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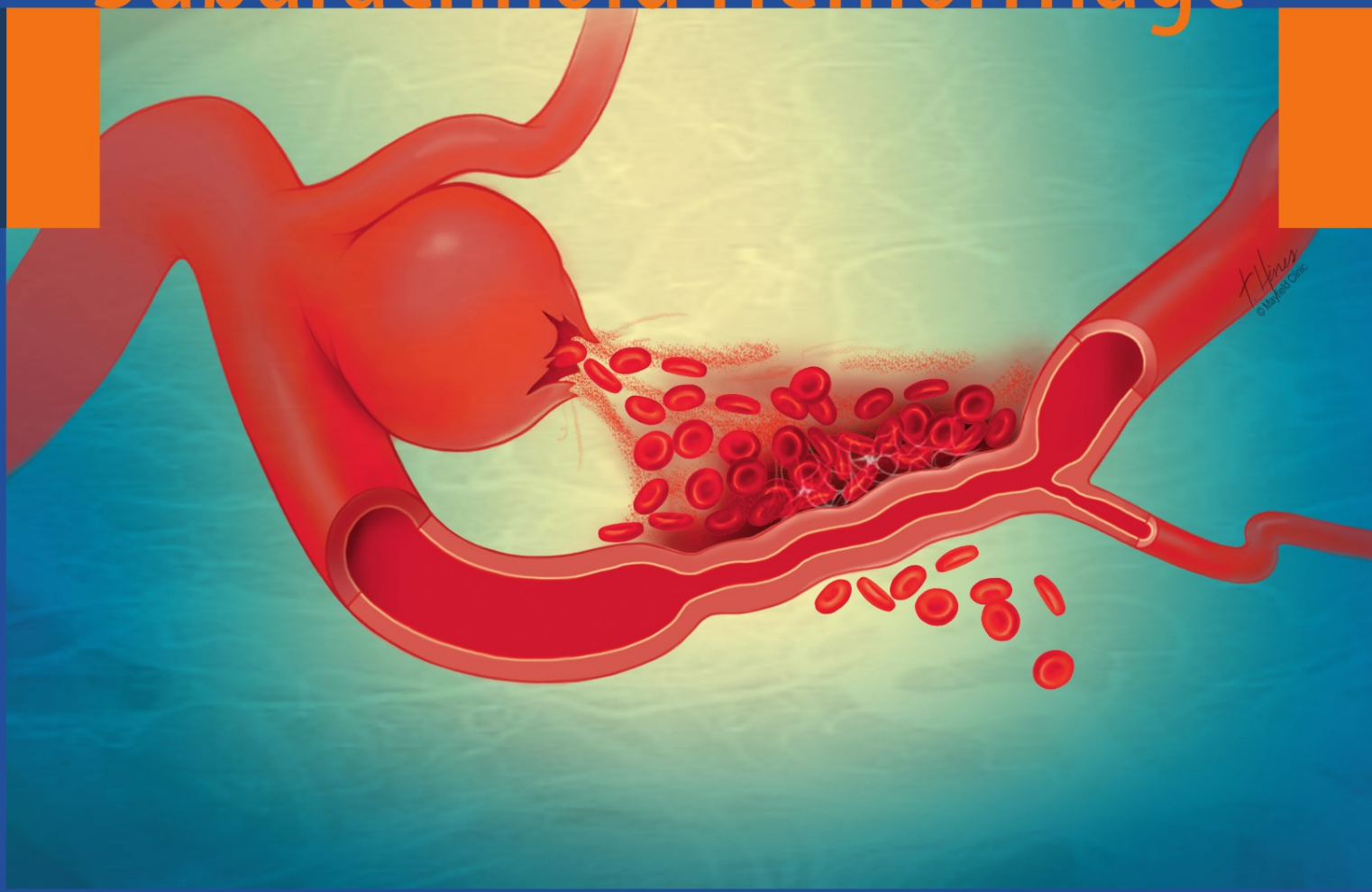
Gail Pyne-Geithman · Norberto Andaluz
Jed A. Hartings · Opeolu M. Adeoye *Editors*

Cerebral Vasospasm
Neurovascular Events After Subarachnoid Hemorrhage



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Cerebral Vasospasm: Neurovascular Events After Subarachnoid Hemorrhage

Edited by
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Preface

This book contains the proceedings of the *11th International Conference on Cerebral Vasospasm: Neurovascular Events after Subarachnoid Hemorrhage*. The conference was held in Cincinnati, Ohio, USA, from July 21–23, 2011, with the recurrent goal to share the latest knowledge on the pathophysiologic phenomena that take place after aneurysmal subarachnoid hemorrhage. This collection of papers represents a cross section of the enormous progress that has been made toward a thorough understanding and effective treatment of neurovascular events following aneurysmal subarachnoid hemorrhage, including cerebral vasospasm. The editors would like to extend their gratitude to the many participants of this most recent conference and thank previous participants for setting the stage for continued progress in this field. We also want to acknowledge the authors of the chapters of this book. We are indebted to these contributors for providing such excellent material. Finally, we would like to express our deepest gratitude to all those who made a flawless meeting possible: our sponsors, the scientific committee, and the members of the organizing committee, especially Ms. Christa McAlpin and Ms. Joanie Pope. This book will be of interest to basic scientists wishing to expand their understanding of cerebrovascular and neural pathophysiology related to subarachnoid hemorrhage and to clinicians who wish to apply state-of-the-art knowledge to their management of this devastating condition.

Cincinnati, USA

Mario Zuccarello
Joseph F. Clark

Acknowledgment

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Contents

Frank H. Mayfield, MD, FACS, FAANS (1909–1991): 75th Anniversary Tribute

John M. Tew Jr.

In this special issue, coinciding with Mayfield Clinic's 75th anniversary, we recognize the vision and practical wisdom of Dr. Frank H. Mayfield, who inspired solutions to the devastating problems of cerebral vasospasm after subarachnoid hemorrhage (SAH). This conference proceedings, *Vasospasm 2011*: *11th International Conference on Neurovascular Events after SAH*, is dedicated to Dr. Mayfield, a University of Cincinnati professor and pioneer in neurosurgery who directed the graduate neurosurgical training programs at The Christ Hospital and Good Samaritan Hospital from 1946 to 1977. His lasting, distinguished contributions in aneurysm surgery formed, in part, some of the foundational science for this conference's nearly 200 cerebrovascular experts from 20 nations who are taking important steps toward developing the first optimal clinical management strategy for vasospasm.

Being curious and fascinated by possibilities of the future, Dr. Mayfield would have valued the opportunity to have attended this conference with you—the dedicated scientists, surgeons, neurologists, nurses, students, and practitioners. Your commitment to research in vasospasm and clinical care as well as cortical spreading depolarizations and to your fellow colleagues is exemplified throughout this special supplement. In recognition of Dr. Mayfield's contributions to the advancement of our profession, this dedication highlights specific areas: service to his patients, commitment to education, integrity in science, collaboration, and dedication to improving medicine. He noted, "Throughout all ages and all fields of endeavor, man has sought to overcome the unpredictability of nature by reformulating existing knowledge in search of new principles." He understood that in a slow, orderly process, science inspires and disseminates new concepts. This conference's proceedings represent your creative work as basic scientists and clinicians whose research offers hope in determining the practical applications to human disease, in this case disorders associated with SAH and vasospasm.

Known as a creative thinker, Dr. Mayfield looked at all events constructively and, when faced with a problem, changed them to opportunity. He was well acquainted with the devastation associated with SAH and uniquely aware of the importance of preventing recurrent hemorrhage and the deadly consequences of vasospasm.

Dr. Mayfield's vision and practical wisdom fueled his drive for solutions to the problems of recurrent hemorrhage or during occlusion of parent vessel surgery for saccular aneurysms. With strong focus, he sought resolution to the disaster that often ensued when a surgeon clipped an aneurysm with permanent ligatures of malleable metallic clips. That is, the surgeon could not determine the clip's optimal location, retain its closing force, or safely remove or replace it after positioning. Neurosurgeon and creative thinker Frank Mayfield collaborated with other physicians and scientists to develop a definitive aneurysm clip; his resulting designs forever changed aneurysm and brain surgery [1].

Nearly 60 years ago, Dr. Mayfield and medical artist George Kees, Jr., began working on the clip and forceps, later known as the Mayfield clip [2]. This small, cross-legged clip and its applicator with tweezers-like dexterity enabled the trial-and-error placement needed during aneurysm surgery. The clip's malleability allowed it to be twisted into shape but retain its springy recoil. These first Mayfield clips of 6–15 mm were stainless steel. Testing, first in laboratory animals and later in patients, ensured that there was no evidence of corrosion and only minimal signs of foreign body reaction and verified that the clips remained positioned, even against pulsatile forces of 400 mm mercury. With these encouraging results, Dr. Mayfield and his colleagues applied these clips to saccular aneurysms in patients. Work continued diligently to improve this design as the clip then underwent modifications and additional testing in the United States, Great Britain, and Sweden by a number of noted neurosurgeons (e.g., James Poppen, James Gardner, Eben Alexander, Lawrence Pool, Charles Drake). George Kees developed patents and produced and marketed the Mayfield clips and appliers.

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Modifications to the Mayfield clip followed: The Heifetz modification provided firmer fixation in the applicator; the McFadden design had a round jaw and blunt tips made of 301 stainless steel; and Drs. Sundt and Nofzinger modified the clip for the treatment of intracranial aneurysms. A call from Dr. Drake, who was having difficulty operating on a basilar aneurysm, prompted Dr. Mayfield and Mr. Kees to devote a late night working in the laboratory. By the next morning, the clip was redesigned and shipped to Dr. Drake; such a practical invention was then possible before the Food and Drug Administration oversight and clinical trials. It was eventually called the Drake fenestrated clip.

Today's neurosurgeons and their patients continue to benefit from the innovative work of Dr. Mayfield and those he later inspired. His commitment and compassion led to other far-reaching improvements for patients with spinal disorders and neurological diseases—a collaboration toward the invention of the seat belt and development of professional neurosurgical organizations. Described as the "conscience of neurosurgery" by Eben Alexander, Dr. Mayfield's legacy calls for each to develop one's own creativity for advancing the field of medicine. Dr. Mayfield would be excited to greet all of you today and salute this international commitment to curing vasospasm through collaboration in science and practice.

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History and Definition of Delayed Cerebral Ischemia

R. Loch Macdonald

Abstract A list of the vasospasm meetings is provided. The early descriptions of angiographic vasospasm and delayed cerebral ischemia are presented. Selected advances in knowledge in the field and some controversies are described. A proposal for definitions of neurological deterioration due to delayed cerebral ischemia, of cerebral infarction, and of vasospasm is reviewed.

Keywords Vasospasm • History • Delayed cerebral ischemia Subarachnoid hemorrhage

Introduction

Martin Luther King, Jr., said: "We are not makers of history. We are made by history," meaning that our thinking about the present is influenced by understanding and interpretation of history. But, in medicine it also is important in that history provides the basis for advancing knowledge. As George Santayama said, "Those who cannot remember the past are condemned to repeat it."

The Vasospasm Meetings

Robert R. Smith organized and chaired the first meeting of what would later become the series of meetings focused on cerebral vasospasm. There were 18 participants at the conference in Jackson, Mississippi, in 1972. The attendees were or would become authorities in the field; they included Francis Echlin, John Kapp, James T. Robertson, Frederick A. Simeone, Robert R. Smith, Thoralf M. Sundt, Bryce Weir, Richard White, Robert H. Wilkins, and Nicholas T. Zervas. A series of conferences followed, with titles that have evolved over time because of changes in theories of brain injury after subarachnoid hemorrhage (SAH; Table 1).

Early Descriptions

Perhaps the earliest description of a patient with delayed cerebral ischemia (DCI) was by Gull in 1859 [12]. He wrote of a 30-year-old female, "While walking, she suddenly called out, "Oh my head," and put up her left hand. She vomited and, as her friend thought, fainted. After a brief interval she partially recovered, and was able to walk back to her residence with the support of two men. When admitted to the hospital at noon the following day, only a slight impression could be made by any attempt to rouse her. The right arm was quite paralyzed, the muscles flaccid; the right leg in the same condition." She improved, and by 3 days after the ictus was able to eat. On the 4th day, she spoke some words, but on the 5th day, she worsened; her pupils became fixed and dilated, and she died. At autopsy, there was SAH in the left Sylvian fissure with massive softening of the left hemisphere and two aneurysms on the middle cerebral artery, one of which had ruptured.

Robertson described findings in 27 cases of death from aneurysm rupture in 1949 [17]. He found infarctions in brain irrigated by patent arteries and wrote: "Hence, it seems possible that the ischaemic changes were due to temporary spasm of the supplying vessels."

Ecker and Reimenschneider reported angiographic vasospasm in six patients with known ruptured aneurysms and noted that it was not observed on angiograms done 26 or more days after SAH [7].

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Table 1 Meetings focusing on angiographic vasospasm and delayed cerebral ischemia

Meeting title	Location, organizer(s)	Resulting book
Subarachnoid Hemorrhage and Cerebrovascular Spasm. The First International Workshop	Jackson, Mississippi, USA, 1972, Robert R. Smith, 18 attendees	Smith, R.R., Robertson, J.T., eds. *Subarachnoid Hemorrhage and Cerebrovascular Spasm. The First 'International' Workshop*. Springfield: Charles C. Thomas Publisher, 1975.
Second International Workshop on Cerebral Arterial Spasm	Amsterdam, the Netherlands, 1979, A.J.M. van der Werf, 200 participants	Wilkins, R.H., ed. *Cerebral Arterial Spasm. Proceedings of the Second International Workshop*. Amsterdam, the Netherlands/Baltimore: Williams & Wilkins, 1980.
3rd International Symposium on Cerebral Vasospasm	Charlottesville, Virginia, USA, 1987, Neal Kassell, 197 contributors	Wilkins, R.H., ed. *Cerebral Vasospasm. Proceedings of the III International Symposium in Charlottesville*. New York: Raven Press, 1988.
4th International Conference on Cerebral Vasospasm	Tokyo, Japan, 1990, Keiji Sano, K. Takakura, Tomio Sasaki	Sano, K., Takakura, K., Kassell, N.F., Sasaki, T., eds. *Cerebral Vasospasm. Proceedings of the International Conference on Cerebral Vasospasm*. Tokyo: University of Tokyo Press, 1990.
5th International Conference on Cerebral Vasospasm	Edmonton and Jasper, Alberta, Canada, 1993, Bryce Weir	Findlay, J.M., ed. Cerebral Vasospasm. *Proceedings of the V International Conference on Cerebral Vasospasm, Edmonton*. Amsterdam, the Netherlands: Elsevier Publishing Company, 1993.
6th International Conference on Cerebral Vasospasm	Sydney, Australia, 1997, Nicholas Dorsch	Dorsch, N.W.C., ed. *Cerebral Vasospasm VI. Proceedings of the VIth International Conference on Cerebral Vasospasm*. Oslington, Leichhardt, Australia, 1999.
7th International Conference on Cerebral Vasospasm	Interlaken, Switzerland, 2000, Rolf Seiler, 75 participants	Seiler, R.W., Steiger, H.-J., eds. *Cerebral Vasospasm. Acta Neurochirurgica*, Suppl. 77. Wien, New York: Springer, 2001.
8th International Conference on Cerebral Vasospasm	Chicago, Illinois, USA, R. Loch Macdonald, 90 participants	Macdonald, R.L., ed. *Cerebral Vasospasm. Advances in Research and Treatment*. New York: Thieme Medical Publishers, 2005.
9th International Conference on Cerebral Vasospasm	Istanbul, Turkey, Talat Kiris	Kiris, T., Zhang, J.H., eds. *Cerebral Vasospasm. New Strategies in Research and Treatment*. *Acta Neurochir* Suppl. Wein: Springer-Verlag, 2008.
10th International Conference on	Chongqing, China, Hua Feng, 90 participants	Feng, H., Mao, Y., Zhang, J.H., eds. *Early Brain Injury or Cerebral Vasospasm*. Volume 1: *Pathophysiology*. *Acta Neurochir* Suppl 110/1. New York: Springer, 2011. Feng, H., Mao, Y., Zhang, J.H. eds. *Early Brain Injury or Cerebral Vasospasm*. Volume 2: *Clinical Management. Acta Neurochir* Suppl 110/2. New York: Springer, 2011.
11th International Conference on Neurovascular Events After Subarachnoid Hemorrhage	Cincinnati, Ohio, USA, Mario Zuccarello, Joseph F. Clark	To be published in *Acta Neurochir Suppl*.

Advances

In the past, neurosurgeons wrote about pre- and postoperative vasospasm as if surgery had some impact on the timing of angiographic vasospasm. It is known now that while the severity might be affected by surgery, the time course is related to the time of SAH and has nothing to do with the time of surgery. DCI varies with time of surgery, however, probably due to effects of surgery on the brain, which is already injured by SAH and made even more vulnerable by reduction in cerebral blood flow (CBF) from angiographic vasospasm.

Fisher described clinical characteristics of DCI in 1975 [8]. The onset of DCI was described as is now known 3–13 days after a single SAH and in about one third of patients. Only severe vasospasm tended to be associated with symptoms. Weir and colleagues defined the time course of angiographic vasospasm by measuring the diameters of cerebral arteries on 627 angiograms from 293 patients with ruptured aneurysms [23]. Angiographic vasospasm had its onset at 3 days, was maximal at 6–8 days, and resolved by 12 days after SAH. The invention of computed tomography (CT) was critical to neurosurgery. Within 4 years, Katada et al. discovered there was a

relationship between the volume of SAH on CT and development of angiographic vasospasm [14]. Takemae and colleagues expanded on this concept, showing in a series of 73 patients with SAH studied by CT that high-density areas (blood) in the basal cisterns within 4 days of SAH were associated with the location of where angiographic vasospasm would develop and whether it would [19]. Fisher et al. later described a similar idea but classified the subarachnoid blood into four grades, which could be used to predict whether angiographic vasospasm would develop and how severe it would be [9]. Aaslid and colleagues described transcranial Doppler ultrasound, a now widely used method for determining CBF velocities and other parameters in patients with SAH [1].

Knowledge of the time course of angiographic vasospasm and improved diagnostic tools, principally angiography, CT, and medical tests, made it possible to associate vasospasm and delayed neurological deterioration. The clinical picture was called by various terms, including delayed ischemic deficit [10], DCI, delayed ischemic neurologic deficit, and symptomatic vasospasm.

The incidence of angiographic vasospasm was 67% (1,842 of 2,738 patients) in 38 studies until 1993 as reviewed by Dorsch et al. when angiography was done 7–14 days after SAH [5]. It was 49% (2,077 of 4,238 patients) in 31 references published from 1993 to 2009 [4]. Whether the incidence has declined or detection is lower now that patients do not all have angiograms in 7–14 days after SAH is not known. The incidence of DCI, variously defined, has not been rigorously reviewed, but according to Dorsch et al. the incidence was 32% (10,445 of 32,188 patients) in 297 references published until 1993 and 29% (6,775 of 23,806 patients) in papers published from 1993 to 2009 [4, 5].

Controversies

The existence of angiographic vasospasm and its significance were questioned initially. Millikan wrote in 1975 that he could not find any data to indicate that angiographic vasospasm had any specific clinical presentation, or that it contributed to complications of SAH [15]. He noted many investigations showing no correlation between CBF and angiographic vasospasm, which is not unexpected since only severe vasospasm reduces CBF, and linear correlations will not be obvious. That angiographic vasospasm, even severe in some cases, occurs without symptoms also was evident in some cases. Whether this is due to adequate collateral flow or lack of other secondary processes that must be added to angiographic vasospasm to cause DCI continues to be unknown. This controversy continues in a modified form today (Fig. 1).

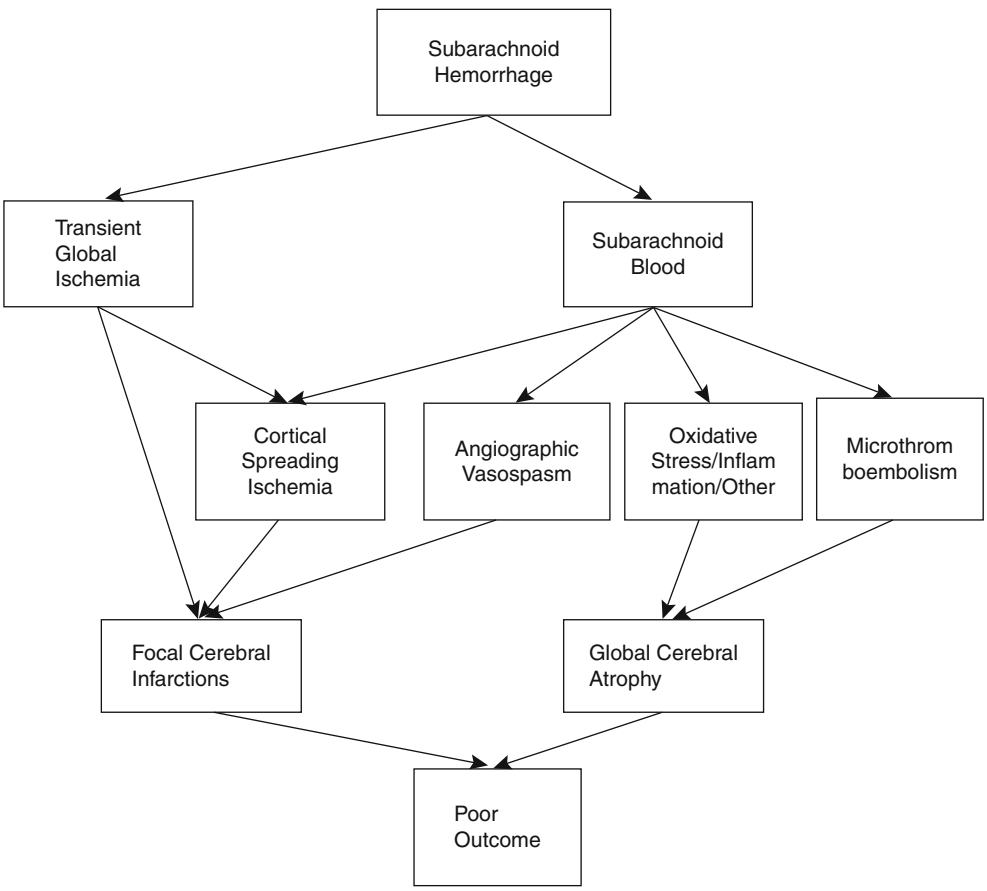

Fig. 1 A simplified scheme of possible processes and pathways leading to focal and global brain injury and ultimately poor outcome after SAH

There is no question about the association of angiographic vasospasm with reduction in CBF and development of DCI and frank infarction [2, 3, 11]. Numerous studies, beginning in the 1960s, reported correlations between reduced CBF and angiographic vasospasm [22]. The correlations are imperfect, which is not surprising considering the multiple contributing factors, technical and imaging issues, and complexities of human disease [20]. What may be more important is if and how multiple pathophysiological processes may contribute to DCI. The existence of these processes in humans is only beginning to be documented, such as cortical spreading ischemia and microthromboemboli [6, 18]. It is not known now how they, along with early brain injury and other delayed effects of the SAH like delayed apoptosis, contribute to DCI.

Definitions

Vergouwen and colleagues proposed definitions of angiographic vasospasm and DCI [21]. Since patients can have angiographic vasospasm without DCI and there may be a few patients with DCI and no angiographic vasospasm, it was recommended to separate the definitions. The pathogenesis of the conditions is not completely defined, so terms implying pathogenesis were avoided, although DCI attributes the deficits to ischemia. The diagnosis of DCI is difficult because of the need to exclude other causes. But in practice, many patients have multiple systemic and nervous system dysfunctions that may or may not contribute to DCI or even be important primarily in the pathogenesis. Diagnosis in sedated or comatose patients is even more challenging. Scales that are used in SAH patients often were not developed specifically for this patient population (e.g., the Glasgow Coma Scale, National Institutes of Health Stroke Scale, and Glasgow Outcome Score). Even defining the etiology of infarctions after SAH is difficult [13].

A proposed definition for clinical deterioration caused by DCI was "the occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia, or neglect), or a decrease of at least 2 points on the Glasgow Coma Scale (either on the total score or on one of its individual components (eye, motor on either side, verbal)). This should last for at least 1 h, is not apparent immediately after aneurysm occlusion and cannot be attributed to other causes by means of clinical assessment, CT or magnetic resonance (MR) scanning of the brain, and appropriate laboratory studies." The diagnosis will be subjective because of the often-complex medical condition of the patient. Cerebral infarction was defined as "the presence of cerebral infarction on CT or MR scan of the brain within 6 weeks after SAH, or on the latest CT or MR scan made before death within 6 weeks, or proven at autopsy, not present on the CT or MR scan between 24 and 48 h after early aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment. Hypodensities on CT imaging resulting from ventricular catheter or intraparenchymal hematoma should not be regarded as cerebral infarctions from DCI." It was recommended that terms like vasospasm or arterial narrowing be applied to descriptions of what the arteries look like on a radiological test (computed tomographic, magnetic resonance or digital subtraction angiography). These terms would not to apply to clinical manifestations of DCI. Transcranial Doppler ultrasound was not included in the diagnostic testing since it has lower sensitivity and specificity to diagnose angiographic arterial narrowing.

Conclusion

In conclusion, the history of angiographic vasospasm and DCI has been briefly summarized. The reader is encouraged to delve deeper into the rich heritage of the field. Indeed, many of the cited papers are among the 100 most cited papers in neurosurgery [1, 9, 16].

Conflicts of Interest RLM receives grant support from the Physicians Services Incorporated Foundation and is a stockholder of Edge Therapeutics. RLM, RTH, EK, SAM, AMo, AR, PV, IW, and NK are consultants for Actelion Pharmaceuticals. SAM is a consultant for Edge Theraputics. RLM is Chief Scientific Officer of Edge Theraputics. EK has been on advisory boards for Roche Diagnostics, and is a stockholder in NeMoDevices. AMo has been a consultant for Micrus Endovascular and Covidien. PV is a consultant for Aesculap. IW is a consultant for Boston Scientific, ev3, and BALT, and receives a departmental grant from Boston Scientific. DB, AF, AMa, and SR are employees and stockholders of Actelion Pharmaceuticals.

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Clinical, Transcranial Doppler Ultrasound, Radiological Features and, Prognostic Significance of Delayed Cerebral Ischemia

George Kwok Chu Wong and Wai Sang Poon

Abstract *Objective*: We aimed to investigate the profiles and prognostic values of delayed cerebral ischemia (DCI) and delayed cerebral infarction.

Methods: IMASH (Intravenous Magnesium Sulphate for Aneurysmal Subarachnoid Hemorrhage) was registered at http://www.strokecenter.org/trials, and http://www.ClinicalTrials.gov (NCT00124150). Data of 327 patients were retrieved for logistic regression analyses.

Results: Seventy-one (22%) patients developed DCI, and 35 (11%) patients developed delayed cerebral infarction. Only 18 (25%) patients with DCI and 7/35 (20%) patients with delayed cerebral infarction had mean middle cerebral artery velocities (transcranial Doppler ultrasound) over 120 cm/s. Regarding the prognostic significance of the components of DCI, delayed cerebral infarction predicted unfavorable outcome in terms of Extended Glasgow Outcome Scale (OR 3.1, 95% [CI] 1.3–7.8), poor outcome in terms of modified Rankin Scale (odds ratio [OR] 3.0, 95% confidence interval CI 1.2–7.7), and dependent activity of daily living in terms of Barthel Index (OR 3.6, 95% CI 1.4–9.2) at 6 months, after adjustments for other prognostic factors. On the other hand, clinical deterioration predicted inpatient mortality (OR 8.8, 95% CI 1.6–48.8) after adjustments for other prognostic factors.

Conclusions: Delayed cerebral ischemia and delayed cerebral infarction carried different prognostic values in aneurysmal subarachnoid hemorrhage.

Keywords Aneurysm • Cerebral infarction • Delayed cerebral ischemia • Stroke • Subarachnoid hemorrhage

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Introduction

Although spontaneous subarachnoid hemorrhage (SAH) accounts for only 3–5% of all strokes and 4.4% of deaths from stroke [1, 2], the relative youth of the affected individuals means that this event is actually responsible for approximately 25% of all years of life lost as a result of stroke [3]. Complications, such as early brain injuries and delayed ischemic neurological deficits, remain a major cause of morbidity and mortality in this group of patients.

The recently completed Asian-Australasian Intravenous Magnesium Sulphate for Aneurysmal Subarachnoid Hemorrhage (IMASH) trial, a randomized, double-blind, placebo-controlled, multicenter, phase III trial, as well as an updated meta-analysis, did not demonstrate any benefits in clinical outcome or clinical vasospasm with magnesium sulfate infusion [4–6]. However, a recently completed German, single-center, randomized, placebo-controlled trial showed no benefit in clinical outcome and delayed ischemic neurological deficit but found a decrease in delayed (after day 3 or 4) cerebral infarction with magnesium infusion [7].

In the current study, we aimed to investigate the profiles and prognostic values of delayed cerebral infarction in the IMASH database.

Methods

Under the IMASH protocol [4], patients who were diagnosed with acute aneurysmal SAH (within 48 h after ictus) were recruited. At 6 months after randomization, the Glasgow Outcome Scale Extended (GOSE) [8], modified Rankin Scale (mRS) [9], and Barthel Index (BI) [10] scores were calculated. The GOSE (primary outcome at 6 months) was stratified into favorable (score 5–8) or unfavorable (score less than or equal to 4) outcomes. The mRS was stratified into 0–2 (good outcome) versus 3–6 (poor outcome). Clinical vasospasm (secondary outcome) was stratified into presence

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or absence. The BI was stratified into at least 85 (independent) or below 85 (dependent).

Delayed cerebral ischemia (DCI) was defined as the occurrence of (1) clinical deterioration, which manifested clinically as new focal neurological deficit (motor or speech deficit) that developed after SAH or a decrease in the Glasgow Coma Scale of 2 or more points for more than 6 h, or (2) delayed cerebral infarction, which was defined as new cerebral infarction, not related to post-treatment (coiling or clipping) complications, rebleed, progressive hydrocephalus, electrolyte or metabolic disturbance, or infection, occurring within the first 3 weeks after ictus. A surrogate outcome, delayed cerebral infarction, was similarly defined as new cerebral infarction not related to post-treatment (coiling or clipping) complications, rebleed, progressive hydrocephalus, electrolyte or metabolic disturbance, or infection, occurring within the first 3 weeks after ictus. These diagnoses were based on clinical, radiological, and laboratory assessments by independent neurosurgeons.

Statistical Analysis

Statistical analyses were generated using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was taken as $p < 0.05$ or 95% CI of OR not including 1. Data were represented using number (percentage) for categorical variables and mean plus or minus standard deviation for the numerical variables. Length of hospital stay and length of intensive care unit stay were compared using the Mann-Whitney U test. Binary logistic regression analyses were carried out using the Enter method, and results were represented using ORs and 95% CIs.

Results

The IMASH trial results were reported recently [4]. Briefly, there was no difference in the incidences of DCI and clinical outcome measures between the magnesium sulfate infusion and placebo saline infusion groups. Three hundred and twenty-seven patients from ten participating centers were included in the trial.

Seventy-one (22%) patients developed DCI, and 35 of the patients developed delayed cerebral infarction. Permanent focal neurological deficits occurred in 17/71 (24%) patients, and there were 7/71 (10%) deaths directly related to DCI. Length of hospital stay (32 ± 22 vs. 28 ± 22 days, $p = 0.003$) and length of intensive care unit stay (13 ± 8 vs. 8 ± 7 days, $p < 0.001$) were significantly longer for patients with DCI. Length of intensive care unit stay (12 ± 9 vs. 9 ± 7 days, $p = 0.030$), but not length of hospital stay, was significantly longer for patients with delayed cerebral infarction. Clinical deterioration per se did not predict the length of either intensive care unit stay or hospital stay. However, only 18 (25%) patients with DCI and 7/35 (20%) patients with delayed cerebral infarction had mean middle cerebral artery velocities (transcranial Doppler ultrasound) over 120 cm/s.

Regarding the prognostic significance of the components of DCI, delayed cerebral infarction, but not clinical deterioration, predicted favorable outcome in terms of GOSE (OR 3.1, 95% CI 1.3–7.8), good outcome in terms of mRS (OR 3.0, 95% CI 1.2–7.7), and independent activity of daily living in terms of BI (OR 3.6, 95% CI 1.4–9.2) at 6 months after adjustments for age, sex, admission WFNS grade, Fisher's scale, intraventricular hemorrhage, and intracerebral hemorrhage. On the other hand, clinical deterioration predicted inpatient mortality (OR 8.8, 95% CI 1.6–48.8) after adjustments for age, sex, admission WFNS grade, Fisher's scale, intraventricular hemorrhage, and intracerebral hemorrhage.

Discussion

Previous studies on prognostic models with adjustments for prognostic factors focused on aneurysmal SAH patients who underwent microsurgical clipping [11–14]. When the results of the ISAT (International Subarachnoid Aneurysm Trial) were published in 2002 [15], most referral neurosurgical centers switched to an endovascular first policy and reserved microsurgical clipping for wide-necked or giant aneurysms. The proportion of patients undergoing endovascular coiling for ruptured aneurysms subsequently reached 70–90%, compared to less than 30% previously. In addition, the standards of neurointensive care and imaging have improved dramatically in the past decade. Accordingly, it appears to be a suitable time to review the prognostic value of DCI such as cerebral infarction.

Of the 21% of patients with DCI, the Columbia University group reported that asymptomatic infarction resulted in more patients with unfavorable outcome than symptomatic DCI [16]. Another recent cohort showed that DCI, including cerebral infarction, was associated with unfavorable outcome [17]. However, no individual analyses of delayed cerebral infarction were conducted in both studies. A recent consensus suggested that cerebral infarction on plain computed tomography (CT) is the most objective measure for DCI based on the British Aneurysm Nimodipine Trial and the four tirilazad SAH trials [18, 19], in which data were obtained mainly from microsurgically treated intracranial aneurysms. Including an equal proportion of aneurysmal SAH patients who have undergone microsurgical clipping and endovascular embolization, we found that cerebral infarction was the

primary component of DCI that predicted unfavorable outcomes at 6 months after adjustments for age, sex, admission WFNS, and initial CT findings. This is similar to the results of the pre-ISAT tirilazad studies [11] and suggests a common etiology to poor neurological outcome.

However, defining delayed cerebral infarction among all the causes of CT hypointensities can be difficult with plain CT alone, and attributing hypodensities on CT to angiographic vasospasm is not sensitive and is unreliable [20, 21]. Further studies are required to define a practical method for clinical evaluation.

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Conflicts of Interest We declare that we have no conflict of interest.

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Identifying Patient Report Outcomes Relevant to Aneurysmal Subarachnoid Hemorrhage Follow-Up

Stuart Ross, Deepti Bhargava, Yahia Al-Tamimi, Tony Goddard, Alan Tennant, and Audrey Quinn

Abstract Patients recovered from subarachnoid hemorrhage can be assessed for neuropsychological dysfunction using postal questionnaires. We assessed 214 patients using various tests of memory, mood and strategic thinking. Patients in good outcome categories (modified Rankin Scale [mRS] 0–1) nevertheless exhibited mood disorder (28%), memory deficit and executive dysfunction (20%). Return to work (49%) was most influenced by previous employment status, Rankin scale and mood.

Keywords Subarachnoid hemorrhage • Outcomes Neuropsychological • Morbidity

Introduction

The outcome after subarachnoid hemorrhage (SAH) has traditionally been measured using the Glasgow Outcome Scale (GOS) or, more recently, the modified Rankin Scale (mRS). The latter has the advantage of having been verified in a telephoned or self-reporting method. A disadvantage of both scales is that over 75% of patients fall into the uppermost category. Whilst this reflects well the overall outcome and management of what can be a devastating disease, patients frequently complain of significant symptoms even though they might be rated as having a good outcome. The symptoms often relate to psychological issues such as poor memory and concentration, mood swings and difficulty dealing with social interaction. Many such patients fail to fit back into society after SAH and cannot return to work [12]. Thus, the topmost category of outcome remains associated with significant loss of economic and social quality of life.

In parallel, studies of SAH and the influence of vasospasm have led to uncertainty that current outcome scales are sensitive to the vagaries of the initial bleed or to the effects of delayed ischaemic neurological deficit (DIND) and its management. Thus, the statement that DIND affects one third of SAH patients and is the most significant cause of secondary morbidity seems to be contradicted by prognostic models, which are greatly influenced by age, initial World Federation of Neurosurgeons (WFNS), a grading scale for subarachnoid hemorrhage.

Methods

Recognising this paradox, utilising a battery of self-reporting measures we attempted to survey neuropsychological morbidity in SAH patients. We selected tools that interrogate the known aspects of psychological dysfunction. Table 1 shows the battery of test questionnaires. We wanted to look at memory and concentration and chose the Everyday Memory Questionnaire (EMQ) to assess it. Mood was Wimbledon Self-Report Scale (WSRS). We reasoned that complexities of social interaction and structured thinking might be assessed using the Dysexecutive (DEX) Questionnaire. The influence of physical disability was rated using the Barthel Index (BI) and the Stroke-Symptom Checklist (SSC), together with a measure of quality of life. It was important to check that patients could read the questionnaires, and those failing the short-sentences test were eliminated from the study.

The questionnaires were posted to participating patients between 6 months and 1 year following the hemorrhage. Until 2005, the Leeds neurosurgical unit had little access to interventional radiology, so over 90% of patients had clipping as their primary method of aneurysm isolation. After

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Table 1 Questionnaires

AKC short sentences test	A simple 10-point test to confirm that the patient is able to read and understand the booklet. Scores range from 0 to 10
Postal Barthel Index (BI)	A 10-item list that assesses various aspects of mobility and activities (ref). The patient rates his or her level of independence in each of the activities according to specified criteria. Scores range from 0 to 100
Self-report Dysexecutive (DEX) Questionnaire	The DEX questionnaire is a recently developed standardised self-report measure of behavioral difficulties associated with executive functioning such as impulsivity, inhibition control, monitoring and planning. Here the patient rates the frequency of difficulties with emotions or personality, motivation, behavior and cognitive problems on a 5-point scale ranging from never to very often. Scores range from 0 to 80
Everyday Memory Questionnaire (EMQ)	A 35-item questionnaire assessing the incidence of memory failures based on different everyday errors. For each of these errors, the patient rates their frequency, ranging from 'never' (score of 0) to 'all the time' (score of 4)
Stroke Symptom Checklist (SSC)	A checklist of 12 common symptoms (including effects of mood, cognition and physical problems) that are associated with brain injury. Patients score a point if symptoms are worse now than prior to their illness. Scores range from 0 to 12
Wimbledon Self-Report Scale (WSRS)	The WSRS was originally standardised on a hospital population with predominantly neurological disorders. It provides a general appraisal of mood rather than being limited to specific symptoms of anxiety or depression. The patient has to rate on a 4-point scale the frequency with which 30 emotions occur, ranging from most of the time to not all. Scored in a 0011 fashion, scores of 0–7 are normal, 8–10 are borderline and 11–30 represent clinically significant mood disturbance
Modified Rankin Score (mRS)	The mRS has been adopted in stroke research as the primary clinical endpoint in most trials. It is a 6-point scale of independence and difficulties with activities of daily living, ranging from needing constant care to complete independence. It has been shown that a mRS score of 0 (no symptoms) often correlates to the perception of complete recovery
Stroke QoL	A new (as-yet-unpublished) needs-based stroke-specific quality-of-life scale for which some of the qualitative interviews included SAH. Comprises 30 dichotomous items; a high score represents poor quality of life

2005, over 80% were treated by endovascular means, so the two cohorts, before and after the introduction of coiling, allow an interesting comparison to gauge the influence of craniotomy on psychological outcome.

Results

Of 376 sent, 214 questionnaires were returned, representing 57% of those participating. For cohort 1, 97 were admitted between 1998 and 2004, whereas 117 were admitted between 2005 and 2008 (cohort 2).

The mean ages were 55.5 (SD 12.37) and 57.40 (SD 9.12), respectively, for cohorts 1 and 2. The cohort 1 and 2 preponderance of female patients was 62% and 73%, respectively, a nonsignificant difference. Of the patients, 86.9% (cohort 1) and 79.9% (cohort 2) were classed as mRS 0 or 1, equivalent to a Good Outcome on the GOS. Similarly, cohorts 1 and 2 were comparable for WFNS grade and previous employment status.

Analysis of the questionnaires showed that 28% had a significant mood disorder. Impairments were reported by 20%; these impairments were of everyday memory, including of speech and reading and writing, and various behavioural difficulties associated with executive functioning, such as impulsivity.

Of 137 patients employed prior to the hemorrhage, only 67 (49%) had returned to work at follow-up.

Structured equation modelling was used to build the interaction between the variables. Cognitive deficits as measured by DEX and mood disorder on the Wimbledon scale explained a third of variance in outcome measured by self-assessed Rankin.

While cognitive deficits and mood disorder themselves directly affect Rankin outcome, there was also an indirect path by which cognitive problems increased the likelihood of mood disorder with an subsequent effect on outcome. Gender acted as a significant independent contextual variable.

Discussion

The age, grade and aneurysm site distribution of the cohorts was similar to literature reports. The high proportion of females in our SAH population has previously been discussed. The outcome of the patients was acceptable, with over 80% in grades 0–1 as measured by mRS.

The rating questionnaires used gave results compatible with expected outcomes and Factor analysis of individual scales lent credence to their validity in this population. There was a hierarchical ordering of items in frequency of

reporting. Thus the scales worked well in assessing cognitive and mood disturbances in the population of patients recovering from subarachnoid hemorrhage.

This study gives another insight into the differences between clipping and coiling treatment of aneurysmal SAH. The International Subarachnoid Aneurysm Trial (ISAT) [1] firmly established coiling as the treatment of choice; nevertheless, clipping is still required in many cases. The action of craniotomy and brain dissection would be expected to be associated with increased deficit initially. This study found that surgery was associated with more impairments and poorer quality of life, although only the SSC showed a significant difference.

As expected, many of those in mRS groups 0 and 1 showed cognitive and mood disturbances.

The recognition of psychological abnormalities after SAH is well established [2, 3]. Various interrogative tools have been used to reflect problems in a wide spectrum of psychosocial categories. Deane et al. [3] noted that, of the respondents with a GOS score of Good Outcome, the SF-36 score was reduced in each domain. The most notable score reduction was in the role-function physical domain, where the mean score was 48% compared with a population norm of 88%. Mood disorder is commonly encountered. Depression was found in as many as 30% of the SAH patients in one study [4]. Post-traumatic stress symptoms, described as intrusive thoughts or avoidance of reminders, have been recognised in SAH patients [5, 6]. Memory deficit may be included with cognitive deficit, which describes more widespread phenomena involving complex brain function. This 'executive function' is predominantly mediated by the frontal lobes and manifests in activities like planning, inhibition, problem solving, attention, and decision making. Ravnik and colleagues [7], investigating patients with clipped anterior communicating artery aneurysms, found that patients with Aneurysmal Subarachnoid Hemorrhage (aSAH) reported attentional deficits most frequently, yet patients performed better on tests of attention than on tests of other cognitive domains.

The cause of psychological disorders remains uncertain. Early studies related cognitive deficit to the initial impact of the hemorrhage [8]. Clinical and radiological parameters reflecting the impact of the bleed were related to memory function, intelligence, and aphasia. However, Haug et al. [9] reported that the site of the aneurysm and mode of treatment could not be linked to neuropsychological outcome. Springer and colleagues reported that after the effects of age, education, and race/ethnicity were controlled for, risk factors for cognitive impairment at 12 months included anemia, treatment with transfusion (adjusted odds ratio [AOR] 3.4; $P=0.006$), any temperature level higher than 38.6°C (AOR 2.7; $P=0.016$), and delayed cerebral ischemia (AOR 3.6; $P=0.01$) [10].

The time course of recovery seems to be longer than physical symptoms. GOS and mRS measure mainly motor skills and are generally assessed at 6 months. Ljunggren estimated that (cognitive) symptoms were still present 3½ years after the ictus [2]. Others suggested that cognitive impairment may improve in a third of affected patients between 3 and 12 months [5, 10]. Factors thought to be associated with persistence of impairment were older age, less education, non-white race, fever, poor initial grade, hyperglycaemia and delayed cerebral ischaemia. Improvement in verbal memory may occur between 6 and 12 months [9]. Post stress symptoms decreased from 60% at 3 months to 30% at 9 months. Mood at 9 months was predicted by prior mental health problems, poor physical health, dysphasia, and impaired prose recall at 3 months [5]. Springer found that 34% of patients who were cognitively impaired at 3 months had improved at 1 year. This was associated with the following factors: more than 12 years of education and the absence of fever higher than 38.6°C during hospitalization [10]. Whilst a 30% recovery over the first year is commonly reported, Haug found the frequency of cognitive impairment was 27% at 3 months and 21% at 12 months [9]. This group also found that verbal memory improved little until after 6 months, whereas Al-Khindi saw improvement of language function as early as 3 months [11].

Conclusions

This study confirmed that patient-reported outcome, measured by postal questionnaire, can be used to assess cognitive and mood disorders in good-grade (mRS 0–1) patients after SAH. A link between physical deficit and mood was established. Return to work was influenced not only by these but also by cognitive deficits, expressed in the DEX questionnaire. The results indicate a clear need for intervention directed specifically to address these problems and improve the socioeconomic well-being of those who are physically well.

Conflicts of Interest We declare that we have no conflict of interest.

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Global Cerebral Atrophy After Subarachnoid Hemorrhage: A Possible Marker of Acute Brain Injury and Assessment of Its Impact on Outcome

Alan K.H. Tam, Don Ilodigwe, Zeyu Li, Tom A. Schweizer, and R. Loch Macdonald

Abstract There is a correlation between poor neuropsychological outcome and focal regions of atrophy in patients with subarachnoid hemorrhage (SAH). No study has investigated the impact of global brain atrophy on outcome after SAH. In other neurological disorders, such as multiple sclerosis, a correlation has been found between global atrophy and outcome. This analysis of patients entered into a randomized clinical trial of clazosentan in patients with SAH (CONSCIOUS-1) investigated the relationship between global cerebral atrophy, clinical factors, and outcome.

The 413 patients in the CONSCIOUS-1 study underwent cranial computed tomography (CT) on admission and 6 weeks after SAH. After patients with large clip/coil artefacts and those with infarctions on CT were excluded, 97 patients remained and had voxel-based volumetric measurements of the baseline and 6-week CT scans. The percentage difference in volume between times was taken and analysed against clinical variables. Relationships were modeled using univariate and multivariate analysis.

Age, female gender, and higher body temperature during the patient's stay in the intensive care unit were significantly correlated with brain atrophy. Greater brain atrophy significantly correlated with poor outcome (modified Rankin scale), more severe neurological deficits on the National Institute of Health Stroke Scale (NIHSS), and poorer health status (EQ-5D).

Keywords Subarachnoid hemorrhage • Outcome • Brain atrophy • CONSCIOUS

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Introduction

Patients who suffer subarachnoid hemorrhage (SAH) experience higher rates of morbidity and mortality compared to those who have other types of strokes [7]. These poor outcomes may be due in part to cerebral infarction secondary to vasospasm or systemic inflammation, although the mechanisms by which poor clinical outcome occur due to these complications is unclear [11, 12]. Global brain atrophy in these patients may play a role as previous studies have linked regional brain atrophy in SAH with poor clinical outcome [2].

Prior research investigating brain atrophy in patients with stroke and SAH has focused on infarct volumes as a supplement to evaluating SAH patients [1, 9]. The results have been inconsistent. The inconsistency may be due to the use of different imaging modalities between studies, with magnetic resonance imaging (MRI) studies demonstrating weak correlation between infarct volume and poor clinical outcome and studies using computed tomography (CT) not finding such a relationship. Although MRI may be a better imaging modality in terms of image quality, it is less practical due to cost, technical difficulties in the acute care setting, and limited access.

In the CONSCIOUS-1 study, all patients had CT scans at baseline, 24–48 h after treatment of the ruptured aneurysm, and 6 weeks post-SAH. Using these data, we were able to study whether global brain atrophy has a relationship to outcome in this patient population by examining the extent of brain atrophy in each patient's CT scan 6 weeks post-SAH compared to their baseline CT. In Addition, we examined patient factors and interventions performed after SAH that may contribute to greater brain atrophy.

Methods

The CONSCIOUS-1 study was a randomized, double-blind, placebo-controlled trial involving 413 patients with aneurysmal SAH. Patients were randomized to treatment with

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placebo or 1 of 3 doses of clazosentan, an endothelin A receptor antagonist. The inclusion and exclusion criteria and study methodology of CONSCIOUS-1 have been described [6].

Of the 413 patients, those with lesions or infarctions on baseline (24 or 48 h after SAH treatment) or week 6 cranial CT scan were excluded to ensure calculated brain volume changes would be due solely to atrophy. Of the remaining 183 patients, additional patients were excluded if they had nondigitalized CT scans (30), there were discrepancies in scanning protocol between baseline and week 6 CT (37), and if there were large clip/coil artefacts that could not be compensated for in the volume analysis (19). This left 97 patients for analysis.

Baseline and week 6 CT images were loaded into MRIcro (version 1.40, Chris Rorden). All CT images were set at a common brightness and contrast value, and a common intensity filter was applied. Images were saved in Analyze format (version 9.0, AnalyzeDirect). They were converted to binary intensity images to allow easier delineation between brain tissue and nontissue space-occupying brain regions (i.e., cerebrospinal fluid [CSF] and ventricles). Converted images were loaded into Analyze. Region-of-interest (ROI) tracings of filtered images for brain tissue were performed in Analyze by outlining the intracranial tissue in each CT slice and outlining nontissue volume (i.e., ventricular volume) as a separate region to remove this from the volume calculation. Voxel-based volumetric measurement was then performed on the outlined intracranial volume in each slice, with summing of volume measurements for all slices from a given time point for each patient.

Clinical outcomes included measurement by National Institute of Health Stroke Scale (NIHSS) at 6 weeks and Glasgow Outcome Scale (GOS), modified Rankin scale (mRS), EuroQol group 5-dimenstion (EQ-5D) score, and Functional Status Examination (FSE). Other demographics, clinical characteristics on admission, medical history, method of aneurysm treatment, radiological data, and complications during hospitalization were extracted from the CONSCIOUS-1 data.

Percentage difference in volume between time points for each patient was calculated with the following formula: ([Week 6 volume − Baseline volume]/Baseline volume) × 100%. Patients were divided into two groups: global brain atrophy greater than median and global brain atrophy less than median. Data comparing the two ROI volumetric measurements are presented as the mean plus or minus the standard error of the mean (SEM). The first analysis examined variables of interest as independent variables with respect to brain atrophy and the percentage of brain volume loss as the dependent and used 2-tailed *t* tests for continuous variables, Mann-Whitney tests for variables with multiple categories, and χ^2 tests for binary variables. Baseline variables and early interventions with $P<0.20$ in univariate analysis were included in the multivariable models of associations with brain atrophy. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Second, an analysis of the correlation between atrophy and outcome measures was performed. The analysis of the relationship between atrophy and clinical outcome was done, with adjustment for confounders age [10], surgical treatment of aneurysm [4], high blood glucose level [3], hypertension [8], systemic inflammatory response syndrome (SIRS) [5] and smoking [13].

Results

Comparing baseline and week 6 volume measurements, the mean brain volume loss was −3.15 ± 0.57%, with a median of −1.65%. The clinical characteristics of the 97 patients divided by those with brain atrophy values greater and less than the median, are outlined in Table 1. Twenty-eight of 97 patients had an increase in their brain volume measurement, with a mean increase of 2.05 ± 0.32%. Factors known to affect brain volume that could be not controlled for in CONSCIOUS-1, such as cerebral edema, nutrition and other systemic illness, may be contributors to/causes of this finding.

In univariate analysis, patients with greater brain volume losses were more likely to have delayed ischemic neurological deficits (DINDs, 10% vs. 0%, $P<0.10$) and angiographic vasospasm (41% vs. 24%, $P<0.10$). Less brain atrophy was observed in females (77% vs. 57%, $P<0.05$) and younger patients (mean age 48 ± 11 vs. 52 ± 10, $P<0.05$). Greater brain atrophy was seen in patients with higher mean systolic blood pressure (168 ± 18 vs. 161 ± 18, $P<0.10$) and elevated mean body temperature in the intensive care unit (ICU) (37.8 ± 0.64 vs. 37.4 ± 0.35, $P<0.01$). A higher SIRS burden was correlated with greater brain atrophy (1.25 vs. 0.88, $P<0.10$).

Clinical variables with $P<0.10$ in univariate analysis from Table 1 were tested for correlation with brain atrophy in a multivariate analysis. Brain atrophy was significantly correlated with age (OR 0.95, CI 0.89–0.997), female gender (OR 3.32, CI 1.06–10.47) and ICU body temperature (OR 0.22, CI 0.07–0.68). Analysis of brain atrophy in relation to clinical outcomes, adjusted for factors associated with brain atrophy (as computed in the univariate analysis; Table 2), along with potential confounders (as listed in the Methods section) revealed a significant correlation between brain atrophy and poor functional outcome (mRS, OR 0.084, CI 0.01–0.72). As well, brain atrophy was correlated with severity of deficits on the NIHSS (OR 0.22, CI 0.07–0.76) and health status/quality of life (EQ-5D score, OR 3.18, CI 1.06–9.56).

Table 1 Patient factors and association with brain atrophy

Characteristic	Brain atrophy less than median	Brain atrophy greater than median	Univariate *P*	Multivariable odds ratio	95% CI for odds ratio	Multivariate *P*
Age	48 ± 11	52 ± 10	0.0455	0.95	0.89–0.997	0.0396
Gender, female	37 (77)	28 (57)	0.0368	3.32	1.06–10.47	0.0401
Hypertension	16 (33)	15 (31)	0.7739			
Diabetes	2 (5)	4 (10)	0.4212			
Smoking	25 (60)	22 (58)	0.8825			
Heart disease	0 (0)	3 (8)	0.1071			
Antiepileptic drug use	14 (29)	10 (20)	0.3176			
Seizures	3 (6)	8 (16)	0.1176			
Hydrocephalus	42 (88)	45 (92)	0.524			
Intraventricular hemorrhage	40 (83)	39 (80)	0.6356			
Intraparenchymal hemorrhage	0 (0)	3 (6)	0.2423			
Aneurysm size ≤5 mm	15 (37)	16 (42)	0.6156			
Aneurysm size >5 mm	26 (63)	22 (58)				
Angiographic vasospasm	11 (24)	19 (41)	0.0752			
DIND	0 (0)	5 (10)	0.0562			
Intensive care						
Body temperature	37.4 ± 0.35	37.8 ± 0.64	0.0003	0.22	0.07–0.68	0.0081
Systolic blood pressure	161 ± 18	168 ± 18	0.0541			
Diastolic blood pressure	75 ± 11	76 ± 9	0.649			
Mean arterial pressure	95 ± 16	95 ± 14	0.969			
SIRS burden, median	0.875	1.25	0.0752			
SIRS	19 (40)	24 (49)	0.3517			
Procedure			0.4456			
Surgical clipping	16 (33)	20 (41)				
Endovascular coiling	32 (67)	29 (59)				
GOSE (dichotomized)	2 (4)	11 (22)	0.0082			
mRS (dichotomized)	2 (4)	11 (22)	0.0145			
NIHSS (dichotomized)	0 (0)	2 (4)	0.4948			
EQTOT (dichotomized)	33 (69)	29 (59)	0.3266			
FSE (dichotomized)	27 (56)	22 (45)	0.2635			
FSE	9.5 ± 5.9	12.4 ± 8.4	0.0549			

DIND delayed ischemic neurological deficit, *EQTOT* EuroQol group 5-dimension, *FSE* Functional Status Examination, *GOSE* Glasgow Outcome Scale, Extended, *mRS* modified Rankin Scale, *NIHSS* National Institute of Health Stroke Scale, *SIRS* systemic inflammatory response syndrome

Table 2 Unadjusted and adjusted odds ratios of brain atrophy on clinical outcome measurements

Outcome	Characteristic	Unadjusted odds ratio, 95% CI	*P*	Adjusted odds ratio, 95% CI	*P*
Poor functional outcome (GOSE, mRS)	Brain volume change, %	0.150 (0.03–0.72)	0.0177	0.084 (0.01–0.72)	0.0238
	Systolic blood pressure			1.05 (1.003–1.10)	0.0381
NIHSS (dichotomized)	Brain volume change, %	0.35 (0.13–0.95)	0.0401	0.22 (0.07–0.76)	0.0163
	Age			0.92 (0.86–0.98)	0.0099
	Systolic blood pressure			1.04 (1.003–1.08)	0.0324
FSE (dichotomized)	Brain volume change, %	1.58 (0.71–3.52)	0.2646	1.91 (0.75–4.86)	0.1747
	Systolic blood pressure			0.97 (0.95–0.999)	0.0419
EQTOT (dichotomized)	Brain volume change, %	1.23 (0.55–2.74)	0.6182	3.18 (1.06–9.56)	0.0396
	Age			1.05 (0.99–1.10)	0.1004
	Gender, female			0.38 (0.13–1.11)	0.0768
	SIRS burden			1.16 (0.60–2.25)	0.6542

GOSE Glasgow Outcome Scale, Extended, *mRS* modified Rankin Scale, *NIHSS* National Institute of Health Stroke Scale, *FSE* Functional Status Examination, *EQTOT* EuroQol group 5-dimension

Conclusion

The results of this study demonstrated a relationship between global brain atrophy in patients with SAH and clinical outcome. More atrophy was associated with poor outcome on the mRS, lower FES scores and lower EQ-5D scores. These findings are consistent with prior studies showing a relationship between brain atrophy and neuropsychological outcome (memory, language, mental processing) [1, 2], although these studies looked at deficits at 1 year post event compared to 3 months in the current analysis. The CONSCIOUS-1 study was not designed to analyze brain volume as it related to outcome. MRI images were not obtained for all patients, and CT images were used to calculate brain volumes in this analysis. Future studies using computer-automated volumetric analysis of MRI images may provide additional insight into the relationship between global brain atrophy and clinical outcome in SAH.

Despite this study's limitations, this analysis of the CONSCIOUS-1 patients provides support for the possibility that SAH patients may suffer functional deficits secondary to brain atrophy independent of focal lesions and effects of angiographic vasospasm and DIND.

Conflicts of Interest RLM receives grant support from the Physicians Services Incorporated Foundation, Brain Aneurysm Foundation and Heart and Stroke Foundation of Canada and is a stockholder of Edge Therapeutics. RLM is a consultant for Actelion Pharmaceuticals and Chief Scientific Officer of Edge Therapeutics.

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Protein Biomarkers in Patients with Subarachnoid Hemorrhage, Vasospasm, and Delayed Ischemic Neurological Deficits

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Abstract Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating neurological disease. It has many sequelae, including vasospasm and delayed ischemic neurological deficits (DINDs). We explored the blood proteome in patients with aSAH using transcranial Doppler (TCD) velocity as a guide to patients who are at risk for symptomatic vasospasm and DIND. Blood was drawn on all days that patients were observed in the neurocritical care unit (NCCU) after aSAH. A team of neurologists and neurosurgeons identified patients with clinical evidence of vasospasm and DIND. Serum was fractionated using protein chips and surface-enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF MS). We detected a pattern of protein expression associated with those at risk for elevated TCD velocities by day 8, compared with blood collected in the presymptomatic stage (days 1–3). We further analyzed serum using pooled samples from study entry to the time of elevated TCD velocities using a protein microarray that analyzed 500 human proteins thematically oriented toward inflammation. After identifying several candidates with elevated concentrations in the pooled samples, we then used reverse protein arrays to quantitate the concentration of potential candidate proteins in the individual samples. Proteins with significantly elevated concentrations included apolipoprotein-E, apolipoprotein-A, serum amyloid protein-4, and serum amyloid protein-P. Future studies in larger sample populations are needed to evaluate these biomarkers further as representative of biosystems involved in vasospasm and DIND or as potential biomarkers predictive of risk associated with disease.

Keywords Subarachnoid hemorrhage • Vasospasm Biomarkers • Delayed ischemic neurological deficit

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Introduction

The morbidity and mortality from aneurysmal subarachnoid hemorrhage (SAH) remains high, with a case fatality rate of 25–50% [4, 5]. Vasospasm is the most common complication of SAH that leads to clinical deterioration; however, its pathophysiology is still poorly understood. The diagnosis of vasospasm is based on clinical signs and symptoms in conjunction with invasive and noninvasive imaging procedures. These include the four-vessel digital angiogram and transcranial Doppler (TCD) [7]. TCD is relatively sensitive and specific for arterial vasoconstriction but less useful in the diagnosis of delayed ischemic neurological deficits (DINDs). Angiography is a sensitive and specific method to demonstrate arterial constriction and vasospasm yet is less useful at predicting the occurrence of DINDs.

Proteomics is the large-scale study of proteins. It is an evolving technology that allows the identification of expressed proteins in any biologic fluid and can facilitate the description of proteins involved in a wide range of biological functions [10]. These methods can help develop candidate proteins that may serve as biomarkers for different disease states [1, 8]. Early experience with proteomics applied to human sera from patients with cancer suggested that this approach has the potential to provide novel information regarding diagnosis, classification, and response to therapy [6]. Protein expression signatures have been used to facilitate early diagnosis of ovarian and prostate cancer and to develop tumor-specific profiles in lung cancer [2, 9, 11].

We explored the application of proteomics to help characterize patients after SAH at risk for vasospasm, DINDs, and

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responses to therapy. We employed three different approaches to screen for candidate biomarkers. SELDI-TOF MS (surface-enhanced laser desorption and ionization time-of-flight mass spectrometry) was used to assess whether serum protein signatures would differentiate between patients with and without vasospasm in the 3 days before vasospasm risk and at peak TCD velocities 8 days after aneurysmal SAH. We also used a protein chip array that evaluated 500 human proteins thematically oriented toward inflammation. Last, we used immunoassays to measure some of these candidate proteins of interest directly.

