alternative in clinical trials.

Conflict of interest statement We declare that we have no conflict of interest.

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Metabolic Reflow as a Therapy for Ischemic Brain Injury

Hiroaki Manabe, Yi Wang, Ryo Yoshimura, Yu Cai, Mark Fitzgerald, Ryon Clarke, and Kevin S. Lee

Abstract Ischemic neuronal damage is a common feature of occlusive strokes, hemorrhagic strokes, and traumatic brain injury. In addition, ischemia can be an anticipated or unanticipated complication of a variety of surgical procedures. Most therapeutic strategies for managing ischemic injury seek to re-establish blood flow, suppress neural metabolism, and/or limit specific cellular injury cascades. An alternative therapeutic approach is to enhance the delivery of metabolic substrates to ischemic tissue. This strategy is typified by efforts to increase tissue oxygenation by elevating the levels of circulating oxygen. Our studies are examining a complementary approach in which the delivery of metabolic substrates is enhanced by facilitating the diffusion of oxygen and glucose from the vasculature into neural tissue during ischemia. This is achieved by increasing the diffusivity of small molecules in aqueous solutions, such as plasma and interstitial fluid. The carotenoid compound, trans-sodium crocetinate (TSC) is capable of increasing oxygen and glucose diffusivity, and our studies demonstrate that TSC increases cerebral tissue oxygenation in the penumbra of a focal ischemic event. In addition, TSC treatment reduces the volume of cerebral infarction in rodent models of both permanent and temporary focal ischemia. This strategy of "metabolic reflow" thus blunts the metabolic challenge in partially-perfused tissue and reduces ischemic neural injury.

Keywords Stroke · Neuroprotection · Oxygen · Diffusion · Metabolic reflow

H. Manabe, Y. Wang, R. Yoshimura, Y. Cai, M. Fitzgerald, R. Clarke, and K.S. Lee (✉)
Department of Neuroscience, University of Virginia, Charlottesville, VA, USA
Department of Neurological Surgery, University of Virginia, Charlottesville, VA, USA
e mail: ksl3h@virginia.edu

Introduction

Ischemic neural damage can occur as a result of most types of stroke and time is of the essence for managing the ischemic insult to affected cells. Consequently, a primary goal for any therapeutic regimen is to reestablish blood flow to the ischemic tissue. In the case of occlusive (i.e. embolic and thrombotic) strokes, disruption of the intravascular impediment to blood flow, typically utilizing tissue plasminogen activator, is the principal therapeutic modality. In the case of hemorrhagic strokes, the situation is more complex because of the intrinsic challenges to stabilizing the hemorrhagic event. Subarachnoid hemorrhages resulting from the rupture of aneurysms on large cerebral arteries can be stabilized by surgical clipping or intravascular coiling. In contrast, intracerebral hemorrhages are less amenable to treatment. The management of hemorrhagic strokes is further complicated by the effects of residual extravascular blood in the subarachnoid and intraparenchymal spaces. Finally, downstream compromise to the microvasculature plays a role in the ischemic challenge produced by both occlusive and hemorrhagic strokes. These smaller vessels, although sometimes not directly impacted by an occlusion or hemorrhage are a key emerging target for optimizing blood supply after stroke.

Differential diagnosis of hemorrhagic versus occlusive stroke is a standard feature of the emergent response to stroke in order to ascertain whether thrombolytic therapy is appropriate to reestablish blood flow. Although this diagnosis consumes crucial time during an ongoing stroke, it is essential to limit the complications of rebleeding and hemorrhagic transformation. Consequently, an important goal for current experimental and clinical studies is to identify therapeutic modalities that can be administered rapidly and safely irrespective of stroke type. The predominant experimental approach to this problem focuses the inhibition of critical injury cascades. Numerous candidate agents targeting a wide variety of injury mechanisms have been

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identified and have shown considerable promise in preclinical studies of stroke. Unfortunately, human studies directed toward the inhibition of specific mechanisms of neural injury have not proven successful in advanced stage clinical trials. The reasons for these failures are several, including inadequate preclinical testing [9, 10, 29, 35, 38, 44, 53], and underpowered or inappropriately designed clinical trials [7, 10, 12, 15, 16, 26, 54]. Another plausible explanation for this lack of efficacy in late stage clinical trials is that drugs targeting a narrow range of injury mechanisms are incapable of overcoming the broad range of injury cascades that are set in motion by prolonged ischemia [60]. The future of neuroprotective therapy in the clinic may thus ultimately require a multimodal, poly-pharmacological approach to achieve success [41].

Two alternative approaches to limiting ischemic cellular injury involve either the suppression of overall metabolic demand [61] or the enhancement of metabolic supply [57]. The former approach seeks to broadly limit mechanisms of cellular injury by reducing metabolic demand and slowing the rates at which injury cascades can progress. The latter approach is also directed toward a broad inhibition of cellular injury, but does so by increasing metabolic supply to ischemic tissue and thus blunting the overall ischemic challenge. The studies described herein will focus on a novel form of the metabolic enhancement strategy using a pharmacologic intervention to increase the delivery of metabolic substrates to ischemic neural tissue.

Historically, therapeutic strategies to enhance the metabolic supply of ischemic tissue have generally utilized increased systemic oxygenation to improve tissue oxygenation. Hyperbaric and normobaric oxygenation have shown promise in limiting cellular injury and neurological function in animals models of stroke [4, 5, 11, 13, 18, 19, 24, 25, 28, 37, 39, 45, 47, 50, 51, 55 57, 59, 62]. In contrast, clinical trials have been more limited and have provided mixed outcomes to date [1 3, 6, 17, 32 34, 43, 48, 49, 63]. These mixed clinical outcomes can be ascribed in part to small sample sizes and relatively long delays prior to initiating treatment.

The underlying concept for the preceding oxygen enhancement studies is that the elevation of vascular oxygen raises the concentration gradient between blood and tissue and thus increases the movement of oxygen into the tissue compartment. However, it is important to note that the rate of movement of a small molecule, such as oxygen or glucose, is also dictated by its diffusion coefficient in the host medium. In the case of circulating blood, the plasma boundary layer is a key resistance for the movement of molecules from the vasculature into tissue [22, 23]. The diffusivity of oxygen and glucose in an aqueous solution, such as plasma, is dictated in part by the number of hydrogen bonds and intermolecular spacing among water molecules. An increase in hydrogen bonding builds the "structure" of aqueous solutions and facilitates the diffusion of small molecules. It is thus possible to increase the access of vascular oxygen and glucose to tissue by modifying the diffusivity of these metabolic substrates. Trans-sodium crocetinate (TSC) is a carotenoid compound that has previously been shown to increase the diffusivity of small molecules, including oxygen and glucose, by facilitating structure building in aqueous media [27, 52]. Moreover, TSC has been shown to increase tissue oxygenation in multiple organs and to improve survival in a model of hemorrhagic shock [36, 42, 46]. The studies described herein examined the ability of TSC to increase cerebral oxygenation in areas of partial ischemia and its effects on neural injury in permanent and temporary models of focal brain ischemia [30].

Materials and Methods

All experimental protocols were approved by the University of Virginia Animal Care and Use Committee. Adult male Sprague-Dawley rats (330 370 g) underwent permanent or temporary focal ischemia [20] by clipping both common carotid arteries and the left middle cerebral artery (three-vessel occlusion: 3-VO). Using the permanent ischemia paradigm, TSC was administered at one of eight dosages, ranging from 0.023 to 4.580 mg/kg (n = 7 animals per group). Equivalent volumes of either Vehicle or TSC were injected into the femoral vein using a "bolus-infusion-bolus protocol", as per the protocol of Okonkwo et al. [36]. Using this protocol, a bolus injection of 0.1 ml was administered 10 min after the onset of ischemia, followed by continuous infusion at 0.01 ml/min for 60 min. Thirty minutes after the end of infusion, another 0.1 ml bolus was injected. The dosages described here represent the total dosage of TSC administered using the bolus-infusion-bolus protocol. After 24 h of permanent ischemia, the animals were euthanized under deep anesthesia. The brains were sectioned coronally at a thickness of 2 mm and the sections were stained in 2% 2,3,5 triphenyltetrazolium chloride (TTC) in phosphate-buffered saline for 5 min at 37°C. Infarct size was measured and the total volume of infarction was corrected for swelling [30].

In the temporary model of focal ischemia, the 3-VO was maintained for 2 h after which the vessels were unclipped and reflow was established. These animals were euthanized at 22 h after establishing reflow. TSC was administered, as described above, beginning 10 min after the onset of ischemia. A dosage of 0.092 mg/kg was used based on the results of the dose-response study using the permanent ischemia model.

The effect of TSC on partial tissue oxygen levels (PtO2) was also examined using the same temporary model of focal ischemia. A Licox probe was placed in the penumbra of the focal ischemic area in order to record tissue oxygenation. TSC was administered at a dosage of 0.92 mg/kg using the bolus-infusion-bolus protocol with the first bolus administered 10 min after the onset of ischemia. The levels of PtO2 were recorded prior to ischemia for at least 20 min to obtain a stable, normoxic baseline. All recorded values were then normalized to this pre-ischemic baseline.

Results

Effect of TSC on Cerebral Infarction After Permanent Focal Ischemia

Treatment with TSC produced a dose-dependent reduction in cerebral injury. The dose-response curve was U-shaped with dosages ranging from 0.023 to 0.229 mg/kg producing significant reductions in infarct volume [30]. The most effective dosage, 0.092 mg/kg, reduced infarct volume by 58%.

Effect of TSC on Cerebral Infarction After Temporary Focal Ischemia

The optimal dosage of TSC for producing neuroprotection in the preceding dose-response experiment was tested for its effect on cerebral infarction produced by temporary (2 h) focal ischemia followed by 22 h of reperfusion. TSC treatment at a dosage of 0.092 mg/kg reduced infarct volume by 45% in this model of ischemia-reperfusion [30].

Effect of TSC on Oxygenation in the Ischemic Penumbra

Partial tissue oxygen levels in the ischemic penumbra of the 3-VO model were reduced by an average of 40 45% from baseline when ischemia was initiated [30]. Ten minutes after the onset of ischemia, animals were treated with either TSC or saline. In the TSC-treated animals, tissue oxygenation began to increase within approximately 10 min of the initial bolus injection. By the end of the second hour of ischemia PtO2 in the penumbra of saline-treated animals was reduced by an average of 41% below baseline, while oxygen levels had recovered to only 20% below baseline in the TSC-treated animals [30]. Upon reperfusion, tissue oxygenation increased well above baseline in both Vehicle-treated and TSC-treated animals. However, tissue hyperoxygenation during reperfusion was significantly less pronounced in TSC-treated animals than in Vehicle-treated animals.

Discussion

During an occlusive stroke, the ischemic penumbra is an area of partial blood flow that contains tissue at risk of being damaged and possibly recruited into cerebral infarction. The penumbra is generally viewed as a region that can be salvaged if vascular reperfusion is achieved within an adequate time frame and/or appropriate measures are taken to protect the tissue. The key determinants of how well penumbral tissue will withstand an ischemic challenge are the duration and depth of the ischemic event. Consequently, when it is feasible, a centerpiece of stroke management includes efforts to restore blood flow in order to limit the duration of metabolic challenge. Thrombolytic therapy can produce complete or partial recanalization with delays to reflow ranging from minutes to several hours after treatment [8, 40]. The delay to recanalization is compounded by the delay to receiving treatment, which typically is on the order of a couple of hours. Therefore, it is not unusual for stroke patients to experience ischemic events that persist for several hours. Although not always effective in establishing reflow, thrombolytic therapy remains the principal medical means for recanalization and a valuable strategy for curtailing the duration of an occlusive stroke.

The depth of an ischemic event can vary widely and the intensity of this challenge dictates the involvement and time course of various injury mechanisms [31]. Our current studies are examining a therapeutic approach designed to blunt the impact of partial ischemia by enhancing metabolic supply to at-risk tissue. Thus, this approach functionally attenuates the depth of ischemic challenge to the penumbra. Trans-sodium crocetinate was shown to substantially and significantly enhance tissue oxygenation in the penumbra of ongoing focal ischemia in the brain [30]. Moreover, TSC reduced neural damage in both permanent and temporary models of focal cerebral ischemia [30]. The protective actions of TSC are predicated on the concept of metabolic reflow, in which ischemic damage is attenuated by facilitating the delivery of metabolic substrates to at-risk tissue. The enhancement of tissue oxygenation is thought to result from the ability of TSC to increase the diffusivity of oxygen [52]. Nonetheless, in studies of this type, it is important to consider alternative explanations for the protective actions of any candidate therapy. Relevant to this issue are previous studies

examining the effects of TSC's structurally-similar parent compound, crocetin. These studies showed that crocetin did not exert significant effects on oxyhemoglobin saturation, oxygen solubility in blood, or blood flow [14, 21]. An increase in the diffusivity of metabolic substrates thus remains a parsimonious explanation for the observed increases in tissue oxygen and the resultant neural protection.

As discussed earlier, a key goal for ongoing and future studies will be to develop therapeutic modalities that can be implemented rapidly and safely irrespective of the type of stroke being treated. In this regard, it is noteworthy that preliminary data from our laboratory indicate that TSC also produces beneficial outcomes in an experimental model of intracranial hemorrhage [58]. It is therefore plausible that metabolic reflow therapy could be effective in treating both occlusive and hemorrhagic strokes. If so, this would obviate the need for a differential diagnosis of stroke type prior to initiating TSC treatment. Another key issue regarding the potential utility of metabolic reflow therapy concerns the time frame over which TSC can be effective. The current studies utilized a protocol in which TSC treatment was initiated soon after the onset of ischemia, which is not practicable in the context of most strokes. Ongoing studies are therefore examining the therapeutic window for TSC treatment in experimental models of stroke to ascertain whether delayed treatment is also effective in limiting ischemic damage.

In summary, our current studies have begun to characterize a novel therapeutic approach for treating ischemic neural injury. Metabolic reflow produced by TSC treatment can enhance the supply of metabolic substrates to at-risk tissue during ongoing partial ischemia. In addition, TSC treatment reduces cerebral infarction associated with permanent and temporary focal ischemia [30]. Future studies will be directed toward defining the utility of this strategy as an early intervention for the treatment of stroke, irrespective of the type of stroke that is occurring.

Conflict of interest statement We declare that we have no conflict of interest.

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The Influence of Cisternal and Ventricular Lavage on Cerebral Vasospasm in Patients Suffering from Subarachnoid Hemorrhage: Analysis of Effectiveness

Daniel Hänggi and Hans-Jakob Steiger

Abstract *Objective*: Within the last decades several clinical trials were performed to analyze the effectiveness of cisternal and ventricular lavage on cerebral vasospasm in patients suffering from subarachnoid hemorrhage. Aim of the present analysis was to review and summarize all documented clinical studies using cisternal or ventricular lavage to prevent vasospasm.

Methods: The MEDLINE Web site (www.pub.med.com) was searched using the clinical query function optimized for clinical therapy. Search terms were subarachnoid hemorrhage, vasospasm, cisternal and ventricular lavage. Results were divided into cisternal and ventricular lavage therapies alone and its combination with additional treatment modalities.

Results: So far the literature search revealed a total of nine clinical trials using cisternal or ventricular lavage alone in patients suffering from subarachnoid hemorrhage. The patients were treated using urokinase or recombinant tissue plasminogen activator. A metaanalysis, investigating a total of 652 included patients revealed a significant reduction of delayed neurological deficits, a significant increase of outcome and a significant decrease of mortality in the treatment group. Additional there was no difference of effectiveness or side effects using urokinase or recombinant tissue plasminogen activator. Hence, only one of these studies was based on a prospective, randomized study design. A combination of cisternal or ventricular lavage with some sort of kinetic treatment was documented in a total of three studies. All of them were designed prospectively. The combined application demonstrated reduced delayed neurological deficits, reduced vasospasm and better outcome in two studies for the treatment group. One study was stopped early due to unexpected complication.

Conclusions: In conclusion, there is strong evidence that cisternal or ventricular lavage alone and in combination with kinetic therapy lead to a reduction of cerebral vasospasm and better outcome in patients suffering from subarachnoid hemorrhage. As a consequence a prospective randomized study would be of great interest.

Keywords Cisternal · Ventricular · Lavage · Vasospasm

Introduction

Cerebral vasospasm and delayed cerebral ischemia contribute the major part of secondary morbidity and mortality following severe subarachnoid hemorrhage (SAH) [7, 8, 14, 17, 18]. Several metaanalysis concluded that orally administered nimodipine is the only proven prophylactic therapy for cerebral vasospasm [3, 22, 28, 31]. In addition, hemodynamic therapy is usually recommended to treat symptomatic vasospasm [23, 26, 29, 31]. Despite these current treatment strategies the rate of related permanent disability is estimated as 10 20% [12].

In general, the treatment strategy could be divided into systemic or local therapy to reduce the incidence of cerebral vasospasm and its consequences after aneurysmal SAH. Additional the local treatment could be performed by intracisternal prolonged release implants [4, 15] or intracisternal thrombolysis with or without the use of the kinetic therapy [1, 10, 11, 16]. The current analysis was performed to review the efficacy of cisternal and ventricular lavage with or without the combination of kinetic therapy in patients suffering from severe aneurysmal SAH.

Materials and Methods

The MEDLINE Web site (www.pub.med.com) was searched using the clinical query function optimized for clinical therapy. Search terms were subarachnoid

D. Hänggi (✉) and H. J. Steiger
Department of Neurosurgery, Heinrich Heine University, Moorenstraße 5, 40225, Düsseldorf, Germany
e mail: Daniel.Haenggi@uni duesseldorf.de

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Table 1 Literature overview of clinical trials using cisternal or ventricular lavage alone

Author	Design	Patients	Type of treatment
Saito et al. 1990 [24]	Retrospective	61	UK
Kanamura et al. 1993 [13]	Prospective Nonrandomized	38	UK
Mizoi et al. 1993 [20]	Prospective Nonrandomized	105	tPA
Usui et al. 1994 [30]	Retrospective	111	UK or tPA
Seifert et al. 1994 [25]	Prospective Nonrandomized	120	tPA
Moriyama et al. 1995 [21]	Retrospective	44	UK
Majchrzak et al. 1995 [19]	Prospective Nonrandomized	28	tPA
Findlay et al. 1995 [5]	Prospective Randomized	100	tPA
Gorski et al. 2000 [6]	Prospective Nonrandomized	45	tPA

hemorrhage, vasospasm, cisternal and ventricular lavage. Results were divided into cisternal and ventricular lavage therapies alone and its combination with additional treatment modalities.

Results

So far the literature search revealed a total of nine clinical trials using cisternal or ventricular lavage alone in patients suffering from subarachnoid hemorrhage (Table 1). The design of the studies was retrospective, prospective nonrandomized and prospective randomized. A total of 652 patients were included in all nine trials (range: 28 120) and treatment was performed using urokinase (UK) and/or tissue plasminogen activator (tPA) intra- or postoperatively. The patients were treated using urokinase or recombinant tissue plasminogen activator. A metaanalysis, investigating all 652 included patients revealed a significant reduction of delayed neurological deficits, a significant increase of outcome and a significant decrease of mortality in the treatment group [1]. Additional there was no difference of effectiveness or side effects using urokinase or recombinant tissue plasminogen activator. Hence, only one of these studies was based on a prospective, randomized study design [1].

A combination of cisternal or ventricular lavage with some sort of kinetic treatment was documented in a total of three studies (Table 2). All of them were designed prospectively. The combined application demonstrated reduced delayed neurological deficits, reduced vasospasm and better outcome in two studies for the treatment group. One study was stopped early due to unexpected complication.

Table 2 Literature overview of clinical trials using cisternal or ventricular lavage in combination with kinetic therapy

Author	Design	Patients	Type of treatment
Kawamoto et al. 2004 [16]	Prospective Randomized	230	UK and headshaking
Hänggi et al. 2008 [10]	Prospective Nonrandomized	40	Ringer solution and lateral rotational therapy
Hänggi et al. 2009 [9]	Prospective Randomized	20	tPA, nimodipine and lateral rotational therapy

Discussion

To our knowledge the present review is the first analysis investigating the effectiveness of cisternal and ventricular lavage in patients after severe aneurysmal SAH.

Clinical studies were divided into purely cisternal and ventricular lavage and its combination with some sort of kinetic treatment.

The effectiveness of purely cisternal and ventricular lavage was investigated in a metaanalysis recently [1]. As mentioned, the inclusion of 652 patients revealed a significant reduction of delayed neurological deficits, a significant increase of outcome and a significant decrease of mortality in the treatment group [1]. Additionally there was no difference of effectiveness or side effects using urokinase or recombinant tissue plasminogen activator. The major limitation was that only one of these studies was based on a prospective, randomized study design [1].

Additionally we performed a further literature search focusing on the documented side effects of clinical trials dealing with alternative treatment regimens such as intracisternal thrombolysis, intrathecal local therapy, and multimodal concepts to reduce cerebral vasospasm after SAH.

Four out of a total of nine clinical trials using central intracisternal thrombolysis do not provide any information about complications of the therapy [6, 13, 19, 25]. In the remaining trials, the rate of hemorrhagic complications analyzed in the meta-analysis by Amin-Hanjani and coworkers resulted in 13 (6%) of 203 patients [1]. These hemorrhagic complications included SAH, intraventricular hemorrhage, intraparenchymal hematoma, epidural hematoma and subgaleal hematoma [5, 20, 21, 24, 30]. Furthermore, only one clear documented case of CSF infection was reported [24], and one trial reported meningitis in 16 of 60 patients, however with negative CSF cultures [30]. Transient meningeal symptoms were reported in seven of ten patients receiving intracisternal thrombolysis [21] but detailed information was not provided.

The first multimodal approaches using a combination of intracisternal thrombolysis or neutral lavage in combination with kinetic therapy demonstrated in all clinical trials a

moderate effectiveness on cerebral vasospasm and outcome of patients after aneurysmal SAH [9 11, 16]. Additionally an increased clot clearance rate was documented in at least one study [10].

The head-shaking method was introduced in 1990 using the Neuroshaker (Mizuho, Tokyo, Japan) and the effectiveness was documented in a prospective trial investigating the combination of cisternal lavage and head-shaking versus cisternal lavage alone [16, 27]. In this study a swinging frequency of 1 cycle per second as motion pattern was chosen. The study demonstrated that a combination of cisternal lavage and head-shaking revealed a decrease of cerebral vasospasm and an increase of patients outcome [16]. Same positive results were documented for the use of neutral lavage in combination with the lateral rotational therapy [10, 11]. Complications such as motion sickness, brain swelling and even hemorrhage were reported in patients treated with the Neuroshaker [2]. For the chosen lateral rotational therapy there was no associated complication of the RotoRest® therapy [10, 11].

However due to observed severe complications with the occurrence of paraparesis in two patients of the study group one multimodal trial was stopped despite promising preliminary results [9].

Conclusion

In conclusion, there is strong evidence that cisternal or ventricular lavage alone and in combination with kinetic therapy lead to a reduction of cerebral vasospasm and better outcome in patients suffering from subarachnoid hemorrhage. As a consequence a prospective randomized study would be of great interest.

Conflict of interest statement We declare that we have no conflict of interest.

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Dural Arteriovenous Fistulae at the Craniocervical Junction: The Relation Between Clinical Symptom and Pattern of Venous Drainage

Gong Chen, Qihong Wang, Yanlong Tian, Yuxinag Gu, Bin Xu, Bing Leng, and Donglei Song

Abstract *Background*: Dural arteriovenous fistula (DAVF) at the craniocervical junction is an unusual condition with alternative presentations and is a rare cause of intracranial subarachnoid hemorrhage (SAH). We performed a retrospective, angiographic study of six consecutive patients to assess the relation between symptom and venous drainage and to predict the risk for SAH.

Methods: There were three females and three males; ages ranged between 37 and 64 with a mean of 52.5. Among them, four had SAH and two had pain. Diagnosis of DVAF was based on CTA, MRA and angiograph.

Results: Three patients (50%, 3/6), with single or main ascending venous route into the intracranial vein, all had intracranial SAH. Among these three patients, varix or pouches was identified in two cases (66.7%, 2/3). Three cases were treated by surgical interventions, while two subjects were endovascular techniques. The overall clinical outcomes were good during an average follow-up period of 13 months. In particular, follow-up angiographs performed 6 months later revealed the complete disappearance of DAVF in three patients.

Conclusions: There was an increased risk of SAH if DAVF at the craniocervical junction manifested an ascending venous route into the intracranial vein and/or presented with varix or pouches.

Keywords Dural arteriovenous fistulas · Craniocervical junction · Clinical symptom · Venous drainage

Introduction

Spinal dural arteriovenous fistulas (sDAVF) most commonly occur at the dorsal thoracolumbar junction and rarely bleed [2, 3, 15]. The sDAVF at the craniocervical junction or upper cervical spine (hereafter referred as to cDAVF) is unusual and is a rare cause of intracranial subarachnoid hemorrhage (SAH) [8]. Symon et al. published the first report in 1984, and Kinouchi et al. reported ten patients in 1998 [11].

Clinically, this kind of DAVF differs from its counterparts in affecting other spinal regions and has various presentations associated with patterns of venous drainage [3, 16]. For instance, the radiculopathy and cranial-nerve disturbance are presented when drainage is through the radial vein [13]. Other rare presentations include occipitalgia [5], transient ischemic attack [14]. In 2004, Aviv et al. analyzed 41 patients divided by SAH (20 patients) and non-SAH groups (21 patients) [1]. There were 12 (60%) cases with intracranial or superior drainage in SAH group compared with only 2 (9.24%) in non-SAH group. The venous varix or pouches were also presented in 7 (35%) patients with SAH group and only in 1 (4.76%) patient in the non-SAH group [10, 12].

We reviewed a consecutive series of six patients from August 2006 until August 2009 in our institution, and paid special attention to analysis venous drainage routes associated with clinical symptom. Treatment methods for these DVAFs were also illustrated in this study.

Clinical Material and Methods

Six consecutive patients (three men and three female) had a median age of 52.5 years (ranging from 37 to 64 years). All patients underwent computed tomography (CT) scan and/or magnetic resonance imaging (MRI), and computed tomographical angiography (CTA) and/or magnetic resonance

G. Chen, Q. Wang, Y. Tian, Y. Gu, B. Xu, B. Leng, and D. Song (✉)
Department of Neurosurgery, Huashan Hospital, Neurosurgical Department, Shanghai Medical College, Fudan University, Shanghai, 200040, China
e mail: onlycgcqy@yahoo.com.cn

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angiography (MRA) as part of an evaluation for DVAF. Selective angiograms of internal carotid artery (ICA), external carotid artery (ECA), vertebral artery (VA), thyrocervical artery, and spinal angiography were used to initially locate a corresponding DVAF. Afterwards, detailed information on the location, arterial supply and venous drainage pattern of a DVAF was reviewed and subsequently identified from three-dimensional (3D) reconstruction of angiography.

Four patients (66.7%, 4/6) showed intracranial SAH with Hunt and Hess (H and H) Grades I II. Two of these four subjects (50%, 2/4), who were referred to our institution from other centers, experienced SAH twice because initial "rout" angiographic findings (i.e., only single VA) were negative in other centers. The remaining two patients (33.3%, 2/6) only had headache and neck pain.

Except for a 64-year-old man (Case 3) with multi organ dysfunction syndrome, interventions were performed for the remaining five subjects. Direct surgery was performed in three patients, endovascular treatment in two cases, and then ventriculoperitoneal shun in two cases with hydrocephalus. The clinical outcome was assessed during follow-up period (ranged from 6 months to 23 months) after treatment as summarized in Table 1.

Results

All six DAVFs located at the craniocervical junction (between foramen magnum and C2) were confirmed by CTA, MRA and angiographies or 3D. Five DAVFs (83.3%, 5/6) were fed by the meningeal branch of VA: form left VA in three cases, right VA in two cases and bilateral VA in one case respectively. The other (Case 4) was fed by postmeningeal artery (PMA) and posterior inferior cerebella artery (PICA).

Five patients (Cases 1 4, and 6) had ascending venous drainages into the intracranial venous system: single ascending route in Case 3 (16.7%, 1/6) with SAH, concurrent ascending and descending routes in four patients (66.7%, 4/6) in which ascending routes were main venous drainage in two patients who had SAH (Cases 1 and 2; 50%, 2/4). Single descending route was found in one patient without SAH (Case 5).

Four patients (Cases 2 5) showed varices or pouch in the draining system: Cases 2 and 3 with ascending venous route had SAH (50%, 2/4); the symptom of Cases 4 and 5 was only pain while single or mainly venous drainage was descending.

Three patients (Cases 1, 2, and 5) underwent foramen magnum craniectomy and upper cervical laminectomy at C1 C2. Therefore, all abnormal vessels including dissected veins were clearly visualized. All identified DVAFs were

Table 1 Summary of the six cases with DAVFs at the craniocervical junction

Patient no.	Age (y)/ Sex	Symptom	Fistula point	Other lesion	Feeder vessels	Varix or pouch	Direction of drainage	Treatment	Outcome	Follow-up angiogram
1	48/F	SAH H&H I	C1	No	Meningeal branch of L-VA	No	Ascending (main) and descending	Surgery	GR	Yes, cure
2	56/M	SAH H&H I	Foramen magnum – C1	Hydrocephalus	Meningeal branch of L-VA	Yes	Ascending (main) and descending	Surgery	GR	Yes, cure
3	64/M	SAH H&H II	C1	Hydrocephalus MODS	Meningeal branch of R-VA	Yes	Ascending	No	MD in nervous system	No
4	37/M	Headache	Foramen magnum	No	L-Postmeningeal artery, L-PICA	Yes	Descending (main) and ascending	Emb	GR, Subtotal Emb	No
5	51/M	Neck pain	Foramen magnum – C1	No	Meningeal branch of R-VA	Yes	Descending	Surgery	GR	Yes, cure
6	59/F	SAH H&H II	Foramen magnum – C1	Dissecting AN of BA	Meningeal branch of bilateral VA	No	Ascending and descending	Stenting for AN	GR	No

SAH subarachnoid hemorrhage, *H&H* Hunt and Hess, *C* cervical vertebra, *MODS* multiorgan dysfunction syndrome, *R* right, *L* left, *AN* aneurysm, *VA* vertebral artery, *BA* basilar artery, *PICA* posterior inferior cerebellar artery, *Emb* embolization, *GR* good recovery, *MD* moderately disabled

then coagulated at a site just distal to the exit zone from the dura mater.

In Case 4, because the lesion was located at the ventral lateral brain stem, the DAVF was subtotally embolized by injection of Ethylene Vinyl Alcohol Copolymer (Onyx) (Micro Therapeutics Inc., Irvine, CA, USA) and then treated with gamma () knife. In Case 6, the identified DAVF was accompanied by a dissected aneurysm (AN) of basilar artery (BA). Because the DAVF had low shunt flow and had no venous pouch, the dissected AN was first treated by stenting (Neuroform, Boston, USA).

Except for the untreated Case 3, treatment outcomes for five subjects were good during an average follow-up period of 13 months. Among five treated patients, the postoperative angiographs performed 6 months later revealed the complete disappearance of DAVF in three patients (Cases 1, 2, and 5). Clinical results are presented in Table 1.

Selected Cases

Case 2: A 56-year-old man experienced a sudden onset of severe headache. His physical examination revealed no neurological deficit except neck stiffness. CT revealed SAH and hydrocephalus (Fig. 1a, b). Angiography showed a DAVF. The hydrocephalus was managed by external ventricular drainage. Repeat cerebral and spinal angiographies, performed 1 week later to exclude other vascular lesions, confirmed a DAVF at the craniocervical junction (Fig. 1c, d).

The patient subsequently underwent a direct surgery. After the left VA was exposed, enlarged arterial feeding vessels were seen running into the varix at C1 level (Fig. 1e). These abnormal vessels were carefully dissected off the left VA and cervical spine, and then were coagulated (Fig. 1f). The ventriculoperitoneal shunt was performed 10 days later. The operative course was uneventful without any post-procedure complications. A follow-up angiogram 6 months post-surgery showed no residual or recurrent DAVF (Fig. 1g, h).

Case 4: A 37-year-old man suffered from headache of foramen magnum for 2 months. CT and MRI showed a vascular lesion in right ventral lateral brain stem (Fig. 2a, b). Angiography (Fig. 2c, d) and 3D (Fig. 2e) of right VA showed a DAVF supplied by right PMA (main) and PICA and draining by both ascending and descending routes (Fig. 2f) without varices or pouch (Fig. 2g).

Due to lesion located in risk area (brain stem), endovascular treatment was used. Under general anesthesia, a Marathon microcatheter (Flow 1.5F, ev3, USA) was navigated to right PMA with aid of a 0.008-in. guidewire (Mirage, ev3, USA). The tip of the microcatheter was placed as close as

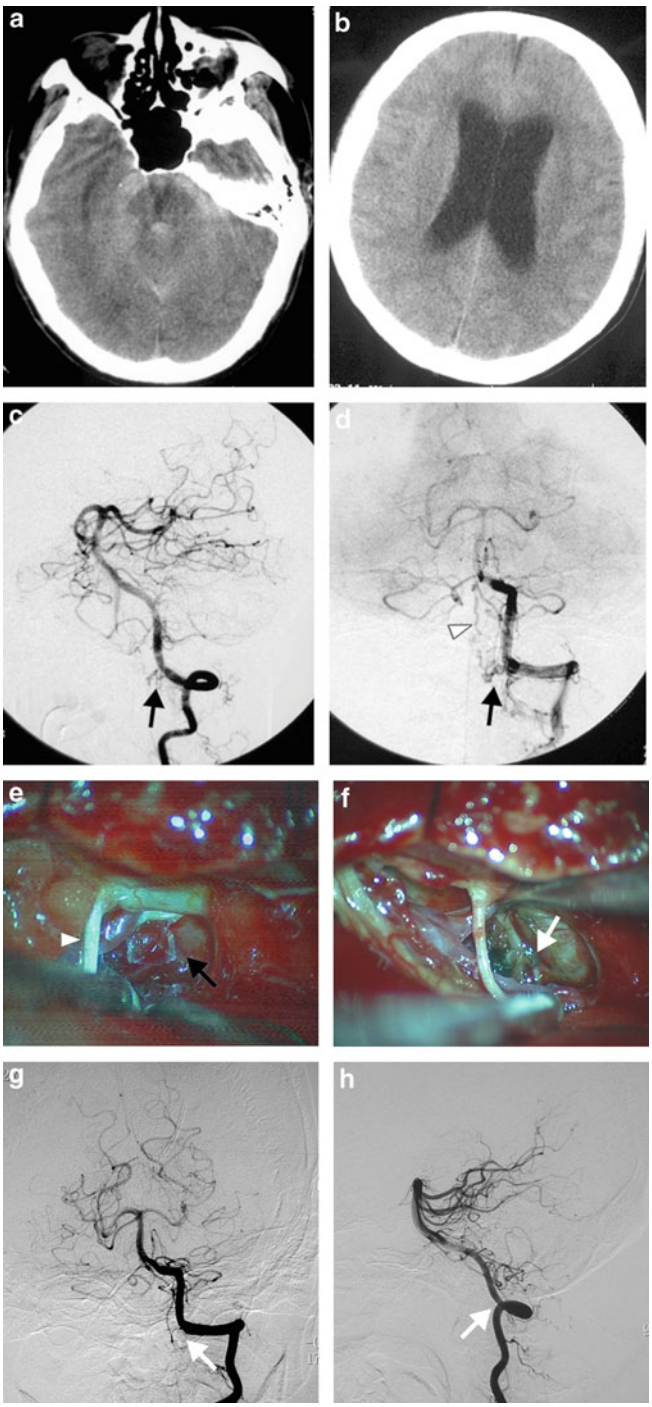

Fig. 1 Case 2: (**a**, **b**) Axial CT showed SAH in the basal cisterns and hydrocephalus. (**c**, **d**) Angiography showed a DAVF (*arrows*) fed by multiple small meningeal branches of left VA. There was early opaci fication of multiple ascending venous routes with varix (*white triangle*). (**e**) The DAVF was seen under and in front of left C2 nerve root (*white triangle*), and enlarged arterial feeding vessels shunt into the varix and enlarged tortuous medullary vein (*black arrows*). (**f**) Both fistula and venous varix of DAVF were divided and coagulated (*white arrows*). (**g**, **h**) Follow up angiography 6 months later confirmed no residual DAVF (*white arrows*)

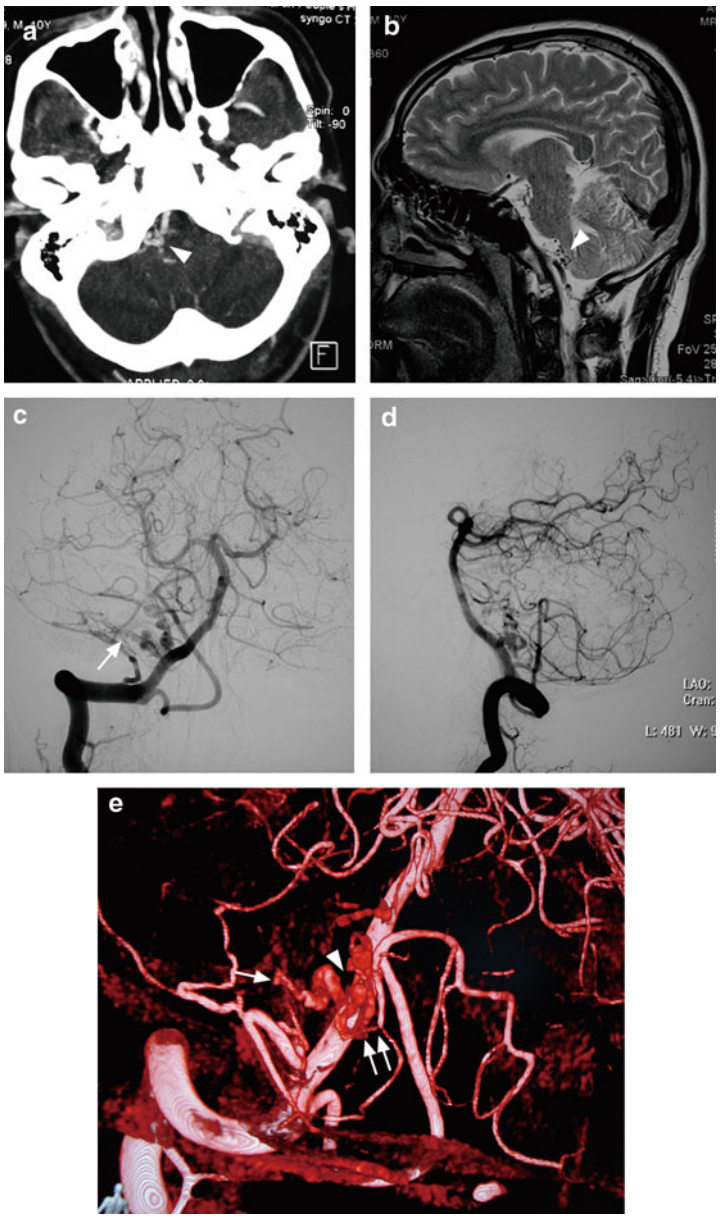

Fig. 2 (continued)

possible to the fistula, and then Onyx-18 was injected (0.4 ml). Post-angiogram demonstrated that DAVF was subtotally embolized without neurological deficits. Only very small residual lesion fed by the PICA (Fig. 2h j). He was then referred to knife for residual lesion.

Discussion

From data in this paper, this kind of DAVF usually occurred in middle-aged people (mean 52.5 years in this study, which is consistent with mean 58 years in literature [1]). SAH caused by these DAVFs has a more benign clinical presentation and than aneurysmal SAH: 95% of patients with DAVF-related SAH had H and H Grades I or II (100% in our series). In contrast, only 70 80% of patients with aneurysmal SAH had H and H Grade I or II [6].

In our series, the feeding arteries of these DAVF were mostly from VA (83.3%, 5/6), and there were no significant differences between left or right side. Although it was reported that right VA supplied DAVF more frequently (a factor of two) compared to the left VA [1], the small number of subjects available to this study made a comparison difficult. The majority of DAVF (66.7%, 4/6) had two

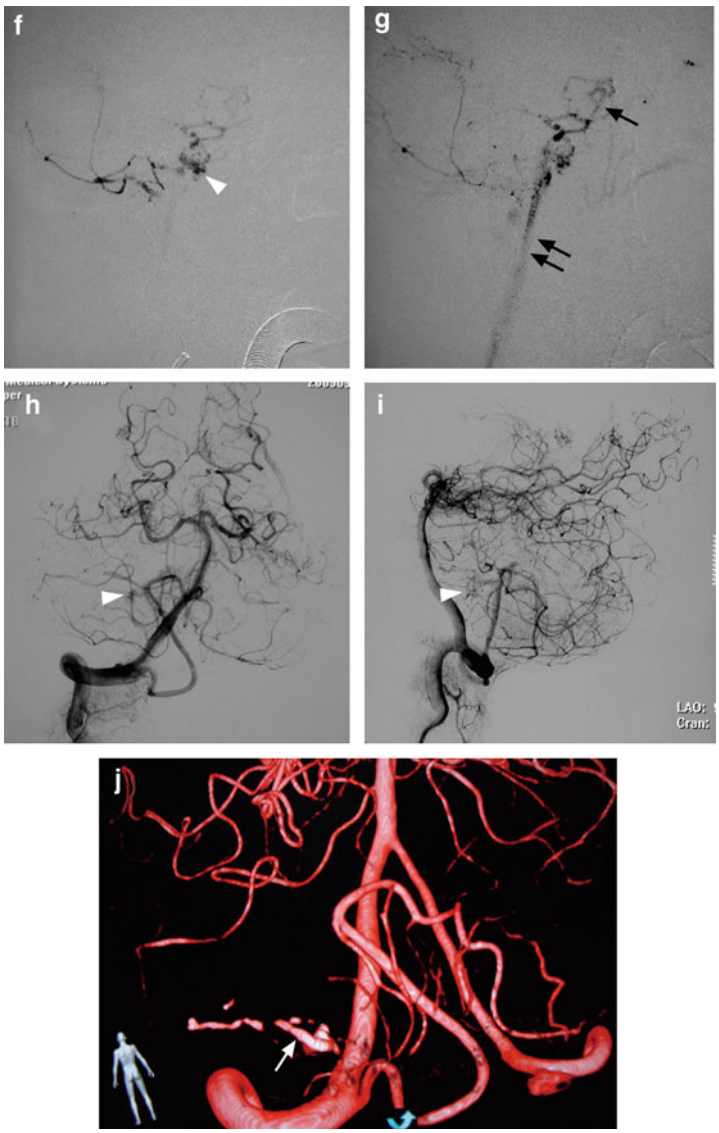

Fig. 2 Case 4: (**a**, **b**) Enhanced CT and T2 weighted MRI showed a vascular lesion (*white triangle*) in right ventral lateral brain stem. (**c** **e**) Angiography and 3D of right VA showed a DAVF supplied by right PMA (*white arrow*) and PICA(*double white arrows*). (**f**, **g**) Concurrent ascending (*black arrow*) and descending routes (*double black arrow*), but descending routes was main (*double black arrow*). (**h** **j**) Post angiogram and 3D demonstrated that DAVF was subtotally embolized, small residual fed by PICA (*white triangle*) and the cast of Onyx in PMA (*white arrow*)

venous drainage routes (ascending and descending routes). Among four patients (Cases 1 3, and 6) with SAH, three patients (Cases 1 3; 75%, 3/4) showed single (or main) ascending routes, two patients (Cases 2 and 3; 50%, 2/4) showed varices or pouch. In Case 6, we were not certain about the relationship between SAH and DVAF, but SAH may be caused by the dissecting AN due to DAVF with low shunt flow and without varices or pouch. Of course, this patient will undergo long follow-up. Although Cases 4 and 5 were associated with varices or pouches, respectively, their venous drainages were identified as descending routes. Hence, their symptoms were only pain of foramen magnum and neck respectively.

None of the patients presented with myelopathy in our series compared with a high percent reported in the literature [1]. However, we found one patient (16.7%, Case 5) had radiculopathy as compared with 8% in literature [1]).

Although these DAVFs or pial arteriovenous malformations have been reported to be an unrecognized cause of SAH [1, 7, 8, 10], it is noteworthy that 22.7 40% of DAVFs were missed at initial angiography in literature [1, 8] (50%, 2/4 in this paper). Our experiences also demonstrate the usefulness of evaluating more segments of relevant arteries, including both bilateral ECAs and Vas, which must be considered in the search for a cause of SAH when initial "rout" angiographic findings are negative. Failure to identify a suspected DAVF after injection of above arteries warrants a thorough evaluation of the thyrocervical, costocervical, and ascending pharyngeal arteries or spinal cord to look for another segmental cervical supply.

Multiple treatment options including surgical removal, endovascular intervention, and radiotherapy or a combination of any above-mentioned techniques, are available for this kind of DAVF.

Surgical intervention is still a very effective treatment [4, 8, 10, 16]. However, the risk of reoccurrence will be still high if not all fistulous tissues are completely removed. Thanks to advances in modern interventional neuroradiology, endovascular technique is becoming more acceptable as the curable or pre-surgical treatment for DAVF. With suitable angioarchitecture for endovascular embolization, complete obliterations were achieved in a few cases with sDAVF [9]. However, sDAVF at the craniocervical junction was usually fed by multiple, minute or tortuous arteries. Consequently, it is difficult to completely cure with endovascular techniques alone. In such circumstances, further treatment such as radiation therapy (e.g., γ knife) will usually be required. Furthermore, even with complete occlusions, the embolic materials (e.g., liquid glue, coils and balloons) might cause or aggravate mass effect. However, if the lesion locates at a high-risk region or is from a high-risk patient group, the advantages of endovascular techniques will significantly outweigh the limitation described above (e.g. Case 4).

Conclusion

The complete angiographic evaluation of a suspected DAVF located at the craniocervical junction or cDAVF should be performed for critical vessel segments. These DAVFs with ascending venous routes into the intracranial circulation or varices or pouches present an increased risk for SAH. The majority of DAVFs were effectively treated by surgical interventions, while a few cases were treated by endovascular treatment. The combination of presurgical embolization and surgery might be an effective method for large, complex and high-flow shunt DAVF.

Conflict of interest statement We declare that we have no conflict of interest

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Surgical Procedure and Results of Cisternal Washing Therapy for the Prevention of Cerebral Vasospasm Following SAH

Tadayoshi Nakagomi, Kazuhide Furuya, Hiroshi Nagashima, Jun-ichi Tanaka, Teruyuki Ishii, Shigehiko Takanashi, Takeyuki Shinohara, Fumihiro Watanabe, Akiko Ogawa, Norio Fujii, and Akira Tamura

Abstract In 1994, we started cisternal washing therapy (CWT) using urokinase combined with head-shaking method in order to prevent cerebral vasospasm. In this paper, we showed the surgical procedure for CWT and reported the effect of this therapy in preventing vasospasm following SAH. A total of 332 consecutive cases with Fisher group 3 SAH since 1988 were analyzed. Of these patients, 118 cases (56 cases before 1994 and 62 cases after 1994) had not CWT, and, 214 cases after 1994 had this therapy. All of these patients had clipping surgery within 3 days following SAH, and had postoperative management both with normovolemia and normal to mild hypertension. In these two groups, the incidence of symptomatic vasospasm (transiently symptomatic vasospasm without infarction), cerebral infarction due to vasospasm on CT, and mortality and morbidity (M&M) due to vasospasm were analyzed. In the group without CWT, the incidences of symptomatic vasospasm, cerebral infarction on CT, and M&M due to vasospasm were 4.2%, 28.8%, and 17.8%, respectively. On the other hand, in the group with CWT, they were 3.7%, 6.5%, and 2.8%, respectively. In the patients with CWT, the incidence of cerebral infarction on CT due to vasospasm and M&M due to vasospasm were significantly ($p < 0.05$) decreased. CWT was effective in preventing cerebral vasospasm.

Keywords Subarachnoid hemorrhage · Vasospasm · Urokinase · Head-shaking · Cisternal irrigation

T. Nakagomi (✉), K. Furuya, H. Nagashima, J. i. Tanaka, T. Ishii, S. Takanashi, T. Shinohara, F. Watanabe, A. Ogawa, N. Fujii, and A. Tamura
Department of Neurosurgery, Teikyo University School of Medicine, 2 11 1 Kaga, Itabashi City, Tokyo, 173 8605, Japan
e mail: nsnaka@med.teikyo u.ac.jp

Introduction

Cerebral vasospasm is still one of the leading causes of mortality and morbidity (M&M) following aneurysmal subarachnoid hemorrhage (SAH) [1]. Clinical studies have clearly demonstrated that occurrence of cerebral vasospasm following SAH is closely associated with the location and volume of the subarachnoid clots [2, 3]. Cisternal administration of thrombolytic agents such as urokinase or tPA has been reported to facilitate dissolution of subarachnoid clot and result in prevention of cerebral vasospasm. In 1994, we started cisternal washing therapy (CWT) using urokinase combined with head-shaking method in order to prevent cerebral vasospasm. In this paper, we showed the surgical procedure for CWT and retrospectively analyzed the effect of this therapy in preventing cerebral vasospasm following SAH.

Surgical Procedure and Postoperative Management

IV mannitol (200 300 ml/patient) is administered at the time of skin incision for the brain relaxation. Then usual pterional craniotomy is carried out for the anterior circulation aneurysms. After dural opening a ventricular drainage tube is placed through the frontal lobe. Subarachnoid clot around the aneurysm and in the cisterns is removed so as not to damage normal brain tissue. After clipping of the ruptured aneurysm, cisternal drainage tube is placed in the carotid or in the chiasmatic cistern (sometimes in the sylvian cistern). After the patients returns to the recovery room, cisternal washing therapy is started by irrigating the subarachnoid space through these two tubes. At the same time patients have postoperative management both with normovolemia and normo to mild hypertension. Procedure for the cisternal washing therapy is usually completed within 72 h.

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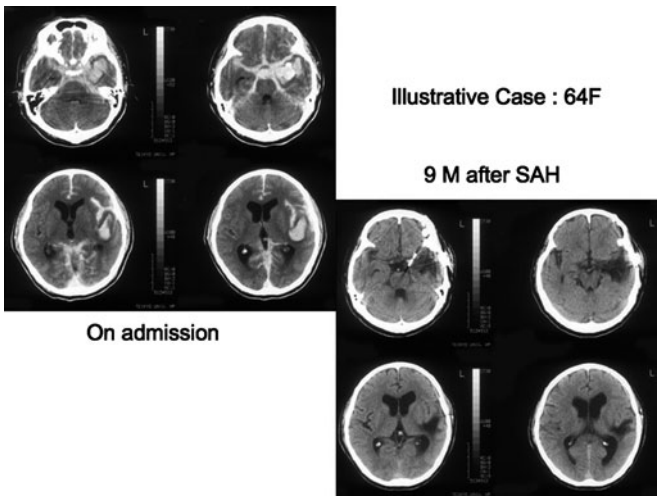

Fig. 1 CT findings on admission (*left*) and at 9 months following SAH (*right*) in the illustrative case

An illustrative case was shown. This case was 64 year's old doctor who suddenly had severe headache and lost consciousness when he was seeing a patient in his clinic. Emergent CT scan revealed Fisher group 3 subarachnoid clots and an intracerebral hematoma in the left temporal lobe (Fig. 1). The patient was intubated under sedation and angiography was done. Angiograms revealed a left IC aneurysm. During the angiography, his brood pressure suddenly rose and the patient had rebleeding from the aneurysm. The patient was transferred to the OR. Preoperative WFNS grade was 4. IV mannitol (300 ml/patient) was administered for the brain relaxation just before skin incision. Skin incision was made in the left fronto-temporal region. Fronto-temporal craniotomy was made after the opening of three burr holes. Just after the opening of dura mater, a ventricular tube was inserted into the anterior horn of the lateral ventricle through the frontal lobe. Then clipping of the aneurysm was achieved using the pterional approach. Removal of subarachnoid clot was carried out not to damage the brain and the cerebral vasculature. Extent of the clot removal was relatively minimal. Before closing the dura mater, draining tube was inserted into the optico-carotid cistern after opening the Liliequist membrane. Both ventricular tube and cisternal tube are used for cisternal washing therapy. The patient had postoperative management with normovolemia and normomild hypertension. Cisternal washing therapy started just after the surgery. CT scan at POD1 shows some disappearance of subarachnoid clots, but still thick clots in the basal cistern and left Sylvian fissure. Cisternal washing therapy was terminated at POD4, because the high density area in the basal cistern almost disappeared. At POD7, angiography was done and revealed vasospasm in the left middle cerebral artery. So, fasudil hydrochloride was administered intraarterially. CT scan at 9 months later following SAH shows no cerebral infarction due to vasospasm (Fig. 1). The patient had returned to his previous work with slight dysphasia and memory disturbance due to an intracerebral hematoma.

Materials and Methods

A total of 332 consecutive cases with Fisher group 3 SAH, who had been admitted to the Teikyo University Hospital from January 1988 to December 2008, were retrospectively analyzed. Of these patients, 118 cases (56 cases before 1994 and 62 cases after 1994 who had not cisternal washing therapy due to medical or surgical complications, problems in placement of the catheter, postoperative obstruction of the catheter with clots, surgeon's choice, and so on.) had not cisternal washing therapy, and, 214 cases after 1994 had this therapy. All of these patients had clipping surgery within 3 days from the onset of SAH. After the patients returned to the recovery room, cisternal washing therapy began by irrigating the subarachnoid space through these two tubes (Fig. 2). Lactated Ringer's solution containing urokinase (60,000 IU/500 ml), was infused from the ventricular tube at a rate of 60 180 ml/h. The pressure for cisternal irrigation was set not to exceed a height of 25 cm H_2O from the external auditory meatus. The intracranial pressure control system usually set at a height of 5 10 cm H_2O. Then the head of the patient was rested on the head-shaking device (Neuroshaker), and was shaken periodically at the rate of 1 1.5 c/s. Almost all patients could tolerate head shaking up to 48 h. Cisternal washing therapy was terminated when the total amounts of urokinase reached 420,000 IU, or the high density area both in the basal cistern and Sylvian fissure disappeared on CT scan. In more than 90% of the patients who had cisternal washing therapy, this procedure was completed within 72 h. After the termination of cisternal

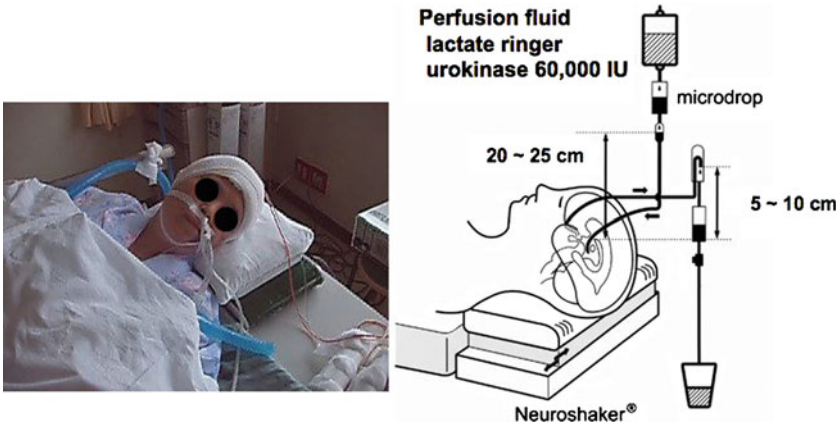

Fig. 2 Schema for the cisternal washing therapy

washing therapy, patients had conventional treatment for cerebral vasospasm. All patients had postoperative management both with normovolemia and normo to mild hypertension. The patients who had not cisternal washing therapy had conventional treatment for cerebral vasospasm just after the surgery.

In these two groups, the incidence of transiently symptomatic vasospasm (without infarction), cerebral infarction due to cerebral vasospasm on CT, and mortality and morbidity (M&M) due to vasospasm were analyzed. Statistical analysis was done using Student's t-test or Chi-square test. The value were considered significantly different when $p < 0.05$.