Methods

Patient Population

Our study included all eligible patients, ages 18–80 years, with aneurysmal SAH who consented for proteomic analysis of blood. All patients had symptom onset within 48 h of presentation. Patients were excluded if they had signs of traumatic SAH, symptom onset >48 h, arteriovenous malformation, tumor, negative angiograms, nonaneurysmal SAH, unknown symptom onset, or pregnancy. The study protocol was approved by our institutional review board, and written informed consent was obtained from each patient or their surrogates.

Diagnosis of Nontraumatic Subarachnoid Hemorrhage, Vasospasm, and DIND

The diagnosis of SAH was based on the presence of an acute bleed on computed tomographic (CT) scan and positive conventional four-vessel cerebral angiogram. If the angiogram was initially positive for aneurysm, then the patient had an aneurysmal bleed. If the angiogram was initially negative for an aneurysm and then a repeat scan at 10–14 days was also negative, the patient was considered angiogram negative. The determination of DINDs was based on the presence of clinical signs of neurological deterioration in the setting of vasospasm. This deficit could include a focal neurological deficit as well as an alteration in Glasgow Coma Scale. Serial TCDs were performed as standard of care to monitor and detect the presence of vasospasm.

Serum Protein Profiles

Serum was collected every day for 15 consecutive days. Each patient had a serum sample obtained within 3 days after the rupture during the asymptomatic period prior to the beginning of the vasospasm risk period. The final analysis required one serum sample from the prevasospasm period and one sample from the days of maximum risk of onset of vasospasm, days 7–9. The serum was denatured in 9 M urea/2% CHAPS for 30 min and diluted to 200 µl final volume. Each sample was run in duplicate on four different protein chips that enhance the retention of different proteins (CM10 – weak cation exchange, Q10 – strong anion exchange, H50, hydrophobic surface; and IMAC 30, metal-binding surface) (Ciphergen Biosystems, Inc., Palo Alto, CA, USA). Samples were run on the Ciphergen PBSIIc at two different laser intensities and instrument settings to optimize peak detection. The spectra were mass aligned, baseline subtracted, and normalized before being subjected to a peak picking algorithm (Ciphergen Express Software 2.1). Mann-Whitney *t* tests of the mean intensity of each individual peak were calculated to compare differences between the groups as previously described [3].

Antibody Array

We screened samples for novel protein expression using an antibody array (RayBiotech Cytokine 507 Array, RayBiotech, Norcross, GA, USA). Four pooled samples were prepared as follows: (a) 14 patients who developed vasospasm: baseline (group 1) and day 8 ± 2.8 at the time of vasospasm (group 2) and (b) 14 patients with SAH who did not develop vasospasm: baseline (group 3) and follow-up at day 8 ± 2.6 (group 4). The chips were run in duplicate and scanned using the GenePix 4000A and GenePix 3.0. We selected the major induced or repressed proteins for further validation. Validation of selected proteins was done using immunoassays and reverse phase arrays.

Results

Mass spectral peak analysis revealed a number of protein peaks that discriminated between patients who developed vasospasm and DINDs from those who did not. Protein peaks that were elevated in the no vasospasm group included the 9.3-kDa peak from the CM10 chip and the 2.1-kDa peak from the Q10 chip. Increased expression of proteins associated with the vasospasm group included a 51.3-kDa and a 3.3-kDa peak from the Q10 chip and a 4.15- and 12.4-kDa peak from the CM10 chip. The pooled samples assayed with the antibody array revealed several candidate proteins associated with the development of vasospasm (Table 1). Several were analyzed further using immunoassays or reverse phase antibody arrays.

Table 1 Proteins of Biomarkers in patients with, Subarachnoid Hemorrhage, Vasospasm, and Delayed Ischemic Neurological Deficits

Antibody array	Validation method	Findings and p value
Thrombospondin-1	ELISA and RPA	G2 ↑, G1/G3, $p=0.15$
Interleukin-9	ELISA	G1/G2, $p=0.48$, G3/G4, $p=0.1$
CTACK (CCL27)	ELISA	G4 ↑, G3/G4, $p=0.02$
C-reactive protein	ELISA and RPA	G3 ↓, G3/G4, $p=0.001$, G1/G3, $p=0.016$
Vitronectin	RPA	G4 ↑, G3/G4, $p=0.005$
Serum amyloid P	RPA	G2 ↑, G1/G2, $p=0.4$, G4 ↑, G3/G4, $p=0.01$
Apolipoprotein A1	RPA	G2 ↓, G1/G2, $p=0.009$, G3 ↑, G1/G3, $p=0.01$
Apolipoprotein E	RPA	G4 ↑, G3/G4, $p=0.006$
Serum amyloid A-4	RPA	G2 ↑, G1/G2, $p=0.05$, G4 ↑, G3/G4, $p=0.001$

Vasospasm: baseline (group 1), day 8 ± 2.8 at the time of vasospasm (group 2)
No vasospasm: baseline (group 3), followup at day 8 ± 2.6 (group 4)
ELISA enzyme-linked immunosorbent assay, *RPA* reverse phase array

Conclusion

Subarachnoid hemorrhage is unique in its association with vasospasm and DINDs. There is a strong serological link between this disorder and the development of vasospasm. Recent studies suggested that injury within the neurological compartment can be detected in the serum. Our initial results demonstrated how proteomic methods can implicate new pathways and suggest potential novel biological markers. With further validation, patients who are at risk for DINDs may be identified in the presymptomatic stages by detection of novel biomarkers. By employing these methodologies, we are capable of identifying proteins of interest that may affect the development of vasospasm and DINDs. Our study identified proteins in the serum amyloid and apolipoprotein-a) (ApoA) and apoprotein-e (ApoE) family as proteins of interest that may yield insight into the pathophysiology of this disorder.

Conflicts of Interest We declare that we have no conflict of interest.

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Randomised Trial of Clazosentan, an Endothelin Receptor Antagonist, in Patients with Aneurysmal Subarachnoid Hemorrhage Undergoing Surgical Clipping (CONSCIOUS-2)

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Abstract We report here results of a randomized, double-blind, placebo-controlled study (http://www.ClinicalTrials.gov, NCT00558311) that investigated the effect of clazosentan (5 mg/h, $n=768$) or placebo ($n=389$) administered for up to 14 days in patients with aneurysmal subarachnoid hemorrhage (SAH) repaired by surgical clipping. The primary endpoint was a composite of all-cause mortality, new cerebral infarction or delayed ischemic neurological deficit due to vasospasm, and rescue therapy for vasospasm. The main secondary endpoint was the Glasgow Outcome Scale Extended (GOSE), which was dichotomized. Twenty-one percent of clazosentan- compared to 25% of placebo-treated patients met the primary endpoint (relative risk reduction [RRR] [95% CI]: 17% [−4% to 33%]; $p=0.10$). Poor outcome (GOSE score ≤ 4) occurred in 29% of clazosentan- and 25% of placebo-treated patients (RRR: −18% [−45% to 4%]; $p=0.10$). In prespecified subgroups, mortality/vasospasm-related morbidity was reduced in clazosentan-treated patients by 33% (8–51%) in poor WFNS (World Federation of Neurological Surgeons) grade (≥III) and 25% (5–41%) in patients with diffuse, thick SAH. Lung complications, anemia and hypotension occurred more frequently with clazosentan. Mortality (week 12) was 6% in both groups. The results showed that clazosentan nonsignificantly decreased mortality/vasospasm-related morbidity and nonsignificantly increased poor functional outcome in patients with aneurysmal SAH undergoing surgical clipping.

Keywords Aneurysmal subarachnoid hemorrhage Clazosentan • Surgical clipping • CONSCIOUS-2

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Introduction

About 40% of patients with aneurysmal subarachnoid hemorrhage (SAH) die, and many of the survivors have permanent cognitive and neurological impairment [1, 13]. Morbidity is associated with increasing age, worse clinical grade on admission and delayed complications, principally delayed ischemic neurological deterioration (DIND). Cerebral angiographic vasospasm is highly associated with DIND and has been an important target for prevention and treatment to improve outcome [3, 4]. Unfortunately, current management for vasospasm and DIND is not effective and is expensive. It

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includes prophylactic nimodipine and rescue therapy with induced hypertension and intra-arterial infusion of vasodilators and angioplasty [5, 7, 14].

Clazosentan is an endothelin A receptor antagonist that has demonstrated significant efficacy against angiographic vasospasm [10, 15]. In a phase 2b study, clazosentan produced a dose-dependent reduction in moderate/severe angiographic vasospasm, amounting to a 65% relative risk reduction (RRR) with the highest dose (15 mg/h) [10]. The current phase 3 study, CONSCIOUS-2, was designed based on the results of the phase 2b study to enrich for patients at risk for vasospasm-related morbidity and targeted patients undergoing surgical clipping.

Methods

This randomized, placebo-controlled, double-blind clinical trial included patients aged 18–75 years with aneurysmal SAH who had the ruptured aneurysm repaired by surgical clipping. Patients had at least diffuse SAH and were World Federation of Neurological Surgeons (WFNS) grade I–IV. Written informed consent was obtained according to local laws, and the trial was registered with http://www.ClinicalTrials.gov (NCT00558311). Additional information on the rationale, study design and methodology is published [11, 12].

Patients were recruited from 102 sites in 27 countries and were assigned (2:1) to receive 5 mg/h clazosentan (Actelion Pharmaceuticals Ltd., Allschwil, Switzerland) or placebo within 56 h of SAH. Clazosentan or placebo was administered intravenously for up to 14 days after SAH. The primary endpoint was vasospasm-related morbidity and all-cause mortality within 6 weeks of SAH, defined by at least one of the following: death; vasospasm-related cerebral infarction (where vasospasm was the primary cause or a relevant contributing factor); DIND due to vasospasm (where vasospasm was the primary cause or a relevant contributing factor); or neurological signs or symptoms, in the presence of a positive angiogram, leading to rescue therapy. DIND was defined as a decrease of ≥2 points on the modified Glasgow coma scale (mGCS) or an increase of ≥2 points on the abbreviated NIHSS (National Institute of Health Stroke Scale) lasting for at least 2 h. Rescue therapy included initiation or increase in dose of an intravenous vasopressor with or without fluid therapy or intra-arterial vasodilator or balloon angioplasty. The main secondary outcome was the Glasgow Outcome Scale Extended (GOSE) 12 weeks after SAH.

Patient management guidelines were followed by the investigators [11]. Clinical and imaging data were evaluated by a blinded, centralised critical events committee. Prespecified subgroups that were analysed were WFNS grade, clot size, age and gender.

Sample size calculations were done, and analysis was done in accordance with the intention-to-treat principle [12]. Treatment effect was tested by logistic regression adjusted for WFNS (I, II, >II) with the Wald chi-square test used to determine treatment effect.

Results

There were 1,157 patients randomised and 1,147 treated (placebo $n=383$; clazosentan $n=764$). Patient demographics were consistent with those of the SAH population. Vasospasm-related morbidity and all-cause mortality occurred in 161/764 (21%) of patients treated with clazosentan and 97/383 (25%) of patients treated with placebo (RRR: 17%, 95% CI: −4% to 33%; $p=0.10$ [logistic regression with WFNS as covariate]; Fig. 1a). This was mainly affected by reduction in rescue therapy from 16% of placebo- to 11% of clazosentan-treated patients (RRR: 36%, 95% CI: 14–53%; Fig. 1b). There was no significant difference between treatment groups in poor outcome (GOSE ≤ 4) at week 12 in the all-treated dataset; poor outcome was reported in 224 (29%) patients receiving clazosentan and 95 (25%) patients receiving placebo (RRR: −18%, 95% CI: −45% to 4%; $p=0.10$ [logistic regression with WFNS as covariate]; Fig. 2).

In the planned subgroup analyses, clazosentan reduced the primary endpoint in patients with poor WFNS grade (≥III) (RRR: 33%, 95% CI: 8–51%; RRR for grade I–II 9%, 95% CI: −21% to 32%) or diffuse, thick SAH (RRR: 25%, 95% CI: 5–41%; RRR for diffuse thin, local thick or local thin SAH 11%, 95% CI: −40% to 43%). The GOSE was not significantly affected by clazosentan in these subgroups.

In terms of adverse effects, there were more lung complications, anaemia, and hypotension in patients treated with clazosentan. Mortality was 6% in placebo and clazosentan groups.

Discussion

We found that 5 mg/h clazosentan for up to 14 days after aneurysmal SAH was associated with a nonsignificant 17% relative reduction in mortality or vasospasm-related morbidity. This was due largely to reduced rescue therapy in the clazosentan group. On the other hand, clazosentan was associated with a nonsignificant 18% increase in poor outcome 12 weeks after SAH. In prespecified subgroups, there was a greater RRR with clazosentan in patients with poor WFNS grade or diffuse, thick SAH. Again, however, the GOSE was not significantly affected. We expected, based on results of phase 2 studies, that since clazosentan significantly reduces angiographic vasospasm, there would be a significant reduction in vasospasm-related morbidity and an improvement in clinical outcome [10, 15].

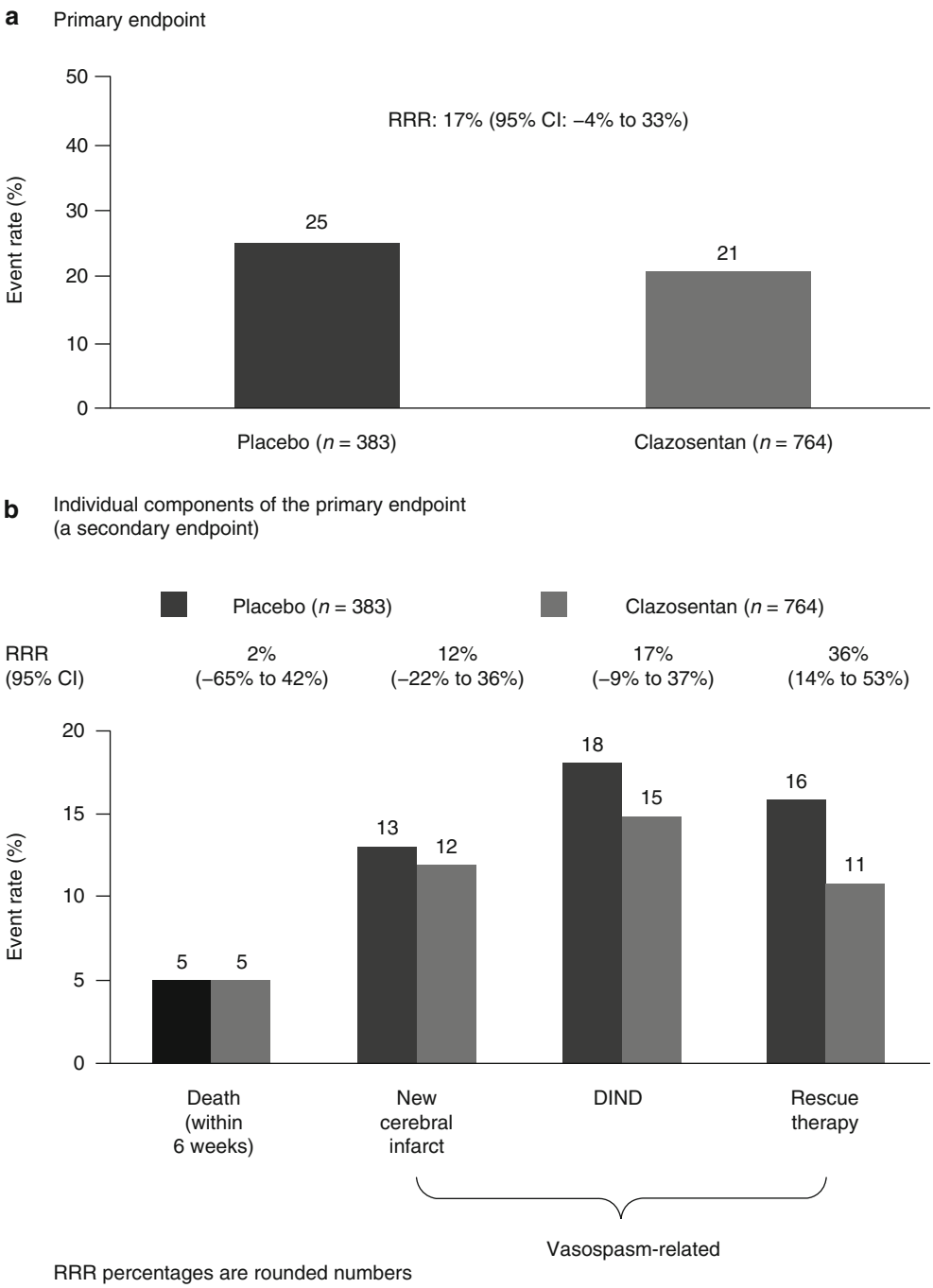

Fig. 1 Event rate (%) for all-cause mortality and vasospasm-related morbidity at 6 weeks (all treated, endpoint substituted; the primary endpoint) (**a**) and for each of the individual components of the primary composite endpoint (alltreated, endpoint substituted; planned analysis, secondary endpoint) (**b**). *RRR* relative risk reduction, *CI* confidence interval, *DIND* delayed ischemic neurological deficit

Conclusion

Explanations for the lack of significant effect of clazosentan on outcome include the possibility that off-target effects of the drug counterbalanced therapeutic benefit. There was a higher frequency of hypotension and lung complications in the clazosentan group, although the exact contribution of these effects to poor outcome remains to be defined. Rescue therapy was used more frequently in the placebo group and could improve outcome in that group to the same extent that clazosentan did, which could obscure treatment effect. Another theory is that the lack of improvement was because processes other than vasospasm contribute to DIND and poor outcome, and these are not improved by preventing angiographic vasospasm [8]. There are robust data showing that clazosentan decreases angiographic vasospasm [10]. However, other processes, such as microthromboembolism, microcirculatory dysfunction, cortical spreading ischemia and delayed neuronal injury have been postulated to contribute to DIND and outcome and may not be prevented by clazosentan [3, 6, 9, 16]. The GOSE was not developed as an outcome measure for SAH and may not be sensitive or appropriate for measuring outcome after SAH. Finally, the severity of the initial hemorrhage is an important

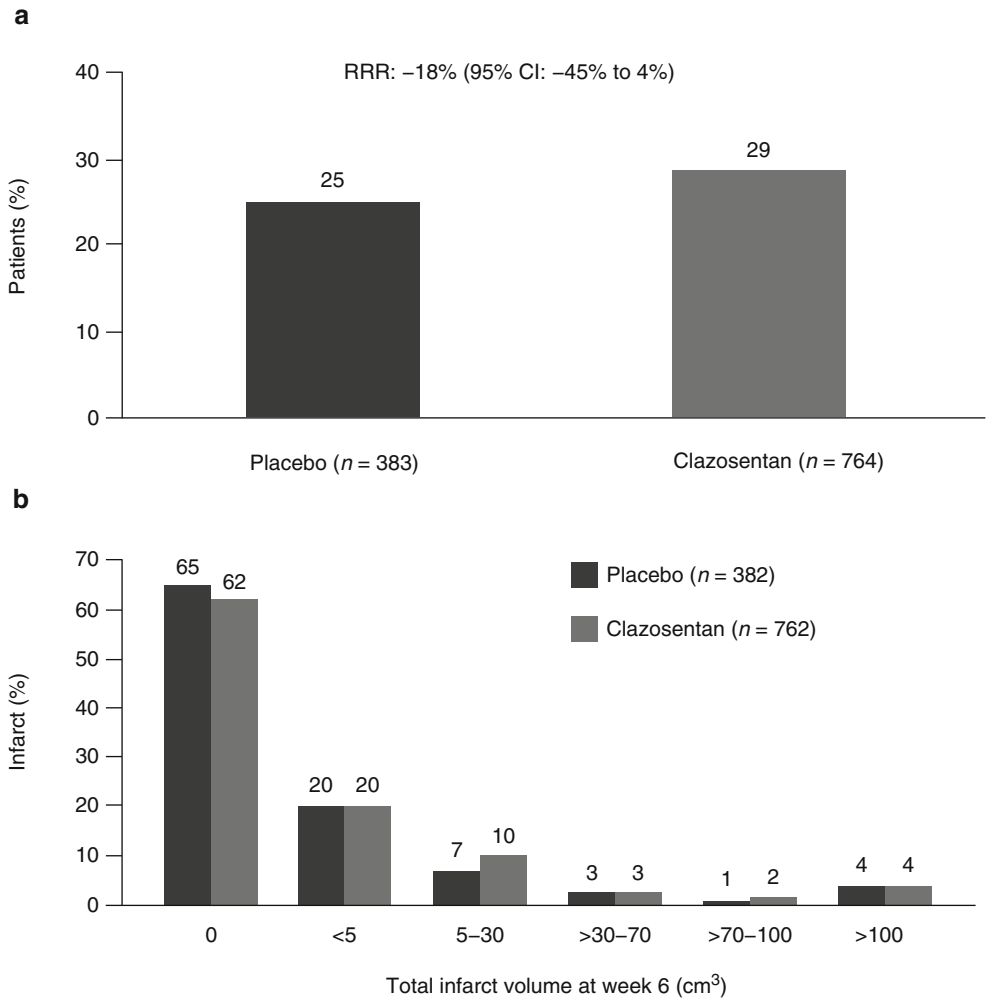

Fig. 2 Key secondary endpoints. Patients (%) with poor outcome (GOSE ≤ 4) at week 12 (dichotomized GOSE, all treated, endpoint substituted) (**a**) and total infarct volume of new/worsened infarcts (all causes) at week 6 (all treated, endpoint substituted) (**b**)

determinant of clinical outcome and may overwhelm the contribution of other processes in some settings and patient populations [2, 9].

Additional studies of clazosentan have been conducted, including a study similar to CONSCIOUS-2 but in patients with aneurysmal SAH undergoing endovascular coiling to repair the ruptured aneurysm. The results of this study are awaited.

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Conflicts of Interest RLM receives grant support from the Physicians Services Incorporated Foundation and is a stockholder of Edge Therapeutics. RLM, RTH, EK, SAM, AMo, AR, PV, IW, and NK are consultants for Actelion Pharmaceuticals. SAM is a consultant for Edge Theraputics. RLM is Chief Scientific Officer of Edge Theraputics. EK has been on advisory boards for Roche Diagnostics, and is a stockholder in NeMoDevices. AMo has been a consultant for Micrus Endovascular and Covidien. PV is a consultant for Aesculap. IW is a consultant for Boston Scientific, ev3, and BALT, and receives a departmental grant from Boston Scientific. DB, AF, AMa, and SR are employees and stockholders of Actelion Pharmaceuticals.

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Angiographic Vasospasm Versus Cerebral Infarction as Outcome Measures After Aneurysmal Subarachnoid Hemorrhage

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Abstract *Background and purpose*: Despite a significant reduction of angiographic vasospasm, the reduction of poor functional outcome in clinical trials on aneurysmal subarachnoid hemorrhage (SAH) remains challenging. While there is general consensus that vasospasm is associated with delayed cerebral ischemia (DCI), cerebral infarction, poor functional outcome, and mortality after SAH, causal relationships are subject to discussion. Therefore, it was the aim of our study to investigate the relationship between various outcome measures and poor functional outcome in clinical trials on pharmaceutical treatment of SAH.

Methods: Based on data from two systematic reviews and a post hoc exploratory analysis, the relationship between the following outcome measures was investigated: (1) radiographic vasospasm, (2) DCI, (3) cerebral infarction, (4) poor functional outcome, and (5) death.

Results: A reduction of angiographic vasospasm did not correlate with an improvement on dichotomous Glasgow Outcome Scale/modified Rankin Scale (GOS/mRS). In contrast, a reduction of cerebral infarction correlated with better neurological outcomes. The heterogeneous definition of DCI in previous clinical trials did not allow pooling of the data. Conclusion: Future clinical trials may use cerebral infarction and functional outcome as main outcome measures to investigate the true impact of an intervention, assuming that the intervention targets cerebral infarction and hereby improves outcome.

Keywords Subarachnoid hemorrhage • Angiographic vasospasm • Cerebral infarction • Poor outcome

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is characterized by high morbidity and mortality rates. While mortality in the course of SAH has been reduced by 50% over the last decades, the reduction of poor neurological outcome remains challenging [15]. The majority of previous clinical trials on pharmaceutical treatment of SAH were designed to reduce the incidence of delayed cerebral ischemia (DCI) with the aim to reduce poor outcome. Only the calcium antagonist nimodipine was found to decrease the incidence of DCI and poor functional outcome after SAH [23]. Many other trials found a significant reduction of angiographic vasospasm but could not demonstrate a significant effect on poor functional outcome. During the last decade, the role of vasospasm in the pathogenesis of DCI has been increasingly discussed. While there is general consensus that vasospasm is associated with DCI, cerebral infarction, poor functional outcome, and mortality after SAH, causal relationships are subject to discussion. An explanation for the failure of previous clinical trials to reduce poor functional outcome might be that the investigated drugs target vasospasm but not DCI. Therefore, two systematic reviews were performed to investigate the relationship between vasospasm and functional outcome and between cerebral infarction and functional outcome [4, 39]. A cohort analysis was performed to identify the relationship between several variables that affect functional outcome after SAH [40].

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Methods

For a detailed description of the methodology, we refer to the individual studies [4, 39, 40].

Meta-analyses

A PubMed search (http://www.ncbi.nlm.nih.gov/pubmed) was used to identify all randomized, placebo-controlled, double-blind clinical trials on pharmaceutical treatment of SAH in a stepwise manner. After identification of valid trials, the individual publications were screened for the incidence of the following predefined outcome measures: (1) radiographic vasospasm, (2) cerebral infarction, (3) DCI, and (4) poor functional outcome. The pooled effect of experimental treatment on outcome measures was then analyzed using Cochrane Review Manager (http://www.cochrane.org) and expressed as pooled risk ratio (RR) estimates.

Radiographic vasospasm was defined as a focal or generalized narrowing of cerebral arterial caliber on cerebral digital subtraction angiography or increased cerebral blood flow velocities as measured by transcranial Doppler (TCD). If angiographic vasospasm was categorized into no/mild, moderate, and severe vasospasm in included studies, the number of patients with moderate-to-severe vasospasm was included. TCD vasospasm was defined as a flow velocity of at least 120 cm/s or peak flow velocity of more than 200 cm/s. If studies reported on both angiographic and TCD vasospasm, only data on angiographic vasospasm were used. Cerebral infarction was defined as "the presence of cerebral infarction on CT [computed tomography] or MR [magnetic resonance] scan of the brain within 6 weeks after SAH, or on the latest CT or MR scan made before death within 6 weeks, or proven at autopsy, not present on the CT or MR scan between 24 and 48 h after early aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment. Hypodensities on CT imaging resulting from ventricular catheter or intraparenchymal hematoma should not be regarded as cerebral infarctions from DCI" [38].

For DCI, the individual definitions of each clinical trial were used. Poor clinical outcome was defined as severe disability, vegetative state, or death, as defined within the individual studies, measured with either the modified Rankin scale (mRS) or (extended) Glasgow Outcome Scale (GOS). An mRS of 3–6 or a GOS of 1–3 were considered poor clinical outcome. In those studies where an inverted GOS scale was used, poor clinical outcome was readjusted to the original scale. For all included studies, we used data from the last blinded outcome measurement. If the study provided the percentage of patients with an outcome event, the actual numbers were calculated.

Exploratory Cohort Analysis

In addition to the aforementioned meta-analyses, an explanatory post hoc analysis of the CONSCIOUS-1 cohort [16] was performed to analyze the relationship between several variables that affect functional outcome after SAH. First, the incidence of WFNS (World Federation of Neurological Societies) grade IV-V, neurological worsening, new cerebral infarction, poor functional outcome, and death in patients with and without angiographic vasospasm was described. Odds ratios (ORs) were calculated for patients with angiographic vasospasm to have neurological worsening, cerebral infarction, poor outcome, and death.

Second, bivariate analyses were performed to study the association of various baseline and clinical variables with poor outcome. The significantly associated variables (at $p<0.10$) were built into a multivariable logistic regression analysis and statistically significant predictor variables for the final model at the 5% significance level were retained through backward selection of these. Also, a path analysis based on structural equation modeling was undertaken to determine the direct and indirect path coefficients for the variables angiographic vasospasm, neurological worsening, and new cerebral infarcts, which were hypothesized to have an influence on poor outcome. Path analysis explores, but does not prove, potential causal relationships by using a single-headed arrow, with the head pointing to the effect and the tail pointing to the cause. The size of correlation is expressed in path coefficients.

Results

Angiographic Vasospasm and Poor Clinical Outcome Meta-analysis

Of 733 articles, 14 trials on pharmaceutical treatment of SAH with angiographic vasospasm, DCI, and poor neurological outcome as outcome events were included in the meta-analysis [2, 5, 7–9, 11, 16, 18, 20–22, 26, 31, 37]. Data from a total of 4,235 patients (2,612 randomized to pharmaceutical treatment and 1,623 to placebo) were analyzed. The analysis of radiographic vasospasm comprised data from 2,819 patients (1,783 patients randomized to experimental treatment and 1,036 to placebo). For the analysis of clinical outcome data from 4,211 (2,597 randomized to pharmaceutical treatment and 1,614 to placebo) were included. The heterogeneous definitions for DCI among the 14 clinical trials did not justify pooling of the data. The pooled analysis of the data of all 14 trials demonstrated a significant reduction of radiographic vasospasm in the pharmaceutical group (pooled RR: 0.80; 95% CI: 0.70–0.92). There was significant heterogeneity between the trials ($I^2=62\%$ and $p=0.001$; Fig. 1), which justified the use of a random-effects model.

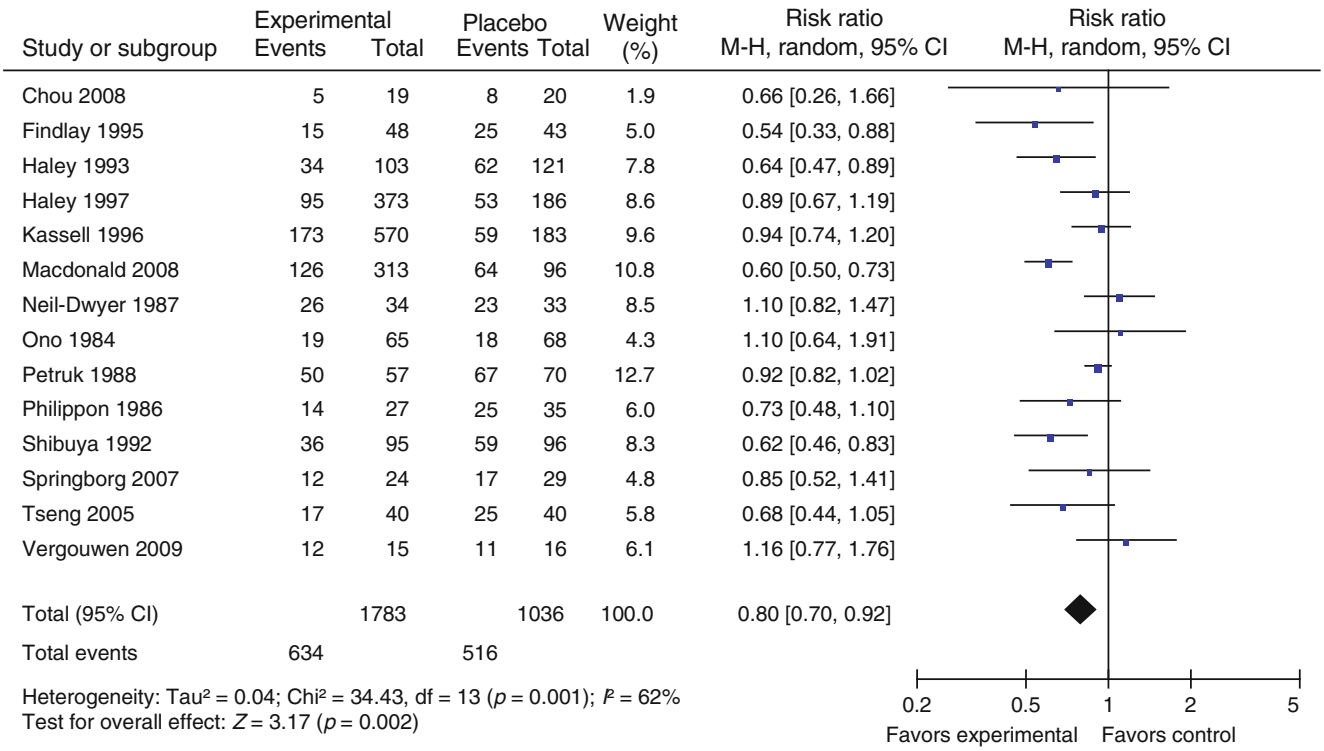

Fig. 1 Pooled risk ratio (*RR*) estimates for patients with pharmaceutical treatment for SAH to have radiographic vasospasm (radiographic vasospasm and poor functional outcome meta-analysis)

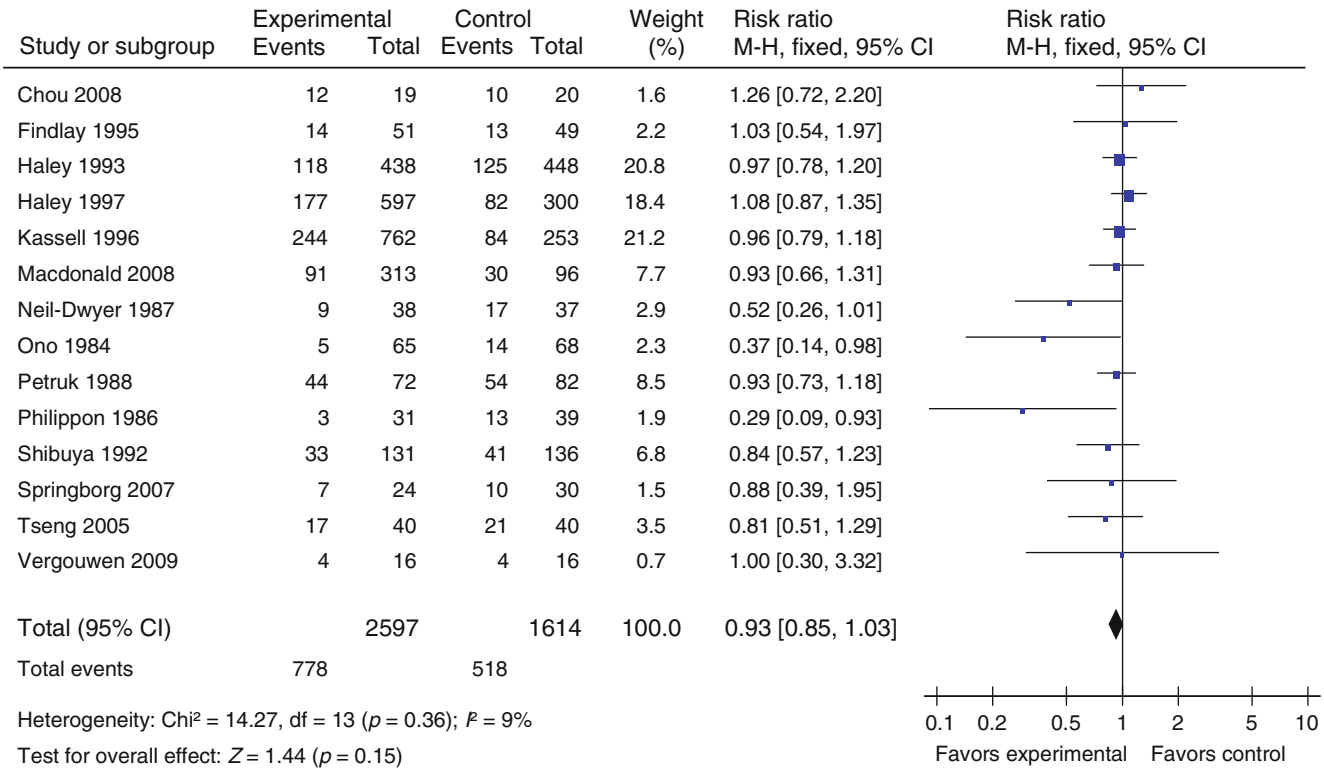

Fig. 2 Pooled risk ratio (*RR*) estimates for patients with pharmaceutical treatment for SAH to have poor functional outcome (radiographic vasospasm and poor functional outcome meta-analysis)

No effect was observed of pharmaceutical treatment on functional outcome (pooled RR 0.93; 95% CI: 0.85–1.03). There was no significant heterogeneity between the trials (I^2=9% and p=0.36; Fig. 2), which justified the use of a fixed-effects model.

Cerebral Infarction and Poor Clinical Outcome Meta-analysis

Of 780 articles, 24 trials on pharmaceutical treatment of SAH with cerebral infarction and clinical outcome as an outcome event were included [1, 2, 6–11, 13, 14, 16, 19, 21, 23–29, 32–34, 41, 42]. For this analysis, data from 8,552 patients (4,818 patients randomized to pharmaceutical treatment and 3,734 to placebo) were available. The analysis of cerebral infarction comprised data from 7,982 patients (4,508 patients randomized to pharmaceutical treatment, 3,474 to placebo). For the analysis of poor clinical outcome, data from 8,489 patients were included (4,784 patients randomized to pharmaceutical treatment, 3,705 to placebo).

The pooled analysis of all 24 trials demonstrated a significant and favorable effect of pharmaceutical treatment on cerebral infarction (pooled RR: 0.83; 95% CI: 0.74–0.93). Heterogeneity between the trials was high (I^2=58% and p=0.0002). This justified the use of a random-effects model (Fig. 3). In addition, there was a favorable effect of pharmaceutical treatment on poor clinical outcome (pooled RR: 0.92; 95% CI: 0.86–0.98). Since there was no significant heterogeneity between the trials (I^2=11% and p=0.30), a fixed-effects model was used (Fig. 4).

Exploratory Cohort Analysis

The analysis comprised data from a total of 413 patients; 194 suffered from moderate-to-severe angiographic vasospasm. Of the 194 patients with moderate/severe vasospasm, 43% had neurological worsening of any cause, 20% had cerebral infarction, 46% had poor outcome, and 12% died. Of the 219 patients with no/mild vasospasm, 14% had neurological worsening, 3% had cerebral infarction, 16% had poor outcome, and 0.5% died. The ORs for patients with moderate/severe

Study or subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight (%)	Risk ratio M-H, random, 95% CI
Asano 1996	20	78	30	81	3.4	0.69 [0.43, 1.11]
Chou 2008	2	19	5	20	0.5	0.42 [0.09, 1.92]
Gomis 2010	14	49	12	46	2.1	1.10 [0.57, 2.11]
Haley 1993	129	267	148	289	7.3	0.94 [0.80, 1.12]
Haley 1997	203	597	81	300	6.5	1.26 [1.01, 1.56]
Hop 2000	4	24	5	26	0.8	0.87 [0.26, 2.85]
Kassell 1996	206	762	81	253	6.6	0.84 [0.68, 1.05]
Lanzino I 1999	90	404	105	412	6.1	0.87 [0.68, 1.12]
Lanzino II 1999	114	410	98	412	6.3	1.17 [0.93, 1.48]
Macdonald 2008	26	313	17	96	2.7	0.47 [0.27, 0.83]
Ohman 1988	34	88	49	92	5.0	0.73 [0.52, 1.00]
Petruk 1988	21	33	32	45	5.1	0.89 [0.65, 1.23]
Pickard 1989	61	278	92	276	5.6	0.66 [0.50, 0.87]
Saito 1998	20	145	34	141	3.1	0.57 [0.35, 0.94]
Shaw 2000	79	207	81	213	6.1	1.00 [0.79, 1.28]
Shibuya 1992	56	127	83	135	6.2	0.72 [0.57, 0.91]
Siironen 2003	22	76	25	80	3.3	0.93 [0.57, 1.50]
Springborg 2007	8	21	13	25	2.1	0.73 [0.38, 1.42]
Suzuki 1989	38	121	31	63	4.5	0.64 [0.44, 0.92]
Tseng 2009	3	40	16	40	0.8	0.19 [0.06, 0.59]
van den Bergh 2005	62	139	67	144	6.0	0.96 [0.74, 1.24]
van den Bergh 2006	41	87	32	74	4.8	1.09 [0.77, 1.54]
Westermaier 2010	12	54	27	53	2.7	0.44 [0.25, 0.77]
Wong 2010	17	169	18	158	2.3	0.88 [0.47, 1.65]
Total (95% CI)		4508		3474	100.0	0.83 [0.74, 0.93]
Total events	1282		1182			

Heterogeneity: Tau² = 0.04; Chi² = 54.57, df = 23 (p = 0.0002); I^2 = 58%
Test for overall effect: Z = 3.26 (p = 0.001)

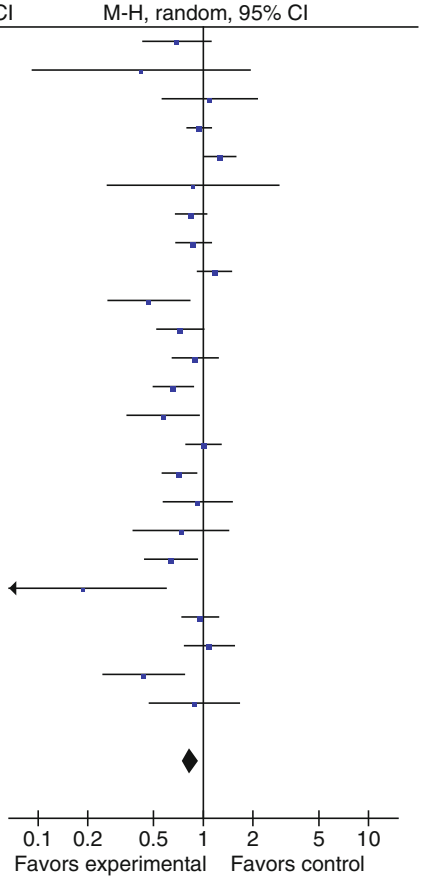

Fig. 3 Pooled risk ratio (*RR*) estimates for patients with pharmaceutical treatment for SAH to have cerebral infarction (cerebral infarction and poor functional outcome meta-analysis)

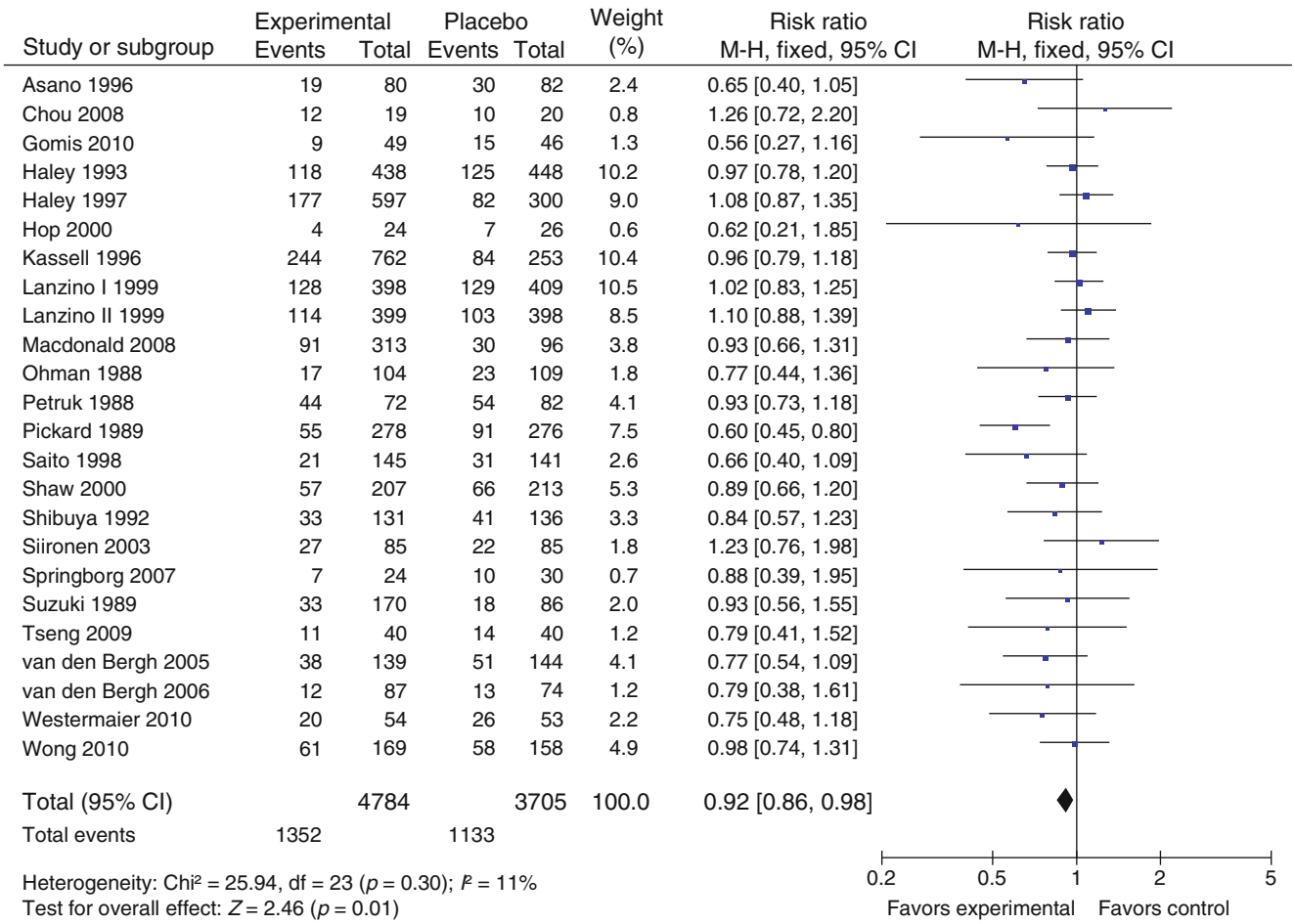

Fig. 4 Pooled risk ratio (*RR*) estimates for patients with pharmaceutical treatment for SAH to have poor functional outcome (cerebral infarction and poor functional outcome meta-analysis)

angiographic vasospasm were 4.53 (95% CI: 2.75–7.50) for having neurological deterioration, 8.93 (95% CI: 3.51–24.10) for developing cerebral infarction, 4.31 (95% CI: 2.67–6.98) for having a poor outcome, and 30.78 (95% CI: 4.38–617.47) for mortality.

The main independent predictors of poor outcome were (1) WFNS grade IV-V (OR 2.93; 95% CI: 1.74–4.94); (2) moderate-severe vasospasm (OR: 2.62; 95% CI: 1.58–4.36); (3) neurological worsening (OR: 2.29; 95% CI: 1.37–3.86); (4) new cerebral infarction (OR: 2.13; 95% CI: 1.58–4.36); and (5) history of hypertension (OR: 1.94; 95% CI: 1.19–3.13). The relationship between WFNS grade, angiographic vasospasm, neurological worsening, new cerebral infarction, and history of hypertension and poor outcome was further tested using a path analysis.

Exogenous independent variables were WFNS grade and history of hypertension; endogenous independent variables were angiographic vasospasm, neurological worsening, and new cerebral infarction. Poor outcome was the dependent variable. The size of correlations is represented by path coefficients, as illustrated in Fig. 5.

Discussion

The main findings of our studies on the relationship between various outcome measures and poor functional outcome in clinical trials on pharmaceutical treatment of SAH can be summarized as follows: A significant reduction of angiographic vasospasm did not correlate with better functional outcomes. In contrast, a lower incidence of cerebral infarction corresponded with improved outcomes. Cerebral infarction contributes to poor neurological outcome by vasospasm-dependent and -independent effects. The heterogeneous definition of DCI among previous clinical trials did not allow pooling of the data.

Our findings underline more recent studies on the pathogenesis of DCI. Despite an undisputed association between angiographic vasospasm and DCI, additional pathophysiological mechanisms, such as cortical spreading ischemia/cortical spreading depression, microthromboembolism, and disturbed autoregulation are likely involved in the pathogenesis of DCI [3, 36]. This is also supported by our cohort analysis as 57% of the patients with moderate-severe

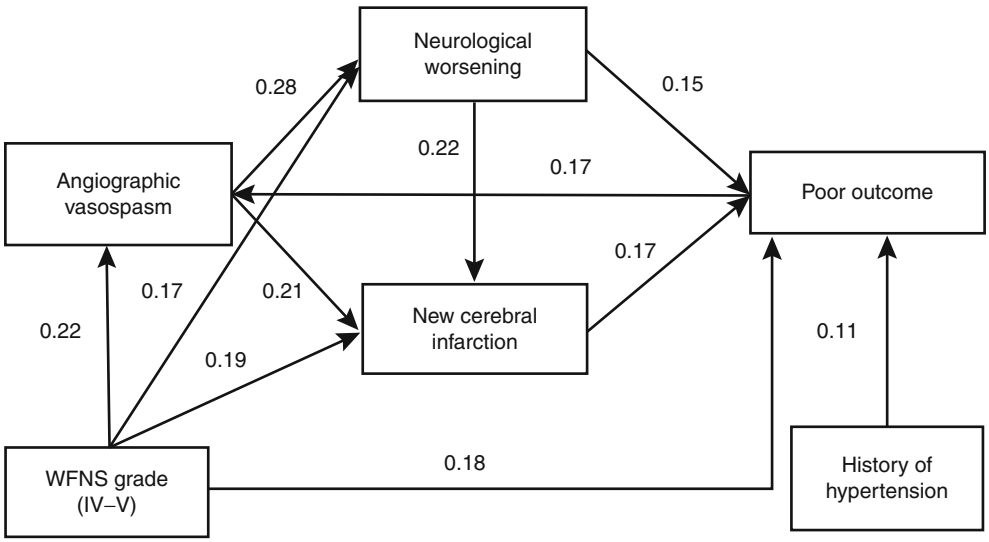

Fig. 5 Path analysis of SAH-related variables contributing to poor functional outcome with direct and indirect path coefficients

vasospasm did not have neurological worsening of any kind. The causal relationship between angiographic vasospasm, DCI, cerebral infarction, and poor functional outcome remains incompletely understood.

There are several explanations for our findings. First, the dichotomous GOS/mRS might be too insensitive to detect meaningful changes in clinical outcome measures [17]. Second, pharmaceutical treatment in SAH patients might affect outcome, such as by causing detrimental cardiopulmonary complications [30]. Third, the sample size in clinical trials might be too small, as it was suggested that more than 5,000 patients may be needed to achieve a significant effect on 3-month mRS when angiographic vasospasm or possibly DCI is reduced by 50% [12]. Last, the aforementioned additional pathophysiological mechanisms contributing to DCI and cerebral infarction may not be targeted by previous pharmaceutical treatments in patients with SAH [35].

In summary, various drug treatments decrease angiographic vasospasm but have no detectable effect on dichotomous GOS/mRS. Importantly, the heterogeneous definition of DCI in previous in clinical studies does not allow pooling of DCI data. There is a strong association between reduction of cerebral infarction and reduction of poor neurological outcome. As cerebral infarction reflects the ultimate endpoint of the ischemic event, it might be a better target for future pharmaceutical intervention in SAH. Finally, primary brain injury (i.e., initial WFNS grade) remains a significant determinant of poor outcome.

Conclusion

Future studies need to determine if the dissociation between angiographic vasospasm and clinical outcome is due to methodological problems, sample size, or insensitivity of clinical outcome measures or because pathophysiological mechanisms other than vasospasm contribute to poor functional outcome. Moreover, future clinical trials may use cerebral infarction and functional outcome as main outcome measures to investigate the true impact of an intervention, assuming that the intervention targets cerebral infarction and thereby improves outcome.

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Conflicts of Interest We declare that we have no conflict of interest.

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Development of Nicardipine Prolonged-Release Implants After Clipping for Preventing Cerebral Vasospasm: From Laboratory to Clinical Trial

Hidetoshi Kasuya

Abstract We have developed a drug delivery system using a vasodilating drug that can be implanted intracranially at the time of surgery for aneurysm clipping, without systemic side effects or side effects associated with long-term intrathecal drug administration. We started our project on 1994 for making a slowly releasing drug delivery system in vitro because cerebral vasospasm occurs 4–14 days following subarachnoid hemorrhage (SAH). A rod-shaped pellet containing 1 mg of nicardipine for animal study was prepared by heat compression. We presented the efficacy and safety of this drug delivery system using both canine double-hemorrhage and clot placement models. Since October 1999, nicardipine prolonged-release implants (NPRIs) containing 4 mg of nicardipine have been used to prevent vasospasm in patients with SAH. NPRIs were placed in the cistern of the cerebral arteries, where thick clots existed; therefore, vasospasm related to delayed ischemic neurological deficits (DINDs) was highly probable. Vasospasm was completely prevented in the arteries by placing NPRIs adjacent to the arteries during surgery. No complications were experienced. We have performed three studies (a single-center study with consecutive patients; a single-center, randomized, double-blind trial; and a multicenter cooperative study) and have proved that implantation of NPRIs reduces the incidence of cerebral vasospasm and DINDs and improves clinical outcome after SAH.

Keywords Cerebral vasospasm • Drug delivery system • Nicardipine • Subarachnoid hemorrhage

Introduction

Despite extensive investigative efforts, the pathogenesis and pathophysiology of delayed cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) remain far from clear. Vasospasm continues to be one of the primary causes of mortality and neurological morbidity and an important cause of cerebral ischemia and stroke, despite the establishment of early surgical obliteration of aneurysm. Although there have been numerous reports describing the prevention of delayed ischemic neurological deficits (DIND), such as intrathecal administration of urokinase, cisternal irrigation with drainage, endovascular treatment, head shaking, and arterial injection of vasodilatory drug, 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 for SAH patients since 1994. The current report presents how we have been developing this drug delivery system from laboratory to clinical trial.

Preliminary Study Using Papaverine

A drug delivery system using copoly(lactic/glycolic acid) (PLGA) was developed for the intracranial administration of papaverine [14]. A rod-shaped implant prepared by a heat compression method was tested to determine its efficacy in preventing cerebral vasospasm in dogs. Sixteen dogs were randomly assigned to one of two groups: placebo or papaverine. Control angiography was performed, followed by right craniectomy and the induction of SAH by the placement of a clot in the sylvian fissure. Two pellets, containing either 25 mg of papaverine or no papaverine, were placed in the cistern. In the in vitro studies, 56% of the actual papaverine loading was released in the first 4 days and 78% within 8 days. On day 7, angiography was repeated, and the animals were killed. A similar experiment using low-dose pellets containing 5 mg of papaverine, half of which was released within 7 days, was performed with 16 mongrel dogs.

There were significant differences between the papaverine- and placebo-treated groups in the reductions of vessel diameters of the internal carotid, middle cerebral, and anterior cerebral

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arteries on the clot side. The mean concentration of papaverine in the clot was 4.5×10^{-4} mol/L. The low-dose pellet failed to prevent cerebral vasospasm, although the mean concentration of papaverine in the clot was 2.3×10^{-5} mol/L. A prolonged-release preparation of papaverine implanted intracranially at the time of surgery prevented vasospasm significantly while maintaining an appropriate concentration of papaverine in the cistern. However, the effective dose was much higher than we expected from the data in vitro, and we concluded that papaverine is not a practical vasodilating drug for clinical use.

In Vitro Study

A rod-shaped pellet (2 mm in diameter, 10 mm in length, containing 4 mg of nicardipine; for animal model 1 mm in diameter, 10 mm in length, containing 1 mg of nicardipine) was prepared by heat compression. PLGA (PLG1600ML; molecular weight 4,000, lactic acid ratio 0.5) was purchased from Taki Company. (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. 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 (5*N*) was added to the solution to shift the pH above 10. The nicardipine free base was extracted with dichloromethane [6, 7, 10, 11, 13].

The release of nicardipine from the pellet was examined in a mixture of 0.02 mol/L phosphate buffer, pH 7.3, and saline (1:1 v/v) in a flask. The flask was shaken in a water bath at a frequency of 20 strokes/min. Five milliliters of the release medium were withdrawn periodically and replaced with an equivalent volume of fresh buffer. The amount of papaverine released was analyzed by high-performance liquid chromatography (Hitachi L6000; Hitachi, Tokyo, Japan) under the following conditions: YMC-Pack ODS AM-312 (150 × 6.0-mm inner diameter) column; column temperature 40°C; 10 mmol/L KH2PO4/acetonitrile (6:4) mobile phase; 1.5 mL/min flow rate; detection with ultraviolet absorbance at 240 nm; pyrilamine internal standard (obtained from Sigma Chemical).

Release curve from the pellets was adjusted similar to the time course of cerebral vasospasm by changing the combination of molecular weight and lactic acid ratio of PLGA.

Animal Model

Canine Clot Placement Model

The purpose of this study was to determine the efficacy of nicardipine prolonged-release implants (NPRIs) for preventing vasospasm in a canine SAH model in a dose-escalating, placebo-controlled, blind fashion. Drug release kinetics of the PLGA pellet containing nicardipine was evaluated in vitro. In vivo, 18 dogs were randomly assigned to one of three groups: placebo or low-dose (0.8 mg) or high-dose (8 mg) nicardipine. Angiography was performed, followed by right craniectomy, the induction of SAH, and the placement of the pellets in the sylvian fissure. On days 7 and 14, the angiography was repeated. In the first 4 days, 61.9% of the actual nicardipine loaded was released and within 10 days, 96%. The average percentage reductions of vessel diameters in the middle cerebral artery on day 7 were 43%, 14%, and 7% in the placebo, low-dose, and high-dose groups, respectively ($p = 0.0319$). The mean concentration of nicardipine in the clots on Day 14 was 9.7×10^{-7} and 5.1×10^{-6} mol/L in the low-dose and high-dose group, respectively. This drug delivery system prevented vasospasm in dogs significantly even at low dose while maintaining an appropriate concentration of nicardipine in the clot adjacent to the arteries [10, 11].

Canine Double-Hemorrhage Model

Ten dogs were assigned to two groups: double-hemorrhage group and double-hemorrhage group treated with implants. Vertebral angiography and arterial blood injection into the cisterna magna were performed, followed by midline suboccipital craniectomy and laminectomy of the atlas and placement of nicardipine implants in the cisterna magna. On day 2, arterial blood injection into the cisterna magna was repeated (double-hemorrhage model). On day 7, vertebral angiography was repeated. The animals were then sacrificed, and the brain and blood clot were taken out. All the animals involved in both groups had been clinically well. Although five animals of the control group showed severe vasospasm, no vasospasm was observed in three animals and only very mild vasospasm in two of the nicardipine-treated group. There was a statistically significant difference in diameter between the two groups (0.5 vs. 1.1 mm, $p = 0.009$). Histological examination showed no specific changes related to implants. Neither clinical symptoms related to implants nor specific histological changes were observed (e.g., hypotension, seizure). These results suggested that the

nicardipine-prolonged-release preparation is safe as well as effective for cerebral vasospasm [13].

Clinical Trial

The First Consecutive 100 Patients

NPRIs have been used to prevent vasospasm in patients with SAH since October 1999. The study analyzed the efficacy and safety of NPRIs in 100 patients with SAH and thick subarachnoid clot (mainly Fisher group 3) treated with NPRIs during surgery after clipping of the aneurysm. The number and location of pellets depended on the amount and site of the subarachnoid clot on preoperative computed tomography and on the type of craniotomy. Two to 12 pellets were implanted in the cisterns of the internal carotid artery, middle cerebral artery, or anterior cerebral artery, where thick clots were present and vasospasm related to DIND was highly likely. Only seven patients developed DIND, and five patients suffered cerebral infarction. Angiography performed on days 7–12 revealed no vasospasm in any of the arteries close to the site of NPRI placement. NPRI placement can completely prevent vasospasm in arteries within the cisterns containing thick clots but is less effective in remote locations [3, 5–9, 12].

Single-Center, Randomized, Double-Blind Trial

Thirty-two patients with severe SAH and undergoing aneurysm clipping were included in this single-center, randomized, double-blind trial. Sixteen patients received NPRIs implanted into the basal cisterns in direct contact to the exposed proximal blood vessels; in 16 control patients, the basal cisterns were opened and washed out only without leaving implants. Angiography was performed preoperatively and at day 8 ± 1. Computed tomographic imaging was analyzed for the incidence of territorial infarcts unrelated to surgery. Patient outcome was assessed using the modified Rankin and National Institute of Health Stroke scales. The incidence of angiographic vasospasm in proximal vessel segments was significantly reduced after implantation of NPRIs (73% control vs. 7% NPRIs). Significant differences occurred also for the majority of distal vessel segments. Computed tomographic scans revealed a lower incidence of delayed ischemic lesions (47% control vs. 14% NPRIs). The NPRI group demonstrated more favorable modified Rankin and National Institute of Health Stroke scales as well as a significantly lower incidence of deaths (38% control vs. 6% NPRIs). Implantation of NPRIs reduced the incidence of cerebral vasospasm and DIND and improved clinical outcome after severe SAH [1, 2].

Multicenter Cooperative Study in Tokyo

We started a multicenter cooperative study on January 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. The patients with SAH were treated with NPRIs during surgery after clipping of their aneurysms. The study included 87 female patients, 38 over 70 years old, 34 in WFNS 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 12 pellets were implanted in the cistern where thick clots existed, and vasospasm was highly likely. DIND, angiographic vasospasm, and cerebral infarctions were seen in 11 of 134 (8.2%), 32 of 130 (24.6%), and 16 of 129 (12.4%) patients, respectively. No complications were experienced. Independent rate at 3 months was 78%. The incidence of cerebral vasospasm in this multicenter trial was similar to that of our first trial performed in a single center [4].

Discussion

Currently, there are no drugs supported by sufficient evidence of efficacy for cerebral vasospasm in patients with SAH, despite abundant evidence of antivasospasm drugs at an experimental level. The problem could not be solved by developing new drugs but by developing methods to maintain an appropriate concentration of the drug in the target cerebral artery and its surrounding environment. We consider that this could not be achieved by systemic administration of drugs but rather by local treatment. It is, however, also difficult to maintain an effective concentration by intrathecal administration of vasodilating drugs if the agent is water soluble because cerebrospinal fluid (CSF) circulation dilutes and washes out the drug. We think that the success of our drug delivery system is influenced by choosing the drug: nicardipine [3].

Vasospasm was completely prevented in arteries in cisterns with thick clots, where vasospasm is highly expected, by placing pellets adjacent to the arteries during surgery. Fewer efficacies were found for arteries remote from the placement of pellets [7]. This was expected from our in vitro findings of the high lipophilicity of nicardipine. Nicardipine was probably adsorbed to the clot and arterial tissue near the pellets because of its high lipophilicity and did not affect the remote arteries since nicardipine was not detected in any cerebrospinal fluid samples in our experimental model [10, 11, 13]. We may find ideal drugs with less lipophilicity that work in the very local region and more remote arteries, or a more effective cocktail of drugs with different characteristics may be developed.

Conclusion

Since the literature describes DIND to be much more common and because of the associated poor outcome, our results suggest that the application of NPRIs 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 NPRIs 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 who were treated by coiling. This problem may be solved by developing a new drug delivery system that allows the maintenance of an appropriate concentration of nicardipine in the target artery since local application of nicardipine is able to prevent vasospasm [3] completely.

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

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Magnesium Sulphate for Aneurysmal Subarachnoid Hemorrhage: Why, How, and Current Controversy

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Abstract The neuroprotective effect of magnesium sulphate infusion has been confirmed in experimental models. Pilot clinical trials using magnesium sulphate in patients with acute aneurysmal subarachnoid hemorrhage (SAH) have reported a trend toward a reduction in clinical deterioration due to delayed cerebral ischemia (DCI) and an improvement in clinical outcomes. However, our recent multicenter trials and systemic review failed to confirm benefit in neurological outcome. In post hoc analysis, data also did not support that a higher dose of magnesium sulphate infusion might improve clinical outcome. We here review the current literature, highlight these discrepancies, and explore alternatives.

Keywords Clinical outcome • Delayed cerebral ischemia • Magnesium • Stroke • Subarachnoid hemorrhage

Introduction

The neuroprotective effect of magnesium sulphate infusion has been confirmed in experimental models. Whereas pilot clinical trials using magnesium sulphate in patients with acute aneurysmal subarachnoid hemorrhage (SAH) have reported a trend toward a reduction in clinical deterioration due to delayed cerebral ischemia (DCI) and an improvement in clinical outcomes, our recent multicenter trials and systemic review failed to confirm benefit in neurological outcome. Furthermore, post hoc analysis data also did not support that a higher dose of magnesium sulphate infusion might improve clinical outcome. In this chapter we review the current literature, highlight these discrepancies, and explore alternatives.

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Proposed Mechanism of Action

The recognition by Grew N, in 1965, of magnesium sulphate as one of the essential constituents of Epson salts, marked the entry of magnesium into medicines [1]. Magnesium is primarily an intracellular ion and an essential cofactor in numerous cellular functions. Magnesium is a well recognized *N*-methyl-D-aspartate (NMDA) receptor antagonist. In hypoxic-ischemic injury, there is a massive synaptic release of glutamate. When bound with postsynaptic ionotropic NMDA receptors, glutamate facilitates the entry of calcium and sodium, which in turn triggers cell death. Magnesium ion blocks the NMDA ion channel in a noncompetitive voltage-dependent fashion and hence prevents cell death. Thus, magnesium protects neurons from hyperactive situations as a neuromodulator. Magnesium is also nature's physiological calcium antagonist. Parenteral magnesium was first used to treat tetanus and eclampsia [1]. With additional perceived similar pathophysiology of cerebral vasospasm between eclampsia and aneurysmal SAH, magnesium sulphate infusion was applied to prevent cerebral vasospasm for aneurysmal SAH patients and hence improve clinical outcome with encouraging pilot results [2–19].

Veyna et al. [4] reported the results of a 40-patient prospective single-blinded clinical trial of high-dose magnesium sulphate infusion therapy (bolus of 6 g followed by 2 g/h intravenous infusion, with a target magnesium level of 4–5.5 mg/dL) following spontaneous SAH. In the magnesium treatment group, they maintained the magnesium sulphate infusion for 10 days. Symptomatic vasospasm, confirmed by angiography, occurred in 6/20 (30%) patients receiving magnesium sulphate infusion and 5/20 (25%) patients receiving the placebo, (odds ratio [OR] 1.3; 95%

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confidence interval [CI] 0.3–5.2). A favourable outcome (good recovery or moderate disability, as defined by Glasgow Outcome Scale [GOS] score of 4–5) was achieved in 13/20 (65%) patients receiving magnesium sulphate infusion and 10/20 (50%) patients receiving placebo treatment (OR 1.9; 95% CI, 0.5–6.6).

Van den Bergh et al. [6] reported the magnesium group results of the Magnesium and Acetylsalicylic Acid in Subarachnoid Hemorrhage (MASH) trial. The MASH trial was a randomized, double-blind, placebo-controlled multicenter trial with a factorial design. The salicylic acid-related data were not complete at that stage. A total of 283 patients were randomized within 4 days after aneurysmal SAH. Magnesium treatment consisted of a continuous intravenous dose of 64 mmol/day, started within 4 days after SAH and continued until 14 days after occlusion of the aneurysm. DCI (computed tomographic [CT] hypointensities with clinical features of decreased consciousness level or new focal neurological deficit) happened in 22/139 (16%) of magnesium-treated patients and 35/144 (24%) of placebo-treated patients (OR 1.3; 95% CI 0.3–1.1). All CT hypointensities (cerebral ischemia, caused by aneurysm treatment, edema associated with intracerebral hematoma, caused by placement of ventricular catheter, others) happened in 62/139 (45%) of magnesium-treated patients and 67/144 (47%) of placebo-treated patients (OR 0.9; 95% CI 0.6–1.5). Poor outcome, defined as a modified Rankin Scale (mRS) score of 3–6 at 3 months, occurred in 38/139 (27%) of magnesium-treated patients and 51/144 (35%) of placebo-treated patients (OR 1.5; 95% CI 0.9–2.4).

In our initial pilot study [7], 60 patients were randomly allocated to receive either magnesium sulphate infusion of 80 mmol/day or saline infusion for 14 days. The incidence of symptomatic vasospasm was 13/30 (43%) in the saline group and 7/30 (23%) in the patients receiving magnesium sulphate infusion (OR 0.4; 95% CI 0.1–1.2). A favourable outcome (good recovery or moderate disability, as defined by GOS 4–5) was achieved in 20/30 (67%) patients receiving magnesium sulphate infusion and 16/30 (53%) patients receiving placebo treatment (OR 1.8; 95% CI 0.6–5.0).

Muroi et al. [9] reported a study of 58 patients with aneurysmal SAH predominantly treated by microsurgical clipping (97%). This was a prospective, randomized, patient-blinded, placebo-controlled pilot study. Patients allocated to the treatment group received a bolus of 16 mmol $MgSO_4$ 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 SAH, subsequent dosage adjustments were made every 12 h. Delayed ischemic neurological deficit happened in 4/31 (13%) patients in the treatment group and 4/27 (15%) of those in the placebo group (OR 0.9; 95% CI 0.2–3.8). New ischemia on CT happened in 3/31 (10%) patients in the treatment group and 6/27 (22%) of those in the placebo group (OR 0.4; 95% CI 0.1–1.7). A favourable neurological outcome (good recovery or moderate disability as defined by GOS 4–5) at 3 months was achieved in 20/31 (64%) patients in the treatment group and 13/27 (48%) patients in the placebo group (OR 2.0; 95% CI 0.7–5.6).

Westermaier et al. [10] recently reported another single-center randomized, controlled clinical trial. Patients were randomly allocated to receive either magnesium sulphate infusion (with a target level of 2.0–2.5 mmol/L) or saline infusion for 14 days. The study was negative in clinical outcomes but showed reduction in DCI and delayed cerebral infarction with magnesium sulphate infusion. A favourable outcome (good recovery or moderate disability as defined by GOS 4–5) at 6 months was reported for 34/54 (63%) patients in the treatment group and 27/53 (51%) patients in the placebo group (OR 1.6; 95% CI 0.8–3.5). DCI occurred in 20/53 (37%) patients in the magnesium group and 35/53 (66%) patients in the control group (OR 0.3; 95% CI 0.1–0.7). Delayed cerebral infarction occurred in 12/54 (22%) patients in the magnesium group and 27/53 (51%) patients in the control group (OR 0.3; 95% CI 0.1–0.6).

Administration Routes

Intravenous administration is the most convenient and in the literature suitable-for-all route and is the route of application in other diseases. Animal studies on focal and global cerebral ischemia suggested a serum magnesium concentration over 1.4–1.5 and 2 mmol/L to achieve maximal neuroprotective effect [20–22]. Toxicity in terms of hypotension was seen only when the serum magnesium level was over 3 mmol/L. From experimental studies, the target plasma magnesium level would be compatible with twice the baseline level. The 10-to 14-day duration was conceptualized from coverage during the at-risk period of cerebral vasospasm. This route, target concentrations, and duration thus formed the basis of most magnesium sulphate studies for aneurysmal SAH.

Conclusion

Our negative multicenter study results surprised most researchers in the field [23]. Patients with aneurysmal SAH were recruited within 48 h of onset from ten participating centers from June 2002 to December 2008, with 6-month data completed in June 2009. Patients were randomly assigned to receive magnesium sulphate infusion, titrated to a serum magnesium concentration level twice the baseline value, or a saline

placebo for 10–14 days. Both patients and assessors were blinded to the treatment allocation. Of the 327 patients recruited, 169 were randomized to receive treatment with intravenous magnesium sulphate and 158 to receive saline (placebo). In the primary outcome analysis, the proportions of patients with a favourable outcome at 6 months (Glasgow Outcome Scale Extended [GOSE] score of 5–8) were similar: 64% in the magnesium sulphate group and 63% in the saline group (OR 1.0, 0.7–1.6). The data for the GOSE and modified Rankin Scale (mRS) are shown in [23]. There were no significant differences in the distributions or results of the proportional odds analyses between the two groups. The secondary outcome analyses also revealed no significant differences between the two groups. The proportions of patients with clinical vasospasm were similar, 25% (42/169) in the magnesium sulphate group and 18% (29/158) in the saline group (OR 1.5, 0.9–2.5), as were the proportions of those with a good outcome at 6 months (mRS 0–2), 57% (97/169) in the magnesium sulphate group and 58% (91/158) in the saline group (OR 1.0, 0.6–1.5); those with an excellent outcome (mRS 0–1) at 6 months, 46% (77/169) in the magnesium sulphate group and 45% (71/158) in the saline group (OR 1.0, 0.7–1.6); and those able to carry out basic activities of daily living independently (Barthel Index [BI] score of at least 85) at 6 months, 57% (97/169) in the magnesium sulphate group and 61% (96/158) in the saline group (OR 0.9, 0.6–1.4). Completion of the Short Form 36 (SF-36) questionnaire was feasible among 189 (58%) communicable survivors at 6 months, with 99 patients from the magnesium sulphate group and 90 from the saline group. Their SF-36 physical scores were similar (mean ± SD), 67.3 ± 26.1 in the magnesium sulphate group and 65.5 ± 25.3 in the saline group (mean difference 3.8; 95% CI −5.6 to 9.2), as were their SF-36 mental scores, 65.4 ± 22.0 in the $MgSO_4$ group and 64.5 ± 24.1 in the saline group (mean difference 3.4; 95% CI −5.7 to 7.6). The daily maximum middle cerebral artery velocities were collected throughout the drug infusion period of the study, and the mean values for the magnesium sulphate and saline infusion groups were similar. Analysis of the plasma magnesium concentration levels did not suggest that higher levels result in better clinical outcomes [24]. An updated meta-analysis also did not support a clinical benefit of intravenous magnesium sulphate infusion in patients with acute aneurysmal SAH [25]. A similar Dutch multicenter randomized clinical trial, the Magnesium in Aneurysmal Subarachnoid Haemorrhage (MASH II) phase III clinical trial, with a lower dosage regimen, completed patient recruitment in the second half of 2011 and results are expected to further clarify the role of intravenous magnesium sulphate infusion for aneurysmal SAH (personal communications with MASH II investigators).

There is another line of magnesium research in aneurysmal SAH led by Kentaro Mori and his team. They evaluated the vasodilatory effect of intracisternal infusion of magnesium sulphate solution in ten patients with symptomatic vasospasm after aneurysmal SAH who underwent early clipping surgery [26]. Cisternal drainage was installed in the prepontine or sylvian fissures. Carotid angiography was performed immediately after the onset of symptomatic vasospasm, and then intracisternal infusion of 15 mmol/L magnesium sulphate in Ringer solution was started at 20 mL/h and continued until day 14. Irrigation was performed from the cisternal tube (inlet) to the spinal drainage (outlet). The cerebrospinal fluid magnesium ion concentration (1.2 ± 0.2 mEq/L) significantly increased after the infusion therapy (6.0 ± 1.7 mEq/L, $p<0.001$), which is much higher than the achievable concentration by intravenous infusion. Repeat angiography showed vasodilatory effect on the spastic cerebral arteries at 3 h after the infusion, especially in the arteries near the site of cisternal drainage placement. The magnesium infusion also caused decreased mean arterial blood velocity in the spastic arteries in six of the seven measured patients (162 ± 38 to 114 ± 42 cm/s, $p<0.001$). Finally, five of the ten patients achieved good recovery, one patient had moderate disability, one patient became severely disabled due to meningitis, and three patients were vegetative or dead due to failure of magnesium irrigation in one patient and advanced age in the other two (more than 80 years old). The results are encouraging, but translation into better clinical outcome needs to be further assessed in randomized clinical trials in both clipping and coiling patients with standard medical treatment including oral nimodipine.

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Conflicts of Interest We declare that we have no conflict of interest.

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Prolonged Intravenous Infusion of Sodium Nitrite Delivers Nitric Oxide (NO) in Humans

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Abstract In preclinical studies, infusion of sodium nitrite delivers nitric oxide (NO) as treatment of vasospasm after subarachnoid hemorrhage. We evaluated safety and toxicity of intravenous nitrite administration in healthy volunteers infused with increasing doses of sodium nitrite for 48 h. Twelve volunteers (5 men, 7 women; mean age was 38.8 years, range 27–56 years) participated in the study. The starting sodium nitrite dose was 4.2 mg/kg/h, and it was doubled for each subsequent volunteer up to a maximal dose of 533.8 mg/kg/h at which a clinically silent dose-limiting toxicity (DLT) was observed. Toxicity included a transient decrease of mean arterial blood pressure or asymptomatic increase of methemoglobin level above 5%. The maximal tolerated dose (MTD) was 267 mg/kg/h. *S*-Nitrosothiols increased significantly in plasma, confirming in vivo sodium nitrite reduction to NO and encouraging its use against vasospasm and ischemia-reperfusion injury to the brain, kidneys, liver, and heart.

Keywords Nitric oxide • Nitrite • Vasospasm • Methemoglobin

Introduction

Preclinical studies indicated that nitric oxide (NO) limits tissue injury in experimental brain, cardiac, and renal ischemia [6]. It has also been shown—when delivered intra-arterially—to prevent the development of delayed cerebral vasospasm after subarachnoid hemorrhage in a primate model [1, 8]. These preclinical findings have not been confirmed in clinical trials because of the lack of systemic NO donors that are safe for prolonged systemic administration.

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Nitrite anion (NO_2) has long been considered a biologically inert, short-living product of NO oxidation. However, an increasing body of evidence indicates that NO_2 is an intravascular storage pool for NO [2, 5, 9] and that it can be reduced to NO under specific local conditions [2]. Preclinical studies have shown that NO_2 provides therapeutic benefits similar to that produced by NO, preventing and reversing delayed vasospasm in a primate model of subarachnoid hemorrhage [4, 9] and limiting ischemic damage to the heart, brain, and kidneys [3, 7] without causing a drop in blood pressure or causing other forms of systemic toxicities associated with systemic administration of nitroglycerin or nitroprusside. Despite these encouraging experimental findings, the prolonged intravenous administration of sodium nitrite, which is crucial in clinical settings, was not studied. Thus, the phase 1, single-center, open-label, nonrandomized, dose-escalation clinical trial was conducted to determine the safety and toxicity of a 48-h intravenous infusion of sodium nitrite in healthy volunteers [10].

Materials and Methods

Healthy volunteers were recruited from the National Institutes of Health (NIH) recruitment program. All volunteers underwent screening procedures that included a complete medical history, physical examination, hematology, serum comprehensive metabolic panel, and methemoglobin via CO oximetry, and women had a pregnancy test. This dose- and-toxicity seeking, single-center, open-label, nonrandomized, dose-escalation clinical trial was approved by the National Institute of Neurological Disorders and Stroke (NINDS) internal review board. The volunteers enrolled in this trial were healthy nonsmoking people, 21–60 years of age of both sexes in good health who did not use any medication; women of childbearing age were allowed but could not be pregnant or breast feeding. After initial assessments, volunteers were admitted to the Intensive Care Unit (ICU), where the infusion of 0.9% sodium chloride (NaCl)

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was initiated for 12 h to establish steady state. Blood pressure, electrocardiograms (ECGs), and methemoglobin were monitored, and additional blood samples were drawn for assessments of hematology, serum comprehensive metabolic panel, platelet functions, chromosomal aberrations, NO_2, nitrate (NO_3) in plasma, whole blood, red blood cells(RBCs), and the protein and lipid-bound NO (nitrosothiols, SNO). Blood samples were collected preinfusion, during infusion, and following the cessation of the 48-h sodium nitrite infusion that started immediately after saline infusion was stopped. All the procedures are described in details in Pluta et al. [10].

The dose-limiting toxicity (DLT) was defined as methemoglobin blood levels approaching 5% or a drop of Mean Arterial Blood Pressure (MABP) by 15–20 mmHg for a prolonged time. The maximal tolerated dose (MTD) was defined as the highest dose studied in at least two of three volunteers without the incidence of DLT.

The starting sodium nitrite dose was 4.2 mg/kg/h, and it was doubled for each subsequent volunteer.

Results

Twelve healthy volunteers (five male and seven female) were enrolled in this study. The mean age was 38.8 ± 9.2 years (range 27–56 years). Two subjects were Caucasian and the rest of volunteers were African American. The mean weight was 77.8 ± 19 kg (range 49–115 kg). The baseline (day 1) mean MABP and methemoglobin were 89 ± 12 mmHg (range: 69–101 mmHg) and 0.7 ± 0.2 % (range: 0.5–1%), respectively.

The mean baseline values for NO metabolome were 17 ± 17 ng/mL plasma NO_2, 1,613 ± 1,029 ng/mL plasma NO_3, 29 ± 11 nmol/L plasma SNO, 17 ± 9 ng/mL whole-blood NO_2, 2,354 ± 1,493 ng/mL whole-blood NO_3, 25 ± 8 ng/mL RBC NO_2, and 1,323 ± 47 ng/mL RBC NO_3.

Sodium Nitrite Infusion

Whole blood and plasma levels of NO_3, RBC levels of NO_2 and NO_3, and plasma levels of SNO increased during the infusion in all evaluable subjects and returned to baseline values within 12 h after stopping the infusion. The mean $t_{1/2}$ of NO_2 was 45.3 ± 1.0 min and 51.4 ± 22.6 min for plasma and whole blood, respectively.

The MTD for a 48-h intravenous infusion of sodium nitrite in healthy volunteers was 267 mg/kg/h (12.8 mg/kg/48 h).

There were no deaths or serious adverse events (SAEs). DLTs were experienced by three volunteers, the first at 534 mg/kg/h and the second and third at the deescalation dose of 446 mg/kg/h. Two of the volunteers had their 48-h intravenous infusion of sodium nitrite stopped early due to clinically significant decreases in MABP.

Discussion

Sodium nitrite injection was well tolerated in healthy volunteers. The MTD for a 48-h intravenous infusion was determined to be 267 μg/kg/h (12.8 mg/kg/48 h). DLTs were restricted to asymptomatic transient decreases in MABP and asymptomatic transient increases in methemoglobin. These changes were not considered clinically significant and did not require any additional intervention other than stopping the infusion.

Conclusion

Our study showed that prolonged intravenous infusion of sodium nitrite is safe and has a limited and only transient clinically silent toxicity. Toxicity was limited to decreases of MABP and increase of methemoglobin level above 5%. After expected increase during sodium nitrite infusion, NO metabolome values returned to baseline levels within 12 h; the only exceptions were S-nitrosothiols of subjects experiencing DLT. The meaning of this finding is unclear, but because of the unexpected drop in blood pressure in one volunteer after 6 h of rest in response to infusion of sodium nitrite at the MTD suggests that the S-nitrosothiols may play a role in regulating the vascular response, and their concentrations may need to be monitored during long-term sodium nitrite infusions.

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Disclosure Results of this study has been published in Pluta et al. [10].

Conflicts of interest I declare that I do not have conflict of interest.

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Proposed Mechanism of Cerebral Vasospasm: Our Hypothesis and Current Topics

Tomio Sasaki and Yuichiro Kikkawa

Abstract Increased vascular contractility plays an important role in the development of cerebral vasospasm following subarachnoid hemorrhage (SAH). Increased vascular contractility can be attributed to either endothelial dysfunction or increased contractility of vascular smooth muscle. Endothelial damage and dysfunction cause impairment of endothelium-dependent vasodilation of the cerebral artery after SAH. In addition to endothelial damage and dysfunction, receptor upregulation in vascular smooth muscle contributes to the induction and enhancement of contractile responses to agonists. Our recent data revealed that feedback regulation of the activity of the G protein-coupled receptor and myofilament Ca^{2+} sensitivity is impaired after SAH. This impaired feedback regulation is suggested to cause a sustained contractile response to various agonists, thereby contributing to increased vascular contractility. In addition, three current topics are reviewed: endothelin type A receptor antagonists, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors for treatment, and cortical spreading depolarization for the mechanism of cerebral vasospasm.

Keywords Cerebral vasospasm • Subarachnoid hemorrhage • Vascular contractility • Endothelium • Vascular smooth muscle

Introduction

For clarifying the mechanism of cerebral vasospasm, it is important to determine the clinical characteristics. During the phase of vasospasm, a reduction in the outer diameter of the spastic artery is observed intraoperatively. This observation suggests the possibility that contraction of vascular smooth muscle may be a major cause of cerebral vasospasm. Papaverine hydrochloride or Ca^{2+} channel blockers cause vasodilation of the spastic artery in the early phase of spasm, whereas they do not exert vasodilatory effects in the acme phase of spasm. Therefore, it is difficult to explain the mechanism of cerebral vasospasm in a simple flow of elevation of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) followed by myosin light chain phosphorylation and contraction. Our experimental data support the notion that vascular contractility is increased after subarachnoid hemorrhage. It is hoped our understanding of the molecular mechanism of increased vascular contractility will be useful to develop a new therapeutic strategy for the prevention and treatment of cerebral vasospasm following subarachnoid hemorrhage (SAH).

Mechanism Underlying Increased Vascular Contractility After SAH

Endothelial Damage and Dysfunction

In canine basilar arteries, endothelial damage can be observed by electron micrographs 3 days after SAH [21]. Degenerative endothelial changes cause the decreased synthesis of prostacyclin (PGI_2) [22]. PGI_2 is mainly generated in the endothelium and acts as a defense hormone that inhibits platelet aggregation and maintains normal peripheral blood flow by its vasodilatory effect [15, 16]. The synthetic activity of PGI_2 is significantly diminished on days 3 and 8 after SAH in canine basilar arteries [22]. In addition, endothelium-dependent vasodilation induced by both adenosine triphosphate (ATP) and acetylcholine is impaired 4 days after SAH [18]. It almost recovers 3 weeks after SAH [18]. Impairment of endothelium-dependent vasodilation of the cerebral artery is an important mechanism of increased vascular contractility after SAH.

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Upregulation of Receptor Expression

Thrombin induced only a small contraction at 1 unit/ml in the basilar artery of a rabbit control model, whereas it significantly enhanced contractions after SAH [9, 10, 14]. Enhanced contractility was also observed with an agonist peptide for the thrombin receptor after SAH [14]. Consistent with the increased contractile response to thrombin, expression of the thrombin receptor proteinase-activated receptor 1 (PAR_1) is upregulated 5 and 7 days after SAH [9, 14]. The intrathecal administration of a PAR_1 antagonist prevents the upregulation of PAR_1 expression and enhancement of the contractile response to thrombin [9], suggesting that thrombin-mediated activation of PAR_1 plays a critical role in upregulating the expression of PAR_1 itself, thereby enhancing the contractile response to thrombin after SAH. A similar enhancement of contractility is also observed with platelet-derived growth factor, phenylephrine and endothelin-1 (ET-1), but not for high K^+ depolarization or phorbol ester [10, 14]. The expression of PAR_1, α1-adrenoceptor, and ET_A receptor has been found to be upregulated after SAH [10]. Accordingly, receptor upregulation is suggested to play an important role in the increased vascular reactivity to agonists.

Impairment of Feedback Regulation of Receptor Activity and Myofilament Ca^{2+} Sensitivity

In rabbit basilar arteries, ET-1, thrombin, and phenylephrine induce a transient contraction, which reaches a peak and gradually declines to a significantly lower level. On the other hand, a transient contractile response is converted to a sustained response after SAH. The conversion of a transient response to a sustained response has been observed with $[Ca^{2+}]_i$, myosin light chain phosphorylation, and contraction. Furthermore, it was found that when the artery was consecutively stimulated with PAR_1-activating peptide or phenylephrine, a second response was significantly reduced in controls, whereas the second response was well preserved in SAH. These observations suggest that a feedback regulation mechanism of the contractile response is impaired, presumably at the receptor level, after SAH, thereby causing a sustained contraction and a continued response to the second stimulation.

Impaired feedback regulation may cause a significant effect on the contractile effect of thrombin because of the unique activation mechanism of PAR_1. The activation of PAR_1 by thrombin is initiated by proteolytic cleavage of the N-terminal region, which covers the region that acts as a tethered ligand and activates the receptor [2]. Therefore, feedback regulation plays an important role in terminating the activity of proteolytically activated PAR_1. In SAH, thrombin-induced sustained contraction has been found to persist even after terminating thrombin stimulation [10]. Trypsin is known to remove the ligand region of PAR_1, thereby converting the active conformation of PAR_1 to an inactive conformation [19]. The addition of trypsin during thrombin-induced sustained contraction completely inhibits the contraction [10]. Furthermore, an inhibitor of Gαq protein also inhibits thrombin-induced sustained contraction [10]. Therefore, these observations suggest that this persistent contraction is associated with persistent activation of PAR_1, and that feedback inactivation of PAR_1 is impaired following SAH. A Gαq inhibitor has also been found to inhibit the sustained phase of contraction induced by ET-1 and phenylephrine [10]. These observations suggest that impairment of feedback regulation of receptor activity is not limited to PAR_1 but also extends to other receptors. This general impairment of receptor inactivation may explain the enhanced contractility to various agonists after SAH.

In an α-toxin permeabilized preparation, GTPγS, a non-hydrolyzable GTP (guanosine triphosphate) analog that is known to directly activate G proteins by skipping receptor-mediated activation, induces a transient response in controls, whereas it induces a sustained response after SAH [10]. This suggests that feedback regulation at the step regulating myofilament Ca^{2+} sensitivity is also impaired after SAH.

Current Topics

ET_A Receptor Antagonist: Clazosentan

Among various substances proposed for a spasmogen, ET-1 has been implicated to be a critical mediator in the pathogenesis of cerebral vasospasm because of its inherent properties to produce a potent and prolonged vasoconstriction [7, 17]. ET-1 primarily exerts its vasoconstrictive activity via the ET_A receptor, which is localized on vascular smooth muscle cells. Therefore, the ET_A receptor has been considered to be a potentially useful therapeutic target for cerebral vasospasm. In 1993, the preventive effect of continuous intravenous administration of an ET_A receptor antagonist (BQ-485) on experimental cerebral vasospasm was shown for the first time using canine basilar artery [8]. In 2005, a phase IIa clinical trial showed that continuous intravenous administration of clazosentan, a selective ET_A receptor antagonist, significantly reduced angiographic vasospasm by 48% compared with the placebo group [24]. In 2008, a phase IIb clinical trial, CONSCIOUS-1, showed that clazosentan significantly and dose dependently reduced moderate or severe angiographic vasospasm, with a

65% relative risk reduction with the highest dose [13]. However, CONSCIOUS-1 was not powered to detect changes in morbidity or mortality, whereas post hoc analyses showed some evidence for improved outcome [13]. A high incidence of pulmonary complications, hypotension, and anemia was considered to be the cause of the negative aspects of clazosentan [13]. In 2011, a phase III clinical trial, the CONSCIOUS-2 study, in patients undergoing surgical clipping revealed that clazosentan at 5 mg/h had no significant effect on mortality and vasospasm-related morbidity or functional outcome [12]. Further investigation is needed to fully understand the potential usefulness of clazosentan in patients with aneurysmal SAH. There are two ongoing studies. One is the CONSCIOUS-3 study in patients undergoing endovascular coiling of ruptured aneurysms, and the other is a clinical study that has been carried out mainly in Japan and South Korea. These studies should provide some answers for the efficacy of clazosentan.

3-Hydroxy-3-Methylglutaryl (HMG)-Coenzyme A Reductase Inhibitor: Statins

In addition to their cholesterol-lowering effect, statins have been shown to possess some pleiotropic effects, such as improvement of endothelial function, anti-inflammation, plaque stabilization, and reduction of thrombosis. Therefore, statins are expected to become therapeutic agents of cerebral vasospasm. In 2005, Lynch et al. and Tseng et al. reported the preventive effect of simvastatin and pravastatin on vasospasm, respectively [11, 23]. Thereafter, many neurosurgeons have been interested in the preventive effect of statins on vasospasm. However, recent studies could not confirm the beneficial effects of statins [1, 25]. To determine the efficacy of statins, larger multicenter clinical trials are required because the number of patients in previous studies was too small to draw meaningful conclusions regarding the efficacy of statins.

Cortical Spreading Depolarization

Cortical spreading depolarization (CSD) is the wave of neuronal depolarization associated with the influx of cations and water. Under physiological conditions, in response to spreading depolarization, there is a normal neurovascular response defined by vasodilation and increased regional cerebral blood flow [3]. However, under pathological conditions, spreading depolarization induces severe spreading hypoperfusion that leads to a prolonged slow potential change [3], which is called spreading ischemia. In recent years, animal and human studies have indicated that CSDs occur early and late after SAH [4, 6]. Investigations regarding the role of CSD in cerebral vasospasm and subsequent delayed ischemic neurological deficits (DINDs) have mainly been performed by Dreier's group. In 2006, they revealed in clinical studies that DINDs in SAH patients are associated with CSD [6]. In SAH, many factors, including oxy-Hb, an increase in extracellular potassium, a decrease in nitric oxide availability, an increase in the release of glutamate, and an increase in the synthesis of ET-1, are suggested to induce CSD [5, 20].

The relationship between CSDs and DINDs may be as follows: After SAH, cerebral vasospasm is induced in large arteries. In the early phase of vasospasm, compensatory vasodilation occurs in small pial arteries. This may be related to spreading hyperemia. In the acme phase of vasospasm, regional cerebral blood flow decreases, extracellular potassium increases, oxy-Hb is released, ET-1 is increased, nitric oxide production is decreased, and release of glutamate is increased. These factors induce CSD in the acme phase of vasospasm, and then cortical spreading ischemia occurs. Cortical spreading ischemia is suggested to contribute to the induction of DINDs. However, whether CSD is the main causative factor of DINDs in SAH patients and to what extent CSD contributes to DINDs still remains to be elucidated.

Conclusion

We presented our understanding of the molecular mechanism of increased vascular contractility following SAH based on our experimental data and reviewed three current topics. Our hypothesis and these topics will be useful to develop a new therapeutic strategy for the prevention and treatment of cerebral vasospasm following SAH.

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

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Monitoring of Cerebral Hemodynamics and Oxygenation to Detect Delayed Ischemic Neurological Deficit After Aneurysmal Subarachnoid Hemorrhage

Martin Seule, Carl Muroi, Christopher Sikorski, and Emanuela Keller

Abstract One of the major goals in the treatment of patients with aneurysmal subarachnoid hemorrhage (aSAH) is early detection and treatment of delayed ischemic neurologic deficits (DINDs) to prevent cerebral infarction and thus poor outcome or even death. The complex changes of cerebral metabolism, hemodynamics, and oxygenation after SAH are underestimated if they are considered exclusively based on angiographic cerebral vasospasm (CVS). The discrepancies on one hand may arise from the heterogeneous and complex pathophysiology of DINDs. On the other hand, the occurrence of DINDs may depend on the relationship between local cerebral oxygen delivery and demand, which can only be determined if cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$) can be measured. We briefly review the most relevant methods for monitoring cerebral hemodynamics and oxygenation and discuss the limitations associated with early diagnosis of DINDs in patients with severe aSAH not amenable for clinical neurological examination.

Keywords Subarachnoid hemorrhage • Delayed ischemic neurological deficit • Neuromonitoring • Cerebral blood flow • Cerebral oxygenation

Introduction

Delayed ischemic neurological deficits (DIND) after aneurysmal subarachnoid hemorrhage (aSAH) may occur in the absence of angiographic cerebral vasospasm (CVS) and vice versa. Further, it could be demonstrated that the distribution of angiographic CVS failed to predict reliably the subsequent pattern of cerebral infarction [22]. Based on the accumulating evidence that arterial narrowing is not the only cause of DINDs, there has been a marked shift to new concepts involved in the development of DINDs, including acute brain injury, microthrombosis, inflammation, and cortical spreading depression [16, 21]. The entire picture of DINDs might be multifactorial, and a clear distinction from angiographic CVS is essential to give insights of major importance in the pathophysiology of DINDs and new neuroprotective strategies. Besides the heterogeneous and complex pathophysiology of DINDs, the occurrence may depend on the relationship between local cerebral oxygen delivery and demand, which can only be determined if cerebral blood flow (CBF) and the cerebral metabolic rate of oxygen ($CMRO_2$) can be measured. Monitoring CBF may allow identifying patients with increased risk of secondary ischemic events after SAH [4, 25, 30]. The complex changes of cerebral hemodynamics and oxygenation pattern are underestimated if they are considered exclusively based on transcranial Doppler (TCD) and angiography.

Several monitoring techniques to assess cerebral hemodynamics and oxygenation have been introduced. However, it has been shown that each monitoring technology has its limitations in clinical practice. The established methods for bedside monitoring of CBF with inert tracers such as nitrous oxide and ^{133}xenon dilution techniques are difficult to perform clinically and time consuming. Imaging studies such as perfusion-weighted magnetic resonance imaging (MRI), ^{133}xenon computed tomography (CT), $H_2{}^{15}O$ positron emission tomography (PET), and single-photon emission computed tomography (SPECT) are powerful research and clinical tools but require a transport, which carries a potentially high risk for critically ill patients. Further, the information obtained is only a snapshot of the patient's condition, not accounting for the dynamic changes during hemodynamically relevant CVS. On this account, continuous bedside monitoring offering direct or indirect assessment of CBF and oxygenation may allow for early detection of DINDs and help guide therapy [28].

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Noninvasive Methods

Transcranial Doppler Sonography

TCD measures blood flow velocities, and CBF may be calculated if the vessel cross-sectional area and the angle of insonation are known and constant. However, in pathological states, these parameters may vary over time, and flow can be increased due to vasospasm or high-flow states as in fever, anemia, hypoxia, and vasopressor therapy. The Lindegaard ratio can help to differentiate between elevated flow velocities caused by hyperperfusion and vasospasm [15]. However, it has to be taken into account that CBF indices are derived from measurements based on flow velocities, so that TCD findings should never be used in insolation. New methods to quantify cerebral perfusion have been developed using contrast agent-specific Doppler imaging modes [5]. The major disadvantage that all ultrasound techniques have is the fact that physical properties like the nonlinear relationship between contrast agent and optic intensities, the depth-dependent attenuation of the ultrasound signal, and inhomogeneous temporal bone window make the quantification of absolute values impossible [18]. Nevertheless, TCD is easy to perform at the bedside and should be used to complement other monitoring techniques available.

Near-Infrared Spectroscopy

Near-infrared spectroscopy (NIRS) provides continuous assessment of a regional tissue oxygen saturation (TO_2S). The principle is based on the finding that light in the near-infrared region (700–950 nm) penetrates biological tissue and is absorbed differently by chromophores of oxygenated (HbO_2) and deoxygenated (HHb) hemoglobin. NIRS sensors containing the light source and detector are generally attached to the forehead and measure the attenuation of light as it travels through tissue in an ellipsoid shaped path. The absorption of photons is determined by the modified Beer-Lambert law, which allows calculating changes of HbO_2 and HHb. Recently, two small case series have shown that NIRS technology can be applied as a continuous bedside monitor over multiple vascular territories, assisting the detection and treatment of DINDs [20, 33]. However, contamination of the NIRS signal by extracerebral tissues such as skin, skull, and cerebrospinal fluid remains a major concern over the clinical application. Possible solutions to quantify and subtract the extracerebral contamination are provided by spatial resolved spectroscopy and time-of-flight or frequency domain technologies [14, 32].

Combination of Near-Infrared Spectroscopy and Indocyanine Green Dye Dilution

Indocyanine green (ICG) is widely used in medical diagnosis with excellent safety records and absorbs infrared light maximally between 790 and 805 nm. New algorithms have been developed to analyze the ICG dye dilution curve and to calculate absolute values of the mean transit time of ICG, CBF, and cerebral blood volume (CBV) without invasive quantification of the input signal to the head by arterial fiber-optic catheters. The practability of this technology has been demonstrated in the environment of a neurointensive care unit, and the values obtained were validated by perfusion-weighted MRI in healthy volunteers and in patients with angiographic vasospasm before and after superselective papaverine installation [8, 12]. To detect secondary ischemic events, normal values and critical thresholds for CBF values obtained by combined NIRS and ICG dye dilution have to be defined in future clinical studies.

Minimally Invasive Methods

Jugular Venous Oximetry

Jugular bulb oximetry provides a continuous and easy-to-perform estimate of the balance between cerebral metabolism and blood flow [23, 26]. A fiber-optic catheter is inserted retrograde into the internal jugular vein and advanced into the jugular bulb at the base of the skull. Monitoring jugular venous oxygen saturation (SvO_2) is used directly to guide therapy and allows calculating the arteriovenous difference in oxygenation ($AVDO_2$). AVDO2 is inversely proportional to CBF if the $CMRO_2$ consumption is constant. It has been shown that episodes of desaturation ($SvO_2 < 50\%$) are indicative of cerebral ischemia, and that additional measurement of the arteriovenous lactate difference (AVDL ≥0.2 μmol/l) might improve the predictive value to detect DINDs after SAH [1]. However, jugular bulb oximetry is a global measurement method, and its limitation has to be considered especially in patients with secondary focal ischemic events [11]. The clinical value of this method has to be seen in following individual trends during systemic complications such as fever and anemia or after initiation of therapeutic interventions such as induced hypertension, barbiturate coma, and intra-arterial spasmolysis [2, 10].

Partial Pressure of Brain Tissue Oxygen

Continuous monitoring of the partial pressure of brain tissue oxygen (PtiO2) involves insertion of a microcatheter directly

into the brain tissue and reflects the balance between regional oxygen supply and consumption. The brain tissue probe is based on a Clark electrode (Licox probe) and may either be tunneled after craniotomy or placed through a bolt system fixed to the skull. It has been shown that episodes of local tissue hypoxia (PtiO2 values ≤10 mmHg; Licox probe) are associated with critical elevated intracranial pressure (ICP) and cerebral infarction [17, 31]. However, the sensitivity to detect secondary ischemic events is limited. The main reason for failure to predict cerebral infarction is the small sample volume of a few cubic millimeters. The placement of several catheters in different vascular territories and the combination of local with global monitoring techniques such as jugular bulb oximetry might overcome this problem at least partially.

Microdialysis

Microdialysis allows monitoring of changes in brain tissue chemistry by inserting a microcatheter lined with a polyamide dialysis membrane into the brain parenchyma. The catheter is perfused with a physiological solution, and the dialysate is analyzed every 30–60 min at bedside. The key substances involved in the development of cerebral ischemia include energy-related metabolites (glucose, lactate, and pyruvate) and neurotransmitters (glutamate). During cerebral ischemia, glucose levels may decrease, and cerebral metabolism may shift from aerobic to anaerobic, resulting in an accumulation of lactate and increase of the lactate-pyruvate ratio. The combination of glucose levels below 0.7 mmol/l and lactate-pyruvate ratio >40 is defined as metabolic crisis [24]. It could be shown in patients with aSAH that reoccurring episodes of metabolic crisis are associated with a less-favorable outcome [13]. Comparing microdialysis with TCD has demonstrated a similar sensitivity, but higher specificity in the detection of CVS and DINDs in patients with aSAH [29]. Cerebral metabolic derangement and increase in glutamate levels may even precede neurological deterioration due to symptomatic CVS, therefore widening the therapeutic window to initiate further diagnostic and therapeutic interventions [24, 27]. The main disadvantage of this technique is the small sample volume. Therefore, cerebral ischemia might be missed just beneath the tip of the probe, even if positioned in the "tissue at risk" (most likely the parent vessel territory) (Fig. 1).

Thermal Diffusion Method

The principle of thermal diffusion flowmetry (TDF) relies on detection of the temperature gradient between a plate generating heat and a detector plate. The thermal conductivity of the brain tissue is directly proportional to blood flow. Commercially available TDF devices involve insertion of a microprobe into the brain parenchyma, and the obtained CBF values have been validated using xenon CT [30]. Although this technique allows for bedside monitoring of absolute CBF values, several limitations have to be considered over clinical application. It has been shown that there is a significant baseline shift of CBF values after recalibration and instabilities in the thermal field within the brain tissue (e.g., fever, induction of hypothermia) influence the accuracy of the measurement method [6].

Combined Probe for NIRS and Intracranial Pressure Monitoring

New techniques combining NIRS and ICG dye dilution to estimate cerebral hemodynamics and oxygenation are available [8]. However, extracerebral contamination of the NIRS

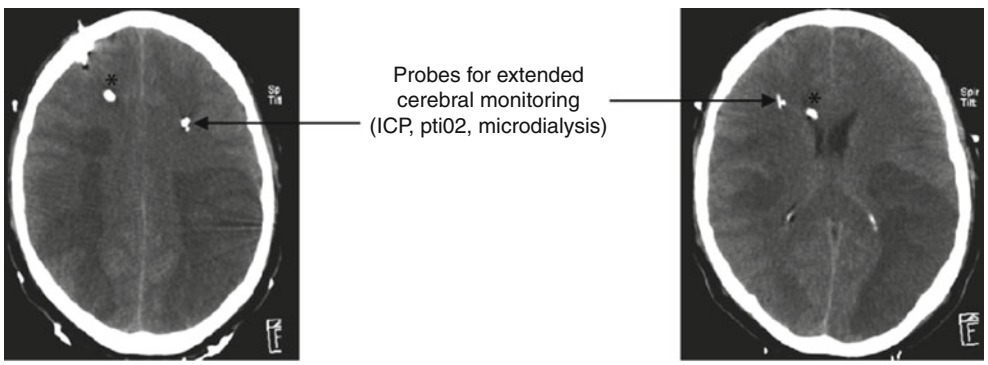

Fig. 1 Computed tomographic (CT) scan of a patient with extensive bilateral infarctions in the middle cerebral artery territories on day 16 after aneurysmal subarachnoid hemorrhage (aSAH). There were no signs of hypoxic episodes or metabolic crisis indicating secondary cerebral ischemia or infarction during the entire monitoring period. Note: * indicates external ventricular drainage

signal using a strictly noninvasive approach limits the clinical application of optical methodologies. To obtain measurement values directly from the brain tissue, a conventional probe for ICP monitoring has been supplied with optical fibers for NIRS (NIRS ICP probe) [7, 9]. In ongoing investigations, parallel measurements using transcutaneous and intraparenchymatous NIRS will offer a completely new approach to quantify and subtract extracerebral contamination of the NIRS signal [19]. In patients with severe acute brain injuries, for whom ICP probes are installed anyway due to brain edema and intracranial hypertension, the new combined NIRS ICP probe offers enhanced modality modes (ICP, mttICG, CBF, CBV, HbO_2, HHb, and total hemoglobin) without an additional surgical intervention. It has been shown that the NIRS-ICP probe can be applied as a bedside monitor providing reproducible measurements of absolute CBF and CBV values [7]. Recently, it could be shown in animals that NIRS probes may detect brain edema formation even before ICP increases [3]. Like other methods applying brain tissue probes such as microdialysis or PtiO2 monitoring, the major restriction of 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.

Conclusion

Continuous bedside monitoring of cerebral hemodynamics and oxygenation can provide clinically important insights into the brain region at risk for DINDs and enhances the potential of early and effective interventions. Based on the limitations of the available monitoring methods, it is reasonable to combine global as well as different focal monitoring devices to provide data at the bedside within minutes. Developments in bioinformatics, delivering statistical and mathematical tools, will be essential to reduce the quantity and increase the quality of the data obtained by extended cerebral monitoring, thus offering reliable decision support to involved clinicians.

Conflicts of Interest We declare that we have no conflict of interest.

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Usefulness of Three-Dimensional Computed Tomography to Quantify the Subarachnoid Hemorrhage Volume: Prediction of Symptomatic Vasospasm

Taku Sato, Tatsuya Sasaki, Jun Sakuma, Tadashi Watanabe, Masahiro Ichikawa, Eiji Ito, Keiko Oda, Yuka Matsumoto, Hitoshi Ando, Michiharu Nishijima, and Kiyoshi Saito

Abstract We quantified the subarachnoid hemorrhage (SAH) volume in 64 patients on three-dimensional computed tomography (3D-CT) scans and studied the correlation between the SAH volume and the occurrence of symptomatic vasospasm (SVS). We studied 64 patients with SAH onset (day 0) and on days 1, 4, 7, and 14. We compared the hematoma volume by 3D-CT with 2D-CT on day 0 and examined the correlation between the hematoma volume and the occurrence of SVS. The hematoma volume, including the volume of normal structures, was automatically calculated (V1). The volume of normal structures manifesting identical CT numbers was previously calculated in patients without intracranial lesions (V2). The total hematoma volume was defined as V1 minus mean value of V2 (= 12 ml). The mean hematoma volume by 3D-CT was 48 ± 12 ml and by 2D-CT was 31 ± 45 ml (mean ± SD, $n=64$). The hematoma volume was significantly larger by 3D-CT than by 2D-CT ($p<0.05$). At all time points, the hematoma volumes were significantly larger in patients with than without SVS. We developed a new method for the quantitative determination of the SAH volume by 3D-CT. This method may allow us to quantify the volume of SAH in clinical studies of cerebral vasospasm.

Keywords Cerebral vasospasm • 3D-CT • Subarachnoid hemorrhage • Hematoma volume

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Introduction

Symptomatic vasospasm (SVS) continues to be a major cause of morbidity and mortality after aneurysmal subarachnoid hemorrhage (SAH). Earlier qualitative assessments indicated that patients with a large SAH volume on computed tomography (CT) scans manifested a high incidence of SVS. The volumetric quantification of SAH on two-dimensional CT (2D-CT) scans using a novel software-based technique encountered problems posed by the partial volume effect and the neglected SAH volume in the convexity or posterior fossa [1, 2, 5]. In our earlier efforts to surmount these difficulties [7], we quantitatively analysed the SAH volume on three-dimensional CT (3D-CT) scans. To confirm the usefulness of our method, in the current study we compared the hematoma volume on 3D- and 2D-CT scans and examined the correlation between the hematoma volume and the occurrence of SVS.

Patients and Methods

Our study population consisted of 64 patients with SAH who underwent initial 3D-CT study within 24 h of SAH onset. They were 21 men and 43 women ranging in age from 31 to 84 years (mean 65.1 years). Informed consent was obtained from all patients or their legal representatives before enrolment in this study. All 64 patients underwent direct surgery to the aneurysm within 48 h of SAH onset. It is policy at our institution first to attempt aneurysmal clipping; therefore, few patients underwent endovascular coil occlusion. This study did not include the latter patients.

All 64 patients underwent 3D-CT on a 4-channel MDCT (Aquilion, Toshiba Corp., Tokyo, Japan) scanner on the day of admission (day 0) and on days 1, 4, 7, and 14 postinsult. CT data were reconstructed on a computer workstation (ZAIO M900, AMIN Corp., Tokyo, Japan). Since the

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reported CT number of SAH ranges from 40 to 80 hounsfield units (HU) [7, 9], we used volume data with HU values between 40 and 80 for volume estimation. As the CT number of the skull bone is 1,000 HU, it was not included in volume data with CT numbers between 40 and 80 HU.

After manually excluding the scalp and subcutaneous tissue from the volume data using the space of the skull bone, the volume (V1) was estimated. This procedure required 2 min. As it is difficult to remove normal structures such as the venous sinus and falx manually, their volumes were included in acquired V1 value. To correct for their inclusion, we obtained informed consent from patients with no intracranial lesions to determine the volume of these normal structures (V2) on CT scans. From their scans, we manually excluded the scalp and subcutaneous tissue to arrive at the V2 value. Then, to determine the total hematoma volume in our 64 patients with SAH, we subtracted the mean V2 value reported elsewhere as 12 ml [7] from V1.

In patients with intraventricular or intracranial hemorrhage (IVH, ICH), their volume was estimated separately. On the screen, their different location made it easy to distinguish between IVH and ICH volumes and the SAH volume. We defined the SAH volumes as the total hematoma volume minus the volume of IVH or ICH. Because the number of patients with IVH or ICH was small, we could not perform statistical analysis; consequently, in our study we excluded IVH and ICH volumes. The total SAH volume on the day of admission (day 0) was also estimated on 2D-CT scans using a novel software-based technique [1, 5].

Of our 64 patients, 12 received continuous irrigation therapy with urokinase and ascorbic acid [3, 4, 6] to prevent SVS, 29 underwent cisternal drainage, and in 30 we administered fasudil chloride [8]. Patients with SVS received hypertensive, hypervolemic, and hemodilution (triple H) therapy. We used the Mann-Whitney *U* test to evaluate the association between the hematoma volume and SVS. Statistical analysis was performed with a commercially available statistical software program (StatMate 3, ATMS Co., Ltd., Tokyo, Japan). The *p* values less than 0.05 were considered statistically significant.

Results

The mean haematoma volume in the 64 SAH patients was significantly larger on 3D-CT than 2D-CT scans (48 ± 12 vs. 31 ± 45 ml, $p<0.05$). As the volume data yielded by 3D-CT can be observed from any direction, the location of the SAH could be easily identified (Fig. 1). In addition, chronological changes in the SAH volume can be followed readily on 3D images (Fig. 2). Of the 64 patients, 4 developed SVS; the spasms were transient in 2 patients. In the other two patients, they were persistent, and these patients manifested a newly developed low-density area. At all time points examined (days 0, 1, 4, 7, 14), the average SAH volume was significantly smaller in patients without than with SVS ($p<0.01$).

Discussion

Attempts to quantify SAH volumetrically have been reported. Friedman et al. [1] developed a software-based volumetric quantification method using 2D-CT. They quantified the hemorrhagic volume in the sylvian and interhemispheric fissures and in the suprasellar, ambient, quadrigeminal, and prepontine cisterns. They determined the amount of blood in each discrete region of interest (ROI) visually based on its density and outlined the ROI manually on each CT slice. Then, they calculated the hemorrhagic volumes in cubic centimeters based on the slice thickness and in-plane resolution of the CT scans. They found that at admission, the hematoma volume in their 40 patients was 2.27–112.3 ml. Their method to estimate the SAH volume requires 20 min compared to 2 min using our method. In a series of 75 patients reported by Reilly et al. [5], the SAH volume at admission was 0.2–38 ml (mean 10.4 ml). We found that the hematoma volume was significantly larger on 3D- than 2D-CT scans ($p<0.05$). This difference may be attributable to the partial volume effect on 2D-CT scans and to the neglected SAH volume in the convexity and posterior fossa.

Reilly et al. [5] used univariate logistic model and multivariate analysis; they reported that the initial clot volume and the percentage of clot cleared each day were significant predictors of vasospasm. We found that at all time points examined, the SAH volume was significantly lower in patients without than with SVS. We delivered continuous cisternal irrigation to prevent SVS [3, 4, 6]. In our efforts to dissolve the clot, we used urokinase, and we delivered ascorbic acid to degrade oxyhemoglobin, one of the strongest spasmogenic substances [3, 4, 6]. Even if the SAH volume on day 0 is large, it may be possible to prevent SVS by intraoperative clot evacuation and postoperative irrigation.

When using our method, care must be taken to avoid errors attributable to normal structures with CT numbers ranging from 40 to 80 HU. Elsewhere, we documented that the mean volume of normal structures was 12 ml [7]; we used this value in the assessment of the true SAH volume in our present patients. In cases where the normal structures are of larger volume, the calculated hematoma volume can be expected to be larger. Nonetheless, we were able to monitor chronological volume changes in 64 patients.

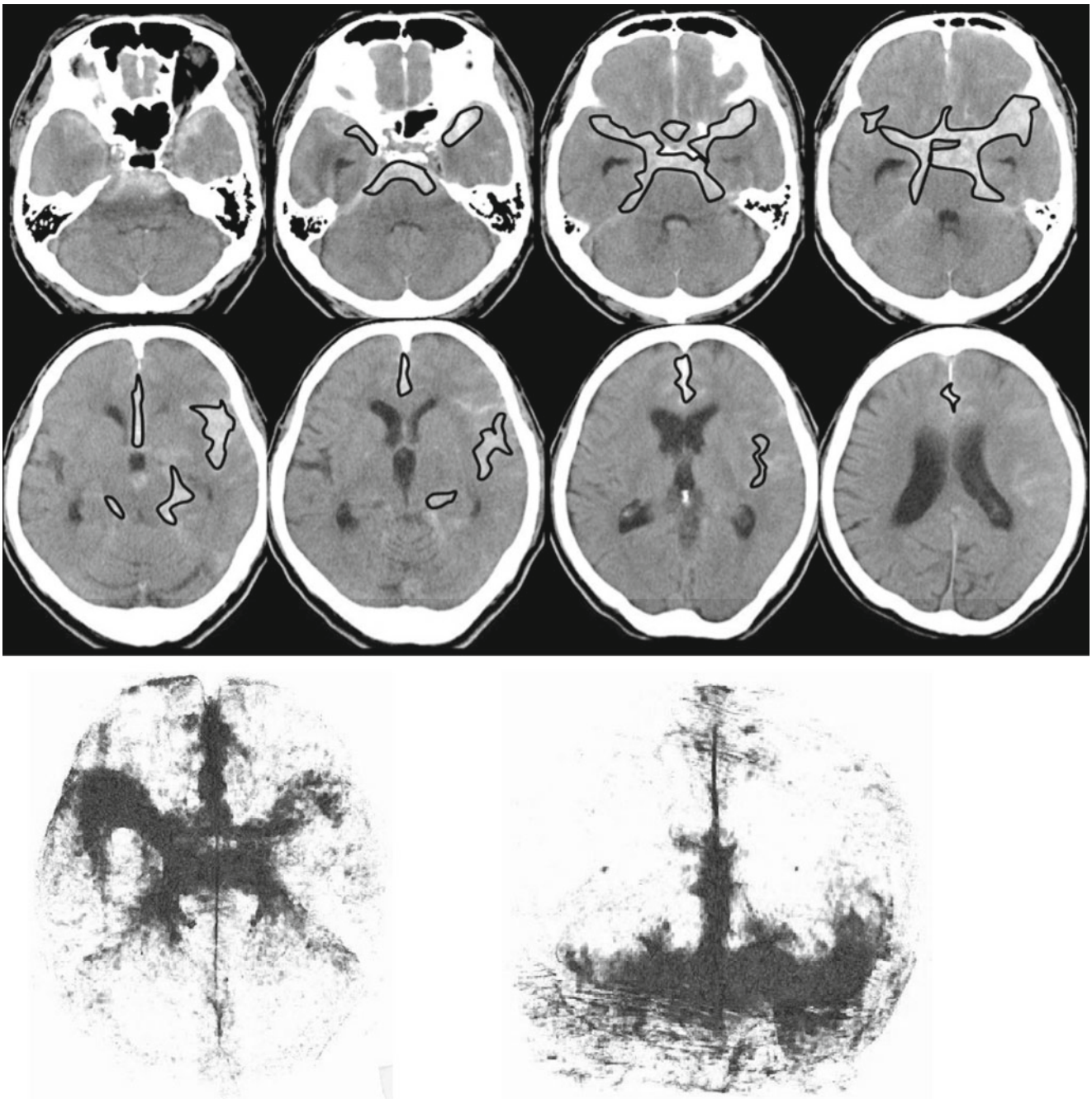

Fig. 1 *Top*: Axial views of conventional computed tomographic (CT) images. After discrete ROIs are manually outlined on each axial slice, the software calculates the two-dimensional CT (2D-CT) volume within that region. *Bottom*: Volume data acquired by three-dimensional CT (3D-CT): *left*, superior view; *right*, anterior view. The localization of the subarachnoid hemorrhage (SAH) can be estimated from all directions. The SAH can be visualized in the convexity and posterior fossa

Conclusion

We developed a new method that makes it possible to quantify the SAH volume quickly on 3D-CT images. 3D-CT facilitates the observation of the hematoma from any direction and helps to evaluate the localization of the SAH. In addition, chronological changes in the SAH volume can be monitored easily on 3D-CT images. Our method may be a useful tool in clinical studies of cerebral vasospasm, although further investigation is necessary.

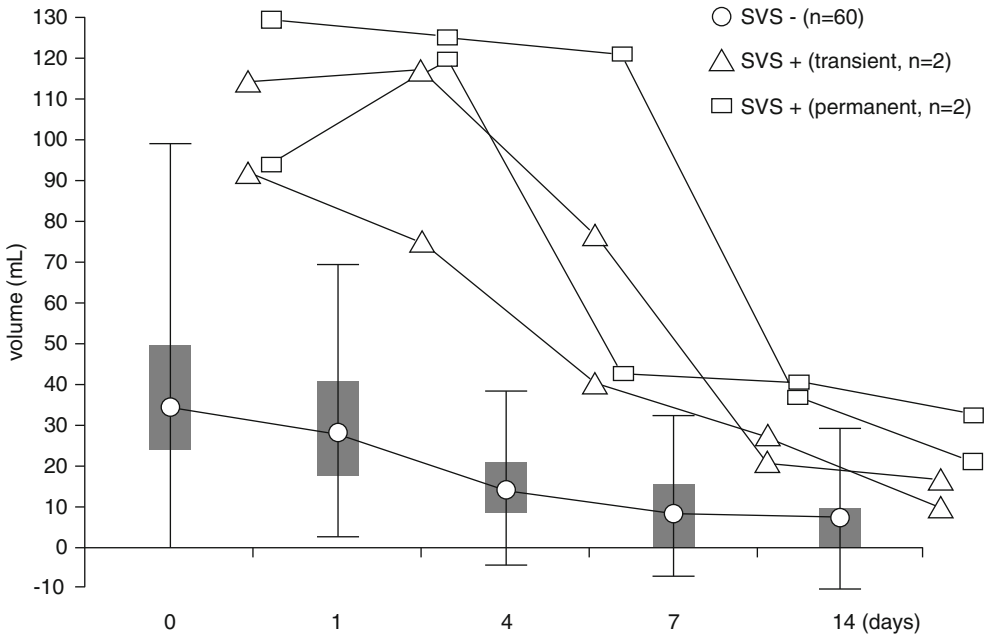

Fig. 2 Chronological changes in the volume of subarachnoid hemorrhage (SAH) in patients with ($n=4$) and without symptomatic vasospasm (SVS) ($n=60$). At all time points examined, the SAH volume was significantly smaller in patients without than with SVS ($p<0.01$ on days 0, 1, 4, 7, and 14)

Conflicts of Interest We declare that we have no conflict of interest.

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COSBID-M3: A Platform for Multimodal Monitoring, Data Collection, and Research in Neurocritical Care

J. Adam Wilson, Lori A. Shutter, and Jed A. Hartings

Abstract Neuromonitoring in patients with severe brain trauma and stroke is often limited to intracranial pressure (ICP); advanced neuroscience intensive care units may also monitor brain oxygenation (partial pressure of brain tissue oxygen, $P_{bt}O_2$), electroencephalogram (EEG), cerebral blood flow (CBF), or neurochemistry. For example, cortical spreading depolarizations (CSDs) recorded by electrocorticography (ECoG) are associated with delayed cerebral ischemia after subarachnoid hemorrhage and are an attractive target for novel therapeutic approaches. However, to better understand pathophysiologic relations and realize the potential of multimodal monitoring, a common platform for data collection and integration is needed. We have developed a multimodal system that integrates clinical, research, and imaging data into a single research and development (R&D) platform. Our system is adapted from the widely used BCI2000, a brain-computer interface tool which is written in the C++ language and supports over 20 data acquisition systems. It is optimized for real-time analysis of multimodal data using advanced time and frequency domain analyses and is extensible for research development using a combination of C++, MATLAB, and Python languages. Continuous streams of raw and processed data, including BP (blood pressure), ICP, PtiO2, CBF, ECoG, EEG, and patient video are stored in an open binary data format. Selected events identified in raw (e.g., ICP) or processed (e.g., CSD) measures are displayed graphically, can trigger alarms, or can be sent to researchers or clinicians via text message. For instance, algorithms for automated detection of CSD have been incorporated, and processed ECoG signals are projected onto three-dimensional (3D) brain models based on patient magnetic resonance imaging (MRI) and computed tomographic (CT) scans, allowing real-time correlation of pathoanatomy and cortical function. This platform will provide clinicians and researchers with an advanced tool to investigate pathophysiologic relationships and novel measures of cerebral status, as well as implement treatment algorithms based on such multimodal measures.

Keywords Cortical spreading depolarizations • Electrocorticography • Multimodality monitoring • Neurocritical care • Subarachnoid hemorrhage

Introduction

Delayed cerebral ischemia, including clinical deterioration and the development of new infarcts, is the leading potentially treatable cause of mortality and disability in patients with aneurysmal subarachnoid hemorrhage (SAH). Cerebral vasospasm has been presumed to be a main contributing cause, and periodic assessment of vascular flow by transcranial Doppler and computed tomographic angiography [1] are central to its diagnosis. Methods of continuous monitoring to detect ischemic changes, such as brain tissue oxygenation ($P_{bt}O_2$), cerebral microdialysis [2], quantitative electroencephalography (EEG) [3, 4], thermal diffusion flowmetry [5], and near-infrared spectroscopy [6] have also been investigated. These techniques hold promise not only for early detection of cerebral vasospasm, but also for fresh insight into the pathophysiology of delayed ischemic complications. Results of recent studies have suggested an dissociation between vasospasm and neurologic outcome, prompting a paradigm shift with renewed search for other factors involved in delayed cerebral ischemia [7, 8].