Results

There were no significant differences in age, sex, WFNS grade, timing of surgery, and site of the aneurysm between two groups (Table 1). In the group without cisternal washing therapy, the incidences of transiently symptomatic vasospasm, cerebral infarction on CT, and M&M due to vasospasm were 4.2%, 28.8%, and 17.8%, respectively (Table 1). On the other hand, in the group with cisternal washing therapy, they were 3.7%, 6.5%, and 2.8%, respectively (Table 1). In the patients with cisternal washing therapy, the incidence of cerebral infarction on CT due to vasospasm and M&M due to vasospasm were significantly ($p < 0.05$) decreased. Favorable outcome (GR + MD = 83.1%) was also obtained in the group with cisternal washing therapy.

Discussion

According to the literature by Dorsch and King [1], symptomatic vasospasm or delayed ischemic neurological deficits (DINDs) occurred in 32.5%. Thirty percent of the patients with DINDs died, and permanent neurological deficits occurred in 34% in the patients. In 1988, Kodama et al. reported that cisternal irrigation therapy with urokinase and ascorbic acid was effective in preventing cerebral vasospasm [4]. In 1990, Suzuki et al. demonstrated that head-shaking method enhanced the fibrinolysis in cisternal irrigation [5]. In January 1994, we combined these two methods in order to get better outcome of the patients with Fisher group 3 SAH and started cisternal irrigation therapy using urokinase combined with head-shaking, i.e., cisternal washing therapy (CWT). Surgical Procedure and Postoperative Management were relatively simple as stated before and CWT was can be easily achieved.

In the present study, the incidences of transiently symptomatic vasospasm, cerebral infarction on CT in the group without cisternal washing therapy were 4.2% and 28.8%, respectively. The incidence of total symptomatic vasospasm (transiently symptomatic vasospasm without cerebral infarction and cerebral infarction confirmed by CT scan) in the patients without cisternal washing therapy was almost the same as the incidence of symptomatic vasospasm reported by Dorsch and King. On the other hand, in the group with cisternal washing therapy, the incidences of transiently symptomatic vasospasm, cerebral infarction on CT due to cerebral vasospasm were 3.7% and 6.5%, respectively. Although there was no significant difference in the transiently symptomatic vasospasm between the two groups, incidence of cerebral infarction on CT decreased significantly ($p < 0.05$) in the group with cisternal washing therapy.

The present study also demonstrated that M&M due to cerebral vasospasm at 6 months following SAH was 17.8% in the group without cisternal washing therapy and 2.8% in the group with cisternal washing therapy. Favorable outcome (GR + MD = 83.1%) was also obtained in the group with cisternal washing therapy.

Table 1 Summary of the cases with Fischer group 3 SAH

	Patients Without Cisternal Washing		Patients With Cisternal Washing		
Age					
Range	16–87		Range	33–83	
Mean	55.5		Mean	58.9	
Sex					
M	41	34.7%	M	84	39.3%
F	77	65.3%	F	130	60.7%
Total	118		Total	214	
Vasospasm					
Symp only	5	4.2%	Symp only	8	3.7%
Infarction	34	28.8%	Infarction	14	6.5%
Total	38	33.0%	Total	22	10.2%
GOS					
GR	63	53.4%	GR	155	72.4%
MD	20	16.9%	MD	23	10.7%
SD	9	7.6%	SD	15	7.0%
V	9	7.6%	V	9	4.2%
D	17	14.4%	D	12	5.6%
M&M					
PBD	17	14.4%	PBD	20	9.3%
Med C	5	4.2%	Med C	17	7.9%
Surg C	10	8.5%	Surg C	12	5.6%
Vasospasm	21	17.8%	Vasospasm	6	2.8%
Total	53	44.9%	Total	55	25.7%

GOS glasgow outcome scale, *M&M* mortality and morbidity, *GR* good recovery, *MD* moderately disabled, *SD* severely disabled, *V* vegetative, *D* dead, *PBD* primary brain damage, *Med C* medical complication, *Surg C* surgical complication

Cisternal washing therapy always carries the risk of hemorrhage, because urokinase is a fibrinolytic agent. In the present study, complications occurred in only six patients (2.8%). Four patients developed hemorrhagic complications. However, follow-up study disclosed that these patients had no neurological deficits. It can be considered that cisternal washing therapy could minimize the risk of bleeding through the use of relatively low dose of urokinase and continuous irrigation.

Conclusion

In conclusion, since the introduction of cisternal washing therapy, the incidence of cerebral infarction on CT, and M&M due to cerebral vasospasm was significantly ($p < 0.05$) decreased in Fisher group 3 patients with SAH. Cisternal washing therapy is strongly recommended as a potent fibrinolytic therapy for the prevention of cerebral vasospasm in the patients with Fisher group 3 SAH.

Conflict of interest statement We declare that we have no conflict of interest.

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Objective Evaluation of the Treatment Methods of Intracranial Aneurysm Surgery

Rui Xu, Ji Zhu, Xiao-chuan Sun, Zhao-hui He, and Xiao-dong Zhang

Abstract *Objective*: This study evaluated the clinical value of craniotomy and intravascular embotherapy in the treatment of intracranial aneurysms.

Methods: The clinical data of 126 cases of intracranial aneurysms from July 2008 to July 2009 was analyzed retrospectively, 86 cases of all were clipped and other 40 cases were coiled.

Results: In 86 cases with craniotomy (according to Hunt-Hess classification, 71 cases belong to grade I III and 15 cases belong to grade IV V), 1 case died, 3 cases recovered with serious nervous system symptoms, 9 cases recovered with Mild neurological symptoms, and the remaining 73 cases recovered with normal life and work. In 40 cases with intravascular embotherapy (according to Hunt-Hess classification, 33 cases belong to grade I III and 7 cases belong to grade IV V), 2 cases recovered with serious nervous system symptoms, 5 cases recovered with mild neurological symptoms, the remaining 33 cases recovered with normal life and work; no death case.

Conclusions: The situation is different in patients according to aneurysm size, shape, and location; if treatment for intracranial aneurysms is to achieve a satisfactory effect, two treatments must complement each other.

Keywords Intracranial aneurysm · Craniotomy · Intravascular embotherapy · Subarachnoid hemorrhage

Introduction

Intracranial aneurysm is a hemangioma-like protuberance caused by abnormal changes in local blood vessels. Clinically, the most frequent initial symptom is subarachnoid hemorrhage (SAH). The symptoms are usually acute with a high rate of fatality and disability and bad prognosis. Correct and timely diagnosis and proper treatment can directly affect the prognosis of the patients. In this article, patients receiving craniotomy and intravascular embotherapy from July 2008 to July 2009 in our department were analyzed, and the results were reported as follows.

R. Xu, J. Zhu (✉), X. c. Sun, Z. h. He, and X. d. Zhang
Department of Neuro surgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P.R. China
e mail: a68690569@sina.com

Materials and Methods

Patient Population

In the last year, 126 patients received surgery on intracranial aneurysm in our department, among which 86 cases were treated with craniotomy and 40 cases were treated with intravascular embotherapy. There were 76 male cases and 50 female cases aging from (19 84) with an average of 49. As to the clinical manifestation, 98 cases suffered from sudden severe headache, 46 cases suffered from conscious disturbance to different degrees, 15 cases suffered from oculomotor paralysis, 16 cases suffered from hemiplegy and two cases were diagnosed through physical examination. Before the surgery started, 15 cases were classified as grade I, 55 cases were classified as grade II, 34 cases were classified as grade III, 16 cases were classified as grade IV and six cases were classified as grade V according to the rules of Hunt-Hess classification. One hundred and twenty-six patients were confirmed by receiving the computed tomography angiography (CTA) and digital subtraction angiography (DSA). Forty-one cases suffered from anterior communicating aneurysm, 52 cases suffered from posterior communicating aneurysm of internal carotid, two cases suffered from anterior cerebral artery aneurysm, 14 cases suffered from middle cerebral artery aneurysm, three cases suffered from vertebrobasilar aneurysm, six cases suffered from siphon aneurysm of internal carotid and eight cases

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suffered from multiple aneurysm. Eighty-three cases had an aneurysm size smaller than 0.5 cm, 52 cases had an aneurysm size ranging from 0.6 to 1.4 cm, four cases had an aneurysm size ranging from 1.5 to 2.5 cm and one case had an aneurysm size larger than 2.5 cm.

Surgical Methods

The transperitoneal approach was introduced in the craniotomy to protect the temporalis. The parent artery and aneurysm neck were firstly exposed. Subsequently, the aneurysm neck was clipped after the aneurysm and the neighboring blood vessels were separated. The parent artery can be occluded temporarily when it was necessary. The occlusion clip was removed after the aneurysm was clipped. The clipped part on the aneurysm was covered by gelatin sponge which had been immersed in narceine. The Seldinger puncture through the femoral artery was adopted in the intravascular embotherapy. After the 6F or 8F vagina blocker was inserted, the cerebral angiography was carried out in order to measure the size of the aneurysm and the aneurysm neck. The width of the parent artery will be measured if necessary. After the heparinization throughout the body, the aneurysm was embolized directly under the tracing route. Under the assistance of bracket sacculus, the aneurysm can also be embolized by taking advantage of the double microtubule.

After the surgery, the spasmolytic, anti-infective and brain cell active medicine were used to prevent the hemorrhage of digestive tract and epilepsy. After endovascular interventional therapy under the assistance of bracket, we carried out the anticoagulation for 3 days by low molecular through conventional subcutaneous injection. Plavix was applied for 6 weeks while enteric-coated aspirin was applied for 6 months through oral administration. As for the patients who suffered from significant subarachnoid hemorrhage and ventricular hemorrhage, the lumbar cistern drainage should be carried out for 3 5 days after the surgery.

Results

Seventy-one patients of the grade I III and 15 patients of the grade IV V were cured using craniotomy with one death case (acute hemorrhage of posterior communicating aneurysm of the left internal carotid accompanied by intracerebral hematoma and cerebral hernia; the aneurysm was clipped and the intracerebral hematoma was removed; secondary infarct occurred after the surgery; the patient died of cardio-pulmonary failure eventually). Three patients suffered from severe nervous system symptoms (seriously disabled), nine patients suffered from mild nervous system symptoms (moderately disabled), and 73 patients returned to their normal life and work. Thirty-three patients of the grade I III and seven patients of the grade IV V were cured using intravascular embotherapy without death case. Two patients suffered from severe nervous system symptoms (seriously disabled), five patients suffered from mild nervous system symptoms (moderately disabled), and 33 patients returned to their normal life and work.

Discussion

Intracranial aneurysm is a common cerebrovascular disease. In recent years, the diagnosis rate of aneurysm and success ratio in curing aneurysm became much higher and the disability rate as well as the mortality rate decreased significantly with the development of image technology such as CT and CTA (CT angiography), the application of magnetic resonance imaging (MRI) and digital subtraction angiography (DSA) and the development of microneurosurgery and nerve interventional therapy.

As to the therapy of intracranial aneurysm using craniotomy and intravascular embotherapy, not only should the pathological changes be eradicated, but also the parent artery and its branch vessels should be kept unobstructed. The development of imageology enables the success ratio of the surgery on intracranial aneurysm to become higher and higher. One hundred and twenty-six cases who suffered from aneurysm were reported in this article. Before the surgery started, the 3D-CTA inspection was carried out firstly to learn about the position, size and form of aneurysm, the width, position of the aneurysm neck, the direction of aneurysm top, relationship between aneurysm/aneurysm neck with parent artery, neighboring blood vessels and osseous structure. Meanwhile, a suitable therapeutic schedule was selected based on the observation of the characteristics of aneurysm on the operation position through rotation and incision.

As to anterior communicating aneurysm, the width and position of the aneurysm neck, the direction of the aneurysm top, A1 on both sides, the distal blood vessel and the neighboring perforating vessel should be examined in order to know if there is any defect or maldevelopment. That was an important index that determined the selection of therapeutic method and the success of the therapy on aneurysm [1]. However, the direction of aneurysm was the primary morphological standard for the selection of the therapeutic method on anterior communicating aneurysm [2]. If the direction of the aneurysm was at the back, the exposing process of the craniotomy became difficult with a higher occurrence rate of harmful damage and complicating disease caused by the

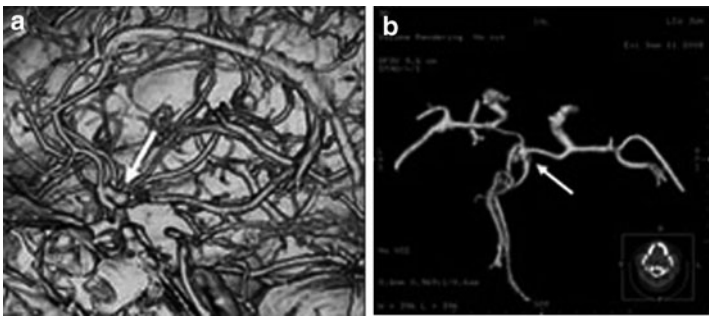

Fig. 1 (**a**) CTA showed that the anterior communicating aneurysm pointed at back. (**b**) Imitation of the aneurysm neck being sheltered at the operation position

surgery. Most aneurysms were located at the back of the A2 section of arteria cerebri anterior and below the A1 section and the Heubner reverse artery. The separation of the A1 section and the Heubner reverse artery were extremely difficult. Furthermore, the top of the aneurysm growing at the back can hide behind the straight gyrus at the same side. Thus, the separation of aneurysm and the excision of the straight gyrus can easily result in the cracking and hemorrhage of the aneurysm during the surgery. Therefore, interventional therapy was adopted by our department in case the aneurysm grew in this direction (Fig. 1). As to the aneurysm that grew in the front lower place and at the front, interventional therapy and surgery can act excellently. However, the application of bracket and sacculus increased the risk of surgery, and the occurrence rate of complicating disease increased as a result [3]. Especially for arteries that have an aneurysm with a large volume and a wide aneurysm neck, the success ratio of embolization was low [4]. For this type of aneurysm, surgery therapy was selected in order to decrease the recurrence rate of aneurysm. As to the anterior communicating aneurysm that had to be cured through surgery therapy, the hemodynamics relationship should be learned through DSA before the surgery starts in order to select a safe and reliable operative approach. During surgery, the preponderant blood supply approach was selected, and that was good for the temporary blockage of parent artery. As to the cases with two symmetrical sides, transperitoneal approach at the opposite side of the direction of the aneurysm top was selected in order to prevent the risk increase of hemorrhage caused by touching the artery during the surgery.

As to aneurysm of internal carotid, the width and position of the aneurysm neck, the direction of aneurysm top should be learned at the same time. In particular, the relationship with osseous structure and the growth position of aneurysm were critical for us to select the proper therapeutic method. As to internal carotid-posterior communicating aneurysm, especially the case when the aneurysm grew to the back (frequently accompanied by oculomotor paralysis), and when the anterior clinoid process did not shelter the aneu-

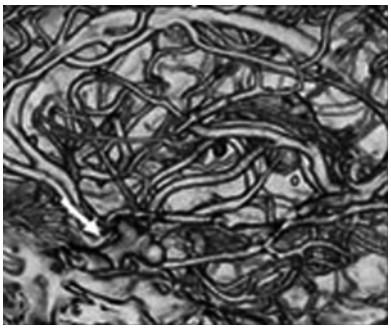

Fig. 2 Relationship between aneurysm and anterior clinoid process, a certain distance between aneurysm and anterior clinoid process was shown

rysm (Fig. 2), craniotomy was selected in order to cure aneurysm effectively and reduce the recurrence rate. Great attention should be paid to the relationship between aneurysm and posterior communicating artery, arteria choroidea, thalamic perforating branch artery and oculomotor nerve during the clipping of the aneurysm. As to the aneurysm of the cavernous sinus section of internal carotid or the clinoid section, the aneurysm was often sheltered by the osseous structure, especially when the aneurysm grew towards the inner side and was related to the clinoid process closely. If the osseous protuberance covered the starting section of the aneurysm neck, the risk of cracking of the aneurysm will be increased during the surgery when the osseous structure should be grinded during craniotomy. It was extremely troublesome to cope with the cracking and hemorrhage during the surgery. Meanwhile, as to the aneurysm that grew towards the inner side (Fig. 3), the aneurysm was often sheltered by the parent artery and the optic nerve and the aneurysm neck cannot be fully exposed during the surgery. It was very hard to clip the aneurysm with a higher risk of damaging the optic nerve. Thus, interventional therapy was adopted to treat this kind of aneurysm.

As to the therapy of the aneurysm of the middle cerebral artery, craniotomy was adopted by our department towards

the 14 cases suffering from aneurysm of the middle cerebral artery because most aneurysms in the middle cerebral artery were located in the neighboring part of critical blood vessels, particularly where there were a large number of vascular loops in the middle cerebral artery M1 and its bifurcating region. The shape of the aneurysm, the size of aneurysm neck and the direction of aneurysm top can only be fully learned when a specific direction was selected using CTA and DSA. Furthermore, the form of aneurysm at this part was usually irregular. In particular, those aneurysms that had a volume not very large and the indistinct display of aneurysm neck and the aneurysm of parent artery can bring great difficulties to the therapy of embolization. Important branches might be occluded. Most cases were not suitable to be cured by interventional therapy. Instead, direct craniotomy should be adopted [4]. Thus, surgery therapy was adopted by our department to treat aneurysm of middle cerebral artery. The information about the aneurysm was learned using CTA and DSA before the surgery started and the relationship between aneurysm and distal proximal blood vessels was dissected and analyzed carefully. Thus, the aneurysm can be clipped successfully and the parent artery as well as its branch vessels can be kept unobstructed (Fig. 4).

As to posterior circulation aneurysm (aneurysm on the vertebrobasilar artery and its branches) and multiple aneurysm, the endovascular interventional therapy will be the first choice. The therapy risk of the posterior circulation aneurysm was high, the operation was difficult and the number of critical structures was larger with a high mortality rate and disability rate. The endovascular interventional therapy can provide a safe and reliable therapy method compared to the craniotomy [5, 6]. Moreover, the embolization rate of aneurysm became higher and higher with the development of bracket and sacculus in recent years; in particular, the development of the soft and safe bracket transfer system and the interventional therapy of wide-neck aneurysm. Interventional therapy will also be the first choice as to the multiple aneurysm because the embolization of several aneurysms can be completed each time [7], particularly for multiple aneurysms on both sides. As to multiple aneurysms, interventional therapy can not only prevent the recurrent hemorrhage of aneurysm but also can eliminate the risk of incorrect judgment caused by the failure to diagnose aneurysm. In case of embolization therapy, the aneurysm with embolization hemorrhage will be the first choice. The principle of easy-first and difficult-later should be adopted if no signs of hemorrhage can be found in aneurysm or

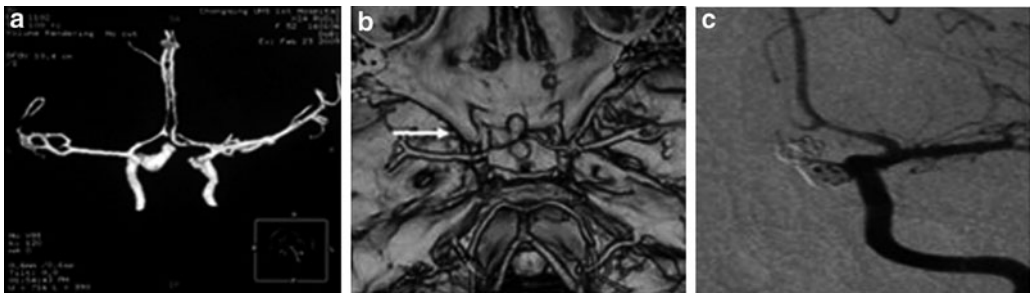

Fig. 3 (**a**) CTA showed the aneurysm pointed to the inner side. (**b**) The aneurysm closely related to the osseous structure anterior clinoid process sheltered the aneurysm neck. (**c**) 3D DSA showed the aneurysm after embolization

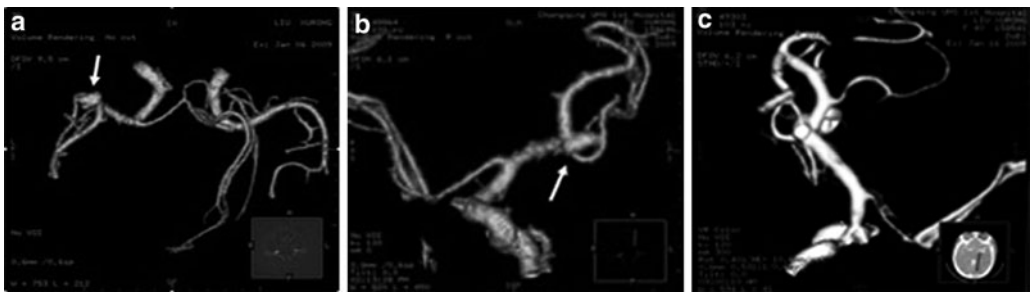

Fig. 4 (**a**) CTA showed the aneurysm in the left middle cerebral artery, the aneurysm was lobulated with many branch vessels around. (**b**) CTA showed the relationship between the aneurysm and parent artery. (**c**) The aneurysm clipped completely, the neighboring vessels were unobstructed

no judgment can be made on the aneurysm suffered from hemorrhage.

The operation time after the cracking of aneurysm has always been the dispute in the circle of neurosurgery. Statistical data from the international cooperative research clearly showed that the middle period (4 7 days) of subarachnoid hemorrhage was not an ideal operation time because the recovery rate was lower than that of the early and later period [8]. Therefore, the major dispute at present: which was the ideal operation time, in the early period or in the late period? Based on the observation in our hospital, the occurrence of cerebrovascular spasm and hydrocephalus were not significant in patients classified as grade I III according to Hunt-Hess classification. The effect of operation was definite. It was advocated to have an operation as soon as possible in order to avoid the influence of recurrent hemorrhage. Patients classified as grade IV V according to Hunt-Hess classification suffered from severe cerebrovascular spasm and hydrocephalus. The operation taken in the early period aggravated the cerebrovascular spasm and hydrocephalus. Even if the aneurysm was occluded anatomically, the occurrence rate of all kinds of complicating disease still increased because of the poor recovery of consciousness. Thus, operation in the late period had a significantly better effect than operation in early period. However, it was not advocated that patients have the operation too early, namely, having the operation within 24 h after entering the hospital, because China is still a developing country and doctors are compelled to undertake more social responsibilities in addition to medical service. The blind emphasis on super early operation can easily result in medical dispute because both the doctors and patients are lacking in preparation.

Conclusion

The most common methods to cure aneurysm are craniotomy and intravascular embotherapy. These two methods are not independent and opposite, instead, they can supplement each other mutually through the analysis on the cases described in this article and the literature reports. The most suitable therapeutic schedule should be selected according to the position, form and size of the aneurysm and the specific situation of the patient in order to enhance the therapeutic effect on aneurysm and reduce the disability rate and mortality rate.

Conflict of interest statement We declare that we have no conflict of interest.

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Recurrent Vasospasm After Endovascular Treatment in Subarachnoid Hemorrhage

Jennifer A. Frontera, Arjun Gowda, Christina Grilo, Errol Gordon, David Johnson, H. Richard Winn, Joshua B. Bederson, and Aman Patel

Abstract *Objectives*: The frequency and predictors of recurrent symptomatic and angiographic vasospasm after angioplasty or intra-arterial chemical vasodilatation (IACV) in patients with subarachnoid hemorrhage (SAH) are not well characterized.

Methods: A retrospective review of serial clinical and angiographic data was conducted between 7/2001 6/2008 on spontaneous SAH patients who underwent endovascular therapy for symptomatic vasospasm.

Results: Of 318 SAH patients, symptomatic vasospasm occurred in 80 (25%) and endovascular intervention was performed on 69 (22%) patients. Of these 69 patients, all received IACV in 274 vessels and 33 also underwent angioplasty in a total of 76 vessels. Recurrent angiographic vasospasm occurred in the same vessel segment in 9/23 (39%) patients who received both angioplasty + IACV compared to 40/49 (82%) of patients who received IACV alone ($P < 0.001$). Recurrent symptomatic vasospasm occurred in 10/26 (38%) angioplasty + IACV patients compared to 28/37 (76%) patients who received IACV alone ($P = 0.003$). The modified-Fisher Score, A1 spasm, distal and multi-vessel vasospasm predicted recurrent angiographic spasm after IACV alone ($P < 0.05$). Procedural complications occurred in 4% of IACV alone patients and 6% of angioplasty + IACV patients ($P = 0.599$).

Conclusions: Recurrent angiographic or symptomatic vasospasm is not uncommon after angioplasty + IACV, but appears to occur significantly less than after IACV alone, without any increase in procedural complications.

J.A. Frontera (✉)
Departments of Neurosurgery and Neurology, Neuroscience Intensive Care Unit, Mount Sinai School of Medicine, New York, NY, USA
e mail: Jennifer.frontera@mountsinai.org
Department of Neurosurgery, Mount Sinai School of Medicine, New York, NY, USA
E. Gordon
One Gustave Levy Place, Box 1136, NY 10029, New York
A. Gowda, C. Grilo, D. Johnson, H.R. Winn, J.B. Bederson, and A. Patel
Department of Neurosurgery, Mount Sinai School of Medicine, New York, NY, USA

Keywords Angiography · Angioplasty · Intra-arterial therapy · Endovascular treatment · SAH · Subarachnoid hemorrhage · Symptomatic vasospasm · Therapy · Vasospasm

Introduction

Clinically significant cerebral vasospasm occurs in 20 40% of subarachnoid hemorrhage patients and is associated with significant morbidity [7]. Over the decades various medical therapies for vasospasm have evolved including hypertensive hypervolemic therapy, first introduced in the 1970s, and oral nimodipine, which remains the only medication used in subarachnoid hemorrhage validated in several randomized controlled trials [4]. Endovascular treatments for vasospasm include both intra-arterial chemovasodilators (IACV) and angioplasty. Clinical improvement after intra-arterial papaverine has been documented in 40 80% of vasospasm patients [8]. Verapamil has been shown to improve angiographic vessel diameter and has been associated with a 30% clinical improvement [5] Similarly, intra-arterial nicardipine has been associated with a 40 90% clinical improvement rate [1, 20]. Unfortunately, most IACV have a short half life and a transient effect, requiring re-treatment [10].

Transluminal balloon angioplasty was first reported as a means of vasospasm treatment in 1984 [22] and its efficacy in improving clinical symptoms has been reported to range between 11 93% [14, 16]. Significant improvements in TCD velocities and CBF have been described following angioplasty [18], and it appears that patients have better long term outcomes when angioplasty is performed within 2 h of symptom onset [17]. Angioplasty is also thought to be more durable than IACV. In this study we sought to determine the frequency of recurrent symptomatic and angiographic vasospasm after endovascular treatment of symptomatic vasospasm with either IACV alone or angioplasty with IACV.

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Methods

Patient Population

A retrospective study of all spontaneous aneurysmal subarachnoid hemorrhage (SAH) patients admitted to the Neuroscience ICU at Mount Sinai Hospital between 7/2001 6/2008 was conducted. Patients were identified through a prospective database maintained by two endovascular physicians and then additional information was obtained through a review of medical records, digitalized computed tomography, and digital subtraction angiography. The study was approved by the hospital Institutional Review Board. The diagnosis of SAH was established on the basis of admission computed tomographic (CT) scans or by xanthochromia of the cerebrospinal fluid. Exclusion criteria included secondary SAH from trauma, dissection, vasculitis, arteriovenous malformation, or other causes, and age <18 years.

Definitions of Vasospasm

Symptomatic vasospasm was defined as the development of new focal neurological signs, deterioration in level of consciousness, or both, when the cause was felt to be ischemia due to vasospasm after other possible causes of worsening had been excluded (such as medication effect, elevated ICP, cerebral edema, intracranial hemorrhage, seizures, metabolic encephalopathy, or infection). Angiographic vasospasm was defined as reversible moderate-to-severe arterial narrowing (>50% of baseline vessel diameter) on digital subtraction angiography not due to atherosclerosis, catheter induced spasm or vessel hypoplasia.

Medical Management

All patients received nimodipine every 4 h for 21 days and phenytoin for 7 days for seizure prophylaxis. All patients received 0.9% normal saline at a rate of 1 ml/kg/h to maintain euvolemia. Patients who developed symptomatic vasospasm were treated with vasopressors to maintain a systolic blood pressure between 180 220 mmHg, adjusted to clinical response, and/or inotropes to maintain a cardiac index > 4.0 L/min/m^2 (as monitored by a pulmonary artery catheter or non-invasive pulse contour analysis). Normal saline and/or 5% albumin solution was given to maintain euvolemia or positive fluid balance. Hemodilution was not routinely performed apart from phlebotomy required for laboratory testing. All patients with symptomatic vasospasm were referred for angiographic confirmation of vasospasm and possible endovascular treatment.

Endovascular Management

All patients underwent digital subtraction angiography on admission prior to aneurysm repair. Follow-up angiography was performed after aneurysm clipping but, apart from this, routine screening angiography was not performed. Repeat angiography was performed for symptomatic vasospasm as soon as possible after symptom onset [17]. All vessels with moderate-to-severe vasospasm were treated with intra-arterial chemodilator (either papaverine or verapamil). Vessels which completely responded to initial chemical vasodilation or vessels which were not accessible or technically feasible for angioplasty were treated with IACV alone. Accessible vessels with residual vasospasm after injection of an intra-arterial chemodilator were treated with balloon angioplasty. Early in the study period balloon angioplasty was performed with a silica Commodore balloon (Cordis, Miami, FL) and then later with a silica HyperGlide balloon (EV3, Inc, Plymouth, MN). All vessels were angioplastied until TIMI 3 flow (defined as full perfusion of the target vessel territory with normal flow) was achieved. Intra-arterial papaverine was used early in the study period (until 2001) and then intra-arterial verapamil was preferentially used. Verapamil was injected into the ipsilateral internal carotid artery (ICA) or vertebral artery, while papaverine was superselectively injected. Intra-arterial nicardipine was used per physician preference in patients who demonstrated angiographic spasm refractory to verapamil. A repeat angiographic run was performed in the same treated vessel segment 10 min after administration of IACV. Initial doses of 5 mg of verapamil and 75 mg of papaverine were used. Repeated doses of verapamil (30 mg maximum per territory) or papaverine (300 mg maximum per territory) were given if necessary. The amount of IACV given for each territory was recorded. Response to IACV alone was coded as: mild improvement in vessel caliber and some delay in distal flow, moderate improvement in vessel caliber with normal flow, complete improvement in vessel caliber and normal flow or no improvement in vessel caliber or flow. Angiographic results were coded by two endovascular physicians. Per our protocol, patients who underwent any endovascular treatment (angioplasty or IACV) for vasospasm had follow-up digital subtraction angiography within 24 h, if feasible.

Clinical and Radiographic Assessment

Baseline demographic data including age and gender, and admission radiographic data including modified Fisher Score [2, 6], the presence of intracerebral or intraventricular hemorrhage, and aneurysm size were recorded. Admission neurological status was evaluated with the Hunt-Hess scale

[9]. A history of hypertension and the type of aneurysm repair (clip, coil, both or neither) was recorded. Angiographic data including the location of vasospasm [coded as left or right, M1, M2, A1, A2, internal carotid artery, posterior cerebral artery, vertebral artery, basilar artery, or distal spasm (≥2nd branch)], the type of intra-arterial treatment (papaverine, verapamil or nicardipine) and the location of angioplasty were documented for each angiogram performed on each patient.

Outcome Measures

Recurrent angiographic vasospasm was coded if it occurred in the same vessel segment that had previously treated (i.e. if the M2 segment was angioplastied and recurrent moderate-to-severe spasm occurred in the M2 it was coded as recurrent angiographic vasospasm). Similarly, recurrent symptomatic vasospasm was coded as occurring only if clinical symptoms (recorded as new motor or speech deficits or mental status decline) were referable to the territory supplied by the same vessel segment that had been previously treated. Recurrent angiographic vasospasm (defined as moderate or severe vessel narrowing) or symptomatic vasospasm was coded dichotomously as present or absent. In patients who underwent both angioplasty + IACV and IACV alone in different vessel segments or during different angiograms, each event of recurrent angiographic vasospasm was coded separately. Hospital and ICU length of stay was recorded. Hospital discharge disposition was dichotomized as poor (expired, discharged to a nursing home or subacute care facility) vs. good (discharged to home, an acute rehabilitation facility, or home with a health aide).

Statistical Analysis

Two by two tables were constructed to assess the association between angioplasty + IACV vs. IACV alone and the outcomes of recurrent angiographic or symptomatic vasospasm and complications related to either procedure. A two-tailed Fisher Exact test was applied when the sample size was small and the Pearson's Chi Squared test was applied for larger sample sizes. Univariate logistic regression analysis was performed to calculate the odds ratios and 95% confidence intervals for the association of demographic and clinical variables and the outcomes of recurrent angiographic or symptomatic vasospasm. Length of stay (LOS) was dichotomized at the median and binary logistic regression analyses were performed to assess the effect of angioplasty + IACV vs. IACV alone on LOS and discharge disposition. Significance was set at the $P \leq 0.05$ level for all analyses.

Results

Of 318 patients with SAH, 80 (25%) developed symptomatic vasospasm. The median time to vasospasm onset was on SAH day 7 (range 2 16). An endovascular intervention to treat symptomatic vasospasm was performed in 69 patients in a total of 274 vessel segments. Data was missing on five patients with symptomatic vasospasm, four patients had symptomatic spasm but no angiographic spasm and two patients with symptomatic spasm had distal angiographic spasm that was not treated endovascularly. Angioplasty + IACV was performed in 33 patients in 76 vessel segments while 56 patients received IACV alone in 197 vessel segments (Table 1). Twenty patients had both angioplasty + IACV and IACV alone in different vessel segments. Demographics and angiographic data of those who received angioplasty + IACV for symptomatic vasospasm is listed in Table 2. Patients with M1, A1 and multi-territory vasospasm were more likely to undergo angioplasty. The most commonly angioplastied vessel segment was M1 (N = 38), followed by ICA (N = 21), A1 (N = 10), M2 (N = 4), PCA (N = 1), basilar (N = 1), and vertebral artery (N = 1).

All patients who underwent IACV + angioplasty achieved TIMI 3 flow. Among patients who received IACV alone, 87% of vessel segments had moderate or complete improvement in blood flow, 12% had only mild improvement in blood flow and 1% had no improvement in blood flow. Among those who received verapamil alone, 86% had moderate to complete improvement, while 90% of those who received papaverine had moderate to complete improvement.

Follow-up digital subtraction angiography after an endovascular intervention for vasospasm was performed in 50/69

Table 1 Endovascular intervention for treatment of symptomatic vasospasm among all patients with symptomatic vasospasm (N 80)

	Total number of patients N (%)	Total number of vessel segments (median, range)
Any endovascular procedure for vasospasm[a]	69 (86)	274 (3, 0 18)
Angioplasty + IA medication	33 (41)	76 (0, 0 8)
IA medication alone	56 (70)	197 (2, 0 15)
Verapamil alone	46 (56)	162 (1, 0 15)
Papverine alone	13 (16)	32 (0, 0 6)
Nicardipine alone	2 (3)	3 (0, 0 3)

[a]Twenty patients received both angioplasty + IACV and IACV alone in different vessel segments or during different angiograms

Table 2 Descriptive characteristics of patients who received angio plasty for symptomatic vasospasm

	Angioplasty + Intra arterial chemodilation	Intra arterial chemodilation Alone[a]	P
Age (median, range)	51 (25 79)	57 (22 89)	0.089
Gender, female, N (%)	19 (58)	26 (72)	0.219
Hunt Hess Grade, (median, range)	3 (1 5)	4 (1 5)	0.673
Aneurysm size (mm), (median, range)	6.4 (2 17)	4.8 (1 43)	0.288
Coil, N (%)	19 (58)	18 (56)	0.799
Modified Fisher Score, (median, range)	4 (1 4)	3 (1 4)	0.247
M1 spasm, N (%)	31 (94)	16 (44)	**<0.001**
A1 spasm, N (%)	27 (82)	21 (58)	**0.040**
ICA spasm, N (%)	16 (49)	12 (33)	0.228
Posterior circulation spasm, N (%)	8 (24)	4 (11)	0.207
Distal spasm, N (%)	19 (58)	21 (58)	1.00
Multi territory spasm, N (%)	28 (85)	21 (58)	**0.019**

Mann Whitney U for continuous/categorical nonparametric data, and Chi Squared for dichotomous data.

[a]angioplasty + IACV not performed on any vessel segment during any angiogram

(72%) of patients within a median of 1 day (range 1 8). The median number of follow up angiograms was 2 (range 0 7). Of patients who underwent angioplasty + IACV, 23/33 (70%) had a follow-up angiogram while 39/56 (70%) of patients who received IACV alone had a follow-up angiogram (P = 0.195). There was no difference in the rate or timing of follow-up angiography after angioplasty + IACV vs. IACV alone. The only predictor of whether a patient received follow up angiography after endovascular treatment of vasospasm was if multi-territory vasospasm was present (OR 11.3, 95% CI 1.9 66.7, P = 0.008). Age, gender, admission Hunt-Hess grade, aneurysm size, modified Fisher score, the presence of ICH or IVH, the timing of vasospasm onset from the initial SAH and repair procedure type were not significant predictors. The time to follow up angiography after a procedure was not significantly associated with recurrent angiographic or symptomatic vasospasm after IACV alone or IACV + angioplasty.

Among the 50 patients who underwent an endovascular treatment for symptomatic vasospasm and had a follow up angiogram, recurrent moderate to severe angiographic vasospasm in a previously treated vessel segment occurred in 39% (N = 9) of patients who received angioplasty + IACV vs. 82% (N = 40) of patients who received IACV alone (Fisher exact p = 0.0008). Similarly, 27% (N = 16) of vessel segments had recurrent angiographic spasm after angioplasty compared to 63% (N = 160) of vessels treated with IACV alone (Chi-squared P < 0.001). Because patients with proximal spasm were more likely to undergo angioplasty, we independently examined recurrent proximal angiographic vasospasm after IACV alone vs. angioplasty + IACV in order to directly compare similar cerebrovascular territories treated with different modalities. Of those who received angioplasty + IACV for proximal spasm, 19% had recurrent proximal spasm compared to 51% of those who received IACV alone for proximal spasm (Fisher exact P < 0.001). Similarly, of those who received angioplasty + IACV in a vessel territory with distal spasm (M2 or distal), only 6% had recurrent distal spasm compared to 25% of those who received IACV alone for distal spasm (Fisher Exact P < 0.001).

Table 3 The association of recurrent angiographic and symptomatic vasospasm after angioplasty with intra arterial chemodilation vs. intra arterial chemodilation alone

	Angioplasty + Intra arterial chemodilation N (%)	Intra arterial chemodilation Alone N (%)	P
Patients with recurrent angiographic vasospasm	9 (39)	41 (82)	<0.001
Patients with recurrent symptomatic vasospasm	10 (38)	28 (76)	0.004
Vessel segments with recurrent angiographic vasospasm	16 (27)	160 (63)	<0.001

When examining recurrent symptomatic vasospasm 38% (N = 10) of patients who received angioplasty + IACV vs. 76% (N = 28) who received IACV alone had persistent symptoms of vasospasm (Table 3). Repeat angioplasty was performed in 3/33 (9%) of patients in six vessels.

We were unable to identify any predictors of recurrent angiographic or symptomatic vasospasm after angioplasty + IACV. After IACV alone initial A1 spasm, distal and multi-territory spasm predicted both recurrent angiographic and symptomatic vasospasm and the modified Fisher score additionally predicted recurrent angiographic spasm.

Procedural complications occurred in 6% (N = 2) of patients who underwent angioplasty with IACV vs. 4% (N = 2) of patients who underwent IACV alone, though this difference was not significant (Fisher exact p = 0.629). One patient suffered a ruptured A1 from the guidewire for a balloon and another patient had a retained balloon fragment in a distal ACA. Two patients had elevated intracranial pressure after papaverine injection.

At the time of hospital discharge, 11 (14%) of patients with vasospasm died; 52 (66%) had a good discharge disposition

and 27 (34%) had a poor discharge disposition (including death). There was no difference in discharge disposition between those who had angioplasty + IACV vs. those who had IACV alone (OR 0.6, 95% CI 0.2 1.7, P = 0.351). Among all patients with vasospasm, the median ICU LOS was 17 days (range 1 91) and the median hospital LOS was 24 days (range 1 97). Again, there was no difference in LOS among patients who received angioplasty compared to those who did not.

Discussion

In this study, we found that after angioplasty + IACV for symptomatic vasospasm, recurrent angiographic and symptomatic vasospasm was not uncommon (39 and 38% of patients, respectively), but was significantly less frequent than after IACV alone. These rates of recurrence after angioplasty + IACV are higher than what has been documented in the literature. In a study of 75 patients undergoing angioplasty in 85 vessels, 15/116 (13%) of vessels had recurrent vasospasm, primarily in the A1 territory [21] However, only 51% of patients had a follow-up angiogram and the time period to follow-up is unclear. Though the authors postulated that it is unlikely that the recurrent vasospasm rate is higher than their observation, it is possible that poor grade patients, who are unlikely to manifest gross clinical signs of decline, had undetected recurrent vasospasm because follow-up angiography either did not occur or was too distant from the time of initial vasospasm treatment. In another study comparing angioplasty to intra-arterial papaverine, 39 patients underwent angioplasty in 101 vessels [3] There was a 45% decrease in TCD velocities and only one vessel segment had recurrent spasm by TCD criteria. Angioplasty was found to be more durable than papaverine alone as measured by TCD recurrence of vasospasm. TCD, however, has important limitations in predicting symptomatic and angiographic vasospasm [7, 13, 19] Because 72% of the patients in our study had a follow-up digital subtraction angiogram within a median of one day from an endovascular intervention, it is likely that the rate of recurrence captured in this cohort represents a more realistic value than what has been previously reported. Since TIMI 3 flow was achieved in all vessels angioplastied, it is unlikely that undertreatment led to a higher than expected recurrence rate. Since vessel rupture following transluminal balloon dilation can be catastrophic, overly aggressive treatment should, similarly, be discouraged.

Recurrent symptomatic and angiographic vasospasm occurred at significantly higher rates in patients treated with IACV alone (76 and 82%, respectively) than with combined angioplasty + IACV. Since 87% of patients in this cohort had moderate or complete angiographic improvement of blood flow after initial treatment with IACV, it appears that IACV treatment is adequate, but not durable. Though patients with proximal spasm were more likely to undergo angioplasty, our data suggests that recurrence of both proximal and distal spasm is more common after IACV alone than with angioplasty, when examining specific vessel segments. Other studies support the limited durability of IACV. Papaverine infusion has previously been associated with recurrent and persistent angiographic and clinical vasospasm in multiple studies [11, 12, 15]. In a study of intra-arterial papaverine using serial transcranial Doppler (TCD), mean flow velocities decreased by 20% the first day after papaverine infusion, but returned to pre-treatment levels in 42% of patients within 48 h of treatment [3]. Different agents used for IACV may have longer durations of action, though definitive studies have yet to be performed.

When examining predictors of recurrent vasospasm, patients with thick clot on CT, A1 spasm and multi-territory spasm are least likely to have durable improvement after intra-arterial medication alone. Angioplasty may be considered in these patients, particularly.

We did not detect a significant difference in complication rates between angioplasty + IACV vs. IACV alone though the complications after angioplasty were more catastrophic than after IACV. We did not find a significant difference in LOS or discharge disposition. Discharge disposition is a crude surrogate for outcome and this may be why no difference was detected. Additionally, symptomatic vasospasm is more closely related to higher level cognitive and functional outcomes than death or severe disability and effects of treatment may not be noted on discharge disposition [7]. Three month and 1 year functional and cognitive outcomes are essential to determining whether angioplasty incurs a long term functional benefit.

Other limitations should be mentioned. First, this was a retrospective study and though the majority of patients had follow up angiography within a day, not all patients did and the reasons for no angiographic follow-up were not available. Symptomatic vasospasm was not coded according to a specific scale such as the NIHSS, but rather in descriptive terms. Similarly, since poor grade patients have marginal exams, recurrent symptomatic vasospasm in this group may have been under-diagnosed and, hence, the rates of recurrence may be higher than we described. We did not perform serial imaging studies to identify infarcts related to vasospasm. Since the duration of time from symptom onset to endovascular intervention has been shown to affect outcomes [17], data on the time to endovascular intervention would be important to factor in to our results, but was not available. We did not have enough power to determine if there are different recurrence rates related to different IACV agents. A prospective study integrating imaging, angiography, exam findings and long term clinical outcomes would be useful.

Conclusion

In conclusion, the recurrence rates of angiographic and symptomatic vasospasm are higher than previously documented after angioplasty + IACV, and are very high in patients receiving IACV alone. Vigilance for vasospasm recurrence should be maintained after endovascular treatment. Our data support the concept that, when feasible, early angioplasty is associated with less recurrence of vasospasm and does not incur additional risks when compared to IACV alone.

Conflict of Interest Statement We declare that we have no conflict of interest.

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Endovascular Embolization for Intracranial Aneurysms: Report of 162 Cases

Weihua Tang, Hua Feng, Zhi Chen, Hongping Miu, Jiexiang Pan, Jiangkai Lin, and Gang Zhu

Abstract *Objective*: To summarize the experiences of endovascular embolization for intracranial aneurysms and emphatically discuss techniques, complications and preventions.

Methods: 171 aneurysms in 162 patients were treated by detachable coil embolization. Among them, 38 cases were treated by GDC, 35 by DCS, and 89 by EDC.

Results: 137 aneurysms were 100% occluded, 27 were 90 95% occluded, and 7 were 80% occluded. Complications associated with operation occurred in nine patients. The coil escaping from the sac of aneurysm into its parent artery was seen in five patients. Six patients suffered from rupture of aneurysm during the operation. Serious vasospasm was seen in five patients. Two patients died of complications.

Conclusions: Endovascular embolization is a safe, effective and minimally invasive method for treating intracranial aneurysms. Choosing exact embolization techniques is very important to improve the therapeutic effect and decrease the complications of embolization of intracranial aneurysms.

Keywords Intracranial aneurysm · Embolization · Therapeutic · Intraoperative complications

Endovascular embolization is less invasive and has fewer complications than surgery. The endovascular treatment of intracranial aneurysms has gradually become popular since detachable coil (GDC) has been applied in clinics. From December 1998 to February 2008, micro-coil embolization was attempted in 162 patients with 171 aneurysms; analysis reports are as follows.

W. Tang, H. Feng, Z. Chen, H. Miu, J. Pan, J. Lin, and G. Zhu (✉)
Department of Neurosurgery, Southwest Hospital, Third Military Medical University of PLA, 20 Gao Tan Yan Street, Sha Ping Ba District, Chongqing, 400038, P.R., China
e mail: zhugang666@yahoo.com.cn

Subjects and Methods

General Information

There were 94 males (58%) and 77 females (42%) aged 10 76 years (mean age 50.3 year). All patients were diagnosed by head CT scanning and cerebral digital subtraction angiography (DSA). DSA examination showed that 153 cases had single aneurysms, and 9 cases had two aneurysms each. Aneurysm sites: posterior communicating artery (74), anterior communicating artery (56), middle cerebral artery (14), ophthalmic artery (9), carotid cavernous (4), carotid clinoid (4), anterior cerebral artery (2), posterior cerebral artery (1), basilar artery bifurcation (1), vertebral artery (1), vertebral artery dissection (3), posterior cerebral artery dissection (1), and carotid traumatic pseudo aneurysm (1). Aneurysm sizes: diameter less than 0.5 cm (15), 0.5 1.0 cm (132), 1.0 1.5 cm (17), greater than 1.5 cm (7), and 22 wide-necked aneurysms. 148 patients were hospitalized for SAH resulting from aneurysmal rupture. Clinical grading according to the Hunt and Hess scale for the 148 patients with SAH was as follows: grade 0 or 1 in 112 cases (76%), grade 2 in 25 cases (17%), and grade 3 in 11 cases (7%). Patients classified as either grade 4 or 5 were not submitted to endovascular embolization. Main symptoms: sudden headache and neck stiffness in 148 cases, 11 cases of disturbance of consciousness, mental disorder in 19 cases, and oculomotor nerve palsy in 58 cases.

Preoperative Treatment

Intravenous administration of Nimodipine (2 4 mg/h) for 1 3 days. Controlled hypotension was given if patients were associated with hypertension. sedation was given in cases of tension and irritability.

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Embolization Materials

Emergency operations in 23 cases. GDC embolization in 38 cases, DCS embolization in 35 cases, multi-point detachable coil (EDC) in 89 cases. Basketry techniques was used in 13 cases of wide-necked aneurysms, nine patients with stent-assisted embolization.

Postoperative Treatment

All cases were given 3H treatment and Nimodipine. As for the cases of serious subarachnoid hematoma or intraventricular hemorrhage, postoperative lumbar puncture and drainage were set for 3 7 days. Postoperative ventricular drainage was done in cases where intraventricular hemorrhage occurred with impaired consciousness. For the stent-assisted coil embolization cases, heparin was given for 3 days. Postoperative vasospasm, cerebral ischemia symptoms or balloon-assisted neck remodeling, an appropriate extension for anticoagulation, and oral aspirin were given for 3 months.

Results

Hundred percent occlusion were observed in 134 cases, 90 95% occlusion in 21 cases, 80% occlusion in 7 cases. 160 patients were cured. Two patients died, both emergency surgery patients; one case died due to gastrointestinal bleeding associated with multiple organ dysfunction caused by severe SAH, and another one case died due to aneurysm rebleeding after 2 weeks. Procedure-related complications occurred in nine cases. Intraoperative aneurysm ruptures in six cases, and five cases of severe cerebral vasospasm. Micro-coil displacement in five cases, including two cases of less prominence that had anticoagulant therapy for 3 months; follow-up showed no symptoms. Two cases using gooseneck fishing device removed. One case of middle cerebral artery occlusion led to emergent craniotomy. One hundred and thirty-seven patients were followed up 3 20 months later. There was no further bleeding or aneurysm recurrence. There were three cases of less dense packing embolization. Oculomotor nerve palsy was fully restored after operation for 3 12 months.

Discussion

With the development of embolization materials and embolization skills, endovascular coil embolization has been increasingly used in the treatment of intracranial aneurysms and is especially suitable for the emergency and patients in serious condition. The International Subarachnoid Aneurysm Trial (ISAT) compared microsurgical clipping and endovascular coil occlusion in patients with ruptured aneurysms felt to be suitable for either technique. Endovascular coiling resulted in a 23.9% relative risk reduction for death or dependency at 1 year, an absolute reduction of 7.4% [1, 2]. Multi-center clinical studies have shown that complications of coil embolization decreased by 15% compared with surgical clipping. While in the prevention of recurrence, there are no significant differences between the two methods [3, 4]. Our experience also shows that coil embolization of intracranial aneurysms with simple operation and high security is especially suitable for the acute and critically affected patients.

Overall complication rate of aneurysm embolization is about 10%, with permanent complication rate of 3 5%, mainly as follows: intracranial arterial occlusion, intraoperative aneurysm rupture, aneurysm re-bleeding, puncture point bleeding, coil shift, vasospasm [5]. Cerebral vasospasm is one of the most common complications of aneurysmal SAH. It can be found in 2/3 SAH patients with cerebral angiography. 1/3 of the patients fully recovered, 1/3 had permanent neurological dysfunction, and 1/3 died of refractory cerebral vasospasm [1]. Therefore, patients with aneurysms associated with SAH should be given anti-vasospasm therapy as soon as possible. In this study, preoperative treatment significantly reduced the degree of cerebral vasospasm, but there were still seven patients with serious vasospasm; angiography showed interruption of blood flow, which was alleviated after emergency thrombolysis and anti-vasospasm therapy. It is generally believed that the cause of intraoperative vasospasm would be as follows: (1) stimulating effect on the vascular wall by the catheter, which includes guiding catheters, micro catheters, and micro-guide wire. Therefore, we should minimize catheter and guide wire to the stimulation of blood vessel walls. (2) Overuse of hypertonic contrast agent has a certain stimulating effect on vessel wall. This may further aggravate cerebral vasospasm and even lead to vascular occlusion. Therefore, isotonic contrast agents are preferred [6, 7].

Intraoperative aneurysm rupture is the most serious complication. The causes include tension, blood pressure increase and micro-catheters, and micro-wire over-intubation into the aneurysm cavity; it is usually required that the end of the microcatheter be maintained in the outer 1/3 of aneurysm cavity [6, 8]. From our experience, the surgeon should shape the end of the micro-catheter in accordance with the angle of aneurysm and parent artery. Timely adjustment of the force depends on the resistance; this is an effective way of preventing aneurysm rupture. In this study, six patients had aneurysm rupture and showed a sudden increase in blood pressure during operation; angiography showed leakage of contrast agents. Two cases of poor micro-catheter shaping; two cases due to excessive force when posting the coils. Filling multi-angle

observation is helpful for preventing the micro-coils from penetrating the aneurysm sac.

Intraoperative micro-coil shift is a common and dangerous complication, including micro-coil shift to the subarachnoid space and to the parent artery. The former, associated with aneurysm rupture, can lead to SAH and is life-threatening; the latter can lead to distal artery occlusion, resulting in neurological dysfunction, and can also be life-threatening [9, 10]. Selecting the appropriate diameter and length of the coil and embolization technique are key points to preventing micro-coil shift. Five cases in this group were shifted to the parent artery, of which two cases were due to the micro-coil being too large for the aneurysm sac and part of it shifting into the parent artery; the other three cases were due to a wide neck and the first coils being too small.

Over the past 18 years, endovascular occlusion of cerebral aneurysms has emerged as an alternative to microsurgical clipping, and the relative merits and shortcomings of these approaches have been the subject of considerable discussion in the neurosurgical literature and at scientific meetings. The minimally invasive nature of endovascular treatment is inherently appealing, but in spite of technical improvements and adjuncts, such as balloons, stents, and biologically modified coils, complete and durable repair, especially in the case of large aneurysms and those with complex geometry or wide necks, remains a challenge.

Conflict of interest statement We declare that we have no conflict of interest.

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Treatment of Post-hemorrhagic Cerebral Vasospasm: Role of Endovascular Therapy

Andrew Grande, Christopher Nichols, Usman Khan, Gail Pyne-Geithman, Todd Abruzzo, Andrew Ringer, and Mario Zuccarello

Abstract In this review, the current role of intracranial angioplasty and intra-arterial vasodilators for post-hemorrhagic vasospasm is described with an emphasis on the rationale for its use and the supporting data from published scientific and clinical studies. Current clinical indications and specific techniques are highlighted. Special attention is given to the evolution of these techniques over time. A discussion of acute and chronic complications, short and long-term treatment results, device specific trends and controversies are outlined.

Keywords Vasospasm · Subarachnoid hemorrhage · Intracranial aneurysm · Percutaneous transarterial balloon angioplasty · Verapamil

Clinical Significance

Rupture of an intracranial aneurysm is the most common cause of non-traumatic subarachnoid hemorrhage (SAH), accounting for about 80% of such cases [1]. Aneurysmal SAH affects approximately 28,000 individuals annually in North America alone [2]. While the initial effects of hemorrhage are usually the most serious, development of cerebral vasospasm and delayed ischemic neurologic deficit (DIND) is the second leading cause of morbidity and mortality [3, 4]. Cerebral vasospasm is angiographically demonstrable in 40 70% of patients who suffer aneurysmal SAH and is associated with symptomatic cerebral ischemia in 20 30% [2, 5]. In early studies of aneurysmal SAH, vasospasm permanently affected about 14% of patients and caused one third of all death and disability [6, 7]. Unfortunately, despite improved diagnostic and therapeutic strategies, over two-thirds of patients will develop vasospasm after aneurysmal SAH, 30 50% of which will develop DIND which carries the risk of permanent disability or death [8 11].