Development and investigation of neuromonitoring methods is therefore critical for understanding cerebral pathophysiology following SAH and for determining best management practices. Unfortunately, however, realization of the full potential of multimodal techniques has been

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limited by the lack of integration of diverse modalities. Each modality typically requires a separate monitor and data collection system; therefore, subsequent downloading, importing, and time synchronizing in an additional system is necessary for multimodal data review. The Component Neuromonitoring System (CNS Technology, LLC, Ambler, PA) is a rare example of a clinical system for integration of multimodal data. However, monitors approved for clinical use are highly restricted in their capabilities to display and process data in new ways, while a system for research requires this ability to prototype and test algorithms for acquisition, processing, display, and real-time decision support. As one example, to discover the clinical meaning and utility of nonseizure EEG activities such as periodic epileptiform discharges, pattern recognition routines could be developed and then displayed alongside processed metrics of other modalities in a common platform. Clearly, advances are limited by our ability to interpret and integrate complex, multimodal data streams.

Our aim was to develop and test a multimodal neuromonitoring system that integrates clinical and research data into a single data collection, analysis, and detection platform. Our specific motivation was to support clinical studies of spreading depolarizations by the CoOperative Study on Brain Injury Depolarizations (http://www.cosbid.org). Spreading depolarizations are pathologic waves of mass neuronal depolarization that are measured by electrocorticography (ECoG) in human cerebral cortex after brain trauma and ischemic and hemorrhagic stroke [9, 10]. In SAH, they are associated with development of delayed cerebral ischemia and infarction and are a potential future target for neuromonitoring and treatment [11–15]. Evidence suggests that spreading depolarizations may be triggered by low blood pressure and high temperature [16], low regional cerebral blood flow (rCBF) [17], and low plasma glucose [18] and may in turn cause changes in cerebral lactate, glucose [18, 19], rCBF [12], and local $P_{bt}O_2$ [20]. To best understand these pathophysiologic relationships, a platform that integrates these multiple modalities is needed. Furthermore, interventional studies using spreading depolarizations as a treatment indication might require a system capable of predicting and detecting their occurrence, monitoring their evolution, and providing user feedback based on depolarizations and possibly criteria from other monitored modalities. A treatment protocol, for instance, might be triggered when depolarizations occur >1/h and CPP is >70 mmHg.

We developed a system, the COSBID-M3, that is adapted from the widely used BCI2000 platform, a brain-computer interface tool for real-time analysis of EEG and ECoG data [21]. BCI2000 has been in use for more than a decade and has been distributed to more than 600 labs worldwide for research. Importantly, it provides a flexible and adaptable research platform capable of performing tasks beyond standard BCI experiments, including functional cortical mapping for epilepsy surgery [22], behavioral experiments in both humans and animals [23], and human-computer interface research. Therefore, we adapted the BCI2000 system to function as a multimodal research monitor capable of acquiring continuous streams of raw data, including arterial blood pressure (ABP), intracranial pressure (ICP), $P_{bt}O_2$, rCBF, EEG, ECoG, and continuous video. It is capable of advanced signal-processing techniques and decision support and is designed in a flexible, open development format. Furthermore, it can be used for researching any signal type or computational algorithm and is not specific to the COSBID group or ECoG signals.

Software System Design

The COSBID-M3 maintains core design principles of the BCI2000 system [21]. These are (1) a common model that can describe any multimodal monitoring system, such that multiple data streams can be split, processed, and recombined to study the interaction of any arbitrary combination of signals; (2) interchangeability and independence of modules, with logical programmatic separation of data acquisition, signal processing, data visualization, system configuration, and decision support; (3) scalability of experimental parameters with no restraints on signal sampling rates, number of channels, or signal-processing complexity; (4) real-time capability such that the time from signal acquisition to processing and display is on the order of milliseconds; and (5) support for offline analysis via a custom data storage format that stores all acquired data, records of all events, and the experimental operating protocol, thus allowing the entire experiment to be replicated offline and in future recording sessions.

The software system is comprised of four independent programs or modules. First, the *Acquisition* module digitizes signals, stores them to disk, and passes them to the processing module. The *Processing* module performs signal analysis routines, ranging from simple mathematical operations (e.g., digital filtering, decimation, matrix multiplication, squaring, etc.) to more complex algorithms (e.g., fast Fourier transforms [FFTs], autoregressive power spectral analysis, seizure detection, etc.). The processed results are passed to the *Decision Support and Feedback* module, which provides useful feedback to the researcher or clinician. The final module is the *Operator*, which handles synchronizing and communication between the other modules, provides an experimental user interface for recording configuration, and handles data visualization (Fig. 1)

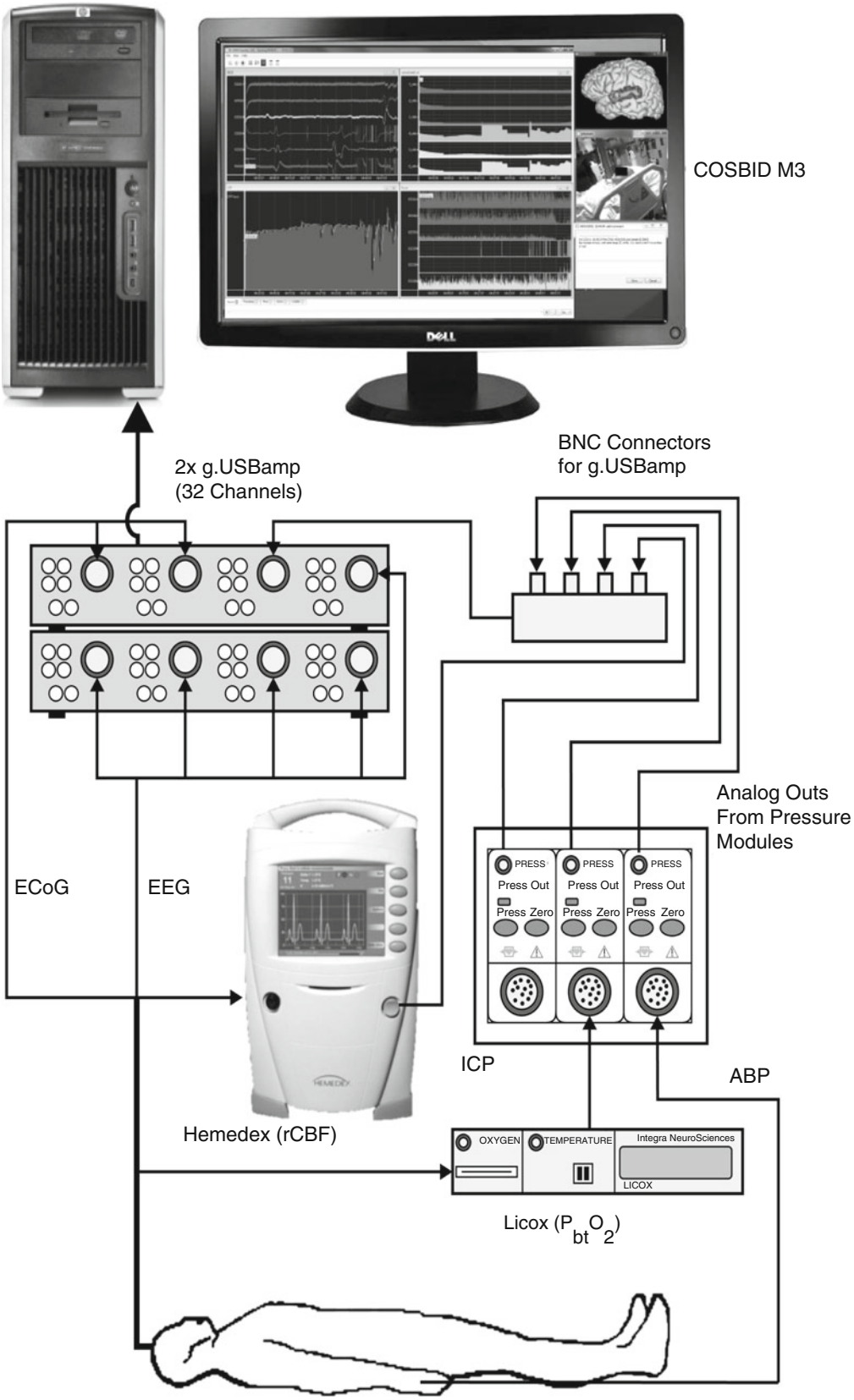


Fig. 1 The COSBID M3 system diagram. The M3 acquires signals from various bedside monitors, including arterial blood pressure (*ABP*), intracranial pressure (*ICP*), brain tissue oxygenation ($P_{bt}O_2$), regional cerebral blood flow (*rCBF*), *EEG* (electroencephalogram), and *ECoG* (electrocorticography). EEG and ECoG signals are acquired directly into the g.USBamps. The ABP, ICP, $P_{bt}O_2$, and rCBF are collected from the analog outputs of the pressure sensor modules and Hemedex monitors. These analog signals are input to a box with four BNC connectors. The box uses a voltage divider to decrease the signal amplitude 500 times so that it is in the appropriate input range for the g.USBamp

Data Acquisition

The Acquisition module collects data from one or more sources; currently more than 20 amplifiers and analog-to-digital converters (ADCs) are supported. The primary data source for COSBID studies is full-band ECoG. Therefore, for the core acquisition hardware we have used the g.USBamp (g.tec, Graz, Austria), a 24-bit direct-current amplifier and ADC with a ±250-mV input range and sampling rate of up to 38.6 kHz per channel. Each g.USBamp has 16 analog input channels, and multiple units can be stacked to allow up to 256 channels. Therefore, our basic setup consists of 2 g.USBamps that acquire 6 ECoG channels, 20 EEG channels, ICP, ABP, $P_{bt}O_2$, and rCBF, for a total of 30 data channels (Fig. 1). However, additional channels could be acquired by adding additional g.USBamps or other ADCs.

Data are sampled at configurable rates, which can be set differently for various signals in the same recording. Blocks of data samples are acquired from the ADC, typically every 30–50 ms; for example, a 50-ms block of data sampled at 1,200 Hz will contain 60 samples. The raw, unfiltered data are stored to disk in a single file in the BCI2000 format, sent to the Operator module for visualization, and finally sent to the Processing module. The data file is saved using the BCI2000 format, which is open source and provides interfaces with a number of programming languages, including C++, MATLAB, and Python. The file can also be down-sampled and converted to other formats, such as binary or European data format (EDF), for importing to other data analysis programs such as LabChart (ADInstruments, New South Wales, Australia).

Signal Processing

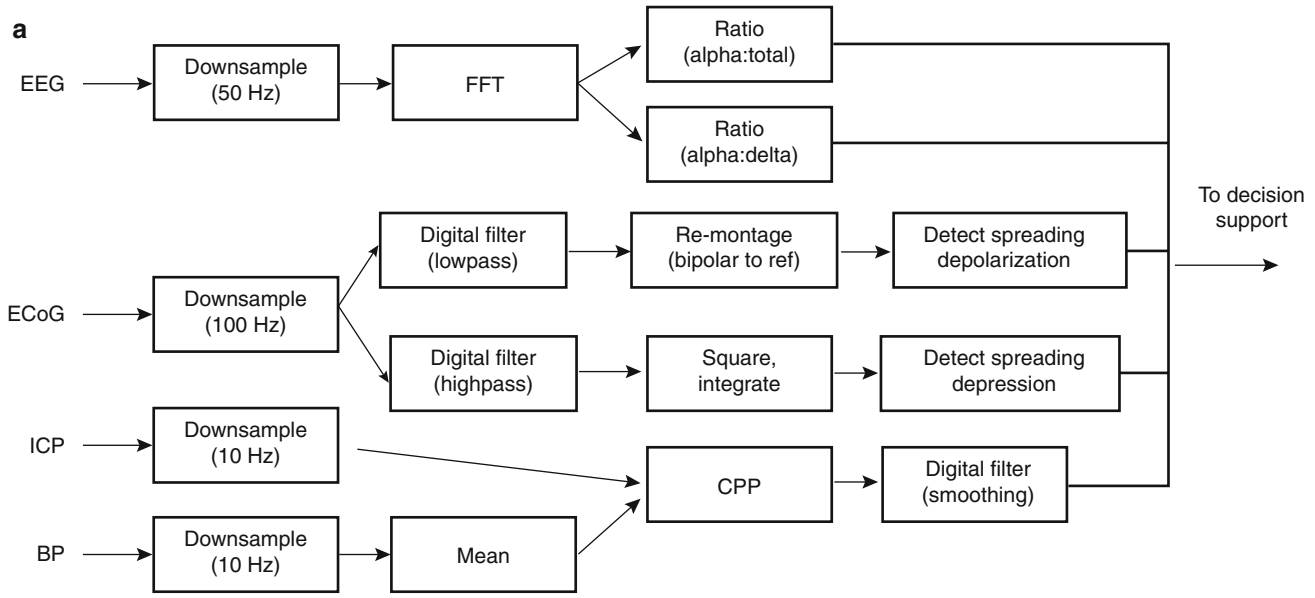
The Processing module uses a *plug-in* architecture for signal analysis and event detection. Plug-ins serve as algorithmic building blocks that allow complex analysis routines to be constructed. Examples of existing plug-ins are digital filtering, data remontaging, down-sampling, FFT, and threshold detection; new plug-ins may be written using the plug-in framework, described in the following. Prior to monitoring, a signal-processing network of plug-ins is designed in a simple text file in which specific subsets of channels are passed to plug-ins for processing (Fig. 2b). The results of each plug-in are passed to subsequent plug-ins for further processing as needed until all of the designated analyses are complete. For example, to detect depressions of 0.5–100 Hz ECoG brain activity induced by spreading depolarizations, a sequence of plug-ins would include a down-sampler, digital filter, squaring and integration filter, and finally a relative threshold filter that detects decreases in this computed power integral. Similarly, cerebral perfusion pressure (CPP) is calculated by building a chain including down-sampling of the ICP and ABP channels, calculating mean values, and finally subtracting ICP from ABP. An example signal-processing chain is shown in Fig. 2a, and a portion of the chain definition is in Fig. 2b.

Although many commonly used plug-ins already exist and are distributed with the COSBID-M3, the plug-in framework was designed to make the M3 extensible and configurable according to specific data-processing needs of individual researchers. Thus, new plug-ins can be created simply using the C++, MATLAB, or Python programming languages. While C++ is the preferred method due to its speed and direct interface with the M3, MATLAB and Python support allows signal-processing prototypes to be tested directly within the real-time system and provides access to the extensive numerical libraries available on these platforms. The M3 framework provides support for data trending, efficient data buffering, and multiple visualization techniques, in addition to many other features. Figure 3 shows an example of the main processing functions for both C++ and MATLAB.

Decision Support and Feedback

The fourth module is the Decision Support and Feedback module. This module is designed to summarize the processed results and relay important information to the researcher or clinicians and caregivers. Using a relatively simple syntax, a variety of displays and alarms can be triggered for any number of conditions based on raw or processed data. For example, to trigger an event when the ICP exceeds 20 mmHg for more than 5 min, the syntax would be ICP >20, 360 s. For a trigger based on multiple conditions, ICP >20 AND CPP <60, 360 s would trigger an event when both ICP is above 20 mmHg and CPP is less than 60 mmHg for longer than 360 s. Another example of using processed data would be detection of decreases in the ECoG power integral, as described previously.

Several default event types have been defined, although these can be extended using the plug-in architecture as well. Currently, events can trigger (1) a visual alert in which flashing text is shown on the display, (2) an audio alert that continuously plays a selected alarm until it is disabled, or (3) an electronic alert that sends an e-mail or text message to a list of recipients. Alerts are useful not



b

```
PluginName ; Unique Identifier ; Input Signals
1. DownsamplePlugin; BPDownsample; Source=BP
2. DownsamplePlugin; ICPDownsample; Source=ICP
3. MeanPlugin; MAP; BP
4. CPPlugin; CPP; ICP+MAP

5. DownsamplePlugin; EEGDownsample; Source=EEG*
6. FFTPlugin; EEGFFT; EEGDownsample
7. RatioPlugin; ADR; EEGFFT
8. RatioPlugin; ATR; EEGFFT
```

Fig. 2 (**a**) An example processing chain involving *ECoG* (electrocorticography), *EEG* (electroencephalogram), *ICP* (intracranial pressure), and *BP* (blood pressure). This chain down-samples each data stream appropriately using the *Downsample* plug-in. The EEG is then processed using the *FFT* (fast Fourier transform) plug-in, and the power spectrum from the FFT is passed to the ratio plug-in, which calculates quantitative EEG measures such as the alpha-delta ratio and alpha-total power ratio. The ECoG signal is split into two separate chains that detect both spreading depolarizations and spreading depressions. The ICP and BP are both down-sampled to 10 Hz; the *MAP* (mean arterial pressure http://en.wikipedia.org/wiki/Mean_arterial_pressure) is calculated with a *Mean* plug-in that calculates the mean of the blood pressure over the previous cardiac cycle. The ICP and MAP are recombined in the *CPP* (cerebral perfusion pressure) plug-in, which subtracts the ICP from the MAP to find the CPP. Finally, the CPP is smoothed with a digital filter. It is important to note that many of these plug-ins are reused (e.g., the Downsample and Digital Filter plug-ins), and that the plug-ins do not depend on any particular data type; that is, they are general-purpose plug-ins. (**b**) A portion of the processing definitions for (**a**). *Lines 1–4* define the CPP plug-ins, and *lines 5–8* define the quantitative EEG plug-ins. The general format for a plug-in line is the name of the plug-in, a unique identifier for the plug-in, and a list of input signals, all separated by semicolons. In line 1, the down-sampler is used; it is named "BPDownsample," and it is taking the signal named "BP" as its input. The ICP is down-sampled similarly. On line 3, the output from BPDownsample is passed into the MeanPlugin, which calculates the mean over some period and is named MAP. Finally, on line 4 the CPPPlugin, named CPP, takes the ICP and MAP signals as input to find the CPP. On line 5, the DownsamplePlugin, named EEGDownsample, takes all channels with "EEG" in the name as input; the "*" character is used as a wild card for string matching, so that channels named EEG1, EEG2, … , EEG20 will all match as input for this plug-in. On line 6, the FFTPlugin takes the FFT of all EEG channels over a specified duration (e.g., 10 s). On lines 7 and 8, the QEEG plug-ins are defined for the alpha-delta ratio (*line 7*) and alpha-total power ratio (*line 8*)

only for routine clinical care but also particularly for research protocols. They could be used, for instance, to alert staff to collect more detailed clinical data surrounding particular events in observational research or to activate a treatment protocol in an interventional trial that targets neuromonitoring variables. As a practical benefit, they can also alert staff to malfunctions or unintended data interruptions.

a

```
    void
    CPPplugin::Process (const GenericSignal& Input,
                        GenericSignal& Output)
    {
1....  float mCPP = Input (mMAPch,0) -  Input (mICPch,0);
2....  mCPPtrend. Insert (mCPP);
3....  Output (0,0) = mCPP;
4....  mCPPvis . Send (Output);  // send results to graph
    }
```

b

```
function out_signal = bci_Process (in_signal)
out_signal = in_signal * refToBipolarMatrix;
```

Fig. 3 Example plug-in code written in C++ (**a**) and MATLAB (**b**). (**a**) The *Input* signal contains the mean arterial pressure (MAP; calculated in the preceding plug-in) and the intracranial pressure (ICP). This plug-in calculates the cerebral perfusion pressure by subtracting the ICP channel from the MAP channel (*line 1*). The CPP (cerebral perfusion pressure) is inserted into a trending buffer (*line 2*) and written to the Output signal (*line 3*). Last, the *Output* signal is sent to the visualization window for the CPP. (**b**) This MATLAB function rereferences the input signal (*in_signal*) using matrix multiplication. The result is saved to *out_signal* in a single line of code and returns the result to the M3

Configuration and Data Visualization

The Operator module handles the most common user interface and visualization tasks. Prior to starting recording, the system is set up via a Configuration Tool, in which hundreds of variables describing the experiment are set. These variables typically have intelligent preset values, and the default values are loaded from a configuration file. Therefore, once a standard experimental and monitoring paradigm is defined and saved in the configuration file, the setup time can be rapid, requiring only entry of subject-specific information (e.g., subject identifiers). In addition, a graphical tool is provided to configure new signals, processing chains, and decision support quickly.

The Acquisition and Processing modules send processed signals to the Operator for visualization. This can consist of raw time series data or any processed data as computed by the Processing module. The monitoring display contains all of the visualization and log windows (Fig. 4). These windows are grouped into tabs for organization and can be moved from tab to tab to compare multiple data streams. For example, Fig. 4 shows three different processed ECoG windows (low-pass filtered, high-pass filtered and squared, and the alpha-delta ratio for each channel) along with the CPP. There are many options for configuring the displays, such as different colors, line fills, and signal clipping, and axes can be changed to display the desired amplitudes and timescales. Elementary data processing, such as filtering and squaring, is also available; this processing is performed for visualization only, does not interrupt data acquisition or affect recorded data, and does not need to be set in the Configuration tool.

Conclusion

The COSBID-M3 provides researchers with a flexible, real-time system for multimodal data acquisition, processing, and display at the bedside. Over 20 amplifier systems are currently supported, and any system that provides a programming interface can be added as well. The g.USBamp was chosen for its previously discussed acquisition characteristics designed for EEG and ECoG; however, alternative, less-expensive amplifiers such as those available from DataTranslation could be used instead for other research purposes, such as computation of autoregulatory indices, rapid microdialysis [24], near-infrared spectroscopy, or laser Doppler flowmetry.

Development of the M3 is ongoing, and future versions will include a full review system integrated with the signal-processing frame to allow review of processed data, inclusion of additional signals such as microdialysis, and better incorporation of imaging results. We also plan to add support for efficient annotations at the bedside, including clinical exam notes, comments, and other notes and results. The COSBID-M3 system is freely available for download. More information may be found at http://www.cosbidm3.com. We are actively seeking developers to contribute to this project and need clinicians to help drive development. Therefore, this highly customizable and extensible platform will provide clinicians and researchers with an advanced tool to investigate pathophysiologic relationships and implement advanced multimodal treatment algorithms.

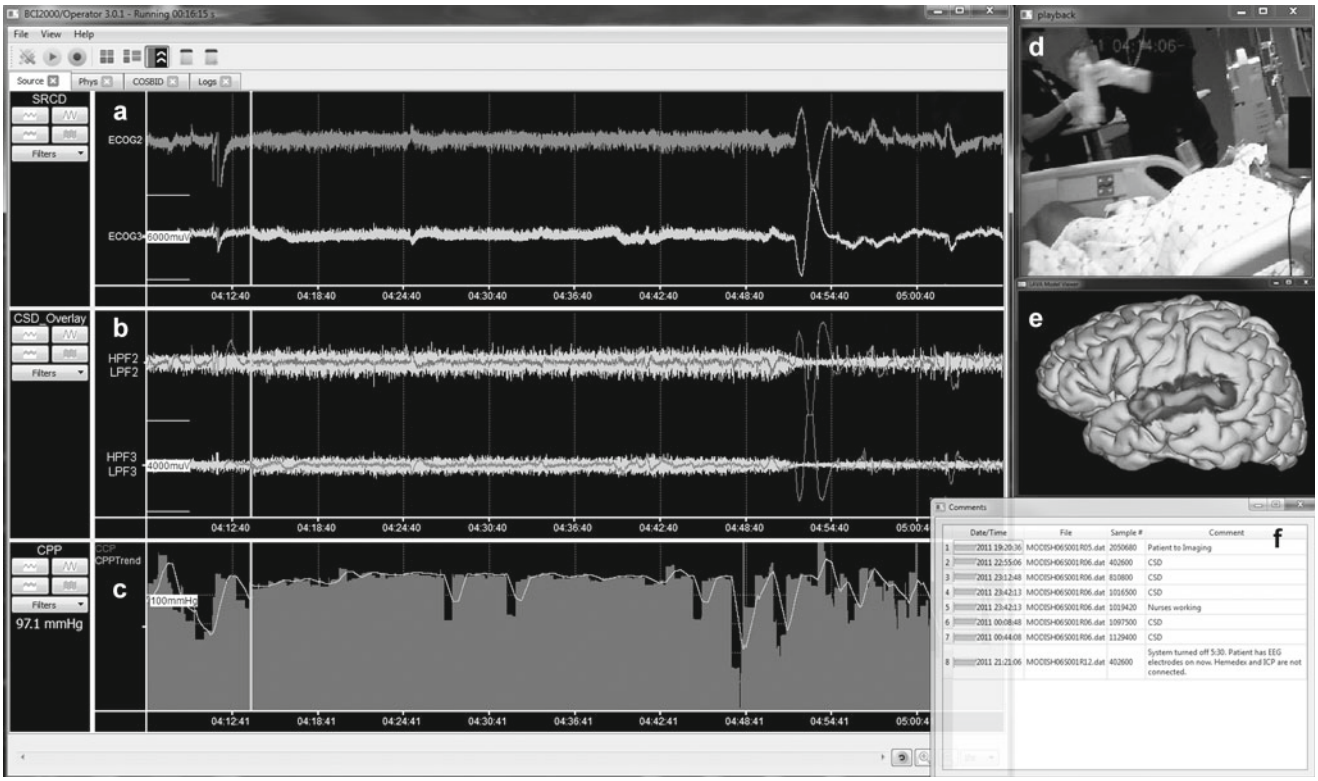


Fig. 4 Example display during monitoring. The main display shows two raw ECoG signals during a spreading depolarization (**a**), the same two channels with the low-pass filtered signal overlaid on the high-pass filtered signal (**b**), and the cerebral perfusion pressure (**c**). The secondary windows on the right contain the synchronized patient video (**d**), the 3D MRI model co-registered with CT data (**e**), and the comment and event log (**f**)

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Transcranial Doppler as a Routine in the Treatment of Vasospasm Following Subarachanoid Hemorrhage (SAH)

Renata F. Simm, Paulo H. Pires de Aguiar, M. de Oliveira Lima, and Bernardo L. Paiva

Abstract *Background*: Vasospasm is an important complication observed after subarachnoid hemorrhage (SAH) and is a frequent cause of mortality and morbidity. We present our routine management of vasospasm after SAH and emphasize the importance of transcranial Doppler (TCD) ultrasonography in this management.

Method: Historical records and images were sampled from June 2005 to September 2011 for 110 patients with SAH due to ruptured aneurysm in the anterior circulation. All surviving patients were followed after discharge. Vasospasm was defined as mild (Lindegaard index 3–4), moderate (Lindegaard index 4–5), and severe (Lindegaard index greater than 5). We excluded patients treated after 72 h of symptom onset. TCD was performed twice per day.

Findings: Ninety-nine patients had surgical clipping of the aneurysm, and 11 had endovascular treatment. Seventy patients treated by clipping and six treated by endovascular procedure had vasospasm. Of the 70 clipped patients with vasospasm, 40 had mild vasospasm, 13 had moderate vasospasm, and 17 had severe vasospasm. All six patients treated by coils had moderate vasospasm. The average duration of vasospasm was 9 days (from 7 to 32 days).

Conclusions: TCD was crucial for monitoring patients with SAH, and to identify which patients will have a higher risk of developing vasospasm.

Keywords Cerebral vasospasm • Subarachnoid hemorrhage • Brain aneurysm • Transcranial Doppler

Introduction

The diagnosis and treatment of vasospasm remains clinically challenging.

Neither clinical signs and symptoms nor neuroimaging including computerized tomography [CT] scan and magnetic resonance imaging [MRI] are sufficient to diagnose vasospasm. While arteriography may be useful to detect, qualify, and treat vasospasm, it is invasive and impractical to perform without good clinical justification.

Transcranial Doppler (TCD) ultrasonography detects blood flow velocity to monitor vasospastic arterial narrowing and is routine in the evaluation and monitoring of patients with subarachnoid hemorrhage (SAH) [1–7].

We present our clinical experience with the routine use of the TCD for monitoring and guiding treatment of symptomatic vasospasm in patients with SAH. We discuss the role of TCD monitoring in conjunction with the clinical exam for inpatients who underwent intracranial pressure (ICP) monitoring, induced coma, as well as decompressive craniotomy or angioplasty in advanced stages of vasospasm.

Methods

Historical records and images were obtained from June 2005 to September 2011 for 110 patients with SAH due to ruptured aneurysm in the anterior circulation. All surviving patients were followed after discharge.

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Vasospasm was defined as mild (Lindegaard index 3–4), moderate (Lindegaard index 4–5), and severe (Lindegaard index greater than 5). We excluded patients treated after 72 h of symptom onset [7]. TCD was performed twice per day during hospitalization.

Results

Ninety-nine patients had surgical clipping of the aneurysm, and 11 had endovascular treatment. Among patients with clipped aneurysms, the Hunt Hess Scale scores were I ($n=25$), II ($n=24$), III ($n=49$), and IV ($n=1$). Among patients treated by endovascular procedures, the scores were I ($n=2$), II ($n=1$), III ($n=3$), and IV ($n=5$). Seventy patients treated by clipping and six treated by endovascular procedure had vasospasm. Of the 70 clipped patients with vasospasm, 40 had mild vasospasm, 13 had moderate vasospasm, and 17 had severe vasospasm. All six patients treated by coils had moderate vasospasm. Of the 17 patients with severe vasospasm by TCD, 12 patients ultimately underwent decompressive craniotomy due to brain ischemia, and an additional two underwent angioplasty. The average duration of vasospasm for all patients was 9 days (range 7–32 days).

Discussion

We found twice-daily TCDs to be a useful screening tool in conjunction with the clinical exam for identifying SAH patients experiencing symptomatic vasospasm. Depending on the grade of vasospasm, we used specific therapies, such as triple H (hypertensive, hypervolemic, and hemodilution) treatment, statins, and correction of magnesium serum levels. Patients with mild vasospasm received only nimodipine. Fasudil is not approved in Brazil.

TCD is useful in the first 24 h to characterize baseline cerebral blood flow, at 24–48 h to monitor for any hyperemia, and after 48 h for early identification of vasospasm.

TCD ensures early diagnosis of vasospasm, classification of vasospasm intensity, monitoring of progression of vasospasm and detection of vasospasm, in multiple arterial segments.

Conclusions

Transcranial Doppler is a useful tool for monitoring patients with SAH and for identifying which patients will have a higher risk of developing vasospasm.

Conflicts of Interest We declare that we have no conflict of interest.

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Systemic Interleukin-6 Levels Reflect Illness Course and Prognosis of Patients with Spontaneous Nonaneurysmal Subarachnoid Hemorrhage

Carl Muroi, Martin Seule, Christopher Sikorski, Wolfgang Dent, and Emanuela Keller

Abstract *Background*: Patients with nonaneurysmal subarachnoid hemorrhage (SAH) show either perimesencephal (pm)SAH or nonperimesencephalic (non-pm)SAH, with hemorrhage extending into adjacent cisterns. Patients with non-pmSAH have higher risk for a complicated clinical course with cerebral vasospasm (CVS) and worse outcome. Systemic inflammatory response has been linked to CVS occurrence and worse outcome in aneurysmal SAH. We analyzed whether levels of interleukin (IL)-6, a proinflammatory cytokine, differ in patients with pmSAH compared with non-pmSAH.

Methods: The clinical course with attention to symptomatic CVS occurrence and clinical outcome was assessed. Daily systemic IL-6 levels and leukocyte counts (Lc) were measured in the acute phase in 11 patients with pmSAH and in 9 patients with non-pmSAH.

Results: Patients with non-pmSAH had significantly higher IL-6 levels compared to patients with pmSAH (14.7 ± 3.2 vs. 3.0 ± 0.6 pg/ml, $p=0.001$). Lc counts did not differ (11.5 ± 0.5 vs. 11.2 ± 0.6 × 10^3/µl, $p=0.485$). Patients with non-pmSAH stayed significantly longer in the neurocritical care unit (16.4 ± 2.1 vs. 10.2 ± 1.1 days, $p=0.012$). Symptomatic CVS occurred in two patients with non-pmSAH. Patients with pmSAH had a significantly more favorable outcome, defined as Glasgow Outcome Scale 5.

Conclusion: Higher IL-6 levels in patients with non-pmSAH supports the common observation of more complicated illness course with higher incidence of CVS compared to patients with pmSAH.

Keywords Interleukin-6 • Cerebral vasospasm • Systemic inflammation • Nonaneurysmal subarachnoid hemorrhage • Perimesencephalic subarachnoid hemorrhage

Background

Despite considerable advances in imaging techniques, a bleeding source cannot be found in a tiny subgroup of patients with spontaneous subarachnoid hemorrhage (SAH) [1, 6]. These patients are considered to suffer from a nonaneurysmal SAH. Nonaneurysmal SAHs are further subdivided in perimesencephalic (pmSAH) and nonperimesencephalic (non-pm)SAH, based on the systematic description by van Gijin et al. [11]. In patients with pmSAH, the blood is located strictly around the midbrain or brain stem, whereas in patients with non-pmSAH the hemorrhage extends into adjacent cisterns [1, 6]. Patients with pmSAH have an uncomplicated illness course with favorable outcome. In particular, the risk of developing cerebral vasospasm (CVS) and delayed cerebral ischemia (DCI) is negligible [1, 3, 4, 6]. Patients with non-pmSAH have a higher risk for a complicated clinical course, with occurrence of CVS/DCI and worse outcome [1, 4, 5, 12]. As well local as systemic inflammatory response is associated with severity of illness, clinical outcome, and occurrence of CVS/DCI in patients with aneurysmal SAH [9]. The possible significance of systemic leukocyte count (Lc) in this context has been discussed [9]. More recently, higher levels of interleukin (IL)-6, a proinflammatory cytokine, have been linked to the occurrence of symptomatic CVS and worse outcome [9, 10]. Systemic IL-6 levels could reflect the severity of illness and risk of CVS to some extent. Recently, we described the difference in systemic IL-6 levels between patients with aneurysmal and nonaneurysmal SAH. The results showed a significantly higher concentration in

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patients with aneurysmal SAH [8]. In the current chapter, we refine our subgroup analysis between patients with pmSAH and non-pmSAH.

Methods

All patients presenting with nonaneurysmal SAH during a 1-year period were included in the current study. The diagnoses of pmSAH and non-pmSAH were based on the initial computed tomographic (CT) scan. Exclusion of a bleeding source was based on cerebral angiography. If the subarachnoid blood was strictly located in the interpeduncular or prepontine cistern, the patient was considered to suffer from pmSAH [8]. If the blood was located in the adjacent basal cisterns, the patient was considered to have a non-pmSAH [8]. In the latter group of patients, repeated angiography was performed after the acute phase to definitely exclude a bleeding source. Clinical assessment at time of admission was carried out according to the World Federation of Neurosurgical Societies (WFNS). WFNS grade was dichotomized between WFNS 1–3 and WFNS 4–5. The clinical illness course with attention to symptomatic CVS occurrence [7] and clinical outcome after 3 months, based on the Glasgow Outcome Scale (GOS), was assessed. Favorable outcome was defined as GOS value of 5. Systemic IL-6 concentrations and Lc were measured daily until day 12 after ictus. IL-6 levels were measured by chemiluminescence enzyme immunoassay. Lc was performed by automated photometric measurement. The current study was of a prospective observational nature. Variables are given as mean plus or minus standard error of the mean. Numeric variables were compared by independent *t* test and nominal variables by Fisher's exact test. A *p* value less than 0.05 was assumed to be statistically significant.

Results

A total of 20 patients with nonaneurysmal SAH were identified, 11 patients presenting with pmSAH and 9 patients with non-pmSAH. Gender and age did not differ between patients with pmSAH and non-pmSAH (Table 1). Although two patients with non-pmSAH showed a WFNS grade 4, dichotomized WFNS grade at time of admission did not differ between the two groups (Table 1). Patients with pmSAH had statistically significant lower IL-6 levels during the illness course compared to patients with non-pmSAH (3.1 ± 0.6 vs. 14.7 ± 3.2 pg/ml, $p=0.001$), while the Lc did not differ (11.2 ± 0.6 vs. 11.5 ± 0.5 × 10^3/μl, $p=0.636$) (Table 1, Fig. 1). Patients with non-pmSAH had statistically longer stay in the neurocritical care unit (16.4 ± 2.1 vs. 10.2 ± 1.1 days, $p=0.012$) (Table 1, Fig. 1). In two of nine patients with non-pmSAH, symptomatic CVS occurred in the illness course. Concerning outcome, ten patients with pmSAH had a favorable outcome, with a GOS value of 5. One patient had a GOS of 4. In patients with non-pmSAH, 3 were considered to have a favorable outcome after 3 months, while the remaining 6 patients had a GOS of 4 and 1 patient a GOS of 1. The cause of death (GOS 1) was not directly related to the bleeding. The outcome was statistically different in a dichotomized manner ($p=0.017$) (Table 1).

Table 1 Characteristics of patients with nonaneurysmal subarachnoid hemorrhage (SAH) and results

	pmSAH ($n=11$)			non-pmSAH ($n=9$)	
Sex (male:female)	3:8		ns[a]	4:5	
Age (mean ± SEM) (years)	53.9	±2.4	ns[b]	59.9	±4.3
WFNS grade					
1–3	11		ns[a]	7	
4–5	0			2	
IL-6 (mean ± SEM) (pg/ml)	3.1	±0.6	$p=0.001$[b]	14.7	±3.2
Lc counts (mean ± SEM) (×10^3/μl)	11.2	±0.6	ns[b]	11.5	±0.5
Stay in the NCU (mean ± SEM) (days)	10.2	±1.1	$p=0.012$[b]	16.4	±2.1
Symptomatic CVS occurrence	0		ns[a]	2	
Outcome					
Favorable (GOS 5)	10		$p=0.017$[a]	3	
Unfavorable (GOS <5)	1			6	

pmSAH perimesencephalic subarachnoid hemorrhage, *n* number of patients, *ns* statistically not significant, *SEM* standard error of the mean, *WFNS* World Federation of Neurosurgical Societies, *IL* interleukin, *Lc* leukocytes, *NCU* neurocritical care unit, *CVS* cerebral vasospasm, *GOS* Glasgow Outcome Scale

[a]Fisher's exact test

[b]Independent *t* test

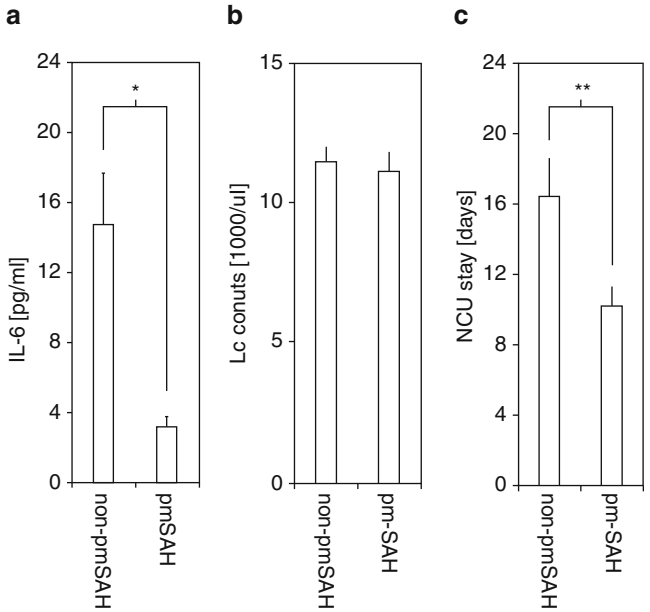

Fig. 1 Results shown as bar graphs. (**a**) Systemic interleukin 6-(IL-6) levels; (**b**) systemic leukocyte counts (Lc); (**c**) stay in the neurocritical care unit (NCU). Statistically significant: $*p=0.001$, $**p=0.017$

Discussion

In the present study, lower systemic IL-6 levels could be shown in patients with pmSAH compared to patients with non-pmSAH. The Lc counts did not differ between these groups. Clinically relevant CVS was observed in two patients presenting with non-pmSAH. No patient with pmSAH had any signs of CVS during the illness course. However, the incidence was not statistically significant, probably due to the small sample size. Further, patients with non-pmSAH had a significantly longer stay in the neurocritical care unit. Although overall outcome could be considered as good in both groups (almost all patients presenting with GOS 4 or 5), statistically significantly more patients with pmSAH had a favorable outcome (defined as aGOS value of 5) compared to patients with non-pmSAH (Table 1).

IL-6 is a pleiotropic proinflammatory cytokine and acts as an acute-phase reactant. Pathologically high IL-6 levels have been found systemically as well as in the cerebrospinal fluid (CSF) in patients with aneurysmal SAH. In particular, higher levels in the CSF have been linked with the occurrence of CVS/DCI and subsequent worse outcome [9, 10]. Systemic IL-6 has been shown to be an independent predictor of outcome in unselected critically ill patients [2]. Pathological levels have also been found in patients with aneurysmal SAH [9, 10]. Significantly higher systemic IL-6 levels have been reported in patients with symptomatic CVS [10]. Recently, we have shown higher systemic IL-6 levels in patients with aneurysmal SAH compared to nonaneurysmal SAH [8]. The current results support our previous assumption that systemic IL-6 concentration reflects the severity of inflammatory stress response and the severity of the illness in patients with nonaneurysmal SAH.

The shortcomings of this study are the small patient number with limited statistical perspectives. Further, the question of whether systemic IL-6 has a direct impact on the development of CVS in patients with nonaneurysmal SAH cannot be answered. A consecutive study with a larger patient population might answer the question of whether systemic IL-6 levels have a predictive value for poor outcome and development of CVS/DCI in patients with nonaneurysmal SAH, in particular in patients with non-pmSAH who are considered to have a higher risk.

Conclusion

In conclusion, the current results indicate that systemic IL-6 concentration reflects the severity of inflammatory stress response and underline the already-known fact of the more complicated illness course with possible poor outcome and higher incidence of CVS/DCI in patients with non-pmSAH compared to pmSAH.

Note The results were published in part as indicated in the text [8].

Conflicts of Interest We declare that we have no conflict of interest.

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Beneficial Effect of Selective Intra-arterial Infusion of Fasudil Hydrochloride as a Treatment of Symptomatic Vasospasm Following SAH

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Abstract *Introduction*: We envisage the efficacy and safety of intra-arterial infusion of fasudil hydrochloride (IAF) for symptomatic vasospasm (SVS) after subarachnoid hemorrhage (SAH). We compared results obtained from the groups that received selective IAF (a microcatheter inserted in intracranial arteries) and nonselective IAF (a microcatheter inserted in the cervical arteries). Glasgow Outcome Scale (GOS) value and computed tomographic (CT) score were used to evaluate clinical outcome and the extent of infarction due to delayed vasospasm.

Material and methods: Over 2 years, 113 patients with SAH underwent clipping or coiling. Among them, 31 patients (27.4%) developed SVS. We performed nonselective IAF in 10 patients and selective IAF in 10 other patients. Eleven patients with SVS were treated without IAF. The data were statistically analyzed.

Result: By univariate linear regression analysis, IAF negatively correlated with CT score ($p=0.016$), but IAF was significantly correlated with GOS ($p=0.035$). By multiple regression analysis, Hunt and Kosnik grade and CT score significantly correlated with GOS.

Discussion: CT score significantly correlated with functional outcome. Although IAF, both selective and nonselective, was significantly effective for the treatment of delayed vasospasm, the former seemed to be more beneficial.

Keywords Aneurysm • Subarachnoid hemorrhage • Symptomatic vasospasm • Intra-arterial infusion • Fasudil hydrochloride (FA)

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Introduction

Recently, fasudil hydrochloride (IAF) for treatment of symptomatic vasospasm (SVS) following subarachnoid hemorrhage (SAH) has been widely used in Japan [1, 2, 5, 7–9]. Fasudil hydrochloride (FA) is a Rho kinase inhibitor (RKI), attenuating smooth muscle contraction of cerebral artery. In Japan, intravenous administration of RKI is widely accepted for prevention of delayed cerebral vasospasm after SAH [6]. From a retrospective point of view, we have analyzed a correlation between intra-arterial infusion of FA (IAF) and final outcome of the symptomatic vasospasm (SVS) cases to envisage the usefulness of IAF in treatment of SVS. In addition, we compared results obtained from the groups that received selective IAF (In these patients, a catheter was inserted into the A1, M1, M2, C1, C2 segment close to the spastic arteries) and nonselective IAF (in these patients, a catheter was placed in the extradural portion of the internal carotid artery or vertebral artery). In this report, we demonstrate more beneficial effect of IAF in the former group than in the latter group as a treatment of SVS following SAH.

Materials and Methods

Treatment Strategy for SVS

Our clinical path for patients with SAH is as follows: We performed clipping or coiling of ruptured aneurysms within 72 h from the onset of SAH. We routinely administered FA intravenously during the period of vasospasm. If patients began to develop SVS, we went to angiogram and infuse FA into the affected vessel at the rate of 3 mg/min. We routinely started intravenous administration of the free radical scavenger Edarabon, which has been accepted for a treatment of acute cerebral ischemia [4], as soon as possible when the patient was diagnosed with SVS.

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From January 2009 to March 2011, 113 patients with aneurysmal SAH were treated with clipping or coiling. Among them, 31 patients (27.4%) developed SVS. We performed IAF in 20 patients; 10 patients were treated nonselectively (group 2) and the other 10 patients selectively (group 3). Eleven patients with SVS did not undergo IAF (group 1).

In group 2, IAF was performed with the catheter introduced into the unilateral cervical portion of the internal carotid artery (ICA) in 6 patients (60.0%), bilateral cervical ICA in 2 cases (20.0%), unilateral ICA and unilateral vertebral artery (VA) in 1 case (10.0%), and bilateral cervical ICA and unilateral VA in 1 case (10.0%). In group 2, all patients received a single IAF procedure except for a case who received IAF twice during the period of vasospasm (at days 8 and 10).

In group 3, IAF into the M1 segment was performed in 7 patients (70.0%), the A1 segment in 2 patients (20.0%), and the C2 segment in 1 patient (10.0%). Balloon angioplasty was done in only 1 case.

Functional outcome was evaluated by final Glasgow Outcome Scale (GOS) value at discharge to home or other hospital. For statistical evaluation, we referred to GOS value as follows: good recovery as 1, moderately disabled as 2, severely disabled as 3, vegetative survival as 4, and dead as 5.

In addition, we graded the extent of cerebral infarction due to delayed vasospasm by an original scoring system called the computed tomographic (CT) score (see Table 1).

We analyzed the correlation between functional outcome and IAF as well as CT score and IAF by univariate linear regression analysis to evaluate the efficacy of IAF for reducing the extent of infarction due to delayed vasospasm and improving the final outcome. In addition, we investigated the factors that have significant influence on GOS value by multiple regression analysis. A value of $p<0.05$ was considered statistically significant.

Table 1 Computed tomographic (CT) score to analyze the extent of cerebral infarction due to vasospasm in a simple fashion

	CT scoring system: Extent of infarction due to delayed vasospasm
None (1)	No definite infarction due to delayed vasospasm
Small (2)	Less than 1 cm in major axis
Medium (3)	Limited in the territory of one cortical branch
Large (4)	Extending to the territory of two or more cortical branches but limited unilaterally
Extensive (5)	Extending to bilateral hemispheres widely

We demonstrated a significant correlation between computed tomographic (CT) score and Glasgow Outcome Score (GOS) by univariate linear regression analysis in this chapter.

Representative Case

A 72-year old female with SAH Hunt and Kosnik (HK) grade II underwent clipping of the right distal anterior cerebral artery (ACA) ruptured aneurysm. At day 10, this patient developed severe left hemiparesis. Angiography showed severe vasospasm in the bilateral ACAs (Fig. 1a). We introduced a microcatheter into the left A1 segment, and 30 mg of FA was infused at the rate of 3 mg/min (Fig. 1b). After IAF, angiographical vasospasm rapidly and remarkably improved, and the left hemiparesis recovered fully after the treatment (Fig. 1c). CT at day 44 showed a small low-density area in the right cingulate gyrus (Fig. 2). The CT score and GOS value of the patient were 1 and 2, respectively.

Results

Profile and Clinical Features of Patients

The age and female/male ratio are recorded in Table 2.

In group 1, the HK grade was II in 3 cases (27.3%), grade III in 3 cases (27.3%), grade IV in 1 case (9.1%), and grade V in 4 cases (36.4%). In group 2, the HK grade was I in 1 case (10.0%), grade II in 2 cases (20.0%), grade III in 3 cases (30.0%), grade IV in 2 cases (20.0%), and grade V in 2 cases (20.0%). In group 3, the HK grade was I in 1 case (10.0%), grade II in 4 cases (40.0%), grade III in 3 cases (30.0%), grade IV in 1 case (10.0%), and grade V in 1 case (10.0%). Mean ± SD HK grade of groups 1, 2, and 3 was 3.5 ± 1.5, 3.2 ± 1.3, and 2.7 ± 1.2, respectively.

CT Score

In group 1, the CT score was 1 in 2 cases (18.2%), 3 in 1 case (9.1%), 4 in 2 cases (18.2%), and 5 in 5 cases (45.5%). In group 2 , the CT score was 1 in 4 cases (40.0%), 4 in 2 cases (20.0%), and 5 in 4 cases (40.0%). In group 3, the CT score was 1 in 4 cases (40.0%), 2 in 2 cases (20.0%), 3 in 3 cases (30.0%), and 4 in 1 case (10.0%). Mean plus or minus standard deviation (SD) of the CT score of groups 1, 2, and 3 was 3.8 ± 1.5, 3.2 ± 1.9, and 2.1 ± 1.1, respectively (Table 2).

GOS Value

In group 1, the GOS value was 1 in 1 case (9.1%), 2 in 2 cases (18.2%), 3 in 1 case (9.1%), 4 in 4 cases (36.4%), and 5 in 3 cases (27.3%). In group 2, the GOS value was 1 in 1

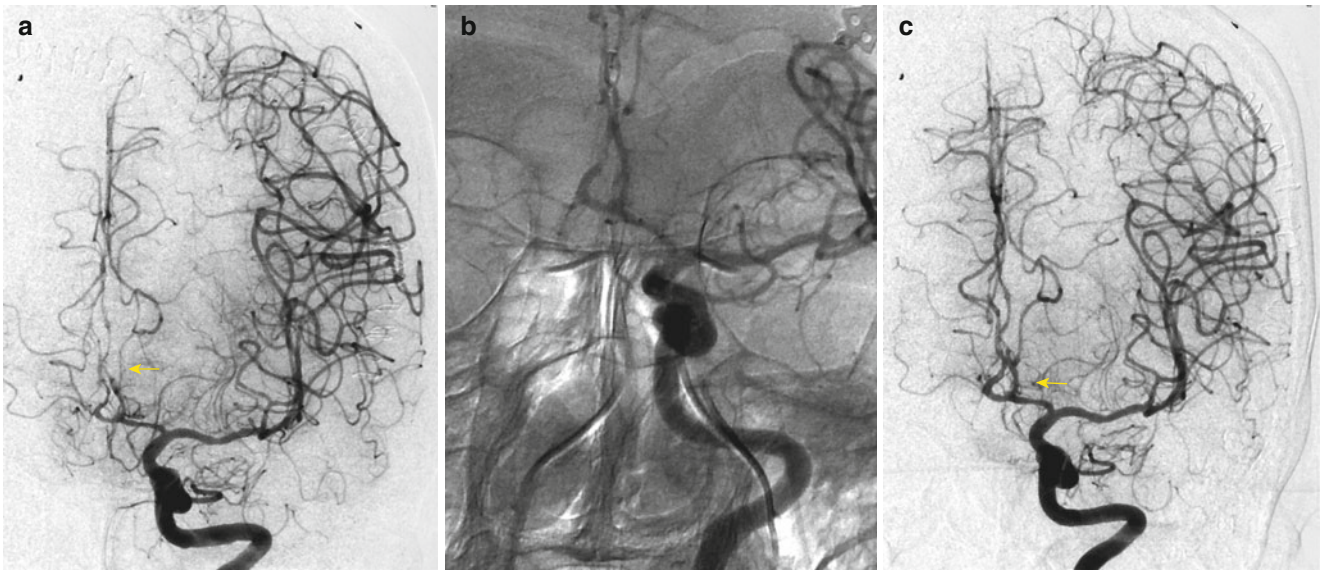

Fig. 1 The anteroposterior view of the left internal carotid angiogram showed severe vasospasm (*yellow arrow*) in the bilateral anterior cerebral arteries (ACAs) (**a**). A microcatheter was introduced into the left A1 segment, then 30 mg of fasudil were infused at the rate of 3 mg/min (**b**). (*yellow arrow*) after selective IAF, angiographical vasospasm improved immediately (**c**)

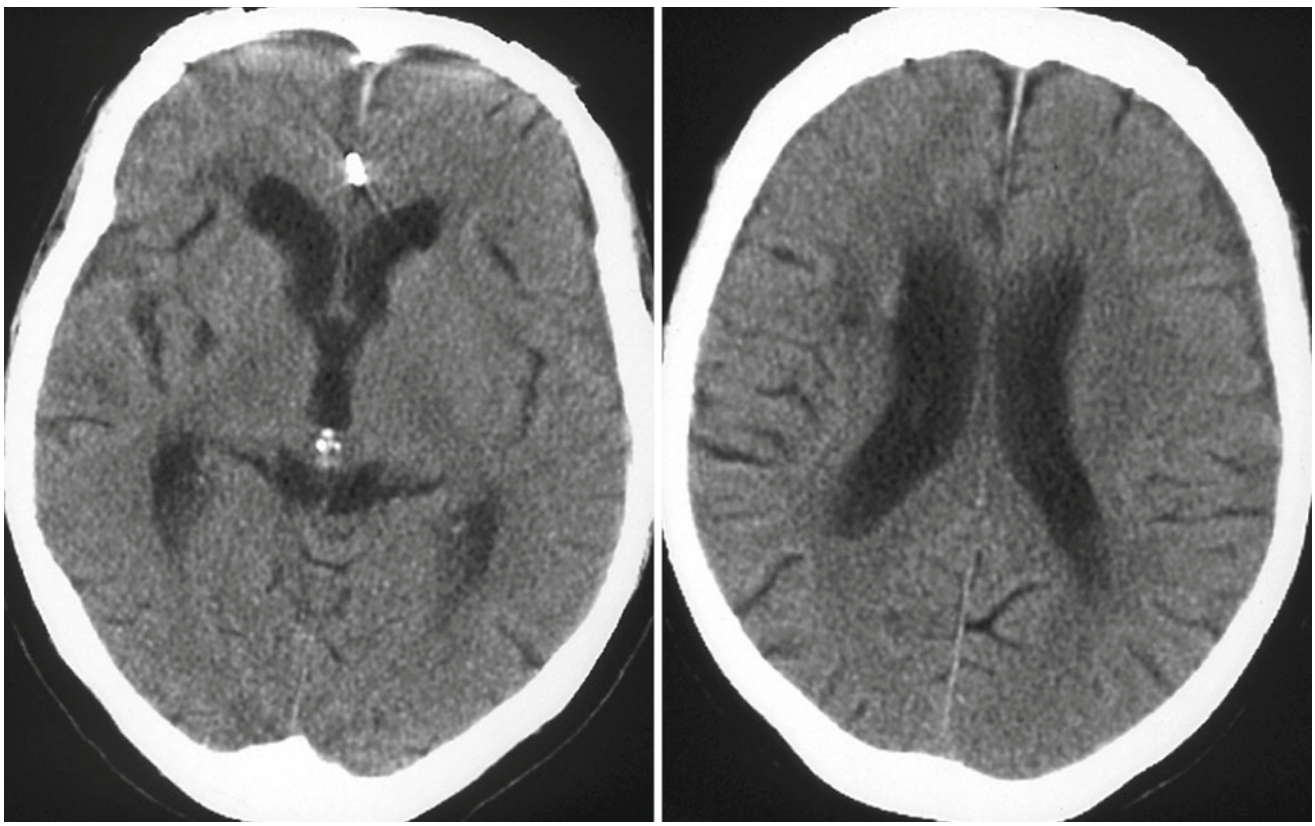

Fig. 2 Computed tomography at day 44 showed a small low = density area in the right cingulate gyrus. The left hemiparesis recovered fully after the intra-arterial infusion of fasudil hydrochloride (IAF)

Table 2 Profiles and clinical features of three groups

	Group 1	Group 2	Group 3
Age(mean ± SD)	65.1 ± 11.8	63.5 ± 15.4	51.8 ± 14.3
Female	10 (90.9%)	9 (90.0%)	7 (70.0%)
H&K grade			
Grade 1	0 (0%)	1 (10.0%)	1 (10.0%)
Grade 2	3 (27.3%)	2 (20.0%)	4 (40.0%)
Grade 3	3(27.3%)	3 (30.0%)	3 (30.0%)
Grade 4	1 (9.1%)	2 (20.0%)	1 (10.0%)
Grade 5	4(36.4%)	2 (20.0%)	1 (10.0%)
Location of aneurysm			
Anterior circulation	10 (90.9%)	7 (70.0%)	9 (90.0%)
Acom	2 (18.2%)	2 (20.0%)	1 (10.0%)
Distal ACA	1 (9.1%)	0 (0%)	1 (10.0%)
IC	5 (45.5%)	4 (40.0%)	4 (40.0%)
MCA	2 (18.2%)	1 (10.0%)	3 (30.0%)
Posterior circulation	1 (9.1%)	3 (30.0%)	1 (10.0%)
VA	0 (0%)	1 (10.0%)	1 (10.0%)
BA	1 (9.1%)	2 (20.0%)	0 (0%)
Mean CT score	3.8 ± 1.5	3.2 ± 1.9	2.1 ± 1.1
Mean GOS score	3.5 ± 1.4	3.6 ± 1.6	2.2 ± 0.9

ACA anterior cerebral artery, *CT* computed tomography, *GOS* Glasgow Outcome Scale, *IC* internal carotid, *H&K* Hunt and Kosnik, *VA* vertebral artery, *MCA* middle cerebral artery, *BA* basilar artery, *Acom* anterior communicating artery

case (10.0%), 2 in 1 case (10.0%), 4 in 3 cases (30.0%), and 5 in 4 cases (40.0%). In group 3, the GOS value was 1 in 3 cases (30.0%), 2 in 2 cases (20.0%), and 3 in 5 cases (50.0%). Mean plus or minus SD of the GOS value of groups 1, 2, and 3 was 3.5 ± 1.4, 3.6 ± 1.6, and 2.2 ± 0.9, respectively (Table 2).

Univariate Linear Regression and Multivariate Regression Analysis

Univariate linear regression analysis showed negative correlation between GOS and IAF as well as between CT score and IAF (Table 2). A correlation coefficient between CT score and IAF was −0.86 (−1.54 to −0.17; 95% confidence interval [CI], $p = 0.016$.). A correlation coefficient between GOS and IAF was −0.66 (−1.27 to −0.05; 95% CI, $p = 0.035$).

In multiple regression analysis, HK grade and CT score significantly correlated with GOS value (correlation coefficient=0.641, $p=0.000$, and correlation coefficient=0.5159, $p=0.000$, respectively).

Adverse Effect of IAF

Epileptic seizures that seemed to be related to the IAF procedure occurred in some cases. These seizures started during the IAF or the next day. An IAF-related epileptic seizure occurred in one patient (10.0%) in groups 2 and in 3 (30.0%) in group 3. Therefore, sufficient administration of antiepileptic drug prior to the IAF procedure is recommended. No other adverse effects related to the IAF procedure were appreciated.

Discussion

The pathogenesis of delayed vasospasm after SAH has not been fully elucidated [3]. Therefore, it is still a major cause of disability and death in patients with SAH. In the present study, 27.4% of patients with SAH developed SVS and required intensive treatment in spite of prophylactic intravenous administration of fasudil. In such situations, IAF is thought to be one of the best additional treatment options because it enables us to deliver RKI to the affected vessel directly. Recently, IAF for SVS has become popular in Japan [1, 2, 7–9]. We analyzed the cases with IAF retrospectively and envisaged the efficacy and safety of IAF in the treatment of SVS.

Adverse Effects

The major adverse effect of intravenous fasudil infusion is intracranial bleeding and hypotension [1]. A postmarketing surveillance study of 1,462 patients reported that intracranial bleeding occurred in 1.6% and hypotension in 0.1%. In the present study, we encountered neither intracranial bleeding nor hypotension after IAF, possibly because of the small size of our study [2]. We did encounter epileptic seizure after IAF procedure in 4 cases. Among them, 3 cases with epileptic seizure were included in group 3. Thus, attention should be paid to the possibility of seizures, especially in cases with SVS who undergo selective IAF.

CT Score

We introduced a CT score system (Table 1) to analyze the extent of cerebral infarction originating from delayed vasospasm after SAH. According to multiple regression analysis,

CT score significantly correlated with GOS value. We believe that our simple, inexpensive CT scoring system may be a good tool for prediction of final GOS value.

Efficacy of IAF

In this study, IAF significantly correlated with CT score and GOS value by univariate linear regression analysis. This result indicated that IAF may have a beneficial effect in the treatment of SVS, and selective IAF seemed to be more effective than nonselective IAF.

Thus, the present investigation supported the usefulness of selective IAF for ultimate treatment of SVS.

Conclusion

CT score significantly correlated with functional outcome by multiple regression analysis. IAF, both selective and nonselective, was significantly effective in treatment of delayed vasospasm. Since the present study was analyzed retrospectively and included a small number of cases, the findings merit further prospective randomized controlled trials to investigate the efficacy of IAF for treatment of SVS.

Conflicts of Interest We declare that we have no conflict of interest.

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Cerebral Hemodynamic Changes After Wartime Traumatic Brain Injury

Alexander Razumovsky, Teodoro Tigno, Sven M. Hochheimer, Fred L. Stephens, Randy Bell, Alexander H. Vo, Meryl A. Severson, Scott A. Marshall, Stephen M. Oppenheimer, Robert Ecker, and Rocco A. Armonda

Abstract Traumatic brain injury (TBI) is associated with the severest casualties from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). From October 1, 2008, the U.S. Army Medical Department initiated a transcranial Doppler (TCD) ultrasound service for TBI; included patients were retrospectively evaluated for TCD-determined incidence of post-traumatic cerebral vasospasm and intracranial hypertension after wartime TBI. Ninety patients were investigated with daily TCD studies and a comprehensive TCD protocol, and published diagnostic criteria for vasospasm and increased intracranial pressure (ICP) were applied. TCD signs of mild, moderate, and severe vasospasms were observed in 37%, 22%, and 12% of patients, respectively. TCD signs of intracranial hypertension were recorded in 62.2%; 5 patients (4.5%) underwent transluminal angioplasty for post-traumatic clinical vasospasm treatment, and 16 (14.4%) had cranioplasty. These findings demonstrate that cerebral arterial spasm and intracranial hypertension are frequent and significant complications of combat TBI; therefore, daily TCD monitoring is recommended for their recognition and subsequent management.

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Introduction

Cerebral vasospasm is a frequent complication after aneurysmal and traumatic subarachnoid hemorrhage (SAH) and carries significant morbidity and mortality [9, 11, 13, 19]. Armonda and co-authors indicated that vasospasm occurred in a substantial number of patients with wartime traumatic brain injury (TBI), and clinical outcomes were worse in such patients [2]. Cerebral angiography remains the standard diagnostic test in this setting; however, this procedure is invasive, expensive, not always available, and not without risk [8]. In contrast, transcranial Doppler (TCD) ultrasonography has been increasingly used over the past few years for diagnosis and monitoring cerebral vasospasm and implementing therapeutic interventions [21]. TBI and cerebrovascular injury are associated with the severest casualties from Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) [3]. From October 1, 2008, the U.S. Army Medical Department TBI program initiated a TCD protocol for examination of head-injured patients who were evacuated from the combat theater to receive care at the National Naval Medical Center, the San Antonio Military Medical Center, and the Walter Reed Army Medical Center. The purpose of this retrospective analysis was to evaluate the TCD determined incidence of post-traumatic cerebral vasospasm and intracranial hypertension after wartime TBI in these patients.

Materials and Methods

TCD data were retrospectively analyzed in 90 patients (two females) aged 18–50 years (mean 25.9 years) who had suffered wartime TBI (with Glasgow Coma Scale scores ranging from

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3 to 15). The patients were categorized according to injury: 18 patients with closed head injury (CHI), 19 patients with CHI due to improvised explosive device (CHI/IED), 33 patients with penetrating head injury (PHI), and 20 patients with PHI due to IED (PHI/IED). A total of 567 TCD studies were made after admission. Patients were identified using a computerized registry and a prospective TCD database maintained in the Sentient NeuroCare Services. TCD recordings of mean cerebral blood flow velocities (CBFV, in cm/s) and Pulsatility Indices (PI) of the anterior and posterior circulation vessels were recorded using a 2-MHz transducer (Doppler Box, DWL/Compumedics, USA). A comprehensive TCD protocol was applied in all cases [1]: If mean CBFV equaled or exceeded 100, 140, and 200 cm/s, the TCD signs of mild vasospasm, moderate vasospasm, and severe vasospasm, respectively, were considered present [20]. Lindegaard ratio was measured when the CBFV exceeded 100 cm/s [15]. On average, patients received 6.4 TCD examinations (range 1–30).

The primary purpose of TCD methodology is to determine the CBFV by quantitative interpretation of Doppler spectrum waveforms. Although the qualitative contour of the TCD waveform during intracranial pressure (ICP) elevation falls into a recognizable pattern, their interpretation depends on the experience and expertise of the TCD examiner/interpreter. Objective, reproducible, and verifiable measures of TCD waveform changes are necessary for TCD findings to be used with certainty for evaluation of intracranial hypertension. One method of quantifying these changes is the utilization of the PI [4], which is a reflection of downstream resistance. The PI takes into account the peak systolic CBFV (pCBFV) and the end-diastolic CBFV (edCBFV) and compares changes in these variables against the change in the standard measure of the entire waveform, such as mean CBFV. Changes in arterial pulsatility, especially occurring during intracranial hypertension, will affect both pCBFV and edCBFV, which are easily identified in the TCD waveform and are reflected by the equation PI = pCBFV − edCBFV/mean CBFV.

The SAS statistical package was used for data analysis (SAS/STAT® 9.3 Software, SAS Institute, Inc., USA). All data were tested for normal distribution using the Shapiro Wilk test: Nonparametric statistics were used if determined appropriate. All data were described using median and interquartile range (25th and 75th percentiles). Spearman rank correlations of MAP (mean arterial pressure), Hct (hematocrit), ICP, and $PaCO_2$ (arterial partial pressure of carbon dioxide) with measures of the CBFV were calculated. Anterior and posterior CBFV data were compared between groups defined by severity of vasospasm (mild, moderate, and severe) using the Wilcoxon rank sum test for each diagnostic group. General linear models were employed to test between diagnostic group differences, adjusting for severity of vasospasm. Statistical significance was assumed on the 5% level.

Study and analysis of the data were done according to the IRBNet protocol 363439-4.

Results

TCD signs of vasospasm were observed in 57 cases (63.3%): 13 (14.4%) in CHI, 12 (13.3%) in CHI/IED, 21 (23.3%) in PHI, and 11 (12.3%) in PHI/IED groups ($p = 0.732$). Different degree of TCD signs of vasospasm presented in Table 1. In PHI patients, there were 75%, 35.7%, and 14.3% TCD signs of mild, moderate, and severe vasospasm, respectively. In the PHI/IED group, there were 36.8%, 5.2%, and 5.2% TCD signs of mild, moderate, and severe vasospasm, respectively. In the CHI group, there were 68.4%, 31.5%, and 15.7% TCD signs of mild, moderate, and severe vasospasm, respectively. Last, in the CHI/IED group, there were 29%, 23.5%, and 17.6% TCD signs of mild, moderate, and severe vasospasm, respectively. TCD evidence of intracranial hypertension was seen in 57.1% of PHI patients, in 63% of PHI/IED patients, in 63.1% of CHI patients, and in 50% of CHI/IED patients. While there were no overall differences in presence of vasospasm, there were statistically significant differences between frequency of degrees of TCD signs of vasospasm between different TBI groups ($p < 0.001$). Post hoc analysis revealed that PHI and CHI groups had higher frequency of mild vasospasm compared to both CHI/IED and PHI/IED ($p < 0.05$).The PHI/IED group had a higher frequency of moderate vasospasm compared to the CHI, PHI, and CHI/IED groups ($p < 0.05$).

Discussion

These results suggest that abnormal TCD findings are frequent in patients with wartime TBI and indicate post-traumatic vasospasm and intracranial hypertension in a significant number of patients. In addition, delayed cerebral arterial spasm is a frequent complication of combat TBI, and severity of cerebral vasospasm is comparable to that seen in aneurysmal SAH. This confirms earlier data that traumatic SAH is associated with a high incidence of cerebral vasospasm with a higher probability in patients with severe TBI [2, 9, 19]. Another cause of abnormally high CBFVs could be reactive hyperemia after TBI; however, the literature suggests that global post-trauma malignant hyperemia is present primarily in the acute stage of TBI [18], although more recent data showed that post-TBI focal hyperemia can be present up to 3 weeks [7]. In our study, utilization of the Lindegaard ratio and qualitative evaluation of the Doppler spectrum were helpful in differentiating between hyperemia and vasospasm.

Table 1 Different degree of transcranial Doppler (TCD) signs of vasospasm in wartime patients with traumatic brain injury (TBI)

	TBI groups			
TCD signs of vasospasm	PHI	PHI/IED	CHI	CHI/IED
Mild vasospasm				
Anterior	114 (106,124)	112 (105,121)	114 (106,124)	116 (107,129)
Posterior	66 (63,71)	(63.5,71)	68 (63,74.5)	67 (62,72)
Moderate vasospasm				
Anterior	159 (149,172)	162 (148,181)	154 (146,173)	150 (146,164)
Posterior	86 (83.5,90)	85.5 (83,89)	89 (85,97)	87 (85,92)
Severe vasospasm				
Anterior	220.5 (210,234)	216 (206,248)	214 (210,225)	214 (209,237)
Posterior	114 (108,124)	124 (104.5,161)	115 (107,132.5)	113 (106.5,124)

Numbers represent median cerebral blood flow velocity (CBFV) in centimeters/second (interquartile range, 25th and 75th percentile)
CHI closed head injury, *IED* improvised explosive device, *PHI* penetrating head injury

Of interest is the finding that the PHI/IED TBI group had a higher frequency of TCD signs of moderate vasospasm when compared to other TBI groups. This result emphasizes the point that explosive blast TBI is one of the more serious wounds suffered by U.S. service members injured in the current conflicts in Iraq and Afghanistan. Observations suggest that the mechanism by which explosive blast injures the central nervous system may be more complex than initially assumed [16].

The purpose of monitoring patients with TBI is to detect treatable and reversible causes of neurological deterioration. There are numerous causes of such deterioration after TBI, and frequent neurological examinations, the availability of urgent neuroimaging, and electroencephalograms (EEGs) are standards in the management of patients with traumatic SAH. Physiological monitoring modalities include TCD, electroencephalography, brain tissue oxygen monitoring, cerebral microdialysis, and near-infrared spectroscopy. TCD has long been used for monitoring patients with SAH, but studies of diagnostic accuracy for detection of vasospasm and cerebral angiography vary widely with regard to sensitivity and specificity of TCD. Ability of TCD to predict clinical deterioration and infarction from delayed cerebral ischemia is not yet validated in a prospective trial. In spite of this, TCD examination is noninvasive and inexpensive, and the pattern of CBFVs observed in patients after SAH of different etiology is very distinctive, enabling immediate detection of abnormally high CBFVs and it appears to be predictive of vasospasm [10, 14].

Recent evidence suggests TCD holds promise for the detection of critical elevations of ICP and decreases in cerebral perfusion pressure (CPP). Using the PI, Bellner et al. [4] demonstrated that an ICP of 20 mmHg can be determined with a sensitivity of 0.89 and specificity of 0.92. They concluded that the PI may provide guidance in those patients with suspected intracranial hypertension, and that repeated measurements may be of use in the neurocritical care unit. There is significant evidence that independent of the type of intracranial pathology, a strong correlation between PI and ICP exists [4, 5, 12, 17]. A recent study indicated that TCD had 94% of sensitivity for identifying high ICP/low CPP at admission and a negative predictive value of 95% to identify normal ICP at admission; the sensitivity for predicting abnormal CPP was 80% [17]. In 2011, Bouzat and co-authors showed that in patients with mild-to-moderate TBI, the TCD test on admission, together with brain CT scan, could accurately screen patients at risk for secondary neurological damage [6]. At the same time, to the best of our knowledge, no one as yet has suggested using the PI as an accurate method to assess ICP quantitatively. Nevertheless, even at this juncture, quantitative and qualitative changes in CBFV values and TCD waveform morphologies may persuade physicians to undertake other diagnostic steps or change medical treatment that will improve care of these patients and their outcomes. At the moment, TCD appears to be useful for following PIs trends, and it is a practical ancillary technique for estimating the direction of CBFV changes in response to increasing ICP or falling CPP. It may also reveal whether there is a response to therapeutic interventions, although further sophistication of TCD data analysis is essential before it may be used with confidence to measure ICP and CPP in the intensive care unit (ICU).

This study had some limitations. First, we were not able to correlate clinical vasospasm with angiographic vasospasm and combine TCD data with other neuroimaging methods that help to identify vasospasm and impaired cerebral perfusion in patients with traumatic SAH. Second, current data should be validated prospectively. In addition, the lack of established TCD criteria for vasospasm in younger patients presents interpretative issues.

The high sensitivity of TCD to identify abnormally high CBFVs and PIs due to the onset of vasospasm and intracranial hypertension, respectively, demonstrates that TCD is an excellent first-line examination to determine those patients

who may need urgent aggressive treatment and continuous invasive ICP monitoring. Because vasospasm and intracranial hypertension represent significant events in a high proportion of patients after wartime TBI, daily TCD monitoring is recommended for the management of such patients.

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Conflicts of Interest We declare that we have no conflict of interest.

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Removal of Clots in Subarachnoid Space Could Reduce the Vasospasm After Subarachnoid Hemorrhage

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Abstract *Background*: Cerebral vasospasm after subarachnoid hemorrhage (SAH) is a major cause of morbidity and mortality. We studied the effects of clot removal on multiple outcome variables following the clipping of ruptured anterior communicating aneurysms.

Methods: From 2007 to 2011, 30 patients with Fisher grade III aneurysmal SAH underwent clipping of an anterior communicating artery aneurysm before SAH day 3. There were 20 women and 10 men, mean age 53.4, range 28–80 years. Seventeen underwent fenestration of lamina terminalis and cisternal removal of clots (group A), and 13 did not (Group B). We compared clinical grades, presence of hydrocephalus at admission, treatment modality, occurrence of clinical vasospasm, the need for interventional vasospasm therapy, and need for ventriculoperitoneal shunting.

Findings: Vasospasm affected 5 of 17 (29%) in group A and 8 of 13 (61.5%) in group B ($p<0.05$). Endovascular treatment for vasospasm was required in one patient in group A (5.8% of 17, 20% of 5) and in five from group B (38.4% of 13, 62.5% of 8) ($p<0.05$). Mortality was observed in one case in group A (5.8% of 17, 20% of 5) and in two cases in group B (15.3% of 13, 25% of 8) and was related to vasospasm after SAH. Ventriculoperitonal shunt (VPS) was required in one case in group A (5.8%) and in five cases in group B (38.4%).

Conclusions: Fenestration of the lamina terminalis and removal of cisternal clots significantly decreased the incidence of post-SAH hydrocephalus and was associated with better outcomes in our series.

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Keyword Subarachnoid hemorrhage • Vasospasm • Cerebral aneurysms • Cisternal irrigation • Lamina terminalis fenestration

Introduction

Cerebral vasospasm after subarachnoid hemorrhage (SAH) is a major cause of morbidity and mortality [1]. Many studies have shown that subarachnoid clot thickness is an important factor in the development of vasospasm, and several authors demonstrated that rapid clot removal, lamina terminalis and Liliequist membrane fenestration, as well as cisternal irrigation reduce the incidence of cerebral vasospasm [1–6]. Experiments in monkeys also showed that removal of cisternal clots avoided severe vasospasm [7].

Many papers have been published suggesting that fenestration of the lamina terminalis during aneurysm surgery in patients with SAH may improve outcome and may decrease the rate of ventriculoperitoneal shunting for post-SAH hydrocephalus and incidence of vasospasm [5, 6, 8]. Studies have shown that the incidences of vasospasm and post-SAH hydrocephalus are proportional to the amount of blood in the subarachnoid spaces, occurring mostly in patients with a Fisher grade 3 SAH [9]. Anterior communicating artery aneurysms are associated with the highest incidence of hydrocephalus, vasospasm, and poor outcomes among anterior circulation aneurysms. The lamina terminalis, anatomically related to the anterior communicating artery complex, is always exposed during the surgical approach to this area. The presence of clots and hydrocephalus may be treated by microsurgically removing the clots and fenestrating the lamina terminalis, especially in patients with Fisher grade 3 SAH, who are more susceptible to poor prognosis. This is a safe and easy adjuvant operative procedure.

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Materials and Methods

From 2007 to 2011, 30 Fisher grade 3 patients underwent clipping of anterior communicating artery aneurysm before SAH day 3. There were 20 women and 10 men, mean age 53.4, range 28–80 years. Seventeen patients underwent fenestration of the lamina terminalis and removal of cisternal clots (group A), and 13 did not (Group B).

The records about clinical grades, presence of hydrocephalus at admission, treatment modality, occurrence of clinical vasospasm, the need for interventional vasospasm therapy, and the need for ventriculoperitoneal shunting were reviewed and analyzed. Patients in group A underwent removal of clots in the sylvian and opticocarotid cisterns. Routine transcranial Doppler studies were performed on all patients from SAH days 1 through to 20. Interventional neuroradiological procedures were performed according to the severity of symptoms and in patients with a Lindengaard score greater than 6.

Results

All aneurysms in both groups were clipped. Both groups were similar regarding age and Hunt-Hess scores. Vasospasm affected 5 of 17 (29%) in group A and 8 of 13 (61.5%) in group B ($p<0.05$). After surgical clipping, endovascular treatment for vasospasm was required in one patient in group A (5.8% of 17, 20% of 5) and in five in group B (38.4% of 13, 62.5% of 8) ($p<0.05$). Mortality was observed in one case in group A (5.8% of 17, 20% of 5) and in two cases in group B (15.3% of 13, 25% of 8). All deaths were related to vasospasm. Ventriculoperitonal shunt (VPS) was required in one case in group A (5.8%) and in five cases in group B (38.4%). The results are summarized in Table 20.1.

Discussion

Insufficient clot clearance in the sylvian cistern is likely to increase the occurrence of severe vasospasm and infarction [1]. Cisternal drainage has been proposed as a mechanism to reduce the incidence of vasospasm and the frequency of hydrocephalus and cerebral vasospasm, which are associated in almost 60% of cases [10, 11].

Decreased rates of cerebral vasospasm and improved outcomes were observed by means of cisternal drainage or fenestration of the lamina terminalis with or without the additional fenestration of the membrane of Liliequist and removal of clots [3, 5, 6, 8, 12, 13]. The proposed mechanism is a reduction in the concentration of blood-derived spasmogenic agents in the cerebrospinal fluid surrounding the basal brain arteries. Continuous cisternal irrigation may lead to clearance of blood volumes of up to 100 ml/day and hemoglobin of up to 1 g/day. Furthermore, a drainage volume-dependent effect has been demonstrated. A very high incidence of VPS (up to 48% in some series) [12] and infection rates of up to 9% [3] have also been noted with the use of this approach. Cisternal irrigation with urokinase in patients with SAH Fisher 3 or 4 was proven to reduce the incidence of vasospasm [4]. The rates of symptomatic and angiographic vasospasm were reduced when meticulous surgical toilette of the basal cisterns, fenestration of the lamina terminalis, and insertion of a continuous cisternal lavage system with or without fibrinolytic agents (chiefly urokinase) were employed [3, 12–14].

A third ventriculostomy through the lamina terminalis to establish an internal cisternal drainage mechanism has been proposed [5]. This physiopathological mechanism would then bypass obstructions of the ventricular system and provide a continuous irrigation mechanism generated by the cerebrospinal fluid pulse pressure, consequently improving cerebrospinal fluid dynamics and reducing concentrations of blood-derived spasmogenic substances. Using this approach, European authors [5, 6] reduced shunting rates and improved outcomes when compared with a normal control group. This effect can be amplified if a microsurgical fenestration of the membrane of Liliequist also is performed [5].

To initiate early triple H (hypertensive, hypervolemic, and hemodilution) therapy in patients with vasospasm after SAH, we perform surgery before SAH day 3. The opportunity to remove cisternal clots and fenestrate the lamina terminalis and Liliequist membrane is considered in the early surgical approach, either before or after aneurysm clipping.

In our series, vasospasm developed in 61.5% of patients who did not undergo lamina terminalis fenestration and in 29% who had fenestration and removal of clots ($p<0.05$).

Table 20.1 Results of the group of patients after SAH treated surgically with fenestration of lamina terminalis and removal of clots both with and without

	Vasospasm diagnosis	Endovascular intervention	Ventriculoperitoneal shunt	Death and disability
Group A, $N=17$	5 (29%)	1 (5.8%/17.2%)	1 (5.8%, 17.2%)	1 (5.8%/17.2%)
Group B, $N=13$	8 (61.5%)	5 (38.4%/62.5%)	5 (38.4%, 62.5%)	2 (15.3%/25%)
	$p<0.05$	$p<0.05$	$p<0.05$	$p<0.05$

We observed a decreased need for multiple interventional neuroradiology sessions for clinical vasospasm in patients undergoing fenestration of the lamina terminalis. Other authors published similar results previously [2, 15]. Better outcomes were also statistically more likely compared to the nonfenestration group. Previously suggested potential benefits of fenestration of the lamina terminalis and removal of clots, including reduction of the incidence of vasospasm, are proven.

Conclusion

Fenestration of the lamina terminalis and cisternal clot removal significantly decreased the incidence of post-SAH hydrocephalus and were associated with better outcomes in our series. They are safe and effective microsurgical maneuvers that can be used during the surgical treatment of anterior communicating artery aneurysms.

Conflicts of Interest We declare that we have no conflict of interest.

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Effect of Lumbar Puncture in Patients with Aneurysmal Subarachnoid Hemorrhage Treated Microsurgically or Endovascularly

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Abstract *Objective*: The aim of this study was to evaluate the effect of treatment modality (surgical clipping vs. endovascular coiling) and lumbar puncture (LP) in patients with aneurysmal subarachnoid hemorrhage (SAH) based on neurologic status on admission and clinical outcome.

Patients and methods: One hundred forty-eight consecutive patients with ruptured intracranial aneurysms treated via endovascular or surgical methods were included in our study. Patients who refused further therapy or received only supportive therapy because of bad neurologic status were excluded. Severity of SAH was evaluated using the Fisher score. World Federation of Neurosurgical Societies (WFNS) and Hunt and Hess (H&H) scores were used for evaluation of neurologic status. Glasgow Outcome Scale scores and modified Rankin scores were used for outcome evaluation.

Results: We found that modified Rankin scores were significantly lower in the surgical clipping group (1.1 ± 1.4) than in the endovascular coiling group (1.7 ± 1.8) (*p*: 0.04). The positive lumbar puncture [LP(+)] group had similar outcome scores as the negative lumbar puncture [LP(−)] group, although the LP(+) group had worse initial SAH evaluation scores (WFNS 1.64 ± 0.95–1.23 ± 0.61, *p*: 0.0004 and H&H 2.18 ± 1.07–1.65 ± 0.88, *p*: 0.001).

Conclusion: Surgical clipping might improve clinical outcome better than endovascular coiling, although a more confident conclusion requires absolute randomization of patients for both treatments. LP could also improve clinical outcome in patients with high initial SAH evaluation scores.

Keyword Intracranial aneurysm • Subarachnoid hemorrhage • Lumbar puncture • Cerebral vasospasm • Clipping • Coiling

Introduction

Delayed cerebral ischemia (DCI) due to vasospasm is still a major problem after aneurysmal subarachnoid hemorrhage (aSAH). Despite significant achievements in research of vasospasm management, like magnesium sulfate, statins, endothelin 1 receptor antagonists, and administration of intra-arterial vasoactive agents like papaverine or angioplasty, only nimodipine has shown a modest improvement in functional outcome [1]. Estimated incidence of cerebral vasospasm after SAH is between 16% and 71%, depending on the definition used [2–4]. While angiographic vasospasm occurs in 21–53% of patients, clinical vasospasm is observed only in 17–21% of patients [2–5]. Effective treatment strategies need to be formulated for preventing or reversing this devastating condition due to delayed cerebral vasospasm. Cerebrospinal fluid (CSF) drainage for vasospasm after treatment of the ruptured aneurysm has been used by some investigators [6–8]. The better outcome of these patients may be related to the reduction of blood degradation products and inflammatory mediator levels in CSF due to continuous spinal or intraventricular drainage. CSF volume reduction by lumbar punctures (LPs) may also play a role by lowering the intracranial pressure and increasing the cerebral perfusion pressure.

The aim of this study was to evaluate the effect of treatment modality (surgical clipping vs. endovascular coiling) and LP in aSAH patients based on neurologic status, initial SAH evaluation scores on admission, and clinical outcome.

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Methods

Patients who were admitted to the Istanbul Medical Faculty neurointensive care unit (ICU) for treatment of aSAH between January 2007 and April 2011 were included in the study. All patients were treated by either surgical clipping or endovascular coiling and had continued follow-up in the neuro-ICU. Permission to use patients' clinical data was obtained from either the patient or the closest relative. Patients with SAH who refused further therapy or had incidental aneurysms without SAH and patients in very poor condition (high Word Federation of Neurosurgical Societies [WFNS] scores of 5 with low Glasgow Coma Scale [GCS] score less than 4) whose aneurysms had not been treated were excluded from the study.

All patients' WFNS and Fisher scores and Hunt and Hess (H&H) grades were recorded on admission to the ICU, and their systolic blood pressures were kept under 140 mmHg until their aneurysms were secured. Nimodipine, anticonvulsive, analgesic, and fluid replacement therapies (100 ml/h 0.09% NaCl) were also started immediately as routine treatment of SAH. The choice between microsurgical clipping and endovascular coiling was collaboratively discussed by the neurosurgeons and interventional neuroradiologists. The optimal method of aneurysm treatment on a patient-to-patient basis was proposed to the patient and relatives after discussion. The aneurysm was then treated as soon as possible after completing all other investigations in the early stage. After the aneurysm was secured, systolic blood pressure was left to progress normally until reaching 200 mmHg. Hydration and hypertension (HH) therapy and CSF drainage with LP were started liberally due to at least the two of the following findings or clinicians' decision: headache, agitation, elevated leukocyte level (without infection), new motor deficit, and worsening GCS score. Patients were also evaluated due to (1) diagnosis of clinical vasospasm, which was diagnosed according to the existence of three signs (headache, neurological deficit, and worsening) (>2) at GCS score; (2) mortality; (3) modified Rankin score; (4) Glasgow Outcome Scale (GOS) score after ICU discharge. Secondary outcomes included length of ICU stay.

All results are shown in mean and standard deviation. A chi-square test was used for mortality and vasospasm evaluation between groups, and the nonparametric Mann-Whitney *U* test was used for other statistical evaluations.

Results

We have evaluated 148 of 350 consecutive patients with ruptured aneurysms since January 2007. All patients' aneurysms were secured either endovascularly or surgically. All patients were treated in the neuro-ICU. Patients' demographic data and their aneurysm locations are shown in Tables 1 and 2, respectively. According to our criteria, 46 of the 148 patients demonstrated clinical vasospasm. While 29 of them were in the clipping group, and the other 17 were in the coiling group (Table 3). HH therapy and CSF drainage with LP were started more liberally, to 99/148 and 65/148 patients, respectively, due to at least the two of the following findings: headache, agitation, elevated leukocyte level (without infection), new motor deficit, and worsening of GCS score or clinicians' decision. Although the mortality rate was higher in the coiling group, there was no statistical difference in both groups. Modified Rankin score was significantly higher in the coiling group as a morbidity measure.

When we divided our patients into two groups based on LP performed (LP+) and no LP performed during the treatment period (LP−), we saw that the LP(+) group's initial neurologic and radiologic evaluation scores were significantly higher than the LP(−) group. Although during follow-up the DCI ratio and HH therapy ratios were significantly higher in the LP(+) group, both groups' outcome scores were similar (Table 4).

Table 1 Demographic data

Age	51. 51.1 ± 10.6
Gender	93F/55M
WFNS	1.4 ± 0.8
Fisher	2.3 ± 1.0
H&H	1.9 ± 1.0
ICU stay	16.6 ± 11.5
HH therapy	66.0%
Clinical vasospasm	31.0%
Clipping	68.9% (102)
Coiling	33.7% (50)
Clip + coil	2.7% (4)
GOS	4.5 ± 1.1
Rankin	1.3 ± 1.6
Mortality	5.4% (8)
n	148

WFNS World Federation of Neurosurgical Societies score, *H&H* Hunt and Hess, *HH* hydration and hypertension, *GOS* Glasgow Outcome Scale score, *n* number of patients

Table 2 Aneurysm locations

	Clipping	Coiling
ACoA	41	24
MCA	48	10
ICA	18	15
Basilar A.	1	5
PICA	3	0
n	111	54

ACoA anterior communicating artery, *MCA* middle cerebral artery, *ICA* internal carotid artery, *PICA* posterior inferior cerebellar artery

Table 3 Groups according to treatment choice: surgical clipping or endovascular coiling

	Clipping	Coiling	*p*
Age	50.5 ± 10.7	52.6 ± 10.1	0.22
Gender	64F/38M	30F/20M	
WFNS	1.4 ± 0.8	1.4 ± 0.7	0.78
Fisher	2.3 ± 1.0	2.2 ± 0.9	0.70
H&H	1.9 ± 1.0	1.7 ± 0.9	0.25
ICU stay	17.6 ± 11.5	14.9 ± 11.7	0.28
Clinical vasospasm	28.4% (29)	34.0% (17)	0.55
Modified Rankin	1.1 ± 1.4	1.7 ± 1.8	0.04
GOS	4.6 ± 0.9	4.3 ± 1.3	0.13
Mortality	2.9% (3)	10.0% (5)	0.06 odds 0.27
n	102	50	

WFNS World Federation of Neurosurgical Societies score, *H&H* Hunt and Hess, *GOS* Glasgow Outcome Scale score, *n* number of patients

Table 4 Groups according to lumbar puncture treatment

	LP(+)	LP(−)	*p*
Clip/Coil	41/23	57/27	
WFNS	1.64 ± 0.95	1.23 ± 0.61	0.0004
H&H grade	2.18 ± 1.07	1.65 ± 0.88	0.001
Fisher	2.60 ± 0.84	2.02 ± 1.04	0.0002
Clinical vasospasm	46.87% (30/64)	15.47% (13/84)	<0.0001
HH (%)	90.62% (58/64)	48.80% (41/84)	<0.0001
ICU stay	17.90 ± 11.02	15.62 ± 11.76	0.31
GOS	4.28 ± 1.33	4.65 ± 0.88	0.10
Modified Rankin	1.56 ± 1.76	1.12 ± 1.39	0.12
Mortality	7.81% (5/64)	3.57% (3/84)	0.25
n	64	84	

p nonparametric Mann-Whitney *U* test, *WFNS* World Federation of Neurosurgical Societies score, *H&H* Hunt and Hess, *HH* hydration and hypertension, *GOS* Glasgow Outcome Scale score, *n* number of patients

Discussion

We found that surgical clipping might improve clinical outcome better than endovascular coiling, and LP could also improve clinical outcome in patients with high evaluation grades after SAH. This may be due to the cleaning of the subarachnoid cisternas from blood clots and blood lysis products during surgery. Serial LPs may also act in a similar way.

Despite a number of clinical trials, little progress has been achieved and understood about vasospasm and DCI after aSAH. Although, many treatment approaches were used to reduce angiographic vasospasm after a SAH and improve clinical outcome, vasodilation was not enough to improve clinical outcome by itself. Many other controversial factors, such as endothelial cell proliferation and nitric oxide depletion, play a role in neuroprotection and clinical outcome. There is almost a consensus on the direct relationship between increasing amount of clot and more severe vasospasm and worse outcome. Therefore, clearance of blood from the subarachnoid space would decrease the inflammation at least regionally in the brain and improve clinical outcome. In our study, surgical clipping with irrigation of the subarachnoid space with as much blood cleansing as possible may have improved the modified Rankin score more significantly than endovascular coiling.

Research on the cerebral vasospasm pathophysiology has been centered on arterial narrowing, which is initiated by blood lysis in the subarachnoid space. Factors that have been implicated in this process include oxyhemoglobin, iron, nitrous oxide, endothelins, intracellular adhesion molecule 1, vascular endothelial growth factor, and arachidonic acid derivatives. Fibrinolytic agents such as urokinase and recombinant tissue plasminogen activator(rt-PA) have been tested in many studies. In a multicenter randomized trial [9], Findlay and coworkers showed that intraoperative rt-PA injection in patients with thick SAH decreased the relative risk of vasospasm up to 56% in comparison with the control group. Although Kodama et al. showed the beneficial effect of a thrombolytic in a large series, such treatment has not yet become widely incorporated into current clinical treatment strategies. However, it is widely accepted that more blood in the subarachnoid space increases DCI and causes a worse clinical outcome. Similarly, as shown by Klimo et al. [6] and Ochiai et al. [7], cleaning the subarachnoid space from blood and blood lysis products by lumbar CSF drainage improves clinical outcome. In a recently published pilot study, they showed that there is no difference in vasospasm between continuous and intermittent LP [10]. We performed intermittent LP in a selected group of patients, while the clearance of CSF from blood and its lytic products was facilitated, transmural tension on cerebral vessels also increased, so that cerebral perfusion increased while consciousness increased. Existing agitation and delirious mental status also resolved in 30% of patients treated with LPs. While we were reaching similar outcome results in LP(+) and LP(−) groups, neurologic status and severity of SAH were significantly worse in the LP(+) group. There was a three times higher incidence of clinical vasospasm in the LP(+) group, and HH therapy was also performed in 90% of this group, but these invasive treatment strategies did not cause any additional increase in the ICU stay.

Conclusion

One of the major limitations of our study about SAH management is that it does not represent management results of patients with all kinds of aSAH. First, we included only

neuro-ICU patients in our study who needed intensive care treatment after SAH. Second, we included only surgically treated patients (either open microsurgery or endovascular surgery). Third, those with incidentally found aneurysms without SAH and patients in very poor condition (high WFNS value of 5 with low GCS <4) whose aneurysms had not been treated were excluded from our study. Also, patient selection (for microsurgical or endovascular therapy) might explain the seemingly more favorable results of microsurgical therapy. Larger series are needed to show the beneficial effect of microsurgery on clinical vasospasm, if there is any, and serial LPs may have an improving effect on clinical vasospasm in selected patients.

Conflicts of Interest We declare that we have no conflict of interest.

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Effect of Aneurysm Treatment Modalities on Cerebral Vasospasm After Aneurysmal Subarachnoid Hemorrhage

Hidenori Suzuki, Waro Taki, and Prospective Registry of Subarachnoid Aneurysms Treatment (PRESAT) Group

Abstract *Introduction*: It is still controversial if the selection of treatment modality (clip or coil) affects cerebral vasospasm development following aneurysmal subarachnoid hemorrhage (SAH).

Materials and Methods: We enrolled 579 SAH patients in the Prospective Registry of Subarachnoid Aneurysms Treatment project, and these patients were treated either microsurgically or endovascularly within 12 days of onset. The incidence of vasospasm was compared between patients treated with clipping and coiling.

Results: Clipping (282 patients) was preferably performed for small aneurysms with a wide neck or middle cerebral artery aneurysms and was followed by cerebrospinal fluid drainage; coiling (297 patients) was preferred for older patients, larger, internal carotid artery and posterior circulation aneurysms, or treatment during a nonacute stage and more frequently followed by antithrombotic treatment. Univariate analyses showed that vasospasm-induced cerebral infarct occurred more frequently in clipped patients than in coiled patients, but this difference disappeared after multivariate analyses. Higher incidence of vasospasm-induced cerebral infarct after clipping was explained by the fact that clipping was selected more for the ruptured middle cerebral artery aneurysm with massive SAH or hematoma, in which vasospasm more frequently occurred.

Conclusions: Treatment modalities (clip or coil) may not significantly affect the incidence of vasospasm.

Keywords Cerebral aneurysm • Cerebral vasospasm • Endovascular treatment • Microsurgery • Subarachnoid hemorrhage

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Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is a common and devastating neurological disorder [1]. Treatment requires early aneurysm repair to prevent rebleeding and intensive medical care to manage the associated problems, including cerebral vasospasm, which remains a major cause of morbidity and mortality after SAH [2]. The International Subarachnoid Aneurysm Trial (ISAT), the only multicenter, randomized clinical trial comparing neurosurgical clipping and endovascular coiling, has had a dramatic impact on the management of aneurysmal SAH by showing that coiling had better 1-year outcomes than clipping, resulting in an increase in the proportion of patients undergoing endovascular coiling [3, 4]. However, effects of aneurysm treatment modality (clipping or coiling) on the incidence of cerebral vasospasm have not been clearly defined.

Materials and Methods

Patient and Clinical Variables

This is a prospective cohort study (Prospective Registry of Subarachnoid Aneurysms Treatment) performed at 29 tertiary referral centers in Japan (listed in the footnote), which provided both microsurgical clipping and endovascular coiling, depending on the characteristics of each case, between March 2006 and February 2007. The study was approved by the institutional ethics committee. Seven hundred and sixty SAH patients reached the centers, and 614 patients met the following inclusion criteria: older than 20 years of age at onset, SAH on computed tomographic (CT) scans or lumbar puncture, saccular aneurysm as the cause of SAH confirmed on three-dimensional CT angiography (3D-CTA) or digital subtraction angiography (DSA), and aneurysmal obliteration by clipping or coiling within 14 days of onset. Excluded from the study were patients with ruptured fusiform, dissecting, traumatic, mycotic, and arteriovenous malformation-related aneurysms or SAH of unknown etiology and patients who were treated using medical instruments or drugs that were not approved by the Japanese Ministry of Health, Labor, and Welfare; thus, none of the patients in this series were treated with nimodipine, surface-modified or bioactive coils, or intracranial stents. There were 26 patients who did not give written informed consent. Therefore, the remaining 588 patients were registered to the data center within 2 days of the initial treatment. Timing of aneurysmal obliteration, selection of clipping or coiling, and other medical management or treatment were decided by site investigators and not limited.

Baseline demographic and clinical data included age, gender, World Federation of Neurosurgical Societies (WFNS) grade on admission [5], Fisher CT group on admission [6],

interval from ictus to aneurysmal obliteration, modality used for aneurysmal obliteration, and location and size of the ruptured aneurysm. The aneurysms were classified into four groups: small size/small neck, small size/wide neck, large, and giant. Small-size/small-neck aneurysms were defined as aneurysms having a maximum aneurysmal diameter (A) less than 10 mm, neck size (N) less than 4 mm, and $A/N \geq 1.5$; the small-size/wide-neck aneurysms were set as $A < 10$ mm, $N \geq 4$ mm, and $A/N < 1.5$; large aneurysms were set as $A \geq 10$ mm but <25 mm; and giant aneurysms were those with $A \geq 25$ mm.

Data on other treatments and complications included cerebrospinal fluid (CSF) drainage, antiplatelet administration, endovascular therapy for vasospasm, symptomatic vasospasm, and vasospasm-induced cerebral infarction. Ventricular, cisternal, or spinal CSF drainage was established to control acute hydrocephalus or to promote subarachnoid blood clearance. Antiplatelets were administered to prevent overthrombosis after coiling or to prevent symptomatic vasospasm. Symptomatic vasospasm was defined as otherwise unexplained clinical deterioration (i.e., a new focal deficit, decrease in the level of consciousness, or both) or a new infarct on CT that was not visible on admission or immediate postoperative scan (vasospasm-induced cerebral infarction) or both. Other potential causes of clinical deterioration, such as hydrocephalus, rebleeding, or seizures, were rigorously excluded. Determination of these complications was made at each center, and the organizing and protocol committee qualified them [7].

Outcome was blindly assessed using modified Rankin Scale (mRS) [8] at 3 and 12 months post-SAH.

Statistics

Categorical variables were reported as a proportion and percentile and were analyzed using chi-square or Fisher's exact test, as appropriate. Continuous variables were reported as a mean plus or minus standard deviation and compared using unpaired t test. The impact of each variable on vasospasm-induced cerebral infarction was determined by multivariate unconditional logistic regression analyses using the dichotomous status (presence or absence) as the dependent variable. All variables were considered independent variables irrespective of the significance on univariate analysis, although only the variable with the smallest probability value was used as a candidate variable among similar clinical variables that were intercorrelated. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated, and independence of variables was tested using the likelihood ratio test on reduced models. Two-tailed probability values less than 0.05 were considered significant.

Results

Characteristics of Patients and Aneurysms Treated with Clipping or Coiling

Nine patients were excluded because data regarding vasospasm were missing. The remaining 579 patients were treated by clipping (282 patients) or coiling (297 patients). Clipping was preferably performed for small aneurysms with a wide neck and for middle cerebral artery (MCA) aneurysms, while coiling (297 patients) was preferred for older patients and for larger dome size, internal carotid artery, and posterior circulation aneurysms or treatment during a nonacute stage (Table 1). Patient gender, WFNS grade, and Fisher CT group on admission were similar between the treatment modalities. After aneurysmal obliteration, CSF drainage was performed more in clipped patients, and antithrombotic treatment was performed more in coiled patients.

Incidence of Vasospasm and Overall Outcome

The incidence of symptomatic vasospasm and endovascular therapy for vasospasm was not significantly different between clipping and coiling (Table 2). Vasospasm-induced cerebral infarct occurred more frequently after clipping; the differences were observed only in the ruptured MCA aneurysm cases regarding the location of aneurysms (Table 2). Compared to non-MCA aneurysm cases, MCA aneurysm cases had significantly higher incidence of Fisher CT group 4 (20.6% vs. 12.3%, $p < 0.05$) and were treated more with clipping (84.0% vs. 38.4%, $p < 0.001$) and less with antithrombotic treatment (16.0% vs. 27.0%, $p < 0.05$), associated with higher incidence of symptomatic vasospasm, endovascular therapy for vasospasm, and vasospasm-induced cerebral infarction (31.3% vs. 19.0%, $p < 0.005$; 18.3% vs. 9.2%, $p < 0.005$; 25.2% vs. 15.2%, $p < 0.01$, respectively). When patients were compared between clipping and coiling in ruptured MCA cases, clipping was preferably performed in patients with worse clinical conditions or more severe SAH in terms of admission WFNS grade and Fisher CT group (Table 3).

Multivariate analyses showed that only younger age (OR 0.981, 95% CI 0.964–0.999, $p < 0.05$) and admission WFNS grade IV (2.421, 1.215–4.821, $p < 0.05$) or V (2.366, 1.068–5.241, $p < 0.05$) were significant factors for vasospasm-induced cerebral infarction. The location of ruptured aneurysms, aneurysm treatment modalities (clipping or coiling), CSF drainage, and antithrombotic therapy did not affect the occurrence of vasospasm. With regard to outcomes, the incidence of independent survival (mRS 0–2) was similar between clipping and coiling at both 3 (62.7% vs. 67.2%) and 12 (67.7% vs. 71.1%) months post-SAH.

Table 1 Summary of patient characteristics stratified by treatment modality

Characteristics	Clip ($n=282$)	Coil ($n=297$)	p value
Age, years	60.2 ± 12.5	62.4 ± 14.6	<0.05[a]
Sex			ns[b]
Female	197 (69.9%)	195 (65.7%)	
Male	85 (30.1)	32 (34.3)	
WFNS grade			
I	93 (33.0)	78 (26.3)	ns[b]
II	68 (24.1)	87 (29.3)	ns[b]
III	29 (10.3)	30 (10.1)	ns[b]
IV	57 (20.2)	66 (22.2)	ns[b]
V	35 (12.4)	36 (12.1)	ns[b]
Fisher CT group			
1	4 (1.4)	3 (1.0)	ns[c]
2	49 (17.4)	68 (22.9)	ns[b]
3	187 (66.3)	186 (62.6)	ns[b]
4	42 (14.9)	40 (13.5)	ns[b]
Date of aneurysm obliteration			<0.001[b]
Days 0–3	273 (96.8)	264 (88.9)	
Days 4–12	9 (3.2)	33 (11.1)	
Aneurysm dome, mm	6.1 ± 3.2	6.8 ± 3.8	<0.05[a]
Neck, mm	3.4 ± 1.5	3.5 ± 1.6	ns[a]
Aneurysm classification			
Small size/small neck	131 (46.6)	158 (53.2)	ns[b]
Small size/wide neck	115 (40.9)	93 (31.3)	<0.05[b]
Large	35 (12.5)	45 (15.2)	ns[b]
Giant	0 (0)	1 (0.3)	ns[c]
Aneurysm location			
Internal carotid artery	71 (25.2)	104 (35.0)	<0.01[b]
Middle cerebral artery	110 (39.0)	21 (7.1)	<0.001[b]
Anterior communicating artery	71 (25.2)	91 (30.6)	ns[b]
Distal anterior cerebral artery	14 (5.0)	14 (4.7)	ns[b]
Posterior circulation	13 (4.6)	64 (21.5)	<0.001[b]
Other	3 (1.1)	3 (1.0)	ns[c]
Cerebrospinal fluid drainage	212 (75.2)	171 (57.6)	<0.001[b]
Antiplatelet therapy	22 (7.8)	120 (40.4)	<0.001[b]

WFNS World Federation of Neurosurgical Societies, *CT* computed tomography, *ns* not significant
Day 0 refers to the calendar day of subarachnoid hemorrhage onset
[a]Unpaired *t* test
[b]Chi-square test
[c]Fisher's exact test

Table 2 Incidence of cerebral vasospasm stratified by treatment modality

Variable	Clip ($n=282$)	Coil ($n=297$)	p value
Symptomatic vasospasm	69 (24.5%)	57 (19.2)	ns[a]
Endovascular therapy for vasospasm	35 (12.4)	30 (10.1)	ns[a]
Vasospasm-induced cerebral infarct	59 (20.9)	42 (14.1)	<0.05[a]
Internal carotid artery	10/71 (14.1)	14/104 (13.5)	ns[a]
Middle cerebral artery	32/110 (29.1)	1/21 (4.8)	<0.05[b]
Anterior communicating artery	12/71 (16.9)	16/91 (17.6)	ns[a]
Distal anterior cerebral artery	3/14 (21.4)	1/14 (7.1)	ns[b]
Posterior circulation	1/13 (7.7)	10/64 (15.6)	ns[b]
Other	1/3 (33.3)	0/3 (0)	ns[b]

ns not significant
[a]Chi-square test
[b]Fisher's exact test

Table 3 Summary of patients with ruptured middle cerebral artery aneurysm stratified by treatment modality

Characteristics	Clip ($n=110$)	Coil ($n=21$)	p value
Age, years	59.7 ± 10.9	59.4 ± 13.0	ns[a]
Sex			ns[b]
Female	80 (72.7%)	12 (57.1%)	
Male	30 (27.3)	9 (42.9)	
WFNS grade			
I	38 (34.5)	9 (42.9)	ns[b]
II	20 (18.2)	7 (33.3)	ns[c]
III	15 (13.6)	1 (4.8)	ns[c]
IV	23 (20.9)	4 (19.0)	ns[c]
V	14 (12.7)	0 (0)	ns[c]
III–V	52 (47.2)	5 (23.8)	<0.05[c]
Fisher CT group			
1	1 (0.9)	0 (0)	ns[c]
2	13 (11.8)	12 (57.1)	<0.001[b]
3	71 (64.5)	7 (33.3)	<0.01[c]
4	25 (22.7)	2 (9.5)	ns[c]
3–4	96 (87.2)	9 (42.8)	<0.001[b]
Date of aneurysm obliteration			ns[c]
Days 0–3	106 (96.4)	16 (76.2)	
Days 4–12	4 (3.6)	5 (23.8)	
Aneurysm dome, mm	6.1 ± 2.8	7.8 ± 5.6	ns[a]
Neck, mm	3.3 ± 1.4	3.4 ± 1.4	ns[a]
Aneurysm classification			
Small size/small neck	51 (46.8)	10 (47.6)	ns[b]
Small size/wide neck	44 (40.4)	8 (38.1)	ns[c]
Large	14 (12.8)	2 (9.5)	ns[c]
Giant	0 (0)	1 (4.8)	<0.05[c]
Large/giant	14 (12.8)	3 (14.3)	ns[c]
Cerebrospinal fluid drainage	81 (73.6)	8 (38.1)	<0.005[c]
Antiplatelet therapy	10 (9.1)	11 (52.4)	<0.001[b]
Symptomatic vasospasm	37 (33.6)	6 (28.6)	ns[c]

(continued)

Table 3 (continued)

Characteristics	Clip ($n=110$)	Coil ($n=21$)	p value
Endovascular therapy for vasospasm	19 (17.3)	5 (23.8)	ns[c]
Vasospasm-induced cerebral infarct	32 (29.1)	1 (4.8)	<0.05[c]
Modified Rankin scale at 1 year			
0	41 (37.6)	17 (85.0)	<0.001[b]
1	19 (17.4)	2 (10.0)	ns[c]
2	11 (10.1)	0 (0)	ns[c]
3	11 (10.1)	1 (5.0)	ns[c]
4	11 (10.1)	0 (0)	ns[c]
5	9 (8.3)	0 (0)	ns[c]
6	7 (6.4)	0 (0)	ns[c]
0–2	71 (65.1)	19 (95.0)	<0.05[b]

WFNS World Federation of Neurosurgical Societies, *CT* computed tomography, *ns* not significant
Day 0 refers to the calendar day of subarachnoid hemorrhage onset
[a]Unpaired *t* test
[b]Chi-square test
[c]Fisher's exact test

Discussion

This study showed that the incidence of cerebral vasospasm was similar between clipping and coiling, although this was not a randomized study, and the characteristics of patients and postprocedural management were different between the treatment modalities. Ruptured MCA aneurysm cases most likely had symptomatic vasospasm and its induced cerebral infarction, possibly due to sylvian hematoma. Clipping was preferably performed for ruptured MCA aneurysms with massive hematoma and poor clinical grades. However, surgical evaluation of the hematoma with postoperative CSF drainage was inadequate for preventing vasospasm; therefore, a new treatment strategy is especially necessary for these aneurysms.

Whether clipping or coiling is associated with an increased risk of cerebral vasospasm has been debated. Theoretically, early clot removal during clipping may decrease the incidence and severity of vasospasm, while this beneficial effect could be negated by other effects associated with surgery, such as brain retraction, vessel manipulation, and impaired subarachnoid space or CSF circulation [9, 10]. In fact, a meta-analysis study showed no significant differences between the treatment modalities on the risk of cerebral vasospasm development and its consequences [9]. ISAT randomized 22.4% of SAH patients who were preoperatively judged to be treated successfully by either modality into coiling or clipping groups and showed that coiling was associated with significantly better outcomes at 1 year post-SAH than clipping [3, 4]. In a subgroup of elderly patients within the ISAT cohort, the patients who received coiling for internal carotid artery aneurysms had better outcomes than patients who were treated by clipping, while the patients with MCA aneurysms who received clipping had better outcomes than the coiling patients [11]. However, ISAT, up to now, has not published any results regarding post-treatment vasospasm incidence. In this study, although the treatment decisions were not protocolized and depended on each investigator's judgment, clipping was preferably selected for small aneurysms with a wide neck and for MCA aneurysms and was associated with CSF drainage. In contrast, coiling was preferred for older patients and those with larger, internal carotid artery, and posterior circulation aneurysms or treatment during a nonacute stage and was associated more with antithrombotic treatment. As a result, vasospasm development and outcomes were similar between clipping and coiling.

Conclusion

In summary, this study showed that selection of aneurysm treatment modalities had no significant impact on cerebral vasospasm development when we selected either treatment modality that would offer lower procedural risks depending on patient and aneurysm characteristics. However, vasospasm still occurred in a relatively high incidence irrespective of treatment modalities. Development of new therapies or strategies against vasospasm suitable for each treatment modality is needed.

Conflicts of Interest We declare that we have no conflict of interest.

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Endovascular Management of Posthemorrhagic Cerebral Vasospasm: Indications, Technical Nuances, and Results

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Abstract Posthemorrhagic cerebral vasospasm (PHCV) is a common problem and a significant cause of mortality and permanent disability following aneurysmal subarachnoid hemorrhage. While medical therapy remains the mainstay of prevention against PHCV and the first-line treatment for symptomatic patients, endovascular options should not be delayed in medically refractory cases. Although both transluminal balloon angioplasty (TBA) and intra-arterial vasodilator therapy (IAVT) can be effective in relieving proximal symptomatic PHCV, only IAVT is a viable treatment option for distal vasospasm. The main advantage of TBA is its long-lasting therapeutic effect and the very low rate of retreatment. However, its use has been associated with a significant risk of serious complications, particularly vessel rupture and reperfusion hemorrhage. Conversely, IAVT is generally considered an effective and low-risk procedure, despite the transient nature of its therapeutic effects and the risk of intracranial hypertension associated with its use. Moreover, newer vasodilator agents appear to have a longer duration of action and a much better safety profile than papaverine, which is rarely used in current clinical practice. Although endovascular treatment of PHCV has been reported to be effective in clinical series, whether it ultimately improves patient outcomes has yet to be demonstrated in a randomized controlled trial.

Keywords Angioplasty • Cerebral infarction • Delayed cerebral ischemia • Delayed ischemic neurological deficit • Endovascular treatment • Subarachnoid hemorrhage • Vasodilator • Vasospasm

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Definition and Terminology

Posthemorrhagic cerebral vasospasm (PHCV) is a major cause of mortality and permanent disability in patients with aneurysmal subarachnoid hemorrhage (aSAH) and may account for almost 50% of deaths among those surviving the initial ictus [1]. Angiographic vasospasm is very common, affecting up to 70% of aSAH patients, and has a predictable time course: delayed onset between days 3 and 5, maximal narrowing between days 5 and 14, then gradual resolution over 2–4 weeks [1–3]. Nearly half of these patients, about 30% of all aSAH survivors, will develop a delayed ischemic neurological deficit (DIND), the so-called symptomatic vasospasm [1]. However, recent studies have suggested that other coexisting factors than vasospasm, such as microvascular dysfunction and cortical spreading depolarizations, may contribute to the pathogenesis of DIND [4]. An international group of experts has recommended using the term *delayed cerebral ischemia* (DCI) as an end-organ phenomenon requiring clinical evidence of neurological impairment or evidence of cerebral infarction on brain imaging or autopsy, irrespective of the presence and severity of angiographic vasospasm [5]. Nevertheless, angiographically demonstrable vessel narrowing remains the most important modifiable risk factor for neurological deterioration and poor outcome in patients with DCI. In fact, DCI correlates strongly with the angiographic severity of vasospasm, and nearly 50% of patients experiencing severe vasospasm will develop territory-specific cerebral infarction [1, 6].

Pathophysiology of PHCV

The precise events that lead to arterial narrowing in PHCV remain unknown. Numerous inciting factors have been implicated, including erythrocyte degradation products, serum-derived lipids, and hematogenic proteins. Presumably, these factors trigger a cascade of biochemical and

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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, vessel wall fibrosis can lead to irreversible stenosis if the process is sustained [7, 8]. The likelihood of developing PHCV and its severity most strongly correlate with the amount of blood entering the subarachnoid space [9, 10]. Although large subarachnoid hematomas will affect adjacent arteries most severely, PHCV is often multifocal and frequently involves remote vascular territories.

Medical Management and Indications of Endovascular Therapy

Oral administration of nimodipine, maintenance of a normotensive normovolemic state, and avoidance of systemic and metabolic insults constitute the mainstay of preventive medical measures against DCI [1]. DCI should be suspected whenever either a focal neurological deficit or loss of two points or more on the Glasgow Coma Scale, which were not immediately present after therapeutic aneurysm occlusion, develop in a patient with aSAH and are sustained for at least 1 h [5]. Other causes of neurological deterioration (e.g., aneurysm rerupture, hydrocephalus, seizures, hyponatremia) should be ruled out based on clinical, neuroimaging, and laboratory data [1, 5].

Medical therapy (i.e., hypertensive hypervolemic or triple-H therapy) is generally considered first-line management for DCI since treatment-related risks are usually much lower than those of neurointervention [1]. The purpose of medical treatment is to optimize cerebral perfusion by increasing mean arterial and central venous pressures through intravenous administration of vasopressors and intravascular volume expansion [1, 11]. However, patients with extensive myocardial infarction, decompensated heart failure, or intestinal ischemia may not tolerate aggressive medical therapy. Similarly, patients with unsecured ruptured aneurysms may be at increased risk of rerupture. Therefore, early aneurysm occlusion is recommended to allow aggressive hypertensive therapy for DCI [1, 11].

In many patients, medical therapy will restore a compensated state of cerebral perfusion and reverse neurological deficits. Failure to respond or inability to tolerate medical treatment should prompt aggressive neurointerventional management [8]. The last has been shown to be most effective when initiated within 2 h of symptom onset [12] and therefore should probably not be delayed when a satisfactory response to medical therapy is not observed within 1 h. However, it should be kept in mind that, although the ability of endovascular therapy to reverse neurological deficits has been documented in clinical case series, whether it ultimately improves patient outcomes remains unclear. For this purpose, a multicenter European randomized controlled trial is currently under way [13].

In very poor-grade aSAH patients, it may be difficult to document neurological worsening. Therefore, treatment of moderate or severe angiographic vasospasm revealed by noninvasive imaging, such as transcranial Doppler (TCD) ultrasonography and computed tomographic (CT) angiography, may be warranted after confirmation by digital subtraction angiography.

Endovascular Options and Preoperative Considerations

The two established neurointerventional modalities for the treatment of symptomatic PHCV are intra-arterial vasodilator therapy (IAVT) and transluminal balloon angioplasty (TBA). While IAVT can be used to treat both proximal and distal vasospasm, the use of TBA should be solely reserved for treating large artery spasm within or proximal to the circle of Willis [1, 8].

Prior to transfer to the neuroangiography suite, noncontrast head CT should be obtained to rule out other causes of neurological deterioration and determine the extent of irreversible cerebral infarction. In addition, the risk of increased intracranial pressure (ICP), as a result of lying flat on the neuroangiography table and the administration of intra-arterial vasodilators, should be anticipated [14, 15]. Thus, ICP should be well controlled and consideration should be given for an external ventricular drain. Similarly, an arterial line can be helpful in this setting, given the risk of systemic hypotension during IAVT, which may negatively impact cerebral perfusion [15, 16]. If TBA is being contemplated, consideration may be given to general anesthesia to minimize patient motion during the procedure.

Is Prophylactic Endovascular Treatment Indicated?

There is currently no place for prophylactic endovascular therapy in the management of PHCV. Although prophylactic TBA was shown to prevent PHCV in animal studies [17], clinical experience has been disappointing, with procedural mortality rates as high as 8% and lack of clinical benefit [18, 19]. During prophylactic TBA, vessels are stretched beyond their baseline normal diameter, which likely accounts for the poor results. Prophylactic IAVT has not been attempted and would be counterintuitive, given the usually transient and short-lived nature of its therapeutic effects.

Transluminal Balloon Angioplasty

Technical Aspects

The mechanism of action of TBA likely involves mechanical disruption of smooth muscle cells and connective tissue fibers in the vessel wall, which results in a paralytic injury preventing vasoconstriction [8, 20]. TBA is likely to be most effective when actively contracting muscle fibers are forcefully stretched and thus should be ideally performed prior to administration of vasodilators. However, if the vessel lumen is too small to allow safe microwire or balloon catheter passage, it may be necessary to administer IAVT in preparation for TBA [8].

Suitable vessels for TBA include the internal carotid arteries, vertebral arteries, basilar trunk, and proximal segments of the middle (M1), anterior (A1), and posterior (P1) cerebral arteries. We approach A1 and P1 with caution since their wall is relatively deficient in tunica media and elastic tissue, theoretically making them more prone to rupture [8]. Although successful TBA for distal vasospasm was recently reported [21], data regarding its safety remain insufficient. In general, we do not advise performing TBA on vessels less than 1.5 mm in baseline diameter.

The major advantage of TBA is that, unlike IAVT, it confers a long-lasting therapeutic effect, making retreatment rarely necessary [8, 22, 23]. Moreover, TBA does not increase ICP, even when multiple vessels are treated. However, given the significant procedural risks, particularly vessel rupture and reperfusion hemorrhage, we typically reserve this option for hemodynamically significant (>50% reduction in vessel diameter), symptomatic proximal vasospasm that either does not respond or responds poorly to IAVT.

We apply extreme caution when a baseline angiogram is not available, making it difficult to determine the severity of PHCV reliably. In such cases, the safety of TBA becomes questionable, particularly for the A1 and P1 segments, which are frequently prone to congenital hypoplasia. Likewise, fenestrated vessels in severe spasm may appear to have a single lumen, and failure to recognize this fenestration on a baseline angiogram can lead to overdilation and vessel rupture. Although small hypodensities on CT may resolve following successful TBA [24], large completed infarcts should discourage attempts to restore cerebral perfusion, given the significant risk of reperfusion hemorrhage. Fatal intracranial hemorrhage has been reported up to 24 h after TBA [22]. Moreover, TBA adjacent to clipped ruptured aneurysms has been associated with fatal rupture of the parent artery [25].

TBA is usually performed under full systemic heparinization. Both single- and dual-lumen balloon catheters that track over 0.010- and 0.014-in. microwires, respectively, may be used. While single-lumen balloons are all compliant, dual-lumen balloons may be compliant, semicompliant, or noncompliant. Most dual-lumen balloon catheters suitable for intracranial TBA were designed for coronary angioplasty. These balloons reach nominal diameters at pressures in the range of 6–18 atm, which are much higher than those required for successful intracranial TBA. Overdilation of vessels should be avoided, and balloons should be undersized, typically to 80–85% of the baseline vessel diameter. To avoid multiple dilations, balloon length should be selected to cover the longest contiguous straight segment of vessel. Balloon inflations around sharply angulated curves may cause forced, traumatic straightening of the artery and thus, should be avoided. If needed, successive dilations may be performed from distal to proximal to avoid crossing a fresh angioplasty site and inadvertently raising a dissection flap [8].

Angiographic and Clinical Results

The success rate of TBA in reversing PHCV and restoring normal or near-normal luminal caliber is in the 80–100% range [12, 22, 26–29]. This equally translates into improved TCD velocities and cerebral blood flow [23, 29]. However, clinical success (i.e., reversal of DCI symptoms) rates are variable, ranging from 30% to 80% [22, 29–31]. This is likely due to differences in disease severity and timing of intervention. In one study, TBA performed within 2 h of symptom onset was associated with 70% clinical improvement compared to only 40% when done later [12]. Retreatment is rarely necessary, with only 3–4% of patients requiring additional procedures [8, 22, 23].

Complications

Complications of TBA include iatrogenic vessel injury (dissection, perforation, occlusion, rupture), ischemic stroke, reperfusion hemorrhage, rebleeding from unsecured aneurysms, and displacement of aneurysm clips [1, 8]. Rupture of an overdilated cerebral artery is a serious and frequently lethal complication. Although older series had documented rupture rates as high as 4–5% [32], recent studies suggested that, with technical advancements, this rate has decreased significantly, to about 1% [33]. Likewise, the rate of thromboembolic complications lies around 4–5% [34]. Although the exact long-term incidence of flow-limiting stenosis at the site of TBA has not been systematically assessed, this complication has been reported [35].