Pathophysiology

Vasospasm is most often a self-limited process that does not develop immediately after subarachnoid hemorrhage, but rather is observed several days after the ictus. Clinically, vasospasm is seen between 3 to 14 days after subarachnoid hemorrhage [12]. Unfortunately, despite a fairly well defined clinical window, the precise series of events that lead to vasospasm remains unknown. Numerous inciting factors have been implicated including erythrocyte degradation products, and serum derived lipid and protein metabolites. Presumably, the inciting factor or factors trigger a cascade of biochemical and immunoinflammatory reactions that ultimately lead to unopposed activation of the contractile apparatus within cerebrovascular smooth muscle cells. Although loss of luminal caliber is initially reversible, if the process is sustained, vessel wall fibrosis can lead to irreversible stenosis [13].

While percutaneous transarterial balloon angioplasty (PTA) is a frequently used therapy for vasospasm its mechanisms have not been completely elucidated. It is probable that

A. Grande, C. Nichols, and U. Khan
Department of Neurosurgery, University of Cincinnati (UC) Neuroscience Institute and UC College of Medicine, Cincinnati, OH, USA
G. Pyne Geithman
Department of Neurology, University of Cincinnati (UC) Neuroscience Institute and UC College of Medicine, Cincinnati, OH, USA
T. Abruzzo and A. Ringer
Department of Neurosurgery, University of Cincinnati (UC) Neuroscience Institute and UC College of Medicine, Cincinnati, OH, USA
Mayfield Clinic, Cincinnati, OH, USA
M. Zuccarello (✉)
Department of Neurosurgery, University of Cincinnati (UC) Neuroscience Institute and UC College of Medicine, Cincinnati, OH, USA
Mayfield Clinic, Cincinnati, OH, USA
Department of Neurosurgery, c/o Editorial Office, ML 0515, 260 Stetson Street, Suite 2200, Cincinnati, OH, USA
e mail: editor@mayfieldclinic.com

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PTA acutely restores luminal caliber by mechanically countering the contractile forces generated by activated cerebrovascular smooth muscle cells. Interestingly, however, the effects of PTA are frequently long lasting and the need for recurrent PTA is rarely encountered. In clinical series in which PTA is used to treat vasospasm, persistent improvements in TCD velocities are reported and in the majority retreatment is not needed [14]. Furthermore, in animal models of vasospasm, vessels prophylactically treated by PTA fail to vasoconstrict in response to peri-adventitial hemorrhage or vasoconstrictor substances [15]. Some evidence suggests that this long lasting effect is a result of mechanical damage to the contractile apparatus within cerebrovascular smooth muscle cells of the vessels subjected to PTA. Histological studies of vessels treated with PTA demonstrate tunica intima and tunica media injury. Animal studies have also demonstrated disruption of connective tissue fibers in the extracellular matrix of the vessel wall [16]. Others suggest that there is stretching and plastic deformation of contractile filaments which un-couples actin and myosin filaments.

Diagnosis

Many attempts have been made to accurately identify and pre-emptively treat patients at risk of developing symptomatic vasospasm. While several clinical risk factors have been associated with the development of symptomatic vasospasm, it is not currently possible to reliably predict which patients will be affected. Of the risk factors identified to date, the distribution and volume of subarachnoid hemorrhage is most important. Other factors that appear to be associated are age, sex, and history of tobacco or cocaine abuse [17]. Since any patient who suffers aneurysmal SAH is at risk for the development of cerebral vasospasm, most centers including our own have adopted a diagnostic surveillance program for all patients. These programs include frequent neurological examination in an intensive care setting with non-invasive vascular studies to detect the presence of symptomatic and clinically silent vasospasm. We routinely use transcranial doppler (TCD) as a non-invasive vascular study performed daily or every other day. When evidence of vasospasm is seen clinically or by TCD examination we next proceed to catheter angiography to confirm vasospasm. Other centers may use computed tomographic angiography (CTA) to confirm the results of a positive TCD study before proceeding to catheter angiography. More recently, cerebral perfusion imaging studies have emerged as a diagnostic tool in the assessment of patients with suspected vasospasm [18]. With these newer tools it may be possible in the future to differentiate which patients have reversible ischemic injury from those patients with non-reversible ischemic injury.

Medical Management

A comprehensive discussion of practices aimed at preventing vasospasm is beyond the scope of this review. However, of the many methods employed, those with the most evidence favoring their use include oral administration of Nimodipine, induced hypertension and augmentation of cardiac output [19, 20]. Preventative therapies on the horizon include intravenous magnesium, intracisternal thrombolysis, oral statins, and novel agents such as endothelin receptor antagonists. In addition, several therapeutic strategies directed at improving cellular tolerance to cerebral ischemia, and enhancements of cerebral oxygenation have been developed. While some of these approaches have shown more promise than others in clinical trials, none should be considered primary options. In current clinical practice, risk-benefit analysis favors medical management as the initial treatment of choice for clinically silent and symptomatic post-hemorrhagic cerebral vasospasm. Although hypertension, hypervolemia and hemodilution (so called HHH therapy) have all been incorporated into commonly used medical management strategies, induced hypertension appears to play the most important role in preventing irreversible brain infarction.

Interventional Treatment of Vasospasm

Clinical Indications and Efficacy

Endovascular therapy including transcatheter intraarterial administration of vasodilators and percutaneous transluminal balloon angioplasty (PTA) is reserved for patients who experience symptomatic vasospasm or DIND refractory to maximal medical management, as outlined above. In many cases of symptomatic vasospasm, progressively worsening focal neurological deficits are unequivocal manifestations of cerebral ischemia demanding therapeutic intervention. In these cases the patient's condition should be regarded with the same urgency as one would regard an acute ischemic stroke. In other cases however, non-lateralizing symptoms such as cognitive impairment, altered mentation, or even fever may be the only symptoms. Recognition of vasospasm in this setting requires a high level of suspicion. Other common conditions such as hydrocephalus, respiratory failure and infection may produce similar clinical manifestations. It is safest to assume that any unexplained neurological deterioration, not attributable to these causes, is the result of cerebral vasospasm, until disproven by cerebral angiography.

Patients with clinically symptomatic vasospasm who fail maximal medical therapy should be considered for endovascular therapy. Options include PTA and transcatheter

intra-arterial vasodilator infusion. PTA remains the most effective treatment with almost immediate vasodilation and long lasting effects, rarely requiring retreatment. Disadvantages, however, are that it is limited to only large proximal vessels and can have more significant complications such as vessel rupture. When treating distal vasospasm or when PTA is ineffective or contraindicated intra-arterial infusion of vasodilators is sometimes employed. Historically, Papaverine has been the most commonly used vasodilator. While it successfully reversed proximal vessel spasm, in most cases the onset of action was often delayed and the therapeutic effects were short lived, not lasting beyond 3 h [21]. In addition, our group and others have reported that multi-vessel infusions of Papaverine can result in harmful elevations of intracranial pressure due to augmentation of cerebral blood volume [22 24]. For these reasons we have now changed our treatment paradigm and no longer use Papaverine but rather use Verapamil a calcium channel blocker.

Since our last publication [22] we have developed a standard treatment paradigm utilizing both PTA and Verapamil. For grade I (0 25% narrowing) or grade II (26 50%) vasospasm we use Verapamil infusion. For grade III (51 75%) or grade IV (>75%) proximal vessel vasospasm we use PTA. This includes vasospasm of the following vessels: internal cerebral artery (ICA), proximal middle cerebral (M1), proximal anterior cerebral (A1), vertebral artery (VA), and the proximal posterior cerebral artery (P1). We approach A1 and P1 with caution since they are relatively deficient in tunica media and elastic tissue, theoretically making them more prone to rupture. When severe vasospasm precludes PTA due to inadequate luminal diameter we will first administer Verapamil. Within 15 20 min we usually see enough vasodilation to attempt PTA. For distal vasospasm we use Verapamil infusion.

While there are no randomized clinical trials demonstrating the efficacy of either PTA or intra-arterial infusion of vasodilators there are many case series, which suggests their efficacy. PTA treatment of cerebral vasospasm restores luminal caliber in almost all cases [18]. In addition mean TCD velocities have been shown to return to baseline and are sustained for 48 h. Cerebral blood flow (CBF), assessed by SPEC scans, also has been shown to be improved in 71% of vessel territories 24 h after treatment [25]. Another series demonstrated improve absolute CBF measured by Xe-CT in all patients treated [18]. Clinical improvement occurs in 61 92% of patients [18, 26 28] with complete resolution of symptoms in up to 58% of patients [18]. Repeat angioplasty is rarely required with only 3 4% of patients requiring additional procedures [25, 27, 28].

There are no reports of long term follow up after intracranial PTA for vasospasm. Consequently, little is known about the long term clinical or morphological sequelae. We have rarely observed long term development of flow limiting, albeit asymptomatic, intimal hyperplasia in our own patients who have returned for angiographic follow up of aneurysms treated by coil embolization. The true incidence and prevalence of intimal hyperplasia (symptomatic or asymptomatic) has not been systematically evaluated.

It seems, however, that the phenomenon of intimal hyperplasia after angioplasty should not be as common after vasospasm as it is in the setting of steno-occlusive atherosclerotic disease as it is generally performed on healthy cerebral vessels, and involves relatively less vessel wall trauma.

Verapamil administered intra-arterially has been demonstrated to be safe [17, 29, 30]. Several case series report dosages between 2 and 20 mg per vessel [17, 29, 30]. In these series no elevation of ICP was seen and angiographic improvement in vasospasm was noted within 5 20 min. One series reported improved vessel diameter in all treated vessels [29], another reported a 44% increase in luminal diameter of stenotic segments [30] and another reported a trend demonstrating improved vessel diameter in intermediate-sized vessels [17]. Following treatment neurologic improvement was seen in 30 66% of patients [17, 29, 30] Furthermore, repeat treatment was necessary in only 15% of patients in one series and only one of the five patients was for recurrent vasospasm in the same vascular territory [30]. In another study with ten patients, no patient required repeat infusion [29].

Prophylactic PTA in patients at high risk of developing life-threatening cerebral vasospasm has been proposed and reported but remains very controversial. Animal studies have shown very promising results for the prevention of vasospasm using prophylactic PTA. In canine models of post-hemorrhagic vasospasm, prophylactic PTA on day 0 prevented the development of vasospasm in all animals on day 7 [15]. Similar results have been seen in small case series. In one such series in which Fisher grade 3 SAH patients were prophylactically treated by PTA, within 3 days of aneurysmal SAH, none developed DIND or clinically significant TCD abnormalities [31]. Compared to historical control data, these results strongly suggest effective prevention of symptomatic vasospasm by PTA. Unfortunately the procedure related mortality secondary to vessel rupture was 8% in this small pilot study. Proponents of prophylactic PTA argue that the powerful effects seen in these animal studies and case series justify its use in patients at high risk for vasospasm. Opponents, however, argue that because of the high mortality of prophylactic PTA it is unethical to use this as a therapy especially when it is so difficult to predict which patients will develop DIND as a result of vasospasm. If prophylactic PTA is to be used therapeutically in the future, further investigation is needed. Nevertheless, the concept of prophylactic angioplasty is purely investigational and has no role in current clinical practice.

Techniques and Devices

Comparative imaging is reviewed prior to any endovascular treatment for vasospasm. Particular attention is given to the baseline caliber of vessels requiring PTA to avoid over dilating. It is also important to identify any vessel hypoplasia or fenestrations, which are contraindications for PTA. A non-contrast head CT can identify irreversible cerebral ischemia, which may be at risk of reperfusion hemorrhage following any endovascular intervention.

General endotracheal anesthesia (GETA) or conscious sedation (CS) can be used for endovascular treatment of vasospasm. GETA is advantages for control of patient movement. This allows for better roadmap navigation of microwires, catheters and balloons and is most important to avoid over inflation of balloons. The disadvantage is that there is no neurological exam to follow. CS, while allowing for monitoring of the neurological exam is prone to patient non-compliance and movement.

Transarterial access is gained using a 6F sheath. A flexible soft-tipped guiding catheter such as the Envoy® or Guider soft tip® is then used for diagnostic studies. If mild or moderate vasospasm is encountered we advance the guide catheter into the internal carotid artery or the vertebral artery and deliver 10 20 mg of Verapamil over 2 min. Careful attention is given to the patient's blood pressure, which can precipitously drop. We then move to the next vessel in the diagnostic study. Once all vessels have been studied we return to the treated vessels to analyze the effect of Verapamil and determine if angioplasty is also needed. When severe vasospasm is encountered requiring PTA we fist anticoagulate the patient with heparin to achieve an activated clotting time (ACT) of 250 300 s. The guide catheter is than advanced in the parent artery towards the skull base. Under roadmap guidance the balloon microcatheter is then positioned within the symptomatic vessel.

In general there are two types of balloon microcatheters used for PTA of vasospasm: the single lumen microcatheter with bipolar balloon fixation and the double lumen balloon microcatheters with bipolar balloon fixation. Single lumen balloon microcatheters include the Hyperglide® and Hyperform® balloon occlusion catheters (EV3, Irvine CA) A companion 0.010″ microwire is required to obstruct the distal end hole for balloon inflation. Because of their high compliance these balloons have less potential risk of vessel rupture. Double lumen balloons dedicate one lumen to a guidewire, and the other lumen to the balloon. In general, these are coronary balloon catheters that are used off label. These devices, which are designed to treat atherosclerotic coronary artery disease, are less compliant and achieve higher nominal pressures. Some interventionalists feel that double lumen balloons possess technical advantages. One advantage is that most double lumen catheters accommodate 0.014″ microwires, which may provide addition guidewire support to reach difficult to cannulate vessels. Another advantage is that it permits the operator to remove and reshape the microwire without giving up balloon catheter position in the case of a difficult stepwise catheterization. In single lumen balloon catheters this same maneuver results in catheter back bleeding and loss of radio opacity decreasing balloon visibility under fluoroscopy. For this same reason double lumen coronary balloons can undergo multiple inflations without losing radio-opacity allowing for more efficient PTA when multiple segments need treatment. Disadvantages to double lumen coronary balloons are that they are stiffer and don't track as well as single lumen balloons.

When using compliant vs. non-compliant balloons it is essential to understand how these different balloons perform in mismatched vessel segments. When using non-compliant balloons, the dilated radius of the vessel regardless of balloon pressure determines the critical threshold for vessel rupture. When bridging mismatched arterial segments, non-compliant balloons will disproportionately stretch the smaller caliber segment, placing it at greatest risk of rupture. Conversely, when a compliant balloon bridges mismatched arterial segments, the balloon will preferentially expand into the larger caliber segment. According to LaPlace's law, a higher wall tension is required in the larger caliber segment to resist balloon expansion, placing it at greatest risk of rupture. Therefore when using a compliant balloon, angioplasty should be stopped when the larger caliber segment approaches 90% of its baseline luminal diameter.

Our practice is to size balloons to achieve a diameter, which is 80 85%, the baseline diameter of the index vessel. Balloon length is chosen to cover the longest contiguous straight segment so that multiple dilations can be avoided. If the target segment is contiguous around a sharply angulated curve, a shorter balloon is chosen and serial distal to proximal dilations are performed. When successive dilations are required these are performed from distal to proximal to avoid crossing a fresh angioplasty site, which may inadvertently raise a dissection flap. Inflations are performed very slowly with as little volume as needed. The operator should conceptualize the process as a slow controlled stretch of a relatively compliant vessel in contrast to angioplasty for atherosclerotic occlusive disease, where the objective is to compress, collapse and redistribute non-compliant intimal plaque.

Complication Avoidance and Management

Complications of intracranial PTA for vasospasm include vessel perforation (with microwire, microcatheter or balloon), vessel rupture (balloon), dissection, occlusion,

ischemic stroke, hemorrhagic transformation of infarcted tissue, rebleeding from unsecured aneurysms and displacement of aneurysm clips.

Most vessel perforations during PTA are from microwire breeches of the vessel wall. While most result in mild self limited subarachnoid bleeding, occasionally lethal or severely disabling intracranial hemorrhage can occur. If a microwire perforation is suspected, the wire should not be retracted, as this will exacerbate bleeding from the puncture site. Protamine must be given as soon as possible to reverse anticoagulation. Restoration of normal coagulation will often produce hemostasis, demonstrated on control angiograms. Intracranial pressure should be controlled medically and with ventricular drainage as necessary. If control angiography demonstrates hemostasis with the wire in place, the balloon catheter should be positioned over the arteriotomy to control bleeding when the wire is retracted. If hemostasis cannot be achieved it may be necessary to sacrifice the perforated vessel by embolization with microcoils or liquid agents. This is achieved by inflating the balloon and then introducing a second microcatheter, into the injured vessel, which is then used for coil or glue embolization.

Fortunately, microwire perforations are not frequent and can be avoided with strict adherence to proper technique. Small penetrating arteries and small cortical branch arteries should be avoided with the wire. If spasm around the wire tip is encountered, the wire should be retracted until the tip bounces freely with arterial pulsations under fluoroscopy and control angiography demonstrates satisfactory flow in the instrumented vessel.

Arterial rupture from over dilation is life threatening. In large case series, the rate of arterial rupture ranges from 4 to 5% [32]. Vessel rupture due to over dilation is demonstrated under live fluoroscopy as an abrupt, rapid increase in balloon diameter, which is disproportionate to the inflation increment, delivered by the operator. If vessel rupture is suspected, the balloon should not be deflated. As with all types of hemorrhagic complications, anticoagulation should be reversed as rapidly as possible with Protamine. Intracranial hypertension should be controlled medically and with ventricular drainage as necessary. Control angiography should be performed through the guidecatheter to assess hemostasis while the balloon is inflated. If persistent bleeding occurs around the balloon, repositioning the balloon or placing a second balloon should be considered. Vessel sacrifice by coil embolization may be necessary, but may not be compatible with an acceptable outcome, depending on the affected vessel. If necessary, a second embolization capable microcatheter should be used while the balloon is left in place. In some cases, sustained balloon occlusion for 30 60 min will produce durable hemostasis, which continues after the balloon is deflated. In other cases, the rupture is converted to a pseudoaneurysm, which may be amenable to coil embolization with or without a stent for vessel reconstruction. In our experience, such pseudoaneurysms are difficult to control by coil embolization alone. Embolization with a combination of coils and liquid embolic agent using a balloon remodeling technique is more likely to be successful.

Conclusion

Vasospasm continues to be a significant component of the morbidity and mortality associated with SAH. While angioplasty remains the best treatment for medically refractory vasospasm there remains significant risk associated with it. As new medications become available, new diagnostic modalities are discovered and the pathophysiology of vasospasm is better understood our treatment paradigms will continue to change. It is imperative that the vascular neurosurgeon or interventionalist of the future remain attuned to these changes and tailor their treatment regiment accordingly.

Conflict of interest statement We declare that we have no conflict of interest.

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Delayed Intracranial Hemorrhage Associated with Antiplatelet Therapy in Stent-Assisted Coil Embolized Cerebral Aneurysms

Xiao-dong Zhang, Hai-tao Wu, Ji Zhu, Zhao-hui He, Wei-na Chai, and Xiao-chuan Sun

Abstract Administration of oral clopidogrel plus aspirin is the most important regimen to reduce thromboembolic complications in stent-assisted coil embolization of cerebral aneurysm. However, such therapy may increase the risk of hemorrhage. The purpose of this study is to analyze the effect of two different antiplatelet regimens on hemorrhagic and thromboembolic complication rates around the stent-assisted coil embolization period. Records over a 2-year period were reviewed in a retrospective cohort study. For 49 consecutive stent-assisted coil embolization procedures over 41 patients, nine patients received routine antiplatelet drugs (300 mg aspirin and 75 mg clopidogrel) for 3 days before embolization, and 32 received a loading dose of antiplatelet drugs (300 mg aspirin and 300 mg clopidogrel) just before induction of anesthesia. Delayed intracerebral hemorrhage (DIH) was observed more often in the routine antiplatelet group (2/9 cases, 22.2%) in comparison with the loading group (0/32 cases, 0%; $P = 0.044$; Fisher exact test). The two hemorrhagic cases were both female, and occurred within 24 h of postembolization. The thromboembolic complication rates were not significantly different between the two groups. Oral administration of routine antiplatelet drugs for 3 days before stent-assisted coil embolization possibly increases the risk of delayed intracranial hemorrhage, compared to loading group. Symptomatic thromboembolic complications have no significant difference in the two different regimens.

Keywords Intracranial aneurysm · Antiplatelet treatment · Intracranial hemorrhages · Embolization · Therapeutic · Aspirin · Clopidogrel · Stents · Brain ischemia

X. d. Zhang, H. t. Wu, J. Zhu, Z. h. He, W. n. Chai, and X. c. Sun (✉)
Department of Neurosurgery, the First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P.R. China
e mail: sunxch1445@gmail.com

Introduction

Most complications resulting from stent-assisted coil embolization of cerebral aneurysms are thromboembolic. Antiplatelet drugs, including aspirin and clopidogrel (Plavix), are widely prescribed to lower the thromboembolic complication rate around stent-assisted coil embolization period [12]. However, bleeding complications are increased during such therapy, especially when dual antiplatelet agents are taken. Although the intracranial hemorrhagic rate is very low, once it occurs, it can cause servere morbidity and mortality [1, 5, 7, 9, 24, 28, 33]. The rational use of antiplatelet drugs still needs to be researched in stent-assisted coil embolized aneurysmal cases. We sought to determine whether the administration of two different oral antiplatelet regimens influences the hemorrhagic and thromboembolic complication rate resulting from stent-assisted coil embolization of cerebral aneurysms through a retrospective review of clinical data.

Patients and Methods

Patient Population

Forty-one patients with 49 stent-assisted coil embolized cerebral aneurysms were enrolled between March 2007 and June 2009 in our hospital. All medical records were reviewed carefully, and all cases meet the following criteria: (1) Intracranial aneurysm was identified by digital subtraction angiography (DSA), computed tomography angiography (CTA) or magnetic resonance angiography (MRA). (2) All aneurysms were treated by stent-assisted coil embolization. (3) Oral antiplatelet drugs were administrated before and after embolization in all cases.

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Table 1 Clinical and procedure-related characteristics of patients with intracerebral hemorrhage and thromboembolic complications

Cases	Gender	Age (y)	Weight (Kg)	Hypertension	Diabetes	Number of aneurysm	Location of aneurysm	Hunt Hess	Teatment group	Stent type	Intraprocedure aneurysm rupture	Delayed intracranial hemorrhage	Thromboembolic complications
1	M	67	63	Yes	No	1	Pcom	1	Routine	NF			
2	M	42	71	No	No	1	Oph	2	Routine	NF			
3	M	51	67	No	No	1	Pcom	2	Routine	NF			
4	F	53	62	No	No	1	Pcom	1	Routine	NF			
5	F	54	56	No	No	1	Oph	1	Routine	NF		Yes	
6	F	56	53	Yes	No	2	L + R Pcom	1	Routine	NF			Yes
7	F	63	57	No	No	1	Pcom	1	Routine	NF			
8	M	37	74	No	Yes	1	Ca	2	Routine	NF			
9	F	54	60	No	No	1	Pcom	2	Routine	NF	Yes	Yes	
10	M	66	72	Yes	No	1	Pcom	1	Loading	NF			
11	F	58	66	Yes	No	2	L + R Pcom	1	Loading	NF			
12	F	44	68	No	No	1	Oph	1	Loading	NF			
13	F	64	51	No	No	1	Pcom	1	Loading	NF			
14	M	42	75	No	No	1	Pcom	2	Loading	NF			
15	M	52	71	Yes	No	1	Oph	2	Loading	NF			
16	F	56	57	No	Yes	2	L + R Pcom	2	Loading	NF			
17	M	54	69	No	No	1	Cl	1	Loading	NF			
18	F	49	65	Yes	No	1	Oph	2	Loading	NF			
19	F	53	66	No	No	2	L + R Pcom	2	Loading	NF			
20	M	39	73	No	Yes	1	Pcom	2	Loading	NF	Yes		
21	F	55	63	No	No	1	Pcom	2	Loading	LEO			
22	F	49	53	No	No	1	Pcom	2	Loading	LEO			
23	F	38	57	Yes	No	1	Pcom	2	Loading	LEO			
24	F	49	55	Yes	No	1	Pcom	2	Loading	LEO			
25	F	44	65	No	No	1	Pcom	2	Loading	LEO			
26	F	43	67	No	Yes	2	L + R Pcom	1	Loading	LEO			
27	F	45	62	Yes	No	1	Pcom	1	Loading	LEO			
28	F	53	66	Yes	Yes	1	Pcom	1	Loading	LEO			
29	M	64	72	No	No	2	L + R Pcom	2	Loading	LEO			
30	F	44	64	No	Yes	2	L + R Pcom	2	Loading	LEO			
31	F	59	62	No	No	1	Pcom	2	Loading	LEO	Yes		
32	F	64	59	Yes	No	1	Pcom	2	Loading	LEO			
33	M	59	73	No	No	1	Pcom	2	Loading	LEO			
34	F	46	63	No	No	1	Pcom	2	Loading	LEO			
35	F	75	60	No	No	1	Pcom	2	Loading	LEO			Yes
36	F	44	56	Yes	No	1	Oph	1	Loading	LEO			
37	M	50	71	Yes	No	1	Cl	1	Loading	EP			
38	F	78	55	No	No	2	L + R Pcom	1	Loading	EP + NF			
39	M	40	75	Yes	No	1	Ca	1	Loading	EP			
40	M	47	71	No	Yes	1	Pcom	2	Loading	EP			
41	F	70	63	No	No	1	Pcom	2	Loading	EP			

Note: *NF* indicates Neroform stent, *LEO* LEO stent, *EP* Enterprise stent, *Pcom* posterior communicating artery, *Oph* Ophthalmic artery, *Ca* Cavernous segment, *Cl* Clinoid segment, *L+R* Left and right

Drug Regimens

All patients were administered oral antiplatelet drugs before embolization. According to dosage of antiplatelet drugs, subjects were divided into routine group (300 mg aspirin and 75 mg clopidogrel for 3 days before embolization) and loading group (300 mg aspirin and 300 mg clopidogrel just before induction of anesthesia). All patients were systemically anticoagulated with intravenous heparin during coil embolization procedures with initial boluses of 70 100 U/kg followed by continuous infusions of 7 10 U/kg/h. Activated clotting times on heparin were monitored and maintained at least twice baseline, usually at or near 300 s, by administration of additional boluses or by adjusting infusion rates, as needed. Heparin was generally discontinued at the conclusion of the procedures. After embolization, patients received oral clopidogrel 75 mg and aspirin 100 mg for 6 weeks and 6 months respectively. Proton pump inhibitor (Losec 40 mg) was administered in each patient for 5 days to prevent gastrointestinal bleeding.

Result Comparison

Indicators such as gender, age, weight, diabetes and hypertension at hospital admission, number of aneurysms, location of aneurysms, Hunt-Hess grade, stent type, intraembolization aneurysm rupture, postprocedure delayed intracranial hemorrhage, and symptomatic thromboembolic complications were described. Statistical analysis in corresponding indicators was performed between the two groups.

Comparative study focused on two major indicators: (1) Delayed intracranial hemorrhage, and (2) Symptomatic thromboembolic complications. Diagnostic criteria for delayed intracranial hemorrhage were: an initially negative computerized tomographic (CT) scan of the head, and repeated CT scan revealing intracranial hemorrhage. Symptomatic thromboembolic complications were determined by the following clinical signs: Onset of new symptom, or aggravation of original symptoms without other reasons (for example: hydrocephalus, intracranial hemorrhage, epilepsy, metabolic disorders, etc.).

Statistical Analysis

Data analysis was performed using SPSS software (Version 13.0 Chicago, IL). Quantitative data were described as mean ± standard deviation. Chi-square and rank-sum test were used to test for differences in multiple groups, and T-test or rank-sum test was used to test for differences in two different groups. Chi-square and Fisher exact test were used to test for differences in frequencies. Statistical significance was accepted at a $P < 0.05$ (Tables 1 and 2).

Results

A 54-year-old female was admitted in hospital because of subarachnoid hemorrhage. All preoperative examinations were normal. CT scan showed subarachnoid hemorrhage in the left sylvian fissure (Fig. 1a). CT angiography demonstrated a wide neck aneurysm located at the junction of the internal carotid artery (ICA) and posterior communicating artery (PcomA) (Fig. 1b). Neuroform was used to assist coil embolization (Fig. 1c). Aneurysm was ruptured during procedure, emergency packing was performed successfully (Fig. 1d). Immediately postembolization CT scan showed blood and contrast medium leaked into subarachnoid spaces, and the patient's consciousness recovered within 1 h (Fig. 1e). The patient had sudden onset of coma 5 h later; follow-up CT revealed a delayed intracerebral hematoma in the left temporal lobe (Fig. 1f).

A 54-year-old female who complained of mild headache was admitted in hospital because CT angiography revealed an aneurysm in the ophthalmic segment of left internal carotid artery (Fig. 2a). All preoperative examinations were

Table 2 Treatment group demographics

Variable	Routine group	Loding group	P	Statistical test	Value
Female	4	10	0.461	X^2	0.544[a]
Male	5	22			
Age	53.00 ± 9.274	52.91 ± 10.446	0.981	T test	0.024
Weight	62.56 ± 7.020	64.53 ± 6.720	0.466	T test	0.753
Hypertension	2/9	12/32	0.393	X^2	0.729[a]
Diabietes	1/9	6/32	0.591	X^2	0.290[b]
Intraprocedure aneurysm rupture	1/10	2/39	0.504	Fisher's Exact Test	
Delayed intracranial hemorrhage	2/9	0/32	0.044	Fisher's Exact Test	
Thromboembolic complications	1/9	1/32	0.395	Fisher's Exact Test	

[a] 1 cells (25.0%) have expected count less than 5. The minimum expected count is 1.54
[b] 1 cells (25.0%) have expected count less than 5. The minimum expected count is 3.07

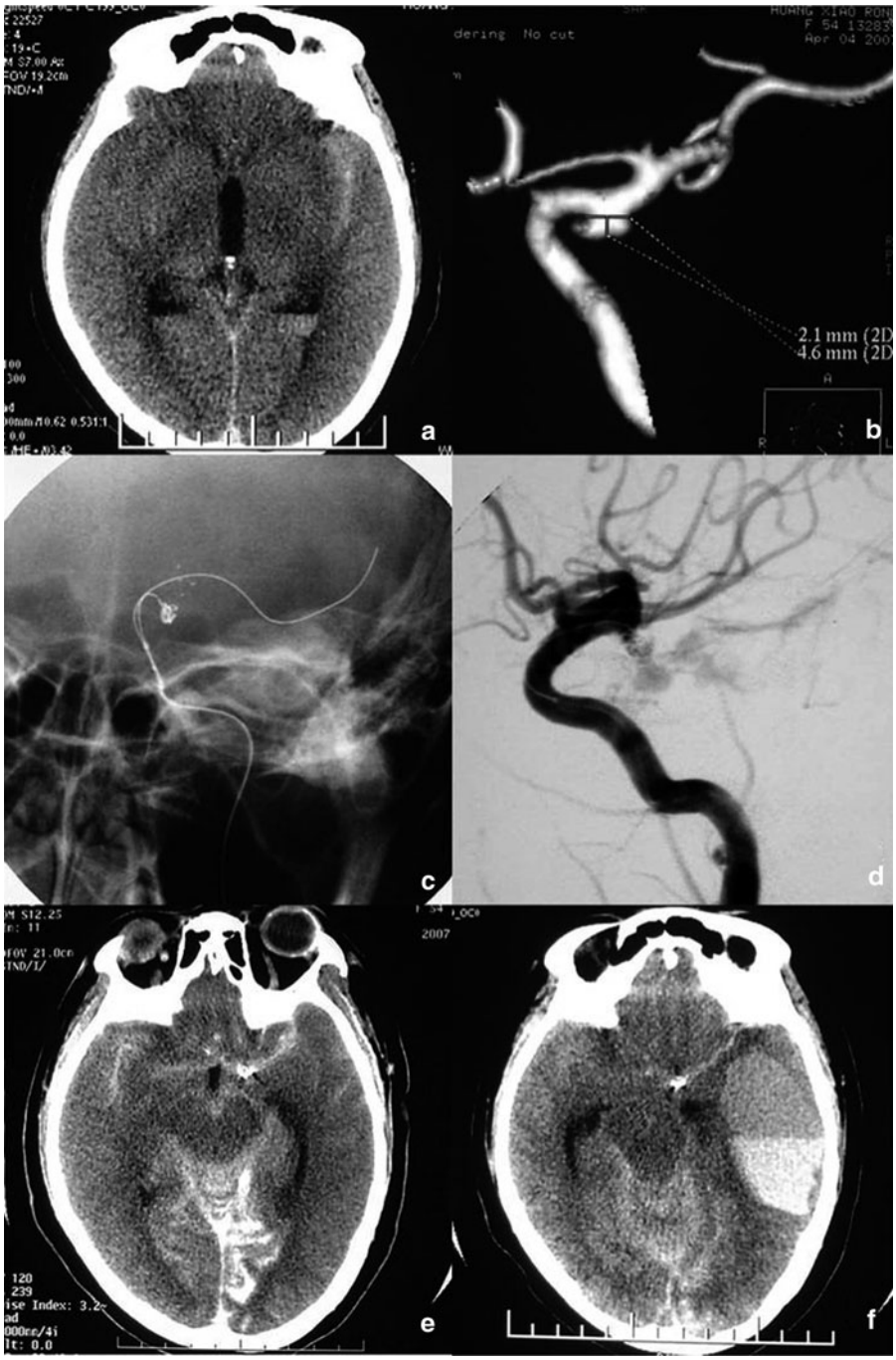

Fig. 1 Images obtained in a 54 year old woman with SAH. **(a)** CT image, axial view, demonstrates subarachnoid hemorrhage in the left sylvian fissure. **(b)** 3D CTA image, left oblique view, shows a wide neck aneurysm located at the junction of the internal carotid artery (ICA) and posterior communicating artery (PcomA). **(c)** Unsubtraction images, left oblique view, demonstrates Neuroform stent was used to assisted coil embolization. **(d)** Intraarterial angiogram, left oblique view, shows aneurysm rupture during embolization, emergency packing was performed successfully. **(e)** Immediately postembolization CT image, axial view, shows blood and contrast medium leaked into subarachnoid spaces, and the patient's consciousness recovered within 1 h. **(f)** Follow up CT image, axial view, reveals a delayed intracerebral hematoma in the left temporal lobe after a sudden coma occurred 5 hours later

normal. Stent assisted coil embolization was performed, the proximal end of Neuroform was fall into the sac of aneurysm because of stent migration. Embolization had to be called off since microcatheter was blocked by Neuroform and unable to be delivered into the aneurysm (Fig. 2b). No evidence of hemorrhage was seen in the immediately postembolization CT scan, and the patient's consciousness recovered soon (Fig. 2c). A sudden onset of coma occurred 8 hours later; Emergency CT revealed delayed intracerebral hematoma in the left temporal and right occipital lobe (Fig. 2d).

Discussion

Besides surgical clipping, endovascular embolization has become a standard treatment of aneurysms [18]. Among all techniques, stent-assisted coil embolization technique is playing an increasingly important role in the treatment of wide-necked aneurysms, small aneurysms, and some refractory aneurysms [8, 17, 25, 29]. Anti-platelet aggregation therapy is the major method of preventing thromboembolic complications of stenting, and oral administration of a duo of

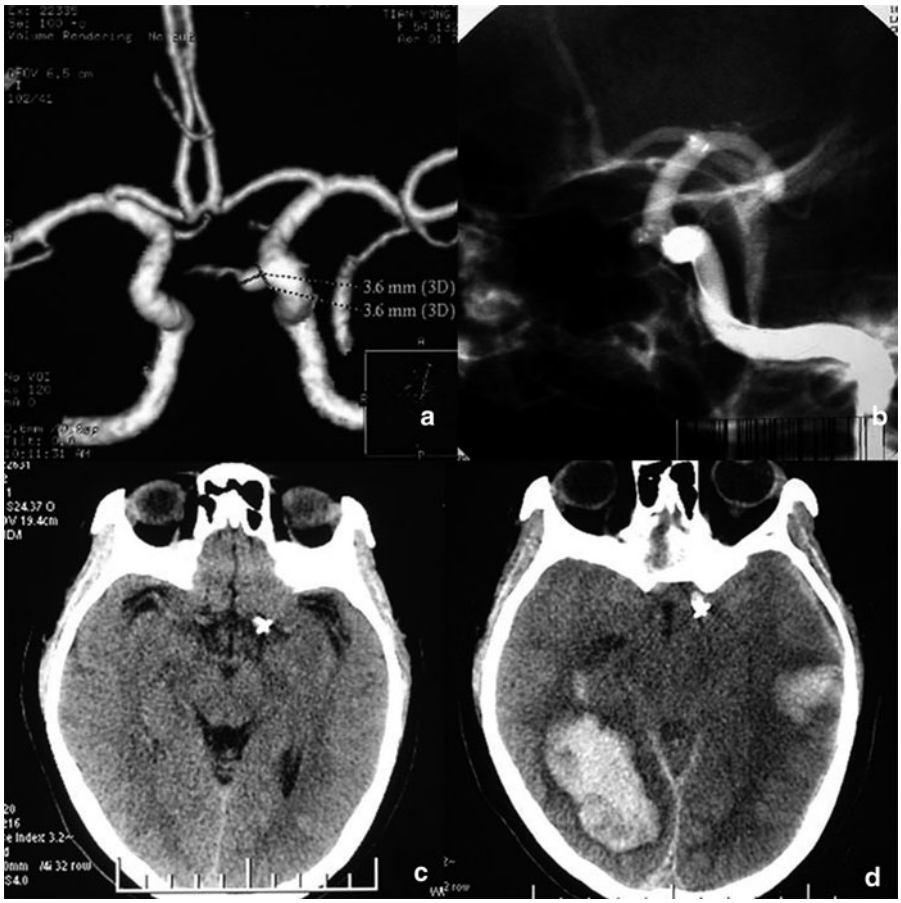

Fig. 2 Images obtained in a 54 year old woman with unruptered aneurysm. **(a)** 3D CTA image, frontal view, reveals an aneurysm in the ophthalmic segment of left internal carotid artery. **(b)** Intraarterial angiogram image, frontal view, the proximal end of the stent Neuroform was fall into the sac of aneurysm because of stent migration. **(c)** Immediately postembolization CT image, axial view, shows no evidence of intracranial hemorrhage, and the patient's consciousness recovered soon. **(d)** Follow up CT image, axial view, reveals delayed intracerebral hematoma in the left temporal and right occipital lobe after a sudden coma occurred 8 hours later

anti-platelet aggregation drugs, clopidogrel and aspirin, has become a recommended regimen to prevent stent thrombosis complications [12]. Anti-platelet related bleeding complications, especially intracranial hemorrhage, have also increased correspondingly [1, 5, 7, 9, 24, 28, 33]. For this reason, correct anti-platelet dosing, better identification of higher risk patients, appropriate monitoring, and incorporation of various periprocedural strategies in routine clinical practice should be paid more attention.

In this retrospective study, we found that there was no significant difference between the two anti-platelet groups in thromboembolic complications, and no patients had ischemic symptoms. In the aspect of delayed intracranial hemorrhage, the routine anti-platelet group tended to present more hemorrhagic complications. Both patients with delayed intracranial hemotoma were female, and both occurred within 12 h. The location of hemotomas happened to be in temporal and occipital lobes.

Asian or Mexican-American ethnicity is a possible risk factor for intracranial hemorrhage (ICH) [13]. The Japanese population is known to have a high incidence of ICH; the incidence in the Hisayama study [14] (130/100,000 person-years in men and 70/100,000 person-years in women) and the Shibata study [31] (61/100,000 person-years) was five times higher than the incidence in Western countries (7 12/100,000 person-years). The difference in the incidence between Japanese and Western populations is in part due to the high prevalences of small artery cerebrovascular lesions and hypertension due to high salt intake, especially among the elderly Japanese population [32]. These findings seem consistent with our observation. Moreover, for anti-platelet related intracranial hemorrhage in stent-assisted aneurysm cases, Western reports [23, 34] are few and all have relationships with external ventricular drainage, lumbar drainage or ventriculo-peritoneal shunt in addition to anti-platelet therapy.

The delayed intracranial hemorrhage cases were all female in our study, which is consistent with some studies [3, 6, 10, 15, 19, 21, 35]. Presbitero's study [21] showed that intracranial hemorrhage rate of females was significantly higher than males in tissue-type plasminogen activator therapy. Alexandar's study also demonstrated that women experience more bleeding than men whether or not they are treated with GP IIb/IIIa inhibitors. The female sex has also been linked to bleeding risk in other clinical settings with a variety of antithrombotic agents (e.g., thrombolytics, unfractionated heparin, and low-molecularweight heparin) [6, 10, 15, 19, 35]. This may be due to females' smaller body

size, their differences in platelet reactivity in relation to levels of sex hormones [2, 20, 27], and the difference in pharmacological responses to therapeutic agents between the sexes [22, 26].

Toyoda [33] found that oral anti-platelet agents were predictive of cerebellar hemorrhage, hematoma enlargement, and early death in Japanese ICH patients, and were independently related to hematoma enlargement within the initial 24 h. In our observation, the two hemorrhagic complications both occurred within 12 h. The time window of both studies conformed with each other, but the hemorrhagic location in this study are the temporal and occipital lobes.

We also noticed that some research has different results from ours [4, 11, 13, 16, 30]. In Hart's [13] review, advanced age and elevated blood pressure are salient risk factors of antithrombotic related ICH. In Lovelock's [16] investigation, incidence of intracerebral haemorrhage associated with antithrombotic use was increased in the population aged over 75 years. In Armstrong's study [4], patient-related risk factors for bleeding with oral anticoagulants include: trauma, invasive procedures, history of bleeding disorder, high anticoagulant intensity, concomitant use of antiplatelet drugs, presence of underlying severe disease, advanced age, and prior history of cerebrovascular accident, or gastrointestinal bleeding, and the risks of intracranial haemorrhage increased with age $>$ 65 years, weight under 70 kg, hypertension on admission and the use of tissue plasminogen activator. Some researchers also pointed out that cerebral atrophy, hypertension, intracranial vasculopathies such as Moyamoya and the combination of clopidogrel and ASA were potential risk factors for development of intracranial hemorrhage [30]. The two hemorrhagic cases of this study were less than 60-years-old, without hypertension, diabetes, and no significant brain atrophy and ischemic cerebrovascular disease; body weight was slightly lower compared to the average weight.

However, in this study, patients were not randomly assigned in the two anti-platelet groups, and the result may also be influenced by the limitations of retrospective study. The number of patients in this study is relatively less only 9 cases in the routine anti-platelet group. This may affect the statistical result, and further analyzing in different Hunt and Hess scale and Fisher grade cannot be performed. In addition, this study only focused on clinical outcomes during hospitalization. The long-term hemorragic and ischemic complications still need further studies.

Conclusion

Oral administration of anti-platelet drugs before stent-assisted coil embolization possibly increases the risk of delayed intracranial hemorrhage. Rational use of anti-platelet drugs, identifying and monitoring the bleeding risk factors and related clinical indicators still need further exploration.

Conflict of interest statement We declare that we have no conflict of interest.

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Microsurgical Treatment of Ruptured Intracranial Aneurysm: A 120-Case Analysis

Weihua Tang, Hua Feng, Zhi Chen, Hongping Miu, Jiexiang Pan, Jiangkai Lin, and Gang Zhu

Abstract *Objective*: To investigate the influence of pre-operative conditions and microsurgical skill on the post-operative outcomes of intracranial aneurysms by retrospective analysis of 120 cases with microsurgical treatment.

Methods: 120 patients with 134 intracranial aneurysms received microsurgical treatment via pterional approach or improved pterional approach.

Results: Of 134 aneurysms, 122 were clipped, one was coated, three were isolated and there was parent artery deligation in one case. 111 Patients were cured, seven cases gave up therapy post-operation, and two died. According to GOS standard, the outcome in the discharge stage was good in 94 cases, mild disability in 12 cases, moderate disability in three cases and severe disability in two cases. Long-term follow-up was performed in all patients, of whom 95 recovered well, mild disability in 12 cases, moderate disability in two cases and severe disability in one case.

Conclusion: Surgical clipping was the most effective method to treat intracranial aneurysm. Optimal chance and microsurgical technique, as well as microanatomical knowledge, are keys for successful treatment.

Keywords Pterional approach · Intracranial aneurysm · Microsurgical clipping

Intracranial aneurysm has high morbidity and mortality. The main clinical manifestation is subarachnoid hemorrhage (SAH), accompanied with severe headache, disturbance of consciousness, with or without associated neurological dysfunction, and so on. Timely and reasonable surgical clipping could significantly improve patient prognosis. With microsurgical techniques, anesthesia, imaging, and equipment advances, the risk of craniotomy clipping continues to decline, and has become a typical method of aneurysm treatment. From January 2000 to February 2008, micro-neurosurgery clipping was attempted in 120 patients with 134 aneurysms; analysis reports are as follows.

W. Tang, H. Feng, Z. Chen, H. Miu, J. Pan, J. Lin, and G. Zhu (✉)
Department of Neurosurgery, Southwest Hospital, Third Military Medi cal University of PLA, 20 Gao Tan Yan Street, Sha Ping Ba district, Chongqing 400038, P.R. China
e mail: zhugang666@yahoo.com.cn

Subjects and Methods

General Information

There were 47 males (39%) and 73 females (61%) aged 32 65 years (mean age 45.7 years). All patients were diagnosed by head CT scanning and cerebral digital subtraction angiography (DSA). DSA examination showed that 118 cases had single aneurysms, and ten cases had two aneurysms each. Aneurysm sites: posterior communicating artery (72), anterior communicating artery (37), middle cerebral artery (17), carotid furcation (3), anterior cerebral artery (2), posterior cerebral artery (1), parietal cortex (1), anterior cerebral artery dissection (1). Aneurysm sizes: diameter less than 0.5 cm (19), 0.5 1.0 cm (81), 1.0 1.5 cm (24), greater than 1.5 cm (10). All patients were hospitalized for SAH resulting from aneurysmal rupture. Clinical grading according to the Hunt and Hess scale (13) for the 120 patients with SAH was as follows: grade 0 or 1 in 43 cases (36%), grade 2 in 55cases (46%), and grade 3 in 22 cases (18%). Patients classified as either grade 4 or 5 were not submitted to craniotomy clipping. Main symptoms: sudden headache and neck stiffness in 120 cases. Eight cases of disturbance of consciousness, mental disorder in 17 cases and oculomotor nerve palsy in 46 cases.

Preoperative Treatment

Intravenous administration of Nimodipine(2 4 mg/h) for 1 3 days. Controlled hypotension was given if patients had

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hypertension. Sedation was given in cases of tension and irritability.

Surgical Procedures

All patients had pterional craniotomy or modified pterional craniotomy with microsurgery.

Postoperative Treatment

All cases were given 3H treatment and Nimodipine. As for the cases of serious subarachnoid hematoma or intraventricular hemorrhage, postoperative lumbar puncture and drainage were set for 3 7 days. Postoperative ventricular drainage was done in cases where intraventricular hemorrhage occurred with impaired consciousness. For the stent-assisted coil embolization cases, heparin was given for 3 days. For postoperative vasospasm, cerebral ischemia symptoms or balloon-assisted neck remodeling, an appropriate extension for anticoagulation, and oral aspirin were given for 3 months.

Results

The group of 120 patients had 134 aneurysms, microsurgical clipping in 122 cases, package for one case, isolate in three cases, and parent artery ligation in one case. 118 cases were cured, two patients died. Discharge evaluation results: good in 94 cases, 15 cases with mild disability, moderate disability in six cases, severe disability in three cases. In postoperative follow-up from 2 months to 7 years, good in 97 cases, 17 cases with mild disability, moderate disability in two cases, severe disability in two cases.

Discussion

Early aneurysm repair to prevent recurrent bleeding is the core to the management of patients with subarachnoid hemorrhage. Dandy successfully carried out the intracranial aneurysm surgery as early as 1937, but due to limitations of the early period, most patients still used conservative treatment [1]. In the 1960s, McKissock made a series of prospective randomized studies showing that for some intracranial aneurysm, surgery to benefit over the risk of aneurysm, clipping surgery of intracranial aneurysms has gradually become the standard treatment [2].

Surgical timing is still controversial, but has gradually become clear. Early surgery (48 72 h after SAH) helps to avoid further bleeding; subarachnoid hemorrhage also can be removed to alleviate the fatal artery spasm [3, 4]. However, for early surgery, brain edema made it difficult to exposure the aneurysms; more brain tissue damage and high risk of intraoperative aneurysm rupture cause high mortality and morbidity. Delayed surgery did not significantly reduce the incidence of rebleeding, but the effect of other aspects is equivalent to early surgery. Although early surgery can reduce the re-bleeding, it cannot reduce ischemic neurological deficits or other complications. In Kassel's [5] summary of 3,521 cases of intracranial aneurysm patients, the results found that delayed operation rebleeding rate was 9%, significantly higher than 4% of early surgery group, but other results of the two groups were similar, such as the fact that the mortality rate was 20% vs. 24%; good result rate was 62% vs. 56%. The rate of vasospasm, surgical complications, hydrocephalus and the first bleeding damage is also similar. Therefore, as in our experience, for the patients to be in good condition or for surgery not to be difficult, surgery should be as soon as possible. Delayed surgery is suitable for the others.

During surgery, if the patients have high ICP, fast intravenous infusion of 20% mannitol should be done to reduce intracranial pressure. If preoperative CT show the patients have hydrocephalus, ventricle puncture should be done to lower intracranial pressure and facilitate the exposure of aneurysms. When in the process of ventricular puncture, a stable blood pressure should be maintained so as to avoid aneurysm re-rupture. During surgery, the cistern should be fully open for release of cerebrospinal fluid [6, 7]. Intraoperative aneurysm rupture is the most dangerous complication; the impact factors include aneurysm itself, surgical factors and other factors. Aneurysm itself includes: (1) thin-walled aneurysm; intraoperative rupture-prone. (2) irregular-shaped aneurysms rupture easily. (3) rupture before surgery has higher rate of intraoperative rupture. (4) the aneurysms difficult to expose, in the neck exposure and clamping operation, are easy to rupture [8, 9].

Cerebral vasospasm is an important factor affecting the prognosis of patients. Removal of subarachnoid hemorrhage as much as possible is the most effective way to prevent and reduce cerebral vasospasm [10, 11]. Wet dressing the parent artery with cotton piece containing papaverine is also helpful. Washing the exposed cistern and subarachnoid space with diluted papaverine solution for 2 3 min could induce vascular dilate. All patients were using 3H therapy, calcium channel antagonists, lumbar external drainage, etc.; all can effectively reduce cerebral vasospasm, prevent secondary ischemic brain damage, cerebral edema and brain swelling.

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Conflict of interest statement We declare that we have no conflict of interest.

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Clazosentan: Prevention of Cerebral Vasospasm and the Potential to Overcome Infarction

Juergen Beck and Andreas Raabe

Abstract Cerebral vasospasm is a common complication occurring after aneurysmal subarachnoid hemorrhage (SAH). It is recognized as a leading preventable cause of morbidity and mortality in this patient group, but its management is challenging, and new treatments are needed. Clazosentan is an endothelin receptor antagonist designed to prevent endothelin-mediated cerebral vasospasm. Vajkoczy et al. (Neurosurg 103:9 17, 2005) initially demonstrated that clazosentan reduced moderate/severe angiographically proven vasospasm by 55% relative to placebo. These findings led to the initiation of the CONSCIOUS trial program to further examine the efficacy and safety of clazosentan in reducing angiographic vasospasm and improving clinical outcome after aneurysmal SAH. In the first of these studies, CONSCIOUS-1, 413 patients were randomized to placebo or clazosentan 1, 5 or 15 mg/h. Clazosentan reduced angiographic vasospasm dose-dependently relative to placebo with a maximum risk reduction of 65% with the highest dose. Despite this, there was no benefit of clazosentan on the secondary protocol-defined morbidity/mortality endpoint; however, additional post-hoc and modified endpoint analyses provided some evidence for a potential clinical benefit. Two additional large-scale studies (CONSCIOUS-2 and CONSCIOUS-3) are now underway to further investigate the potential of clazosentan to improve long-term clinical outcome.

Keywords Aneurysmal subarachnoid hemorrhage · Cerebral vasospasm · Clazosentan · CONSCIOUS-1 study

J. Beck (✉) and A. Raabe
Department of Neurosurgery, Inselspital, University of Bern, Bern, Switzerland
e mail: Juergen.Beck@insel.ch

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) affects approximately 0.01% of the population every year [3, 7]. Although rare, it is a major medical emergency with approximately 50% of sufferers dying shortly after aneurysm rupture. If the patient survives the initial bleed and the burst aneurysm is successfully secured, one of the most serious potential complications is cerebral vasospasm, the constriction of blood vessels in the brain. Vasospasm after aneurysmal SAH can be detected angiographically in up to 70% of patients [1, 2, 6], and results in clinical symptoms such as disturbed language, confusion and muscle weakness in 20 30% of individuals [2, 4]. Vasospasm-related cerebral infarcts have been reported to occur in approximately 50% of patients when magnetic resonance imaging (MRI) is used for assessment [10]. Currently, management of vasospasm is challenging, with few treatment options and limited evidence of efficacy.

Cerebral vasospasm is thought to result from an increase in vasoconstrictor mediators released by the breakdown of blood accumulated in the subarachnoid space [2]. The onset of vasospasm is usually 3 5 days after the initial bleed, with maximal arterial narrowing at 5 14 days [1]. Mediators implicated in vasoconstriction include oxyhemoglobin, bilirubin oxidation products, nitric oxide, and endothelin-1. Clazosentan is an endothelin receptor antagonist designed to block the effect of endothelin. It is under investigation for use in the prevention of vasospasm and associated morbidity and mortality. Following intravenous administration of clazosentan, steady-state levels are achieved within 2 h and, on cessation of infusion, the drug is rapidly cleared [9]. The potential of clazosentan in the prevention of cerebral vasospasm was initially demonstrated clinically by Vajkoczy et al. [8] in a double-blind study of 34 patients. Following aneurysmal SAH, patients were randomized to placebo or clazosentan (0.2 mg/kg/h) infusion initiated within 48 h of microsurgical aneurysm clipping and continuing for 14 days. Angiographs at day 8(±1) post-aneurysm rupture showed

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that, relative to placebo, clazosentan reduced the incidence of moderate/severe vasospasm by 55% (88% vs. 40% for placebo and clazosentan respectively, $p = 0.008$). In a planned post-hoc analysis, 15% of clazosentan- and 44% of placebo-treated patients had new cerebral infarcts within 14 days of SAH. This difference was not significant ($p = 0.13$; the study was not powered to this endpoint) but is broadly consistent with the reduction in angiographic vasospasm reported with clazosentan.

Materials and Methods

The promising Vajkoczy et al. [8] data led to the initiation of the Clazosentan to Overcome Neurological iSChemia and Infarct OccUrring after Subarachnoid hemorrhage program (CONSCIOUS), a series of clinical trials to further investigate the use of clazosentan in cerebral vasospasm prevention. The first of these, CONSCIOUS-1, was a large, double-blind study (52 centers in 11 countries) to assess the effect of clazosentan on angiographically proven moderate/severe vasospasm within 14 days of SAH (primary endpoint), and morbidity and mortality within 6 weeks (secondary endpoint) [6]. Patients ($n = 413$) who had experienced an aneurysmal SAH (secured by clipping or coiling) were randomized to clazosentan (1, 5, or 15 mg/h) or placebo initiated within 56 h of SAH in a ratio of 1:1:1:1. Patients were 18 70 years old with a ruptured saccular aneurysm confirmed by digital subtraction angiography and a World Federation of Neurosurgeons Scale grade of I IV (or grade V that had improved to grade IV). Additionally, to enrich the population for high risk of vasospasm and capacity to improve, only patients with thick or diffuse subarachnoid clot on baseline computed tomography (CT) scan (prior to securing procedure) were included. Regardless of randomization, all patients were treated according to standard care protocols (e.g. oral nimodipine, infusion of fluids).

Results

Vasospasm 9 (±2) days after aneurysm rupture was assessed angiographically. Relative to placebo, clazosentan resulted in a dose-dependent reduction in moderate/severe vasospasm [defined as a reduction in arterial diameter of 34 66% (moderate) or > 66% (severe)] (Fig. 1). The highest dose of clazosentan (15 mg/h) resulted in a 65% risk reduction relative to placebo ($p < 0.0001$). There were no between-group differences in percentage of patients experiencing

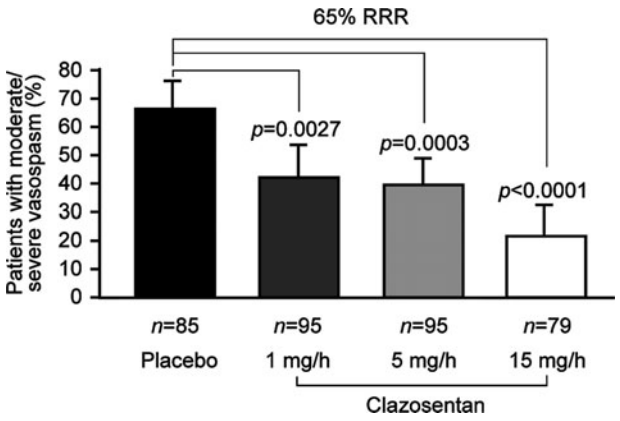

Fig. 1 CONSCIOUS 1 primary endpoint [6]. Clazosentan significantly and dose dependently reduced the incidence of moderate/severe vaso spasm. RRR, relative risk reduction. Per protocol analysis. Printed with permission from Macdonald et al. [6]

morbidity and mortality using the protocol-defined investigator-assessed composite endpoint (31, 37, 31, and 38% for placebo and 1, 5, and 15 mg/h clazosentan, respectively, $p > 0.1$ for all comparisons, Fig. 2). This endpoint included all infarcts regardless of whether they were vasospasm related. In a subsequent post-hoc analysis of all-cause mortality and vasospasm-related morbidity (including only vasospasm-related infarcts and centrally assessed in patients with a post-baseline CT scan), a non-significant dose-dependent trend for a reduction in morbidity and mortality was observed with clazosentan (Fig. 2). This trend was supported by additional post-hoc analyses of individual elements of this composite (Fig. 2). There was no effect of clazosentan on extended Glasgow Outcome Scale. During the trial, mortality rates were 4, 4, 8, and 7% in the placebo and clazosentan 1, 5, and 15 mg/h groups, respectively. Low blood pressure, anemia and pulmonary complications occurred more frequently with clazosentan than placebo.

Discussion

Like Vajkoczy et al. [8], the findings from CONSCIOUS-1 indicate that clazosentan has a marked beneficial effect on angiographic vasospasm. CONSCIOUS-1 also found some evidence of a related clinical benefit, even though the findings were not significant. There may be several reasons for this. For example, it has been proposed that vasospasm is only one factor involved in delayed infarction and poor outcome, and that a multifactorial approach is therefore required to improve outcome following aneurysmal SAH [5, 7]. Alternatively, it is possible that the design of CONSCIOUS-1 masked clinical effects of clazosentan. For

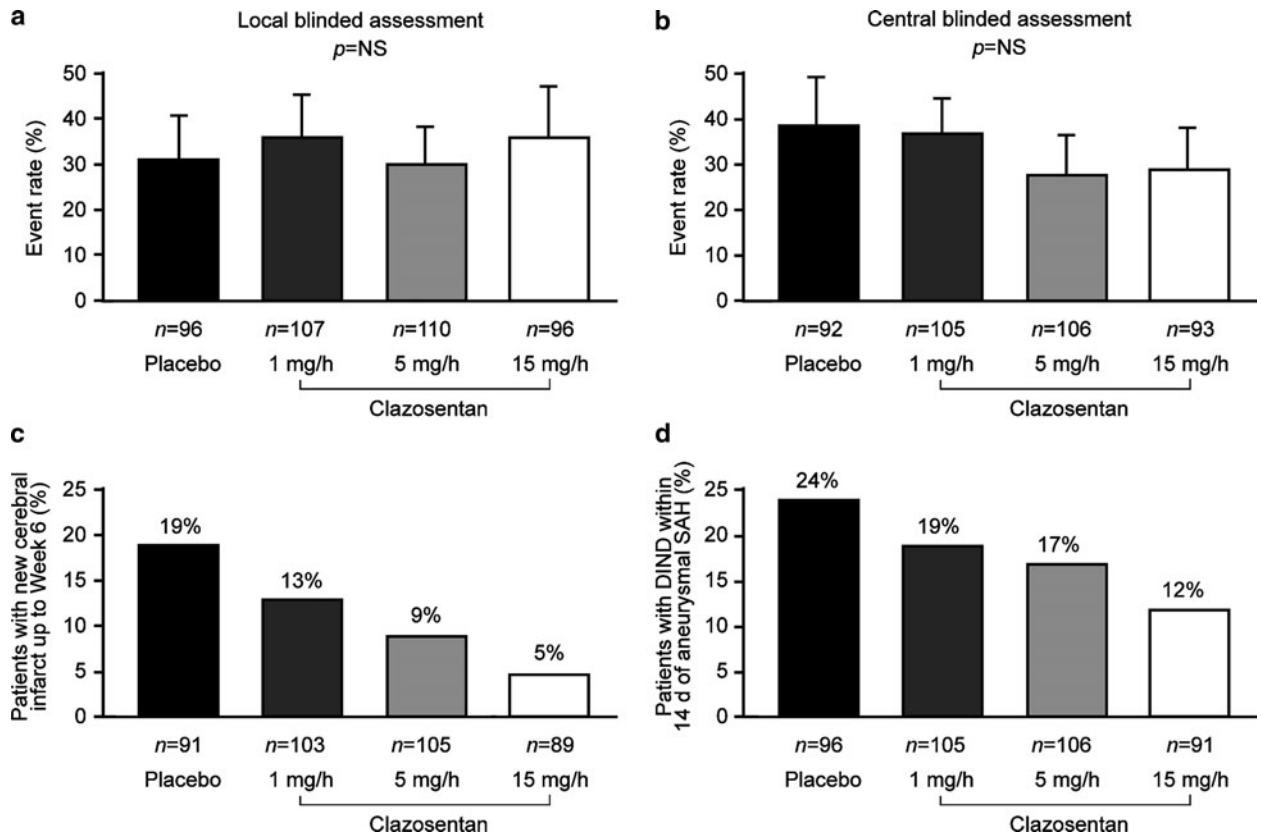

Fig. 2 CONSCIOUS 1 morbidity and mortality analyses [6]. (**a**) protocol defined morbidity and mortality endpoint. Includes death within 6 weeks, new cerebral infarct within 6 weeks (assessed by local investigator), vasospasm related delayed ischemic neurologi cal deficit (DIND) within 14 days of SAH (assessed by local investigator), use of rescue therapy for vasospasm within 14 days of SAH. All treated dataset. 95% CIs. Printed with permission from Macdonald et al. [6]. (**b**) modified morbidity and mortality endpoint. Includes death within 6 weeks, vasospasm related new cerebral infarct within 6 weeks (assessed by central panel blind to treatment group), vasospasm related DIND (centrally assessed by digital sub traction angiography), any rescue therapy within 14 days of SAH. All treated dataset. 95% CIs. (**c** and **d**) individual elements of composite endpoint, post hoc analysis (central assessment of patients with CT scans). All treated dataset

example, even though many baseline characteristics were matched in patients, there are potential risk factors for vasospasm that may have confounded the results (e.g., securing procedure, aneurysm location). Additionally, to investigate vasospasm-related clinical outcome, surrogate markers such as presence of a new infarct must be used. However, there are many potential causes of infarcts and neurological damage (including peri-procedural damage), so it is important that only those related to vasospasm are included. The modified endpoint in CONSCIOUS-1 accounted for this and involved review of all clinical and imaging data by a central panel to facilitate consistent interpretation. Using this measure, there was a trend for improvements in patients receiving clazosentan. However, the study was not designed for this endpoint and this was a post-hoc analysis. Finally, clinical scales such as the Glasgow Outcome Scale are based on functional physical changes and are not designed to identify subtle neurological changes; more sensitive measures may therefore be needed. These observations need to be taken into account when designing studies investigating vasospasm.

Following the CONSCIOUS-1 findings, two additional large-scale, multi-center studies are now under way (Fig. 3). CONSCIOUS-2 is investigating the efficacy and safety of 5 mg/h clazosentan compared with placebo in reducing vasospasm-related morbidity and all cause-mortality in patients with aneurysmal SAH secured by surgical clipping. CONSCIOUS-3 is following a similar protocol but involves patients secured by endovascular coiling randomized to clazosentan 5 mg/h, clazosentan 15 mg/h or placebo. Both of these studies are utilizing a centralized critical event committee. This committee involves an image review subcommittee (8 neuroradiologists to review and evaluate CT scans and angiographs) and a clinical review subcommittee (four

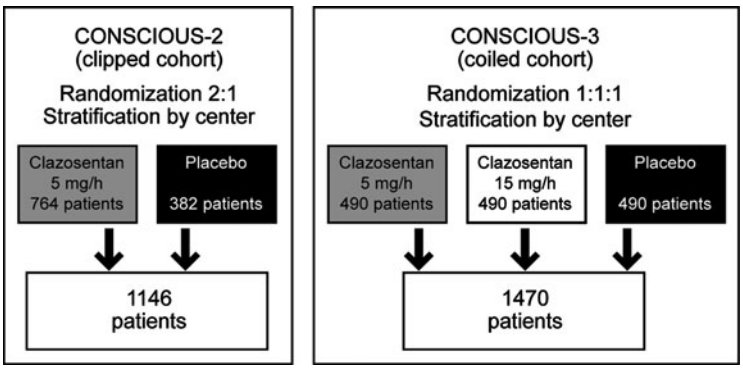

Fig. 3 CONSCIOUS 2 and CONSCIOUS 3 overview. Patient numbers are estimated

neurosurgeons and two neurointensivists) to review clinical data and assess clinical events and their causes. Both studies also focus on vasospasm-related morbidity. In particular, the protocol-defined morbidity and mortality endpoint includes any of the following within 6 weeks of aneurysmal SAH: death (all causes); new cerebral infarct due to vasospasm; delayed ischemic neurological deficit (DIND) due to vasospasm; neurological signs and symptoms in the presence of confirmed angiographic vasospasm leading to use of rescue therapy. Additionally, as anemia and pulmonary events may be related to fluid retention, these studies are utilizing fluid management guidelines.

Conclusion

In summary, it is clear that new treatments are needed to help improve outcome after aneurysmal SAH. Clazosentan is an endothelin receptor antagonist under investigation for the prevention of cerebral vasospasm after aneurysmal SAH. Studies to date report a significant effect of clazosentan on the reduction of angiographically proven cerebral vasospasm, but a benefit in clinical outcome (e.g., infarcts, DIND and functional outcome) is yet to be shown. Two additional studies are under way designed to specifically investigate the effect of clazosentan on clinical outcome following aneurysmal SAH.

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Conflict of Interest Statement We declare that we have no conflict of interest.

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Current Management of Subarachnoid Hemorrhage in Advanced Age

Norihito Shimamura, Akira Munakata, and Hiroki Ohkuma

Abstract *Purpose*: We focused on the recent management of aneurysmal subarachnoid hemorrhage (SAH) in patients over 70 years old (advanced age).

Methods: From January 2001 through July 2009 we treated 372 aneurysmal SAH cases including 123 patients of advanced age. Since 2006 we have been selecting primarily interventional treatment in advanced age. We divided patients into two groups: from 2001 to 2005 and from 2006 to 2009. We analyzed Hunt-Kosnik (HK) grade, treatment methods, rate of vasospasm, Glasgow Outcome Scale at 30 days after onset of SAH, and the ratio of shunt operations. Statistical analyses were done with chi-square analysis or the t-test.

Results: The ratio of procedures in advanced age increased statistically from 28% (51/183) to 38% (72/189). Mean age of patients increased from 76.2 to 77.7. HK grade and proportion of radical surgeries were similar. But the proportion of acute stage surgery and coil embolization increased significantly from 79 to 95% and from 8 to 24%, respectively. Incidence of symptomatic vasospasm increased from 35 to 37%, while asymptomatic vasospasm decreased from 4.7 to 3.2%. Rate of angioplasty increased significantly from 22 to 76%. The proportion of shunt surgeries decreased from 33 to 19% and favorable outcomes increased from 47 to 51%.

Conclusions: Mean age and proportion of procedures in advanced age are increasing, but outcomes have improved. These results depend on radical surgery for aneurysm in the acute stage and aggressive prevention and treatment of vasospasm. Interventional treatment is necessary to improve the outcome in cases of advanced age.

Keywords Clipping · Coiling · High aged patient · Interventional treatment · Subarachnoid hemorrhage · Vasospasm

N. Shimamura (✉), A. Munakata, and H. Ohkuma
Department of Neurosurgery, Hirosaki University School of Medicine, 5 Zaihuchou, Hirosaki Aomori pref. 036 8562, Japan
e mail: shimab@cc.hirosaki u.ac.jp

Introduction

As a result of the ISAT trial, ruptured aneurysms that are suitable for both clipping and coiling have often been treated by coiling [7]. Less invasive treatment has become more desirable recently in a society with advancing age. But the follow up paper of the ISAT reported that clipping was favorable for subarachnoid hemorrhage (SAH) patients over 70 years old. [8]. Many papers reported that outcomes for advanced age SAH patients treated by clipping or coiling were poor [1, 3, 13, 14]. In Japan, the number of elderly patients with aneurysmal SAH is increasing. Since 2006 we have been primarily selecting interventional treatment for patients of advanced age. We focused on current management and outcome of SAH in patients who were over 70 years old (advanced age) in our institute in a retrospective study.

Material and Methods

From January 2001 through July 2009 we treated 372 aneurysmal SAH patients, including 123 patients of advanced age. From 2006 onward we selected interventional treatment for SAH patients of advanced age, primarily under local anesthesia. We divided patients into two groups according to treatment strategy: from 2001 to 2005 and from 2006 to 2009.

Postoperative treatment strategy was as follows: We routinely maintain the normovolemia, intravenous administration of fasudil hydrochloride, and avoidance of bed rest to prevent vasospasm. Post operative angiography was done from day 7 through day 11 after the onset of SAH. When neurological deterioration occurred, we immediately examined the blood, including electrolytes, and performed a brain CT. When symptomatic vasospasm was suspected as the prime concern, high dose hydrocortisone (500 mg) and mannitol (300 ml) were infused. Triple H therapy was also carried out. Angiography was performed simultaneously and, for etiologic spastic artery, intra-arterial injection of drugs

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(papaverine, fasudil, nicardipine) and/or balloon angioplasty were carried-out.

We analyzed Hunt-Kosnik (HK) grade, treatment methods, rate of symptomatic vasospasm, Glasgow Outcome Scale (GOS) at 30 days after the onset of SAH, and rate of shunt surgery. Statistical analyses were chi-square analysis or the t-test (JMP 8.01, SAS institute Inc. Cary, NC). And a p-value below 0.05 was accepted as statistically significant.

Illustrative Cases

Case 1. A 79-year-old female suffering with SAH was transferred to our department on day 0. HK grade was 3 and Fisher group was 3. Ruptured right internal carotid artery aneurysm and un-ruptured right middle cerebral artery aneurysm were diagnosed by angiography (Fig. 1a). Liver cirrhosis and thrombocytopenia were complicating factors, leading to coil embolization of the ruptured aneurysm under local anesthesia on the same day (Fig. 1b). Complete obliteration of aneurysm was achieved and the postoperative course was uneventful. She did not suffer vasospasm and achieved good recovery of GOS.

Case 2. A 70-year-old female suffering with SAH was transferred to our department. HK grade was 3 and Fisher group was 3. Ruptured right internal carotid artery aneurysm was diagnosed by angiography (Fig. 2a), and neck clipping was performed on day 0. Left hemiparesis and consciousness disturbance occurred on day 8, and conservative therapy was not effective for vasospasm (Fig. 2b). We did intra-arterial injection (ia) of papaverine (Fig. 2c), balloon angioplasty (Fig. 2d, e) and ia of fasudil (Fig. 2f). Her symptoms reverted, but finally she achieved a moderately disturbed GOS.

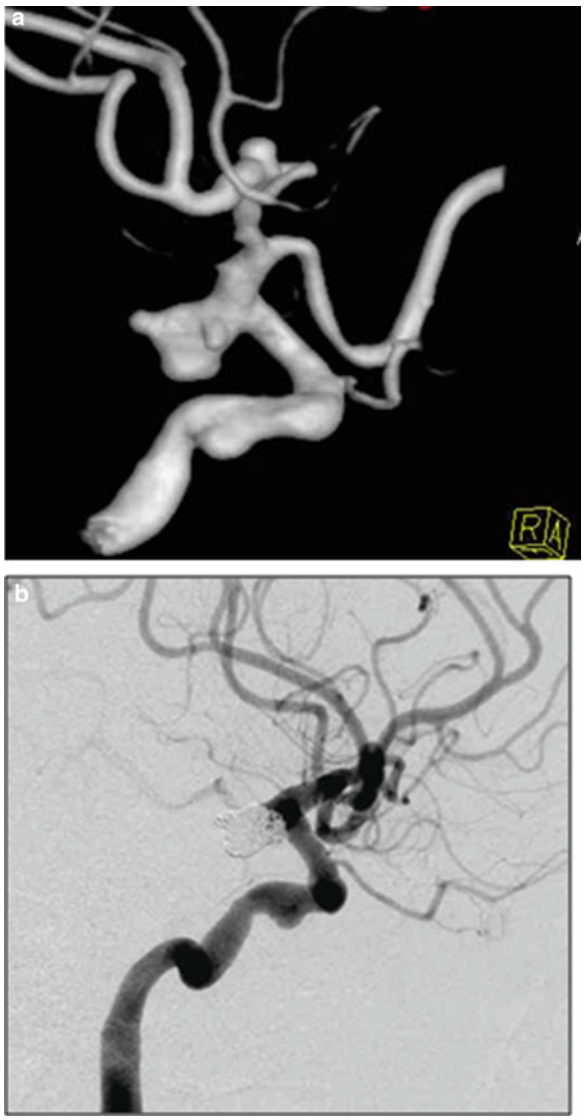

Fig. 1 Seventy nine year old female subarachnoid hemorrhage case combined with thrombocytopenia due to liver cirrhosis. (**a**) Preoperative 3D rotational angiography. Multi blebed large right internal carotid artery aneurysm and un ruptured right middle cerebral artery aneurysm were diagnosed. (**b**) Post operative digital subtraction angiography. Coil embolization of ruptured aneurysm under local anesthesia was done on day 0. She did not suffer vasospasm and she achieved good recovery

Results

The number of patients of advanced age from 2001 to 2005 was 51 and the number from 2006 to 2009 was 72 (Table 1). The proportion of patients of advanced age increased statistically from 28 to 38% during those periods. Mean age of patients increased from 76.2 to 77.7 years old. Locations of aneurysms were similar. HK grade and proportion of radical surgeries performed were similar (Fig. 3a and Table 1). But the proportion of acute stage surgery (within 72 h) and coil embolization increased statistically from 79 to 95% and from 7.7 to 23.7%, respectively. Incidence of symptomatic vasospasm in patients on whom assessment was possible increased from 35% (15/43) to 37% (23/62) (Fig. 3b, c), while asymptomatic vasospasm decreased from 4.7% to 3.2%. The rate of intra-arterial injection of medicine and/or angioplasty increased significantly from 24 to 76%. Proportion of shunt surgeries decreased from 33 (14/43) to 19% (13/63). The favorable outcome (GR and MD) increased from 47 to 51% (Fig. 3d).

Discussion

Recently the debate over clipping versus coiling has acquired less meaning. Both treatments achieve similar outcomes and should complement each other [10, 14, 15].

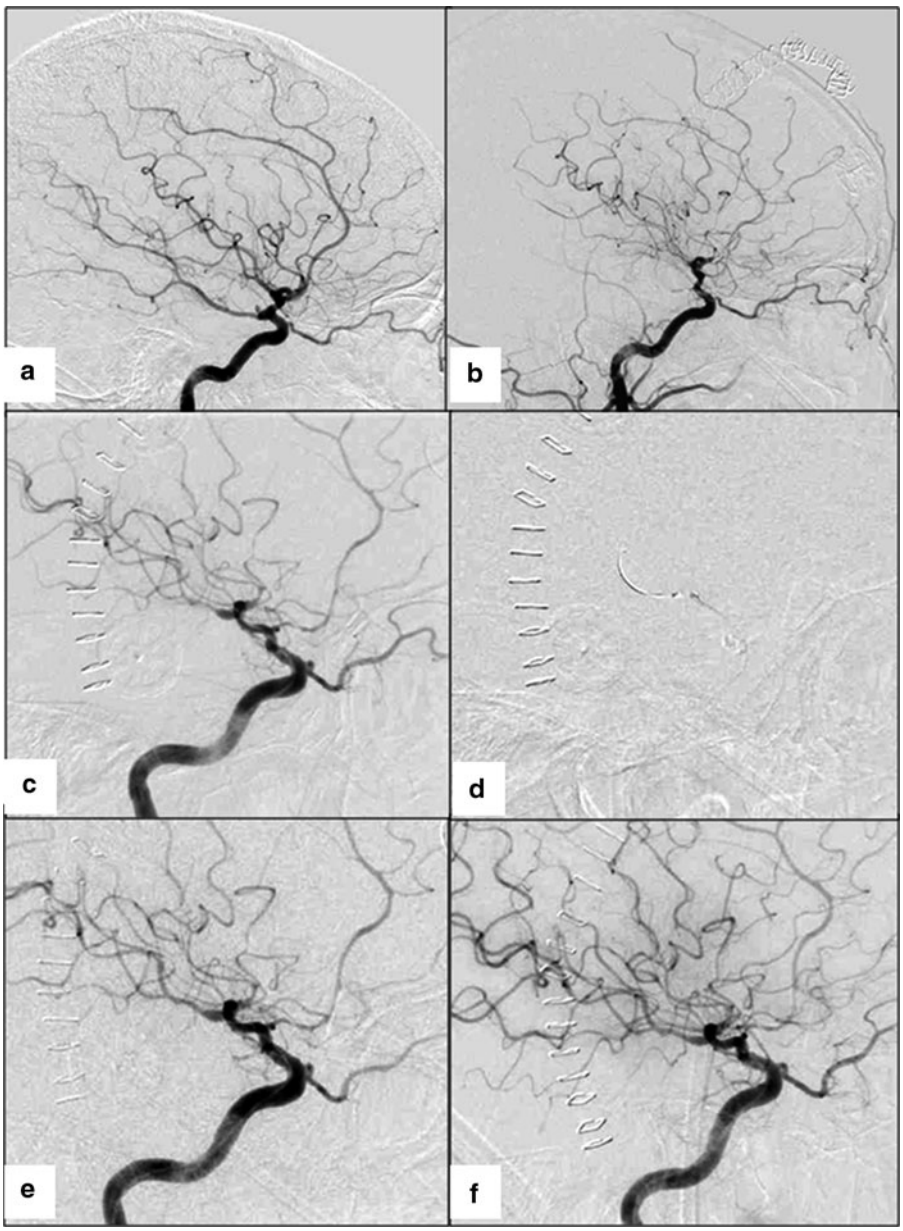

Fig. 2 Seventy year old female subarachnoid hemorrhage case. (**a**) Ruptured right internal carotid artery aneurysm. Neck clipping was done on day 0. (**b**) Vasospasm occurred on day 8. (**c**) After intra arterial injection of papaverine. (**d**) Balloon angioplasty of middle cerebral artery was done. (**e**) Peripheral arteries did not dilate. (**f**) Intra arterial injection of fasudil hydrochloride was done. Diffuse vasodilatation occurred. She achieved moderately disabled

Favorable outcome in elderly SAH patients ranges from 35 to 58% [2, 11, 12, 14].

Effective since 2006, coil embolization of ruptured aneurysms has been primarily selected for patients of advanced age. After the strategy changed, outcomes for elderly SAH patients improved, although the mean age and the proportion of patients of advanced age are increased. And the proportion of ventriculoperitoneal shunt surgeries decreased, because of the conservation of cerebro-spinal fluid circulation. The increase in symptomatic vasospasm after 2006 depended on the high ratio of vasospasm in clipped patients (46.5%, 20/43 pt), in coil-embolized patients the rate of vasospasm was 16.7% (2/12 pt). Nimodipine is not approved in Japan, so we cannot use nimodipine for SAH patients. On the other hand, we can use fasudil hydrochloride for prevention and release of vasospasm [4, 15]. Also, we medicate with edaravone to rescue the ischemic penumbra, and this drug reduces the risk of vasospasm [9]. Coil embolization decreases the incidence of vasospasm [10], and angioplasty rescues cases of symptomatic vasospasm [15].

One serious problem is the indication in elderly SAH patients. The indication of ruptured aneurysm depends on the patient's clinical condition, agreement of the family, religious inclination, and social customs. Many papers from Europe and North American define elder as over 65 years old [2, 3, 5, 6, 14]. And a ratio of elderly SAH patients

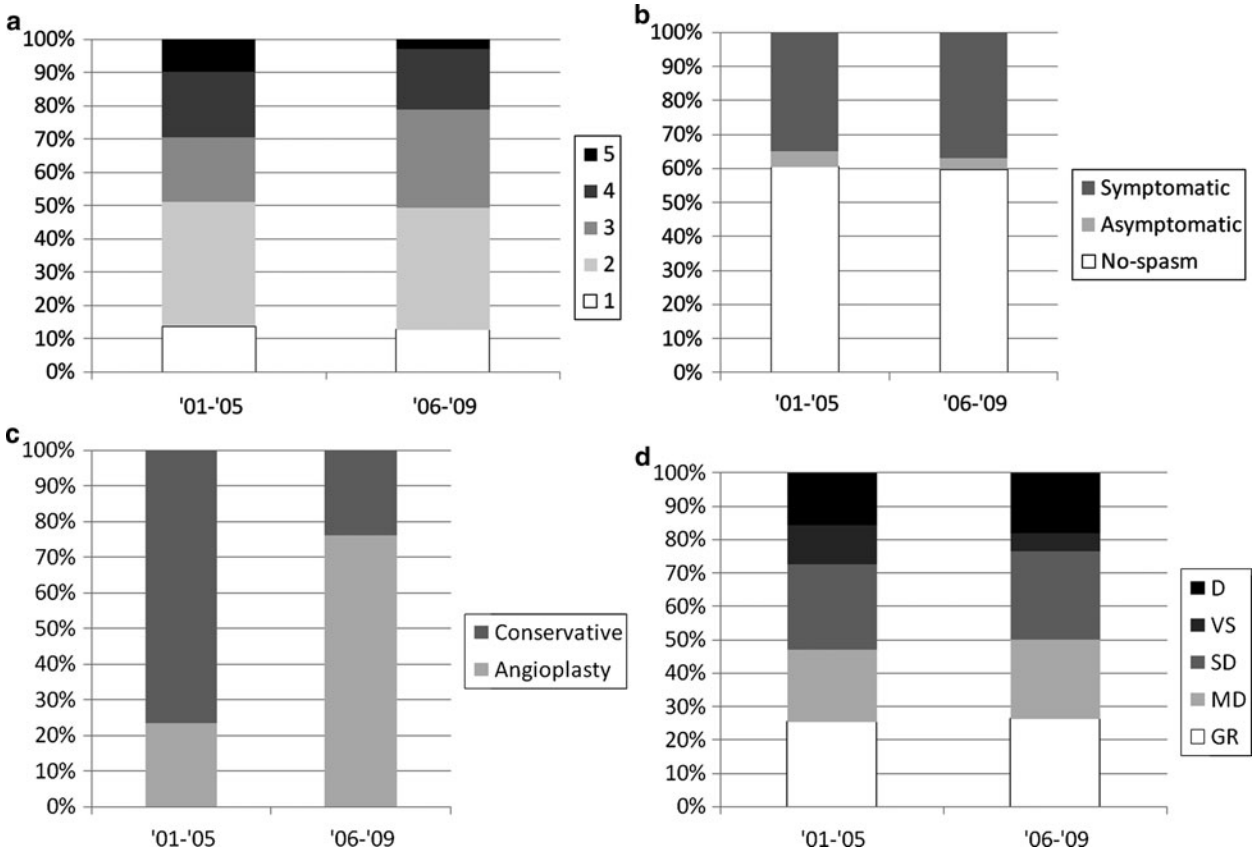

Fig. 3 Distribution of analyzed factors. (**a**) Hunt Kosnik grade. Change of HK grade was not significant. (**b**) Rate of vasospasm. Incidence of symptomatic vasospasm changed from 29 (15/51) to 32% (25/72). And asymptomatic vasospasm changed from 4 to 3%. (**c**): Treatment methods for vasospasm. Rate of angioplasty significantly increased from 22 to 76%. (**d**) Glasgow outcome scale at 30 days after the onset. Rate of favorable outcome (GR and MD) increased from 47 to 51%

Table 1 Characters of patients

Periods	2001 2005	2006 2009
Number of patients* (Total amount of pt. and percent)	51 (183 pt, 27.9%)	72 (189 pt, 38.1%)
Male/Female	7/44	6/66
Mean Age (95% C.I.)	76.2 (74.8 77.7)	77.7 (76.6 78.8)
Rate of radical operation[a]	76.4% (39/51)	81.9% (59/72)
Rate of acute stage operation[b,*]	78.9% (30/39)	94.9% (56/59)
Treatment type* (Clip: Coil: Wrap)	36:3:0	44:14:1

*$p < 0.05$, qui square test
[a]1: radical operation means clipping, coiling or wrapping
[b]2: acute stage define as within 72 h after the ictus

who have undergone surgery have been reported from 15 to 75% [1, 11, 12]. Mean life expectancy for the Japanese is 79 years for males and 86 years for females. And Japanese people especially persevere to save the lives of patients even if the patients will be vegetative state, so radical surgery was performed in over 80% of cases even if the ages of patients were extremely high.

Clinical care of elderly patients is hard due to less stamina, complication, cardiopulmonary dysfunction, and so on. But the number of elderly SAH patients certainly is increasing in advanced aging, while half of those of advanced age can be discharged on foot if treated adequately. All stroke care teams should establish a strategy for care of elderly patients.

Conclusions

Mean age and proportion of patients of advanced age are increasing, but outcomes for such patients have improved. This result is a function of radical surgery for aneurysm (clipping or coiling) and aggressive prevention and treatment of vasospasm. Interventional treatment is necessary to improve the outcome of SAH patients of advanced age.

Conflict of interest statement We declare that we have no conflict of interest.

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A Numerical Approach to Patient-Specific Cerebral Vasospasm Research

Harvey Ho, Changwei Zhang, Xiaodong Xie, and Peter Hunter

Abstract *Background*: Cerebral vasospasm (CVS) is a devastating sequela of subarachnoid hemorrhage (SAH). Among the many factors that are associated with the pathogenesis of CVS, the cerebral blood flow (CBF) and underlying haemodynamics play an important role. In this paper we present an integrated clinical-engineering approach to CVS research.

Method: After an admission CT scan, CT angiography (CTA) and/or Digital Subtraction Angiography (DSA) scans are performed for SAH patients. The anatomy of cerebral vasculature and its geometric parameters are monitored and compared with follow-up CTA and/or DSA scans. The governing equations for blood flow are numerically solved for the arteries and the computational results are analysed.

Findings: In the particular CVS case presented in this paper, the numerical results indicate that blood flow velocity increases in moderate and severely spastic vessels (A1, M1 segment) that perfuse brain tissues. However, decreased vessel diameters in A1 and M1 arteries create larger resistance to CBF and hence lead to reduced flow in the inner carotid artery (ICA).

Conclusions: A numerical approach to patient-specific CVS analysis has been established, and some initial results are achieved via application to an actual spasm case. The undergoing and future work include applying the approach to more CVS cases and incorporating computational models of different scales into the current framework for CVS and SAH research.

Keywords Cerebral vasospasm · Cerebral haemorrhage · Haemodynamics · Blood flow model

H. Ho (✉) and P. Hunter
Bioengineering Institute, University of Auckland, Auckland, New Zealand
e mail: harvey.ho@auckland.ac.nz
C. Zhang and X. Xie
Department of Neurosurgery, West China Hospital, Sichuan University, Chendu, China

Introduction

Cerebral vasospasm (CVS) i.e. overconstriction of cerebral arteries is a serious complication of subarachnoid haemorrhage (SAH), a neurological emergency usually caused by aneurysm rupture. CVS, which occurs about 3 7 days after SAH, can lead to delayed ischaemic neuro-deficit (DIND) and contribute to the mortality and morbidity rate of SAH patients [1 3]. A tremendous amount of effort has been made over the last several decades to tackle this severe complication and some effective methods such as clazosentan in medication and triple-H therapy (hypertensive/hypervolumic/hemodilution) in clinical practice have been established to prevent or reverse CVS [1, 2]. However, the improvement in CVS has not been translated into clinical outcome: there was only a small reduction in delayed neurological deterioration and no effect on clinical outcome at 3 months after SAH [1]. Hence, some new pharmaceutical therapies and theoretical hypotheses are proposed in literature. For example, Pluta suggested that nitric oxide (NO) plays a central role in CVS development and proposed treatment modalities at different phases of CVS [2]. Humphrey et al. hypothesized that the onset and resolving of CVS is due to a series of biochemomechanic events that surround the blood clot [3].

Whichever the case, the cerebral blood flow (CBF) and its variation before and after SAH, and during the full time course of CVS must be carefully examined. This includes CBF's relationship with other vaso-regulation factors such as NO, endothelin and cerebralspinal fluid (CSF) pressure. Therefore, a reliable way of evaluating cerebral haemodynamics is important because it not only provides insights into the overall cerebral response to SAH, but also acts as a basis for testing and validating various hypotheses.

The current in vivo modalities for measuring cerebral blood flow include transcranial Doppler (TCD), phase contrast MR angiography (PC MRA), etc. In addition, CT perfusion imaging (CTP) measures cerebral perfusion at tissue level and is therefore useful for detecting cerebral

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infarction [4]. Each of these methods has its application domain and has greatly aided clinical diagnosis and theoretical analysis. However, none of these modalities are able to directly measure wall shear stress (WSS), which stimulates endothelial nitric oxide synthase (eNOS) and is therefore important for interpreting the mechanisms of CVS and DIND [2].

In this work we introduce a numerical and patient-specific approach to haemodynamics study. This approach is able to simulate blood flow from a vasculature level (e.g., the flow in an arterial tree) to a local structure level (e.g., to capture the flow patterns such as WSS in aneurysms, spastic vessels, etc.). We apply these numerical methods to the actual angiographic vasospasm cases which are detected by CT angiography (CTA) or digital subtraction angiogram (DSA), in the hopes that some novel prognostic mechanisms can be revealed.

Methods

Our approach involves both clinical examination of CVS and biomechanic analysis. The former is performed at the Endovascular Intervention Unit of the West China Hospital (WCH), the latter i.e. the work of vascular model reconstruction, flow simulation is performed at the Auckland Bioengineering Institute (ABI). The analysis procedures are described below:

1. Medical images (CTA, DSA) are acquired for SAH patients upon admission
2. Vasospasm is monitored, recorded and measured in follow up scans (CTA or DSA)
3. Patient-specific geometries are either digitized from medical images directly, or mapped to a generic cerebral vasculature model
4. The governing equations for blood flow are numerically solved
5. The quantitative data are analysed, postprocessed.

Steps (1) and (2) are performed in WCH, where the research protocol is approved by the ethics committee of WCH, and informed consent are obtained from patients. The scanning protocols for SAH patients are well established in WCH and are effectively applied as routine clinical practices (CTA scanner: PHILIPS Brilliance 64, DSA suite: PHILIPS FD20). Steps (3) and (4) are implemented in ABI where the blood flow is solved and validated by in vivo data. In particular, Step (4) is implemented similar to that of Smith et al. [5] and has been employed in a series of cerebral haemodynamics studies such as in Ho et al. [6]. In Step (5) the CVS is analysed by neurosurgeons, interventional radiologists, and bioengineers at both institutions.

Results

As a preliminary work, a single CVS case was analysed according to the procedures described above: a 52-years-old female patient who suffered from SAH received admission CT scanning on the first day. The follow-up DSA scanning on the same day revealed multiple (5) aneurysms in her brain (a ruptured MCA aneurysm is indicated by the triangle in Fig. 1a). Endovascular coiling treatment was performed on the fourth day and another DSA scanning was performed (Fig. 1b). It is obvious that vasospasm occurred at the M1, A1 cerebral arteries and at the distal end of the internal carotid artery (ICA) (indicated by arrows 1, 2 and 3 in Fig. 1b, respectively). These spastic vessel diameters are mapped to the generic cerebral vasculature (Fig. 1c) and the modified vasculature is shown in Fig. 1d.

For the classification of vasospasm, we follow the rules of Dankbaar et al. [4] i.e.: no spasm (0 25% decrease in vessel diameter), moderate spasm (25 50% decrease), and severe spasm ($>$ 50% decrease). Hence, moderate to severe spasm occurred at the M1 and A1 segment (marked 1 and 2), and minor (no) to moderate spasm occurred at the ICA.

The computer simulation for blood flow in the generic arterial tree took 6 min to calculate on a desktop computer (Intel Pentium Dual-Core 2 GHz). The results show that the flow velocity increases in M1 (by approximately 20%, from 41 cm/s to 49 cm/s) and A1 (by approximately 70%, from 33 cm/s to 56 cm/s). This is understandable because the cardiovascular system attempts to maintain the perfusion rate through the spastic arteries by increasing the flow velocity. The flow velocity in the ICA, on the other hand, decreased by about 30% (from 56 cm/s to 40 cm/s). This may be interpreted as increased resistance from A1 and M1 segments due to decreased diameter.

The computational results in the arterial tree without spasm (Fig. 1c) were validated using Doppler ultrasonic measurements in our previous studies [6]. Since no TCD measurements were taken for this patient, the computational results for spastic arteries remain to be validated in future work.

Discussion

Although the exact mechanism for CVS and its correlation with DIND are still not fully understood, it has been suggested that many synergistic and competitive processes contribute to its developing and dissolving [1 3]. One of the central players in these processes is the haemodynamics factor (which regulates CBF). In this pilot study, we introduced a numerical approach to evaluate blood flow in a

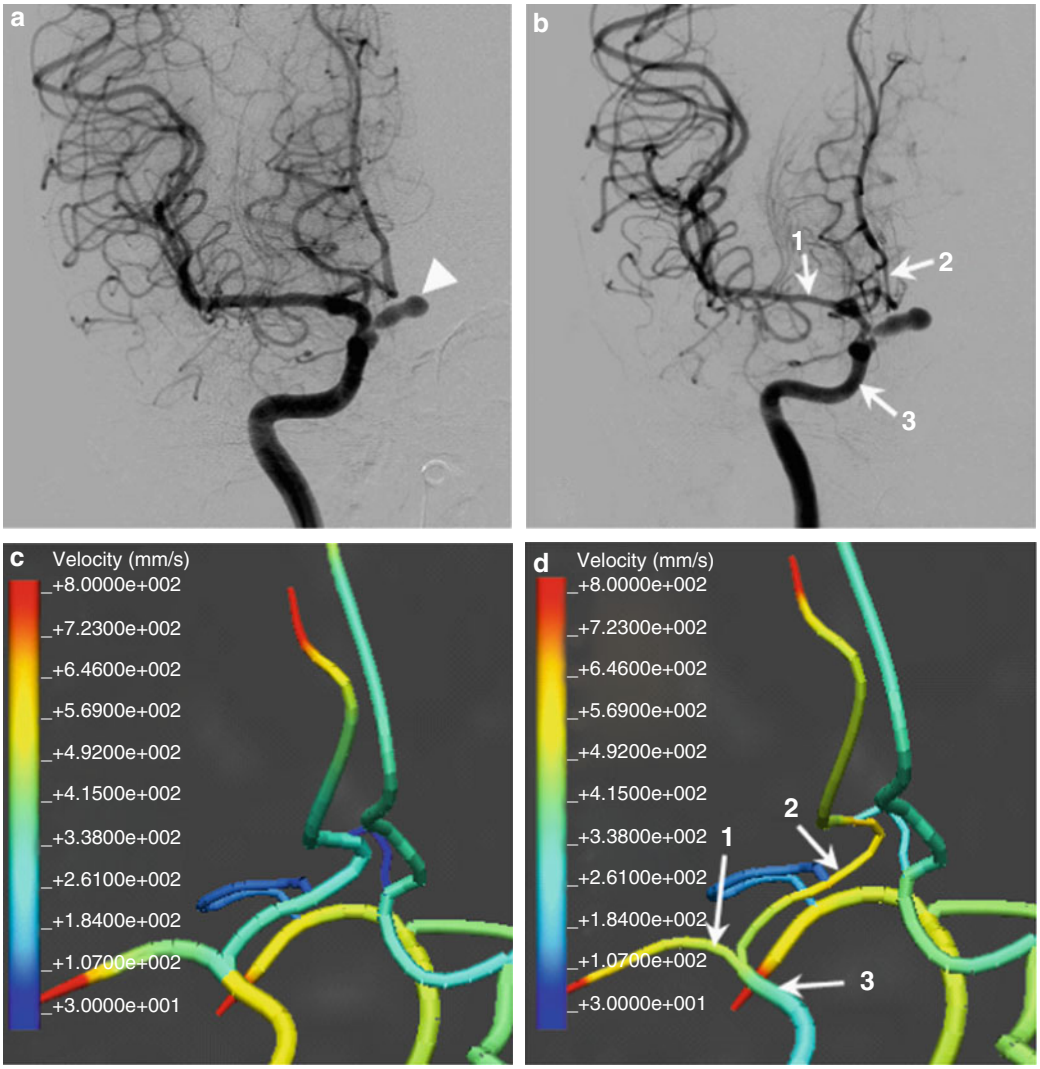

Fig. 1 Numerical analysis of blood flow in spastic vessels: (**a**) DSA scanning on admission day; (**b**) another DSA scanning on the 4th day after admission: vasospasm was evident at arterial segments indicated by *arrows* 1, 2, and 3; (**c**) numerical simulation of the blood flow in Circle of Willis without CVS; (**d**) numerical simulation of blood flow in spastic arteries: the *arrows* 1, 2, and 3 are equivalent to that of **b**. The velocity data are taken at 0.5 s (one cardiac cycle ≈ 1 s). Note: the high flow velocities shown at arterial terminals are numerical out boundary treatment

patient-specific spastic vascular network. We showed the preliminary results by comparing the flow velocity in a spasm case with a normal case. Undergoing work include improving the current generic model by adding more efferent arteries to the tree, and collecting and analyzing more CVS patient cases.

It should be emphasized that although the haemodynamic model alone may give explanations to some spastic phenomena, it does not provide a comprehensive framework for decoding the intricate processes involved in CVS and DIND after SAH. To work towards this direction, many other mechanisms, such as vessel wall constituent turnover, microcirculation dysfunction, blood-brain barrier breakdown, and NO regulation induced by WSS, need to be taken into account [1 3].

Conclusion

The current work established an initial platform, to which the new insights and knowledge gained from our clinical observations, laboratory experiments, and computational models can be added. The ultimate objective is to find novel mechanisms that can improve the clinical outcome of subarachnoid haemorrhage.

Conflict of interest statement We declare that we have no conflict of interest.

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Evidenced Based Guidelines for the Management of Good Grade Subarachnoid Haemorrhage Patients in Leeds, UK

A.C. Quinn, G. Hall, S. Marsh, M. Clark, and S. Ross

Abstract Aneurysmal Subarachnoid Haemorrhage (SAH) is a common neurosurgical condition with high morbidity and mortality, with our trust treating over 120 patients annually. Although there are recommendations for the management of some aspects of subarachnoid haemorrhage, a comprehensive guideline document has not been produced. Our guidelines seek to address all aspects of acute patient care in our neurosurgical unit, using evidence based medicine with a multi-disciplinary team to produce care pathways establishing a standard of care for our patients.

Keywords Aneurysm · Subarachnoid haemorrhage · Guidelines · Evidence based · Vasospasm

Introduction

The following paper describes multidisciplinary guidelines that have been formulated for the management of subarachnoid haemorrhage (SAH) patients for use within our neurosurgical region.

Although there are recommendations for the management of some aspects of SAH a comprehensive set of guidelines for the total care of such patients has not been produced. The American Heart Association Guidelines published in 2009 [1] made recommendations for the medical management of subarachnoid haemorrhage and its complications, but did not address many other more practical aspects of patient care. Furthermore, few prospective randomised controlled trials have been performed in patients with subarachnoid haemorrhage.

We have sought to produce a comprehensive guideline document that seeks to address all aspects of acute patient care from the time of presentation within the peripheral hospitals to definitive aneurysmal management and post-intervention care within our tertiary centre and transfer back to the base hospital.

The guidelines will be on our hospital intranet Health sciences Care Pathway, available to all staff involved.

Methods

In order to produce a comprehensive document in 2004 we set up a multidisciplinary team with regular team meetings to produce care pathways. Our unit treats approximately 120 aneurysmal SAH per year and the following specialties are involved: neurosurgeons, neuroanaesthetists, intensivists, neuroradiologists staff nurses team leaders and matrons. We also involved our pharmacy and physiotherapy departments. In the latter stages clinical management were invited to participate not only to see the problems that we face day to day but also as a catalyst to ensure that the guidelines moved forward appropriately. Our first goal was to agree on the topics, then delegation and responsibilities to different staff within the specialty. We attempted to grade our recommendations according to the Oxford system of classification [2].

Results

The full set of guidelines are available from audrey.quinn@leedsth.nhs.uk. Figure 1 summarises the contents of the document.

The following four paragraphs give a summary and examples of the major clinical areas.

Nursing and Physiotherapy

We have included the following: position, environment, symptom control, nutrition, bowel care and psychological

A.C. Quinn (✉), G. Hall, S. Marsh, M. Clark, and S. Ross
Leeds General Infirmary, Leeds, UK
e mail: Audrey.Quinn@leedsth.nhs.uk

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Fig. 1 Contents of guidelines document

support. Interestingly, as part of the process during our multi-disciplinary group meetings we were able to modify our daily care routines e.g. patient position where we changed our firm policy of nursing all SAH patients in the supine position to a more relaxed system that accommodated patient request.

For this particular section the guidelines were difficult to evidence base, so a consensus was reached at group level for best practice. This allows care to be rationalised and unified from the nursing point of view.

Surgery

We felt that it was important to be prescriptive in terms of the way in which referrals were made, investigated, resuscitated transferred and managed. There are sections on early referral and advice, investigation of SAH, optimisation and transfer to our centre and definitive aneurysm treatment clipping vs. coiling as well as postoperative care. For neurosurgical considerations, our guidelines have a sufficient evidence base to confidently recommend our current practice with regard to for example the choice and timing of treatment discussed at a daily (multi-disciplinary group) MDT.

Critical Care

The following areas are discussed in detail as we felt that the following medical conditions were those in which deficiencies in care commonly arose: BP (blood pressure) control, fluid balance and electrolytes, cerebral salt wasting vs. SIADH (syndrome of inappropriate ADH secretion), vasospasm/DIND (delayed ischaemic neurological deficit), analgesia, thrombo-embolism prophylaxis, diabetic control.

We attempted to be as prescriptive as possible particularly for our junior members of staff who are confronted with these issues on the front line usually out-of-hours. Figure 2 is an example of one of the care pathways we have produced. This chart represents our first line management for DIND once the diagnosis has been made, the flow chart should be consulted and the recommendations instituted where appropriate. There are evidenced based recommendations for many of the points included in the flow chart for example nimodipine use, sodium homeostasis and avoidance of hypovolaemia. We recognize that there is no level one evidence for triple H therapy, but in line with common clinical practice, we have adopted this technique as a first line step in reversing a clinical deficit. There are sections leading on from this detailing hypertension and sodium therapy.

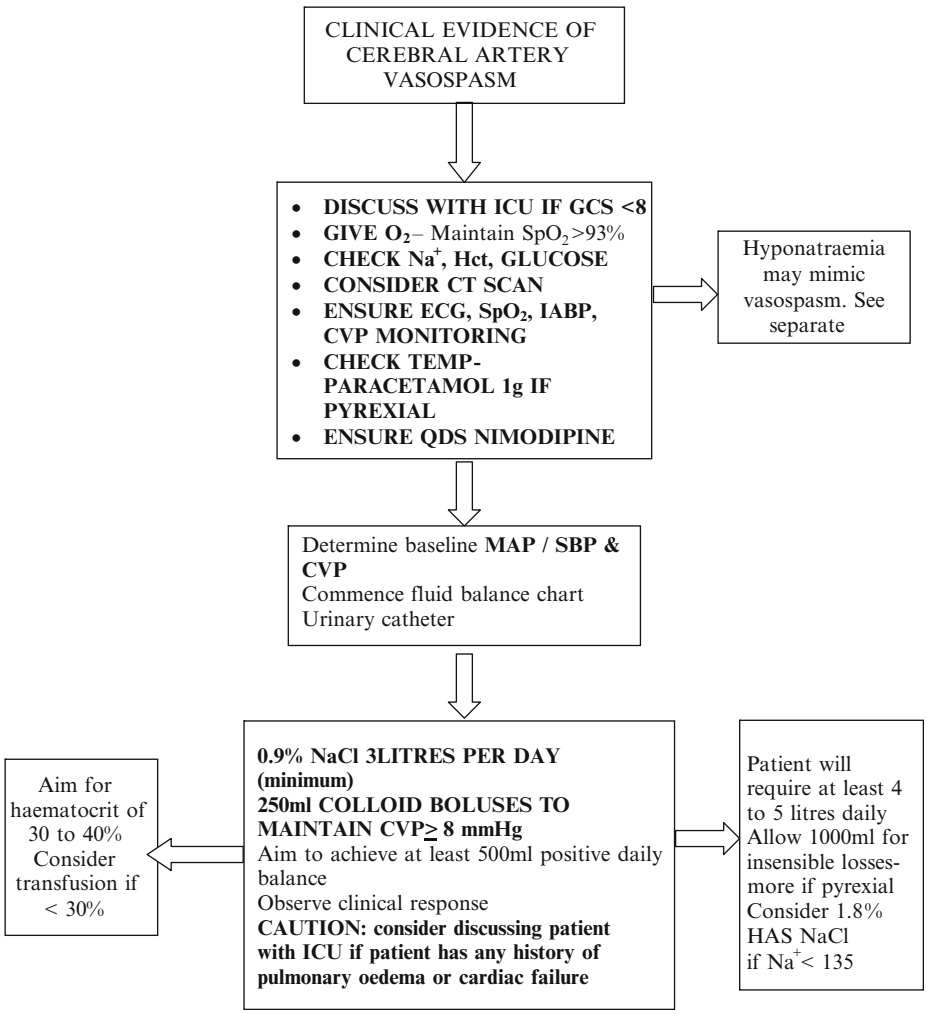

Fig. 2 First line management of vasospasm (DND) hypervolaemia and haemodilution

Interventional Neuro-radiology

Following the ISAT trial [3], our management of good grade, anterior circulation aneurysms is usually by endovascular coiling.

We have included information on ward care, relevant nursing issues with regard to haemostasis and position, information for patients, anticoagulation issues and how the patients should be followed up.

For the dissemination of the guidelines, it is important that they are available in a variety of accessible formats. For this reason as well as hard copies being available in relevant areas, the guidelines will go onto our trust database to allow instant 24 h access for front line staff. To ensure updated information, each section has a nominated lead responsible for the contents and regular review.

As a tertiary referral centre we recognised the need to involve the peripheral hospitals to ensure high levels of care, and that it is our responsibility to ensure this happens. Following the publication of these guidelines, there will be dissemination of the information to the peripheral units to ensure consistency of care irrespective of geographical situation. We recognise that his will require proper organisation and follow-through. We are also in the process of developing an outreach programme whereby we link directly with nominated staff in peripheral hospitals to provide information and support for SAH patents.

Discussion

We initiated our guidelines group in 2004 following concerns that our patient population were not receiving appropriate levels of care. At that time we referred to the literature: pre-nimodipine work (Awad) [4] reported an incidence rate of vasospasm of 42% with a less than 7%

incidence of mortality from vasospasm. Studies in the 1990s post nimodipine from (Charpentier and Qureshi) [5, 6] reported a reduction in this incidence to around 20% with a reduction in mortality also. Our own figures in 2003 showed not only a higher incidence rate 27.4% but also a higher mortality rate 7.9% from vasospasm. However, our overall mortality was comparable and we did question whether we could be using a more inclusive definition of vasospasm. Of concern to us was that our management of SAH could be deficient in some areas e.g. delay in diagnosing vasospasm on the ward or initiating HHH therapy.

Another aim was to provide a consistent level of care to facilitate comparative research studies within the unit in the future. For example we have a number of current research interests ongoing at the moment with relation to vasospasm including the impact of lumbar drains and stellate ganglion blockade. The results of these interventions could be easily affected by simple deficiencies in clinical care e.g. attention to sodium or poor fluid replacement.

In producing the guidelines it has not only generated conversation between specialties with regard to patient care and management, but stimulated discussion regarding shaping the service for the future. At a time of financial constraints this process has been slow and at times frustrating. However we hope this will lead to organization and development of local and regional teaching days and as well as highlighting training issues for junior staff such as running formal theatre lists where trainee surgical staff can learn central venous access techniques.

After the guidelines are established we hope to improve our risk management profile by charting reviewing and acting on deficiencies that are highlighted by these guidelines. It also allows the development of a framework for departmental audit, to continue to improve care and enable ongoing data collection. This first draft will require to undergo reassessment and updating. Our hope is that following management intervention we will be able to secure funding for a dedicated SAH nurse.

It is important to remember that these are guidelines and not protocols, for patients with good grade SAH care. Care for our poorer grade patients in ICU will still be individualised.

Finally, we are acutely aware of the financial constraints within our health care system in particular the shortage of basic resources such as high dependency beds and trained staff. Having established a comprehensive group to manage and produce these guidelines should assist us in highlighting these deficiencies with the hope of directing resources appropriately.

We hope that this document will allow rationalisation of care within our region, lead to educational course and facilitate further training of staff within our region. We should gain a solid base of standardised care for our patients we can build on in the future with ongoing audit and research opportunities.

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Conflict of interest statement We declare that we have no conflict of interest.

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Clinical Trial of Nicardipine Prolonged-Release Implants for Preventing Cerebral Vasospasm: Multicenter Cooperative Study in Tokyo

H. Kasuya

Abstract *Background*: Since October 1999, nicardipine pellets (NP) have been used to prevent vasospasm in patients with subarachnoid hemorrhage (SAH). We started a multicenter cooperative study on Jan 1, 2007, and 136 patients in six hospitals were enrolled to this trial in 2 years. The incidence of cerebral vasospasm and outcome were examined in these patients.

Methods: The patients with SAH were treated with NP during surgery after clipping of their aneurysms.

Findings: The study included 87 female patients, 38 over 70 years old, 34 in grades 4 and 5, and 46 of Fisher group 2 or 4. Aneurysms were located on anterior circulation in 133, posterior in 3. All patients were treated with Fasudil hydrochloride except for 3. Two to twelve pellets were implanted in the cistern where thick clots existed and vasospasm was highly likely. Delayed ischemic neurological deficits (DIND), angiographical vasospasm and cerebral infarctions were seen in 11 of 134 (8.2%), 32 of 130 patients (24.6%), and 16 of 129 (12.4%), respectively. No complications were experienced. Independent rate at 3 months was 78%.

Conclusions: The incidence of cerebral vasospasm in this multicenter trial is similar to that of our first trial performed in a single center.

Keywords Drug delivery system · Nicardipine · Subarachnoid hemorrhage · Cerebral vasospasm

Introduction

Delayed ischemic neurological deficits (DIND) resulting from the development of cerebral vasospasm is an important cause of disastrous complications following aneurysmal subarachnoid hemorrhage (SAH). Although there have been numerous reports describing the prevention of DIND such as intrathecal administration of urokinase, cisternal irrigation with drainage, endovascular treatment, head shaking, arterial injection of vasodilatory drug [8], most of them are complicated even if they are effective.