Intra-arterial Vasodilator Infusion Therapy

Technical Aspects

A variety of vasodilators has been used intra-arterially to treat PHCV, including (1) the phosphodiesterase inhibitors papaverine and milrinone; (2) the calcium channel blockers nimodipine, nicardipine, and verapamil; and (3) the rho kinase inhibitor fasudil hydrochloride [36–52]. It is believed that the mechanism of action of IAVT involves vasodilation via a direct pharmacological effect on the vascular smooth muscle cells, although a neuroprotective effect cannot be completely excluded. IAVT can be used as a stand-alone therapy to treat both proximal and distal vasospasm, regardless of the size of the vessels and their position in the arterial tree [1, 8]. Alternatively, IAVT can be used to manage residual distal vasospasm following TBA. The technique of IAVT involves either bolus injection of vasodilators or slow infusion over a period of 30–90 min. In addition, IAVT can be administered through either selective (≥4 French) or superselective (≤3 French) catheters. Selective IAVT through a catheter in the extracranial internal carotid or vertebral artery is usually sufficient in most patients. However, superselective infusions may be advantageous when there is competing flow at a bifurcation to ensure that most of the drug is delivered into the high-resistance, spastic cerebral vessels rather than the low-resistance, normal ones. Although generally safer than TBA, IAVT has several disadvantages, including delayed onset of action, transient therapeutic effect, and risk of increased ICP, particularly when multiple vessels are treated.

Angiographic and Clinical Results

Phosphodiesterase Inhibitors: Papaverine, Milrinone

Angiographic improvement is observed in 60–90% of patients following intra-arterial papaverine infusion [36–38]. However, clinical improvement occurs in only 25–50% [36, 38–40]. Moreover, the beneficial effect of papaverine on cerebral blood flow does not seem to last longer than 3 h [41]. Milrinone has also been shown to result in angiographic improvement and enhanced cerebral blood flow in patients with PHCV, although clinical improvement has yet to be demonstrated [42, 43].

Calcium Channel Blockers: Nimodipine, Nicardipine, Verapamil

Intra-arterial nimodipine leads to angiographic response in 40–80% of patients, although clinical improvement occurs in as many as 70–75% [44, 45]. Conversely, nicardipine has been associated with angiographic improvement in all treated patients, but the rates of clinical improvement have been dose-dependent, ranging from 42% to 91% [46, 47]. Moreover, high-dose nicardipine has been associated with significant effects on systemic blood pressure [16, 47]. Likewise, verapamil has been shown to result in an angiographic response in all treated vessels, with a mean increase in vessel diameter of 29–44% [48–50]. However, the rates of clinical improvement also appear to be dose-dependent, ranging from 30% to 70% [48, 49]. Verapamil-induced cerebral vasodilation seems to develop gradually and stabilize over 15–30 min [50]. Interestingly, the positive effects of high-dose intra-arterial verapamil on cerebral hemodynamics and brain metabolism may last as long as 12 h [15]. For this reason and given its favorable safety profile, we routinely use verapamil as a first-line agent for IAVT.

Rho Kinase Inhibitors: Fasudil Hydrochloride

Fasudil hydrochloride is widely used in Japan. It has been shown to result in angiographic improvement in all treated patients and variable clinical response, ranging from 45% to 70% [51, 52].

Complications

In addition to the usual risks of cerebral angiography (e.g., arterial dissection, ischemic stroke), adverse events can be caused by the IAVT agent itself. Intracranial hypertension, systemic hypotension, and seizures have been reported with all agents [14–16, 23, 43, 46, 52, 53]. Intracranial hypertension is presumably the result of increased cerebral blood volume resulting from widespread intracranial vasodilation. Furthermore, given their short-lived therapeutic effect, multiple IAVT procedures are often necessary; therefore, the overall risk may become cumulative.

For more than 20 years, papaverine has been widely employed in IAVT procedures. However, in current clinical practice, it is infrequently used because of concerns about potential neurotoxicity, including transient or permanent monocular blindness, mydriasis, transient hemiparesis, seizures, gray matter necrosis, cardiac dysfunction, and respiratory arrest [1, 54]. Moreover, intracranial hypertension tends to be a significant problem when multiple vascular territories are being treated [14, 53]. Papaverine-related serious complications occur in 2–5% [23, 30, 53].

Conclusion

While medical management remains the first-line treatment for symptomatic PHCV, endovascular therapy should not be delayed in medically refractory cases. Proximal vasospasm can be managed by either TBA or IAVT. In contrast, only

IAVT is a widely accepted treatment for distal vasospasm. Although the therapeutic effects of TBA are long-lasting and reatreatment is rarely necessary, potential serious procedural risks should limit its use to severe cases of symptomatic PHCV. Conversely, despite its transient therapeutic effects and the risk of increased ICP, IAVT has become an effective and low-risk treatment for most cases of PHCV. This is partly due to the advent of newer vasodilators with longer duration of action and better safety profile than papaverine. Finally, it should be kept in mind that the clinical benefit of endovascular therapy for PHCV has only been reported in case series and that a positive effect on ultimate patient outcome has yet to be proven.

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

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The Concept of a Hybrid Operating Room: Applications in Cerebrovascular Surgery

Javier Fandino, Philipp Taussky, Serge Marbacher, Carl Muroi, Michael Diepers, Ali-Reza Fathi, and Luca Remonda

Abstract The use of intraoperative digital substraction angiography (iDSA) is a tool in cerebrovascular surgery. According to recent studies, iDSA has been shown to alter surgical treatment in approximately 12% of cases. Moreover, it has been demonstrated that even experienced cerebrovascular surgeons might not accurately predict the need for iDSA. Intraoperative DSA prevents unnecessary surgical manipulations after occlusion of aneurysms and accurately demonstrates occlusion rates. We present our preliminary experience using routine iDSA within the concept of a hybrid operating room for cerebrovascular surgery. A total of 99 patients underwent iDSA in our hybrid operating room. Indications included intraoperative evaluation of occlusion rate of clipped aneurysms and patency of vicinity vessels ($n=82$), chemical angioplasty with papaverin ($n=4$), and balloon angioplasty ($n=1$). In four (5%) patients, a reposition of the clip was needed due to neck remnant and perfusion of the aneurysm sack after clipping. A total of five cases underwent combined microsurgical and endovascular treatment of ruptured aneurysms and arteriovenous malformations (AVMs). The concept of a hybrid operating room has been considered in the planning and design of operation rooms dedicated to cerebrovascular surgery. Hybrid procedures combining endovascular with microsurgical strategies within the same surgical session are feasible and safe. These procedures are associated with cost-benefit advantages.

Keywords Intraoperative angiography • Cerebrovascular surgery • Hybrid operation room

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Introduction

Intraoperative digital subtraction angiography (iDSA) is still a rarely applied tool in cerebrovascular surgery. In recent studies, iDSA has been shown to alter surgical treatment in approximately 12% of cases [1–4]. It prevents unnecessary surgical manipulations after occlusion of aneurysms and accurately demonstrates occlusion rates. The introduction of iDSA within a hybrid operation room enables the combinations of endovascular and microsurgical techniques in cerebrovascular surgery. We present our experience and concept of a hybrid operating room for interdisciplinary treatment of cerebral aneurysms and arteriovenous malformations (AVMs).

Materials and Methods

The hybrid operating room is defined as a surgical theater that enables the combination of endovascular and surgical treatment of cerebrovascular pathologies and the use of interventional tools such as temporary occlusion during clipping in a single procedure. In our experience, the combination of mono- or biplanar angiography apparatus with standard surgical tables is crucial to allow standard positioning of the patient during surgery. The surgical table (Alphamaquet 1150, Maquet AG, Switzerland) and C-arm (Xper FD20, Philips, Netherlands) are coupled, and a collision alarm system prevents independent or uncontrolled movements of the table or C-arm. The carbon fiber top of the table is 360 radio translucent. Radiolucent head pins and head holders are required for optimal acquisition of angiograms and intraoperative CT scans. Surgical personnel have been trained to assist endovascular procedures. Neurosurgeons are responsible for all technical issues during the procedures. This setting allows the utilization of the operating room by vascular surgeons and other surgical specialties with or without performance of angiograms (Fig. 1).

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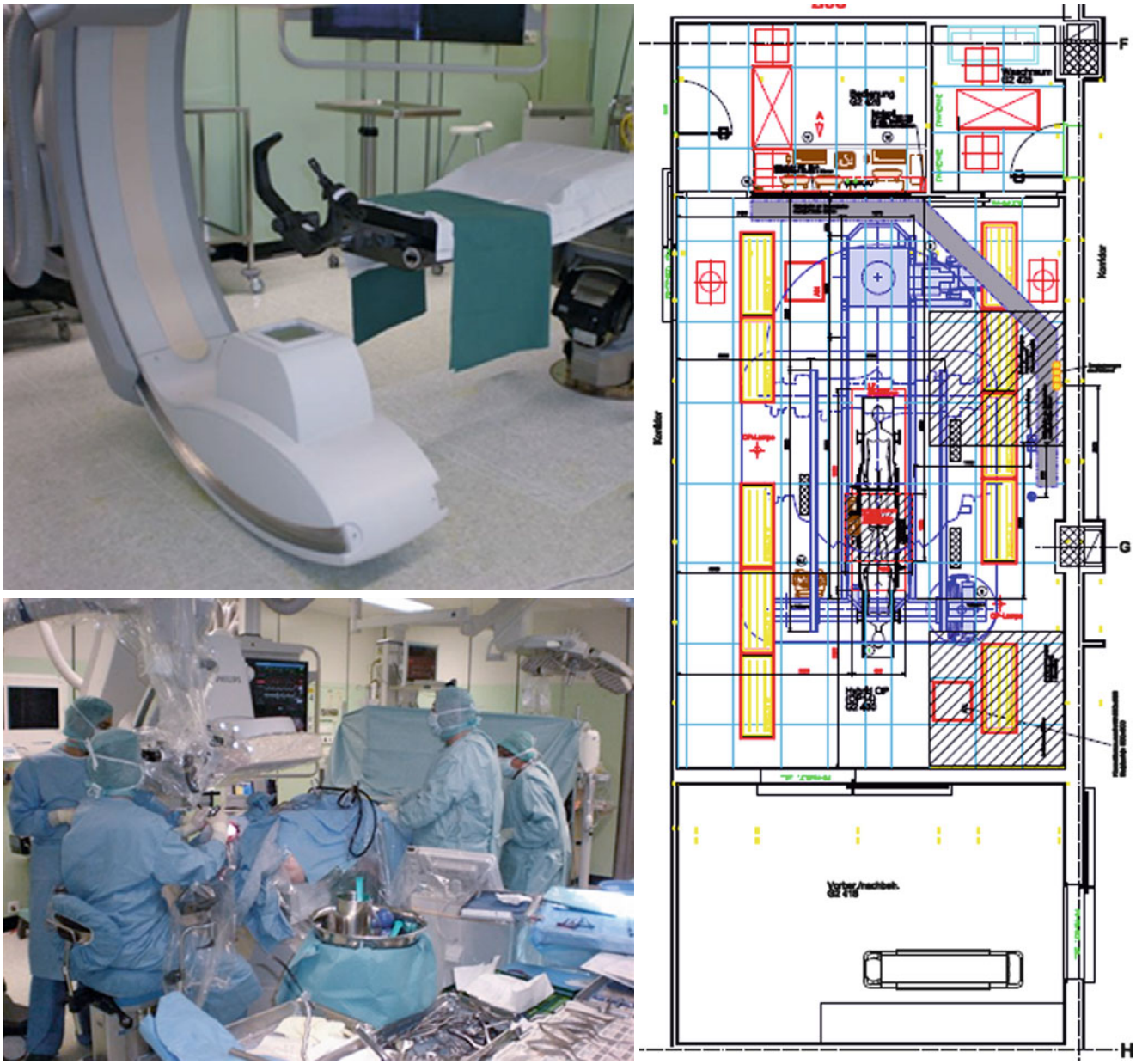

Fig. 1 The hybrid operating room allows combined microsurgical and endovascular approaches in cerebrovascular surgery. Radiolucent head pins and head holders are necessary for high-quality intraoperative imaging, which includes intraoperative digital substraction angiography (*iDSA*) and three-dimensional computed tomographic angiography (*3D-CTA*)

Results

A total of 99 patients underwent operations in our hybrid operating room. Indications included intraoperative evaluation of occlusion rate of clipped aneurysms and patency of vicinity vessels ($n=82$), chemical angioplasty with papaverin ($n=4$), balloon angioplasty ($n=2$), and primarily endovascular coiling with option of neurosurgical intervention ($n=4$). In four (5%) patients, a reposition of the clip was needed due to neck remnant and perfusion of the aneurysm sack after clipping. In one case, the clip was repositioned due to occlusion of a vessel near the aneurysm. In one case, a suboptimal occlusion of an aneurysm (anterior communicating aneurysm) could be documented. In this case, reposition of the clip was unsuccessful in reaching a total occlusion of the aneurysm, and the postoperative follow-up conventional angiogram and coiling of the remnant aneurysm were uneventfully performed. All endovascular aneurysm embolizations were successful; in two cases, coiling of ruptured aneurysms was performed after emergency craniotomy for evacuation of an intracerebral hematoma. A total of five cases underwent combined microsurgical and endovascular treatment of ruptured aneurysms and AVMs. Three illustrative cases are presented (Figs. 2, 3, and 4).

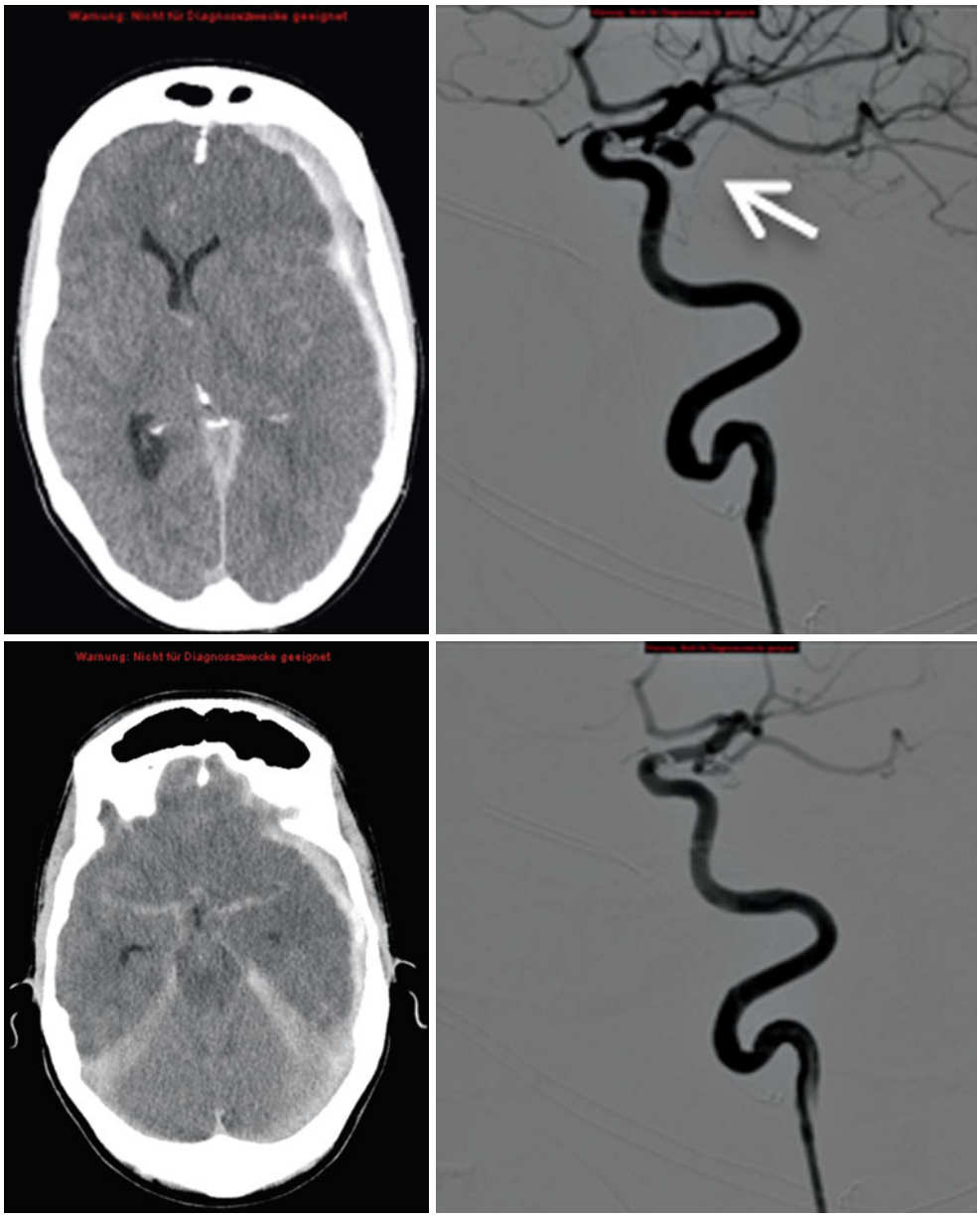

Fig. 2 Illustrative case 1. Acute subdural hematoma after aneurysm rupture of a left posterior communicating artery aneurysm (*arrow*). The patient underwent emergency craniotomy for evacuation of the hematoma followed by coiling of the aneurysm. The duration between hospital admission and final angiogram was 4 h

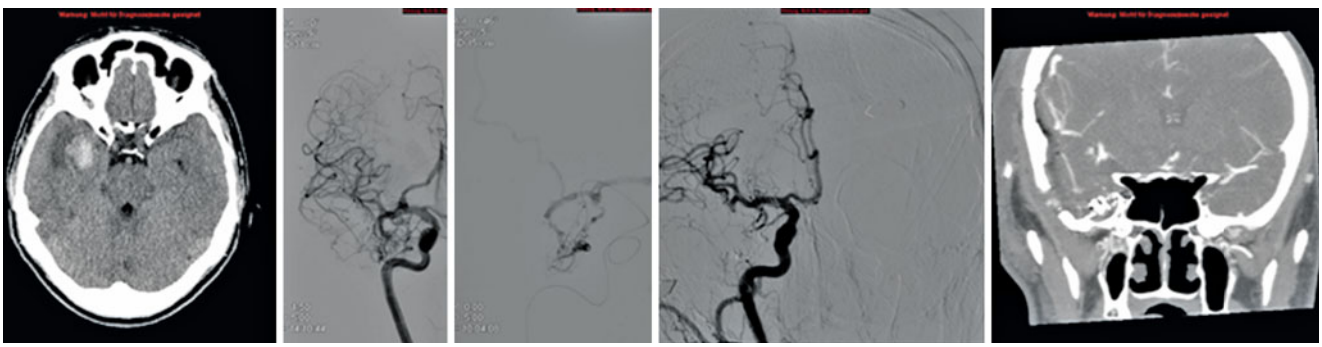

Fig. 3 Illustrative case 2. Combined endovascular and microsurgical treatment of a right temporal arteriovenous malformation (*AVM*). After embolization of two main feeders, complete resection of the AVM could be achieved. Postinterventional angiogram and computed tomography (*CT*) showed no remnant of the AVM

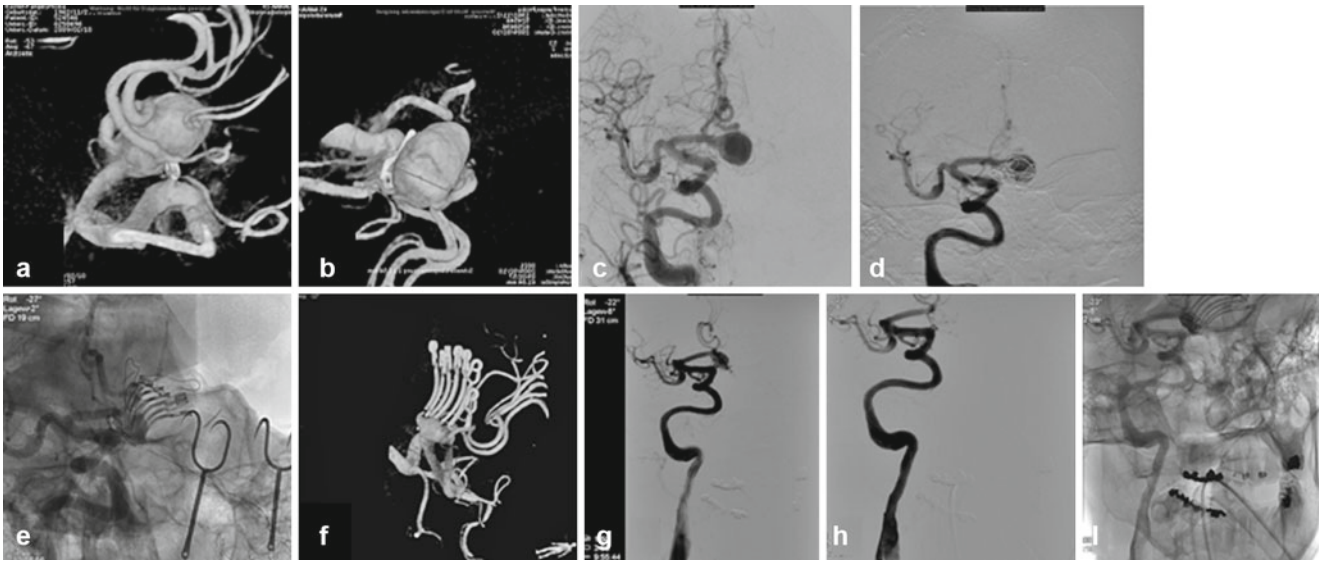

Fig. 4 Illustrative case 3. Combined endovascular and microsurgical treatment of a complex recurrent right internal carotid artery (ICA) aneurysm treated in the 1970s (**a**–**c**). Primary endovascular occlusion was not possible (**d**). The aneurysm was clipped using a left-sided pterional approach under endovascular temporary occlusion of the right anterior communicating artery (ACA) (**e**–**g**). The remnant aneurysm could be coiled (**h**, **i**). No postoperative neurological deficit could be documented

Discussion

Intraoperative imaging in neurosurgery allows the early identification of complications and optimizes the treatment objectives. Intraoperative DSA [1–4] and near-infrared indocyanine green video angiography for the assessment of vascular flow during cerebrovascular surgery have contributed to safety and the prevention of severe complications during cerebrovascular procedures [5]. The concept of a hybrid operating room not only allows the performance of routine iDSA in all cerebrovascular procedures but also opens the possibility for combined approaches in a sterile environment. Both microsurgical and endovascular techniques can be applied to achieve excellent results and prevent complications, whose possible causes might be detected during surgery. From our point of view, the following important issues have to be included in planning for a hybrid operation room for cerebrovascular procedures: location, table, multidisciplinary application, operating room technicians, and surgeon's capability for operating the equipment. The hybrid operating room should be planned within the concept of a conventional surgical environment that allows sterile procedures and personnel flexibility. According to our experience, a surgical table that allows all standard positions is crucial not only for neurosurgeons but also for other surgical specialties; this can contribute to optimal utilization of the operation room during the slots when neurosurgical procedures are not planned. In our concept, operating room technicians have to be trained for endovascular techniques to integrate them in combined procedures and facilitate the surgical processes. Finally, we consider it crucial that the neurosurgeon be trained for operating all the equipment installed in the hybrid operating room, including the angiogram apparatus and software for the acquisition and evaluation of three-dimensional rotational angiography.

Conclusion

The concept of a hybrid operating room should be considered in the planning and design of operating rooms dedicated to cerebrovascular surgery. Hybrid procedures combining endovascular with microsurgical strategies in the same surgical session are feasible and safe and have cost-benefit advantages.

Conflicts of Interest Statement The authors do not have financial interests with the manufacturing companies of the equipment mentioned in this manuscript.

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Computerized Occlusion Rating of Embolized Ruptured Intracranial Aneurysms: Relation to Intra- and Postinterventional Aneurysm Rehemorrhage

Helena Milavec, Andreas Gruber, Ernst Schuster, Eva Lahnsteiner, Christian Dorfer, Maximilian Krawagna, Engelbert Knosp, and Camillo Sherif

Abstract Endovascular coil occlusion of ruptured cerebral aneurysms has a higher rate of rebleeding compared to surgical clipping. Initial aneurysm coil occlusion rate (OR) is the strongest available predictor of aneurysm rebleeding. Standard clinical subjective occlusion rating (SOR) is limited by subjective bias. Therefore, computerized occlusion rating (COR) was introduced. Its superiority was established for experimental and human aneurysms. In the present clinical study, we aimed to evaluate COR as a risk factor for postprocedural reruptures (PPRs) and intraprocedural reruptures (IPRs). In our series of 249 consecutive patients treated in our institution, we observed 7 (2.8%) cases with IPR and 7 (2.8%) cases with PPR. These patients were analyzed in the present study. Mean COR value was 85% (range 71–96%). In 12 (85.7%) cases, COR was lower than SOR. In aneurysms with a COR of 95% or higher, no PPR occurred. All patients with IPR harbored multiple aneurysms. In conclusion, our data showed a distinct tendency of potentially dangerous overestimations when using SOR compared to the objectively measured COR values. IPR was always associated with multiple aneurysms.

Keywords Endovascular coiling • Occlusion rate • Intracranial aneurysm • Rehemorrhage • Intrainterventional rerupture • Postinterventional rerupture • Computerized occlusion rating

Introduction

Coil occlusion of intracranial aneurysms (IAs) has evolved as the first-line treatment for the majority of ruptured cerebral aneurysms [1]. Postprocedural aneurysm recurrence, rebleeding, and late rupture of incompletely occluded IAs remain drawbacks of endovascular coil embolization when compared to surgical clipping. The initial degree of aneurysm occlusion, referred to as occlusion rate (OR), is the only available predictor of aneurysm rebleeding with level 1 evidence [2]. Patients with an OR above 90% are at relatively low risk of recurrent bleeding in the long term [3].

In clinical routine, OR is only subjectively estimated. This subjective estimation is theoretically limited by considerable subjective bias. In an effort to address this issue, Sherif et al. [4] reported a new computer-assisted method for two-dimensional digital subtraction angiography (DSA) images providing a more objective quantification of aneurysm occlusion on angiographic images. For experimental and human aneurysms, they showed the superiority of COR compared to angiographic subjective occlusion rating (SOR) estimations by examining the angiographic OR values in comparison with histometric computerized occlusion rating (COR) of postmortem-retrieved aneurysms [5]. In a subsequent study, the authors transferred these methods to a clinical series of 249 consecutive patients and hence also demonstrated, in a clinical setting, the superiority of COR over the present

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standard SOR. COR minimizes the unacceptable statistically significant interobserver variability [6].

The objective of the present study was to focus on COR along with other risk factors affecting intraprocedural rerupture (IPR) and postprocedural rerupture (PPR) and to describe the minimum COR rate that avoided rebleeding in this patient series.

Materials and Methods

This retrospective, single-center study included 14 patients with IPR or PPR, selected from a consecutive series of 249 patients with ruptured embolized IAs treated at the Department of Neurosurgery of the Medical University of Vienna in a 7-year period (1998–2004). Demographic and clinical evaluation included age at first treatment, sex, Hunt and Hess grade [7] at admission, aneurysm multiplicity, and treatment details such as time between first bleeding and first treatment.

All IAs were embolized with Guglielmi detachable coils (GDCs; Target Therapeutics/Boston Scientific, Fremont, CA, USA) according to the manufacturer's instructions. Pre- and postinterventional DSA was performed and stored in digital imaging and communications in medicine (DICOM) format.

Immediately after coil embolization, the angiographic SOR of each aneurysm had been estimated by an experienced endovascular neurosurgeon according to Roy et al. [8]: Total occlusion (Roy class 1) was defined as 100% SOR, residual neck filling (Roy class 2) was defined as 95% SOR, and residual aneurysm filling (Roy class 3) was rounded to 10% accuracy (90%, 80%, 70%, etc.).

In a second step, a different independent observer performed COR of the DSA images using the new custom-made software CoilControl-2D® (NVtec-Neurovascular Technologies, Ltd., Vienna, Austria) (Fig. 1).

We used a binary logistic regression model to evaluate the influence of initial COR, aneurysm multiplicity, sex, and age on IPR and PPR. The paired *t* test was used to compare COR with SOR values.

Results

The findings of the quantitative evaluation are summarized in Table 1. In the present series (nine females and five males), we observed seven (2.8%) cases of IPR and seven (2.8%) cases of PPR.

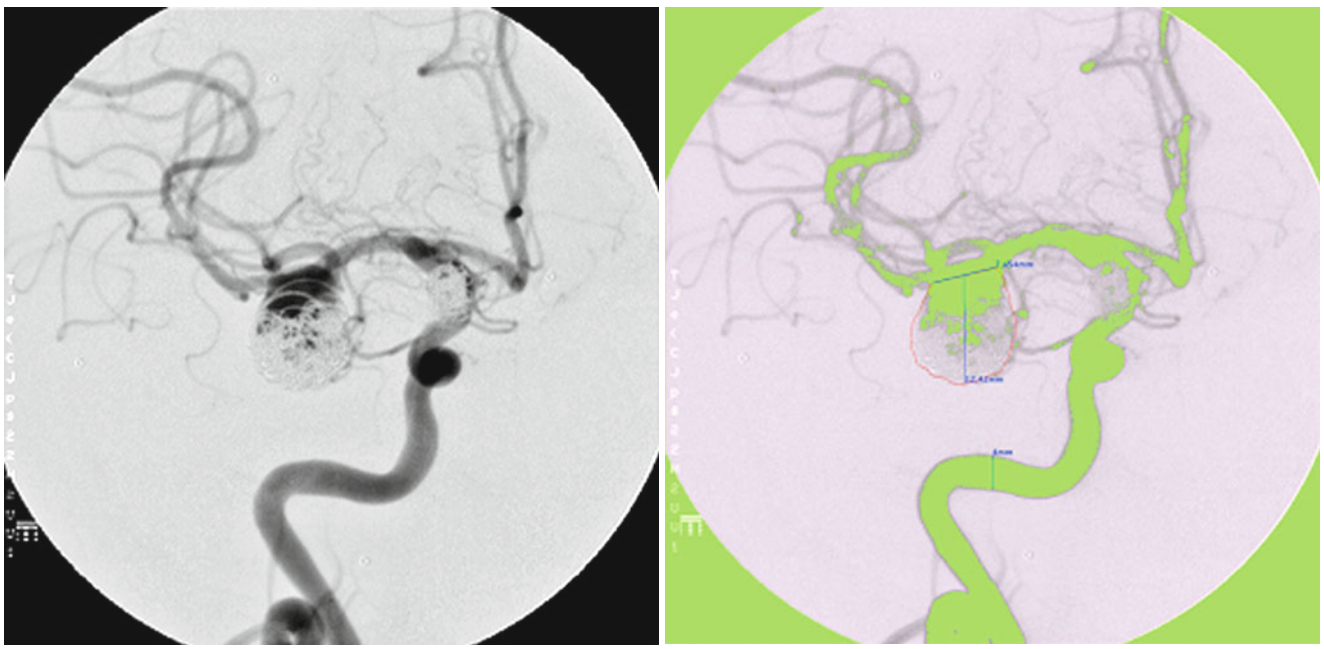

Fig. 1 Image processing using CoilControl-2D®. *Left* Digital subtraction angiographic (*DSA*) image of a female patient, 58 years old, initially Hunt and Hess grade 1: Subjective occlusion rating (*SOR*) resulted in Roy class 3 estimation (<90%). The DSA shows a subtotally coiled aneurysm of the right M1/M2 bifurcation (middle cerebral artery, *MCA*). *Note*: The image shows a second coiled aneurysm of the posterior communicating artery (*Pcom*) *Right:* Image after processing with the CoilControl-2D software. The total area of the aneurysm was defined. Based on a gray-level distinction of the contrast medium in the parent arteries, the blood-perfused areas within the aneurysm were defined and false-color labeled. The relation of blood-perfused areas within the aneurysm to total aneurysm area is given as percentage computerized occlusion rating (*COR*). The COR was notably lower, amounting to 64%

Table 1 Findings of the quantitative evaluation

	Age (years)	Aneurysm multiplicity	H&H grade	Time to treatment (days)	Aneurysm rerupture (days)	SOR (%)	COR (%)	Aneurysm total area (mm^2)
Mean	52.83	0.71	3.00	6.21	41.64	91.43	84.86	61.593
SEM	3.274	0.125	0.363	1.594	39.204	1.522	2.355	16.9667
SD	12.251	0.469	1.359	5.964	146.689	5.694	8.813	63.4836
Minimum	36	0	1	0	0	80	64	18.6
Maximum	74	1	5	19	551	100	96	256.9

COR computerized occlusion rating, *SOR* subjective occlusion rating, *H&H* Hunt and Hess, *SEM* standard error of the mean, *SD* standard deviation

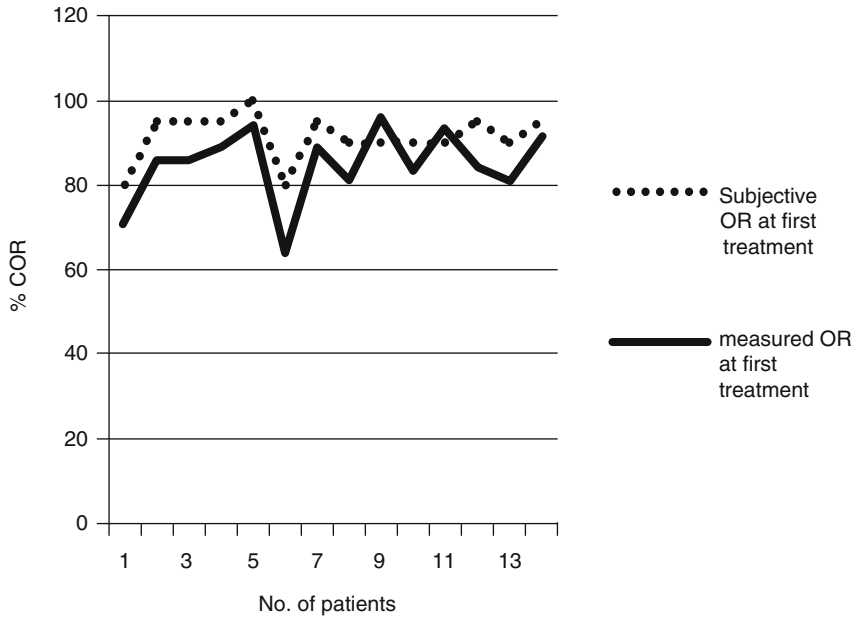

Fig. 2 Subjective occlusion rating (*SOR*) versus computerized occlusion rating (*COR*). *x axis* Patient number, *y axis* % COR. In 12 (85.7%) cases, COR values were lower than SOR estimations. The *t* test showed that the mean of COR values was significantly lower than the mean of SOR estimations ($p=0.01$)

Mean age at the time of the first treatment was 53 years, ranging from 36 to 74 years. Ten (71.4%) patients harbored multiple aneurysms.

The symptom duration before treatment ranged from 0 to 20 days. One (7.1%) patient was treated within 24 h, six (42.9%) patients received a treatment within the first 3 days, and seven (50%) patients were treated after the third day.

Of the seven aneurysms with PPR, two (28.6%) reruptured the day after initial treatment, one (14.3%) reruptured 2 days after treatment, and four (57.1%) aneurysms reruptured 3 days after treatment.

Subjective Occlusion Rating

The SOR estimations ranged from 80% to 100% (Fig. 2). SORs resulted in 100% occlusion for one (7.1%) aneurysm, in 95% occlusion for six (42.9%) aneurysms, in 90% occlusion for five (35.7%) aneurysms, and in less than 90% occlusion for two (14.3%) aneurysms.

Computerized Occlusion Rating

Mean COR was 85%, ranging from 71% to 96%. Four (28.6%) patients were graded from 91% to 99% COR. Nine (64.3%) patients had COR values ranging from 70% to 90%. One (7.1%) patient had a COR of 64%. The *t* test showed a significant difference between estimated SOR and measured COR ($p=0.01$). In 12 (85.7%) cases, COR values were lower than SOR estimations.

Intra- and Postprocedural Ruptures

We observed IPR in patients with a COR value from 81% to 96%. PPR occurred in patients with COR values ranging from 71% to 94%. Six (85.7%) of seven patients suffering from PPRs had a COR of less than 90% (Fig. 2). There was no PPR in aneurysms with a COR of 95% or higher.

All 7 patients who suffered IPR harbored additional unruptured aneurysms. There were significantly more females than males who presented with PPR ($p=0.094$). Aneurysm location, total aneurysm area, and initial Hunt and Hess grade [7] did not influence the occurrence of IPR or PPR in this series. Regressing the four variables COR, sex, age, and presence of multiple aneurysms led to correct predictions of IPR/PPR in 100%.

Discussion

Intraprocedural Reruptures

In our series, we could demonstrate that IPR remains a relevant risk of endovascular coiling procedures. According to the literature, the overall risk of IPR is 1–5% [9–11]. Elijovich et al. [10] reported potential predictors of IPR evaluating patients treated in the Cerebral Aneurysm Rerupture After Treatment (CARAT) study [2]. The authors documented IPR in 5% of their 299 patients; 63% of patients with IPR suffered incurring periprocedural death or disability as compared to only 15% of the patients without IPR. IPR occurred more frequently in clipping (19%) than in coiling (5%). In the present study, we could find a high risk of IPR for patients harboring multiple aneurysms, a condition that had not been analyzed in the CARAT study. Contrary to CARAT reporting a nonsignificant female IPR predominance, we did not observe any gender predominance for IPR. We feel this likely to be due to the smaller sample size of the present evaluations.

Postprocedural Reruptures

In a series of 1,001 patients, Johnston et al. [2] studied the predictors of early and late rehemorrhage after surgical and endovascular treatment of ruptured intracranial aneurysms. They reported that SOR estimation was the only statistically significant predictor with level 1 evidence for PPR. In their study, 75.9% of the aneurysms showed complete occlusion (SOR 100%), 17.3% were subtotally occluded (SOR 91–99%), 5.1% were partially occluded (SOR 70–90%), and 1.7% showed SOR values less than 70%. The authors concluded that complete aneurysm occlusion would not prevent aneurysm rerupture but minimize its risk. In the present study, we had no patient presenting with PPR above 95% COR. We would not dare, however, to conclude that 100% COR definitely prevents PPR. We feel it reasonable that this finding is related to the lower sample size of our study population as compared to the CARAT study.

Subjective Overestimation of Occlusion Rate

Analyzing the results of SOR, we observed in the present study a distinct tendency to overestimate aneurysmal occlusion compared to the more objective COR method. Previous findings support these results [4, 5]. The reasons for the superiority of COR are better aneurysmal border definition and better recognition of the contrast agent gray values. These factors result in minimized intra- and interobserver variability [6].

Raymond et al. [12] showed that Roy class 1 and 2 aneurysms are at a low risk of recurrence. The SOR overestimation documented in our data might lead to wrong follow-up decisions with a high risk of aneurysm recurrence. Thus, it might ultimately lead to rebleeding of COR Roy class 3 aneurysms that had been subjectively overestimated to SOR Roy class 1 and 2. This finding has a potentially great clinical impact as we may miss exactly these patients in our actual clinical routine.

Limitations

The small sample size of our study population is the main limitation to the present findings. A minimum of ten events per independent variable has been recommended [13], yet our study did not meet this requirement.

Three-dimensional (3D) structures are difficult to evaluate on two-dimensional (2D) images. Particularly for aneurysms with a complex anatomy, this is the major methodological limitation of 2D-COR. We tried to minimize this limitation by means of careful selection of representative images, displaying not only the aneurysm neck but also the whole extent of the aneurysm dome. = To avoid bias of 2D imaging evaluations, (...), 3D DSA or noninvasive 3D MRA will be required in future human series. These techniques are already available. Their superiority over 2D-SOR and even 2D-COR has been already established for experimental aneurysms [14].

Conclusion

In comparison to the objective COR values, SOR estimations showed the potential danger of overestimating aneurysm coil occlusion. These overestimations might result in wrong patient follow-ups in our actual clinical routine. An objectively assessed COR of 100% minimizes the risk of rebleeding. IPR was always associated with multiple aneurysms.

Conflicts of Interest Statement Sherif C is shareholder of NVtec ltd., Vienna, Austria. The other authors have no conflict of interests.

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Spreading Ischemia After Aneurysmal Subarachnoid Hemorrhage

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Abstract Spreading depolarization (SD) is a wave of mass neuronal and glial depolarization associated with net influx of cations and water. Prolonged SDs facilitate neuronal death. SD induces tone alterations in cerebral resistance arterioles, leading to either transient hyperperfusion (physiological neurovascular coupling) in healthy tissue or hypoperfusion (inverse neurovascular coupling = spreading ischemia) in tissue at risk for progressive damage. Spreading ischemia has been shown experimentally in an animal model replicating the conditions present following aneurysmal subarachnoid hemorrhage (aSAH), in animal models of the ischemic core and penumbra following middle cerebral artery occlusion, and in patients with aSAH. In animals, spreading ischemia produced widespread cortical necrosis. In patients, spreading ischemia occurred in temporal correlation with ischemic lesion development early and late after aSAH. We briefly review important features of SD and spreading ischemia following aSAH.

Keywords Aneurysmal subarachnoid hemorrhage • Spreading depression • Delayed cerebral ischemia

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Introduction

Spreading depolarization (SD) is the generic term for depolarization waves in the central nervous system characterized by abrupt, near-complete sustained depolarization of neurons, observed as a large change of the slow electrical field potential. Moreover, SD is characterized by (1) near-complete breakdown of the transcellular ion gradients [23, 41], (2) extreme shunt of neuronal membrane resistance [3], (3) electrical activity loss (spreading depression) [26], and (4) neuron swelling with dendritic spine distortion [40]. Thus, the term SD describes a near-complete short circuit between neurons and extracellular space electrically, and a cytotoxic edema biochemically and morphologically [23, 40].

Synchronous near-complete sustained depolarization of thousands or millions of neurons with similar spatial orientation produces a large change of the slow extracellular potential as a direct electrocorticographic (ECoG) summary measure

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for SD [3]. In addition to the duration of the slow potential change, pattern and duration of the depression of spontaneous brain electrical activity and the polarity of ultraslow potential components can be assessed in the ECoG to determine the biological significance of a given SD [31].

SD is termed terminal SD in regions where neurons do not repolarize [15]. Terminal SD is always observed in response to heavily noxious situations, such as severe persistent hypoxia, severe focal ischemia (less than about 15 ml/100 g/min) or cardiac arrest. However, strictly speaking, only the initial segment of terminal SD represents the SD process, while the later ultraslow negative potential is assumed to reflect mechanisms of injury and death [31]. In contrast to heavily noxious conditions, mild ischemia induces a cluster of recurrent SDs with intermediate durations as observed experimentally using the endothelin-1 (ET-1) model of mild focal ischemia [8, 9, 31]. Very mild ischemia seems to trigger single, short-lasting events [30] whose patterns are almost identical to those recorded in normally perfused tissue around an ischemic zone where tissue recovers from SD within 1 or 2 min [29]. If oxygen, glucose, and regional cerebral blood flow (rCBF) show tissue gradients, a gradual transition in space from terminal to short-lasting SD via intermediate SD patterns is measured when the depolarization wave travels along those gradients.

While terminal SD is always associated with neuronal death [34], neurons survive short-lasting SD [29, 30]. SDs of intermediate duration are typically observed in the ischemic penumbra after middle cerebral artery occlusion, defined as a region of constrained blood supply in which membrane ion homeostasis is preserved [20]. In those studies, SD occurrence and cumulative duration were correlated with infarct size and dynamics of infarct expansion [4, 18, 27]. Moreover, SDs that were chemically triggered outside the penumbra and that invaded it caused stepwise enlargement of the necrotic core [8, 40]. This suggested that SDs of intermediate duration initiate cascades leading to cell death similar to terminal SDs.

SD presumably entails triggers that activate intracellular signals causing neuronal death. However, this requires a certain minimum duration of a single SD or possibly cumulative duration of multiple SDs. The commitment point has been defined as the time point after onset of SD at which neurons undergoing SD start to be irreversibly damaged [36]. Of note, the neuronal network may transiently recover from such a deleteriously prolonged SD, and cell death may only occur in a delayed fashion. Potential mechanisms by which long-lasting SDs recruit tissue into cell death include the intracellular calcium overload, mitochondrial depolarization, and excessive release of glutamate [6, 33].

Using ECoG, SDs have been shown in abundance in patients with aneurismal subarachnoid hemorrhage (aSAH) and delayed ischemic stroke after aSAH [11, 13], malignant hemispheric stroke [5], spontaneous intracerebral hemorrhage, and traumatic brain injury (TBI) [14, 19, 38]. The full spectrum of SD from short-lasting to very long-lasting events was recorded in patients with aSAH [31] and TBI [19]. Interestingly, SDs also occurred in abundance in patients with delayed cerebral ischemia after aSAH in whom angiographic vasospasm was prevented by nicardipine prolonged-release implants [42]. There is increasing evidence that SDs represent an independent risk factor for poor outcome in patients with TBI [17] and aSAH [12].

The Normal Neurovascular Response to SD

In the normal neurovascular response to physiological neuronal activation and deactivation, neurotransmitter and neuropeptide release during synaptic transmission is involved [1]. Neuronal activation is associated with glutamate-evoked calcium influx in postsynaptic neurons, which activates production of nitric oxide (NO) and arachidonic acid metabolites. This results in vasodilation that reflects both presynaptic activity and the degree of postsynaptic depolarization. Furthermore, astrocytes sense glutamatergic transmission via metabotropic glutamate receptors, and signal vascular smooth muscle via end feet through arachidonic acid pathways [22]. SD is associated with more pronounced changes than physiological neuronal activation, but principles underlying the neurovascular response to SD may be remarkably similar to those underlying the neurovascular response to physiological neuronal activation [6]. This applies to the release of glutamate and vasoeffectors like NO and arachidonic acid metabolites, ion flux directions, and increase in metabolism and energy demand [25, 41].

Thus, rCBF increases in response to SD by more than 100%. This increase in rCBF typically propagates in the tissue together with the depolarization wave [28]. Therefore, it is termed *spreading hyperemia*. The spreading hyperemia seems to serve several purposes, including increased delivery of energy substrates such as glucose and oxygen to the tissue and increased clearance of metabolites from the extracellular space [39]. It outlasts SD and only ends after about 2 min. After the spreading hyperemia, rCBF shows a mild decrease for up to 2 h. This normal mild rCBF reduction after SD is called spreading oligemia [24]. During spreading oligemia, vascular responses to arterial pCO_2 changes and functional activation are attenuated [32].

The Inverse Neurovascular Response to SD

The inverse neurovascular response to SD is a marked, prolonged hypoperfusion due to severe arteriolar constriction that is evoked by SD under specific pathological conditions [6]. The inverse response occurs when there is local dysfunction of the microvasculature. With the inverse response, severe microarterial spasm instead of vasodilation is coupled to the

neuronal depolarization phase of SD [10, 35, 37]. The resulting spreading perfusion deficit prolongs the neuronal depolarization phase since there is no energy for neuronal repolarization. This is observed as a prolonged negative slow potential change, also referred to as a negative direct current (DC) shift. During the inverse neurovascular response, neurons are particularly challenged as energy demand is increased and energy delivery is simultaneously decreased. The resulting prolonged depolarization is therefore more likely to cause lasting neuronal damage, as explained previously. In other words, the inverse neurovascular response to SD can convert a relatively harmless short-lasting SD into a harmful intermediate or even terminal SD. The inverse neurovascular response can thus cause widespread cortical necrosis in animals [7] in contrast to SD under physiological conditions to which hyperemia is coupled [29].

The hypoperfusion as a consequence of the inverse rCBF response to SD is often called spreading ischemia [10] to distinguish it from the harmless physiological spreading oligemia that occurs following SD-induced hyperemia under physiological conditions. Current experimental findings suggest that spreading ischemia results from a vicious cycle due to disturbed microvascular reactivity. This vicious cycle has been explained in more detail elsewhere [6]. Most important, the classic condition that causes this vicious cycle is a decrease of cortical NO availability combined with an increase of baseline extracellular potassium. Both conditions occur when blood lyses in the subarachnoid space after aSAH. Therefore, based on animal experiments, it was suggested that spreading ischemia may be a pathophysiological mechanism involved in the development of delayed cerebral ischemia after aSAH [10].

Spreading Ischemia in Patients After aSAH

Based on this experimental hypothesis, a prospective, multicenter study of the Co-Operative Studies on Brain Injury Depolarizations (COSBID) group was performed [11]. In this study, 13 patients with aSAH were investigated using subdural optoelectrode technology for simultaneous laser-Doppler flowmetry and DC-ECoG, combined with recordings of tissue partial pressure of oxygen. In total, 603 SDs were recorded in 2,467 h of ECoG recording time. SDs were characterized by a DC shift of −10.8 (−9,7, −13,7) mV (median, first, and third quartile, respectively) and a propagation velocity of 2.1 (1.8, 2.9) mm/min. Simultaneous ECoG and rCBF were measured in 1,953 h of recording time, during which 417 SDs were recorded. Isolated SDs were seen in 12 patients. Physiological, absent, or inverse rCBF responses were coupled to such isolated SDs. The normal hyperemic neurovascular response was associated with tissue hyperoxia, whereas tissue hypoxia accompanied the inverse response. In association with structural brain damage, temporal clusters of prolonged SDs were measured in five patients. These clusters were typically associated with the inverse neurovascular response, spreading ischemia. Spreading ischemia could last for over 2 h during such clusters. Progressively hypoxic responses during clusters of SDs were also found in a subsequent study by Bosche and colleagues [2].

Pooling of all SDs with simultaneous recordings of ECoG and rCBF showed that 295 (71%) of 417 SDs had no initial hypoperfusion longer than 0.5 min, 78 (19%) SDs had an initial hypoperfusion of 0.5–2 min, and 16 (4%) had initial hypoperfusions of 2–5 min. The remaining 28 (7%) SDs showed spreading ischemia of 5–144 min in at least one optoelectrode pair. Importantly, the duration of SD-induced hypoperfusion correlated strongly with the duration of depolarization measured by the negative DC shift. These studies therefore suggested that, as in experimental animals, inverse coupling/spreading ischemia is a mechanism in the human brain that converts relatively harmless, short-lasting into deleterious long-lasting SDs.

Low-Frequency Vascular Fluctuations

Combined ECoG and rCBF monitoring also uncovered a characteristic vascular signature that might be used for noninvasive detection of SDs [6]. Low-frequency vascular fluctuations with a frequency of less than 0.1 Hz are determined by the resting spontaneous activity of the normal brain and can be detected by imaging methods such as near-infrared spectroscopy or functional magnetic resonance imaging [16]. SD causes a depolarization block of action potential generation, which disrupts this resting state and results in a spreading wave of depressed spontaneous activity [21]. Using subdural optoelectrode technology for simultaneous laser-Doppler flowmetry and ECoG, it was found that the SD-induced spreading depression of activity was accompanied by a spreading suppression of low-frequency vascular fluctuations in aSAH patients [11]. This characteristic vascular signature was associated with all neurovascular responses to SD, including physiological, absent, or inverse coupling. Importantly, the duration of the suppression of the low-frequency vascular fluctuation correlated significantly ($R = 0.91$) with the duration of suppression of spontaneous ECoG activity.

Conclusion

Because the duration of suppression of spontaneous ECoG activity also correlates with the duration of the negative DC shift during SD, the spreading suppression of low-frequency vascular fluctuations may serve as a novel functional index of SDs and a noninvasive, real-time measure of ischemic cell damage in the human brain.

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

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Full-Band Electrocorticography of Spreading Depolarizations in Patients with Aneurysmal Subarachnoid Hemorrhage

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Abstract Cortical spreading depolarizations (CSDs) are a pathologic mechanism occurring in patients with aneurysmal subarachnoid hemorrhage and may contribute to delayed cerebral ischemia. We conducted a pilot study to determine the durations of depolarizations as measured by the negative direct current shifts in electrocorticography. Cortical electrode strips were placed in six patients (aged 35–63 years, Fisher grade 4, World Federation of Neurosurgical Societies [WFNS] 3–4) with ruptured aneurysms treated by clip ligation. Full-band electrocorticography was performed by direct current amplification (g.USBamp, Guger Tec, Graz, Austria) with ±250-mV range, 24-bit digitization, and recording/display with a customized BCI2000 platform. We recorded 191 CSDs in 4 patients, and direct current shifts of CSD ($n=403$) were measured at 20 electrodes. Amplitudes were 7.2 mV (median; quartiles 6.2, 7.9), and durations were 2 min 14 s (1:53, 2:45). Ten direct current shifts in two patients with delayed infarcts were longer than 10 min, ranging up to 28 min. Taken together with previous studies, results suggest a threshold of 3–3.5 min to distinguish a normally distributed class of short CSDs with spreading hyperemia from prolonged CSDs with initial spreading ischemia. Results further demonstrate the clinical feasibility of direct current electrocorticography to monitor CSDs and assess their role in the pathology and management of subarachnoid hemorrhage.

Keywords Cortical spreading depression • Spreading depolarization • Electrocorticography • Electroencephalography • Stroke • Subarachnoid hemorrhage • Delayed cerebral ischemia • Vasospasm • Neuromonitoring • Neurocritical care

Introduction

Delayed cerebral ischemia (DCI) is the leading cause of mortality and disability in patients with aneurysmal subarachnoid hemorrhage (SAH) [21]. Occurring from days 4 to 14 after hemorrhage, DCI is evidenced by clinical deterioration, such as new onset of focal neurological impairment, and may result in the appearance of new infarcts on imaging that were not present after securing the aneurysm [25]. DCI is presumably a consequence of extravasation of blood as its toxic by-products exert effects on proximal arteries, microvasculature, or underlying cerebral cortex. However, cortical lesions represent by far the most abundant pathomorphological finding in autopsy studies of DCI after SAH [23] and are more common than territorial infarcts [8, 22]. These patterns suggest that DCI cannot be explained only by proximal vasospasm as diagnosed through angiography.

A novel pathologic mechanism described in experimental and clinical studies in SAH is cortical spreading depolarization (CSD), as reviewed recently [1, 4, 16]. CSD describes a class of pathologic electrochemical waves that slowly propagate through cerebral gray matter at 2–5 mm/min. CSD is elicited when the Na^+/K^+ ATP-ase (adenosine triphosphatase) fails to compensate for cation influx of Na^+ and Ca^{2+}, resulting in near-complete sustained depolarization of

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neurons and glia. Water follows the net gain of intracellular solutes, resulting in abruptly developing cytotoxic edema. CSD is observed as an abrupt, large, negative potential shift in the low-frequency or near-direct-current (DC) range of the electrocorticogram (ECoG) [2, 13, 19]. The negative DC shift closely relates to the near-complete breakdown of electrochemical gradients across cellular membranes during mass depolarization and therefore generally mirrors the rise of $[K^+]_o$ and decline of $[Na^+]_o$ and $[Ca^{2+}]_o$ [11, 17]. In the high-frequency or alternating current (AC) range of the ECoG, CSD causes silencing of spontaneous activity (spreading depression).

Recovery from CSD depends on adequate cerebral blood flow and intact neurovascular coupling to match the increased demand for ATP (adenosine triphosphate) and restore ion gradients by the Na^+/K^+ pump. Thus, with normal neurovascular coupling, the hemodynamic response to CSD is spreading hyperemia, which allows for rapid repolarization, recovery of the negative DC shift, and restoration of spontaneous activity [20]. In the presence of erythrocyte degradation products such as potassium and hemoglobin, however, the hemodynamic response inverts to a cortical spreading ischemia due to profound microvascular constriction in response to depolarization [5, 6]. Cortical spreading ischemia results in prolongation of the depolarization, reflected in the duration of the negative DC shift. Prolonged depolarizations accompanied by spreading ischemia result in necrotic cortical lesions in animals [5, 19] and are a candidate mechanism underlying DCI in humans [9]. Therefore, the negative DC shift duration may serve as an index of the pathogenic effect of CSD.

Most clinical studies of CSD have used AC ECoG in the 0.1–100 Hz range, which precludes a direct assessment of the duration of tissue depolarization reflected by the negative DC shift at frequencies less than 0.1 Hz [3, 9, 10, 12]. Therefore, the durations of CSD-induced depression of spontaneous activity are used as a less-direct, although important, functional measure of the impact of CSD. More recently, however, Dreier and colleagues have successfully implemented full-band (0–100 Hz) ECoG recordings using DC-coupled amplifiers [7, 19] a technique that is ubiquitous in animal studies of CSD. They found that spreading ischemia occurs in the human brain after SAH and is associated with more prolonged DC shifts. Thus, given the importance of DC-ECoG as a direct extracellular measure of cortical depolarization, the objective of the present study was to gain further experience in full-band ECoG using DC-coupled amplifiers. In particular, we used an amplifier and data acquisition system commonly used in clinical epilepsy studies of brain-computer interfaces. In a pilot study of poor-grade SAH, here we report characteristics of the DC potential shifts of CSD and their association with vasospasm and DCI.

Methods

Clinical Protocol

Six patients with aneurysmal SAH were prospectively recruited. Inclusion criteria were (1) ruptured saccular aneurysm demonstrated by computed tomographic (CT) angiography, (2) onset of symptoms less than 72 h before admission, (3) World Federation of Neurosurgical Societies (WFNS) grade III–IV, (4) diffuse thick or localized thick clot greater than 1 mm on initial CT (Fisher grade 3–4), (5) surgical treatment by clip ligation less than 24 h after admission, and (6) age 18–70 years. Research was approved by the local institutional review board and was conducted in accordance with the Declaration of Helsinki. Written informed consents were obtained from legally authorized representatives prior to the start of study procedures.

At the conclusion of surgery, an electrode strip for subsequent ECoG was placed on the surface of viable cortex in the vascular territory of the affected artery. After surgery, patients were transferred to the intensive care unit, where continuous ECoG was acquired for up to 15 days. Thereafter, electrode strips were removed at the bedside by gentle traction. No hemorrhagic or infectious complications of the electrode strip were encountered. Clinical outcome was assessed at 6 months using the Glasgow Outcome Scale Extended (GOSE) by a telephone interview or clinic visit.

Throughout recordings, patients were ventilated and pharmacologically immobilized as required. Sedation was mostly maintained with propofol or midazolam, and analgesia was provided with fentanyl. Intracranial pressure (ICP) was monitored, if clinically indicated, by a ventricular drainage catheter or an intraparenchymal ICP transducer (Codman, Raynham, MA). Glasgow Coma Scale (GCS) score, blood gases, glucose, and electrolytes were documented regularly.

A delayed ischemic neurological deficit (DIND) was defined as a decrease of at least 2 points on the GCS exam or the occurrence of a new focal neurological impairment that persisted for at least 1 h, occurred more than 3 days after SAH, and was not present after aneurysm occlusion. The diagnosis of a DIND required the exclusion of other causes by routine assessments, including CT imaging, physiologic measures, and appropriate laboratory studies [25]. Serial CT scans were performed postoperatively, at the time of clinical deterioration, and after the monitoring period to screen for delayed infarcts. In addition, four patients had magnetic resonance imaging (MRI) studies after termination of ECoG on days 6–16 post-SAH. Imaging was evaluated by a study neuroradiologist (A.V.) for Fisher grade scoring and to determine the presence of delayed infarcts. Significant vasospasm was defined using transcranial Doppler (TCD) sonography as mean velocity of 200 cm/s or greater in at least one middle

cerebral artery (MCA) [26]. Patients with angiogram-proven vasospasm or elevated TCD velocities were treated with hemodynamic therapy (hypertension, hypervolemia) [24]. Patients with DIND but no evidence of vasospasm were maintained normotensive and euvolemic.

Electrocorticography

The ECoG recordings were made from a linear subdural strip that consisted of six electrodes with 10-mm spacing between contacts (Wyler, Ad-Tech Medical, Racine, WI), and ground was provided by a self-adhesive Ag/AgCl patch electrode on the shoulder. Cortical strip electrodes were connected in a sequential bipolar fashion to a full-band, DC-coupled amplifier (g.USBamp, Guger Technologies, Graz, Austria). The amplifier records with a ±250-mV range and uses 24-bit digitization to obtain resolution less than 30 nV; it was set to digitize the analog signal at 1,200 samples/s. No filtering was performed at the recording stage, yielding a frequency range of 0–600 Hz. When monitored, analog signals of ICP, arterial blood pressure, partial pressure of brain tissue oxygen ($P_{ti}O_2$; Licox, Plainsboro, NJ), and local cerebral blood flow (Hemedex, Inc., Boston) were acquired by the g.USBamp without signal conditioning through a custom interface. Digital ECoG and physiologic signals were recorded and displayed at bedside using the COSBID (CoOperative Study on Brain Injury Depolarisations) Multi-Modal Monitor (M3). The M3 was developed based on the BCI2000 platform, a brain-computer interface tool written in C++ to support a variety of data acquisition systems, which was adapted to meet the needs of multimodal data acquisition and research in neurocritical care (see the chapter by Wilson et al., this volume).

Data Processing and Analysis

For review and scoring, ECoG and physiologic signals were down-sampled to 200 Hz, imported into LabChart software (ADInstruments, New South Wales, Australia), and analyzed off line for CSD. In DC recordings, the signature of spreading depolarization is a sustained negative shift of the slow potential of 2–15 mV lasting more than 1 min [14]. When spontaneous brain electrical activity is present (0.5–100 Hz band), the depolarization also causes electrocorticographic depression, which spreads with the negative DC shift. Thus, spreading depolarizations were identified by (1) the simultaneous occurrence of the negative DC shift and depression of spontaneous activity and (2) the sequential occurrence of DC shifts and depressions on adjacent channels, evidencing the propagation of CSD across the cortex.

DC shifts in bipolar recordings may reflect the superposition (subtraction) of DC shifts occurring individually at each of the two electrodes connected in a recording channel [14]. This distortion precludes analysis of DC shift durations and amplitudes, which can only be interpreted as monopolar signals of an active channel against a neutral reference. Therefore, for analysis of DC shifts, pseudoreferential channels were derived from the original bipolar recordings. Pseudoreferential channels are derived by summing adjacent channels so that the derived channel reflects the potential difference between an active electrode of interest (i.e., with depolarizations) and a silent pseudoreference electrode [13]. An appropriate reference electrode is identified as one with a stable baseline and lack of depolarization activity.

Data are given as median (first, third quartile); $p<0.05$ was considered statistically significant.

Results

ECoG was obtained from six patients who were surgically treated by clip ligation for ruptured saccular aneurysms. Patient characteristics are given in Table 1. All patients were operated within 24 h after admission, and ECoG recordings began within 3 days of onset of SAH symptoms. Long-duration recordings through the period of risk for DCI were possible in only two patients (9.9- and 12.9-day durations). In other patients, recordings were terminated early due to damage to electrode lead wires ($n=3$) and based on clinical decision of a favorable recovery ($n=1$). CSDs were observed in four patients.

A total of nine DINDs occurred in six patients, and two were associated with concurrent vasospasm. ECoG recordings were obtained during only one DIND, which precludes assessment of their association with CSD. Delayed infarcts occurred in four patients. Three of these had both CSD and significant vasospasm; the fourth had neither. Only one delayed infarct was directly attributable to vasospasm. In addition to CSD, two patients had focal electrographic status epilepticus based on ECoG. Patient 1 had 53 seizures lasting 17.2 h, and patient 6 had 28 seizures lasting 8.6 h.

The majority of CSDs (148 of 191, 77%) occurred in 3 patients through day 5 post-SAH. Both patients with recordings through days 6–13 had a total of 43 CSDs in this period. Amplitudes and durations of DC shifts were measured in pseudoreferential derivations of recordings from each electrode. For each CSD, the DC shift was measured at each electrode participating in the wave and having a stable baseline. Thus, 403 DC shifts total were measured for the 148 events, providing an average of 2.72 electrodes analyzed for each depolarization wave. These occurred at 20 of the total possible 24 electrodes of 4 patients. Electrode impedances ranged from 6 to 110 kΩ, with the majority

Table 1 Summary of Patient Characteristics

Patient no.	Age, sex	Fisher grade	WFNS	Aneurysm location, size (mm)	ECoG start (days post-SAH)	ECoG duration (days)	Number of CSD	Max DC shift duration (min)	DIND (days post-SAH)	Vasospasm (days post-SAH)[a]	Delayed infarct	6-month GOSE
1	63, F	4	4	ACoP, 3	0.5	2.5	97	8.3	6.4, 7.7	No	No	4
2	35, F	4	4	MCA, 5	3.0	9.9	17	28.1	15.4	Yes, 4–8	Yes	2
3	49, F	4	4	BCA, 3	1.3	0.9	0	–	7.6	No	Yes	7
4	61, M	4	3	ACA, 2	0.6	3.8	0	–	7.2, 9.3	No data	No	8
5	35, F	4	3	ICA, 4	1.4	12.9	31	7.8	17.5	Yes, 9–17	Yes[b]	8
6	51, F	4	4	ICA, 3	1.5	2.7	46	10.9	3.2, 6.0	Yes, 6–9	Yes	8

ACoP posterior communicating artery, *MCA* middle cerebral artery, *BCA* basal cerebral artery, *ACA* anterior cerebral artery, *ICA* internal carotid artery, *DC* direct current, *CSD* cortical spreading depolarization, *WFNS* World Federation of Neurological Societies scale, *DIND* delayed ischemic neurologic deficit, *GOSE* Glasgow Outcome Scale Extended, *SAH* subarachnoid hemorrhage, *ECoG* electrocorticography

[a]Based on transcranial Doppler (TCD) sonography. Computed tomographic (CT) angiography data were available for patients 2 and 5 and confirmed TCD findings

[b]Vasospasm-related territorial infarct of posterior cerebral artery (PCA)-MCA distribution

(68%) in the 20–40 kΩ range, and offset potentials were typically less than 100 mV.

DC Shift Durations

The median duration of DC shifts at the 20 electrodes was 2 min 14 s (quartiles 1:53, 2:45). Maximum and minimum durations observed at each electrode were 4:35 (3:16, 7:56) and 1:26 (1:10, 1:53), respectively. Median amplitude at the 20 electrodes was 7.2 mV (6.2, 7.9). Figure 1a shows the frequency distribution of all 403 DC shift durations. This distribution differs significantly from normality, with a skew toward larger values (Kolmogorov-Smirnov tests, $p<0.01$). We assumed that within this distribution there is a population of shorter events reflecting CSD as occurs in normal tissue with spreading hyperemia, and that these conform to a normal distribution, whereas longer CSDs in tissue with impaired metabolism or inverse vascular reactivity (spreading ischemia) account for the positive skew. To distinguish these groups, tests for fit to a normal distribution were performed as the longest-duration DC shifts were truncated from the distribution. At a cutoff of 3 min 13 s, the distribution no longer differed from

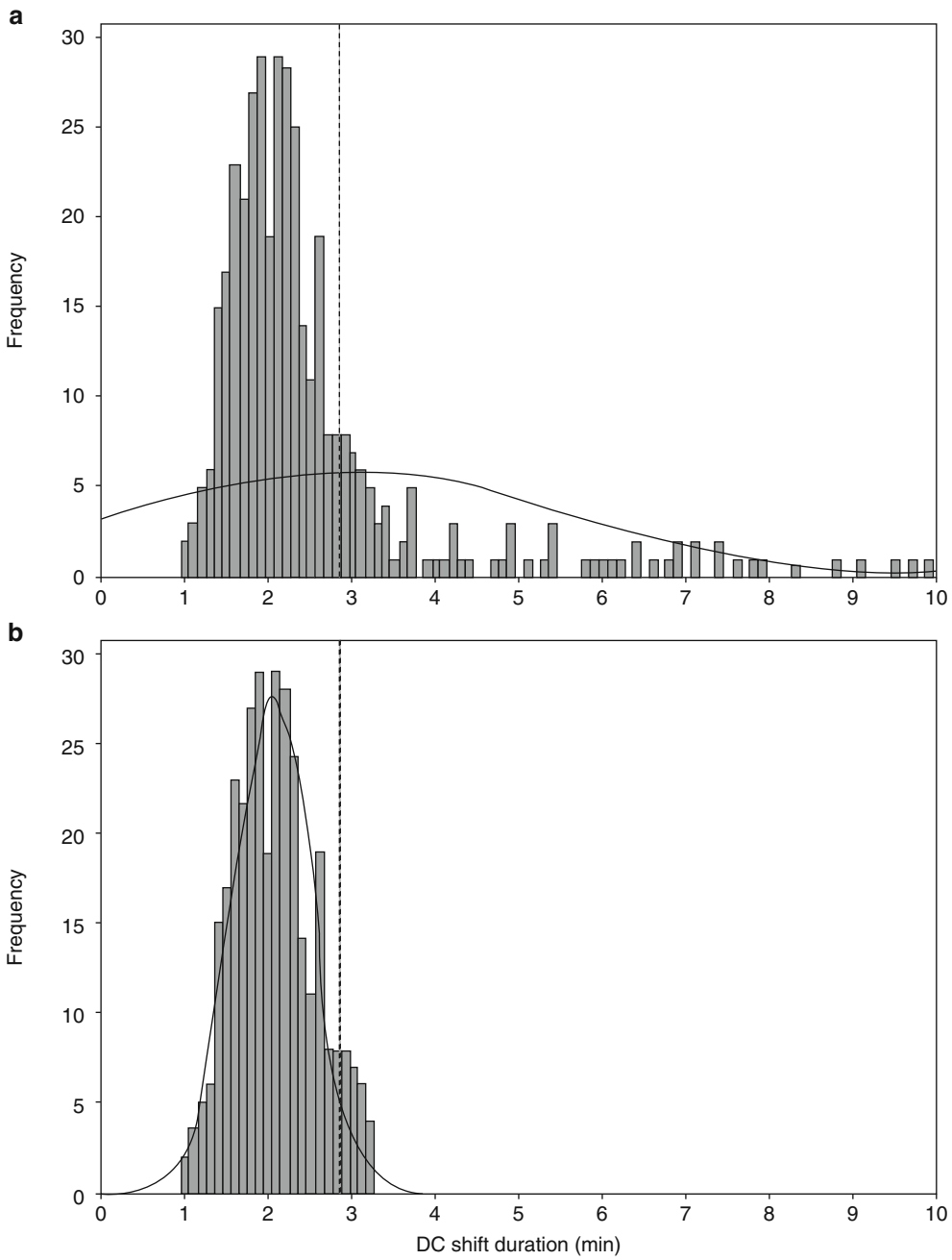

Fig. 1 Histograms of direct current (*DC*) shift durations. Frequency distribution of all 403 DC shifts measured at 20 electrodes in 4 patients (**a**) and of 310 DC shifts after removing all values greater than 3 min 13 s (**b**). *Solid lines* show normal fits to the distributions. *Dashed vertical lines* show the 95% confidence value for the distribution in (**b**)

normality (Kolmogorov-Smirnov, $p>0.05$) (Fig. 1b). For the truncated distribution, the mean was 2 min 4 s (SD 0:29), and 95% of values were less than 2 min 52 s. Using this 95% confidence interval of the truncated distribution as a cutoff, 310 of all 403 (77%) DC shifts were of short duration, and 93 (23%) were prolonged. Prolonged DC shifts occurred at 16 of 20 electrodes and in all 4 patients with CSD.

Case Examples of Very Prolonged DC Shifts

While prolonged DC shifts were common, two patients exhibited particularly long DC shifts lasting more than 10 min. The case example of patient 2 is illustrated in Fig. 2. This 35-year-old female had an MCA aneurysm clipped on day 1 and underwent a decompressive craniectomy on day 2 due to rising ICP and mass effect in association with MCA stroke (Fig. 2d). No CSD was observed from the start of ECoG recording at 3 days post-SAH until day 7. Seventeen CSDs then occurred from day 7 to day 13 (Fig. 2a, b). DC shifts at the anteriorly located electrode 3 were mostly less than 4 min in duration and did not increase. By contrast, at posterior electrode 6, DC shifts were initially less than 5 min but progressively increased to 15 min and then 28 min by day 13. Neurologic exam was steady throughout these episodes, improved from GCS 8 to 10 at 5 h after the last CSD, and remained 10 when no CSD occurred on the last day of recording. Vasospasm was diagnosed by TCD and CT angiography on days 4–8 in left proximal MCA and MCA-ACA (anterior cerebral artery) windows, but flow velocities attenuated thereafter, before prolongation of CSDs. On day 16, MRI showed a new infarction of the basal ganglia (Fig. 2e, f).

In patient 6, an electrode strip was placed at the anterior temporal lobe after clipping an ICA (internal carotid artery) aneurysm (Fig. 3a, b). In 2.7 days of recording, 46 CSDs occurred at four electrodes. All DC shift durations were less than 4 min at three electrodes. In the fourth electrode, durations were less than 2 min through 2.0 days post-SAH, but then 5 CSDs became prolonged until day 3.6, ranging from 5 min 20 s to 10 min 55 s. At this time, a DIND occurred with decrease in GCS from 13 to 9. After ECoG ended, a second DIND occurred at day 6, with the onset of vasospasm diagnosed by TCD flow velocity greater than 200 cm/s in the MCA-ACA window. Follow-up MRI on day 7 showed development of a new infarct in the anterior temporal lobe adjacent to the electrode strip (Fig. 3c, d).

Discussion

This pilot study confirmed the technical feasibility of obtaining long-term, full-band ECoG recordings in patients with acute brain injury. The major challenge of recording with DC amplifiers is an unstable, shifting baseline potential, which complicates recording and review for several reasons. First, there is a possibility that the baseline potential would exceed the input range of the amplifier, resulting in clipping of the signal and loss of data. To prevent this, a lower gain setting could be used, but at the cost of reduced resolution of the lower-amplitude signals in the higher-frequency ranges (>0.5 Hz) typically studied in clinical neurophysiology. Finally, shifting baselines complicate data review because frequent adjustment to amplitude scaling and range are required to maintain signals in display view. AC amplifiers, the common choice for clinical neurophysiology studies, avoid these pitfalls with use of capacitative coupling to filter baseline offset potentials, but at the cost of losing low-frequency (<0.1 Hz) signals.

In our study using the DC-coupled g.USBamp (Guger Technologies, Graz, Austria) and a custom platform for data acquisition and display (see the chapter by Wilson et al., this volume), these theoretical problems were not encountered. Offset potentials between platinum cortical electrodes were usually less than 100 mV and never exceeded the ±250 mV recording range of the amplifier. Furthermore, excellent amplitude resolution to less than 30 nV was obtained with 24-bit digitization. Real-time review and display of data were facilitated by the use of various data-processing tabs customized for study of CSD. These included display of raw and filtered data (to view slow potentials with a constant baseline [0.02 Hz high-pass filter] and to view high-frequency activity [0.5–100 Hz band-pass filter]) in addition to processed measures such as spectrograms and power ratios. Thus, demonstration of full-band recording methods with DC amplifiers, both here and in studies of Dreier and colleagues [7, 19], should enable their broader use.

Previously, we used an inverse filter signal-processing technique to recover DC potentials from recordings made with AC-coupled amplifiers [14]. These studies confirmed that complex, multiphasic, slow potential changes in AC recordings do reflect the typical DC potential shifts of spreading depolarizations as recorded in animals with DC amplifiers. Furthermore, using this technique to analyze 295 negative DC shifts in patients with traumatic brain injury, we found a median duration of 2 min 22 s (1:56, 3:04) [13], which is similar to the values found here in SAH patients (median 2:14 [1:53, 2:45]). Based on these studies, it appears that 3 min is a conservative upper limit for the duration of a normally distributed group of shorter CSD events. These are presumed to be depolarizations coupled to a primary hyperemic response as evoked experimentally in the uninjured brain [15, 18, 20]. Human data support this: Depolarizations with initial hypoperfusion (i.e., spreading ischemia), as recorded with laser Doppler flowmetry adjacent to electrodes, were observed mainly when negative DC shift durations exceeded 3.5 min [7]. Thus, a threshold of 3–3.5 min may represent a physiologic principle in the human brain distinguishing different degrees of CSD pathology.

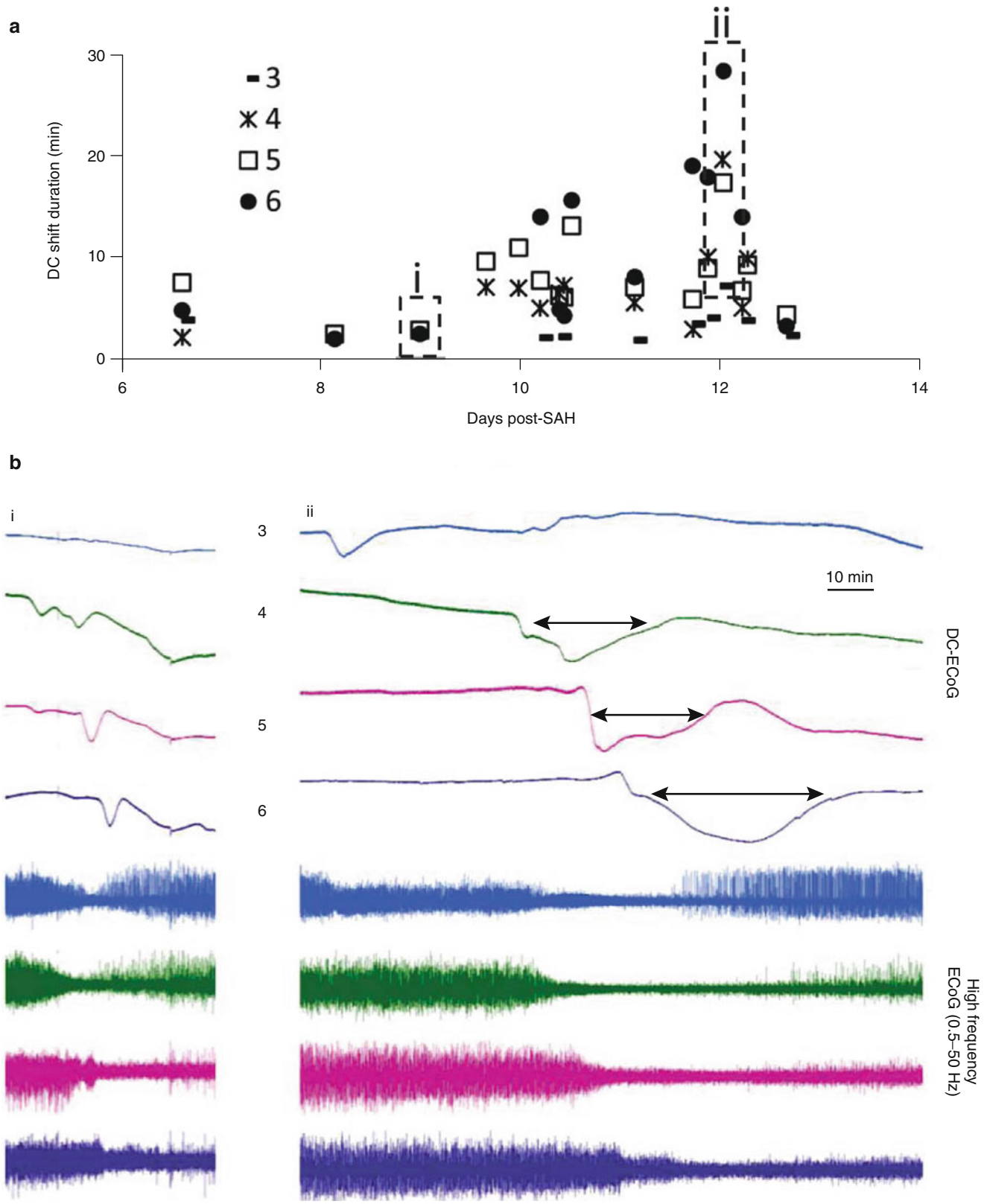

Fig. 2 Prolongation of direct current (*DC*) shifts and delayed infarction. (**a**) The DC shift durations of spreading depolarizations occurring on electrodes 3–6 from days 6 to 13 after subarachnoid hemorrhage (*SAH*). Depolarization waves highlighted with dashed boxes (*i*, *ii*) are shown in (**b**). Top traces in (**b**) show full-band DC-ECoG (*electrocorticography*) and bottom traces show recordings of the same electrodes (3–6) after high-pass filtering (0.5 Hz). Note the prolongation of depolarizations occurring on day 13 (*ii*, *arrows*) compared to day 8 (*i*). (**c**, **d**) Postoperative computed tomographic (*CT*) scans on day 4 after SAH. (**e**, **f**) T2 fluid-attenuated inversion recovery (*FLAIR*) and diffusion-weighted magnetic resonance imaging (*MRI*) scans, respectively, obtained on day 16 after completion of ECoG. Diffusion restriction and hyperintense FLAIR signal in the basal ganglia (*arrows*) are consistent with acute infarction, which is new relative to the postoperative CT

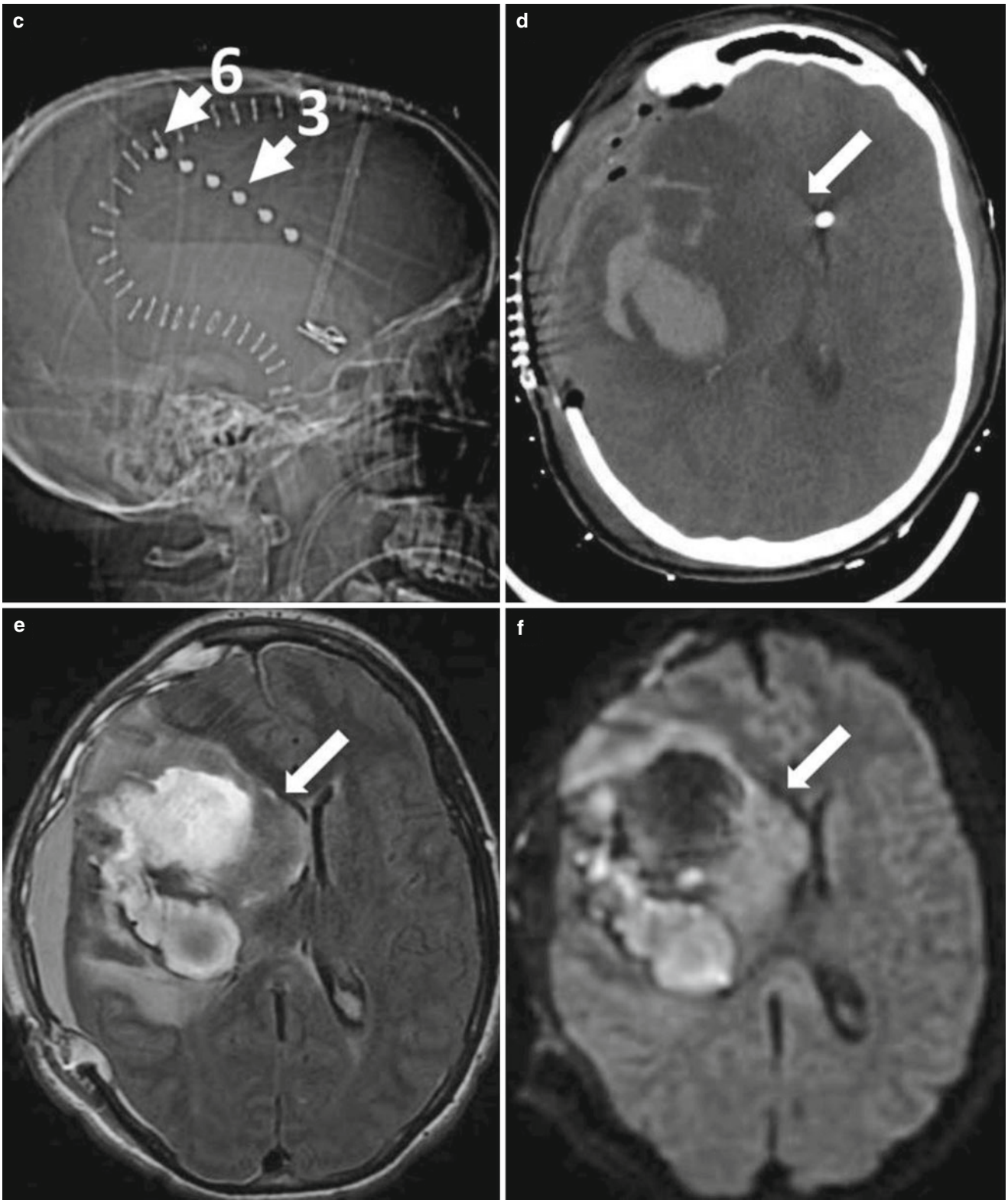

Fig. 2 (continued)

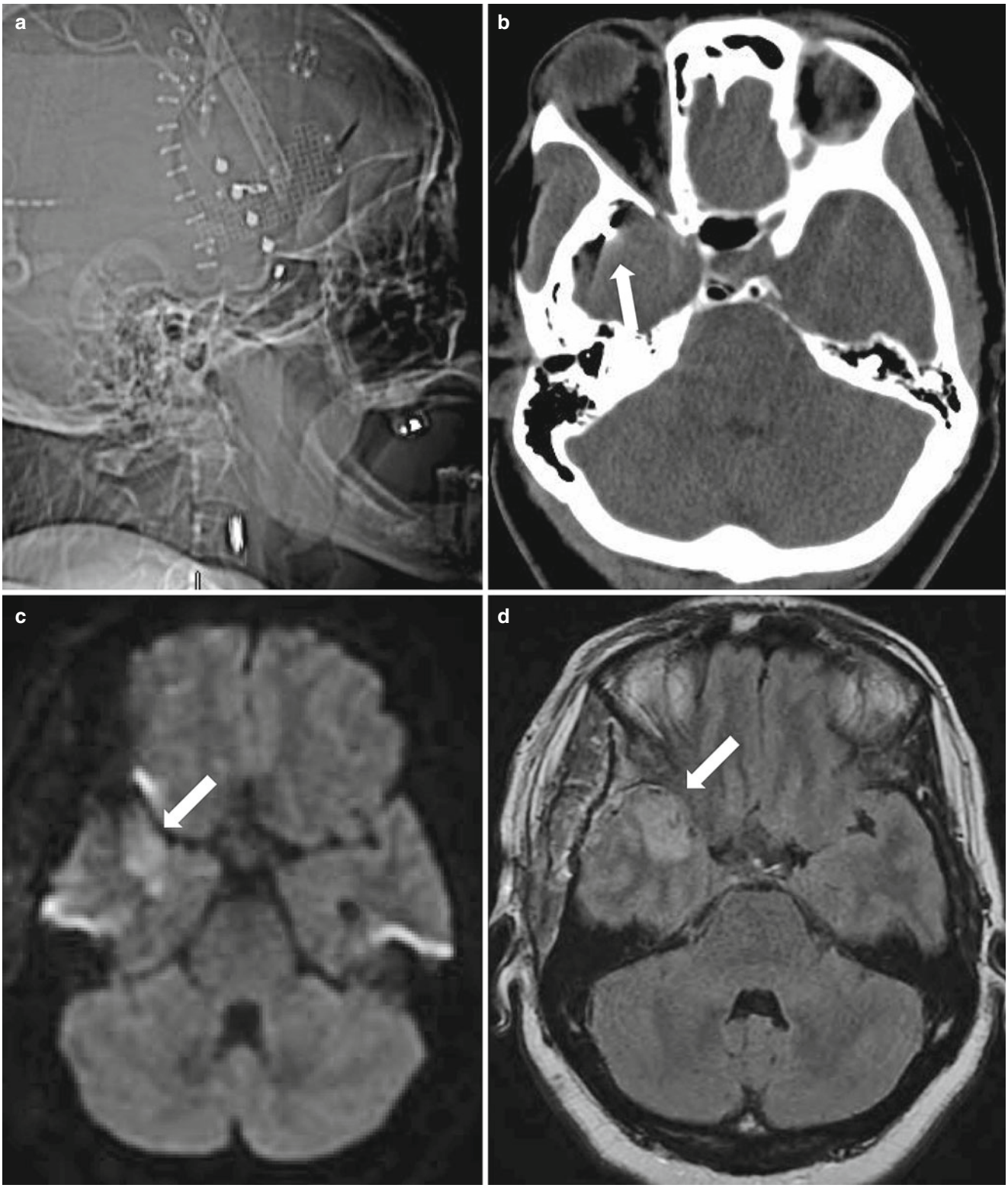

Fig. 3 Development of delayed infarct. (**a**, **b**) The positioning of the electrode strip along the right anterior temporal lobe in postoperative computed tomographic (*CT*) scans on day 2 after subarachnoid hemorrhage (*SAH*). The *arrow* in (**b**) shows the location of electrode artifact. (**c**, **d**) Diffusion-weighted and T2 fluid-attenuated inversion recovery (*FLAIR*) magnetic resonance imaging (*MRI*) scans, respectively, obtained on day 7, after completion of electrocorticography (*ECoG*). Diffusion restriction and hyperintense FLAIR signal in the right anterior temporal lobe (*arrows*) are consistent with acute infarction, which is new relative to the postoperative CT and located adjacent to the electrode strip (**b**)

We found that prolonged negative DC shifts are common in SAH, occurring at 16 of the 20 electrodes that had CSD. This is likely attributable to the uniformly poor grade of patients studied, in whom DIND and delayed infarction occurred with high incidence. Limitations in sample size and ECoG recording durations preclude any assessment of the association of CSD with DCI, vasospasm, or outcome. However, it is noteworthy that two patients with delayed infarct development had CSDs of increasing duration that eventually exceeded 10 min. These results are consistent with a possible role of CSD and ECoG in the pathogenesis and monitoring of DCI, respectively. The value of different measures of CSD, such as high-frequency depression durations, DC shift durations, and their temporal patterns, to monitor DCI is the focus of a larger patient series and deserves further study.

Conclusion

Taken together with previous studies, our results suggest a threshold of 3–3.5 min to distinguish a normally distributed class of short CSDs with spreading hyperemia from prolonged CSDs with initial spreading ischemia. Results further demonstrate the clinical feasibility of direct current electrocorticography to monitor CSDs and assess their role in the pathology and management of subarachnoid hemorrhage.

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Conflicts of Interest We declare that we have no conflict of interest.

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Cerebral Glucose and Spreading Depolarization in Patients with Aneurysmal Subarachnoid Hemorrhage

Asita Sarrafzadeh, Edgar Santos, Dirk Wiesenthal, Peter Martus, Peter Vajkoczy, Marcel Oehmchen, Andreas Unterberg, Jens P. Dreier, and Oliver Sakowitz

Abstract The pathogenesis of delayed cerebral ischemia (DCI) is multifactorial and not completely elucidated. Our objective was to determine if episodes of spreading depolarization (SD) are reflected in compromised levels of extracellular glucose monitored by bedside microdialysis (MD) in aneurysmal subarachnoid hemorrhage (aSAH) patients. Patients with aSAH, prospectively included in the COSBID (CoOperative Study on Brain Injury Depolarisations) protocol (Berlin, Heidelberg), had hourly monitoring of cerebral glucose by MD and in parallel electrocorticographic (ECoG) monitoring for SD detection on day of admission until days 10–14 after aSAH. Cerebral MD probes were placed in the vascular territory at risk for DCI. Twenty-one aSAH patients (53.3 ± 9.1 years; mean ± standard deviation), classified according to the World Federation of Neurosurgical Societies (WFNS) in low (I–III, 11) and high (IV–V, 10) grades, were studied. Of these, 13 patients (62%) presented with DCI. Median glucose was 1.48 (0.00–8.79). Median occurrence of SD was 7 (0–66)/patients. High WFNS grade (WFNS grades IV–V) patients had more SDs ($p=0.027$), while the overall glucose level did not differ. In high-grade SAH patients, SDs were more frequent. Individually, the occurrence of SD was not linked to local deviations (neither high nor low) from the LOWESS (locally weighted scatterplot smoothing) trend curve for extracellular glucose concentrations. Rapid-sampling MD techniques and analyses of SD clusters may elucidate more detail of the relationship between SD and brain energy metabolism.

Keywords Cortical spreading depolarization • Subarachnoid hemorrhage • Electrocorticography • Cerebral microdialysis

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Introduction

Advances in medical and surgical management have resulted in a dramatic decline in hospital mortality after aneurysmal subarachnoid hemorrhage (aSAH) [16]. Nevertheless, aSAH remains a major cause of premature mortality, accounting for 27% of all stroke-related potential years of life lost before age 65 [1]. Treatment of aSAH is based on the prevention and management of secondary complications, mainly secondary deterioration caused by delayed cerebral ischemia (DCI), which occurs in approximately 21% of aSAH patients, most commonly between 4 and 14 days posthemorrhage, and is a leading cause of long-term morbidity [5]. Multivariate analyses revealed that both transcranial Doppler (TCD) sonography and angiographic vasospasm failed to be related to prediction of cerebral infarction [13], suggesting that the pathophysiological model of cerebral ischemia after aSAH may need to consider additional factors other than large-artery spasm.

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Cortical spreading depolarization (SD) waves are associated with dramatic failure of brain ion homeostasis, efflux of excitatory amino acids from nerve cells, increased energy metabolism, and changes in cerebral blood flow [10]. SDs have been monitored in aSAH patients [3] and possibly play an important role in the development of the neuronal injury in both the early and the delayed phase [2]. The consequences of these intrinsic mechanisms are intimately linked to the composition of the brain extracellular microenvironment, the level of brain perfusion, and in consequence brain energy supply [10]. In hypoxic, ischemic, or hypoglycemic brain tissue, SDs will usually occur spontaneously, and recovery occurs with a prolonged time course [9].

As shown experimentally, SD may be more frequent under hypoglycemic conditions. Furthermore, "vasospasm-related" hypoperfusion may lead to a reduction of glucose as the main substrate for the brain cells inducing SDs. We therefore studied the dynamics of glucose using bedside microdialysis (MD) during the occurrence of SDs in patients with aSAH based on the COSBID (CoOperative Study on Brain Injury Depolarisations) protocol (http://www.cosbid.org).

Methods

Multimodal cerebral monitoring, including cerebral MD and subdural electrocorticography (ECoG), was performed in a prospective observational study in 21 patients. Inclusion criteria were age of 18 years or more and a Glasgow Coma Scale (GCS) score of 4 or higher. Exclusion criteria were bilaterally fixed and dilated pupils or other signs of imminent death, as well as a history of trauma/bleed of 5 days or more prior to admission. All research procedures were approved by the Ethics Committee, University of Heidelberg Medical School, and the Ethics Committee, Charité University Medicine Berlin. Informed consent was obtained for all patients.

Operative Procedures

The patients underwent craniotomy for early (<72 h) aneurysm clipping. All patients received an invasive intracranial pressure probe and a flexible cerebral MD probe. In addition to routine monitoring, patients had a subdural 6- or 8-contact linear electrode strip (Wyler, 5/10-mm platinum; Ad-Tech Medical Instrument Corp., Racine, WI) placed on cortex accessible through the craniotomy or a burr hole. Care was taken to insert the MD probe close to the ECoG electrode strip.

Postoperative Care

All patients were treated in the intensive care unit for as long as indicated by their primary disease or other organ system disorders. Treatment of increased intracranial pressure was initiated when necessary. Treatment of DCI with hypertensive, and if necessary, with hypervolemic, and hemodilutional therapy was performed as soon as it was diagnosed. DCI was defined as symptomatic vasospasm (delayed ischemic neurological deficit, DIND), infarction attributable to vasospasm, or both [5]. A DIND was defined as the occurrence of focal neurological impairment, or a decrease of at least 2 points on the GCS. This should have lasted for at least 1 h, not be apparent immediately after aneurysm occlusion, and not be attributed to other causes, such as hydrocephalus, sepsis, or rebleeding as assessed by clinical assessment, computed tomography (CT), and appropriate laboratory studies [17]. A postoperative CT scan was carried out in every patient to confirm the location of the probes. Neurological, GCS, and pupil exams were carried out hourly.

Bedside Microdialysis

An MD catheter with a 10-mm membrane was inserted into the brain parenchyma of the corresponding vascular territory of the aneurysm, with the catheter tip located approximately 1.5 cm from dura level (CMA 71, CMA, Sweden; molecular weight limit of 100 kDa). Care was taken to avoid insertion into macroscopically lesioned brain tissue or into an intracerebral hemorrhage. The correct positioning of the catheter tip was verified postoperatively by CT. Catheters were perfused with sterile Ringer's solution at a flow rate of 0.3 μl/min. Molecules from the cerebral extracellular fluid enter into the catheter with an estimated recovery of 0.65–0.72 [6, 7]. On the outlet tube, perfusates were collected in microvials and analyzed hourly at bedside in a mobile photometric, enzyme-kinetic analyzer (CMA 600). MD was performed for 10–14 days after SAH, and daily medians of the microdialysate concentrations were calculated for each patient. Decreases in glucose below 0.6 mmol [14] and below 1.0 mmol were interpreted as critical and as a deterioration of cerebral metabolism, respectively.

Statistical Analysis

All statistical analyses were performed by statisticians and coauthors of the present study (D.W., P.M.). They were blinded to the clinical details of the patients. The initial 12 h after the placement of the MD catheter were not used for analysis to avoid invalid measurement. The ECoG data for each patient were analyzed using Chart v.7.0 by experienced members of the COSBID group and coauthors of this study (J.D., O.S.) The hourly measurements of MD metabolites were used to explore the relative changes after each SD. One measurement before each SD was used as a

reference (basal value named "value 0") for the next two hourly measurements. Curves of extracellular glucose concentrations were determined by applying local regression (locally weighted scatterplot smoothing, LOWESS). LOWESS curves depict the individual trend of a given parameter. Glucose concentrations above and below the LOWESS curve were analyzed when they coincided with an SD. The sign test was performed to compare SDs associated with glucose levels above or below the LOWESS curve. The SPSS software package was used for statistical analyses and graphical presentation of the results (SPSS v. 17.0, Chicago, IL, USA). We regarded p values less than 0.05 as significant.

Results

Patient Characteristics

In 21 patients, combined ECoG recording and simultaneous MD measures were available. The mean age was 53.3 ± 9.1 years. Twelve patients were female (57%). Patients were classified according to the World Federation of Neurosurgical Societies (WFNS) in low (I–III, 11) and high grade (IV–V, 10). Thirteen patients developed DCI.

SD and Cerebral Glucose Levels

Median occurrence of SD was seven events (0–66, range)/patient, and 3 patients had no SD during the intensive care stay. Median glucose was 1.48 mmol (0.00–8.79, range). As glucose concentrations were difficult to compare between patients, data were categorized according to being above or below the LOWESS curve (Fig. 1). During and 1 h after SD, the levels of cerebral glucose in the interstitial brain fluid of aSAH patients were slightly but not significantly lower than the LOWESS curve ($p=0.210$, sign test). In individual patients, critically low phases of cerebral glucose (<0.6 mmol) [14] were observed. Patients with high WFNS grade had more SDs ($p=0.027$, t test), while the overall glucose level did not differ significantly ($p=0.844$, t test of means of LOWESS curve between high and low WFNS grade patients; Fig. 2).

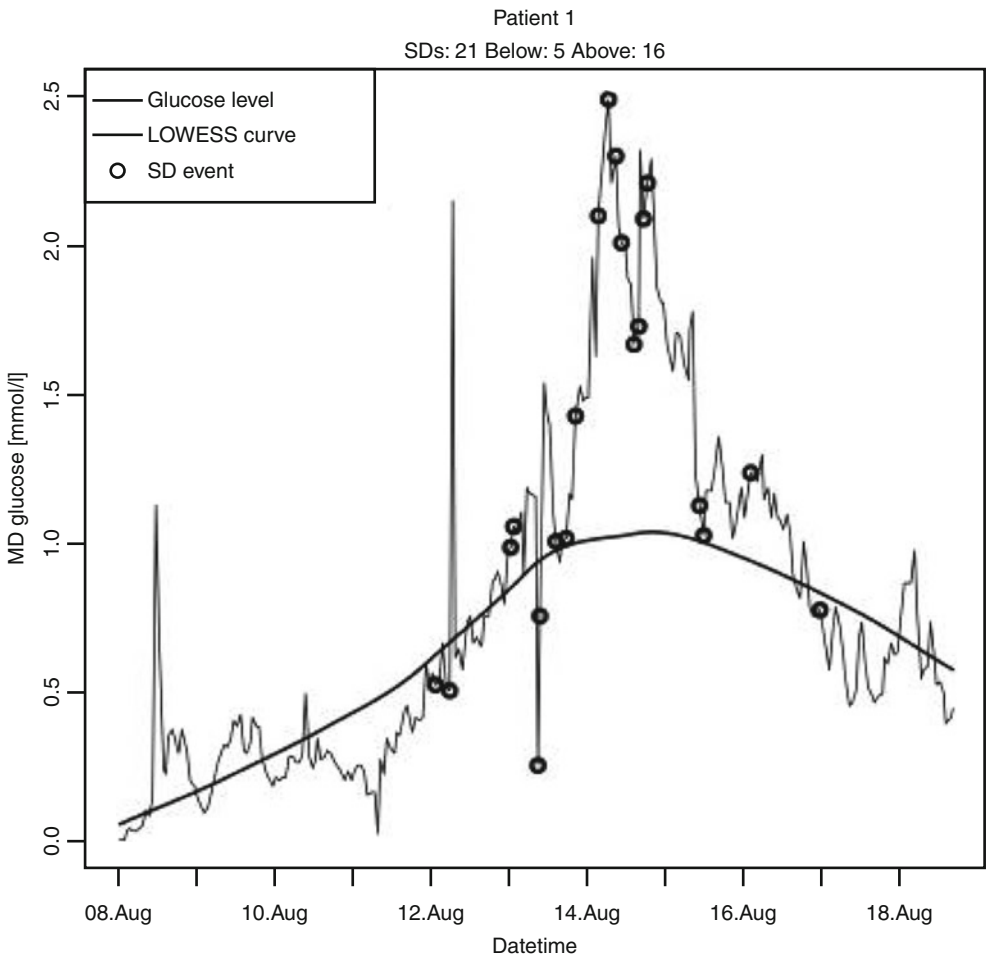

Fig. 1 Individual example showing the variability of cerebral glucose during 11.5 days. The *thicker line* corresponds to the locally weighted scatterplot smoothing (*LOWESS*) curve. The patient had 21 SDs, marked by circles. Sixteen occurred during local "highs" and 5 during local "lows" as compared to the LOWESS curve. Of note, during the first 4 days, cerebral glucose was critically low (<0.6 mmol [14]). *MD* microdialysis, *SD* spreading depolarizations

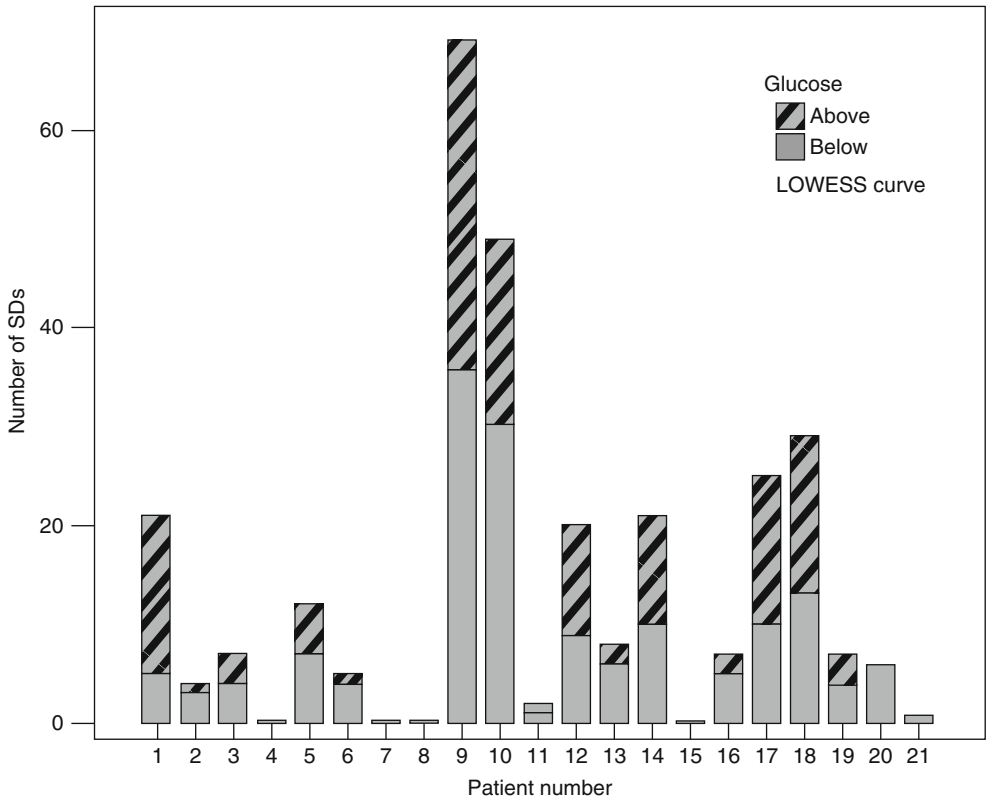

Fig. 2 The number of spreading depolarizations (*SDs*) for each individual patient is shown. In almost all patients, SDs occurred *above* and *below* the locally weighted scatterplot smoothing (*LOWESS*) curve

Discussion

In high-grade aSAH patients, SDs were more frequent, while MD glucose levels remained stable. Critically low phases of cerebral glucose were observed in individual patients. Individually, the occurrence of SD was not linked to local deviations (neither high nor low) from the trend curve for extracellular glucose concentrations.

The brain extracellular fluid maintains nutriment trafficking (oxygen and glucose) among the brain cells. Microdialysis is an in vivo sampling technique used to monitor the variations in the composition of extracellular fluid in a specific tissue or organ. Profound changes in extracellular glucose, lactate, glutamate, and glycerol in patients with signs of acute or DCI have been shown [8, 11]. Hyperglycemia appears not to be automatically reflected in high cerebral glucose levels [14], and critically low cerebral glucose is associated with unfavorable outcome after traumatic brain injury [18] and after aSAH [15]. As shown experimentally, SD may be more frequent when cerebral glucose is low. Furthermore, vasospasm-related hypoperfusion may lead to a reduction of glucose as the main substrate for the brain cells, inducing SDs. Rapid-sampling MD has consistently shown rapid transient decreases of glucose and increases of lactate in the injured human brain in patients with traumatic brain injury [4, 12]. In the present study, the MD technique used employs a longer time interval of monitoring (hourly analysis), limiting the assessment of short SD-associated metabolite changes. Furthermore, spatial resolution may not always be optimal as the distance of the microdialysis probe to the possible ischemic/hypoperfused brain tissue is variable among the patients. This is a common problem of every regional neuromonitoring technique. We also observed a high variability of basal metabolite concentrations before SD. Because of these significant interindividual differences between baseline levels, we used the LOWESS approach and did not analyze absolute concentration changes. Accordingly, we cannot argue that low extracellular brain glucose does not affect SDs, but alterations (either "high" or "low") of glucose in individual patients are not linked to occurrence of SD. Additional limitation stems from the low sampling frequency of MD (i.e., 1/h). Rapid-sampling MD techniques and analyses of SD clusters may elucidate more detail of the relationship between SD and brain energy metabolism.

Conclusion

In high grade aSAH patients, SDs were more frequent while MD-glucose levels remained stable. Though critically low phases of cerebral glucose were observed in individual patients, the occurrence of SD was not linked to local deviations (neither high nor low) from the trend curve for extracellular

glucose concentrations. Rapid sampling MD techniques and analyses of SD clusters may elucidate more detail of the relationship between SD and brain energy metabolism.

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Financial Disclosure We declare that we have no financial interest in the monitoring device described.

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Evaluation of Intracranial Electrocorticography Recording Strips and Tissue Partial Pressure of Oxygen and Temperature Probes for Radio-Frequency-Induced Heating

Michael Scheel, Jens P. Dreier, and G. Bohner

Abstract Spreading depolarization and subsequent cortical spreading ischemia have been recognized as new mechanisms of ischemic damage in patients with subarachnoid hemorrhage. We are investigating these mechanisms using intracranial implanted devices and perform magnetic resonance imaging (MRI) to monitor for early or delayed ischemia. Before patients undergo MRI with intracranially implanted devices, MR safety with respect to heating induced by radio frequency (RF) needs to be carefully considered. We tested an electrocorticography (ECoG) six-contact electrode strip (Adtech TS06R-SP10X-000) at 1.5 T and a tissue oxygenation/temperature Licox™ probe (model CC1.P1) at 3.0 T for RF-induced heating as MRI safety tests were not available at these field strengths. We observed no relevant temperature increases for the ECoG probe at 1.5 T. For the Licox probe, temperature increased beyond 4°C when measurements were performed at 3.0 T. Our data suggest that MRI can be safely performed in patients with an implanted ECoG electrode strip at 1.5 and 3.0 T. For the Licox probe, MRI can be performed at 1.5 T according to safety regulations, but at 3.0 T, temperature increases pose a significant risk for tissue damage due to RF-induced heating.

Keywords Cortical spreading depolarization • Electrocorticography • Cerebral oximetry • Subarachnoid hemorrhage • Magnetic resonance imaging

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Introduction

In patients with subarachnoid hemorrhage (SAH), spreading depolarization has been identified as a new damaging mechanism [8, 9]. The DISCHARGE-1 (Depolarisations in ISCHaemia after subARachnoid haemorrhaGE) study, currently under way at our institution, is part of the COSBID (CoOperative Study on Brain Injury Depolarisations) initiative and investigates the association of spreading depolarization with delayed ischemia in patients with SAH. The study protocol includes continuous intracranial electrocorticographic (ECoG) readings (using an ECoG electrode strip) and partial brain tissue oxygenation measurements (using a Licox™ probe; Integra NeuroSciences™ model CC1.P1) over a period of 2 weeks. During these 2 weeks, we performed three magnetic resonance imaging (MRI) examinations to monitor for the occurrence of early and delayed ischemia.

Before patients with implanted devices undergo MRI examinations, special safety considerations need to be made. MR safety considerations associated with medical devices may be split into three categories: (1) displacement and torque, (2) RF heating, and (3) imaging artifacts. For the two devices that we used in our study, RF-induced heating effects were the primary concern. Adtech™, the manufacturer of the ECoG probe, provided us with (otherwise-unpublished) MR safety tests at 3.0 T, but no test results or documentation for 1.5 T were available. The Licox probe, on the other hand, has been rated at 1.5 T as "MR conditional" (i.e., MRI measurements may be performed when adhering to certain safety regulations). However, no test results or documentation at 3.0 T were available for the Licox probe.

We investigated at which field strength we may safely perform MRI measurements when ECoG and Licox probes are implanted. We therefore performed MR safety measurements with the ECoG probe at 1.5 T and with the Licox probe at 3.0 T. Based on the available reports, we expected no deflection or torque for either device and therefore focused on testing RF-induced heating effects.

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Materials and Methods

We tested the ECoG and tissue temperature/oxygenation probes that we use in the context of the DISCHARGE-1 study: (1) a six-contact electrode strip (4-mm platinum iridium contacts within a silicon sheet with stainless steel internal wires and nickel-chromium tail contacts contained within polyurethane tubing; Adtech TS06R-SP10X-000) and (2) a Licox probe (Integra NeuroSciences, model CC1.P1). Each device was placed in a phantom with the approximate size of a human head (width × height × depth = 13 × 15 × 20 cm). The phantom was filled with gelled saline (1.6 g NaCl/l) simulating electrical conductivity of human tissue. Both devices were placed in a typical configuration that would be used for recording in patients. Temperature recording was performed continuously during the MRI protocol described in the following using a fluoroptic temperature measurement kit (model m3300, Luxtron Corp., accuracy ± 0.5°C at a rate of 0.1 Hz). The two thermal fiber-optic probes were placed at the first (most distant) and third contact of the six-contact electrode strip. While testing the Licox probe, we placed the two thermal fiber-optic probes at the tips of the two internal wires of the Licox probe. These locations were chosen as they will most likely demonstrate the largest temperature change [1, 13]. The MRI protocol included spin echo, inversion recovery, and echo planar imaging sequences. Table 1 describes the full details of the tested scanners and imaging protocol. At both scanners, the body coil served as the transmit coil and the head coil as the receiver coil.

Table 1 Sequence parameter at the 1.5-T and 3.0-T scanner

Sequence	TR (TI)	TE	SL	SAR W/kg
Adtech: *ECOG strip electrode at 1.5-T Phillips Intera*				
FLAIR	6,000 (2,000)	150	6	1.8
T1 spin echo	450	14	6	1.5
DWI	4,330	95	6	0.2
T2*	830	30	6	0.1
3D-T1-FFE	7.3	3.3	1	0.1
LICOX: *temperature/pO2 probe at 3.0-T Siemens Trio*				
DWI	7,600	93	2.5	0.14
FLAIR	8,000	100	5	0.28
3D-TOF	24	3.9	0.6	0.27
T1-SE	500	9	4	0.15
T1-GRE	292	2.5	5	0.31

DWI diffusion-weighted imaging, *FFE* fast field echo, *FLAIR* fluid-attenuated inversion recovery, *TOF* time-of-flight angiography, *SE* spin echo, *GRE* gradient echo, *TR* time of repetition, *TE* time of echo, *SL* slice thickness, *SAR* specific absorption rate

Results

We observed no relevant temperature increases for the ECoG probe. Temperature changes always stayed below 1°C, not exceeding baseline temperature fluctuations (Fig. 1). Testing the Licox probe at 3.0 T, we observed relevant temperature increases up to 4°C. Temperature increases were different for the two temperature probes; the one at the more distant internal wire tip (probe 2) showed more pronounced increases (Fig. 2).

Discussion

When adhering to safety recommendations, MRI can be safely performed in patients with implanted medical devices and is done literally every day at many institutions worldwide. However, various case reports notably demonstrated that implanted devices may pose a significant risk to patients undergoing an MRI examination [7, 12, 14, 15]. Therefore, before an MRI is performed in such patients, careful inquiries and, if necessary, specific testing needs to be done. Otherwise, MRI cannot be performed.

For our setup and study protocol of DISCHARGE-1, we found no relevant temperature increase with respect to the Adtech ECoG electrode strip at 1.5 T. The data add to retrospective case series in patients undergoing MRI with intracranial ECoG electrodes that described no adverse effects in clinical observations [3, 6]. Moreover, previous phantom studies showed that MRI can be safely performed following safety recommendations [2, 4, 5, 11]. It should be noted, however, that due to complex interactions between different factors contributing to RF-induced heating, individual testing may be necessary for the specific setup to be used [4]. It is noted that "Ad-Tech Medical does not have, or claim FDA [Food and Drug Administration] approved MR-Conditional status for these products, or European certification for safe use with MRI. However (otherwise unpublished) test results for these products are reported in a statement provided by the company suggesting that MRI may be safe under certain specific conditions" [4].

Following safety regulations, MRI may also be safely performed with Licox probes at 1.5 T [10]. However, at 3.0 T, our tests showed a significant temperature increase ($\Delta T \sim 4$°C) for the Licox probe, exceeding safety limits. Therefore, in patients with an implanted Licox probe, MRI should not be performed at this field strength or the probe needs to be removed. These results are in agreement with the current regulations as communicated by Integra NeuroSciences, stating that tests "included radio frequency induced heating, magnetically induced displacement force

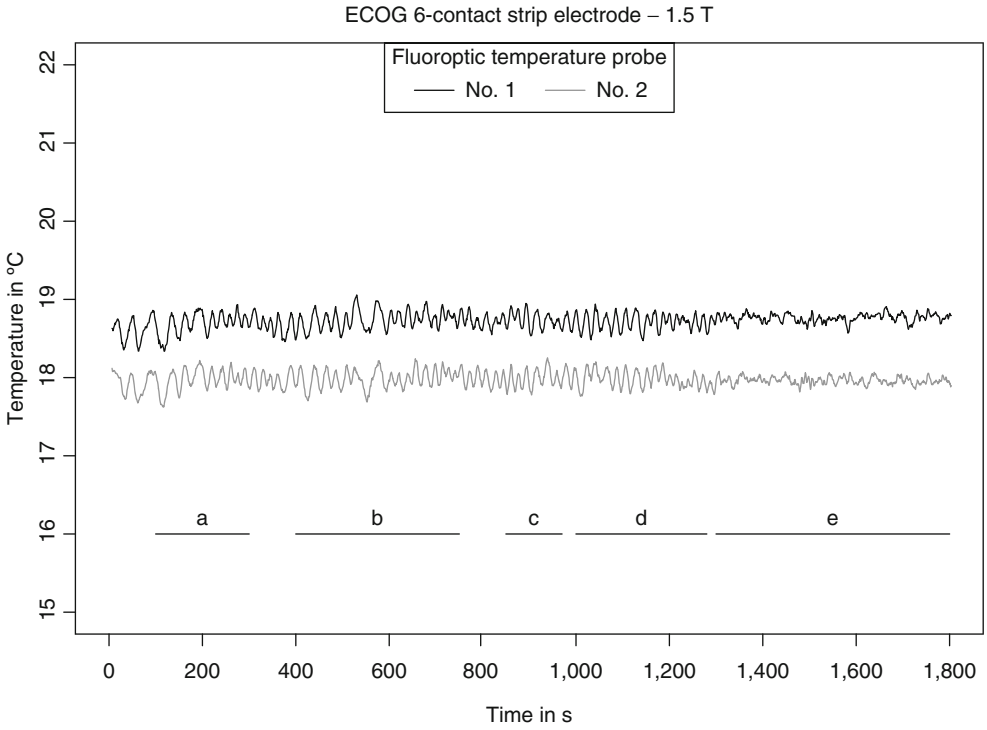

Fig. 1 Temperature changes at the first and third contact of the electrocorticography (*ECoG*) electrode. Letters *a–e* represent the duration of the different sequences: *a* FLAIR (fluid-attenuated inversion recovery), *b* T1-SE (spin echo), *c* DWI (diffusion-weighted imaging), *d* T2*, *e* 3D-T1

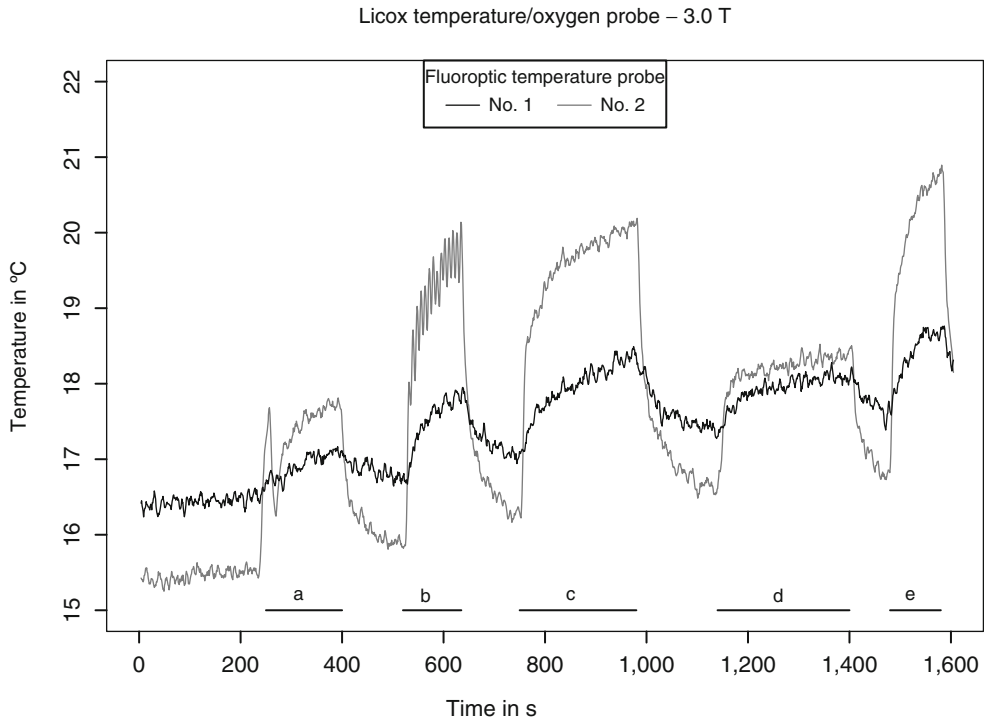

Fig. 2 Temperature changes at tips of the two internal wires of the Licox probe. Probe 2 at the more distant internal wire tip shows pronounced heating. Letters *a–e* represent the duration of the different sequences: *a* DWI (diffusion-weighted imaging), *b* FLAIR (fluid-attenuated inversion recovery), *c* 3D-TOF (three-dimensional time of flight), *d* T1-SE (spin echo), *e* T1-GRE (gradient echo)

and torque to demonstrate that these components were considered MR Conditional as defined in ASTM F2503 … using a 1.5 T MR system, only. Testing has not been performed on other MR Systems and therefore should not be used" [10].

A limitation of our study is that we only tested at one spatial configuration using a body-transmit/head-receive configuration. Using a head-transmit coil reduces the amount of RF energy applied, and it has been shown that by using such a setup, RF-induced heating effects are significantly lower than in the body-transmit/head-receive configuration [4]. We are planning such tests in the near future to investigate different coil configuration and orientations of the devices.

Conclusion

In summary, we performed tests assessing RF-induced heating effects for two intracranial devices (Adtech electrode strip and Licox tissue partial oxygen and temperature probe) demonstrating that at 1.5 T both devices are "MR conditional," that is, MRI can be safely performed when following specific safety regulations. We have now performed more than 50 MRIs at 1.5 T in patients with this electrode strip implanted and never observed any effects that would suggest cerebral damage due to RF-induced heating or other MRI-related adverse events. For the Licox probe at 3.0 T, however, we found temperature increases (4°C) that exceed safety limits; MR examinations should therefore not be performed at 3.0 T without prior removal of the Licox probe.

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Conflicts of Interest We declare that we have no conflict of interest.

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Criteria for the Diagnosis of Noninfectious and Infectious Complications After Aneurysmal Subarachnoid Hemorrhage in DISCHARGE-1

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For the COSBID Study Group

Abstract Patients with aneurysmal subarachnoid hemorrhage (aSAH) frequently develop secondary noninfectious and infectious complications that have an important impact on clinical course and outcome. We here report on criteria for the diagnosis of the most important complications after aSAH based on clinical status, neuroimaging, and laboratory tests, including cerebrospinal fluid parameters. These criteria will be used for a retrospective analysis of aSAH patients who were recruited at the Charité Berlin for the CoOperative Study on Brain Injury Depolarisations (COSBID) before the Depolarisations in Ischaemia after Subarachnoid Haemorrhage-1 (DISCHARGE-1) trial started. Moreover, they serve for the survey of complications in DISCHARGE-1. We also report on a customized, Web-based database that has been developed for the documentation of the clinical course after aSAH. This database is used for the COSBID outcome study on aSAH and for DISCHARGE-1.

Keywords Aneurysmal subarachnoid hemorrhage • Complications • Infections

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Introduction

Subarachnoid hemorrhage has an incidence of 6–11 per 100,000 [1, 18] and accounts for 5% of all strokes [16]. The cause of subarachnoid hemorrhage is a ruptured saccular aneurysm in 85% of cases [24]. Despite improvement in understanding and management of aneurismal subarachnoid hemorrhage (aSAH), the prognosis is still poor. Case-fatality rates vary between 32% and 67%, and one third of the survivors remain dependent on assistance [13].

About 10–15% of patients die immediately after onset of aSAH prior to hospital admission [15]. This is mainly due to the direct impact of the bleeding, which can cause global cerebral ischemia, epileptic seizures, or dysfunction of the cerebrospinal circulation with increase in intracranial pressure and brain herniation. In addition, secondary complications occur in the early and delayed phase after aSAH, such as infections associated with systemic inflammatory response syndrome (SIRS) or sepsis, anemia, electrolyte disorders, cardiac failure and arrhythmia, or lung edema. More than 50% of aSAH patients develop such complications, with important implications for clinical course and outcome [23, 30]. The most important in-hospital complication, however, is delayed cerebral ischemia (DCI), which occurs in at least one third of the patients and is associated with a statistically significant adverse effect on patient outcome [26, 27].