We have developed a drug-delivery system using a vasodilating drug that can be implanted intracranially at the time of surgery for aneurysm clipping and have been using the drug to SAH patients since October 1999. We previously published a report on the efficacy and safety of nicardipine prolonged-release implants [nicardipine pellets (NP)] to prevent vasospasm in SAH patients [5, 6]. Vasospasm was completely prevented in the arteries in cisterns with thick clots, where vasospasm was highly expected, by placing NP adjacent to the arteries during surgery. When we experienced 100 consecutive SAH patients treated with NP, we reported that the incidence of DIND, severe angiographical vasospasm, and cerebral infarctions due to vasospasm were, 7, 11, and 5%, respectively [9].

The current report presents the results of this drug delivery system of multicenter cooperative study held in six hospitals in Tokyo between 2007 and 2008.

Methods and Materials

Development of NP

A rod-shaped pellet (2 mm in diameter, 10 mm in length, containing 4 mg of nicardipine) was prepared by heat compression. Copoly (lactic/glycolic acid) (PLGA) (PLG1600ML; molecular weight 4,000, lactic acid ratio 0.5) was purchased from Taki Co. (Kakogawa, Kobe, Japan). A mixture of PLGA (900 mg) and nicardipine free base (100 mg) was dissolved in dichloromethane (10 ml). The dichloromethane was evaporated with a rotary evaporator, and the resultant mass was dried further under vacuum.

H. Kasuya
Department of Neurosurgery, Medical Center East, Tokyo Women's Medical University, 2 1 10 Nishiogu, Arakawa ku, Tokyo 116 8567, Japan
e mail: kasuyane@dnh.twmu.ac.jp

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The dried powder (40 mg) was charged into a Teflon tube (2-mm inner diameter). The tube was set in a stainless steel cylinder kept at 35 40°C. A pressure of 100 kg/cm^2 was applied between the upper and lower stainless steel dies. The compressed pellet was sterilized by γ-ray (Nippon Shosha Service, Tokai, Ibaraki, Japan). Nicardipine free base was prepared as follows: Nicardipine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in water. NaOH (5N) was added to the solution to shift the pH above 10. The nicardipine free base was extracted with dichloromethane [5 7].

Patient Population and Management

One hundred thirty-six patients were investigated in neurosurgery department in six hospitals (Tokyo Women's Medical University Hospital, Medical Center East, Yachiyo Medical Center, Kofu Neurosurgery Hospital, Asakadai Central Hospital and Itabashi Central Hospital). The study was approved by Ethical Committee of the University and each hospital, and informed consent was obtained. Table 1 lists the clinical aspects of the patients treated. The status of patient on admission was assessed by World Federation Neurological Surgeons (WFNS) grading system [2] and CT scan was by Fisher grouping system [3]. The eligibility criteria for this study were SAH patients treated through craniotomy. NP was applied principally to patients with CT radiographic SAH group of 3 (thick clot). Only that part of the blood clot necessary for exposure and clipping of the aneurysm was removed surgically. We started our protocol on Jan 1, 2007 and 136 patients were enrolled in this study in 2 years. The number of pellets and the location of the placement depended on the amount and site of the subarachnoid clot in the preoperative CT or from the operative field, and in the craniotomy. Cerebral vasospasm was assessed by DIND and angiography on days 7 12 performed in all patients. Deterioration in the level of consciousness, the appearance of motor weakness, sensory deficit, or aphasia was recorded as DIND if there was no other explanation in the postoperative period. All patients were evaluated every 3 h for signs of neurological deterioration up to at least 14 days after SAH. Angiographically demonstrated vasospasm of the anterior circulation artery was classified into four grades: none; mild (minimal or mild change in vessel lumen); moderate (between mild and severe); and severe (threadlike and diffuse narrowing of vessels). Basic management of postoperative patients depended on a policy of each hospital. Clinical outcome at 3 months was measured with the Glasgow Outcome Scale (GOS) [4].

Table 1 Characteristics of 136 patients treated with nicardipine pellets in six hospitals

Characteristics	Number of patients
Sex	
Female	87
Male	49
Age	
< 59	55
< 69	43
≥ 70	38
WFNS garde	
1	42
2, 3	60
4, 5	34
CT on admission (Fisher)	
Group 2, 4	46
Group 3	90
Ruptured aneurysm	
Anterior	133
Posterior	3
Day of surgery	
0	62
1	53
2	6
3	4
4	11
Current smoker[a]	49
Number of pellets used	
2 5	81
6 9	49
10 12	6

[a]Current smoker: smoking every day before SAH

Results

Table 1 shows the characteristics of 136 patients treated with NP between Jan 1, 2007 and Dec 31, 2008. They include 87 female patients, 38 over 70 years old, 34 in grades 4 and 5, and 46 of Fisher group 2 or 4. Location of aneurysms was 50 in anterior cerebral artery (ACA), 46 in middle cerebral artery (MCA), 37 in internal carotid artery (ICA), and 3 in others. Fifteen patients were treated after 3 days of onset. All patients were treated with Fasudil hydrochloride except for three. Two to twelve pellets were implanted in the cistern of the ICA, MCA and/or ACA, where thick clots existed and vasospasm related to DIND was highly likely. Eleven of 134 patients treated with NP developed DIND (8.2%). Of these patients, clinical deterioration with infarction occurred in seven patients (5.2%). Moderate to severe angiographical vasospasm was seen in 32 of 130 patients (24.6%). Cerebral infarctions on CT scan were reported in 16 of 129 (12.4%) (Table 2). No complications were experienced. Independent rate at 3 months was 78% (106/136).

Table 2 Cerebral vasospasm and outcome in 136 patients treated with nicardipine pellets

	Number of patients
DIND[a]	11/134
Angiographical vasospasm (moderate, severe)	32/130
Low density area on CT	16/129
Hydrocephalus[a]	43
Outcome (Glasgow) at 3 months	
Good	85
Moderate disability	21
Severely disability	15
Persistent vegetative	6
Dead	9
Total	136

[a]DIND: delayed ischemic neurological deficits, Hydrocephalus: ventricular dilatation with symptoms requiring shunt surgery

Discussion

This is a summary of 136 patient files of SAH patients surgically treated with NP submitted to the corresponding author from cooperating hospitals. Delayed ischemic neurological deficits occurred in only 8.2%. Of these patients, clinical deterioration with infarction occurred in seven patients (5.2%). The status of 106 patients was independent at 3 months (78%) after treatment. The incidence of cerebral vasospasm in this multicenter trial is similar to that of our first trial performed in a single center. The difference between both trials is the incidence of cerebral infarctions seen on CT scan, which is 12.4% in this trial, a figure that is much higher than that of the past study and higher than that of detected DIND. These results may be possible due to the fact, that it is sometimes difficult to detect DIND in patients who suffered an initial high grade SAH. And on CT scans it can be difficult to distinguish infarctions which are due to vasospasm from other low density areas caused by primary brain damage or surgery. The basic management of postoperative procedures in this series may vary because of the difference in treatment policy of each hospital. This drug delivery system can be used with any other treatment without risking additional complications. It is noted that all patients were treated with Fasudil hydrochloride except for three. Even though they are at low risk of developing DIND, many of the patients treated in this study had SAH graded Fisher 2 and 4, due to the easily applicable NP treatment.

Since the literature describes DIND to be much more common as well as the associated poor outcome, our results suggest that the application of NP to SAH patients may prevent vasospasm related cerebral infarctions and therefore avoid an unfavorable outcome. The efficacy was proven by a randomized double-blinded controlled trial performed in Germany [1]. This drug-delivery system offers a promising approach for preventing vasospasm when a craniotomy is performed as part of the aneurysm treatment. However, the application of NP has its limitations: such as for the arteries on the contralateral side of the craniotomy, or the more distal arteries. We were not able to use these pellets for patients that were treated by coiling. This problem may be solved by developing a new drug delivery system which allows the maintenance of an appropriate concentration of nicardipine in the target artery, since local application of nicardipine is able to completely prevent vasospasm [7].

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Conflict of interest statement We declare that we have no conflict of interest.

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Intravenous Magnesium Sulfate After Aneurysmal Subarachnoid Hemorrhage: Current Status

George Kwok Chu Wong, Matthew Tai Vai Chan, Tony Gin, and Wai Sang Poon

Abstract Delayed ischemic neurological deficit or clinical vasospasm remained a major cause for delayed neurological morbidity and mortality for patients with aneurysmal subarachnoid hemorrhage (SAH). Magnesium is a cerebral vasodilator. In experimental model of drug or SAH-induced vasospasm, magnesium blocks voltage-dependent calcium channels and reverses cerebral vasoconstriction. Furthermore, its antagonistic action on N-methyl-D-aspartate receptor in the brain prevents glutamate stimulation and decreases calcium influx during ischemic injury. Clinically, the protective effect of magnesium has also been found useful in women with preeclampsia, a condition thought to be due to cerebral vasospasm. Initial experimental result in human was found to safe and effective as compared to historical data. In our pilot study, 60 patients were randomly allocated to receive either magnesium sulfate infusion 80 mmol/day or saline infusion for 14 days. The incidence of symptomatic vasospasm decreased from 13/30(43%) in the saline group to 7/30(23%) in the patients receiving magnesium sulfate infusion, p = 0.10, odds ratio 0.398, 95% CI 0.131 1.211. Favorable outcome (Good recovery and moderate disability, as defined by Glasgow Outcome Scale) was achieved in 20 of 30 (67%) patients receiving magnesium sulfate infusion and 16 of 30 (53%) patients receiving placebo treatment, p = 0.292, odds ratio 1.750, 95% CI 0.616 4.974.

From literature review, a total of 441 patients from four studies (including ours) were grouped for analysis. Using random effects model (Mantel-Haenszel, Robins-Breslow-Greenland), the pooled odds ratio for symptomatic vasospasm or delayed cerebral ischemia is, 0.620, 95% CI 0.389 0.987, statistically significant. Similarly, the pooled odds ratio for favorable outcome is 1.598, 95% CI 1.074 2.377, statistically significant. There are two multi-center phase III studies (IMASH and MASH2) being carried out to assess the clinical effects, in which IMASH has finished data collection on 30th June 2009.

Keywords Magnesium sulfate · Vasospasm · Clinical studies

Introduction

Delayed ischemic neurological deficit or clinical vasospasm remained a major cause for delayed neurological morbidity and mortality for patients with aneurysmal subarachnoid hemorrhage (SAH). Magnesium is a cerebral vasodilator. In experimental model of drug or SAH-induced vasospasm, magnesium blocks voltage-dependent calcium channels and reverses cerebral vasoconstriction. Furthermore, its antagonistic action on N-methyl-D-aspartate receptor in the brain prevents glutamate stimulation and decreases calcium influx during ischemic injury [13]. Clinically, the protective effect of magnesium has also been found useful in women with preeclampsia, a condition thought to be due to cerebral vasospasm. Initial experimental result in human was found to safe and effective as compared to historical data [1]. We aimed to investigate the current status of the use of magnesium sulfate after aneurysmal subarachnoid hemorrhage.

Materials and Methods

Literature search for randomized controlled clinical trials on magnesium sulfate after aneurysmal subarachnoid hemorrhage revealed six key publications [5, 6, 9 12]. None of these studies has adequate power to detect a statistically significant improvement in outcome measures. Pilot results

G.K.C. Wong and W.S. Poon (✉)
Division of Neurosurgery, Department of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China
e mail: wpoon@surgery.cuhk.edu.hk
M.T.V. Chan and T. Gin
Department of Anaesthesia and Intensive Care, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

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of iMASH (Intravenous Magnesium sulfate in Aneurysmal Subarachnoid Hemorrhage) trial [12], magnesium component of MASH (Magnesium and Acetylsalicylic acid in Subarachnoid Hemorrhage) study [10], the study published by Veyna et al. [11] and the study published by Muroi et al. [5] were analyzed using standard outcome measures: (1) clinical vasospasm or symptomatic vasospasm or delayed cerebral ischemia; (2) Clinical outcome at 3 6 months, (favorable outcome was Glasgow Outcome Score 4 5 versus unfavorable outcome with Glasgow Outcome Score 1 3). The study by [6] was not included in the analysis because none of the outcome measures (clinical vasospasm and clinical outcome) was reported in the manuscript. The study by [9] was also not included in the analysis due to the unconventional omission of nimodipine in the magnesium group.

Odds ratios and 95% CI of favorable outcome and clinical vasospasm/delayed cerebral ischemia were calculated for specific studies and then pooled by the Mantel-Haenszel method as described below.

Case control studies of dichotomous outcomes were represented by arranging the observed counts into fourfold (2 by 2) tables. The Mantel-Haenszel method provided a pooled odds ratio across the strata of fourfold table. Meta-analysis was used to investigate the combination or interaction of a group of independent studies.

For a single stratum odds ratio was estimated as follows:

		Exposed	Non exposed
Outcome	Cases	a	b
	Non cases	c	d

Sample estimate of the odds ratio = (ad)/(bc)

The Mantel-Haenszel method was used to estimate the pooled odds ratio for all strata, assuming a fixed effects model:

$$\hat{O}R_{MH} = \frac{\sum_{i\ 1}^{k}\left(\frac{a_i d_i}{n_i}\right)}{\sum_{i\ 1}^{k}\left(\frac{b_i c_i}{n_i}\right)}$$

A confidence interval for the Mantel-Haenszel odds ratio was calculated using the Robins, Breslow and Greenland variance formula [8].

The GraphPad Prism 5 and MedCalc software were used for data analysis. Outcome data were given with percentage and odds ratios are displayed with 95% confidence intervals.

Results

Our pilot result was described in previous chapter and published [12]. Sixty patients were randomly allocated to receive either magnesium sulfate infusion 80 mmol/day or saline infusion for 14 days. The incidence of symptomatic vasospasm decreased from 13/30 (43%) in the saline group to 7/30 (23%) in the patients receiving magnesium sulfate infusion, p = 0.10, odds ratio 0.398, 95% CI 0.131 1.211. Favorable outcome (Good recovery and moderate disability, as defined by Glasgow Outcome Scale) was achieved in 20/30 (67%) patients receiving magnesium sulfate infusion and 16/30 (53%) patients receiving placebo treatment, p = 0.292, odds ratio 1.750, 95% CI 0.616 4.974.

Veyna et al. [11] reported a 40-patient prospective single-blinded clinical trial of high dose magnesium sulfate infusion therapy (A bolus of 6 g followed by 2 g/h intravenous infusion, with an aimed magnesium level of 4 5.5 mg/dl) following spontaneous subarachnoid hemorrhage. They enrolled patients with Hunt and Hess Grades II IV and

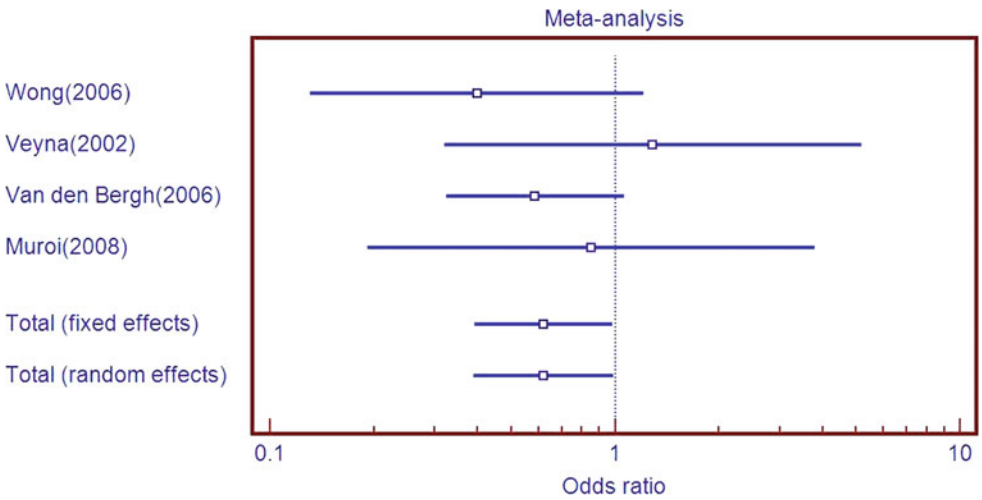

Fig. 1 Pooled odds ratio for clinical vasospasm/delayed cerebral ischemia

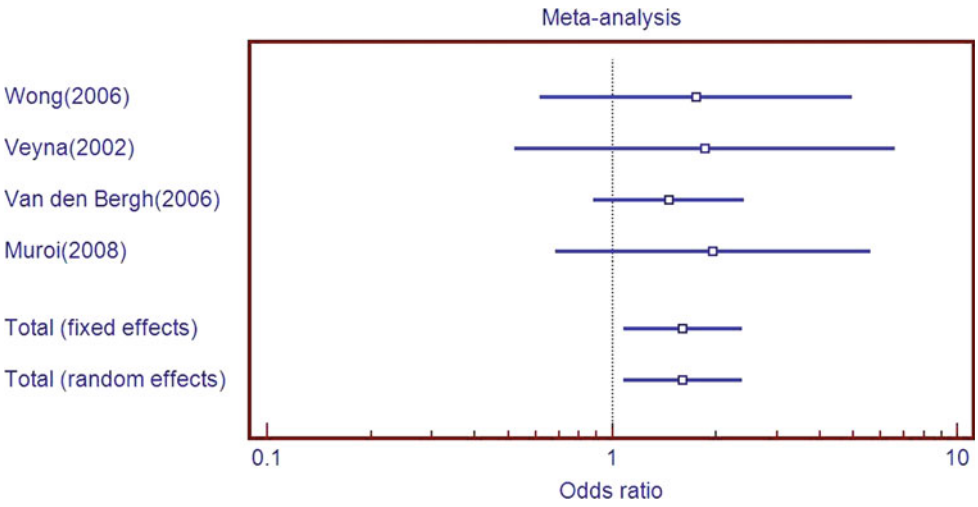

Fig. 2 Pooled odds ratio for favorable outcome

presented within 72 h after spontaneous subarachnoid hemorrhage. In the magnesium treatment arm, they maintained the magnesium sulfate infusion for 10 days. Symptomatic vasospasm, confirmed by angiography, occurred in 6 of 20 patients receiving magnesium sulfate infusion and 5 of 20 patients receiving placebo, p = 0.723, odds ratio 1.286, 95% CI 0.319 5.177. Mean Glasgow Outcome Scale were 3.8 ± 1.6 and 3.6 ± 1.5 (mean ± SD, p = 0.74) in the magnesium and sulfate infusion group and placebo group respectively. Favorable outcome (Good recovery and moderate disability, as defined by Glasgow Outcome Scale) was achieved in 13 of 20 (65%) patients receiving magnesium sulfate infusion and 10 of 20 (50%) patients receiving placebo treatment, p = 0.337, odds ratio 1.857, 95% CI 0.521 6.614.

Van den Bergh et al. [10] reported the magnesium component result of the magnesium and acetylsalicyclic acid in subarachnoid hemorrhage (MASH) trial. MASH trial was a randomized, double-blinded, placebo-controlled multicentre trial with a factorial design. The acetylsalicyclic acid related data were not complete at the time of analysis. A total of 283 patients were randomized within 4 days after aneurysmal subarachnoid hemorrhage. Magnesium treatment consisted of a continuous intravenous dose of 64 mmol/day, to be started within 4 days after subarachnoid hemorrhage and continued until 14 days after occlusion of the aneurysm. Delayed cerebral ischemia happened in 22/139(16%) of magnesium-treated patients and 35/144(24%) of placebo-treated patients, p = 0.075, odds ratio 1.286, 95% CI 0.323 1.060. Poor outcome as defined by modified Rankin Score >3 at 3 months occurred in 38/139 (27%) of magnesium-treated patients and 51/144 (35%) of placebo-treated patient, p = 0.143, odds ratio 1.458, 95% CI 0.879 2.417. 18/139 (13%) patients in the magnesium treatment group and 6/144 (4%) patients in the placebo group had an excellent outcome.

$$n = \frac{\left[Z_\alpha \sqrt{(1+1/m)\,\bar{p}\,(1-\bar{p})} + Z_\beta \sqrt{p_0(1-p_0)/m + p_1(1-p_1)}\right]^2}{(p_0 - p_1)^2}$$

$$\bar{p} = \frac{p_1 + m p_0}{m+1} \qquad n_c = \frac{n}{4}\left(1 + \sqrt{1 + \frac{2(m+1)}{nm|p_0 - p_1|}}\right)^2$$

p0 = Probability of event in Control Group
p1 = Probability of event in Experimental Group
m = Ratio of controls to experiment subjects
nc = Continuity correction factor

Fig. 3 Formulae on which the estimated sample size is calculated

Muroi et al. [5] reported a 58 patient study with aneurysmal subarachnoid hemorrhage predominantly treated by microsurgical clipping (97%). This was a prospective, randomized, patient-blinded, and placebo-controlled pilot study. Patient allocated to the treatment group would receive a bolus of 16 mmol MgSO4 administered over 15 min, followed by a continuous intravenous infusion of 64 mmol/day. To maintain the serum magnesium level at twice the baseline, with a maximum of 2 mmol/L until day 12 after subarachnoid hemorrhage, subsequent dosage adjustments were made every 12 h. Indications to end magnesium treatment included Bradycardia (pulse rate less than 45/min), hypotension (systolic blood pressure less than 110 mmHg, atrioventricular conduction disturbances, asystole (> 2 s), respiratory failure, oliguria, and severe electrolyte disturbance. Delayed ischemic neurological deficit happened in 4/31 (13%) of the treatment group and 4/27 (15%) of the placebo, p = 0.833, odds ratio 0.852, 95% CI 0.191 3.794. There was a trend towards favorable neurological outcome (as defined by independency in activity of daily living in the treatment group (20/31, 64%) as compared to placebo group

(13/27, 48%), p = 0.209, odds ratio 1.958, 95% CI 0.682 5.620 at 3 months.

Prevedello et al. [6] reported a 72 patient study with aneurysmal subarachnoid hemorrhage and microsurgical clipping of aneurysm performed. Patients were placed in two groups in alternate order with a ratio of 2 to 1. Group 1 received prophylactic hypervolemic and hemodilution therapy in addition to nimodipine. Group 2 received the same treatment with the addition of magnesium sulfate in continuous infusion, keeping serum magnesium levels close to double normal values. No statistical difference in vasospasm incidence was found between the two groups. However, no 3 or 6-month clinical outcome data were collected. This study was excluded from subsequent metaanalysis.

Schmid-Elsaesser et al. [9] reported another 130 patient randomized clinical trial comparing intravenous magnesium sulfate infusion to nimodipine infusion. All patients in the magnesium sulfate infusion group did not receive oral or intravenous nimodipine. The design did not take into account that nimodipine was the evidence-based standard treatment for patients after aneurysmal subarachnoid hemorrhage [7]. The trial management was thus dissimilar to the contemporary clinical setting and was excluded from the subsequent metaanalysis.

A total of 441 patients from four studies were grouped for analysis [5, 10 12]. The target of the magnesium arm of the four studies was to produce a similar degree of hypermagnesemia such as doubling the baseline value. All four studies recruited patients with SAH during the acute phase, within 48 96 h after aneurysmal SAH. The magnesium infusion was maintained for 10 14 days. Neurological outcome were measured in Glasgow Outcome Score in 3 months [5, 10, 11] and 6 months [12].

Using fixed effects model [3, 8], the pooled odds ratio for symptomatic vasospasm or delayed cerebral ischemia is, 0.620, 95% CI 0.389 0.987, statistically significant (Fig. 1). Similarly, the pooled odds ratio for favorable outcome is 1.598, 95% CI 1.074 2.377, statistically significant (Fig. 2).

Discussion

From this meta-analysis, magnesium sulfate infusion was safe and effective for neuroprotection in patients with SAH. The result suggested that multi-center trials as IMASH and MASH2 might be able to provide evidence and indication of magnesium sulfate infusion for patients with aneurysmal SAH. For sample size estimation (Fig. 3) [2], we used neurological outcome as primary endpoint and calculated from the pooled data. Allowing type I error of 5% and achieving a power of 80%, to show Mg group has a more favourable over the placebo group, at least 168 patients per group would be required. The targetted sample size would be 353, allowing a 5% loss to follow up. IMASH has finished data collection on 30th June 2009.

Magnesium sulfate infusion was safe and inexpensive as a potential drug for neuroprotection in patients with aneurysmal SAH. The drawback was that it requires an intravenous assess for a long period of time as 10 14 days. This may cause inconvenience in good grade patients. The option of oral magnesium supplement had been tried out [9] but the unknown bioavailability and predictable diarrhea remain as the biggest concerns. One may argue that if one aims to reduce the damage from cerebral vasospasm, one may start magnesium infusion when clinical vasospasm occurs, instead of earlier on. The problem of timing can be reflected from the negative result of the IMAGES (Intravenous MAGnesium Efficacy in Stroke trial) in which magnesium was given within 12 h after ischemic stroke [4]. Given the unpredictable timing and delayed clinical recognition, prophylactic administration as in all these trials should be the way forward. Also, prophylactic administration of magnesium may also help to reduce brain injury arising from etiologies other than cerebral vasospasm.

Conflict of interest statement We declare that we have no conflict of interest.

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Predictors Analysis of Symptomatic Cerebral Vasospasm After Subarachnoid Hemorrhage

L. Yin, C.Y. Ma, Z.K. Li, D.D. Wang, and C.M. Bai

Abstract *Purpose*: Symptomatic cerebral vasospasm (SCVS) is still lacking in reliable early warning methods and often diagnosed after clinical deterioration of neurological function, making prevention and treatment extremely passive. This study investigates the risk factors relevant to SCVS after subarachnoid hemorrhage (SAH) in order to provide useful information for clinical work.

Materials and Methods: Clinical data of 211 patients with SAH was reviewed in this study. 16 risk factors relevant to SCVS were retrospectively analyzed, including univariate analysis and logistic analysis.

Results: Of 211 patients, 81 were found to have SCVS (38.4%). Univariate analysis showed that age, history of hypertension, Hunt-Hess grade at admission, modified Fisher grade, aneurysm location, fever and intraventricular blood clot reached statistical significance ($P < 0.05$), suggesting that they might be risk factors to SCVS. However, logistic analysis showed that only age (OR=1.027, 95% CI=1.002 1.053, $P < 0.05$) and modified Fisher grade (OR=2.985, 95% CI=2.048 4.352, $P < 0.05$) entered the regression equation, suggesting both were independent risk factors.

Conclusions: SCVS is the final result of multiple factors acting together. Age and modified Fisher grades are independent risk factors to SCVS.

Keywords Risk factor · Subarachnoid hemorrhage · Symptomatic cerebral vasospasm

L. Yin (✉), C.Y. Ma, Z.K. Li, and D.D. Wang
Department of Neurology, The Second Affiliated Hospital of Dalian Medical University, 116027 Dalian, China
e mail: andreas2005@vip.sina.com
C.M. Bai
Department of Neurosurgery, The Second Affiliated Hospital of Dalian Medical University, 116027 Dalian, China

Introduction

Symptomatic cerebral vasospasm (SCVS), also known as delayed ischemic neurological deficit (NIND), refers to cerebral vasospasm with clinical symptoms of cerebral ischemia. This is the most frequent complication of subarachnoid hemorrhage (SAH) with high morbidity and high mortality. It is also one of the most important risk factors affecting the SAH prognosis [1]. At present, SCVS is still lacking in reliable early warning methods and is often diagnosed after clinical deterioration of neurological function, making prevention and treatment extremely passive. Therefore it is of great clinical significance to find the risk factors to SCVS. Unfortunately, consistent conclusions could not be found in the literature; sometimes they were even completely contradictory [2 5].

In this study, the complete clinical data of 211 patients with SAH was collected and all risk factors related to SCVS were retrospectively analyzed in order to find out useful predictors for clinical work.

Materials and Methods

Two hundred and eighteen spontaneous SAH patients, admitted to the Second Affiliated Hospital of Dalian Medical University from June 2001 to May 2009, were collected in this study. The diagnosis of SAH was confirmed by cranial CT/MR, or occasionally by lumbar puncture. Seven patients were excluded due to lack of incomplete clinical data or short hospitalization time ($\leq$ 72 h). Two hundred and eleven patients were finally included in the study, including 98 males and 113 females with a mean age of 56.02 $\pm$ 14.27.

All reported risk factors relevant to SCVS were observed in each patient included in the study. The risk factors were age, gender, history of hypertension, diabetes, smoking, drinking, Hunt-Hess grade at admission, modified Fisher grade on CT [6], location of aneurysm, times of SAH,

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Table 1 Observation indexes and their values

Variables	Observation indexes	Values
X1	Gender	Female 0, male 1
X2	Age	Numerical variable
X3	Operation	No 0, within 3 days 1, 4 days 2 weeks 2, in 2 weeks 3
X4	Hypertension	No 0, yes 1
X5	Diabetes	No 0, yes 1
X6	Smoking	No 0, yes 1
X7	Drinking	No 0, yes 1
X8	Times of SAH	First time 0, second time 1, more than 3 times 2
X9	Hunt Hess grade at admission	Grade I 0, grade II 1, grade III 2, grade IV 3, grade V 4
X10	Location of aneurysm	No aneurysm 0, anterior circulation 1, posterior circulation 2, unclear 3
X11	Intracranial hematoma	No 0, yes 1
X12	Intraventricular blood	No 0, yes 1
X13	WBC count	Numerical variable
X14	Platelet count	Numerical variable
X15	Fever	Normal 0, 37 38.5°C 1, over 38.5°C 2
X16	Modified Fisher grade	Grade 0 I 0, grade II 1, grade III 2, grade V 3
Y	SCVS	No 0, yes 1

fever, intracranial hematoma, intraventricular blood clot, operation (surgical or interventional), WBC count, and platelet count.

A patient was defined to have SCVS when he/she presented worsened clinical symptoms within 3 weeks of onset, and these symptoms could not be explained by other reasons such as rebleeding, intracranial hematoma, hydrocephalus, fever, electrolyte disease, etc. [6, 7]. Transcranial Doppler ultrasound showed that the velocity of middle cerebral artery was $\geq$120 cm/s.

Patients were then divided into two groups, i.e., patients with SCVS and patients without SCVS. Each observation index (risk factor) was evaluated and a certain value was given (0 4) (Table 1). SPSS 11.5 was used to compare and analyze each observation index between two groups, including univariate analysis and multivariate analysis. Chi-square test was used for classification variables and t-test was used for numerical variables. $P < 0.05$ was statistically regarded significant.

Results

Of 211 patients with SAH, 81 patients (38.4%) were found to have SCVS, including 32 males and 49 females with an average age of 60.07 ± 13.23, while 130 patients (61.6%) were not found to have SCVS, including 66 males and 64 females with an average age of 53.50 ± 14.36.

Univariate analysis of classification variables showed that there was no significant difference between the two groups on gender, operation, diabetes, smoking, drinking, times of SAH and intracranial hematoma ($P > 0.05$). However, there were significant differences on hypertension, Hunt-Hess grade, modified Fisher grade, location of the aneurysm, fever, and intraventricular blood clot, suggesting that these six factors might be risk factors to SCVS ($P < 0.05$) (Table 2).

Univariate analysis of numerical variables showed that there were no significant differences in WBC and platelet counts between two groups. However, there was significant difference on age between two groups, indicating that age might be a risk factor to SCVS (Table 3).

Multivariate logistic regression analysis was done for all seven variables mentioned above that reached statistical significance, using the method of Backward: LR. At last only age (OR = 1.027, 95% CI = 1.002 1.053, P = 0.037) and modified Fisher grade (OR = 2.985, 95% CI = 2.048 4.352, P = 0.000) entered the regression equation, suggesting that both are independent risk factors to SCVS.

Discussion

Ecker and Riemenschneider first described cerebral vasospasm (CVS) according to their 26-day continuous radiographic observation in patients after aneurysm rupture 50 years ago [8], and it had obtained wide acceptance. After that, people found that CVS had the characteristics of high morbidity and high disability rate, and was one of the important factors affecting SAH prognosis [1].

CVS usually occurs within 4 16 days after SAH, especially within 7 10 days, and may last for more than 2 weeks. Clinically, CVS may be divided into two kinds. One is asymptomatic CVS, which has no clinical symptoms and signs but only abnormal accessory examinations, such as vascular stenosis by angiography, high blood flow speed by Doppler ultrasound, etc. This kind of patient usually recovers well. Another is SCVS, which has not only abnormal accessory examinations, but also deteriorative clinical manifestations, such as conscious disturbance, hemiplegia, hemidysesthesia, etc. SCVS is often difficult to reverse with bad prognosis, and is the main cause of disability and death in patients with SAH. In this study, 81 out of 211 patients (38.4%) had SCVS, strongly suggesting its high incidence.

Reported studies showed that many factors were related to SCVS (see Table 1), but independent risk factors have been controversial. For this reason, we retrospectively analyzed the risk factors in our own 211 SAH patients with

Table 2 Univariate analysis of classification variables for SCVS (chi square test)

Observation index	Non SCVS group	SCVS group	RR	X^2	P
Gender					
Male	66	32	0.633	2.545	0.111
Female	64	49			
Operation					
No	82	53		1.024	0.795
Within 3 days	9	3			
3 days 2 weeks	31	20			
In 2 weeks	8	5			
Hypertension					
No	83	37	2.052	6.261	0.012*
Yes	47	43			
Diabetes					
No	121	74	1.272	0.210	0.646
Yes	9	7			
Smoking					
No	115	65	1.887	2.687	0.101
Yes	15	16			
Drinking					
No	113	70	1.045	0.011	0.917
Yes	17	11			
Times of SAH					
1	125	75		2.242	0.311**
2	5	5			
3	0	1			
Hunt Hess grade					
I	16	4		33.634	0.000*
II	88	30			
III	13	18			
IV	6	17			
V	7	12			
Location of aneurysm					
0	30	4		14.708	0.005*
1	58	40			
2	5	5			
3	37	32			
Intracranial hematoma					
No	112	67	1.300	0.458	0.498
Yes	18	14			
Ventricular blood					
No	116	57	3.489	12.022	0.001*
Yes	14	24			
Modified Fisher grade					
I	36	7		57.296	0.000*
II	58	10			
III	26	32			
IV	10	32			
Fever					
0	48	17		11.032	0.004*
1	71	46			
2	11	18			

*P < 0.05, **Monte Carlo precise probability

complete clinical data in this study. The univariate analysis showed that there were statistically significant differences between SCVS group and non-SCVS group on hypertension,

Table 3 Univariate analysis of numerical variables for SCVS (t test)

Observation index	Non SCVS group	SCVS group	T	P
Age	53.5 ± 14.36	60.07 ± 13.23	3.332	0.001*
WBC count	13.91 ± 34.46	12.11 ± 4.36	0.463	0.644
Platelet count	206.44 ± 63.90	207.06 ± 58.14	0.071	0.944

*P < 0.05

Hunt-Hess grade, modified Fisher grade, location of aneurysm, fever, intraventricular blood clot and age, indicating that these seven factors are risk factors to SCVS. However, the logistic regression analysis showed that only growing age and modified Fisher grade entered the regression equation, suggesting both are the independent risk factors to SCVS.

The relationship between age and SCVS is contradictory in the literature. Charpentier et al. [9] reported that an age of less than 50 years old was the risk factor to SCVS. Rabb et al. [10] found that patients were likely to have SCVS if they were younger than 50 years old. Torbey found that patients older than 68 years had a lower incidence of SCVS [2]. But there were opposite opinions. Through retrospective analysis in their 106 SAH patients, Ferch et al. [3] found that growing age and Hunt-Hess grade were independent risk factors to SCVS. Our study also showed that growing age was an independent risk factor to SCVS (OR = 1.027, P = 0.037), which was quite similar to Ferch's study. The reason why growing age is relevant to SCVS is not clear. It is estimated that following the age increase, the auto-regulation ability of the cerebral arteries is damaged, the angiosclerosis becomes more severe, and the compensatory effect of collateral circulation and tolerance to cerebral ischemia are also decreased. Therefore, we should pay more attention to the elderly patients in order to avoid SCVS despite the fact that age itself is not controllable.

Fisher grade is an index to reflect the relationship between subarachnoid blood volume and CVS. This grading system was first reported by Fisher in 1980 [11]. To overcome pitfalls of the Fisher grade, Claassen revisited the original Fisher grade in 2001 [6]. The modified Fisher grade could predict CVS better and is getting more and more acceptance. However, the relationship between modified Fisher grade and CVS is also controversial. Many researchers found that Fisher grade was a good predictor of CVS; the higher the grade, the higher the incidence of CVS [4, 12]. However, Smith found that there was no definite correlation of Fisher grade to CVS in 134 patients, except to intraventricular blood clot, therefore suggesting the necessity to revisit the original Fisher grade [5]. This study showed that the modified Fisher grade was also an independent risk factor to SCVS (OR = 2.985, P = 0.000). This means that the higher grade of modified Fisher grade, the more tendency to have SCVS.

Hunter-Hess grade is used to reflect the severity of SAH patients. Hirashima found that Hunt-Hess grade III IV is an independent risk factor to SCVS [13]. Ferch reported that Hunt-Hess grade IV V and growing age were independent risk factors of symptomatic stroke [3]. But in this study the Hunter-Hess grade did not entered the logistic model, though univariate analysis showed that there was a statistically significant difference between patients with and without SCVS. The possible explanation is that many factors may raise Hunter-Hess grade, such as rebleeding, acute hydrocephalus, intracranial hypertension, fever, disorder of electrolyte, etc. Therefore, Hunter-Hess grade is not a reliable predictor of SCVS.

In addition, hypertension, location of aneurysm, fever, and intraventricular blood clot could not enter the regression model either in this study, despite significant differences between two groups at univariate analysis. Therefore, they are not as sensitive as modified Fisher grade and age and could not be used to predict SCVS independently. Of course, these factors might be relative to age and modified Fisher grade. The advantage of using logistic analysis is that age and modified Fisher grade might, at some degree, include some information of the other five factors.

Conclusion

In conclusion, SCVS is the final result of multiple factors acting together. Some probable risk factors could not be included because of sampling error, limited sample size, or other reasons. It is necessary to increase sample size and undergo prospective clinical observation in order to establish a precise prediction model. Clinically, doctors should consider the bad prognosis and take active measures to avoid or reduce SCVS when the patient has risk factors mentioned above.

Conflict of interest statement We declared that we have no conflict of interest.

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Intra-arterial Administration of Fasudil Hydrochloride for Vasospasm Following Subarachnoid Haemorrhage: Experience of 90 Cases

S. Iwabuchi, T. Yokouchi, M. Hayashi, K. Sato, N. Saito, Y. Hirata, J. Harashina, H. Nakayama, M. Akahata, K. Ito, H. Kimura, and K. Aoki

Abstract *Background*: We investigated the clinical efficacy of intra-arterial administration of fasudil hydrochloride for cerebral vasospasm.

Method: We reviewed 90 cases treated with intra-arterial administration of fasudil hydrochloride between August 1998 and April 2009 and investigated the clinical efficacy for cerebral vasospasm.

Findings: Angiographic improvement of vasospasm was noted in all procedures. Eight had ischemic lesion on CT at discharge in Group A, which included 39 patients who presented angiographic and symptomatic vasospasm. However, 4 (50%) of these eight were recovered with a condition of GR. No patients showed ischemic lesion on CT in Group B, which included 51 patients who presented angiographic vasospasm without symptoms. Two (3.3%) of 59 patients who presented angiographic vasospasm without symptoms at the initial follow-up angiography had ischemic lesion on CT at discharge. The 1-year follow-up showed 78.9% of GR. No patient showed any adverse effects resulting from intra-arterial administration of fasudil hydrochloride.

Conclusion: Intra-arterial administration of fasudil hydrochloride was an effective and safe management technique for vasospasm.

Keywords Vasospasm · Fasudil hydrochloride · Intra-arterial administration

Introduction

Fasudil hydrochloride is an inhibitor of myosin light chain kinase [1], which is essential in smooth muscle contraction. Fasudil hydrochloride also inhibits Rho-associated protein kinase, which affects vascular smooth muscle contraction by inactivating myosin light chain phosphatase [4, 10, 12]. Fasudil hydrochloride is effective in preventing vasospasm after subarachnoid haemorrhage [7, 9, 11] and is widely administered intravenously in Japan. In the present study, we investigated the clinical efficacy of intra-arterial administration of fasudil hydrochloride for cerebral vasospasm.

Materials and Methods

We reviewed 90 patients treated with intra-arterial administration of fasudil hydrochloride for cerebral vasospasm following subarachnoid haemorrhage between August 1998 and April 2009 and investigated the clinical efficacy for cerebral vasospasm. A total of 90 patients were then divided into two groups; Group A included 39 patients who presented angiographic and symptomatic vasospasm, and Group B included 51 patients who presented angiographic vasospasm without symptoms. A 4- or 5-French catheter was inserted via the femoral artery into the cervical portion of the internal carotid artery. Intra-arterial administration of fasudil hydrochloride entailed injection into the patient of 15 mg of fasudil hydrochloride dissolved in 20 ml of physiological saline through the catheter over a period of approximately 15 min after diagnostic angiography. The procedure was performed one to two times. Overall outcome was assessed at 1 year using the Glasgow Outcome Scale that comprised five levels [6]: good recovery (GR), moderate disability (MD), severe disability (SD), vegetative state (VS), and death (D).

Results

Fifty-six patients underwent surgical clipping, and 34 patients underwent endovascular coiling. Summary of prognosis by the groups are shown in Table 1. In Group A, 26 of

S. Iwabuchi (✉), T. Yokouchi, M. Hayashi, K. Sato, N. Saito, Y. Hirata, J. Harashina, H. Nakayama, M. Akahata, K. Ito, H. Kimura, and K. Aoki
Department of Neurosurgery, Toho University Ohashi Medical Center, 2 17 6 Ohashi, Meguro ku, Tokyo 153 8515, Japan
e mail: iwabuchi@med.toho u.ac.jp

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39 patients had repeated intra-arterial administration, ranging from 2 to 5 times. In Group B, all patients were administered single sessions. Angiographic improvement of vasospasm was noted in all 75 procedures in Group A. Immediate clinical improvement was noted in 26 (67%) of the 39 patients. Seven of these 26 patients had ischemic lesion on CT at discharge. Among the remaining 13 patients who did not demonstrate immediate clinical improvement after intra-arterial administration of fasudil hydrochloride, 8 had ischemic lesion on CT at discharge. However, 4 (50%) of these 8 recovered with a condition of GR. Twenty-six of 39 patients underwent multiple intra-arterial administration of fasudil hydrochloride treatment, and 9 (35%) of these 26 had ischemic lesion on CT. However, 17 (65%) of these 26 showed GR at 1-year follow-up. The shortest angiographic recurrence of vasospasm was 8 h. Despite intra-arterial administration of fasudil hydrochloride performed at follow up DSA for angiographic vasospasm without symptoms, eight patients developed symptomatic vasospasm within a few days after intra-arterial administration of fasudil hydrochloride. They underwent multiple intra-arterial administration of fasudil hydrochloride treatment, and two patients had ischemic lesion on CT at discharge. Angiographic improvement of vasospasm was noted in all 51 procedures in Group B. No patients showed ischemic lesion on CT. Two (3.3%) of 59 patients who presented angiographic vasospasm without symptoms at the initial follow up angiography had ischemic lesion on CT at discharge. Although we encountered 15 patients who developed cerebral infarction due to vasospasm in present series, GR on GOS was seen in 7 of these 15 patients. CT scans of seven GR patients revealed ischemic change of the ACA territory, or small lesion in right MCA territory. Six patients of MD showed ischemic lesion in

Table 1 Summary of results by the groups

Group A; 39 patients presenting angiographic vasospasm with symptom	
Angiographic improvement	75/75 procedures
Immediate clinical improvement (Yes)	26/39
Ischemic change on CT	7/26
GR	3/7
MD	3/7
SD	1/7
Immediate clinical improvement (No)	13/39
Ischemic change on CT	8/13
GR	4/8
MD	3/8
VS	1/8
Group B; 51 patients presenting angiographic vasospasm without symptom	
Angiographic improvement	51/51procedures
Ischemic change on CT	0/51

GR good recovery, *MD* moderate disability, *SD* severe disability, *VS* vegetative state

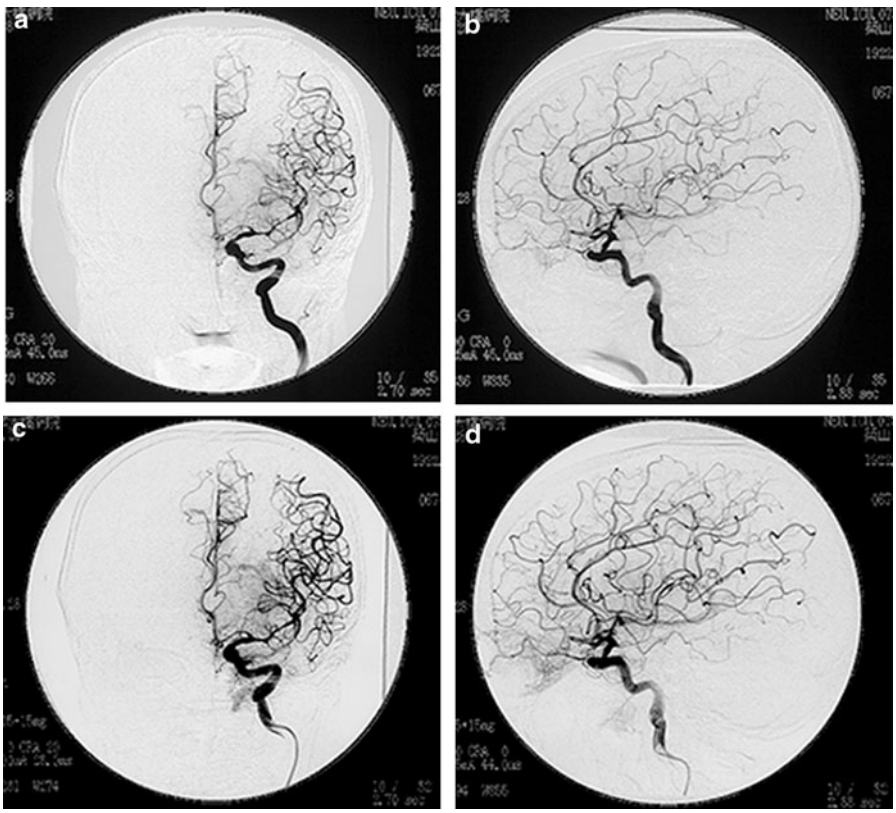

Fig. 1 Angiography before and after intra arterial administration of fasudil hydrochloride. (**a**, **b**) Before administration. (**c**, **d**) After administration. Vasodilation is seen not only in proximal vessels, but also in distal vessels after intra arterial infusion of fasudil hydrochloride

MCA territory. One patient of SD presented SAH with ICH and one patient of VS was 80-years-old. The 1-year follow-up showed 78.9% of GR. No patient showed any significant changes in vital signs, such as lower blood pressure or symptomatic autonomic responses, or any other adverse effects resulting from intra-arterial administration of fasudil hydrochloride.

Discussion

We previously reported the effectiveness of intra-arterial administration of fasudil hydrochloride by measuring cerebral circulatory dynamics, as determined by cerebral angiography performed before and after intra-arterial administration of fasudil hydrochloride (Fig. 1) [5]. We found that the time to peak opacification and the time to half-peak opacification were significantly reduced in the proximal portion of the middle cerebral artery in the early arterial phase after intra-arterial administration of fasudil hydrochloride, and that the time to peak opacification was significantly reduced both in the distal portion of the middle cerebral artery in the late arterial phase and in the transverse sinus in the venous phase. Intra-arterial administration of fasudil hydrochloride induced dilation of the proximal arteries and improved cerebral microcirculation in patients with vasospasm. The clinical review this time is also evidence towards the previous finding. Clinical effectiveness could be expected, even if the patient did not show immediate clinical improvement. To rescue the MCA territory may lead to better prognosis, even if the procedure is repeated. Fasudil hydrochloride resolves symptomatic vasospasm, but the duration of action is limited. Its metabolite, which has spasmolytic activity, remains in the blood for approximately 8 h after infusion [7]. In our series, the shortest angiographic recurrence of vasospasm was 8 h and multiple administrations may be considered for persistent vasospasm. This procedure carried out before presentation of symptomatic vasospasm could improve outcome in patients with subarachnoid haemorrhage. Adverse effects, such as change in pupil size, blindness, seizures, and respiratory arrest, were reported after intra-arterial infusion of papaverine hydrochloride for cerebral vasospasm [2, 3, 8]. We observed no significant changes in vital signs, or any other adverse effects resulting from intra-arterial administration of fasudil hydrochloride.

Conflict of interest statement We declare that we have no conflict of interest.

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Role of Controlled Lumbar CSF Drainage for ICP Control in Aneurysmal SAH

Ali Murad, Samer Ghostine, and Austin R.T. Colohan

Abstract *Background*: A prospective study of lumbar CSF drainage in the setting of raised intra-cranial pressure refractory to medical management and ventriculostomy placement is presented. There has been increasing data that this may be a effective and safe intervention for reduction of ICP.

Method: An IRB approved prospective study was conducted. Six patients with increased intracranial pressure secondary to aneurysm rupture were initially managed with sedation, ventriculostomy placement, mild hyperventilation (pCO_2 = 30 35), and hyperosmolar therapy (Na = 150 155). A lumbar drain was placed if ICP continued to be above 20 mmHg despite optimization of medical therapy.

Findings: After lumbar drain placement, ICP was reduced from 30.2 mmHg ± 6.7 to 9.7 mmHg ± 7.4, an average decrease of 20.5 mm H_2O ($P < 0.001$). There was no significant change in CPP. Requirements for hypertonic saline and/or mannitol boluses and sedation to control ICP were decreased. There was no incidence of CSF infection or cerebral herniation.

Conclusions: We have shown that controlled lumbar drainage is a safe, efficacious and minimally invasive method for treatment of elevated ICP which refractory to medical management. Ventriculostomies are always placed before utilizing lumbar drains to minimize the risk of cerebral herniation. We would advocate making controlled lumbar drainage a standard part of ICP control protocols.

Keywords Sub-arachnoid hemorrhage · Cerebral Edema · Intracranial pressure · Intracranial hypertension · Lumbar drain

A. Murad (✉), S. Ghostine, and A.R.T. Colohan
Department of Neurosurgery, Loma Linda University Medical Center, 11234 Anderson Street, Rm. 2562 B, Loma Linda, CA 92354, USA
e mail: amurad@llu.edu

Introduction

Trauma remains the most common cause of brain injury and elevated intracranial pressure, with other pathologies such as aneurysmal subarachnoid hemorrhage (SAH) and hemorrhagic stroke constituting other causes. The guidelines most commonly employed for control of elevated intracranial pressure are the Brain Trauma Foundation (BTF) guidelines. They recommend use of sedation, mild hyperventilation, hyperosmolar therapy and ventricular cerebrospinal fluid (CSF) drainage as first tier therapies for control of elevated intra-cranial pressure (ICP) in traumatic brain injury (TBI) [6]. Moderate hypothermia, hyperventilation, barbiturate coma, and decompressive craniectomy are used as second tier interventions if first tier therapies fail to normalize ICP. However, a number of these patients develop ICP refractory to all measures above. This results in cerebral ischemia, due to a decrease in cerebral blood flow and oxygenation, leading to increased morbidity and mortality [14].

Vourc' first described the use of an indwelling lumbar intrathecal catheter for CSF drainage in 1963 [26]. This method of CSF drainage is now commonly used as a supplementary method for brain relaxation during cranial procedures and several conditions including cranial and spinal CSF leaks, pseudomeningoceles [23]. However, it has not been a well accepted intervention for ICP control due to concerns about a high risk of cerebral herniation. These concerns have been based on reports from pre-CT era which showed a high incidence of herniation after lumbar puncture the setting of meningitis and mass lesions [9, 16, 19]. We present results on six patients with aSAH treated with lumbar CSF drainage for elevated ICP refractory to medical management and ventriculostomy placement.

Aneurysmal SAH frequently leads to elevated ICP due to CSF outflow obstruction and cerebral ischemia. There is some evidence that increased ICP is related to vasospasm and poor outcome [12]. Use of lumbar cerebrospinal fluid (CSF) drainage is uncommon for control of

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increased intra-cranial pressure (ICP). There have been some recent case series reporting the use of lumbar CSF drainage for this purpose [2, 25]. We present the results of a prospective study to evaluate the efficacy of lumbar CSF drainage to treat increased ICP refractory to medical management.

Methods

Six patients with elevated ICP secondary to aneurysmal SAH had lumbar CSF drains placed for ICP control in an IRB approved prospective study. The purpose of the study was to evaluate the efficacy and safety of lumbar drainage in this clinical setting. The ruptured aneurysms had already been secured with clipping or coiling prior to lumbar drain placement. Three patients had aneurysms clipped and an equal number had them coiled.

All patients had medical management, which was considered to have been maximized once the following criteria were met: a ventriculostomy catheter was placed, serum Na was higher than 150 mEq/L, pCO_2 was between 30 and 35 mmHg, normothermia and deep sedation had been established. A lumbar drain was placed when ICP was above 20 mmHg for an average of 3 h despite optimization of the above parameters.

Data analysis was performed using SigmaStat 3.5 statistical analysis software. Mean ICP, was compared before and after lumbar drain placement as well as differences in requirements for hyperosmolar therapy, sedation and/or chemical paralysis were assessed.

Results

The patients' average age was 48.5 years (range 40 60 years). Four patients were male and two were female. Lumbar drains were placed on average 3.3 days post-admission (range, 2 5 days). The average period of lumbar CSF drainage was 3.8 days (range, 3 5.5 days). Three of the six patients had severe angiographic and clinical vasospasm. These patients had lumbar drain placement 4 5 days post aneurysm rupture, while the ones without evidence of vasospasm had lumbar drains placement 2 3 days post aneurysm rupture.

After lumbar drain placement, ICP was reduced from a mean of 30.2 mmHg $\pm$ 6.7 to 9.7 mmHg $\pm$ 7.4 ($P < 0.001$) (Fig. 1); In the 24-h period preceding lumbar drain placement, five out of six patients required additional boluses of hypertonic saline and/or mannitol for a total of 2.7 boluses per patient. In contrast, in the 24-h period following lumbar drain insertion, only one patient required a total of two boluses. This difference was statistically significant ($P < 0.05$).

Cerebral perfusion pressure (CPP) increased from 76.7 mmHg $\pm$ 19.8 to 81.2 mmHg $\pm$ 10.2. However, this change was not statistically significant. There were also no statistical differences between the mean arterial pressure (MAP) before and after lumbar drain placement, which was 105.4 mmHg $\pm$ 16 and 90.6 mmHg $\pm$ 16.4, respectively.

Requirements for sedatives and paralytics were also significantly decreased ($P < 0.05$) after lumbar drain placement. In the 24-h period prior to lumbar drain insertion, five out of six patients received additional boluses of sedatives, narcotics and/or paralytics for ICP control. After lumbar drain placement, none of the patients needed a bolus of these medications. Requirement for continuous sedation

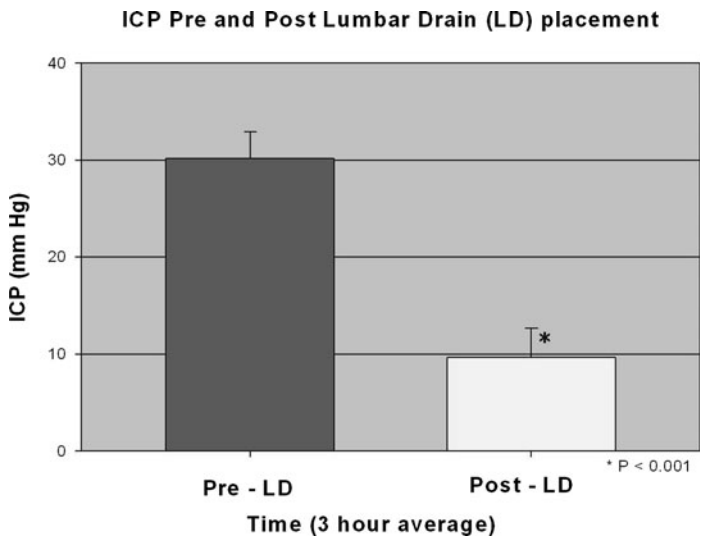

Fig. 1 ICP before and after lumbar drain placement (3 h average)

was also decreased, allowing the use of lower dosages of these medications. This permitted a more rapid weaning of sedation, and performance of more accurate neurological examinations.

There was no incidence of CSF infection or pupillary changes related to lumbar drain placement during the study period. Two of the six patients died during the study period. One patient has severe diffuse vasospasm leading to her death. The other death was due to withdrawal of care by the family as the patient's neurological exam remained poor. One patient had a MCA territory stroke due to vasospasm. He ultimately needed a decompressive craniectomy as he had an abrupt increase in ICP to 74 mmHg and developed a unilateral fixed and dilated pupil. He required a lumbar drain his ICP remained above 20 mmHg despite a large craniectomy.

Discussion

Each year there are 25,000 new cases of subarachnoid hemorrhage (SAH) secondary to rupture of an intracranial aneurysm in the United States alone. This is associated with significant morbidity and mortality with an estimated one-third of patients dying before they get medical attention. Less that half of surviving patients have good functional outcomes [1, 7]. Clinically evident vasospasm occurs in 25 37% of these patients [1, 4] causing further neurological deterioration.

Therapeutic interventions are aimed initially at securing the aneurysm followed by mitigating the effects of SAH and preventing secondary injury due to vasospasm. A combination of cerebral edema, hemorrhage and CSF outflow obstruction often lead to elevated ICP in patients with aSAH. There is evidence showing that intracranial hypertension after aSAH leads to poor outcome [10, 17, 22]. Currently most institutions, including ours, follow BTF guidelines for controlling intracranial hypertension in brain injury patients; therefore similar therapeutic modalities are used for treatment of elevated ICP in both TBI and aSAH patients. This is mainly due to lack of any detailed studies specifically looking at treatment of elevated ICP in aSAH patients.

While global brain injury due to trauma and aneurysm rupture shares similarities such as deranged cerebral autoregulation, cerebral ischemia and cerebral edema; aneurysmal SAH leads to unique vascular and metabolic derangements. While clinically evident vasospasm is a well recognized occurrence in aSAH, its occurrence and significance in TBI is open to debate [3]. Their was a lack of change in CPP before and after initiation of lumbar drainage in our study and was also observed in another study which used decompressive craniectomy for ICP control in aSAH patients [18]. This further indicates the presence of fundamental differences in vascular auto-regulation in the two groups, the mechanism of which is not yet clear. This is in contrast to significant increase in CPP in TBI patients after lumbar placement. A subgroup analysis comparing TBI and aSAH patients from other studies [25, 27] which have used lumbar drainage for ICP may help further validate this observation. Intracerebral microdialysis is a rapidly developing tool to monitor physiological and pathophysiological changes in chemical processes associated with ischemia. Currently most of microdialysis data studies relating to brain injury have been done in TBI patients, with limited data available in aSAH patients; a recent study showed potential differences between the two patient populations [17]. Further microdialysis studies in aSAH patients will help advance our knowledge of pathologic metabolic imbalances which are unique to aSAH and perhaps better understand the derangement in vasoreactivity.

aSAH presents challenges which require special therapeutic considerations for treatment of ICP. Some interventions for control of ICP have to be considered more carefully when using in aSAH patients especially in the setting of vasospasm. The treatment of elevated ICP with mannitol and furosemide, while effective, depletes a patient's fluid volume which counteracts the efforts to maintain hypervolemia and hypertension. This assumes even more importance if there is vasospasm. Hypertonic saline may be a better choice in these circumstances as it helps maintain fluid volume and increases osmolarity.

Another first tier therapy for control of ICP is the use of sedatives, analgesics, anesthetic agents, and/or paralytics. These medications are also very helpful in keeping the patient comfortable as they are ventilated. However, very deep sedation interferes with performing a neurological examination of these patients and can mask neurological decline due to vasospasm. A combination of several such medications at high doses also has a hypotensive effect which counteracts efforts to keep the blood pressure elevated in aSAH patients.

Second tier interventions for control of elevated ICP include use of barbiturate coma, decompressive craniectomy, controlled hyperventilation. Use of barbiturates has been extensively studies in TBI patients. It can be quite effective in controlling ICP but has some disadvantages. They include the loss of ability to carry out a neurological examination on a patient and requires continuous electroencephalographic (EEG) monitoring to titrate effective dosing. It is also associated with a significant risk of hypotension, with as a many as 25% of patients developing clinically significant hypotension [20]. Only one patient in our study was placed in barbiturate induced-coma. The ICP was initially controlled with standard medical interventions,

ventriculostomy, lumbar drainage but she developed diffuse severe vasospasm, causing rebound elevated ICP. The family did not want any further surgical interventions including a craniectomy and care was eventually withdrawn as her neurological exam did not improve.

Controlled hyperventilation with pCO_2 less than 30 mmHg is also a second tier therapy for control of ICP. It can rapidly reduce elevated ICP by causing cerebral vasoconstriction. This effect is generally lasts only a few hours and more importantly reduces cerebral blood flow [21], increasing the potential of secondary brain injury specially when there is vasospasm.

Decompressive craniectomy is a very effective intervention for control of ICP [18, 22]. However, it requires taking the patient to the operating room and commits the patient to two surgeries. It is not without complications with a reported complication rate of 10 50% [8, 28].

We have routinely used lumbar CSF drainage as a measure to control elevated ICP refractory to medical management. At our institution, patients are only considered for lumbar drain placement after a ventriculostomy has been placed. Medical measures include continuous hypertonic saline infusion, furosemide and/or mannitol boluses, mild hyperventilation (pCO_2 = 30 35 mmHg), deep sedation and/or chemical paralysis, and maintenance of normothermia. We have used lumbar CSF drainage in certain patients with elevated ICP refractory to medical management before considering decompressive craniectomy or barbiturate coma. One patient in the study group already had decompressive craniectomies prior to placement of a lumbar drain for ICP control.

While CPP and ICP have been shown to independently impact outcome in patients with severe TBI [5, 11, 13, 15], currently there are no CPP guidelines specifically for aSAH patients. There is some evidence that ICP may be a more important parameter in aSAH patients and related to the development of vasospasm [12].

Since 1998 there have been several case series published on the use of lumbar CSF drainage in adult patients with elevated ICP in TBI and aSAH [2, 24, 25, 27]. While there is no sub-group analysis comparing TBI to aSAH patients, highest reported rate of cerebral herniation is 6% and meningitis 7% [25]. There was no incidence of meningitis on our study. These reported rates of complications, particularly cerebral herniation, are much lower than has been feared in the past, preventing the use of lumbar drainage for ICP control. ICU management and monitoring of has advanced significantly over past few decades, which allow controlled lumbar CSF drainage with relative safety. We feel that the highest risk of herniation is at the time of placing the lumbar drain. This can be minimized by taking great care in not allowing a large amount of CSF leak in an uncontrolled manner.

Conclusion

We demonstrate the effectiveness and safety of lumbar CSF drainage for ICP control in the setting of aneurysmal SAH. It helps reduce the amount of sedation needed to allow better neurological exam, which is important in the setting of vasospasm. It also has several advantages over second tier ICP reduction therapies such as barbiturate coma, hypothermia, and hyperventilation. While the number of patients involved in the study is small, combined with the successful results from other studies, this therapeutic modality should be considered in certain patients with elevated ICP prior to the performance of a craniectomy.

Conflict of interest statement We declare that we have no conflict of interest.

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Chronic Hydrocephalus After Aneurysmal Subarachnoid Space Hemorrhage

Gang Huo, Mao-yuan Tang, Qing-lin Feng, Lv-ping Zheng, and Gang Yang

Abstract To discuss predisposing factors of chronic hydrocephalus after aneurysmal subarachnoid space hemorrhage (aSAH). Clinical data of treating 32 patients with chronic hydrocephalus after aSAH through operations was retrospectively analyzed and processed. The incidence rate of chronic hydrocephalus of patients with an age above 60 years, Hunt-Hess III IV level, posterior circulation aneurysm and anterior communicating aneurysm, hemorrhage twice or more and ventricle hematocele is prominently higher than patients with an age below 60 years, Hunt-Hess I II level, aneurysms on other parts, one hemorrhage and no ventricle hematocele ($P < 0.05$). An age above 60 years, Hunt-Hess III IV level, posterior circulation aneurysm and anterior communicating aneurysm, hemorrhage twice or more and ventricle hematocele are predisposing factors of chronic hydrocephalus after aSAH.

Keywords Aneurysm · Subarachnoid space hemorrhage · Hydrocephalus

Introduction

Aneurysmal subarachnoid space hemorrhage (aSAH) can be complicated by chronic hydrocephalus after 2 weeks and can lead to disease worsening. Thirty two patients with chronic hydrocephalus who were treated by operations were available in 206 aSAH patients who were accepted and treated by our department and had complete data in recent years and accounted for 15.5% of aneurysmal subarachnoid space hemorrhage in corresponding period. The paper pays more attention to the predisposing factors and clinical characteristics of chronic hydrocephalus after aSAH.

G. Huo (✉), M. y. Tang, Q. l. Feng, L. p. Zheng, and G. Yang
Department of Neurosurgerythe First Affiliated Hospital of Chongqing Medical University, Chongqing, 400016, P.R. China
e mail: xiaomin198171@tom.com

Clinical Data and Method

General Information

There were 19 male cases and 13 female cases with ages ranging from 34 years to 73 years and an average age of 61.3 years, wherein 22 cases are more than or equal to 60 years. The cases were graded into 6 cases with I III level and 26 cases with III V level according to Hunt-Hess when they visited the hospital; 15 cases had hemorrhage once, 12 cases had hemorrhage twice and 5 cases had three-time hemorrhage. The cases were divided into 7 cases with posterior circulation aneurysm, 15 cases with anterior communicating aneurysm, and 10 cases with aneurysms on other parts.

Clinical Manifestation

In 16 cases, conscious disturbance did not improve or even worsened, or improved for a time and then worsened. 12 cases had headache, 10 cases had intellectual disturbance, 7 cases had papilledema, 6 cases had unstable walking, 4 cases had urinary incontinence, and 3 cases had oculomotor nerve palsy.

Imaging Examination

All cases displayed subarachnoid space hemorrhage according to skull CT examination, wherein 14 cases had ventricle hematocele. All cases were verified to have intracranial aneurysms according to MRA or cerebral angiography examination, wherein 3 cases had multiple aneurysms. Patients with more than 2 weeks of first subarachnoid space hemorrhage time, in 11 cases, displayed hydrocephalus according to CT and MRI examination data when they visited the

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hospital, wherein 5 cases had acute or subacute hydrocephalus according to CT and MRI examination before they visited the hospital; another 21 cases had hydrocephalus according to CT or MRI examination during hospitalization 2 weeks after visiting the hospital and after leaving the hospital.

Treatment

Thirty two cases accepted ventriculoperitoneal shunting operations, 11 cases who already had hydrocephalus when they visited the hospital accepted ventriculoperitoneal shunting operations for one stage in aneurysm operations or accepted ventriculoperitoneal shunting operations by stages after operations.

Prognosis

The cases were followed for 6 56 months; 29 cases with hydrocephalus symptoms were alleviated, and the ventricle was reduced in follow-up examination according to skull CT. Another 3 cases generated hydrocephalus symptoms again in the period of follow-up study. The ventricle which was reduced after operations was expanded further according to skull CT. Shunt valve jamming was prompted, one case accepted ventriculoperitoneal shunt valve recanalization operation, another two cases accepted ventriculoperitoneal shunting operations again, and the hydrocephalus symptoms after operations were alleviated.

Statistical Treatment

Data processing was carried out on a SAS6.12 software package, and statistical method selects X^2 examination or Fisher exact probability methods.

Result

According to the schedule (Table 1), the chronic hydrocephalus incidence rate of aSAH patients with age of more than 60 years is 27.8% and is prominently higher than patients with age less than 60 years, whose incidence rate is 7.9% ($P < 0.01$). The chronic hydrocephalus incidence rate of patients with Hunt-Hess III V level is 26.5% and is prominently higher than patients with I II level, whose incidence rate is 5.6% ($P < 0.01$). The chronic hydrocephalus incidence rates of posterior circulation aneurysm and anterior communicating aneurysm are 46.7% and 25.9%, respectively, and are prominently higher than patients with aneurysms on other parts, whose incidence rate is 7.5% ($P < 0.01$). The hemorrhage frequency is higher, the incidence rate of chronic hydrocephalus is higher, and the incidence rate of twice-or-more hemorrhage is prominently higher than the incidence rate of once hemorrhage ($P < 0.01$). The chronic hydrocephalus incidence rate of patients with ventricle hematocele is 46.7% and is prominently higher than the incidence rate of patients without ventricle hematocele, whose incidence rate is 10.2% ($P < 0.01$).

Table 1 Supporting schedule chronic hydrocephalus incidence rate of various factors

Factors	Chronic hydrocephalus incidence rate (incidence number/ total cases)	P value
Age (Year)		<0.01
<60	7.9%(10/127)	
≥60	27.8% (22/79)	
Hunt Hess Classification		<0.01
I II level	5.6% (6/108)	
III IV level	26.5% (26/98)	
Part		Comparison between a and b:>0.05
(a) Posterior circulation aneurysm	46.7% (7/15)	Comparison between a and c:<0.01
(b) Anterior communicating aneurysm	25.9% (15/58)	Comparison between b and c:<0.01
(c) Aneurysms on other parts	7.5% (10/133)	
Hemorrhage frequency		Comparison between 1 and 2:<0.01
1	9.5% (15/158)	Comparison between 1 and ≥3:<0.01
2	30.8% (12/39)	Comparison between 2 and ≥3:>0.05
≥3	55.6% (5/9)	
Ventricle hematocele		<0.01
Yes	46.7%(14/30)	
No	10.2%(18/176)	

Discussion

Intracranial aneurysms are usually started by subarachnoid space hemorrhage and can be complicated by chronic hydrocephalus after 2 weeks. Patients' prognosis is directly affected, which should obtain attention at clinics [1 4]. It was thought that chronic hydrocephalus was caused by hematid which is broken into subarachnoid space after SAH blocks arachnoidal granulations. Later studies discovered that hematid, which entered arachnoid villi and subarachnoid

space, was denatured, broken or eliminated by phagocytic cells after 3 5 days, and the hematid number in cerebrospinal fluid did not have any prominent relation with circulation disturbance of cerebrospinal fluid. Some scholars discovered that the fibrosis degree of the subarachnoid space has a relation with chronic hydrocephalus; most agreeable nosogenesis are flexible meningocyte hyperplasia and collagen generation after SAH, and fibrosis of arachnoidal granulation and subarachnoid space, which generate reduction of cerebrospinal fluid circulation and absorption, thereby leading to hydrocephalus [5, 6].

In the typical clinical manifestation, the symptoms do not improve prominently 2 weeks after aSAH or improve for a time and then worsen again; or the manifestation is normal pressure hydrocephalus, which is complicated by papilledema [7]. Sixteen cases in the group manifested as no improvement or even worsening of conscious disturbance, or as improvement for a time and then worsening again, which should obtain more attention. If patients with aSAH gradually generate manifestations such as unstable walking, dementia and urinary incontinence, hydrocephalus can be easily reflected. However, diagnosis of chronic hydrocephalus after aSAH needs definite CT or MRI examinations.

The predisposing factors, which are displayed by the data in the group, comprise the following: first, patients whose ages are more than 60 years; second, patients whose Hunt-Hess levels are III V level; third, patients whose aneurysms are posterior circulation aneurysms and anterior communicating aneurysms; fourth, patients whose hemorrhage frequency is twice; fifth, patients with ventricle hematocele. The aSAH patients should pay attention to the occurrence of chronic hydrocephalus when the factors exist. Yoshioka et al., who researched aSAH patients, discovered that when age is higher, the incidence rate of chronic hydrocephalus is higher. They believed that the subarachnoid space of older patients is wider due to encephalatrophy, the hemorrhage of the subarachnoid space is penetrated and wide, and fibrosis of subarachnoid space is easily generated, thereby leading to the disease. The posterior circulation aneurysms and anterior communicating aneurysms are easily generated due to wider basal cistern of aneurysms. Aneurysms have greater hemorrhage after breaking and cannot be cleaned easily; repeated and multiple hemorrhages of aneurysms can generate chronic hydrocephalus easily due to the same reason. Some scholars think that ventricle hematocele may change circulation dynamics of cerebrospinal fluid and can lead to more serious fibrosis of subarachnoid space in the later period, thereby leading to chronic hydrocephalus. Not all acute hydrocephalus can develop into chronic hydrocephalus, however, and patients with acute hydrocephalus should follow up the occurrence of chronic hydrocephalus closely. The predisposing factors reported by the literature also comprise factors such as being female, blood volume of subarachnoid space, hypertension medical history, symptomatic cerebral vasospasm, cistern flushing drainage time, EVD time [1, 4, 5] and the like.

The treatment method of chronic hydrocephalus after aSAH is mainly ventriculoperitoneal shunting operation. Operation indicators comprise the following: first, no improvement and even worsening of conscious disturbance; second, high intracranial pressure symptoms such as headache, vomit, papilledema and the like; third, typical normal pressure hydrocephalus with shutting indications. The operation time is selected to be after aneurysm clipping operations or simultaneous with aneurysm clipping operations.

Komotar et al. think that operations of endplate windowing in aneurysm microsurgery operations can lower the incidence rate [3, 8] of chronic hydrocephalus. Some scholars report that abokinase can be used to prevent fibrosis of subarachnoid space, thereby lowering the incidence rate of chronic hydrocephalus. Patients with acute hydrocephalus can accept EVD, and patients with SAH can accept lumbar-puncture to release blood cerebrospinal fluid. The operation zones can be repeatedly flushed during operations to eliminate accumulated blood in the subarachnoid space and is beneficial for reducing the occurrence of chronic hydrocephalus.

Conclusion

Through our reasearch, we can make such an inference. The incidence rate of chronic hydrocephalus of patients with an age above 60 years, Hunt-Hess III IV level, posterior circulation aneurysm and anterior communicating aneurysm, hemorrhage twice or more and ventricle hematocele is prominently higher than patients with an age below 60 years, Hunt-Hess I II level, aneurysms on other parts, one hemorrhage and no ventricle hematocele. An age above 60 years, Hunt-Hess III IV level, posterior circulation aneurysm and anterior communicating aneurysm, hemorrhage twice or more and ventricle hematocele are predisposing factors of chronic hydrocephalus after aSAH.

Conflict of interest statement We declare that we have no conflict of interest.

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Statins in the Management of Aneurysmal Subarachnoid Hemorrhage: An Overview of Animal Research, Observational Studies, Randomized Controlled Trials and Meta-analyses

Andreas H. Kramer

Abstract *Background*: The pathophysiology of delayed neurological deficits (DNDs) following aneurysmal subarachnoid hemorrhage (SAH) is complex, and is not limited to arterial narrowing (vasospasm) and classical ischemia. Thus, combined drug approaches, or therapies with multiple effects, may have the greatest potential for benefit. Statins are known to have pleiotropic vascular effects, some of which may interrupt the pathogenesis of DNDs. Based on promising preliminary reports, many clinicians routinely administer statins to prevent DNDs.

Methods: A systematic review was performed to identify and summarize all animal research, observational studies, randomized controlled trials (RCTs) and meta-analyses which have evaluated the use of statins in the management of SAH.

Results: Nine animal studies, nine observational (cohort and case control) studies, six RCTs and three meta-analyses were identified. Animal studies have generally administered statin doses that, when adjusted for body weight, are 10 80 times larger than what is used in humans. Nevertheless, these models have consistently reported statins to reduce vasospasm and to demonstrate additional neuroprotective effects. However, observational studies have not revealed an association between statin-use and reduced DNDs or improved neurological outcomes. Results of RCTs have been inconsistent and limited by small sample size, but together suggest that statins may reduce DNDs, with no clear impact on mortality or neurological recovery. Optimal drug administration strategies (timing of initiation, most effective dose and duration) have not been clarified.

Conclusions: The role of statins in the management of patients with SAH remains unclear. Although promising, statins should not, at this time, be considered standard care.

A.H. Kramer
Departments of Critical Care Medicine and Clinical Neurosciences, Hotchkiss Brain Institute, University of Calgary, Calgary, AB T2N 2T9, Canada
e mail: Andreas.Kramer@albertahealthservices.ca

Keywords Statin · SAH · Vasospasm

Introduction

In recent population-based studies, subarachnoid hemorrhage (SAH) has a worldwide, age-adjusted annual incidence of about 4 7 cases per 100,000 population, comprising approximately 3 7% of strokes [1]. Case-fatality rates have declined somewhat, but remain approximately 30 40%, with about 8% of patients dying prior to hospital arrival [1, 2]. Slightly less than half of patients regain functional independence [2]. Cognitive impairment, depression and even posttraumatic stress disorder contribute to reduced quality of life among survivors [3 7]. The most important prognostic factor is the initial degree of neurological impairment [8, 9], such that recent literature has increasingly emphasized the importance of understanding the pathophysiological events contributing to "early brain injury" [10 12]. However, cerebral infarction, whether occurring early or later in patients' course, is also a major predictor of poor outcomes, the importance of which is emphasized by its potential preventability [8, 13].

Delayed neurological deficits (DNDs) occur in about 20 30% of patients, of which as many as half develop an infarct [14, 15]. Most, but not all, of these patients have evidence of arterial narrowing involving their proximal cerebral vasculature ("vasospasm") [16]. Thus, past research has largely been directed at finding treatments which increase the caliber of cerebral arteries. Vasodilating drugs, such as intravenous endothelin-receptor antagonists and locally-administered nicardipine sustained-release implants remain very promising in reducing DNDs and, ultimately, in improving neurological outcomes [17, 18]. Alternatively, it has become increasingly clear that the pathophysiological mechanisms underlying the development of DNDs and delayed cerebral infarction are considerably more complex than being exclusively due to vasospasm. Additional mechanisms that have been implicated include inflammation,

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cortical spreading depression, thrombosis, microvascular vasospasm, oxidative injury, and endothelial dysfunction [10 12, 19]. Furthermore, factors involved in early brain injury, such as neuronal apoptosis, blood-brain barrier disruption and global cerebral edema, may also predispose to DNDs. It is for these reasons that *combined* drug approaches, or the use of therapies with *multiple* simultaneous effects, may represent the most effective approach to preventing DNDs.

3-Hydroxy-3-methylglutaryl (HMG) coenzyme A reductate inhibitors, or statins, are one class of drugs with numerous, "pleiotropic" effects, which have proven beneficial in the management of vascular disease [20]. Several of these effects may be neuroprotective and directly applicable in disrupting the pathogenesis of DNDs [21].

Literature Review

Search Strategy

The OVID and PUBMED interfaces were used to search MEDLINE, EMBASE and Cochrane databases. The theme "statins" was searched using the Boolean operator "OR" to combine the MESH term "Hydroxymethylglutaryl-CoA Reductase Inhibitors" with keywords "HMG-CoA reductase inhibit$", "statin$", "simvastatin", "pravastatin", "lovastatin", "atorvastatin" and "rosuvastatin". The theme "subarachnoid hemorrhage" was searched by combining the MESH terms "Subarachnoid Hemorrhage", "Intracranial Aneurysm", and "Vasospasm, Intracranial" together with keywords "vasospasm", "delayed neurologic deficit$", "delayed cerebral ischemia" and "delayed ischemic neurologic deficit$". The two themes were then combined using the Boolean operator "AND". The abstracts of retrieved articles were reviewed to identify animal experiments, human studies and review articles dealing with the use of statins in SAH.

Experimental Studies in Animals

Numerous randomized, blinded animal studies have assessed the efficacy of statins in preventing vasospasm and ameliorating cerebral injury in the setting of SAH (Table 1) [22 30]. Statins were invariably found to be beneficial. Specifically, they were reported to raise endothelial nitric oxide synthase (eNOS) expression [22, 26]; reduce neutrophil recruitment [23]; reduce expression of intercellular adhesion molecule (ICAM-1) and nuclear factor kappa-B (NF-κB) [23, 24]; prevent neuronal apoptosis [27, 29]; limit blood brain barrier disruption and resultant cerebral edema [27, 29]; raise CBF [28]; and improve neurological performance [22, 24, 26 28].

To what degree the findings of these experiments can be generalized and applied to patient care is unclear for several reasons. First, the types of animal models have varied somewhat, and may not mimic the actual pathophysiological events that ensue following a ruptured cerebral aneurysm. For example, one common approach has been to inject autologous blood into the cistern magna [23 25, 28 30]. Second, most animal studies have used statin doses that, on a mg/kg basis, greatly exceed what can safely be administered to humans. Clinical studies have generally used 40 80 mg, or 0.5 1 mg/kg, per day of simvastatin or pravastatin. In contrast, animals were typically given 10 80 times more than this. Moreover, drug was administered using various routes, including subcutaneous and intra-peritoneal, which are never used in humans. Third, in several experiments, animals were treated with statins well in advance of SAH. Because SAH typically occurs in relatively young individuals (40 60 years), more often in women, the proportion of patients taking statins prior to aneurysm rupture is actually relatively small. Even in animal studies where statins were introduced after SAH, drug administration commonly occurred much sooner (within 30 min) than what is generally feasible clinically, where enteral drugs are usually not initiated for several hours, in some cases even days. Thus, the tremendously beneficial effects of statins observed in animal models may be impossible to emulate in patients.

Despite these limitations, much has been learned about the effects of statins in SAH. Investigators from China have recently, for the first time, reported that atorvastatin may attenuate early brain injury, both when it is administered before and after the introduction of blood into the subarachnoid space [27, 29]. Specifically, there was preservation of the blood brain barrier and less cerebral edema. Improvements in neurological scores were also observed, although only when atorvastatin was given for two weeks prior to SAH [27]. The mechanism of neuroprotection appeared to be a reduction in caspase-dependent apoptotic cell death, perhaps through upregulation of Akt and GSK3β pathways.

Essentially all studies have also found statins to attenuate the degree of vasospasm, although there was some variation in the response of different blood vessels (basilar artery vs. middle cerebral/internal carotid) [22 28, 30]. Upregulation of eNOS, which again appears to be mediated by the PI3K/Akt pathway, is probably at least partially responsible for this reduction in vasospasm [22, 26]. Importantly, however, it is unclear that these effects are consistently replicated at lower doses, and they are definitely blunted when statin-initiation occurs *after* SAH has already occurred [22, 24, 27].

A recent publication surprisingly suggested that lower doses (1.5 mg/kg/day) in rats may actually improve

Table 1 Animal studies assessing the use of statins in subarachnoid hemorrhage models

Author/Year	Model information	Statin	Dose/Route	Duration/Timing	Prevention of vasospasm	Biochemical effects	Other effects
McGirt (2002) [22]	Mouse	Simva	20 mg/kg/day	14 days before and 3 days after	Yes (72 h) MCA but not BA	eNOS expression increased	Improved neurological scores
	ICA perforation (monofilament)		sc		Less when started *after* SAH	Not significant when started *after* SAH	
McGirt (2006) [23]	Rabbit	Simva	40 mg/kg/day	3 days after	Yes (72 h) BA	Reduced CD18-positive cell count	N/A
	Blood injection into cisterna magna		sc				
Chen (2006) [24]	Rat	Atorva	10 mg/kg/day	15 days before and 2 days after	Yes (48 h) BA	Reduced ICAM-1, LFA-1 and IL-6 expression in pre-SAH treatment group	Improved neurological scores primarily in pre-SAH group
	Blood injection into cisterna magna		enteral		Less when started *after* SAH		
Bulsara (2006) [25]	Canine	Simva	20 mg/kg/day	10 days (after SAH)	Yes (72 h) BA	N/A	N/A
	Blood injection into cisterna magna		enteral				
Sugawara (2008) [26]	Rat	Simva	1 mg/kg /day ("low dose")	3 days after	Yes (24 and 72 h) ICA but only in high dose group	Phosphorylated Akt and eNOS increased but only in high dose group	Improved neurological scores but only in high dose group
	ICA perforation (monofilament)		20 mg/kg/day ("high dose") ip				
Cheng (2009) [27]	Rat	Atorva	20 mg/kg/day	15 days before	Yes (24 h) BA	Reduced expression of caspase-3 and caspase-8 (markers of apoptosis)	Improved neurological scores
	Perforation		enteral			Reduced apoptosis	Reduced brain water content
							Reduced BBB permeability
Takata (2009) [28]	Rat	Simva	1.5 mg/kg/day ("low dose")	14 days after or lower dose × 5 weeks after	Yes (7 days) BA but not MCA; dose-dependent	N/A	Improved neurological scores but only with lower dose
	Blood injection into cisterna magna		10 mg/kg/day ("high dose") enteral		Increased CBF		Benefit lost after cessation of treatment at 14 days
							Improved neuronal survival but only at higher dose
Cheng (2009) [29]	Rabbit	Atorva	10 mg/kg/day ("low dose")	3 days after	N/A	Increased phosphorylated Akt and GSK3β	Reduced brain water content
	Blood injection into cisterna magna		40 mg/kg/day ("high dose") sc			Reduced expression caspase-3; not other apoptotic proteins	Reduced BBB permeability
						Reduced apoptosis	
Duan (2009) [30]	Rabbit	Simva	5 mg/kg/day	7 days after	Yes (7 days) BA	Reduced expression ICAM-1 and NF-κB	N/A
	Blood injection into cisterna magna		enteral				

BA basilar artery, *BBB* blood brain barrier, *CBF* cerebral blood flow, *eNOS* endothelial nitric oxide synthase, *hrs* hours, *ICAM* intercellular adhesion molecule, *ip* intraperitoneal, *LFA* leukocyte function associated antigen, *MCA* middle cerebral artery, *N/A* not assessed, *sc* subcutaneous

neurological scores to a greater degree than higher doses. Importantly, the authors also observed that much of the benefit of statins was lost when they were discontinued 2 weeks after SAH, which is the usual duration of time that these agents are given to patients. Thus, it is possible that in order for long-term outcomes to be improved by statins, they may need to be administered for more than 2 3 weeks. Previous and ongoing RCTs have not considered this possibility. To advance knowledge in this area, it may be necessary for future animal studies to more closely mimic the pathophysiology of SAH and to utilize drug doses that are more relevant in humans.

Biological Effects in Humans

Three randomized controlled trials (RCTs) have reported various biological effects of statins in patients with SAH (Table 2) [31 33]. In summary, the findings of these studies have been considerably less impressive than what has been reported in animals.

Lynch and colleagues reported that 80 mg/day of simvastatin significantly reduced plasma levels of S100β, a marker of astrocyte activation, known to have prognostic significance in SAH [31, 34]. In addition, levels of von Willebrand factor (vWF), a marker of endothelial function were increased in the simvastatin group. Unfortunately, these measurements were not made prior to day 3, such that it is unclear if the baseline values were well balanced between the statin and placebo groups. The other two studies assessed numerous markers of inflammation, endothelial function, coagulation and fibrinolysis. In both cases, despite the relatively short duration of follow-up, LDL and total cholesterol levels were reduced by statin administration. In contrast, statins did not appear to have any meaningful impact on any of the other biomarkers measured. Even the effect of statins on vWF could not be confirmed [33]. Although it seems less likely, Tseng and colleagues concluded that the neuroprotective effects of statins could be mediated through cholesterol-lowering mechanisms [32]. It is important to point out that none of these studies measured biomarkers in the brain (e.g. using microdialysis) or cerebrospinal fluid.

Physiological Effects in Humans

As a surrogate outcome in a RCT assessing the efficacy of 40 mg/day of pravastatin, Tseng and colleagues recorded cerebral blood flow autoregulation using the transient hyperemic response test. It was reported that the use of pravastatin significantly reduced the mean number of days during which abnormal autoregulation was observed, both on the side of the ruptured aneurysm and on the contralateral side. Patients who developed TCD-defined vasospasm, DNDs and unfavorable outcomes had considerably longer periods of time where autoregulation was impaired. Thus, it appears that preservation of autoregulation is one possible manner whereby statins may protect against ischemic insults. This study did not explore the molecular mechanisms of this phenomenon, and the findings have not yet been confirmed by other investigators [35]. An ongoing RCT is evaluating the effects of statins on cerebral blood flow and metabolism using positron emission tomography [36].

Randomized Controlled Trials and Meta-Analyses

To date, four peer-reviewed RCTs evaluating the efficacy of statins in SAH have been published (Table 3) [31, 33, 37, 38]. These studies enrolled a total of 190 patients, of whom 94 have been treated with statins and 96 with placebo. Three

Table 2 Randomized controlled trials assessing the effects of statin use on biochemical markers

Author/Year	Statin	Dose	Duration	Biochemical tests	Results
Lynch (2005) [31]	Simva	80 mg	14 days	Astrocyte activation: S100β Endothelial function: vWF	S100β and vWF consistently lower over initial 12 days in hospital
Tseng (2007) [32]	Prava	40 mg	14 days or discharge	Cholesterol (total, LDL, HDL) and triglycerides D dimer, CRP, fibrinogen	Lower LDL and total:HDL ratio Other markers unchanged
Vergouwen (2009) [33]	Simva	80 mg	14 days	Cholesterol (total, LDL, HDL) and triglycerides *Fibrinolysis*: tPA antigen and activity; PAI 1 antigen and activity *Coagulation*: prothrombin fragment F1+2 *Endothelial function*: vWF, soluble thrombomodulin *Inflammation*: CRP, IL 1, IL 6, IL 8, IL 10, TNFα	Lower LDL, total Other markers largely unchanged Possible increase in thrombomodulin

CRP C reactive protein *HDL*, high density lipoprotein, *IL* interleukin, *LDL*, low density lipoprotein, *PAI* plasminogen activator inhibitor, *TNF* tumour necrosis factor, *tPA* tissue plasminogen activator, *vWF* von Willebrand factor

Table 3 Randomized controlled trials evaluating the efficacy of statins in the management of patients with subarachnoid hemorrhage

Author/Year	N (statin:control)	Drug/dose	Started within	Duration	Identical placebo	Allocation concealment
Peer reviewed						
Tseng (2005) [37]	40:40	Pravastatin 40 mg	72 h	Max 14 days	Yes	Yes
Lynch (2005) [31]	19:20	Simvastatin 80 mg	48 h	14 days	Yes	Not clear
Chou (2008) [38]	19:20	Simvastatin 80 mg	96 h	Max 21 days	Yes	Not clear
Vergouwen (2009) [33]	16:16	Simvastatin 80 mg	72 h	14 days	Yes	Yes
Non peer reviewed						
Jaschinski (2008) [45]	40:58	Pravastatin 40 mg	24 h	Until discharge	No	No
Macedo (2009) [46]	11:10	Simvastatin 80 mg	72 hrs	21 days	No	No

Table 4 Meta analyses evaluating the efficacy of statin in the management of patients with subarachnoid hemorrhage[a]

Author/Year	Number of Studies	Number of Patients	Model	Summary Measure	DINDs	Mortality	Poor Neurological Recovery	TCD Defined Vasospasm
Sillberg (2008) [39]	3	158	Fixed effects	Risk Ratio	0.38 (0.17 0.83) 0.40 (0.23 0.70)	0.22 (0.06 0.82)	N/A	0.83 (0.60 1.14)
Vergouwen (2009) [33] Kramer (2009) [43]	4	190	Random effects	Risk Ratio Odds ratio	0.57 (0.29 1.13)	0.37 (0.13 1.10)	0.92 (0.68 1.24)	0.99 (0.66 1.48)
All RCTs	6	309	Fixed effects		**0.38 (0.23 0.64)**	**0.51 (0.25 1.02)**	**0.81 (0.49 1.32)**	0.83 (0.42 1.61)
			Random effects		0.38 (0.21 0.69)	0.41 (0.16 1.03)	0.81 (0.49 1.32)	**0.98 (0.35 2.78)**
Published RCTs	4	190	Fixed effects		**0.41 (0.20 0.82)**	**0.29 (0.09 0.93)**	0.92 (0.48 1.79)	0.83 (0.42 1.61)
			Random effects		0.40 (0.15 1.05)	0.29 (0.09 0.93)	0.92 (0.48 1.79)	**0.98 (0.35 2.78)**
Observational Studies	6	1,542 (386 with statins)	Fixed effects		**0.96 (0.71 1.31)**	**1.18 (0.78 1.73)**	**1.20 (0.84 1.72)**	N/A
			Random effects		0.96 (0.71 1.31)	1.14 (0.69 1.91)	1.20 (0.84 1.72)	
Total	12	1851	Fixed effects		0.76 (0.58 0.99)	0.95 (0.67 1.34)	**1.05 (0.79 1.40)**	N/A
			Random effects		**0.80 (0.61 1.05)**	**0.89 (0.56 1.40)**	1.05 (0.79 1.40)	

[a]Summary measures presented in bold represent the primary analysis. If there was significant heterogeneity ($I^2 > 50\%$ or Q statistic $p < 0.05$), studies were combined with random effects model. If there was no significant heterogeneity, they were combined with fixed effects model.

of these studies administered 80 mg/day of simvastatin for 14 21 days, while the other used 40 mg/day of pravastatin for a maximum of 14 days (many patients did not complete the full course of therapy). In contrast to the animal studies, where statin-initiation never occurred later than 30 min after the ictus, the RCTs allowed delays of up to 96 h. The proportion of high-grade SAH patients varied from 23 33% [37, 38], and the ratio of clipped vs. coiled patients varied from as high as 85:15% to as low as 23:77% [33, 38]. The first two published studies reported statins to have dramatic effects in the reduction of DNDs, TCD-defined vasospasm, and even mortality [31, 37]. These findings could not be confirmed in the more recent publications [33, 38].

Three meta-analyses summarizing existing RCTs have now been published (Table 4) [39 41]. The first was based on three RCTs, one of which was (at the time) available only in abstract form, and included a total of 158 patients [39]. The authors used a fixed effects model to combine data and produce risk ratios. It was concluded that statins reduce vasospasm (reported in 2/3 studies), DNDs (3/3 studies) and mortality (2/3 studies). This meta-analysis was criticized especially for the definitiveness of its conclusions, given the clear limitations of the data [42, 43].

The more recent meta-analysis by Vergouwen and colleagues included four RCTs [40]. The authors were considerably more conservative in their methodology, in that they applied exclusively random effects models, which

produce summary measures with wider confidence intervals. Random effects models are generally considered more appropriate than fixed effects models when there is significant heterogeneity across studies [44]. It was reported that statins had no statistically significant impact on any relevant outcomes, including DNDs (reported in 4/4 studies), mortality (3/4 studies), poor neurological recovery (3/4 studies) and TCD vasospasm (3/4 studies) [40].

The broader search strategy applied by Kramer and Fletcher identified two additional studies, which were described as RCTs by the authors, but have only been published as abstracts [41, 45 48]. Correspondence with the authors suggested that these had a "pseudo-randomized" design, in that there was no blinding or concealment of treatment allocation. Thus, meta-analysis summary measures (odds ratios) were calculated with and without their inclusion. The primary analysis was performed using fixed effects models in the absence of significant heterogeneity (defined as $I^2 < 50\%$ *and* Q statistic with p value ≥ 0.10); otherwise, random effects models were applied. In either case, the alternative approach was used as part of a sensitivity analysis to determine if the findings remained robust [41].

Using a fixed effects model (because criteria for heterogeneity were not met) to combine data from the six RCTs with 309 patients, the authors reported that statins significantly reduced the incidence DNDs. This effect persisted even with exclusion of the two unpublished studies [45, 46]. However, when a random effects model was used to combine the four published studies (as done by Vergouwen and colleagues), the results were no longer (quite) statistically significant [41].

Previously unpublished mortality data was provided by the principle investigator of one of the four published RCTs [31]; this information was not included in the other meta-analyses. When all six RCTs were combined, statins did not significantly reduce mortality, although there appeared to be a trend (OR 0.51, 95% CI 0.25 1.02). Restricting the analysis only to the four published studies, statins did significantly reduce mortality (OR 0.29, 95% CI 0.09 0.93).

Statins could not be confirmed to have any clear efficacy in improving neurological outcomes. This finding is limited by the variable approaches used to define "poor outcome". This is unfortunate, because neurological recovery is generally the most meaningful outcome to assess in RCTs of SAH, and other neurocritical care patients. Mortality data is more difficult to interpret, since most patients who die do so following withdrawal of mechanical ventilation, when there is a perception of a dismal neurological prognosis. This process is heavily influenced by patients' and surrogate decision makers' preferences, as well as variations in clinicians' practices. For example, even with comparable patient characteristics and similar quality of care, one center might have lower mortality rates, but a higher proportion of patients who survive with severe disabilities [47]. Considerable heterogeneity has been observed in the effects of statins on transcranial Doppler (TCD)-defined vasospasm; however, combining the data does not reveal any significant impact [40, 43]. This is in contrast to animal studies, where (substantially higher dose) statins consistently ameliorated vasospasm.

Observational Studies

Several cohort and case control studies have assessed the effectiveness of statins. Although blinded RCTs represent the optimal study design to assess the efficacy of a treatment, observational studies have several advantages, including that they typically involve consecutive (rather than highly selected) patients, are numerically larger and are reflective of day-to-day practice [48]. After excluding studies which focused entirely on the effects of pre-SAH statin use, the meta-analysis performed by Kramer and Fletcher summarized the findings of six observational studies, which included a total of 1,542 patients, of whom 386 were treated with a statin [49 53]. Unlike the RCTs, there was no suggestion of benefit from statins in improving any of the relevant outcomes, including DNDs, mortality or poor neurological recovery. Also unlike the RCTs, there was little heterogeneity, with individual studies reaching quite consistent conclusions. Indeed, not one of the six studies found statins to be effective.

Kramer and Fletcher also performed subgroup analyses, where randomized and observational studies were further classified into categories based on the statin regimen (high vs. low dose; simvastatin vs. pravastatin), the predominant method of aneurysm treatment (clip vs. coil), the definition of DNDs that was used (clinical deterioration only vs. clinical deterioration plus ancillary evidence of vasospasm), and the initial grade of hemorrhage ["high" (stupor and coma) vs. "low"]. No subgroup of studies could be identified where statins had a preferentially larger effect [41].

Four observational studies have assessed whether the use of statins *prior to* aneurysm rupture is protective against DNDs (Table 5) [49, 54 56]. These studies are of interest when one considers that pre-SAH statin-initiation was particularly beneficial in animal models (Table 1). However, the practical relevance of such information is unclear, since a small minority of patients is actually receiving chronic statin therapy at the time of SAH. Two studies (Parra, McGirt and colleagues, Table 5) reported that pre-SAH use dramatically reduced the occurrence of DNDs, from 43 48% (rates that are unusually high) to 7 10% (rates that are extremely low) [55, 56]. These findings were not confirmed in a subsequent larger study performed by Moskowitz and colleagues [54].

Table 5 Observational studies assessing the association between statin use at the time of subarachnoid hemorrhage and the subsequent development of complications

Author/Year	Statin Treated	Control	Control selection	DINDs	Mortality	Poor neurological outcome	Vasospasm
Parra (2005) [55]	20	40	Matched	2/20 (10%) vs. 17/40 (43%); p = 0.02	20% vs. 13%; p = 0.46	BI 77 vs. 39; p = 0.003; MLPSS 12 vs. 19; p = 0.03	18% vs. 51%; p = 0.03
Singhal (2005) [49]	36	478	Historical	14/36 (39%) vs. 135/478 (28%); p = 0.18 Multivariate OR 1.43 (0.66 3.08); p = 0.36	Multivariate OR 0.44 (0.14 1.40, p = 0.17)	Multivariate OR 1.06 (0.45 2.50); p = 0.90	78% vs. 61%, p = 0.05 Multivariate OR 2.75 (1.16 6.50); p = 0.02
McGirt (2006) [23]	15	100	Historical	1/15 (7%) vs. 48/100 (48%); p = 0.002 Multivariate OR 0.09 (0.01 0.77)	N/A	N/A	N/A
Moskowitz (2009) [54]	26	282	Historical	6/26 (23%) vs. 86/282 (31%); p = 0.43	N/A	N/A	N/A
Total	97	900		23/97 (24%) vs. 286/900 (32%) I^2 = 77%, Q = 13.2, p = 0.004 (indicative of heterogeneity) **OR 0.44 (0.13 1.50); p 0.19**	N/A	N/A	N/A

BI Barthel Index, *MLPSS*, Modified Lawton Physical Self Maintenance Scale, *N/A* not assessed, *OR* odds ratio.

Singh and colleagues actually reported a *higher* rate of DNDs among patients pre-treated with statins, although this finding was attributed to statin withdrawal [49]. In contrast, however, Moskowitz and colleagues [54] did not find any indication that statin cessation is deleterious.

Given the substantial heterogeneity in the findings of these four studies (I^2 = 77%, Q = 13.2, p = 0.004), they have been summarized in Table 5 using a random effects model. The combined results, evaluating 97 treated and 900 control patients, reveal no statistically significant impact of pre-SAH statin use on the occurrence of DINDs (OR 0.44, 95% CI 0.13 1.50; p = 0.19). Similarly, statins did not have an effect on mortality. However, one of the four studies reported better neurological outcomes, recorded using the Barthel index and Modified Lawton Physical Self Maintenance Scale, among statin-treated patients [55]. Divergent results were found in two studies reporting the relationship between pre-SAH statin use and radiographic vasospasm [49, 55].

Conclusions

Statins have pleiotropic effects, which may interrupt several steps in the complex cascade of events that culminate in DNDs and delayed infarction. Recent animal studies have reported that statins may even ameliorate early brain injury following SAH [27, 29]. The combined results of existing RCTs, which have enrolled more than 300 patients, suggest that statins reduce DNDs [41]. Furthermore, statins are relatively safe, cheap, familiar, and easy to administer. It is therefore understandable that many clinicians have already chosen to routinely administer statins, even though such treatment is not supported by a definitive, multi-center randomized controlled trial and is not advocated by recent consensus guidelines [57].

Despite these observations, the role of statins in the care of patients with aneurysmal SAH remains uncertain for several reasons. First, RCTs have all been small and have shown some inconsistency in methodology and results. Some high quality studies have not shown any indication of biochemical or clinical efficacy [33, 38]. Second, despite a reduction in the occurrence of DNDs, statins have not been proven to improve neurological outcomes [40, 41]. Third, observational studies, evaluating hundreds of consecutively treated patients, have consistently failed to show even a trend in favor of statin use [41]. Thus, there should be equipoise among clinicians regarding the efficacy of statins in SAH, justifying the enrolment of patients in ongoing clinical trials [36, 58, 59]. It is, perhaps, unfortunate that the ongoing phase III RCT (STASH trial) was not preceded by true phase II studies, in order to clarify fundamental questions regarding optimal dosing regimens (i.e., how soon should drug be initiated, what is the most efficacious dose, how long should it be continued).

Conflict of interest statement We declare that we have no conflict of interest.

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New Modalities to Assess Efficacy of Triple-H Therapy: Early Experience

Deepti Bhargava, Yahia Al-Tamimi, Audrey Quinn, and Stuart Ross

Abstract The traditional axiom that vasospasm induced reduction of blood flow leads to poor tissue oxygenation and ischaemic cellular injury culminating in delayed neurological deficits has been challenged and the efficacy of triple H therapy in reversal of the above is debated. In this study we assess cerebral physiology before and during onset of DIND and with application of triple H therapy with real time neuro-monitoring tools. Patients with Fisher grade 3/4/3 + 4/rebleed were consented. Probes for measuring rCBF, pTiO2, and Microdialysis parameters glucose, glycerol, lactate, and pyruvate were inserted at time of coiling/clipping. Subsequent monitoring was done in HDU/ITU setting. Return of parameters to baseline was regarded as effective triple H therapy. Study is ongoing and the current paper presents our experience with first five patients. The results suggest safety and feasibility of multimodal monitoring in clinical setting to establish an understanding of relationship between clinical symptoms, brain perfusion, oxygenation, and metabolism in real time to test and guide therapy in future.

Keywords Subarachnoid haemorrhage · Delayed ischaemic neurological deficit · Triple H therapy · Neuromonitoring

Introduction

Triple-H Therapy has been in vogue for over 30 years and remains the most widely accepted treatment for Delayed Ischaemic Neurological Deficit (DIND)/clinical vasospasm [1]. However, each component of the therapy has potentially hazardous adverse effects and over the years numerous studies have tried to establish the efficacy of this therapy in vasospasm both prophylatically and for treatment. While use of the therapy in prophylaxis has been discarded, the evidence to support its use in treatment suffers from lack of randomised control trials and is at best inconclusive [2].

The current understanding [3] is that multifactorial reduction of cerebral blood flow leading to reduced tissue oxygen and culminating in energy crisis is responsible for clinical manifestation of Delayed Ischaemic Neurological deficit. We hypothesised that if this were true, an efficacious therapy should restore cerebral blood flow, oxygenation and metabolic parameters.

With real-time neuromonitoring modalities at hand we wanted to observe the effects of vasospasm and triple-H therapy on cerebral physiology to establish the efficacy of this therapy and to see if it was possible to use these tools to individualise therapy in patients with aneurysmal subarachnoid haemorrhage (SAH).

Materials and Methods

We sought to do a prospective observational study with patients undergoing invasive neuromonitoring while receiving standard therapy during onset and recovery of DIND. This study was based in Neuro-ITU and HDU. It was approved by local Research Ethics Committee and Trust R&D. Inclusion criteria consisted of age 18+ years, aneurysmal SAH, Fisher Grade 3/4/3 + 4, or rebleed and recruitment prior to day 4 post haemorrhage. Exclusion criteria were history of pulmonary edema, congestive cardiac failure, coagulation disorders. Written informed consent was obtained from the patient prior to inclusion in the study. Written assent was sought from relatives where neurological status impaired patient's capacity to consent.

Intervention once consented, the three probes regional cerebral blood flow (rCBF) using thermal-diffusion flowmetry (Hemedex), Brain Tissue Oxygenation (ptiO2) Probe (Licox) and energy metabolism (CMA 70 Brain Microdialysis

D. Bhargava (✉), Y. Al Tamimi, A. Quinn, and S. Ross
Leeds General Infirmary, Leeds, UK
e mail: deeptibhargava7@gmail.com

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Catheter) were placed at the time of coiling or clipping through separate frontal twist drill burr holes on the side of the aneurysm and preferably in the at-risk territory.

Normovolaemia was maintained afterwards with mean arterial pressure $\geq$ 90 mmHg, no prophylactic triple-H therapy was used. If the patient developed symptoms of vasospasm, they were treated with standardised triple-H therapy as per hospital protocol (Box 1).

Analysis Baseline values of cerebral physiology parameters were noted for all patients. Autoregulation indices were calculated. Descriptive statistics were calculated for continuous variables and return to baseline values was regarded as effective triple H therapy. In cases where a complete reversal was not achieved 5% improvement was regarded as a significant response to triple H therapy.

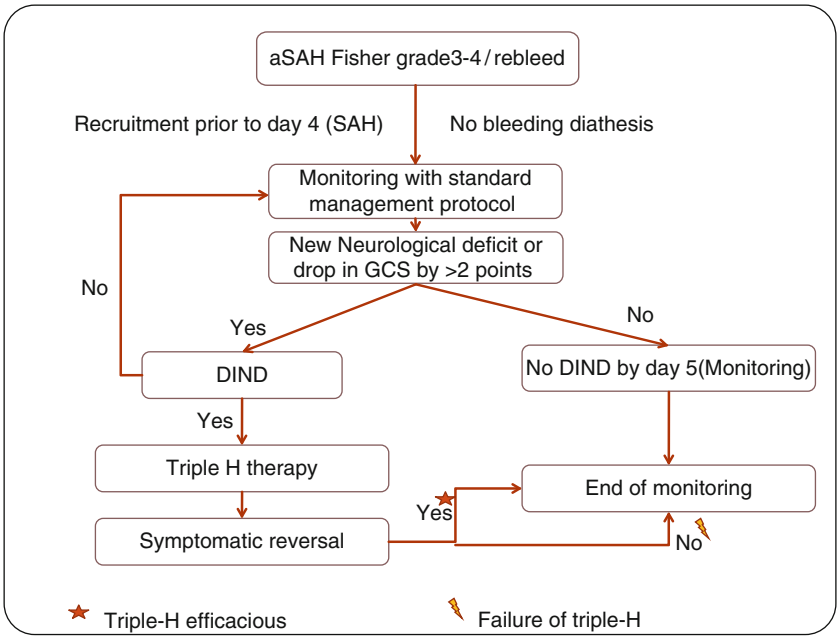
- **Stage 1**
- Cerebral vasospasm has been suspected or diagnosed clinically and/or radiologically
- Discuss with Intensive Care Unit if GCS $\leq$ 8
- Give supplemental oxygen therapy to achieve SpO2 > 93%
- Ensure ECG, SpO2, BP monitoring
- CT scan to exclude differential diagnosis, e.g., hydrocephalus
- Check U & E, FBC, blood glucose
- Ensure patient is receiving Nimodipine
- Document baseline Mean Arterial Pressure (MAP) and Central Venous Pressure (CVP)
- Paracetamol 1 g, if pyrexial (max. 4 g/24 h)
- **Stage 2: Hypervolaemia and Haemodilution**
- Commence fluid balance chart. Ensure urinary catheter has been inserted.
- Ensure adequate hydration. Maintain CVP $\geq$ 8 mmHg. Aim to achieve CI = >5l/min/m2
- Fluid replacement –:
- -Use 0.9% saline 3L per day – consider 1.8% saline if serum Na low or low-normal
- -Give 250 ml colloid boluses to achieve positive fluid balance, e.g., Volplex or Voluven.
- -Aim for a haematocrit of 30 – 35%. Consider blood transfusion if Haematocrit < 30% /Hb < 7.
- Observe for clinical response. If no/incomplete response when patient's fluid status has been optimised commence hypertensive therapy.
- **Stage 2: Hypertension**
- Insert arterial line. Check baseline MAP
- Commence noradrenaline infusion at 2 mcg/min.
- Titrate Noradrenaline to clinical response (up to 10 mcg/min). Aim to increase MAP upto120 mmHg initially. Watch for bradycardia.
- If no response after 2 h (having achieved a MAP of 120 mm Hg) set target for increasing MAP upto130 mmHg. May have to consider additional agents and referral to Intensive Care Unit (ICU)
- If patient has a history of pulmonary oedema or cardiac failure,or is already bradycardic consider dobutamine (2.5 – 10 mcg/kg/min). Watch for tachycardia, arrhythmias. May be given via dedicated peripheral cannula. Discuss with ICU.
- If symptoms persist despite above measures consider invasive cardiac monitoring on ICU, e.g., LiDCO, PulseCO, PA catheter. Further management to be guided by Cardiac Index / Pulmonary Capillary Wedge Pressure. Maintain fluid profile and tailor haemodynamic support to Systemic Vascular Resistance (SVR)
- Cardiac Index monitoring in l/min/m2 can be used from the outset to guide fluid therapy if noninvasive monitoring (task force monitor) is available.

Box 1

Prospective data was collected for three epochs: before onset of DIND, during DIND and after initiation of triple-H therapy. The probes were removed if there were no symptoms of vasospasm by day 5 post insertion, if there was complete reversal of vasospasm or development of established infarct (Box 2).

Results

The study is ongoing and to date five patients have been recruited. Four of these patients were awake while the fifth patient was not roused from anaesthesia within the duration of then monitoring because of poor respiratory function.



Box 2

One patient developed DIND and triple H therapy was administered to one patient. Patient characteristics and individual clinical results are detailed.

The first patient was a 58-year-old, known hypertensive, non smoker caucacian male.

He was admitted with a Fisher 3, WFNS 2 SAH secondary to ruptured anterior communicating artery aneurysm. Neuromonitoring was commenced day 1 post bleed at the time of insertion of external ventricular drain for hydrocephalus. The aneurysm was coiled on day 2. The patient experienced new onset waxing and waning leg weakness on day 3, not attributable to any other cause and the clinical diagnosis of vasospasm was made. However the patient was maintaining his own blood pressure and met departmental protocol parameters for MAP and CVP. Hence no additional triple-H therapy was administered. On day-5 he made a complete symptomatic recovery. The monitoring results are displayed in Fig. 1. The central venous pressure was between 9 and 13 during the entire monitoring period. While no difference in regional cerebral blood flow or tissue oxygenation was observed with DIND, lactate pyruvate ratio seemed to correlate with clinical symptoms. Again with elevation of MAPs from 80 to 112 (mean), the lactate pyruvate ratios improved while the rCBF or oxygen readings did not show any statistical difference.

The second patient was 61 year old, caucasian male. He was a chronic smoker with mild COPD. He presented with Fisher 4, WFNS 3 SAH from anterior communicating artery aneurysm. On the day of bleed (day 0) he had a seizure, aspirated and dropped level of consciousness. At this stage he was intubated and ventilated. Coiling of the aneurysm with subsequent evacuation of hematoma for rising ICP was done on day 1 at which stage neuromonitoring was commenced. On day 5 he had a drop in MAP and with CPP below 60, triple H therapy was started. Target MAPs were achieved and a corresponding significant change in blood flow, tissue oxygenation and lactate pyruvate ratios was observed. On day 6, monitoring was stopped. Sedation withdrawal was possible on day 7 at which stage the patient was extending. He subsequently died on day 9 (Fig. 2).

Third patient was 43 years old female. She presented with Fisher 3, WFNS 1, ruptured right middle cerebral artery aneurysm. She was coiled on day 2 and made an uneventful recovery. Fourth patient was a 71 years old hypertensive female presenting with Fisher 3 + 4, WFNS 2, ruptured anterior communicating artery aneurysm. This was coiled on day 1. She was agitated during the monitoring period and made a slow recovery with mild cognitive deficits. The last patient was a 54 years old male. He presented with Fisher 3, WFNS 4 SAH secondary to rupture of left middle cerebral artery aneurysm. This was clipped on day 2 and he made an uneventful recovery.

All the last three patients had stable neurological and monitoring parameters during the monitoring period (Fig. 3).

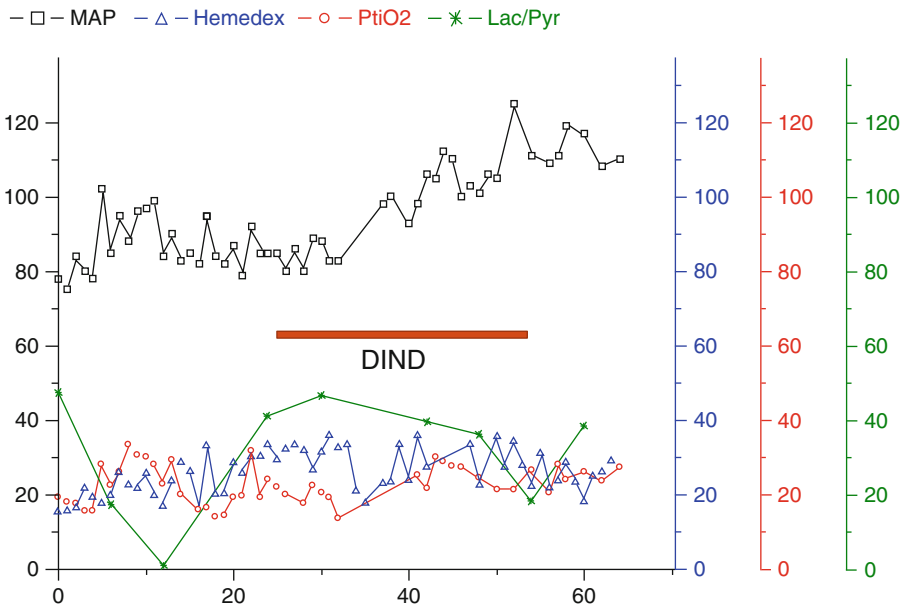


Fig.1 Monitoring results Pt. 1 (DIND). X axis: time in hours, Y axis: MAP and PtiO2 in mmHg, rCBF(Hemedex) in ml/100 g tissue/min, and Lactate/Pyruvate as a ratio

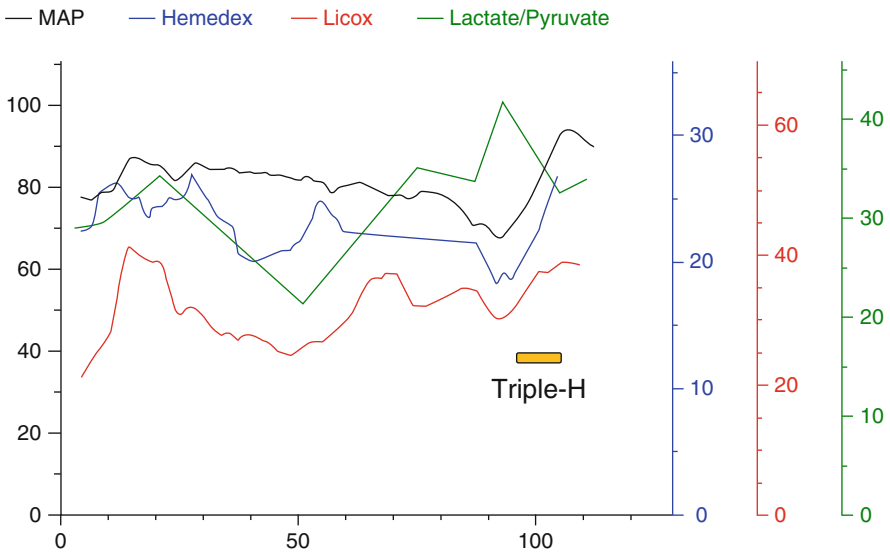


Fig. 2 Monitoring results Pt. 2 (Triple H). X axis: time in hours, Y axis: MAP and Licox in mmHg, rCBF(Hemedex) in ml/100 g tissue/min, and Lactate/Pyruvate as a ratio)

Discussion

This is the initial phases of the study and the patient numbers are very small to draw any conclusions. Nevertheless some interesting observations have been made which we would like to discuss.

No adverse event was noted due to invasive neuromonitoring in any patient.

In the one patient with DIND, only L P ratios were associated with change of clinical condition. The figures were in accordance with previous microdialysis data [4]. We did not observe any baseline differences in lactate or glucose values at the start of monitoring. However, glycerol values were high to begin with in all patients. These gradually tapered if no subsequent ischaemic insults were encountered.

Autoregulation was intact in all our patients to begin with [5]. Only one patient (patient 2) subsequently lost autoregulation.

In this case, triple-H therapy was effective in restoring cerebral physiological parameters, including microdialysis. Unfortunately the clinical correlation of this could not be established. We are yet to come across the clinical situation where triple H is used in context of DIND. As such, the primary objective of the study remains unanswered and we hope to get better picture on the whole with more patients in the study.

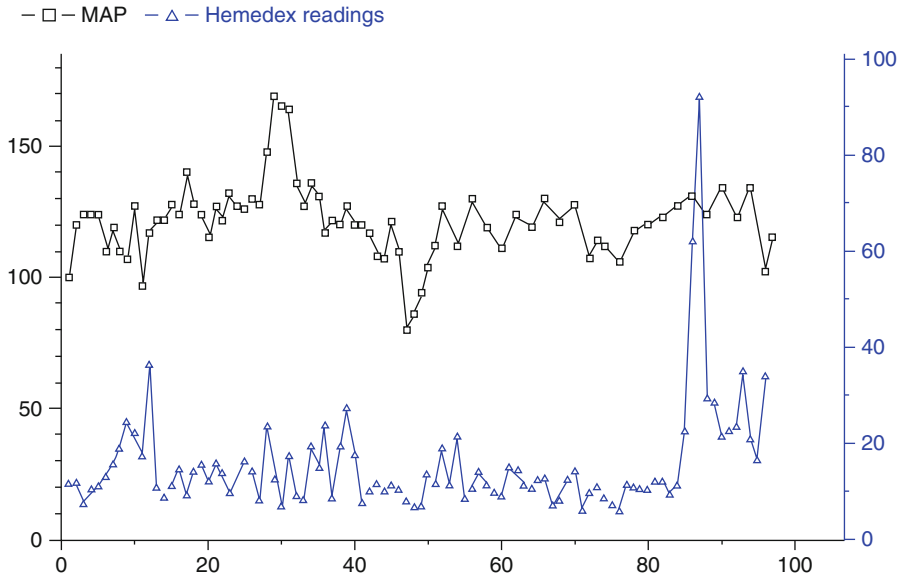


Fig. 3 Example of intact autoregulation patient 3 5. X axis: time in hours, Y axis: MAP in mmHg, rCBF(Hemedex) in ml/100 g tissue/min)

We understand that one major limitation of this study is that the baseline variability for the monitoring parameters has not been well established and as such currently a protocol driven therapy is used. With further enrolment and data analysis we hope to generate some baseline data, especially in awake patients.

Conclusions

The study is ongoing. Early results suggest safety and feasibility of using invasive multimodal neuromonitoring in clinical setting to establish an understanding of relationship between clinical symptoms, brain perfusion, oxygenation and energy metabolism in real time and to test and guide therapy in future.

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Nicardipine Pellets for the Prevention of Cerebral Vasospasm

Claudius Thomé, Marcel Seiz, Gerrit Alexander Schubert, Martin Barth, Peter Vajkoczy, Hidetoshi Kasuya, and Peter Schmiedek

Abstract Regardless of numerous efforts there is no prophylactic treatment proven to be effective in the prevention of cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH). As systemic administration of vasoactive drugs has been associated with significant side effects and insufficient efficacy, intrathecal administration of nicardipine prolonged-release implants (NPRI) has been developed. At the time of surgical clipping of the ruptured aneurysm, NPRIs are positioned next to the large cerebral arteries. Several clinical protocols revealed that NPRIs dramatically reduce the incidence and severity of angiographic vasospasm, which was paralled by a reduction in cerebral infarction and delayed ischemic neurologic deficit. On average, the incidence of angiographic vasospasm decreased from approximately 70% to less than 10%. Efficacy seemed to be dose-dependent and reduced for peripheral vasospasm. Nevertheless, a significant improvement of functional outcome was demonstrated. A separate patient series demonstrated the efficacy of fewer NPRIs in the perichiasmatic cistern. Further investigations were performed in comparison to coiled patients and with intraventricular implantation of NPRIs, which had a less pronounced effect. Overall, NPRIs are a most promising option for the prevention of cerebral vasospasm after SAH and large controlled trials are needed to further confirm these results.

C. Thomé (✉), M. Seiz, and G.A. Schubert
Department of Neurosurgery, Innsbruck Medical University, Anichstr. 35, A 6020 Innsbruck, Austria
e mail: claudius.thome@uki.at
M. Barth and P. Schmiedek
Department of Neurosurgery, Universitätsmedizin Mannheim, University of Heidelberg, Theodor Kutzer Ufer 1 3, 68167 Mannheim, Germany
P. Vajkoczy
Department of Neurosurgery, Charite' Universitätsmedizin Berlin, Berlin, Germany
H. Kasuya
Department of Neurosurgery, Tokyo Women's Medical University, Tokyo, Japan

Keywords Subarachnoid hemorrhage · Cerebral vasospasm · Nicardipine · Prolonged-release implants · Intrathecal

Introduction

Regardless of numerous efforts, the pathophysiology and pathogenesis of delayed ischemic neurological deficits (DIND) following aneurysmal subarachnoid hemorrhage (SAH) remain far from clear and DIND still is an important cause of morbidity and mortality after SAH. Secondary deterioration has been mainly attributed to cerebral vasospasm and calcium is known to play a central role in post-hemorrhagic vasoconstriction. In a subpopulation of SAH patients that is characterized by large amounts of blood in the basal cisterns and in the subarachnoid space (i.e., Fisher grade III), angiographic vasospasm occurs in up to 80% of patients, while cerebral infarction can be detected in up to 40% of patients [8, 10]. Systemic administration of vasoactive drugs, like calcium channel antagonists, has been associated with significant side effects and insufficient efficacy, so that there has not been a prophylactic treatment proven to be effective in the prevention of cerebral vasospasm following SAH so far. In an attempt to avoid the systemic drug effects, intrathecal administration of slow release preparations has been developed. Particularly, nicardipine prolonged-release implants (NPRI) have been investigated in several clinical protocols.

Material and Methods

NPRIs (pellets with a diameter of 2 mm and a length of 10 mm) containing 4 mg of nicardipine have been used as previously described by Kasuya et al. [6]. At the time of surgical clipping of the ruptured aneurysm, NPRIs were

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positioned next to the large cerebral arteries or in the basal cisterns. A Medline-based literature search was conducted to identify all clinical series published on the use of NPRIs.

In a separate series of 14 patients four NPRIs were placed in the perichiasmatic cistern. Inclusion criteria were clinical grades II to IV according to Hunt and Hess and Fisher grades III or IV. Aneurysms were located at the anterior communicating ($n = 6$), middle cerebral ($n = 3$), posterior communicating ($n = 4$) or pericallosal artery ($n = 1$) and were surgically occluded within 48 h after onset. Angiographic vasospasm was analyzed by digital subtraction angiography (DSA) on day 8 ± 1 after ictus or at the time of clinical or neuromonitoring signs of cerebral vasospasm. Outcome was evaluated at 6 weeks using the Glasgow Outcome Scale (GOS), which was dichotomized in favorable (no or moderate disability) and unfavorable outcome.

Results

Clinical use of NPRIs was started in October 1999, by the pioneer of intrathecal administration of calcium channel antagonists, Dr. Kasuya. His group first reported a series of 20 patients in 2002. There was no vasospasm locally at the sites, where the pellets (2 10) were positioned, and only one patient (5%) developed DIND [6]. It is important to note that all patients were characterized as grade III according to Fisher, thus constituting a "high-risk" population with an estimated risk of angiographic vasospasm of 70% and a risk of DIND of 30%. In a prospective 5-year series, 69 "high-risk" patients were prophylactically treated with NPRIs and compared to 28 "low-risk" (good clinical grade, sparse subarachnoid blood) patients. DIND occurred in 6% of "high-risk" and 11% of "low-risk" patients [5]. Summarizing the first 100 patients, Krischek et al. confirmed that NPRIs locally prevented vasospasm, and DIND respectively infarction rates were as low as 7% and 5% in a "high-risk" SAH population [7]. A multi-center cooperative study on NPRIs in Tokyo on 136 patients confirmed these results with rates of angiographic vasospasm, cerebral infarction and DIND of only 25%, 12% and 8% respectively [4].

Based on these encouraging results, our group designed a single-center prospective randomized phase IIa study with 32 "high-risk" patients. In contrast to previous series, a fixed number of ten pellets was applied to all patients and radiological assessment of study and control groups was performed blinded at an outside institution. DSA revealed dilatation (!) of basal arteries at day 8 to an average of approximately 115% of baseline and a positive effect even on distal arteries. Overall, angiographic vasospasm was reduced from 73 to 7% ($p < 0.001$) and cerebral infarction from 47 to 14% ($p = 0.05$). Due to these dramatic effects clinical outcome was significantly improved in the NPRI group [1]. More detailed outcome analysis after 1 year revealed superiority of the NPRI group in functional outcome according to National Institutes of Health Stroke Scale, modified Rankin Scale and Karnofsky Performance Scale, while quality of life showed no difference between groups [3].

In an attempt to clarify dosing and positioning of NPRIs, the above-mentioned series of perichiasmal administration of four NPRIs was prospectively conducted in 14 patients. Angiographic vasospasm was present in 31% and clinical outcome was favorable in 57% of patients. An illustrative example is given in Fig. 1.

In a prospective non-randomized study by Schneider et al., 27 patients receiving NPRIs after surgical clipping were compared with 25 patients without NPRIs treated by endovascular coiling. Angiographic vasospasm occurred in 11% (NPRI) vs. 48% (coiling) and cerebral infarction occurred in 7% (NPRI) vs. 28% (coiling) [9]. Intraventricular application of six or ten NPRIs was prospectively applied in 31 patients after surgical clipping or endovascular coiling by our group. In comparison to the above-cited control group the incidence of angiographic vasospasm was reduced from 73% to 53% (6 NPRIs) and 48% (10 NPRIs). Patients with NPRIs showed less vasospasm-related cerebral infarction and better outcome scores [2].

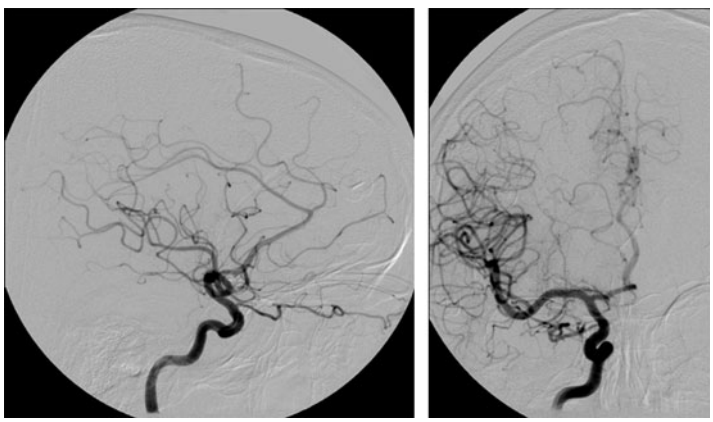

Fig. 1 Illustrative case of a 56 year old male patient with SAH Hunt and Hess grade II and Fisher grade III. Four NPRIs were placed in the perichiasmatic cistern after surgical clipping of the ruptured aneurysm of the anterior communicating artery. Lateral and ap digital subtraction angiogram 8 days after ictus demonstrated no evidence of vasospasm but rather dilatation particularly of the middle cerebral artery

Discussion

NPRIs dramatically reduce the incidence and severity of angiographic vasospasm in the large cerebral arteries, which is paralled by a reduction in cerebral infarction and DIND. The effect seems to be dose-dependent [2] and is most pronounced when ten NPRIs are applied in the basal cisterns. In that case, studies indicate that efficacy expands to the contralateral side and the more peripheral arteries [1]. In patients at high risk for developing cerebral vasospasm the incidence of angiographic vasospasm is decreased from approximately 70% to less than 10%. In this subpopulation the risk of vasospasm-related infarction is reduced from approximately 30 to only 5%, greatly minimizing the associated morbidity and mortality. So far, none of the series demonstrated side effects of NPRI treatment. Recent results indicate that the combination of surgical clipping plus NPRIs is superior to endovascular coiling, which does not allow intrathecal drug administration during the procedure [9]. Intraventricular application of NPRIs is possible and safe, but its efficacy is much lower than cisternal application [2]. This is obviously due to the low local concentration of nicardipine at the basal arteries plus the drug´s lipophilic nature and may be exaggerated by the necessity to drain (drug-containing) cerebrospinal fluid for intracranial pressure control. Thus, local high-dose administration should be preferred.

Conclusion

Implantation of NPRIs reduces the incidence of cerebral vasospasm and delayed ischemic deficits, which led to an improvement of clinical outcome. Thus, NPRIs are a most promising option for the prevention of cerebral vasospasm after aneurysmal SAH and large controlled trials are needed to further confirm these results.

Conflict of interest statement We declare that we have no conflict of interest.

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Part IV: Imaging Studies

Neuromonitoring in Intensive Care: A New Brain Tissue Probe for Combined Monitoring of Intracranial Pressure (ICP) Cerebral Blood Flow (CBF) and Oxygenation

E. Keller, J. Froehlich, C. Muroi, C. Sikorski, and M. Muser

Abstract *Background*: The benefits of monitoring cerebral blood flow (CBF) in stroke patients are apparent. New techniques combining near infrared spectroscopy (NIRS) and indocyanine green (ICG) dye dilution to estimate cerebral hemodynamics are available. However, with transcutaneous NIRS and optodes applied over the skin, the signal is contaminated by extracerebral tissues. The objective is to develop a new brain tissue probe for combined monitoring of intracranial pressure (ICP), CBF and cerebral blood volume (CBV).

Methods: Conventional intraparenchymal probes for ICP monitoring are supplied with optical fibers. The light is coupled into the brain tissue and collected after absorption and scattering with a light detector. Venous injections of 0.2 mg/kgbw ICG are performed. The mean transit time of ICG (mttICG), CBF and CBV are calculated.

Results: With a prototype of the probe in a first patient with subarachnoid hemorrhage 6 pairs of repetitive measurements were performed. Mean values were for mttICG 5.6 ± 0.2 s, CBF 22.3 ± 2.8 ml/100 g/min and CBV 2.1 ± 0.3 ml/100 g.

Conclusions: NIR spectroscopy allows the synchronous determination of multiple parameters with one single device. By measurements in parallel with the NeMo Probe and NIRS optodes placed over the skin, new algorithms can be developed to subtract the extracerebral contamination from the NIRS signal.

Keywords Subarachnoid haemorrhage · Traumatic brain injury · Cerebral blood flow · Brain tissue probe · Intracranial pressure

E. Keller (✉), C. Muroi, and C. Sikorski
Neurocritical Care Unit, Department of Neurosurgery, University Hospital Zurich, Frauenklinikstrasse 10, CH 8091 Zurich, Switzerland
e mail: emanuela.keller@usz.ch
J. Froehlich
Laboratory for Electromagnetic Fields and Microwave Electronics, ETH Zurich, Zurich, Switzerland
M. Muser
NeMoDevices, Zurich, Switzerland

Introduction

The benefits of monitoring cerebral blood flow (CBF) in patients with subarachnoid hemorrhage, severe hemispheric stroke and head injury are apparent. Numerous techniques have been developed to estimate cerebral hemodynamics [1, 5, 10, 11, 14, 19]. However, each method has its own advantages and drawbacks. To date a suitable method for bedside CBF measurement, able to detect smaller areas of ischemia and easy to perform at the bedside is still lacking.

Optical methodologies may be the ideal instrument to monitor disease-related secondary brain injuries in instable patients in the environment of intensive care, emergency care and surgical units. New techniques combining near infrared spectroscopy (NIRS) and indocyanine green (ICG) dye dilution to estimate cerebral hemodynamics are available [3, 7, 8, 13, 15]. However, transcutaneous NIRS with optodes applied over the skin is controversially discussed, because the NIRS signal is contaminated by extracerebral tissues (skin, skull, cerebrospinal fluid layer) [4, 6, 17]. To obtain measurement values directly from the brain tissue a conventional probe for intracranial pressure (ICP) monitoring can be supplied with optical fibers for NIRS. For patients with severe brain injuries and stroke, where ICP probes are installed anyway because of brain oedema and intracranial hypertension, a new combined NIRS ICP probe may offer enhanced modality without an additional surgical intervention.

The objective is to develop a new intraparenchymal probe for combined monitoring of ICP, brain temperature, CBF and cerebral blood volume (CBV) with NIRS and ICG dye dilution.

Methods

The study was approved by the Ethics Committee of the University of Zurich. Measurements were performed in a patient with severe subarachnoid hemorrhage Hunt and Hess grade 5, Fisher 4 with ruptured aneurysm of the anterior communicating artery. Because of occlusive hydrocephalus

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a ventricular catheter (Bactiseal®, Codman, Johnson and Johnson, USA) was inserted to drain cerebrospinal fluid. After coiling the ruptured aneurysm, the patient developed brain edema and ICP monitoring with an intraparenchymal probe was needed. A prototype of the new NIRS ICP probe (NeMo Probe®, NeMoDevices AG, Switzerland) was inserted through a support bolt (Raumedic®, Germany) from a burr hole by an experienced neurosurgeon in the ICU (Fig. 1). The probe was placed into the brain tissue, 2 cm deep from the dura. The correct positioning of the probe was confirmed with computed tomography (CT) (Fig. 2).

For NIRS, conventional intraparenchymal probes for ICP monitoring (diameter 6 French) were supplied with optical fibers (NeMo Probe®, NeMoDevices, Switzerland). The light is coupled out into the brain tissue via a metalized microprism and collected after absorption and scattering with a light detector. A thermistor and an ICP sensor (microminiature strain gauge pressure sensor) were included at the tip of the probe. Before implantation the ICP sensors were extensively tested over 8 days and showed no significant measurable drift. A NIRS apparatus, specifically constructed for the measurement mode (NeMo System®, NeMoDevices, Zuerich, Switzerland) include the light sources, the hardware for data collection and the software to analyze the NIRS data (NeMo View®) (Fig. 3). Regular measurements were performed daily and repeated after 15 min under stable clinical conditions (unchanged ICP, mean arterial pressure, $paCO_2$). Central venous injections of 0.2 mg/kgbw ICG were performed, followed by the injection of 10 ml Glucose 5% flush. The ICG concentrations were calculated based on the changes in OD. Regional values for the mean transit time of ICG (mttICG), CBF and CBV were calculated according to published algorithms [8].

Statistical analysis: Standard deviation and coefficient of variation were calculated for repeated measurements. ICP values were compared using the correlation coefficient and paired t-test.

Results

In a first patient with subarachnoid hemorrhage, with two prototypes of the new brain tissue probe 16 measurements with ICG injections were performed on 4 consecutive days. No complications associated with the measurement technique occurred. The user-acceptance by neurosurgeons and ICU staff was as high as for conventional brain tissue probes for ICP monitoring. Day 3, the first probe had to be

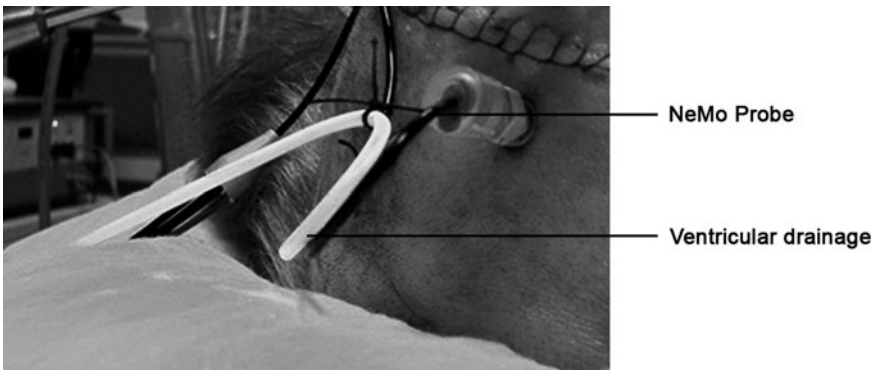

Fig. 1 *Dark*: NeMo Probe inserted through a support bolt from a burr hole; *bright*: ventricular drainage

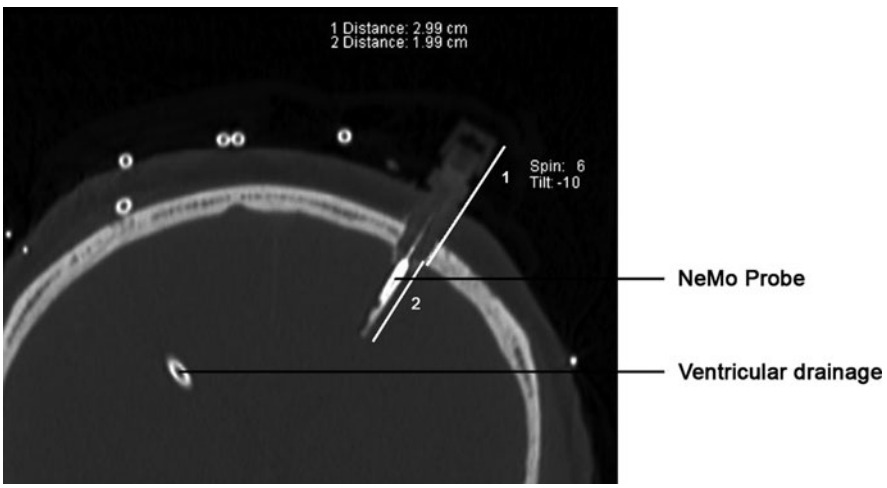

Fig. 2 Computed tomography (CT) scans showing the NeMo Probe and the ventricular drainage (coronar section, bone window)

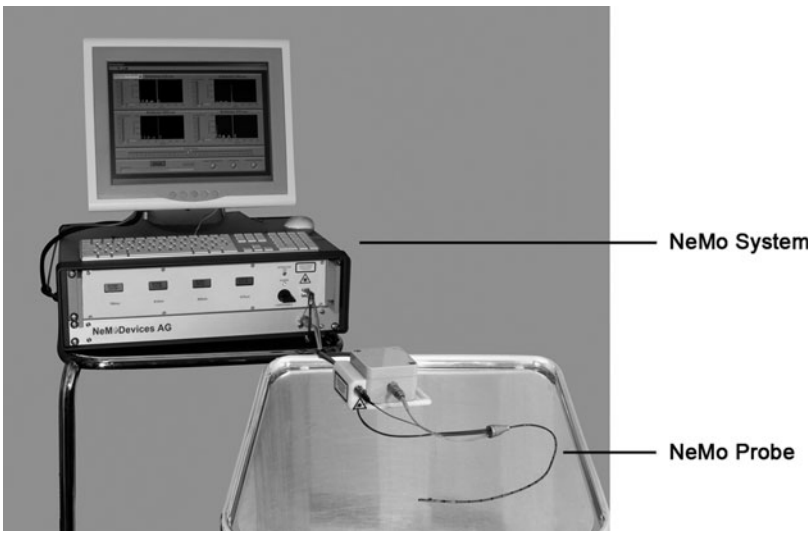

Fig. 3 NeMo System including light sources, data collection unit and software for data analysis

changed because of decreased signal to noise ratio. Day 5, before an additional measurement could be performed, the probe was pulled out by the nurse accidentally. In 12 single, 6 pairs of measurements, data analysis was successful. Mean values were for mttICG 5.6 ± 0.2 s, CBF 22.3 ± 2.8 ml/100 g/min and CBV 2.1 ± 0.3 ml/100 g. The coefficients of variation for repetitive measurements were for mttICG 0.046, CBV 0.18 and CBF 0.14. 12134 ICP values obtained with the NeMo Probe (ICP_{NeMo}) were compared with ICP values measured with a conventional ventricular drainage and an external tranducer (ICP_{VD}). The correlation coefficient between ICP_{NeMo} and ICP_{VD} was 0.7219 (95% CI 0.7132 to 0.7303; $p < 0.0001$).

Discussion

Originally, as scientific prototypes, the optical fibers for combined NIRS and ICP monitoring were first integrated into a conventional subdural probe for ICP-monitoring and tested in two patients [9]. Still, in clinical practice the use of ICP probes constructed as subdural types is not common yet, as intraparenchymal probes are preferred due to their higher measuring accuracy and their smaller diameter which makes their application less invasive [2, 16]. Therefore, the industrialized prototypes are now being produced as probes to be inserted directly into the brain tissue.

Two prototypes of the new intraparenchymal NIRS ICP probe could be tested in a first patient and values for mttICG, CBF and CBV could be determined. The measured values obtained with the NIRS ICP probe with a mean CBF of 22.3 ± 2.8 ml/100 g/min and a mean CBV of 2.1 ± 0.3 ml/100 g correspond to normal values obtained with $H_2{}^{15}O$ positron emission tomography (PET) (22.2 ± 4.9 ml/100 g/min for CBF and 2.7 ± 0.56 ml/100 g for CBV) or with dynamic susceptibility contrast magnetic resonance imaging (MRI) (23 ± 14 ml/100 g/min for CBF and 1.3 ± 0.4 ml/100 g for CBV) in the white matter [12, 18]. To test the accuracy of the measurement method, in a very first step its reproducibility was examined with repeated measurements under unchanged clinical conditions. The standard deviations and coefficients of variation are clinically well acceptable.

For patients with severe brain injuries and stroke, where ICP probes are anyway installed because of brain oedema and intracranial hypertension, the NeMo Probe offers enhanced modality modes (ICP, brain temperature, mttICG, CBF, CBV, changes of oxygenated, desoxygenated and total hemoglobin concentrations) without an additional surgical intervention. The new multimodal monitoring system provides information of cerebral hemodynamics, oxygenation and metabolism synchronously within minutes at the bedside and may allow optimising and individualising new neuroprotective therapies for every single brain injured patient.

Like other methods applying brain tissue probes, as microdialysis or brain tissue oxygen tension pressure ($PbtO_2$) monitoring, the major restriction of the NeMo System applying the NIRS ICP probe is that it is a regional measurement method, giving relevant results only if the probe is inserted into the area of interest. Cerebral vasospasm leading to focal ischemic events after subarachnoid hemorrhage may occur in different and multiple vascular territories, presumably not being observed by the NeMo Probe. Ongoing theoretical examinations applying different model simulations will clarify the extent of the measurement volume of the NeMo Probe, which depends not only on the distance between the light emitter and detector, but on different light scattering properties under specific pathophysiological conditions in the brain tissue.

The advantages of patches with NIRS optodes applied over the skin, is that they are strictly non-invasive. Several vascular

territories, therefore, can be monitored over both hemispheres symmetrically. Furthermore, measurements with the NeMo Probe and optode patches over the skin in parallel, will allow for the development of algorithms to determine and subtract extracerebral contamination. In less severely ill patients, not requiring an ICP monitoring probe and in patients treated with full anticoagulation (e.g. during cardiopulmonary bypass surgery) the non invasive approach with optode patches attached over the skin and optimized algorithms may be the first choice.

Conclusion

In conclusion: The new combined NIRS ICP probe allows the determination of multiple parameters synchronously with one single device. By measurements in parallel with the NeMo Probe and NIRS optodes placed over the skin, new algorithms will be developed to determine the contribution from extracerebral tissues within the cumulative signal obtained by transcranial NIRS.

Disclosures

E. Keller, J. Froehlich and M. Muser have a financial interest as founder, resp. member of staff of NeMoDevices AG.

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Vasospasm After Subarachnoid Hemorrhage: A 3D Rotational Angiography Study

Guo-En Yao, Qi Li, Xiao-Jiang Jiang, Juan Liu, Jia-Lun Li, Li-Li Zhang, Lei-Lei Li, John Zhang, and Peng Xie

Abstract *Background and Purpose*: The purpose of this study was to investigate the clinical value of 3D rotational angiography (3DRA) for evaluation of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage (SAH) by comparison with 2D digital subtraction angiography (DSA).

Methods: Forty-six patients who had undergone 2D DSA and 3DRA for evaluation of cerebral vasospasm following SAH were retrospectively analyzed. 3DRA was routinely performed after standard 2D DSA. 3D volume rendering images were created from 3DRA dataset and compared with DSA for the detection and characterization of vasospasm.

Results: Of the 46 patients investigated, 25 had vasospasm on 2D DSA images. No vasospasm was observed in 21 patients with aneurysmal SAH. According to the reference standard of DSA, 46 spastic segments were found in 25 patients with vasospasms. A total of 51 spastic segments were found on 3DRA volume rendering angiograms. The sensitivity, specificity, positive and negative predictive values of 3DRA for detecting vasospasm were 100, 76, 90, 100%, respectively.

Conclusion: The pseudo-spasm phenomenon was frequently observed on 3DRA volume rendering images. 3DRA was less useful than 2D DSA for evaluation of vasospasm after SAH.

Keywords Cerebral vasospasm · 3D rotational angiography · Digital subtraction angiography · Subarachnoid hemorrhage

G. E. Yao
Department of Neurology, The First Affiliated Hospital, Chongqing Medical University, Chongqing, China
Institute of Neuroscience, Chongqing Medical University, Chongqing, China
Department of Neurology, Daping Hospital, The Third Military Medical University, Chongqing, China
Q. Li and P. Xie (✉)
Department of Neurology, The First Affiliated Hospital, Chongqing Medical University, Chongqing, China
Institute of Neuroscience, Chongqing Medical University, Chongqing, China
e mail: Peng_xie@yahoo.com
X. J. Jiang and J. Liu
Department of Neurology, Daping Hospital, The Third Military Medical University, Chongqing, China
J. L. Li
Department of Medicine, Chongqing Medical and Pharmaceutical College, Chongqing, China
L. L. Zhang and L. L. Li
Institute of Neuroscience, Chongqing Medical University, Chongqing, China
J. Zhang
Department of Neurology, The First Affiliated Hospital, Chongqing Medical University, Chongqing, China
Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA, USA

Introduction

Cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH) is a devastating medical complication that usually occurs within 2 weeks after rupture of an intracranial aneurysm [10, 22]. It remains a major cause of morbidity and mortality and continues to affect a significant proportion of the SAH population. Symptomatic vasospasm secondary to SAH presents in some 17 40% of all cases [7]. Early detection and treatment of severe symptomatic cerebral vasospasm is essential to prevent future ischemic attacks [21]. In clinical practice, cerebral vasospasm is usually demonstrated by the narrowing of arterial vessels on angiograms before the onset of clinical signs and symptoms.

Over the years, transcranial doppler (TCD) has been the most widely used screening method for cerebral vasospasm in many institutions [8, 16]. However, the validity of TCD results could be affected by many factors and it could not be recommended as a diagnostic method for cerebral vasospasm in patients with ruptured aneurysms [11]. In recent

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years, multi-slice CT angiography (CTA) has emerged as an alternative imaging method for evaluation of cerebral vasospasms. However, CTA is less useful than DSA in assessment of postoperative cerebral vasospasm because the visualization of all arterial segments may be hampered by the beam-hardening artifacts of aneurysm clips [12, 15]. Digital subtraction angiography (DSA) has long been considered the gold standard in detection and characterization of aneurysms and cerebral vasospasms in the setting of SAH [4]. Recent reports suggested that DSA is less useful than 3D rotational angiography (3DRA) for detection of intracranial aneurysms [2, 6]. In previous reports, DSA negative small aneurysms confirmed at surgery were consistently reported by using 3DRA [19]. Because of the possibility of reconstruction in any chosen projections, 3DRA has supplemented DSA as a new gold standard in detection of aneurysms. However, the role of 3DR in detection of cerebral vasospasm has not been explored in previous reports.

The purpose of our study was to investigate the clinical value of 3DRA for evaluation of cerebral vasospasm in patients with aneurysmal SAH by comparison with 2D DSA.

Materials and Methods

Institutional review board approval was obtained. A total of 46 patients (32 female, 24 male, average age 51 years) who have undergone 2D DSA and 3DRA for evaluation of cerebral vasospasm following SAH at Daping Hospital were retrospectively analyzed. Patients were referred for DSA and 3DRA if they had clinically suspected ruptured intracranial aneurysms. Intra-arterial DSA was performed transfemorally by using a biplane unit. 3DRA was routinely performed after standard 2D DSA. DSA was performed by selective injection of 8- to 10-mL contrast material in the internal carotid and vertebral arteries. A standard projection format, including anteroposterior, lateral and oblique views was obtained and relevant images were sent to the picture archiving and communication systems (PACS).

When an aneurysm was suggested on DSA, additional 3DRA was routinely performed to delineate by using a biplanar C arm. Rotational angiography was performed with a 200° arc rotational run around the patient's head during intra-arterial injection of contrast material. The raw data was transferred to a dedicated workstation for postprocessing. 3D volume rendering reconstructions were made with a matrix size of 1024 pixels. The reconstructed 3D volume rendering images were sent to a PACS.

DSA images and reconstructed volume rendering rotational angiograms were retrospectively reviewed by separate investigators. The interpretation of DSA and 3DRA angiograms was performed in a blinded manner. In interpretation of the DSA and 3DRA results, the readers were blinded to the history of the patient and they were not aware of the results of the other technique. Images were initially reviewed by independent readers and if they had disagreement regarding the presence and severity of vasospasm, they discussed among themselves until a consensus was reached. In interpretation of DSA and 3DRA angiograms, the intracranial arterial tree was divided as the following anatomic segments: the internal carotid artery (ICA), A1 and A2 segments of anterior cerebral artery (ACA), M1 and M2 segments of middle cerebral artery (MCA), P1 and P2 segments of posterior cerebral artery (PCA) and the vertebrobasilar artery. In order to judge the presence and severity of spasm, hypoplastic vascular segments were excluded from the final analysis. In each segment, the readers had to evaluate the location and presence of a vasospasm. If the vasospasm was considered present, the investigators also had to evaluate the severity of stenosis by using a grading scale as follows: mild vasospasm (<30% luminal narrowing), moderate (30 70% luminal narrowing) and severe (>70% luminal narrowing). In total, the arterial trees were divided into four main categories: no vasospasm, mild vasospasm, moderate and severe vasospasm.

The 2 × 2 contingency tables were constructed from true-positive, false-positive, true-negative and false-negative 3DRA results by using DSA findings as reference. Sensitivity, specificity, positive and negative predictive values of 3DRA for detection of cerebral vasospasm were calculated by using DSA as standard.

Results

A total of 46 patients who had successfully completed DSA and 3DRA for evaluation of vasospasms were included into the final analysis. The average time between onset of symptoms and radiological examination of vasospasm was 6 days, with a range of 2 16 days. 3DRA was performed immediately after DSA examination in all patients. There were no procedure-related complications or technical failures.

Of these 46 patients, 25 had vasospasm on 2D DSA images. No vasospasm was observed in 21 patients with aneurysmal SAH. According to the standard reference DSA results, 46 spastic segments were found in 25 patients with aneurysmal SAH. Of the 46 angiographic spastic segments, 11 segments were classified as mild vasospasm, 16 segments as moderate spasm and the remaining 19 segments was rated as severe vasospasm. The spastic arterial segments were located at the ICA (n = 8), the A1 segment of the ACA (n = 18), the A2 segment of the ACA (n = 8), the M1 segment of the MCA (n = 10) and the M2 segment of the

MCA (n = 2). In our case cohort, none of the spastic segments were located at the arterial tress in the posterior circulation.

At 3DRA examinations, a total of 51 spastic segments were present on 3D volume rendered angiograms. The spastic segments were located at the ICA (n = 8), the A1 segment of the ACA (n = 20), the A2 segment of the ACA (n = 8), the M1 segment of the MCA (n = 13) and the M2 segment of the MCA (n = 2). All spastic segments found at DSA were present on 3DRA. No false-negative spastic segments were found at 3DRA. In most cases, 3DRA was as equal to DSA for delineation of the vasospam (Fig. 1). A total of 5 segments of disagreement were noticed between DSA and 3DRA. Of these five pseudo-spastic segments, two were located at the A1 segment of the ACA and the remaining three were located at the M1 segment of the MCA. In our study, the 3DRA pseudo-spastic artifacts may occupy the whole vessel segment or just part of the vessel. In one patient with anterior communicating artery aneurysm, the whole A1 segment of the ACA was regarded as severe vasospasm on 3DRA angiogram (Fig. 2).

However, no vasospasm was noticed on DSA images. In another patient with a MCA bifurcation aneurysm, the segmental spastic artifact, which was regarded as moderate vasospasm on 3DRA angiogram, was present at the middle portion of the MCA (Fig. 3). The sensitivity, specificity, positive and negative predictive values of 3DRA in detecting vasospasm were 100, 76, 90, 100%, respectively.

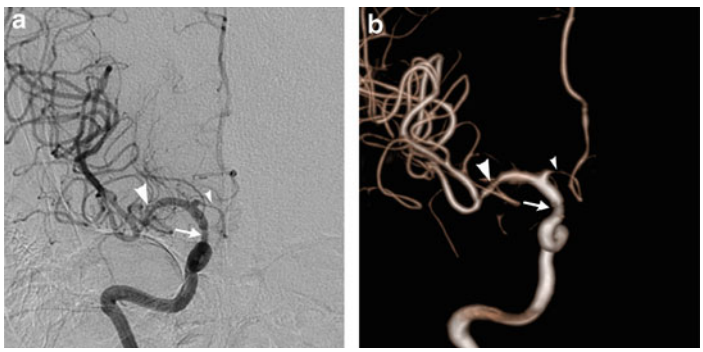

Fig. 1 Multiple vasospasms in a patient with subarachnoid hemorrhage. (**a**) Anteroposterior DSA shows multiple vasospasms located at the ICA (*arrow*), A1 segment of the ACA (*small arrowhead*) and the M1 segment of the MCA (large arrowhead). (**b**) Corresponding 3DRA angiogram showing the same multiple vasospasms

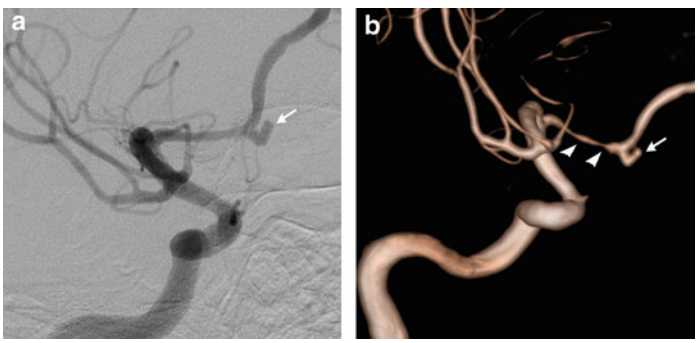

Fig. 2 Illustration of a 3DRA false positive spastic case in a patient with a ruptured anterior communicating artery aneurysm. (**a**) DSA shows the anterior communicating artery aneurysm (*arrow*). No vasospasm was noticed at the ACA. (**b**) 3DRA showing the anterior communicating artery aneurysm (*arrow*) and false positive severe vasospasm of the ACA (*arrowheads*)

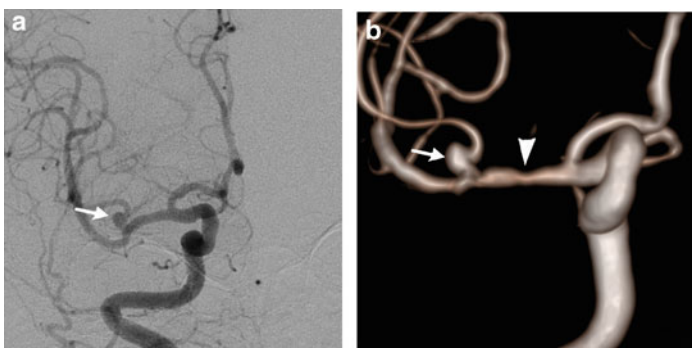

Fig. 3 Segmental pseudo vasospasm in a patient with a MCA aneurysm. (**a**) DSA shows a MCA bifurcation aneurysm (*arrow*). No vasospasm was noticed at DSA. (**b**) 3DRA showing the same MCA aneurysm (*arrow*) and a segmental pseudo spasm located at the M1 segment of the MCA

Discussion

Vasospasm due to rupture of an intracranial aneurysm is a medical condition that requires early diagnosis and management. Traditionally, DSA was the method of choice in the diagnostic workup of patients with ruptured intracranial aneurysms [1]. In previous reports, DSA has also been considered the standard of reference in assessment of the severity and location of the spasm in patients with signs and symptoms suggestive of cerebral vasospasms [9, 18].

In recent years, several alternative imaging techniques for delineation of vasospasm have been introduced into clinical practice. Several studies reported the initial results comparing CTA with DSA for detection and characterization of cerebral vasospasms. However, the technique was still less useful than 2D DSA in detecting vasospastic segments [13].

An inherent limitation of the technique is that it visualizes a real-life 3D vascular tree as a 2D object, which may result in inaccurate diagnosis. In recent studies, DSA false-negative aneurysms have been reported in several different studies [14, 23]. In contrast, 3DRA is a three dimensional imaging technique that allows reconstruction of a rotational angiographic sequence in unlimited projections. The ability to visualize objects in a 3D environment makes 3DRA a revolutionary imaging technique in the diagnostic workup of patient with ruptured intracranial aneurysms. In our study, we evaluated the diagnostic accuracy of 3DRA in detection and delineation of cerebral vasospasms by using DSA as reference standard.

In our present study, we found that 3DRA was capable of detecting vasospastic segments in all DSA positive cases. However, we also observed that five segments of disagreement between DSA and 3DRA results. These five segments were regarded as spastic segments at 3DRA. However, no real stenosis was found at DSA for any of these segments. We noticed that the pseudo-spastic segments were all located at the A1 segment of the ACA and the M1 segment of the MCA. For other segments of the intracranial vascular tree, the sensitivity, specificity, positive and negative predictive values of 3DRA in detecting intracranial vasospasm were 100%. Based on the results of our findings, we speculated that the spastic artifacts were location-dependent. Similar location-dependent sensitivities were also reported in previous studies of cerebral vasospasm with other imaging techniques. In early TCD studies, several investigators reported that the sensitivity of TCD in detecting cerebral vasospasm was remarkably low for spasms located at the ACA and MCA [17, 24]. In a study of 17 patients with suspected cerebral vasospasms, Yoon et al. compared CTA with DSA for evaluation of cerebral vasospastic segments [25]. They reported 94.7% sensitivity for vasospasms located at the proximal segments (A1, M1, ICA etc.) of the intracranial arterial tree. However, the sensitivity of multidetector CTA for detection of vasospasms was 100% for distal segments. In another study comparing CTA with DSA for evaluation of vasospasms, Binaghi et al. found that the sensitivity of CTA was 87.5% for spasms located at the A1 segment [3]. In contrast, 100% sensitivity could be expected for spasms located at other parts of the vascular tree. The results of our study, together with other recent reports, suggested that the alternative imaging techniques were equivalent to DSA for delineation of distal vessel segments. However, the diagnostic accuracy was relatively low for visualization of proximal segments of the intracranial vascular tree.

Although the mechanism underlying the phenomenon was not fully explored, we proposed that it is associated with 3D reconstruction techniques. Recently, 3D reconstruction techniques which allow real-life visualization of vascular trees have been increasingly used in neurovascular diagnostic imaging. In our study, the volume rendering technique was used for 3D reconstruction of rotational acquisition sequences. The technique was also widely used to visualize intracranial aneurysms and cerebral vasospasms at CTA. It is well established that the reconstruction usually requires specific software for postprocessing [20]. Loss of information may occur during postprocessing, which will result in vessel deformity. In previous CTA studies, it was reported that the degree of spasm may be overestimated on volume rendered images [25].

The artifact may also be caused by the influence of several technical factors during the acquisition of the 3DRA datasets. Besides technical factors, aneurysm and parent vessel hemodynamics may also affect 3DRA images. In a recent in vitro study, Ernemann et al. used a silicone model of the cerebral arteries to evaluate the influence of geometric parameters on aneurysm delineation [5]. They found that image quality of rotational angiography may be improved by employing longer rotational runs with more projection images. The filling of contrast material within the vascular structures was considered another important factor in 3DRA acquisition. Optimal 3DRA images would be obtained by homogeneous filling of the vascular structures. Filling deficits or pseudostenosis may occur when the vascular segments were filled by non-homogeneous contrast material.

Conclusion

The results of our present study suggested that 3DRA was less useful than 2D DSA for evaluation of vasospasm after SAH. In our study, we observed that a significant portion of pseudo-spastic artifacts may occur on 3DRA volume rendered angiograms. The artifacts were likely to

present at the proximal segment of the ACA and MCA. Knowledge of the pseudo-spastic phenomenon may help prevent diagnostic inaccuracies in the evaluation of cerebral vasospasm following SAH.

Conflict of interest statement We declare that we have no conflict of interest.

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Value of Noninvasive Imaging in Follow-Up of Intracranial Aneurysm

Li Jiang, Zhao-hui He, Xiao-dong Zhang, Bin Lin, Xiao-hong Yin, and Xiao-chuan Sun

Abstract Follow-up is necessary for treated and untreated aneurysms. The purpose of this study is to assess the results of treated aneurysms, the development of untreated aneurysms and the incidence of new aneurysms through short-term follow-up with noninvasive imaging, including CTA and MRA. More-than-once follow-up imaging with either CTA or MRA was performed in 73 patients, 65 of them suffering SAH. CTA was performed in 46 patients with clipped aneurysms, 9 patients with coiled aneurysms and 8 cases with untreated aneurysms. MRA was performed in ten patients with coiled aneurysms. CTA follow-up demonstrated that in 48 clipped aneurysms, 47 aneurysms completely disappeared; one aneurysm with neck remnant and one new aneurysm was found. No recurrence was found after microsurgical clipping. CTA follow-up provided limited information for ten coiled aneurysms because of poor quality images due to artifacts from coil. MRA follow-up of 12 coiled aneurysms showed there were no recanalization, recurrence or new aneurysm. In 20 untreated aneurysms, 19 stayed unchanged, and one aneurysm automatically disappeared. The newest generation of CTA and MRA can be used for following-up of intracranial aneurysms, and is more readily accepted by Chinese patients because of convenience, non-invasiveness and low price.

Keywords Noninvasive · Follow-up · Intracranial aneurysm · CTA · MRA

Introduction

Intracranial aneurysm often leads to catastrophic events once ruptured, which usually presents with subarachnoid hemorrhage (SAH) [1]. Furthermore, patients with intracranial aneurysms are also at risk for formation of new aneurysms or growth of untreated aneurysms or recurrence of treated aneurysms. Therefore, follow-up with vascular imaging is necessary to assess the outcomes of both treated and untreated aneurysms. In current clinical practice, three imaging modalities are widely used in the diagnosis and assessment of intracranial aneurysm, namely, digital subtraction angiography (DSA), computed tomography angiography (CTA), and magnetic resonance angiography (MRA) [1 3]. Although DSA is considered the "gold standard" both in diagnosis and follow-up of intracranial aneurysm, it is an invasive test with potential risk of complications. With the development of technique, noninvasive methods, such as CTA and MRA [1 6], plays an important role in the preoperative and postoperative evaluation of cerebral aneurysms.

Clinical Materials and Methods

Patients

This study was approved by the ethics committee of Chongqing Medical University, and informed consent was obtained from all patients. We selected all patients who underwent follow-up at least once with CTA or MRA in the period from January 2006, to September 2009. Some patients who experienced follow-up with DSA were excluded. Finally 73 patients (42 female and 31 male) with 90 aneurysms were included in the study, aged between 19 and 77 years (mean 48.5 years). Of the total 73 patients, 65 patients presented with SAH, 46 patients with 48 aneurysms experienced microsurgical clipping, 19 patients with 22 aneurysms underwent endovascular coiling, and eight patients did not get aneurysm treated. CTA was performed in 46 patients with clipped aneurysms, 9 patients with coiled aneurysms and 8 cases with untreated aneurysms. MRA was performed in

L. Jiang, Z. h. He, X. d. Zhang, B. Lin, X. h. Yin, and X. c. Sun (✉)
Department of Neurosurgery, First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P.R. China
e mail: sunxch1445@gmail.com

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Table 1 Locations of intracranial aneurysms

Characteristics	Data
Internal carotid artery (ICA)	7
Ophthalmic artery	4
Internal carotid artery posterior communicating artery	36
Anterior communicating artery	21
The anterior cerebral artery	1
Pericallosal artery	1
Anterior choroidal artery	2
The middle cerebral artery	15
Superior cerebellar artery	2
Vertebral artery	1

ten patients with coiled aneurysms. Mean duration of follow-up was 11.9 months (range 3 33 months).

Follow-up images were compared with previous images. Treated aneurysms were classified as completely disappeared, residual, recurrent and new aneurysms, and untreated aneurysms were classified as unchanged, grown, or new ones. Within the entire sample of aneurysms, aneurysmal locations involved the internal carotid artery (ICA) (n = 7), the ophthalmic artery (n = 4), the internal carotid artery posterior communicating artery (n = 37), the anterior communicating artery (n = 21), the anterior cerebral artery (n = 1), the anterior choroidal artery (n = 2), the middle cerebral artery (n = 14), the superior cerebellar artery (n = 2), the pericallosal artery (n = 1), and the vertebral artery (n = 1) (Table 1).

Image Acquisition

CTA: Titanium clips, which are believed to generate fewer artifacts than cobalt clips, were utilized in treatment of all the clipped aneurysms. To avoid motion artifacts, all patients were examined in supine position with heads fixed by a specialized brain holder during CT imaging. CTA were performed on 64-multidetector row spiral CT machine (Somatom Sensation 64; Siemens Medical Systems, Erlangen, Germany) with following parameters: pitch 0.531; 0.6-mm section collimation; 0.625-mm reconstruction interval; matrix 512 × 512; 180 240-mm field of view; 100 kV, 300 mA (nonenhanced image); 120 kV, 300 mA (contrast-enhanced image). The CT examination extended from the first cervical vertebra to the cranial vault. Each CTA acquisition was performed with intravenous injection of 80 mL of iodinated contrast medium (Ultravist, 370 mg iodine/mL; Schering, Germany) through an 18-gauge needle into the antecubital vein by a power injector at a rate of 4 mL/s. The test bolus method was employed to determine the imaging delay for each patient. All CTA data were transferred to a workstation (Advantage for Windows; GE Medical Systems) for postprocessing. Both conventional and subtraction CTA images were acquired, and clips were subtracted in subtraction CTA, but were displayed in conventional CTA.

MRA: 3D-CE MRA was performed with a 1.5T scanner (SIGNA, GE Healthcare) by using a standard head coil. Three dimensional fast low angle shot (3D-FLASH) was performed. Parameter of CE-MRA was shown, as follows: field of view, 16.8 24 cm; TR/TE, 5.6/1.4 ms; flip angle, 20°; section thickness, 1.4 mm; and acquisition time, 30 s. Abolus of 20 mL of magnevist Solution (Gd-DTPA) was injected at 3 mL/s with a flush of 20 mL of saline, by using a power injector (MEDRAD, SPECTRIS).

Image Interpretation

CTA and MRA images were independently interpreted by two professional neuroradiologists who have rich experience in vascular diagnostic neuroradiology. For CTA, both conventional and subtraction CTA images were acquired, and all follow-up images were compared with previous images, including pre-operative and post-operative images. Treated aneurysms were classified as completely disappeared, residual, recurrent or new aneurysms, and untreated aneurysms were classified as unchanged, grown, or new ones. We also compared the value of CTA and MRA after endovascular coiling.

Result

Seventy-three patients with 90 aneurysms were included in the study, and CTA follow-up demonstrated that of 48 aneurysms treated with titanium clips, 47 aneurysms completely disappeared, and only one clipped aneurysm on the anterior communicating artery was found neck remnant 7 days after clipping, but remained unchanged as showed by CTA follow-up twice at an interval of 9 months (Fig. 1b1 b3). Another aneurysm of anterior communicating artery was considered completely clipped at surgery, but one new aneurysm was found on the anterior communicating artery (just on the opposite side of the treated aneurysm) by CTA 3 months after clipping, and became larger 10 months after clipping; however, treatment for the aneurysm was still not indicated (Fig. 1a1 a3). One 40-year-old male patient was diagnosed with aneurysm in left middle cerebral artery with a history of SAH and underwent microsurgical clipping with a satisfactory effect, and the aneurysm had body remnant identified by CTA 7 days after clipping, but the remnant was confirmed disappeared by CTA follow-up 5 months and 1 year after discharge (Fig. 2d1 d4). Therefore, we consid-

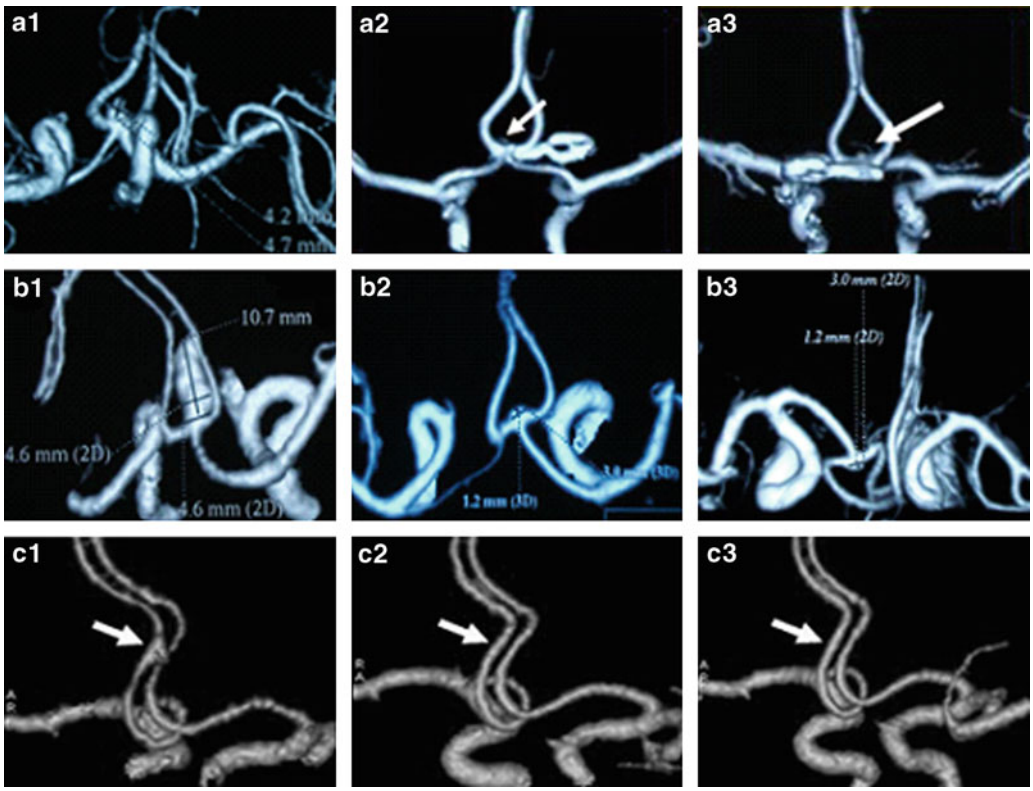

Fig. 1 (**a1**) CTA image obtained before operation, shows an aneurysm of the anterior communicating artery; (**a2**) CTA image obtained 3 months after discharge, shows that the clip remains stable without shift, and no recurrence is found, but there is a small protuberance on the opposite side of clip; (**a3**) CTA image obtained 10 months after discharge, shows the protuberance that was found 7 months before, became larger, which is considered by the neuroradiologists as new formative aneurysm. (**b1**) CTA image obtained before operation, shows a aneurysm of the anterior communicating artery; (**b2**) CTA image obtained 3 months after operation, shows that, the aneurysm had a neck remnant; (**b3**) CTA images obtained 9 months after discharge, shows the remnant remained unchanged. (**c1**) CTA image shows an aneurysm of the anterior cerebral artery, and the patient did not experienced treatment of aneurysm; (**c2**) CTA image obtained 3 months after discharge, shows that the aneurysm is spontaneously disappeared; (**c3**) CTA image obtained 7 months after discharge, shows no aneurysm is found

ered that the aneurysm should not be defined as residual but completely disappeared. No recurrence was found after microsurgical clipping. CTA follow-up provided limited information for ten coiled aneurysms because of poor quality images due to artifacts caused by coil, which render adequate evaluation impossible. One untreated aneurysm spontaneously disappeared, which was confirmed by CTA follow-up twice at an interval of 7 months (Fig. 1c1 c3). The other 19 untreated aneurysms were found unchanged, which was demonstrated by an average 6-month-follow-up with CTA. Of 12 coiled aneurysms follow-up with MRA (Fig. 2a1 a3), no recanalization, recurrence or new aneurysms were found. (Tables 2 and 3)

Discussion

Digital subtraction angiography (DSA) is considered the current gold standard for both diagnosis and evaluation of intracranial aneurysms, because of high spatial resolution [1 3]. However, it is an invasive and time-consuming technique, carrying a series of complications, such as infection of incisions, groin hematoma, arteriovenous fistulas, hemiparesis, confusion, and sometimes even death, although the morbidity and mortality is very low [1, 7, 8]. Furthermore, DSA is not offered in the outpatient department; therefore patients have to be admitted, which brings out a comparatively higher treatment cost. In addition, the DSA procedure is not comfortable, making it a big problem for patient compliance with follow-up examinations, especially in China. At our institution, most aneurysmal patients are inclined to accept follow-up with noninvasive test, including CTA and MRA, rather than DSA.

CTA, which is noninvasive, time-saving, and comparatively low-cost, is frequently used for detection of intracranial aneurysms with high sensitivity and specificity which have been reported to approach that of DSA, and the latest generation of three-dimensional CT angiography is capable of providing important surgical information [1, 4, 9 12]. Some researchers even think that CTA may potentially replace DSA in the emergency setting [13]. At our institution,

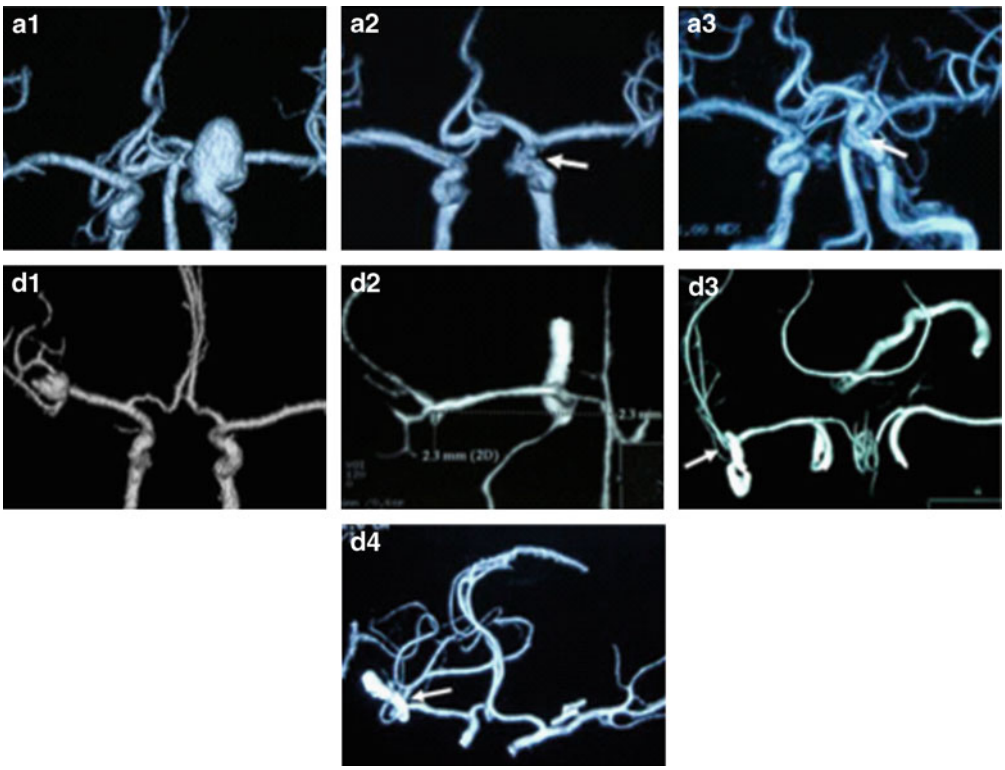

Fig. 2 (**a1**) CTA image obtained before operation, shows a big aneurysm of the ophthalmic artery, whose diameter is over 15 mm; (**a2**, **a3**) MRA images obtained 3 and 9 months after discharge, shows no remnant or recanalization. (**d1**) CTA image obtained before operation, shows a aneurysm in left middle cerebral artery; (**d2**) CTA image obtained 7 days after clipping, shows that the aneurysm had a body remnant; (**d3**, **d4**) CTA images obtained 5 and 12 months after discharge, shows that the body remnant disappears

Table 2 Outcomes of aneurysms (follow up with CTA)

Characteristics	Data
Clipped aneurysms	48
Completely disappeared aneurysms	47
Residual aneurysms	1
New aneurysms	1
Recurrent aneurysms	0
Untreated aneurysms	20
Spontaneously disappeared aneurysms	1
Unchanged aneurysms	19
Coiled aneurysms	10

Table 3 Outcomes of aneurysms (follow up with MRA)

Characteristics	Data
Coiled aneurysms	12
Completely disappeared aneurysms	12
Residual aneurysms	0
New aneurysms	0
Recurrent aneurysms	0

almost all aneurysmal patients experience CTA right after being admitted. Meanwhile, many studies also demonstrate that CTA is a reliable tool which can be used to evaluate the outcome of clipping [2, 14 16]. However, some investigators conclude that CT angiography is still not equivalent to DSA due to artifacts caused by metal clips and patient movement [2, 15, 17 19]. In this study, to obtain optimal images, three methods were adopted: (1) all clipped aneurysms were treated by titanium clips, which are considered to generate fewer artifacts than cobalt clips [2]; (2) all CTA were performed on 64-row multidetector CT and CTA subtraction techniques were involved too; (3) the application of imaging parameter and contrast medium were based on the recommendation of other studies [18, 20 22] as well as our own experience. In addition, all patients' heads were fixed during CT imaging to prevent motion artifacts. Therefore, high quality images with more useful information and fewer artifacts were acquired, providing higher accuracy to the assessment of residual aneurysm filling, the patency of the adjacent parent vessels, the remaining vasculature for de novo aneurysms and the clip position.

In this study, CTA follow-up demonstrated that only one clipped aneurysm on the anterior communicating artery had neck remnant but remained unchanged (Fig. 1b1 b3), and one new aneurysm was found on the anterior communicating artery, just on the opposite side of treated aneurysm, by CTA 3 months after clipping

(Fig. 1a1 a3). There is a strange case: one 40-year-old male patient was diagnosed with aneurysm in the left middle cerebral artery with a history of SAH and underwent microsurgical clipping with a satisfactory effect, and the aneurysm had body remnant identified by CTA 7 days after clipping, but was confirmed disappeared by CTA follow-up 5 months and 1 year after discharge (Fig. 2d1 d4). Unfortunately, the patient refused DSA, so adequate imaging data could not be collected, and the real reasons why the residual body of the aneurysm disappeared remain unclear. As a result, we can only presume that microsurgical clipping changed the hemodynamics of the parent artery, which led to the disappearance of aneurysmal remnants; therefore, we considered that the aneurysm should not be defined as residual but completely disappeared.

Nine patients who experienced endovascular coiling also experienced follow-up with CTA after coiling, but artifacts caused by coils precluded evaluation in the region of the parent vessel aneurysm complex. Twenty aneurysms remained untreated, 19 of which were found unchanged by CTA, and one ruptured aneurysm on A3 segment of the anterior cerebral artery was found to have automatically disappeared by CTA.

MRA is another noninvasive examination, which has good correlation with DSA and is considered a choice for the diagnosis of intracranial aneurysm [5, 13, 23]. Meanwhile, the value of MRA in the follow-up of intracranial aneurysms treated with coils has already been demonstrated by many studies [6, 20, 24 30].

For better visualization of coiled aneurysms and of branch arteries and residual neck with fewer artifacts, contrast-enhanced MRA (CE-MRA) was adopted in this study, which is considered superior to three-dimensional time-of-flight MRA (3D TOF-MRA) for visualization of small remnants and uncovered a larger neck remnant or filling of the coil pack with fewer artifacts [3, 31, 32]. As a result, high quality images with good spatial resolution could be obtained.

To our knowledge, few reports have independently addressed the role of noninvasive techniques (including CTA and MRA) for the follow-up of treated intracranial aneurysms without DSA. In most studies, DSA is performed as the gold standard, and the findings of CTA or MRA are compared with DSA, although the accuracy of CTA and MRA has already been widely demonstrated. Therefore, in this study, only CTA or MRA was performed in the follow-up, and to obtain high quality images, we reviewed literature, some methods that were widely recommended [1, 3, 18, 20 22, 31 33], as well as our own experience, which has been explained in detail above. A noteworthy fact is that the incidence of follow-up of intracranial aneurysm has obviously increased, since CTA and MRA were used for diagnosis and evaluation of intracranial aneurysm at our institution.

We acknowledge several potential limitations of this study: it was a single-center study involving a limited number of operators; also, the sample size of 73 patients is small, and patient compliance with follow-up remained a big problem, although the incidence of follow-up of intracranial aneurysm has obviously increased at our institution in recent years, especially for MRA; only ten patients treated by detachable coils came to follow-up with MRA. In addition, long-term follow-up is still needed to assess the outcomes of intracranial aneurysms.

Conclusion

The latest generation of CTA and MRA is reliable and with noninvasive tools can provide high quality images. Surgically clipped aneurysm should be followed up with CTA, while aneurysm after coiling should be followed up with MRA, and both of them can be used for following-up of intracranial aneurysms independently without DSA. Furthermore, long-term follow-up with larger sample size is still needed.

Conflict of interest statement We declare that we have no conflict of interest.

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Neuroimaging Research on Cerebrovascular Spasm and Its Current Progress

Fang Chen, Xiaoming Wang, and Bihua Wu

Abstract The cerebrovascular spasm is a common complication of subarachnoid hemorrhage. The prognosis is affected severely with regard to quality of life of patients, and earlier determination of the cerebral vasospasm becomes very important. In recent years, there have been many research results in early judgment of cerebrovascular spasm, and imaging technology research is particularly prominent in this area. This article summarizes the advantages and disadvantages and the specific roles of several common imaging technologies to determine the early stage of cerebral vasospasm. Transcranial Doppler (TCD) was the first used to examine cerebral vasospasm and mainly detected vascular hemodynamic changes of cerebrovascular spasm in patients. Digital subtraction angiography is the gold standard for diagnosis of cerebral vasospasm, but its efficacy in determining severity of cerebrovascular spasm indexing is still in dispute. It is invasive, it is difficult to repeat the examination, and it can induce complications, so its clinical application is limited. CT imaging technology is a hot topic in this area. There is an important guiding significance in early diagnosis and treatment of cerebral vasospasm in CT perfusion imaging (PCT) and CT angiography (CTA). PCT mainly performs qualitative and quantitative analysis through hemodynamic parameters such as cerebral blood flow, cerebral blood volume, and mean transit time. CTA is minimally invasive, fast, and reliable as an efficient imaging technology, and will possibly replace DSA for the assessment of vasospasm, particularly in critically ill patients with cerebral vasospasm in an urgent examination. This means it has greater value and helps to improve the prognosis of patients. MR imaging in the early stages to judge cerebral vasospasm has great value. DWI can effectively assess the cerebral vascular spasm earlier to detect trace bleeding sites and reflect the damage of cerebral vasospasm by apparent diffusion coefficient. Combined with DWI and PWI, perfusion can be understood in all regions and can be found in ischemic penumbra, which is a more accurate way to determine cerebral vasospasm and more beneficial in guiding the treatment of patients and improving their prognosis. However, there have been false-negatives and false-positives when detecting cerebrovascular spasm post-SAH. It is of great importance to select accurate, convenient, non-invasive imaging technologies that judge cerebrovascular spasm and guide treatment that improves the prognosis of these patients and other aspects.

Keywords Vasospasm · Imaging · TCD · DSA

Introduction

Cerebrovascular spasm is a common complication of subarachnoid hemorrhage caused by intracranial aneurysm rupture, brain trauma, surgery, etc. It is also one of the significant causes of disability and death in SAH patients. Therefore, early diagnosis and treatment of cerebrovascular spasm is critical. Recent research shows that neuroimaging has great value for early diagnosis and detection of cerebrovascular spasm. The study and its progress in this area are summarized as below.

Transcranial Doppler (TCD)

Aasid et al. first used TCD technology to carry out dynamic monitoring of middle cerebral artery (MCA), anterior cerebral artery (ACA), posterior cerebral artery (PCA), vertebral artery (VA) and the basilar artery (BA) and other vessels for people confirmed as cerebral vasospasm patients through digital subtraction cerebral angiography. They found that the blood flow velocity was significantly increased during

F. Chen, X. Wang (✉), and B. Wu
Department of Neurology, Affiliated Hospital, North Sichuan Medical College, Nanchong, Sichuan 637007, China
e mail: wangxm238@163.com

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vasospasm, and they set the situation when MCA average blood flow velocity (VMCA) was larger than 120 cm/s as the diagnostic criteria for cerebral vasospasm. They also set the severity grading criteria: mild for VMCA between 120 140 cm/s, moderate for VMCA between 140 200 cm/s, severe for VMCA larger than 200 cm/s. Makoto et al. [1] researched the time for average MCA speed to increase to its peak when cerebral vasospasm broke out, and they found that post-SAH, blood flow velocity of the MCA rapidly increased, peaked 8 10 days after bleeding, and then decreased gradually. Soutiel et al. [2] reported that MCA blood flow velocity increased to a peak 4 12 days after bleeding, and this coincides with the peak time of cerebral vasospasm post-SAH found in clinical observation. The faster the blood flow, the more severe the cramps, and the worse the prognosis. When VMCA>200 cm/s delayed ischemic damage and cerebral infarction may even occur clinically. A scholar researched the clinical value of TCD for basilar artery vasospasm, and the results showed that the ratio of basilar artery and extracranial vertebral artery (BA/EVA) has value in diagnosing vertebrobasilar artery spasm. When BA/EVA>2, it is vertebrobasilar artery spasm, when BA/EVA>3, it is severe vertebrobasilar artery spasm [3]. In addition, TCD could also monitor dynamic parameters like pulsatility index and spectrum when cerebral vasospasm bursts. Some scholars reported that when the typical spectrum of a vessel changed, its blood flow velocity would show a change characterized by "slow fast slow" [4].

Okada et al. [5] have studied CVS sensitivity and specificity of TCD and compared them with the DSA findings, and found that sensitivity of TCD in detecting cerebral middle artery spasm was 80% and specificity 89%. Lysakowski et al. [6] found that the sensitivity of TCD in diagnosis of MCA spasm sensitivity was about 99% and specificity about 69%. TCD has advantages like non-invasiveness, test repeatability and ease of dynamic monitoring, and it has become the most commonly-used technology in clinic to diagnose cerebral vasospasm, but the sensitivity in early phase diagnosis of cerebral vasospasm is not high, and it could not easily detect changes in microcirculation spasm. TCD examinations are also affected by intracranial pressure, hematocrit, spastic site, the patient's age and gender and the operator's proficiency, etc., which reduced the accuracy of CVS clinical diagnosis.

Digital Subtraction Angiography (DSA)

DSA is regarded as the gold standard for cerebral vasospasm diagnosis. It can show blood vessels' changes in running, size and collateral circulation very clearly. Bederson et al. [7] found that post-SAH, cerebral vasospasm often occurred within 1 3 days of the acute phase and the subsequent delayed phase, which was biphasic. Yoon et al. [8] did DSA examination in 70 cerebral vasospasm patients and found that the spastic vessel wall was not smooth, and the diameter was apparently shorter than normal. Otawara et al. [9] said that blood vessels narrowed by 60% can be judged as light moderate cramps, and narrowed more than 60% can be judged as severe spasticity. Stetan Weidger et al. [10] took the average diameter of cerebral blood vessels of patients without SAH in the same period with DSA as the reference value to determine the degree of vasospasm: non-spasm for diameter narrowed 0 10%, mild cramps for diameter narrowed 11 33%, severe cramps for diameter narrowed 67 100%, and when the vessel grew thinner and thinner, cerebral infarction ultimately occurred. So DSA can accurately determine cerebral vasospasm, but the severity grading criteria should still be further explored.

DSA can determine CVS with high accuracy, but due to its invasive nature and difficulty in repeated checking, and the possibility of causing re-bleeding and complications like vasospasm, its clinical application is limited.

Perfusion Computed Tomography (PCT) and Computed Tomography Angiography (CTA)

PCT and CTA have significance in guidance of early diagnosis and treatment of cerebral vasospasm. PCT is a quick and relatively cheap imaging technology and uses qualitative and quantitative analysis of hemodynamic parameters such as cerebral blood flow, cerebral blood volume and mean transit time; it can monitor hemodynamic changes and show the dangerous area of brain ischemia when there is a cerebral vasospasm [11]. Laslo et al. [12] reported that PCT can monitor vessels' vasospasm in a short period of time, which has great value to early judgment of cerebral vasospasm. MTT (MTT = CBV/CBF) has the most sensitive perfusion parameters for cerebral vasospasm measurement post-SAH. When there is a mild vasospasm, CBF and CBV begin to decrease, MTT increases gradually and reaches the maximum 10 13 days post-SAH; when there is moderate to severe spasm, the mean values of CBF and CBV reduce remarkably. When there is vertebrobasilar spasm, CBV and CBF begin to decrease on the second day. Wintermark et al. [13] found by PCT that when MTT reached 4.56s, symptomatic cerebrovascular spasm occurred, and when MTT reached 5.58s, avuncular necrosis of the brain occurred. PCT is minimally invasive, operation repeatable and could also provide information on cerebral infarction and ischemic penumbra of the corresponding cerebral hemorrhage domain, so it could show the early cerebral vasospasm after SAH. Ryuzaburo Kanazawa et al. [14] found by PCT that when ACA, MCA and PCA spasm occurred, the mean

values of MTT were 3.49s, 3.80s and 4.34s respectively. When MTT values exceeded the corresponding mean value of MTT by 20%, it indicated a symptomatic spasm, while exceeding by more than 47% may result in spastic infarction. The CBF and CBV value declined and MTT value increased more significantly for patients with delayed spastic infarction than without. Harrigan et al. [15] suggested PCT could be used to recognize patients with delayed cerebral ischemia after SAH, and has great value for drugs and endovascular treatment guidance. CTA has the advantages of being minimally invasive, a shortcut and reliable, and it is increasingly being regarded as an efficient imaging technology. It might replace DSA in vasospasm assessments post-SAH. In particular it has great value for emergency inspections for critical cerebral vasospasm patients, which helps improve the prognosis of these patients. Wintermark et al. [13] reported that when inspecting critical and non-cooperative patients, it could be used to quickly acquire patients' CT data, assess accurately whether the blood vessels are clear or not for patients with acute SAH, and could also be used for intracranial aneurysm screening and intraoperative monitoring.

CTA can judge proximal cerebral artery non-spasm and severe-spasm with extremely high accuracy, 96 and 100% respectively, while accuracy for mild and moderate spasm are 90 and 95% respectively, and 81 and 94% for peripheral vascular mild-moderate and severe spasm respectively [16]. Binghai et al. [17] classified cerebrovascular vasospasm into mild-moderate and severe using CTA. When CBV and CBF were normal, MTT's increasing indicated mild-moderate spasm, and its sensitivity, specificity and accuracy were 86.8, 96.8 and 95.2% respectively; when CBV and CBF decreased, MTT's increasing indicated severe spasm, and its sensitivity, specificity and accuracy were 76.5, 99.5 and 97.5% respectively. When using PCT, the sensitivity, specificity, and accuracy for checking mild-moderate vasospasm were 90, 100 and 92.3% respectively, and 20, 100 and 38.5% respectively for checking severe vasospasm. CTA has low sensitivity (64%), especially in the judgment of mild-moderate cerebral vasospasm, but has high specificity, which was 96%. Combining CTA and PCT can reduce false-positives significantly in judging cerebral vasospasm and has great value particularly in judging vertebrobasilar vasospasm, so combining the two, early cerebral vasospasm can be judged with higher accuracy. [13]

Many scholars have already used CTA/PCT in clinical cerebral vasospasm diagnosis. Ayse Aralamak et al. [18] found with CTA/PCT that after SAH, when the vascular stenosis of microvascular spasm exceeded 50%, CBV and CBF decreased significantly; when local perfusion increased to 83%, ischemic infarction emerged, and when there is watershed perfusion abnormality, microcirculation spasm cannot be detected. Binghai et al. used multi-slice spiral CTA/PCT to assess the location and severity of cerebral vasospasm and related perfusion abnormalities, which can assess the risk of serious delayed cerebral vasospasm and guide minimally invasive therapy. However, CTA/PCT has contrast agent side effects and radiation, and costs much, so its clinical application is limited. It must be noted that throughout the scanning, the braking of patient is very important, as slight movements will affect the dynamic curve.

Diffusion-Weighted Magnetic Resonance Imaging (DWI) and Perfusion-weighted Magnetic Resonance Imaging (PWI)

Grifiths et al. [19] had detected cerebral blood flow reduction and ischemia with some SAH patients before surgical and endovascular treatment using DWI and PWI technology, suggesting that DWI and PWI are simple, non-invasive methods to evaluate cerebral blood flow and SAH complications, whose clinical application could help improve the prognosis of patients with SAH. Abrar et al. [20] reported that DWI could effectively assess cerebral vasospasm post-SAH, and also detect microbleed sites early. Busch et al. [21] thought the decrease of the apparent diffusion coefficient (ADC) value reflected local vasospasm, brain tissue spreading depolarization and CBF decrease, the time and space evolution process of ADC value could reflect the severity of SAH, and DWI could detect brain tissue abnormality in acute phase of SAH, which contributes to early recovery of the injured brain tissue. Condett et al. [22] reported that for patients without cerebral vasospasm, there was no abnormal signal either in the form of DWI or imaging, and distinct ADC value asymmetry was not found outside the central gray matter nuclei; while for patients with cerebral vasospasm, regardless of symptoms, both DWI and imaging showed abnormal high signal and remarkable ADC value reduction; and for patients abnormal in T2 WI and LALR sequences, DWI abnormality is more extensive.

Rordorf et al. [23] reported that perfusion abnormality was more obvious than proliferation for patients with symptomatic vasospasm post-SAH, and they found that the MTT extends extensively on PWI and was characterized by the appearance of small focal ischemia necrosis lesions on DWI. When neuron symptomatic spasm occurred, abnormality on MTT appeared more extensively on PWI than on DWI. DWI can discriminate early brain infarction, while PWI can detect brain perfusion, and their combination could help to detect penumbra, which could improve the prognosis of these patients.

Combining DWI with PWI, we can detect cerebral vasospasm post-SAH rapidly and accurately and could also detect ischemic penumbra, which can better guide treatment and improve prognosis for patients. But the expensive cost limits their clinical application.

Conclusion

Single photon emission computed tomography (SPECT) can judge cerebral ischemia resulting from cerebral vasospasm with higher sensitivity than DSA; 88% for the former, while only 68% for the latter. It is difficult to judge relatively small vasospasms with TCD, while SPECT makes up for the deficiencies. However, there exist some false-negatives and false-positives using SPECT to detect cerebral vasospasm post-SAH, which may be due to the short time period of vasospasm, whose symptoms thus could not be detected in less than 24 h. Whether false-positive vasospasm means subclinical vasospasm is left for further studies [24]. Positron emission tomography (PET) has higher spatial resolution than SPECT. Hayashi et al. [25] used PET to measure the cerebral circulation and metabolism of subarachnoid hemorrhage patients in acute phase, and found that the CBF, average CBF/CBV and cerebral metabolic rate of oxygen decreased significantly.

To sum up, imaging technologies have great value in early cerebral vasospasm judgment. With technology improving, there could be more advanced imaging technologies applied to cerebral vasospasm judgment. Choosing accurate, convenient, and non-invasive imaging technology has significant meaning for cerebral vasospasm judgment, treatment guidance and prognosis improvement of patients and many other aspects.

Conflict of interest statement We declare that we have no conflict of interest.

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Detection and Characterization of Intracranial Aneurysms with Dual-Energy Subtraction CTA: Comparison with DSA

Fajin Lv, Qi Li, Jingmin Liao, Tianyou Luo, Yun Shen, Jialun Li, John Zhang, and Peng Xie, MD

Abstract *Background*: To investigate the diagnostic performance of dual-energy subtraction CTA in evaluating intracranial aneurysms by comparison with DSA.

Methods: Ninety-seven patients with suspected intracranial aneurysms were included into our study and completed both 64-section dual-energy subtraction CTA and DSA examinations. Two independent readers retrospectively reviewed all subtraction CTA images in a blinded manner. Sensitivity, specificity, positive predictive value and negative predictive value of subtraction CTA and DSA were calculated on a per-patient and per-aneurysm basis.

Results: According to the reference standard, 96 aneurysms were present in 81 patients and no aneurysm was found in 16 patients. The overall sensitivity, specificity, positive predictive value and negative predictive value of subtraction CTA on a per-aneurysm basis were 98.9, 100, 100 and 94.1%, respectively. DSA prospectively detected 88 aneurysms in 79 of 81 patients. On a per-aneurysm basis, the sensitivity, specificity, positive predictive value and negative predictive value of DSA were 91.7, 100, 100 and 66.7%, respectively.

Conclusion: The diagnostic accuracy of 64-section dual-energy subtraction CTA is promising in detection and characterization of intracranial aneurysms. In most cases, it may substitute for conventional DSA as the primary imaging method in the diagnostic work-up of intracranial aneurysms.

Keywords Subarachnoid hemorrhage · Digital subtraction angiography · CT angiography · Subtraction

Introduction

Subarachnoid hemorrhage (SAH) due to rupture of an intracranial aneurysm is a medical emergency that requires prompt diagnosis and treatment [1]. Traditionally, digital subtraction angiography (DSA) has been considered the reference standard for the diagnostic work-up of intracranial aneurysms. However, it is an invasive and time-consuming technique that is associated with a 0.5% risk of permanent neurological complications [2]. Recently, CTA has emerged as a promising imaging method for the detection and therapy planning of ruptured intracranial aneurysms [3, 4]. In emergency settings, CTA can be easily performed in a timely manner, thereby allowing rapid detection and therapy planning of ruptured intracranial aneurysms. Since the introduction of multidetector CT scanners, the diagnostic performance of CTA has been approaching that of DSA in evaluation of intracranial aneurysms [5 8]. Nevertheless, multidetector CTA is less useful than DSA in detecting small aneurysms as well as aneurysms at the base of the skull [9 11]. Several subtraction methods have been proposed to remove bone structures in clinical practice. Early studies with subtraction CTA have shown promising results in detecting and delineating intracranial aneurysms.

F. Lv and T. Luo
Department of Radiology, The First Affiliated Hospital, Chongqing Medical University, Chongqing, China
Q. Li and P. Xie, MD (✉)
Department of Neurology, The First Affiliated Hospital, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, 400016, Chongqing, China
Institute of Neuroscience, 1 Yixueyuan Road, Yuzhong District, 400016, Chongqing Medical University, Chongqing, China
e mail: peng_xie@yahoo.com
J. Liao and Y. Shen
GE China CT Imaging Research Center, Beijing, China
J. Li
Department of Medicine, Chongqing Medical and Pharmaceutical College, Chongqing, China
J. Zhang
Department of Neurology, The First Affiliated Hospital, Chongqing Medical University, 1 Yixueyuan Road, Yuzhong District, 400016, Chongqing, China
Institute of Neuroscience, 1 Yixueyuan Road, Yuzhong District, 400016, Chongqing Medical University, Chongqing, China
Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA, USA

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Results of recent studies with subtraction CTA demonstrated that subtraction CTA is superior to conventional CTA for detecting aneurysms adjacent to bone [12, 13]. However, subtraction CTA has limited sensitivity for evaluation of small aneurysms with the use of four-section CT scanners [14]. Recently, the introduction of 64-section multidetector CT scanners, which allow rapid acquisition of isotropic data, has greatly advanced the role of CTA in depicting small aneurysms. The purpose of our study was to investigate the diagnostic performance of 64-section dual-energy subtraction CTA in detection and characterization of intracranial aneurysms.

Materials and Methods

Patients

The study was approved by the ethical committee of Chongqing Medical University. A total of 115 patients who underwent dual-energy subtraction CTA in our hospital for suspected intracranial aneurysms were retrospectively reviewed. Patients were scheduled to perform CTA on the basis of clinical findings, including signs and symptoms suggestive of aneurysm, presentation of SAH confirmed by nonenhanced CT scan or xanthochromia at lumbar puncture. Patients were eligible for the study if they had undergone both dual-energy subtraction CTA and DSA for suspected intracranial aneurysms. Finally, a total of 97 subjects (56 men, 41 women, mean age 49 years, age range 19 78 years) were included into our study. Sixty-seven of these enrolled patients presented with SAH. Eighteen patients were excluded from the study because they refused DSA and were operated on based on the subtraction CTA results alone.

Subtraction CTA Protocol

All patients underwent subtraction CTA with a 64-row multidetector CT scanner (LightSpeed VCT; GE Medical Systems, Milwaukee. WI). Patients were placed supine and the scan volume extended from the first cervical vertebra to the cranial vault. All patients' heads were fixed between pre-contrast and enhanced CTA examinations to prevent motion artifacts. For subtraction CTA, an additional non-enhanced scan (100 kV, 300 mA) was performed to identify bone structures that were subsequently subtracted from the enhanced scan. A total of 80 ml nonionic contrast medium (Ultravist, 370 mg iodine/ml) was injected through a 18-gauge needle via antecubital vein with an automated injector at a flow rate of 4 ml/s. Scanning delay was automatically adjusted for each patient by a bolus tracking technique. To minimize misregistration between the two scans, the contrast enhanced scan was performed using the same x-ray tube start angle and table speed. Enhanced scan was obtained with the following parameters: 120 kV, 300 mA, pitch of 0.531, section thickness of 0.5, 0.5 mm increment, 180 mm field-of-view, 512 × 512 matrix with soft reconstruction kernel. The thickness and interval of source images were both 0.625 mm.

Image postprocessing was performed at a workstation (Advantage for Windows, GE Medical Systems). After loading the CTA datasets in memory, bone removal was achieved by subtracting the nonenhanced scan from the contrast-enhanced data with pixel by pixel subtraction method. Subtraction CTA datasets were reconstructed to generate 3D volume rendering (VR) and maximum intensity projection (MIP) images for interpretation of aneurysms. The average time required for preparation of subtraction 3D images was approximately 6.8 min.

DSA Protocol

DSA was performed in all patients with femoral catheterization by the Seldinger technique with a biplane DSA unit (Coroskop Plus, Siemens, Erlangen, Germany). DSA was performed with selective bilateral internal carotid artery and vertebral artery injections. Standard anteroposterior, lateral DSA views were obtained and additional oblique views were acquired at the discretion of the angiographer.

Image Analysis

All subtraction CTA and DSA images were randomized before interpretation. Subtraction CTA datasets were reconstructed as 3D VR and MIP images for image analysis. DSA results were judged by the radiologist who performed the examination. All the VR and MIP images were prepared by a trained technologist. Two skilled reviewers (F.L., with 10 years of experience performing CTA, and Q.L., with 3 years of experience with CTA), who were blinded to the results of DSA and the other reader's judgments, retrospectively reviewed the subtraction CTA results. All 3D VR and MIP CTA images were evaluated in conjunction with CTA source images. The readers had to evaluate the location, size, shape of the aneurysm as well as its relationship to parent arteries. The image quality of subtraction CTA was rated as excellent, good, moderate and poor for visualization of intracranial aneurysms.

Statistical Analysis

For statistical analysis, 2 × 2 contingency tables were constructed. Sensitivity, specificity, positive predictive value and negative predictive value of subtraction CTA and DSA for aneurysm detection were calculated on a per-aneurysm and per-patient basis.

Results

All enrolled subjects successfully completed both subtraction CTA and DSA examinations. There were no complications or technical failures during subtraction CTA examination. All subtraction CT angiograms were diagnostic. The image quality of subtraction CTA was rated excellent in 95 patients, good in two patients. In two patients with suspected cerebral aneurysms, the image quality of subtraction CTA was rated "good" due to patient movement during the scans. There were no neurological complications related to DSA procedure during the examination.

According to the reference standard, a total of 96 aneurysms were present in 81 patients and no aneurysm was found in 16 patients. A single aneurysm was detected in 68 patients. Eleven patients had two aneurysms, two patients had three aneurysms. The aneurysms were located in the internal carotid artery (n = 47), the anterior cerebral artery (n = 3), the anterior communicating artery (n = 28), the middle cerebral artery (n = 12), and the vertebrobasilar artery (n = 6). Of the 47 internal carotid artery aneurysms, 34 were located in the posterior communicating artery. Of the 96 aneurysms detected, 18 aneurysms (19%) were <3 mm, 29 aneurysms (30%) were 3 5 mm, 42 aneurysms (44%) were 5 10 mm, and 7 aneurysms (7%) were >10 mm in maximal diameter.

In our present study, subtraction CTA prospectively detected 95 of 96 aneurysms. Of the 95 aneurysms detected by CTA, reader one correctly identified 94 aneurysms, reader two correctly identified 95 aneurysms. One aneurysm was not detected by both readers. The patient with false-negative CTA results was a 49-year-old female presented with SAH. The patient had two aneurysms located at the posterior communicating arteries on both sides. The left posterior communicating artery aneurysm, which is the causative aneurysm of SAH, was identified by both readers. The right posterior communicating artery aneurysm measured 0.9 × 2.1 mm was overlooked by both readers during the initial reading. However, both aneurysms were clearly present on 3D VR angiograms and could be correctly identified by both readers in retrospect review of CTA images (Fig. 1).

The diagnostic accuracy of subtraction CTA was calculated on a per-aneurysm and per-patient basis for both readers (Table 1). On a per-aneurysm basis, the overall sensitivity, specificity, positive predictive value and negative predictive value of subtraction CTA were 98.9, 100, 100 and 94.1%, respectively. However, the overall sensitivity of subtraction CTA was 100% on a per-patient basis. For aneurysms less than 3 mm, the sensitivity of subtraction CTA was 88.9% for reader 1 and 94.4% for reader two. However, the sensitivity was 100% for aneurysm larger than 3 mm.

DSA prospectively detected 88 aneurysms in 79 of 81 patients. A total of eight aneurysms were missed by DSA. All DSA false-negative aneurysms were detected with subtraction CTA. There were no DSA false-positive cases. The characteristics and reasons for missing were listed in Table 2. Six of eight missed aneurysms were non-causative aneurysms in patients harboring multiple aneurysms.

In a patient presented with SAH, DSA successfully detected a C2 internal carotid artery aneurysm but failed to depict a second aneurysm located at the C3 segment of the internal carotid artery. However, both aneurysms were present on subtraction CTA performed 4 days before the initial DSA. In retrospective review of the initial DSA results, both readers were not confident about the presence of the second aneurysm. A repeat DSA was performed 5 days after the initial DSA examination and depicted both aneurysms (Fig. 2).

Discussion

Recently, CTA has increasingly been recognized as an important imaging method in detection and characterization of intracranial aneurysms. The diagnostic performance of CTA has significantly improved since the introduction of multidetector CT scanners. In our present study, we demonstrate that the dual-energy subtraction CTA has challenged the role of DSA as a primary imaging method of choice in the diagnostic work-up of suspected intracranial aneurysms.

Numerous studies have compared the diagnostic accuracy of CTA with DSA in detecting and characterizing intracranial aneurysms. Early studies with single-detector CTA have shown limited sensitivity for detection of small aneurysms [15, 16]. Technical innovations from single-detector to multidetector CT scanners have greatly advanced the role of CTA in evaluation of intracranial aneurysms. Recent studies with 16-section multidetector CTA have shown high sensitivity and specificity that were equal to DSA for evaluation of aneurysms larger than 3 mm. In a study of 85 patients with suspected intracranial aneurysms, Yoon et al. [17] reported 100% sensitivity for aneurysms >3 mm. How-

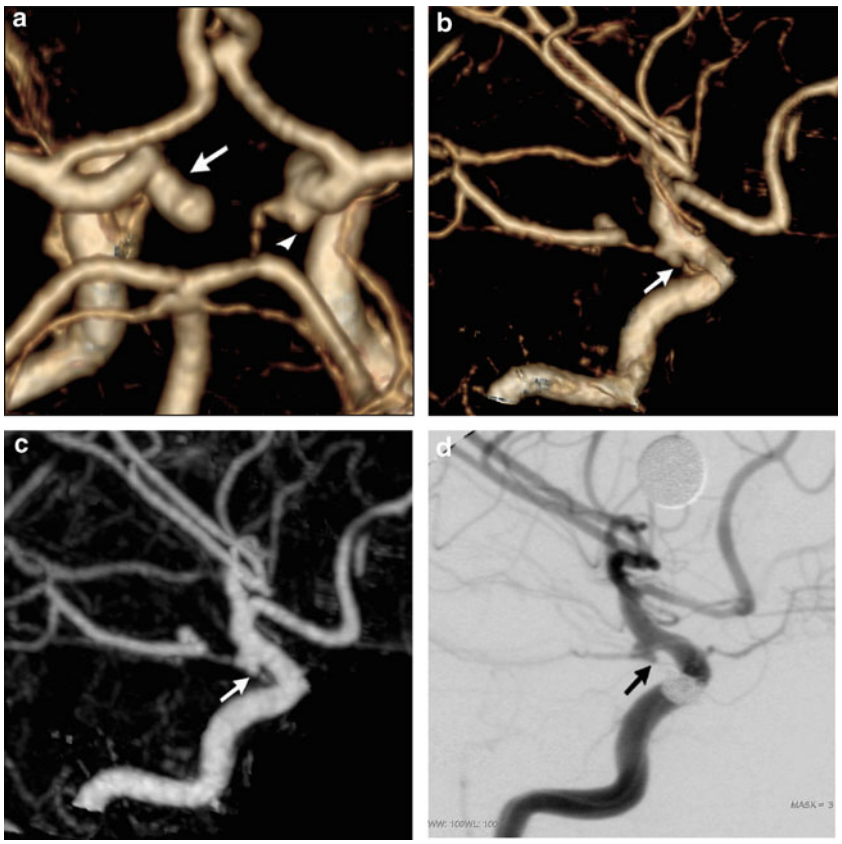

Fig. 1 Images in a patient with acute subarachnoid hemorrhage. (**a**) Both readers correctly identified the left posterior communicating artery aneurysm (*arrow*), but overlooked a second aneurysm located at the right posterior communicating artery (*arrowhead*) during the initial reading. (**b**) The missed aneurysm was clearly present on volume rendering angiogram (*arrow*). (**c**) MIP images with the same projection angle. (**d**) DSA further confirmed the presence of the right posterior communicating artery aneurysm

Table 1 Diagnostic performance of subtraction CTA on a per patient and per aneurysms basis

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Per patient				
Reader 1	98.9	100	100	94.1
Reader 2	100	100	100	100
Overall	100	100	100	100
Per aneurysm				
Reader 1	97.9	100	100	88.9
Reader 2	98.9	100	100	94.1
Overall	98.9	100	100	94.1

ever, the sensitivity was only 74.1% for aneurysms <3 mm. Similar results were reported in a study of 35 patients with multidetector CTA by Jayaraman et al. [18]. Despite technical innovations, 64-section multidetector CTA still has a practical limit of 3 mm for detection of intracranial aneurysms. Preliminary studies with 64-section multidetector CTA found that the mean sensitivity was 70.4% for aneurysms less than 3 mm [19]. In another study with 64-section multidetector CTA, McKinney et al. [20] demonstrated that the sensitivity was 92.3% for aneurysms was less than 4 mm. Although many studies with promising CTA results have been published, conventional multidetector CTA was less useful than DSA for detection of suspected intracranial aneurysms. Detection of small aneurysms located at the skull base is still challenging because bone structures may partly obscure vessels.

Recently, several subtraction methods have been proposed to eliminate bone structures that may interfere with visualization of aneurysms adjacent to bone. Initial attempts to subtract nonenhanced from contrast-enhanced scan were based on section by section subtraction, which is susceptible to patient movement during or between scans [21]. Various subtraction methods as well as vacuum-type head holders that may restrict patient movement were developed for subtraction CTA. Imakita et al. [22] found that subtraction CTA with controlled orbit scanning was superior or equivalent to DSA in depicting 33 of 50 aneurysms. More recently, matched mask bone elimination (MMBE) was developed by Venema et al. [23] to remove bone from CTA source images in an automatic way. In a study of 88 patients, Romijn et al. [14] compared the diagnostic accuracy of MMBE CTA with DSA and 3D rotational angiography. The authors reported 99% sensitivity for aneurysms larger than 3 mm.

In our present study, we reported that the sensitivity for aneurysms <3 mm was 88.9% for reader one, and 94.4% for reader two. For aneurysms larger than 3 mm, the sensitivity and specificity were both 100%. Compared with previous

studies with multidetector CTA, our study reported high sensitivity for detection of aneurysms less than 3 mm. The improved diagnostic accuracy depends, in large part, on technical innovations of 64-section multidetector CT scanners and the dual-energy subtraction imaging method we used in our present study. The major advantage of 64-row multidetector CT scanners is higher spatial resolution, which is an essential prerequisite for good image quality [24]. In our present study, the image quality of subtraction CT angiograms was rated excellent in 95 of 97 patients. In addition, all angiograms could provide detailed information regarding aneurysm anatomy and its relationship to adjacent vessels (Fig. 3). We also noticed that sophisticated postprocessing algorithms such as 3D VR and MIP may offer a perspective that more closely approximates the surgical approach than DSA [25]. This is especially useful in clinical pre-operative decision-making. In this regard, dual-energy subtraction CTA can be used to triage patients between endovascular coiling and surgical clipping [26, 27].

Table 2 Characteristics of aneurysms missed by DSA

Aneurysm location	Size (mm)	Confidence	Main reason for missing
ICA: Supraclinoid	2.1 × 1.5	2	Very small, mistaken for tortuous vessels
ICA: Supraclinoid	3.0 × 2.9	1	Very small, multiple aneurysms
ICA: Terminus	2.7 × 1.8	1	Very small, vascular superimposition
ICA: Periophthalmic	1.7 × 1.6	1	Very small, multiple aneurysms
ACA	1.9 × 1.4	1	Very small, vasospasm
PICA	2.6 × 1.4	2	Very small, unusual location
AcomA	1.8 × 1.0	1	Ideal projection was not obtained
AcomA	2.5 × 1.4	1	Very small, multiple aneurysms

ICA internal carotid artery, *ACA* anterior cerebral artery, *AcomA* anterior communicating artery, *PICA* posterior inferior cerebellar artery
Confidence score refers to the reader's confidence about the presence or absence of an aneurysm.
1 definitely not present, 2 probably not present, 3 equivocal, 4 probably present, 5 definitely present

Interestingly, we also noticed that the diagnostic accuracy was better with dual-energy subtraction CTA than DSA for detection of suspected intracranial aneurysms. In our present study, DSA failed to identify eight very small aneurysms that were suspected on subtraction CTA. However, these missed aneurysms may have been demonstrated at 3D rotational angiography, which allows reconstruction in unlimited projections. The results of our study suggested that three-dimensional visualization of aneurysms with dual-energy subtraction CTA may provide additional information

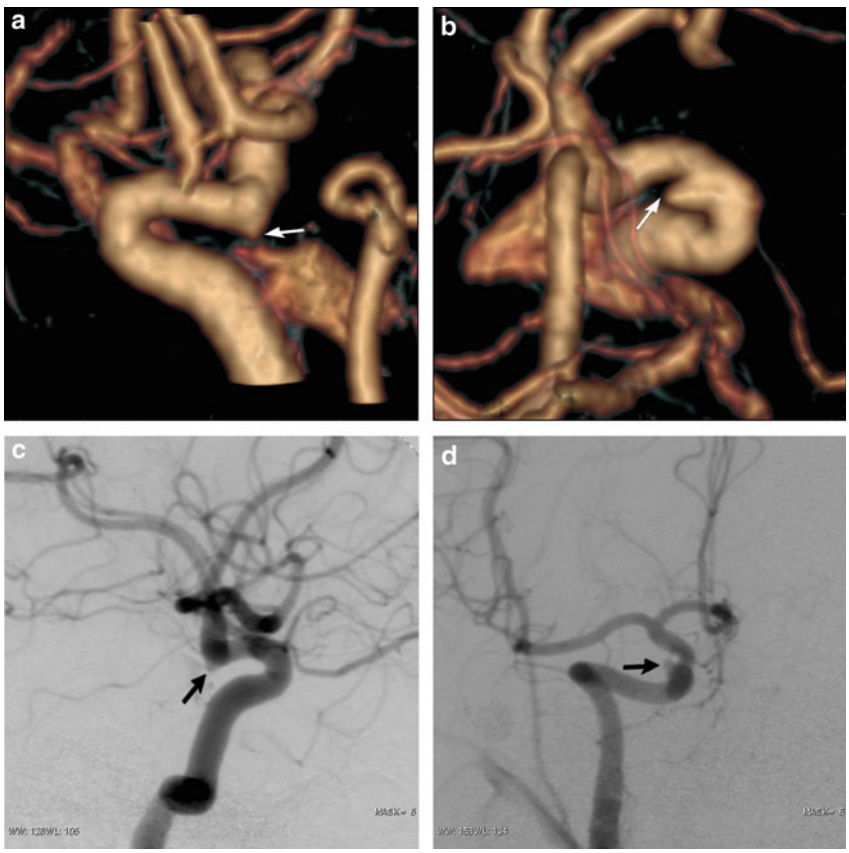

Fig. 2 Images in a patient with multiple internal carotid artery aneurysms. The C3 internal carotid artery aneurysm was missed by initial DSA examination. (**a**) The C2 internal carotid artery aneurysm was clearly visualized on subtraction CTA VR angiogram (*arrow*). (**b**) Subtraction CTA also delineated a second aneurysm located at C3 internal carotid artery (*arrow*). (**c**) Initial DSA depicted the C2 internal carotid artery aneurysm (*arrow*), but failed to detect the C3 internal carotid artery aneurysm due to lack of ideal frontal projection at DSA. (**d**) A repeat DSA examination performed 5 days later revealed the C3 internal carotid artery aneurysm (*arrow*)

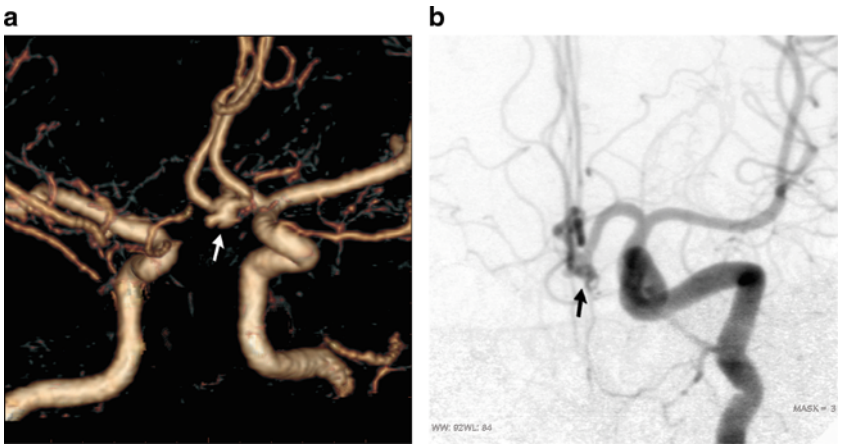

Fig. 3 Anterior communicating artery aneurysm revealed by subtraction CTA and DSA. (**a**) Subtraction CTA VR image allows three dimensional visualization of aneurysm and may offer a perspective that closely approximates surgical approach (*arrow*). (**b**) DSA delineates the anterior communicating artery aneurysm in two dimensions (*arrow*). The shape of the aneurysm was not clearly visualized with 2D DSA

to 2D DSA for evaluation of intracranial aneurysms. This could be partially explained by the fact that DSA provides only two-dimensional projections of cerebral vessels. In some cases, small aneurysms may be mistaken for tortuous vessels at DSA. We also noticed that vascular superimposition occurred in certain cases with DSA false-negative aneurysms. Sometimes, an ideal projection that could delineate aneurysm from adjacent vascular structures could not always be readily obtained in clinical settings because of complex arterial anatomy at the site of aneurysms. In contrast, subtraction CTA allows three-dimensional visualization of aneurysms in any chosen projection, which may improve detection and delineation of small cerebral aneurysms. By rotating reconstructed angiograms, we can easily obtain a perfect projection that may clearly depict small aneurysms as well as adjacent vascular structures.

In previous studies, many authors have used DSA as the gold standard to which CTA was compared. However, in some cases, DSA has missed small aneurysms that were seen on CTA [28]. Consistent with previous reports, we also found DSA false-negative aneurysms in our series. Our results and those of others suggest that conventional 2D DSA may miss small aneurysms when evaluating cerebral aneurysms.

Conclusion

In conclusion, our study suggested that dual-energy subtraction CTA is an accurate and powerful imaging method for detection and characterization of intracranial aneurysms. Furthermore, we also found that dual-energy subtraction CTA may have greatly advanced the role of CTA in detection of small aneurysms as well as aneurysms adjacent to bone. In most cases, the latest generation dual-energy subtraction CTA may substitute conventional 2D DSA as the primary imaging method in the diagnostic work-up of intracranial aneurysms.

Conflict of interest statement We declare that we have no conflict of interest.

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