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COSBID Substudy on aSAH and DISCHARGE-1

The term *cytotoxic edema* describes the "Tsunami"-like process of neuronal swelling in the brain cortex that is ignited when passive cation influx across the cellular membranes exceeds adenosine triphosphate (ATP)-dependent sodium pump activity followed by water influx. Dependent on the capacity to recruit additional pump activity, the cytotoxic edema is reversible or not. Thus, the cytotoxic edema marks the transition from life to neuronal death in those neurological conditions that place the highest health burden on our society in terms of mortality, morbidity, and economic costs: stroke and hypoxia. Diffusion-weighted imaging is clinically used for early detection of cytotoxic edema but is unsuitable for continuous bedside monitoring. Extensive experimental work has established that spreading depolarization (SD) is the mechanism that abruptly causes cytotoxic edema in the cortex [6]. Technology has been developed to record SDs in patients using subdural electrode strips [25]. Using this technology, it was shown that SDs occur abundantly in patients with aSAH [4, 7, 9, 20]. DCI was associated with temporal clusters of recurrent SDs [9]. The CoOperative Study on Brain Injury Depolarisations (COSBID) now investigates how to make clinical use of the monitoring of SDs. Specifically, the COSBID outcome study on aSAH aims at investigating whether SDs are associated with worse outcome. DISCHARGE-1 (Depolarisations in Ischaemia after Subarachnoid Haemorrhage-1) is a prospective, multicenter diagnostic phase III trial [22] that is, in a sense, nested in the COSBID outcome study on aSAH. Inclusion and exclusion criteria are given in Table 1. In DISCHARGE-1, serial magnetic resonance imaging is performed to characterize the relationship between SDs and DCI (International Standard Randomised Controlled Trial Number [ISRCTN]: ISRCTN05667702, http://www.controlled-trials.com/ISRCTN05667702). Specifically, DISCHARGE-1 aims at determining a cutoff value for the duration of SD-induced depression of spontaneous brain activity that would indicate DCI in real time and would thus inform about neuroprotective treatment decisions in the future. This may translate the SD concept into clinical practice and may provide novel targets for treatment development and a novel type of proof-of-concept study on neuroprotectants that allows for early selective intervention. For the purpose of these studies on SD that require an invasive electrocorticography (ECoG) recording strip, it is necessary to monitor the diverse non-infectious and infectious complications after aSAH. In the following, the criteria for the diagnosis of these complications are briefly described. Moreover, our database that has been developed for documentation of the various parameters is briefly explained.

Table 1 Inclusion and exclusion criteria

Key inclusion criteria:
Male or female patients aged 18 years or older (inclusive)
World Federation of Neurosurgery (WFNS) grade I–V unless the clinical state suggests an unfavorable prognosis such as wide, nonreactive pupils for more than 1 h
Ruptured saccular aneurysm demonstrated by computed tomographic (CT) angiography or digital subtraction angiography (DSA)
Onset of aneurysmal subarachnoid hemorrhage (aSAH) clinical symptoms in the preceding 72 h
Treatment of aneurysm (clip ligation or endovascular coil embolisation)
Indication for a ventricular drain or oxygen sensor in coiled patients
Informed consent is obtained from the patient or a legal representative
Key exclusion criteria:
aSAH due to other causes (e.g., trauma, fusiform or mycotic aneurysm)
Admission in a clinical state with unfavorable prognosis (e.g., wide, nonreactive pupils for more than 1 h)
Bleeding diasthesis
Cytostatic therapy in patients with malignant disease
Pregnancy
Special exclusion criteria for magnetic resonance imaging (MRI) such as non-MRI-compatible, nonremovable metals, artificial joints and the like, electronic devices (pacemaker, pumps, etc.)
Interruption of monitoring for more than 48 h during days 3–5, for 24 h during days 6–8, or for 24 h during days 9–11 unless the patient dies after day 5 or develops an MRI-documented delayed ischemic stroke during the monitoring period and recording is only interrupted after those events
Respiratory/hemodynamic instability does not allow MR transport (i.e., transport of the patient to the scanner, patient monitoring during imaging, transport back to the ward)

Definition of Time Frames and Time Points

As *initial status*, we define either the period from onset of the first symptom until surgical intervention or the first 24 h if the surgical intervention is performed later than 24 h. All changes in clinical status or pathological findings in this phase are not considered complications but are related to the initial event. As the *early period*, we define the time period following the initial period until 72 h. All events occurring thereafter until day 15 are defined as *delayed*. Hunt and Hess Scale, World Federation of Neurosurgical Societies (WFNS) scale, and Glasgow Coma Scale (GCS) are scored as documented at first contact with a doctor.

Definition of Complications

As a complication we define any (1) worsening of patient status (medical status, neurological status; vital parameters; requirement for new medication, treatments or interventions); (2) new pathological neuroimaging findings; or (3) new pathological parameters in laboratory or cerebrospinal fluid (CSF) tests. We identify 18 groups of adverse events (AEs) and 4 groups of serious adverse events (SAEs) as shown in Table 2. For any event with onset after implantation of the ECoG recording strip, it is documented whether it occurred in relation to the recording strip.

Table 2 Definition of adverse events (AEs) and serious adverse advents (SAEs)

Definition of AEs:
Impairment of the general condition of the patient
Physical injury, including falls
Surgical/interventional complications
Increase of intracranial pressure/hydrocephalus
Infection, sepsis, or systemic inflammatory response syndrome (SIRS)
Cerebral infarct or intracranial bleeding
Epileptic seizure
Vasospasm of cerebral arteries
New neurological deficit
Electrolyte disorder
Clinical worsening of organ functions (heart, lung, gastrointestinal tract, liver, kidney, blood)
Pathological laboratory parameters (if not covered by other AEs)
Intracranial bleeding in the proximity of electrocorticography (ECoG) recording strip
Bleeding or liquorrhea at the exit point of the ECoG recording strip from the skull
Wound infection or dehiscence at the exit point of the ECoG recording strip from the skull
Intended or accidental manipulation of the ECoG recording strip
Bleeding, liquorrhea, infection, or manipulation at other intracranial probes than the ECoG recording strip
Any suspicious findings that may have relationship to the study
Definition of SAEs:
Fatal events/death
Life-threatening events (cause immediate risk of death from the event as it occurred)
Events that require inpatient hospitalization during follow-up period
Required surgical intervention to prevent permanent impairment

The Most Important Noninfectious Complications

A delayed ischemic neurological deficit (DIND) is defined as the occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia, or neglect) or a decrease of at least 2 GCS points (either on the total score or on one of its individual components [eye, motor on either side, verbal]). Moreover, the diagnosis of a DIND requires that the neurological deficit is not present immediately after aneurysm occlusion; that it lasts for at least 1 h; cannot be attributed to other causes such as hydrocephalus or rebleeding by means of clinical assessment, computed tomography or MRI of the brain, and appropriate laboratory studies; and does not occur earlier than 72 h after the initial haemorrhage [28]. DCI is defined by the occurrence of a DIND or evidence of a new brain infarct on serial neuroimaging studies. Any neurological deficit or cerebral infarct detected prior to 72 h is termed *early ischemic neurological deficit* or *early cerebral infarct*, respectively.

Epileptic seizures are defined according to the criteria of the International League Against Epilepsy (ILAE) [5].

Criteria for proximal vasospasm after aSAH are defined using digital subtraction angiography (DSA) as greater than 30% narrowing of the arterial luminal diameter in one of the following arterial segments: A1, A2, M1, M2, and C1–C2. Magnification errors are corrected by comparing extradural segments of the internal carotid artery (C4–C5). Using transcranial Doppler (TCD) sonography, significant vasospasm is defined by a mean velocity of 200 cm/s or greater in at least one middle cerebral artery (MCA) [29]. Vasospasm is excluded if the MCA mean velocities remain below 120 cm/s throughout the observation period.

Criteria for Infections

For SIRS and sepsis, we use the criteria established in 1992 by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference [3].

The following sites of focal infection are considered according to the definitions by the Centers for Disease Control and Prevention (CDC) [11, 14]: surgical site infection (SSI); bloodstream infection/sepsis (BSI); pneumonia (PNU); urinary tract infection (UTI); central nervous system (CNS) infection; cardiovascular system (CVS) infection; eye, ear, nose, throat (EENT), or mouth infection; lower respiratory tract infection (LRI); and gastrointestinal system (GI) infection. For diagnosis, CDC criteria are applied except for meningitis and ventriculitis (see next section).

Criteria for Acute, Nosocomial Meningitis or Ventriculitis and Differentiation from Noninfectious Changes of CSF Parameters Following aSAH

Bacterial meningitis and ventriculitis are frequent complications in neurocritical care patients. Extraventricular drainage (EVD) is an important risk factor for these infections as the drain represents a communicating tube between brain and patient environment that is ideal for the invasion of microorganisms. A meta-analysis reported an incidence of 5–20% for EVD-associated CNS infections [2]. Other intracranial probes, not representing communicating tubes, such as microdialysis (MD) catheters, electrode strips for ECoG recordin, or oxygen sensors are only rarely associated with ventriculitis or meningitis. Nevertheless, the risk is somewhat increased since contamination is possible due to unsterile conditions during the surgical procedure, or an infection may develop per continuitatem from an extracranial focus (e.g., from wound infection or sinusitis). Based on studies in patients undergoing presurgical diagnostics for epilepsy with invasive electrodes, a risk of infection between 2.6% and 3.9% was estimated [10, 17]. These studies, however, were not restricted to electrode strips but also included electrode grids, which show a higher risk.

The diagnoses of ventriculitis and meningitis are a challenge in patients with aSAH since, similar to meningitis, aSAH is typically associated with fever, headache, neck stiffness, meningeal signs, cranial nerve signs, and irritability (Criterion 2 of the CDC definitions). These symptoms not only are observed with the initial hemorrhage but also can recur as symptoms of DCI. Hence, the CDC definition for meningitis can only be used with limitations in aSAH patients. Moreover, aSAH of itself induces a massive inflammatory reaction in the cerebrospinal compartment [19]. We therefore define a bacterial, nosocomial meningitis or ventriculitis according to the following criteria given in Table 3: (1) cultured organisms from CSF (Criterion 1 of the CDC definitions), (2) development of clinical signs typical of a CNS infection and worsening of CSF parameters in the course of the in-hospital stay (modified Criterion 2 of the CDC definition), or (3) MRI-proven signs for CNS infection. The cell index (ratio of leukocytes to erythrocytes in CSF and leukocytes to erythrocytes in peripheral blood) is also calculated as a potential marker for CNS infections in aSAH patients [21].

Table 3 Criteria for meningitis or ventriculitis

Patient must meet at least one of the following criteria:
Criterion 1: Patient has organisms cultured from cerebrospinal fluid (CSF).
or
Criterion 2: A–C have to be fulfilled.
A: Patient develops at least one of the following signs or symptoms during in-hospital stay that differs from the initial symptoms related to aneurysmal subarachnoid hemorrhage (aSAH) onset:
Fever (>38°C)
Headache
Neck stiffness
Meningeal signs
Cranial nerve signs
Irritability
B: If diagnosis is made ante mortem, physician institutes appropriate antimicrobial therapy.
C: Laboratory tests or magnetic resonance imaging (MRI) scan show at least one of the following:
Secondarily increased white cells, elevated protein, or decreased glucose in CSF
Organisms seen on Gram stain of cerebrospinal fluid (CSF)
Positive antigen test of CSF
MRI-proven signs of acute central nervous system (CNS) infection

Modified from Centers for Disease Control and Prevention (CDC) definitions

Database

For the COSBID substudy on aSAH and DISCHARGE-1, we developed a customized, Web-based database. It uses the Django Web framework (http://www.djangoproject.com/) written in Python (http://www.python.org) and the PostgreSQL database back end (http://www.postgresql.org). For security purposes, all network traffic uses a https protocol with Apache server. SSL certificates are provided by Charité-IT. The database is patient centered, and all other objects are related to the patients. This design allows multiple studies; for example, DISCHARGE-1 is embedded in the COSBID outcome study. All changes to the data are recorded and stored in the database. Users of the database can have different roles with specified access to data. This is done on different levels: Firstly, access can be restricted to a specified group of objects, such as study, study center, set of values; secondly, different users can have the right only to read the data or add or modify items. Currently implemented roles are

- Normal user with read/write access to the patients in his or her recruiting center.
- Study monitor with read access to all patients from the monitored study and ability to write tickets/queries for any item related to monitored patients. Furthermore, the monitor can close cases, which means that data of a closed case can no longer be changed.
- Administrator.
- Analyzer without writing access.

An important part of the database is automated internal data validation:

- Logical validation (e.g., monitoring) cannot start before admission to the hospital.
- Pathophysiological/clinical validation; for example, if the diagnosis of migraine is stated, the headache has to fulfill the criteria of the International Headache Society (IHS) for migraine [12]; if GCS on admission was 3, WFNS score cannot be 1.
- Check for complete data:
  - A subgroup of data has to be inserted immediately.
  - Another subgroup of data has to be entered at the end of the monitoring period to allow the case to be completed.
  - In general, all data are mandatory. Items have to be allowed to be set as "missing data."
  - For repetitive data (e.g., blood pressure, glucose), the smallest required intervals (e.g., daily, hourly) are set.

Special focus was placed on the recording of AEs and SAEs. For each day during the monitoring period, an AE event record has to be set. At the least, it has to be stated that there was no AE on the specified day. Furthermore, interdependence between different AEs has to be documented. If AEs are related to the study, a recommendation for a protocol change can be specified if necessary. Some AEs, such as infective diseases, require further description, such as kind of infection, pathogen (if confirmed), including foreign body where the pathogen was found (e.g., EVD or central venous catheter). For documentation purposes, AEs have to be printed out and stored in the patient file. In case of SAEs, an additional copy is printed that has to be sent to the study sponsor.

Discussion

The criteria described here for the diagnosis of complications will serve as a basis for a retrospective analysis of aSAH patients who were recruited at the Charité Berlin for the COSBID before the DISCHARGE-1 trial started. Moreover, the criteria serve for the survey of complications in DISCHARGE-1.

The aim of the retrospective study will be to determine the incidences of different complications after aSAH, including ventriculitis, meningitis, or local hemorrhage in all patients who received a subdural recording strip versus control patients who did not receive such a strip during the same time period. There may be limitations to the control group of this study. It is aimed for a match regarding age, sex, severity of aSAH and EVD. A bias may result from the fact that the clinical course is less well documented in such a retrospective control group. Moreover, it is likely that neuroimaging was less frequently performed in the control group, so that relevant complications might have been missed.

In DISCHARGE-1, evaluation of safety endpoint occurrence is carried out on the safety set that is defined by all patients in whom a recording device was implanted. Independent data monitoring and a data safety and monitoring board (DSMB) has been established. Adverse events such as shunt-dependent documented up to 6 months post-aSAH. Late epilepsy (>2 weeks postintervention) is documented up to 3 years post-aSAH, similar to a pilot study on patients who were recruited prior to DISCHARGE-1 [8]. Stop criteria in DISCHARGE-1 are (1) local epi-/subdural hemorrhage in the recording area (estimated risk 0–2%) or (2) local infection (estimated risk 2.6–3.9%) [10, 17]. No such complications were observed in the small previous case series of aSAH patients who received a subdural recording strip [4, 6, 7, 20].

Conclusion

We here present the study design for the analysis of noninfectious and infectious complications in patients suffering from aneurismal subarachnoid hemorrhage. The criteria for these complications follow international standards and

definitions. These criteria will be used in a retrospective analysis of aSAH patients who were previously recruited at the Charité Berlin for the COSBID study and in a prospective analysis of patients recruited for the DISCHARGE-1 study. In addition we reported on the database that has been developed for the documentation of the clinical course and complications after aSAH.

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Conflicts of Interest We declare that we have no conflict of interest.

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Neurovascular Coupling During Spreading Depolarizations

Ulrike Hoffmann and Cenk Ayata

Abstract Injury depolarizations akin to spreading depression of Leão are important in the progression of tissue damage in ischemic stroke, intracranial hemorrhage, and trauma. Much of the research on injury depolarizations has been focused on their origins, electrophysiological mechanisms, and metabolic impact. Recent studies showed that injury depolarizations cause vasoconstriction and diminish perfusion, which radically differs from the predominantly hyperemic response to spreading depression in otherwise-normal brain tissue. This adverse hemodynamic effect exacerbates metabolic supply-demand mismatch and worsens the tissue outcome. Although the mechanisms transforming the hemodynamic response from vasodilation into vasoconstriction are unclear, recent data suggest a role for elevated extracellular K^+ and reduced intravascular perfusion pressure, among other factors. Clues from physiological and pharmacological studies in normal or injured brain in different species suggest that the intense pandepolarization evokes multiple opposing vasomotor mechanisms with variable magnitudes and timing, providing a conceptual framework to dissect the complex neurovascular coupling in brain injury.

Keywords Cortical spreading depolarizations • Neurovascular coupling • Cerebral perfusion • Electrocorticography

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Spreading Depression Versus Injury Depolarizations

Almost 70 years after Leão discovered the peculiar, slowly propagating electrocorticogram suppression triggered by intense stimulation in rabbit brain [9], spreading depolarization waves are now recognized as major pathophysiological events in brain injury states, such as stroke, intracranial hemorrhage, and trauma, as well as in migraine [8]. Although originally termed "spreading depression" by Leão based on the depression of cortical electrical activity, the key feature is indeed an overwhelming pandepolarization of most, if not all, cell types in brain tissue. The critical event appears to be a sudden loss of neuronal membrane resistance due to opening of large conductance nonselective cation channels, triggering a massive K^+ and glutamate efflux coupled to Ca^{2+}, Na^+, and water influx and cell swelling. It is believed that the sudden uncontrolled rise in extracellular K^+ ($[K^+]_e$) and glutamate depolarize nearby cells, and in this way the process slowly propagates (~3 mm/min) by way of contiguity regardless of functional or vascular divisions. As severe as it sounds, however, transmembrane potentials and ion and water homeostasis are restored within less than a minute in otherwise-healthy brain tissue, and there is as yet no evidence that spreading depolarizations are injurious in normal brain even when they occur in large numbers over a short period of time.

The situation is different, however, when spreading depolarizations occur in injured brain tissue, such as in ischemic stroke or subarachnoid hemorrhage. This is because the clearance of $[K^+]_e$ and glutamate and the restoration of transmembrane ionic and water homeostasis are to some extent perfusion and energy dependent. Therefore, when injury depolarizations occur in ischemic penumbra, their recovery is delayed and duration prolonged, often by manyfold (Fig. 1). Indeed, some depolarization events may fail to recover altogether and become incorporated into the permanently depolarized ischemic core. Further complicating the picture, the cerebrovascular response to spreading depolarizations radically differs between normal and ischemic or injured brain.

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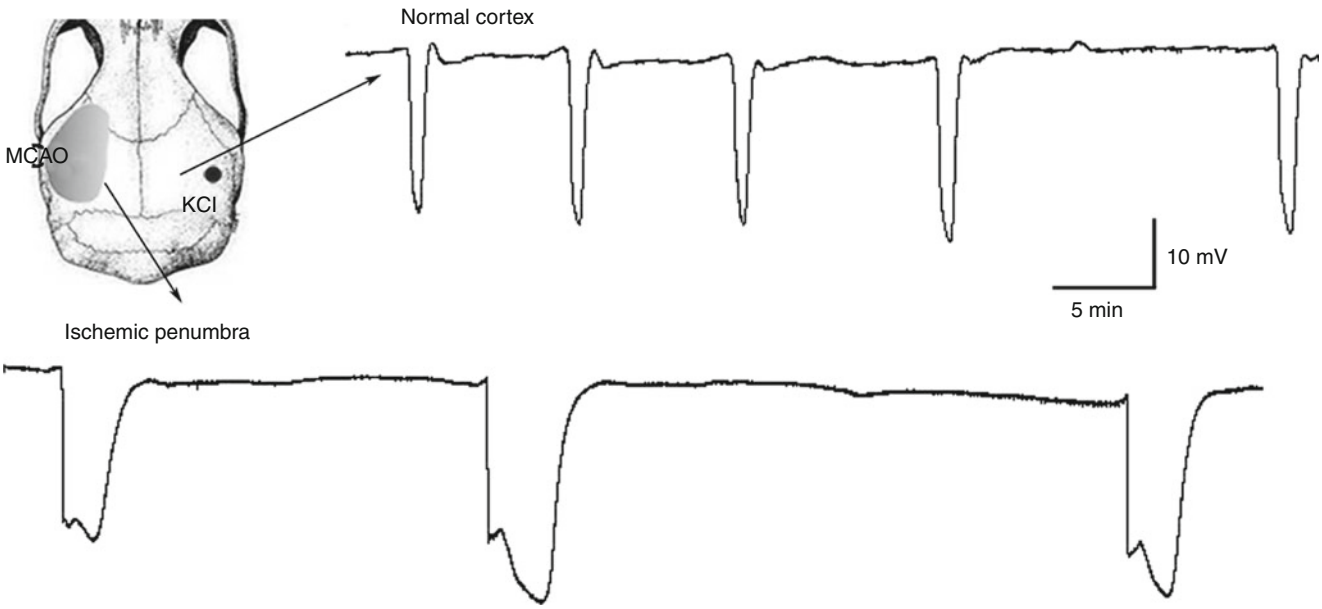

Fig. 1 Spreading depressions versus injury depolarizations. Electrophysiological tracings show the extracellular slow (direct current, *DC*) potential changes recorded by glass intracortical micropipettes. Each negative deflection represents a spreading depolarization wave. In contrast to spreading depolarization waves triggered by topical KCl application in normal brain tissue (*upper tracing*), injury depolarizations spontaneously triggered in focal ischemic brain are significantly prolonged when recorded within the penumbra (*lower tracing*). *MCAO* middle cerebral artery occlusion

Neurovascular Coupling During Spreading Depolarizations

In healthy brain tissue, spreading depolarizations evoke a large hyperemic response (~100% increase), occasionally preceded by a brief and small hypoperfusion in most species [7]. This is indeed one of the largest, if not the largest, hyperemias evoked by any physiological stimulus studied to date. However, the magnitude of hyperemia significantly diminishes in ischemic or injured brain tissue. This is not at all surprising because the classical doctrine of autoregulation dictates that when perfusion pressure drops, cerebral vessels dilate to reduce cerebrovascular resistance and thus keep perfusion unchanged. This diminishes the capacity of vessels to dilate further during the depolarization wave (i.e., ceiling effect). What is surprising in the blood flow response to depolarization in ischemic or injured brain is the emergence of a vasoconstrictive phase at its onset, coincident with the depolarization (i.e., direct current [DC] shift), during which tissue perfusion drops below baseline. In other words, not only the hyperemic component is diminished but also a hypoperfusion component appears preceding and ultimately replacing the hyperemia and is coupled to prolonged depolarizations (Fig. 2).

A detailed spatiotemporal analysis of the hemodynamic response to injury depolarizations has been carried out in focal ischemic brain using high-resolution laser speckle flowmetry in lissencephalic mice and rats, as well as in gyrencephalic cats. The data clearly demonstrate that the magnitudes of hypoperfusion and hyperemia are inversely related, and both change as a function of baseline perfusion [2, 10, 11, 13, 15]. The lower the baseline perfusion is, the larger and longer the initial hypoperfusion and the smaller the subsequent hyperemia will be. Consequently, the magnitude and duration of hypoperfusion during an injury depolarization closely correspond to the duration of the depolarization. Indeed, when the depolarization is permanent, it is associated with a permanent reduction in tissue perfusion. By this way, the perfusion deficit expands in a stepwise fashion coupled to the occurrence of spreading depolarization events [13]. The transformation of the vasodilation response to spreading depolarizations into vasoconstriction in injured brain (i.e., its inversion) has recently been elegantly demonstrated in patients with subarachnoid hemorrhage as well [1, 4].

Factors Transforming the Hemodynamic Response in Ischemic or Injured Brain

The mechanisms responsible for the inversion of the hyperemic response to hypoperfusion are not known but likely relate to tissue homeostasis in ischemic brain. The primary inciting event in ischemia is, of course, a reduction in tissue perfusion pressure. We have therefore tested the impact of reduced perfusion pressure in simulated penumbra conditions using controlled hypotension [16, 17]. We found that

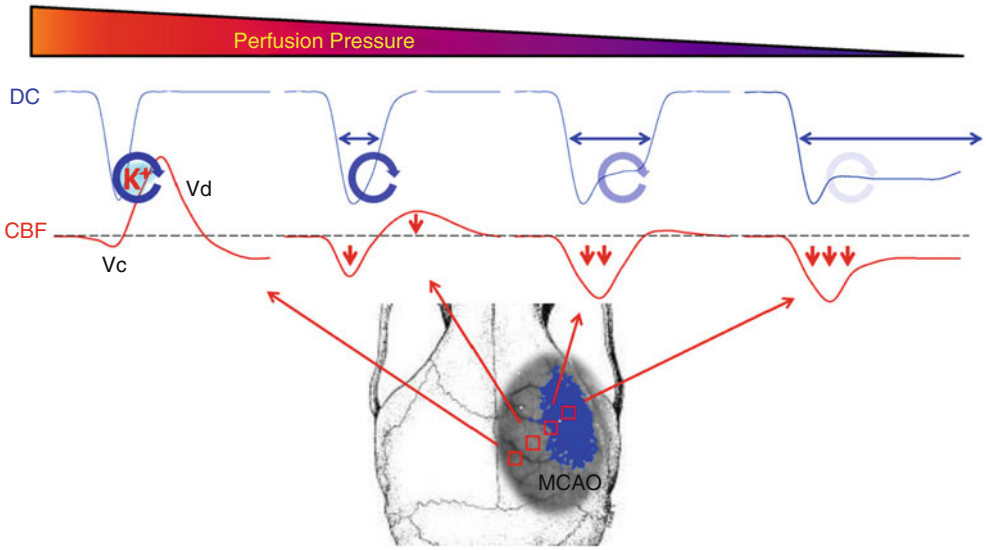

Fig. 2 Transformation of the cerebral blood flow (*CBF*) response to injury depolarizations as a function of reduction in perfusion pressure. Line drawings depict typical slow (*DC*) potential shift of spreading depolarizations (*blue*) and accompanying CBF changes (*red*) in nonischemic, and mild, moderate, or severely ischemic regions of interest (*red squares* from *left* to *right*). Note the gradual prolongation of the depolarization (*blue arrows*) and the emergence of an initial hypoperfusion that ultimately replaces diminished hyperemia (*red arrows*). The repolarization is critically dependent on restoration of ionic and water balances, such as clearance of the surge in $[K^+]_e$, which is gradually diminished in poorly perfused or ischemic tissue (*blue cycle*). When an injury depolarization fails to recover, the associated hypoperfusion also fails to recover completely. The CBF map imaged using laser speckle flowmetry is overlaid on a mouse skull, with superimposed blue pixels showing the spatial extent of the perfusion defect after distal middle cerebral artery occlusion (*MCAO*). *Vc* vasoconstriction, *Vd* vasodilation

simply decreasing systemic arterial blood pressure by approximately 50% doubled the duration of spreading depolarization waves and diminished the magnitude of associated hyperemia by more than 60%; a transient hypoperfusion also appeared preceding the hyperemia (Fig. 3). Interestingly, these changes took place without a major drop in tissue perfusion, suggesting that intraluminal perfusion pressure rather than the tissue metabolic state (i.e., ischemia) was the critical determinant. Conversely, pharmacologically induced hypertension potently opposed the vasoconstrictive effect of periinfarct injury depolarizations during middle cerebral artery occlusion [14], underscoring the importance of perfusion pressure with direct relevance to hypertension, hypervolemia, and hemodilution (HHH) therapy against delayed vasospasm and ischemia after subarachnoid hemorrhage. In contrast, a reduction in tissue pO_2 alone, simulated by systemic hypoxia without a change in perfusion pressure, did not significantly alter the DC shift or the blood flow response to spreading depolarizations. Further, the effect of reduced perfusion pressure on the duration of spreading depolarizations and the transformed blood flow response was not restored when tissue oxygenation was augmented by normobaric hyperoxia, again suggesting that tissue O_2 availability or energy status are not that critical within a wide physiological range.

Another potential mechanism transforming the hemodynamic response to spreading depolarizations in ischemic or injured brain is elevated baseline $[K^+]_e$ (~5–10 mmol) due to the partial suppression of Na^+, K^+ ATPase (adenosine triphosphatase) activity. Indeed, superfusion of cortical surface with artificially elevated $[K^+]$ is sufficient to transform the blood flow response to spreading depolarizations from a monophasic hyperemia into a biphasic wave with an initial hypoperfusion in otherwise-normal brain [5]. An additional factor that is capable of transforming the blood flow response is reduced nitric oxide availability. Systemic administration or topical superfusion of nitric oxide synthase inhibitors or scavengers mimics the effects of reduced perfusion pressure by bringing out an initial hypoperfusion and diminishing the subsequent hyperemia [3, 6, 7]. Indeed, when coupled to elevated resting $[K^+]_e$ (35 mmol), reduced nitric oxide availability is capable of triggering prolonged and severe vasoconstriction, leading to spreading ischemia [2, 5].

Vasomotor Components of the Hemodynamic Response to Spreading Depolarizations

Therefore, a few seemingly unrelated factors (e.g., intraluminal perfusion pressure, resting $[K^+]_e$, and nitric oxide availability) are all capable of transforming the blood flow response in otherwise-normal brain by augmenting the initial vasoconstriction/hypoperfusion and diminishing the subsequent hyperemia, similar to that observed in ischemic or injured brain. Interestingly, the blood flow response to

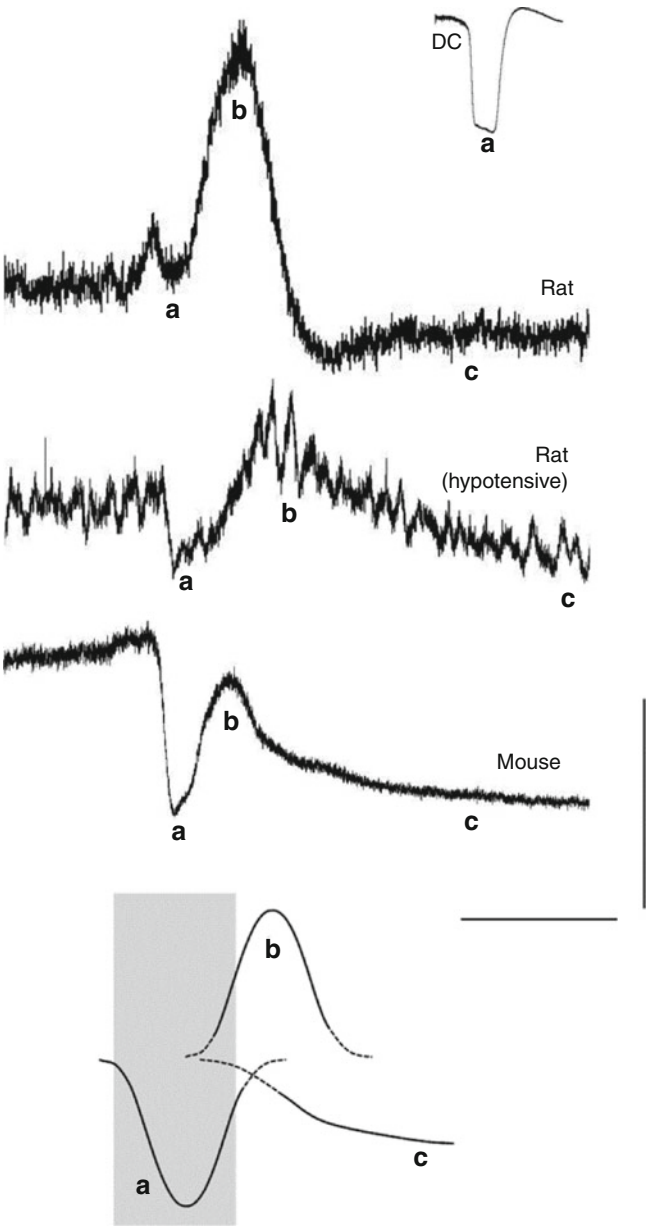

Fig. 3 Representative cortical laser Doppler blood flow tracings during spreading depolarizations in a normal rat (*upper*), in a hypotensive rat (*middle*; arterial blood pressure 45 mmHg), and in a normal mouse (*lower*) showing the initial vasoconstrictive (a) and subsequent hyperemic (b) phases, followed by a longer-lasting oligemia (c). Such physiological manipulations and species differences suggest that the integrated flow response is composed of multiple opposing vasomotor components corresponding to each phase (*bottom drawing*). The magnitude and duration of each component are variable in different species and under different conditions. *Shaded area* indicates the approximate timing of the depolarization as detected by the slow potential shift (direct current, *DC*) shown on the upper-right inset, which temporally coincides with the vasoconstrictive (a) phase. *Vertical bar*: CBF 100%, *horizontal bar*: time 2 min

spreading depolarization in normal mouse brain differs from that in normal rat and human brain: The initial hypoperfusion is more pronounced and temporally corresponds to the constrictive phase in the hypotensive rat (Fig. 3). These diverse vascular responses across species and under different conditions can be explained by proposing multiple opposing vasomotor components that, when integrated, form the cerebral blood flow response to spreading depolarizations. At least three such components are discernible (Fig. 3):

(a) Short-lasting initial vasoconstriction that temporally coincides with the depolarization
(b) Vasodilation that starts toward the end of the depolarization and peaks after repolarization
(c) Slowly developing oligemia that lasts up to an hour

Importantly, the expression of each component appears to be species dependent and can be physiologically and pharmacologically modulated. For instance, mechanisms of the initial vasoconstriction in the normal rat brain are postulated to act throughout the duration of the depolarization but are counteracted by the prevailing vasodilator response that begins later during depolarization. The result is a very brief initial hypoperfusion in the normal brain. During hypotension, the extended vasoconstrictive influence is unmasked due to its enhancement and the relative diminution of the vasodilator influence, which only appears in the net response after some delay. This relative balance of vasoconstriction/dilation may be similar to the normal situation in the mouse brain.

Individual vasomotor components are presumably mechanistically distinct. The most likely mediator for the initial vasoconstrictive component (a) is the dramatic rise in $[K^+]_e$ (>40–80 mmol), which is a strong vasoconstrictor agent, possibly siphoned by astrocyte end feet surrounding the small arteries and arterioles. Subsequent vasodilation (b) likely has multiple mediators (e.g., nitric oxide) and is in part metabolically driven. The postdepolarization oligemia is also likely to have multiple mechanisms and mediators, as recently shown in rats [12]. Of course, this is an oversimplified scheme, but it serves as a conceptual framework to gain insight into physiological and pharmacological modulation of neurovascular coupling during intense tissue depolarizations.

Conclusion

Identifying the precise mechanisms and mediators of the neurovascular coupling during spreading depolarizations has been challenging because of the intense nature of ionic and water shifts, biochemical and metabolic changes (e.g., pH), and massive and uncontrolled release of numerous neurotransmitter and modulator molecules. To further complicate the matter, the depolarization likely has an impact on all cell types in the neurovascular unit, including the endothelium. Nevertheless, resolving the mechanisms and mediators will be critical to target and prevent the adverse hemodynamic

effects of injury depolarizations to curb the delayed secondary worsening of tissue outcome after ischemic stroke, intracranial hemorrhage, and trauma.

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Conflicts of Interest I declare that I have no conflict of interest.

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Subarachnoid Blood Converts Neurally Evoked Vasodilation to Vasoconstriction in Rat Brain Cortex

Masayo Koide, Adrian D. Bonev, Mark T. Nelson, and George C. Wellman

Abstract The matching of blood flow to regional brain function, called functional hyperemia or neurovascular coupling, involves the coordinated activity of neurons, astrocytes, and parenchymal arterioles. Under physiological conditions, localized neuronal activation leads to elevated astrocyte endfoot Ca^{2+} and vasodilation, resulting in an increase in cerebral blood flow. In this study, we examined the impact of subarachnoid hemorrhage (SAH) on neurovascular coupling. SAH model rats received two injections of autologous blood into the cisterna magna 24 h apart. Cortical brain slices from SAH model animals were prepared 4 days after the initial blood injection. Arteriolar diameter and astrocyte endfoot Ca^{2+} were simultaneously measured using two-photon microscopy. As expected, neuronal activity evoked by electrical field stimulation (EFS) caused an elevation in endfoot Ca^{2+} and vasodilation in brain slices from control animals. However, in brain slices from SAH animals, EFS induced a similar increase in astrocyte endfoot Ca^{2+} that caused arteriolar constriction rather than vasodilation. Vasoconstriction was observed in approximately 90% of brain slices from SAH animals in response to EFS, with 40% exhibiting a sustained vasoconstriction, 30% exhibiting a transient vasoconstriction (diameter restored within 1 min after EFS), and 20% responded with a biphasic response (brief vasodilation followed by vasoconstriction). This inversion of neurovascular coupling may play a role in the development of neurological deficits following SAH.

Keywords Subarachnoid hemorrhage • Neurovascular coupling • Cerebral artery • Parenchymal arteriole • Astrocyte • Microcirculation • Cortical brain slice • Vasospasm

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Introduction

Neurovascular coupling forms the basis of functional hyperemia, whereby localized neuronal activity leads to vasodilation and increased blood flow to enhance the supply of oxygen and nutrients to active neurons. This physiologically crucial mechanism is mediated by the coordinated activity of neurons, astrocytes, and parenchymal arterioles. During neurovascular coupling, neurally released glutamate activates astrocytic metabotropic glutamate receptors, leading to an inositol triphosphate (IP_3) receptor-mediated wave of intracellular Ca^{2+} release in astrocytes. Astrocyte endfeet completely encase parenchymal arterioles and evoked increases in endfoot Ca^{2+} lead to the release of vasodilatory substances (e.g., vasodilatory arachidonic acid metabolites, K^+ efflux from large-conductance Ca^{2+}-activated K^+ channels) from astrocytic endfeet onto parenchymal arterioles [1, 4, 7, 24].

For decades, angiographically detected cerebral vasospasm was considered the major cause of delayed ischemic neurological deficits following subarachnoid hemorrhage (SAH) [11, 22]. However, recent studies suggest that prevention of angiographically defined cerebral vasospasm did not improve outcome in SAH patients [16, 17]. Perfusion deficiencies within the microcirculation, which cannot be resolved using conventional angiography, may be one mechanism contributing to delayed ischemic neurological deficits following SAH [8, 9, 15, 22]. Restricted blood flow through intracerebral/parenchymal arterioles could result from microthrombi [25], vasoconstriction due to extravascular blood or blood breakdown products [20, 21], or impaired neurovascular communication [2].

In this study, we examined the impact of SAH on neurovascular coupling using a rat double-injection SAH model. Here, we report that SAH converts neurovascular coupling from vasodilation to vasoconstriction. Local vasoconstriction in response to neuronal activity would promote a decrease in local blood flow and could potentially compromise neuronal viability.

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Materials and Methods

Rat SAH Model

Sprague-Dawley rats (males, 10–12 weeks old) were used in accordance with *The Guide for the Care and Use of Laboratory Animals* (NIH Pub. No. 85–23, revised 1996) following protocols approved by the Institutional Animal Use and Care Committee of the University of Vermont. Autologous and unheparinized arterial blood (0.5 ml) was injected twice into the cisterna magna of anesthetized animals at an interval of 24 h [21]. Animals were euthanized by decapitation under deep anesthesia (pentobarbital, 60 mg/kg, ip) 4 days after the initial blood injection.

Brain Slice Experiments

Cortical brain slices (coronal sections, 160-μm thick) were prepared from the territory of the middle cerebral artery using a vibratome (VT1000S, Leica) and then loaded with the Ca^{2+} indicator dye fluo-4 (10 μM, acetoxymethyl ester) with 0.05% pluronic acid at 29°C for 1 h. Parenchymal arteriolar diameter and astrocytic endfoot Ca^{2+} were simultaneously imaged (~1 Hz) using a BioRad Radiance 2100 MP dedicated multiphoton imaging system. To mimic in vivo conditions, brain slices were superfused with artificial cerebrospinal fluid containing U46619 (100 nM) to induce a modest level of arterial tone. Electrical field stimulation (EFS; 50 Hz, 0.3-ms duration alternating square pulse for 3 s) with a pair of platinum wires placed parallel to the brain slice was used to stimulate neurons and induce an elevation in astrocytic endfoot Ca^{2+}. Arteriolar diameter was determined by averaging the internal diameter obtained from three points along the arteriole on a given image and expressed as percentage change from the diameter recorded prior to EFS (first image of the recording). Astrocyte endfoot Ca^{2+} was quantified using the maximal fluorescent method [5]. All experiments were conducted at 37°C.

Results

In the vast majority (52 of 53) of brain slices from unoperated control animals, neuronal activation by EFS elicited the predicted elevation in astrocyte endfoot Ca^{2+}, followed by parenchymal arteriole vasodilation. In about half of these control brain slices (28/53 brain slices), the vasodilation to EFS was transient in nature, with arterioles returning to their prestimulation diameter within 1 min after EFS. In an additional 19 brain slices from control animals, parenchymal arterioles showed sustained (>1 min) vasodilation to EFS. The remainder of brain slices from control animals (5/53 brain slices, 9.4%) exhibited "biphasic" vascular responses: brief/transient dilation followed by constriction. In one brain slice from a control animal, EFS evoked only vasoconstriction.

Table 1 Summary of arteriolar responses induced by electrical field stimulation (EFS)

	Cont		SAH	
Dilation transient	52.8%	(28/53)	5.1%	(3/59)
Dilation sustained	35.8%	(19/53)	3.4%	(2/59)
Biphasic response	9.4%	(5/53)	20.3%	(12/59)
Constriction transient	1.9%	(1/53)	28.8%	(17/59)
Constriction sustained	0.0%	(0/53)	42.4%	(25/59) slices

cont control, *SAH* subarachnoid hemorrhage

In marked contrast, EFS caused parenchymal arteriolar vasoconstriction in over 90% of brain slices (54/59) from SAH animals. This EFS-induced vasoconstriction after SAH was transient in 17 of 59 brain slices and sustained in 25 of 59 brain slices. In 12 of 59 brain slices, parenchymal arteries showed a biphasic vascular response (brief vasodilation followed by vasoconstriction). The remainder of brain slices (5 of 59) from SAH animals responded with vasodilation after EFS (transient: 3 brain slices; sustained: 2 brain slices). These observations demonstrate an inversion in EFS-induced arteriolar response from dilation to constriction in brain slices from SAH model animals (Table 1). Interestingly, despite the dramatic SAH-induced change in the polarity of the neurovascular response, EFS-induced increases in astrocyte endfoot Ca^{2+} that preceded vasodilation (control animals) and vasoconstriction (SAH model animals) were remarkably similar (Fig. 1).

Discussion

Our observations demonstrate a fundamental change in neurovascular coupling following SAH. In over 90% of cortical brain slices from SAH model animals, evoked neuronal activity caused an elevation in astrocytic endfoot Ca^{2+} followed by vasoconstriction. This vasoconstriction observed after SAH is in marked contrast to neurally evoked vasodilation typically observed in brain slices from control animals. Vasodilation is the predicted physiological response to increased neuronal activity and is the basis of functional hyperemia. On the other hand, vasoconstriction to neuronal activity represents a pathological response that could severely limit blood flow to active neurons. Our work is consistent with recent in vivo and clinical studies that have reported

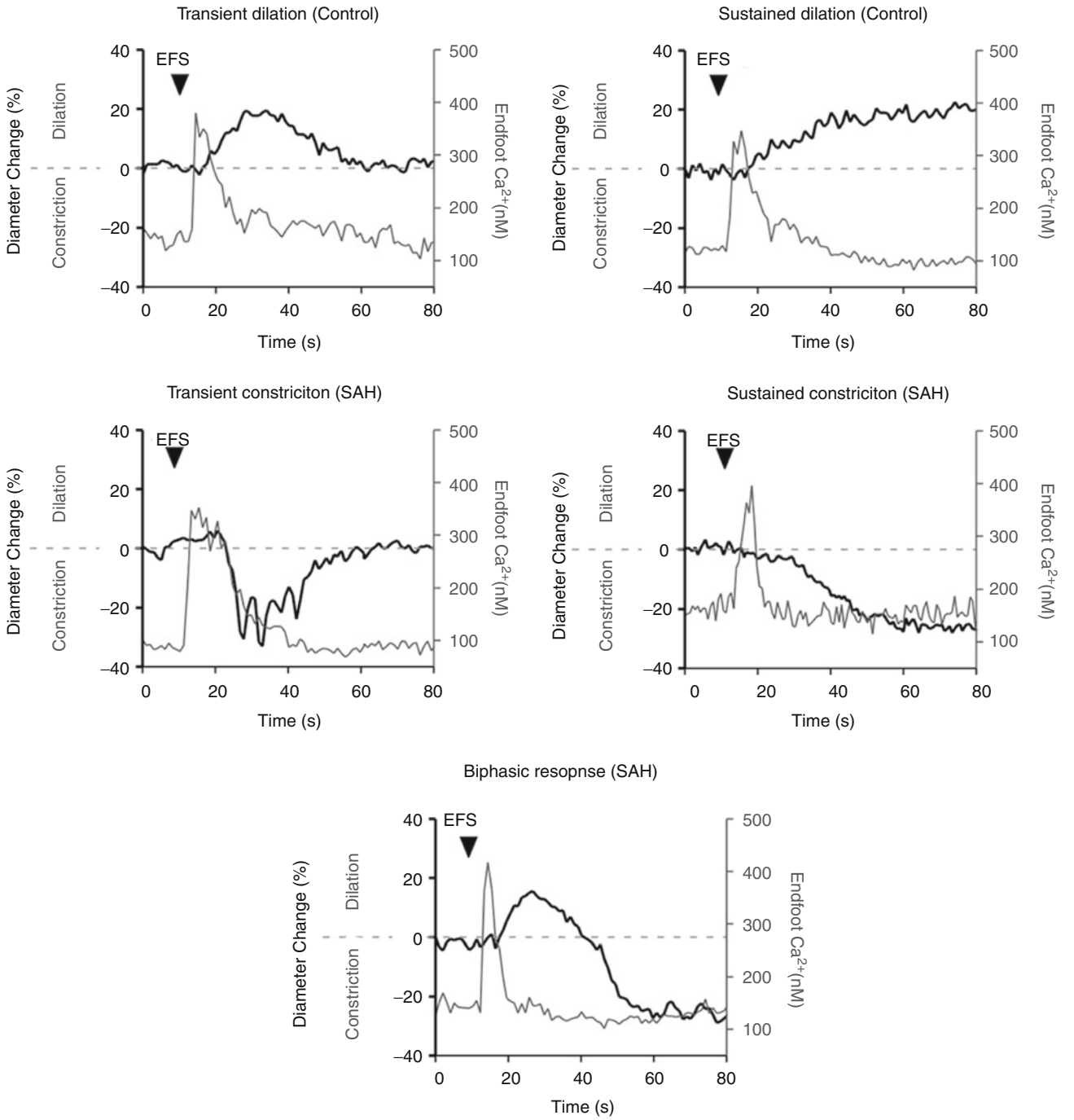

Fig. 1 Parenchymal arteriolar responses in rat cortical brain slices induced by electrical field stimulation (*EFS*). Parenchymal arteriolar diameter and astrocyte endfoot Ca^{2+} concentration were simultaneously measured using two-photon microscopy. Increased neuronal activity evoked by EFS caused an elevation in astrocyte endfoot Ca^{2+}, leading to several patterns of arteriolar responses. Representative traces of changes in arteriolar diameter and astrocyte endfoot Ca^{2+} concentration are shown in brain slices obtained from control and subarachnoid hemorrhage (*SAH*) animals. Responses were defined as "transient" if arteriolar diameter returned to baseline within 1 min after EFS. Arteriolar responses that were maintained for 1 min after EFS were counted as "sustained" responses. Recordings exhibiting both dilation and constriction were classified as "biphasic" responses. All biphasic responses showed an initial brief dilation followed by sustained constriction. Transient dilations and sustained dilations were observed in brain slices from control animals. Traces of transient constriction, sustained constriction, and biphasic response were recorded from brain slices from SAH model animals. All patterns of arteriolar responses were observed after similar increases of astrocytic endfoot Ca^{2+} evoked by EFS

decreased cerebral blood flow in response to global neuronal hyperactivity under certain pathological conditions, such as ischemic depolarizations in mice [23] and cortical spreading depression in human aneurysmal SAH patients [3]. Our studies suggest that SAH can also induce more subtle changes in brain function that have a profound impact on neurovascular coupling.

The majority of neurovascular coupling studies, obtained using healthy animals, have reported neurally evoked vasodilation, consistent with functional hyperemia. However, a few reports have demonstrated that neuronal activation and elevated astrocytic endfoot Ca^{2+} can, under certain conditions, lead to parenchymal arteriolar constriction in healthy "control" animals [5, 6, 18]. Several mechanisms have been found to underlie neurally evoked vasoconstriction in brain slices from control animals, including production of the vasoconstrictor 20-hydroxyeicosatetraenoic acid [18], excessive release of K^+ from large conductance Ca^{2+}-activated K^+ channels located on astrocyte endfeet caused by abnormally high levels of endfoot Ca^{2+} [5] and hyperoxia-induced decrease of vasodilators prostaglandin E_2 and adenosine [6]. Our unpublished observations suggest these mechanisms are not involved in neurally evoked vasoconstriction in brain slices from SAH animals.

Conclusion

We have previously reported that SAH can induce reactive astrogliosis, leading to altered astrocyte structure and function [19]. The cellular mechanisms linking SAH to altered astrocyte function and inversion of neurovascular coupling are currently under investigation and may involve an elevation in perivascular potassium [12]. Inversion of neurovascular coupling may act in concert with SAH-induced enhanced constriction of pial arteries [10, 13, 14, 26] and parenchymal arterioles [21] to induce ischemic neuronal damage. In summary, we report the conversion of neurovascular coupling from vasodilation to vasoconstriction in brain slices from SAH model rats. This phenomenon may play an important role in the development of cortical infarcts following cerebral aneurysm rupture.

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Conflicts of Interest We declare that we have no conflict of interest.

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Impact of Subarachnoid Hemorrhage on Parenchymal Arteriolar Function

George C. Wellman and Masayo Koide

Abstract Intracerebral or parenchymal arterioles play an important role in the regulation of both global and regional blood flow within the brain. Brain cortex lacks significant collateral sources of blood and thus is at risk if blood flow through parenchymal arterioles is restricted. Increasingly, evidence is accumulating that abnormal parenchymal arteriolar constriction contributes to the development of neurological deficits caused by subarachnoid hemorrhage (SAH). For example, parenchymal arterioles isolated from SAH model rats exhibit enhanced constriction in response to increased intravascular pressure. This increased pressure-dependent constriction or myogenic tone would result in a shift in the cerebral autoregulatory response and decreased cerebral perfusion. Here, we summarize our current knowledge regarding cellular mechanisms contributing to enhanced contractility of parenchymal arteriolar myocytes following SAH. Our studies demonstrated that SAH-induced membrane potential depolarization involving altered K^+ homeostasis leads to enhanced voltage-dependent Ca^{2+} channel activity, increased smooth muscle cytosolic Ca^{2+}, and parenchymal arteriolar constriction. In summary, emerging evidence demonstrates that SAH can profoundly affect parenchymal arteriolar tone, promoting decreased cortical blood flow and compromised neuronal viability.

Keywords Ca^{2+} channels • K^+ channels • Microcirculation • Vascular smooth muscle • Vasospasm

Role of Parenchymal Arterioles in the Regulation of Cerebral Blood Flow

Parenchymal arterioles are small-diameter blood vessels containing a single layer of smooth muscle that are located in the brain parenchyma downstream of the Virchow-Robin space. Parenchymal arterioles can be distinguished from pial arteries, surface arterioles, and arterioles located within the transitional Virchow-Robin space by their lack of extrinsic innervation and encasement by astrocytic end feet [2]. Considering Poiseuille's law, which states that flow through a cylinder is proportional to the fourth power of the radius, it is not surprising that small changes in the diameter of these blood vessels will have a profound impact on the delivery of oxygen and nutrients to cells within the brain. Further, because collateral blood supply to the brain cortex is meager, hyperconstriction or occlusion of a parenchymal arteriole will severely limit tissue perfusion and nutrient supply to a given cortical region [12]. Another unique feature of parenchymal arterioles is their role in functional hyperemia, by which focal increases in neuronal activity are coupled to parenchymal arteriolar dilation. This matching of local blood flow to regional brain function involves the coordinated activity of neurons, astrocytes, and parenchymal arterioles (i.e., the neurovascular unit) [1]. Thus, parenchymal arterioles play an important role in local, as well as global, cerebral blood flow.

Enhanced Pressure-Induced Constriction and Elevated Arteriolar Wall Ca^{2+} of Parenchymal Arterioles from Subarachnoid Hemorrhage Model Rats

Pial or brain surface arteries from subarachnoid hemorrhage (SAH) model animals exhibit enhanced constriction, reflecting a combination of enhanced expression of voltage-dependent

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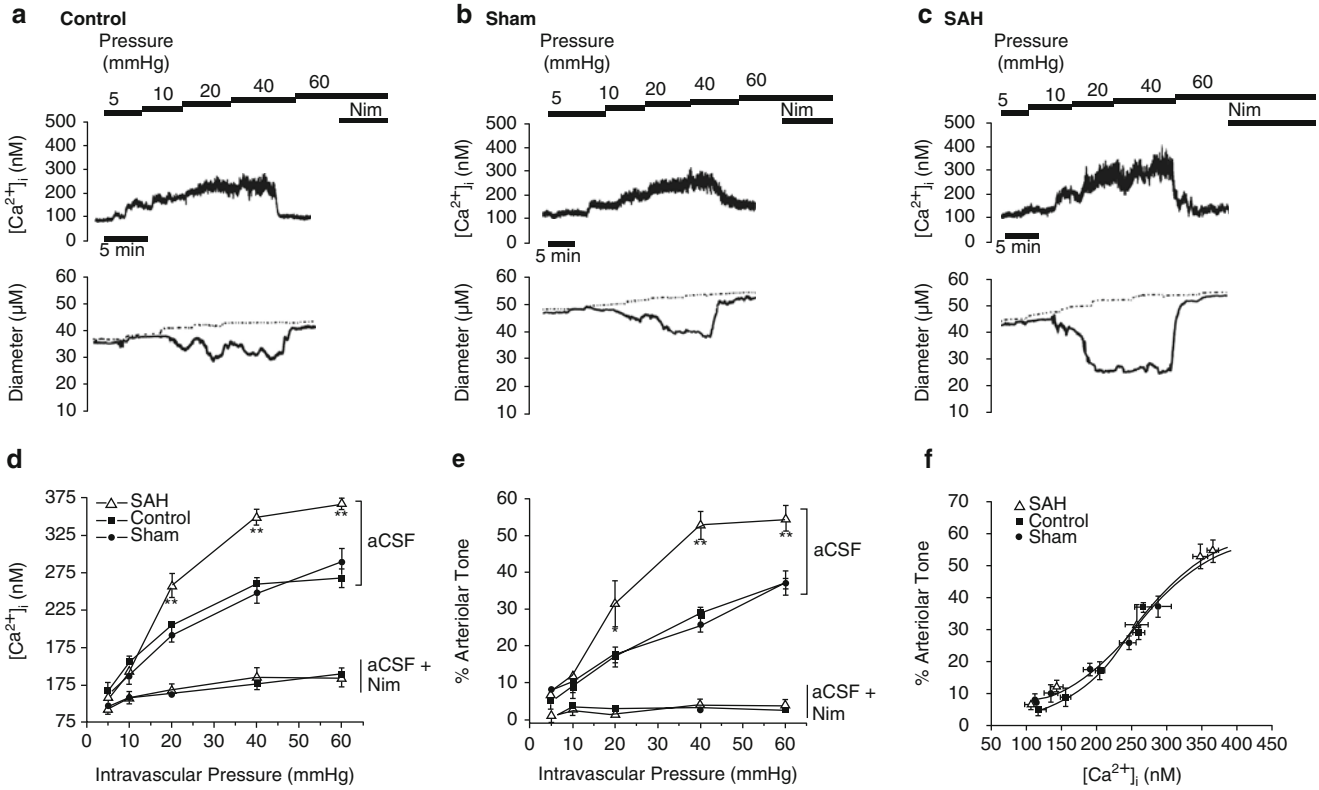

Fig. 33.1 Elevated cytosolic Ca^{2+} and enhanced myogenic tone in parenchymal arterioles from subarachnoid hemorrhage (*SAH*) animals. (**a**–**c**) Representative simultaneous $[Ca^{2+}]_i$ and diameter measurements obtained from intact arterioles isolated from unoperated ((**a**) control), sham-operated ((**b**) sham) and SAH (**c**) animals. Recordings were obtained during stepwise increases in intravascular pressure and subsequent nimodipine application (300 nM) at 60 mmHg. *Dashed traces* represent diameters in Ca^{2+}-free artificial cerebral spinal fluid (aCSF) containing nimodipine (300 nM). (**d**, **e**) Summary of $[Ca^{2+}]_i$ (**d**) and constriction (**e**) obtained in the absence and presence of 300 nM nimodipine. $^*p<0.05$, $^{**}p<0.01$ versus control unoperated and sham operated. (**f**) Relationship between $[Ca^{2+}]_i$ and constriction for arterioles isolated from control, sham-operated, and SAH animals derived from summary data depicted in *panels* (**d**) and (**e**) (Reproduced from Nystoriak et al. [13])

Ca^{2+} channels (VDCCs) and increased activity of these channels due to membrane potential depolarization [4, 5, 21]. Although available information is equivocal, a number of in vivo studies suggested that SAH can also negatively affect the microcirculation within the brain parenchyma [7, 15, 16]. However, because the small size of parenchymal arterioles poses a significant technical challenge, few in vitro studies have directly examined the impact of SAH on the parenchymal vasculature [19]. Most histological studies suggested that parenchymal arterioles from SAH model animals are more constricted [14]. Further, mechanistic information regarding the impact of SAH on the function of parenchymal arteriolar myocytes is limited. Our recent and ongoing work has begun to address these knowledge gaps.

Using the rat "double-injection" SAH model, we have examined the impact of subarachnoid blood on parenchymal arteriolar function and Ca^{2+} signaling [13]. In this study, SAH rats received two intracisternal injections of autologous arterial blood via the cisterna magna at 24-h intervals. This model recapitulates key pathologies observed in human SAH patients, including vasospasm, behavioral deficits, and decreased cortical blood flow [10, 18, 20]. Importantly, we observed extravascular red blood cells along parenchymal arterioles for distances greater than 500 μm into the cerebral cortex, demonstrating that subarachnoid blood can pass beyond the Virchow-Robin space and directly interact with parenchymal arterioles within the brain cortex. These observations are consistent with previous reports of labeled (biotinylated) oxyhemoglobin penetrating a depth of greater than 1 mm into the cerebral cortex in a similar rat SAH model [17].

To examine the relationship between smooth muscle cytosolic Ca^{2+} and pressure-induced myogenic tone, simultaneous measurements of Ca^{2+} and diameter were obtained from isolated parenchymal arterioles using the ratiometric Ca^{2+} indicator fura-2 [13]. Within the physiological range of intravascular pressures (40–60 mmHg), parenchymal arterioles isolated from day 4 SAH rats exhibited significantly elevated arterial wall Ca^{2+} and enhanced vasoconstriction (Fig. 33.1a–e). Interestingly, the relationship between arteriolar Ca^{2+} and constriction (i.e., Ca^{2+} sensitivity) was similar between groups (Fig. 33.1f). Further, selective L-type VDCC antagonists (e.g., nimodipine) caused a near-maximum

decrease in arteriolar Ca^{2+} and vasodilation (Fig. 33.1a–e). In the presence of L-type VDCC inhibitors, the R-type VDCC antagonist SNX-482 and the purported T-type VDCC antagonist mibefradil did not alter cytosolic Ca^{2+} or diameter of parenchymal arterioles isolated from control or SAH model animals. These data demonstrate that elevated $[Ca^{2+}]_i$ due to enhanced L-type VDCC activity underlies SAH-enhanced parenchymal arteriolar constriction.

SAH-Enhanced VDCC Activity in Parenchymal Arteriolar Myocytes Is Due to Suppression of Voltage-Dependent K^+ Channels and Smooth Muscle Membrane Potential Depolarization

Enhanced L-type VDCC activity in parenchymal arteriolar myocytes from SAH animals could result from enhanced L-type VDCC expression or membrane potential depolarization leading to increased activity of existing channels. Our data are consistent with the latter of these two possibilities. First, quantitative real-time polymerase chain reaction (PCR) determined that expression of $Ca_V1.2$ messenger RNA (mRNA), encoding the predominantly expressed L-type VDCC pore-forming α_1 subunit in vascular smooth muscle, was similar in parenchymal arterioles isolated from control and SAH animals. Second, $Ca_V1.2$ protein levels were not different between parenchymal arteriolar homogenates obtained from control and SAH animals. Third, in vitro measurements using intracellular microelectrodes revealed a smooth muscle membrane potential depolarization of approximately 7 mV in pressurized parenchymal arterioles obtained from SAH animals relative to control animals. As the open-state probability of L-type VDCCs is steeply voltage dependent in the physiological range of membrane potentials [11], a membrane potential depolarization of this magnitude (7 mV) would be expected to cause a substantial increase in Ca^{2+} channel activity [13]. These findings demonstrate that smooth muscle membrane potential depolarization, not increased L-type VDCC expression, is responsible for enhanced parenchymal arteriole constriction after SAH.

Voltage-dependent delayed rectifier K^+ (K_V) channels are expressed in the cerebral vasculature and are key regulators of smooth muscle membrane potential and arterial diameter [11]. Decreased K_V channel activity would cause membrane potential depolarization, increased Ca^{2+} influx via VDCCs, and vasoconstriction [6]. Thus, membrane potential depolarization and enhanced constriction of parenchymal arterioles from SAH animals could reflect a reduction in K_V channel activity. We have previously demonstrated in pial cerebral arteries that the blood component oxyhemoglobin suppresses K_V currents in cerebral artery myocytes by 30–40% and causes vasoconstriction within minutes of application [3, 9]. This acute oxyhemoglobin-induced K_V channel suppression is mediated via a cell-signaling pathway involving activation of matrix metalloproteases (MMPs), leading to shedding of heparin-binding epidermal growth factor-like (EGF-like) growth factor (HB-EGF), activation of the tyrosine kinase EGF receptor, and K_V channel internalization [3, 9] (Fig. 33.2). Currently, it is not known if this novel mechanism of K_V channel suppression contributes to the sustained membrane potential depolarization and constriction that we have observed in parenchymal arterioles obtained from 4-day

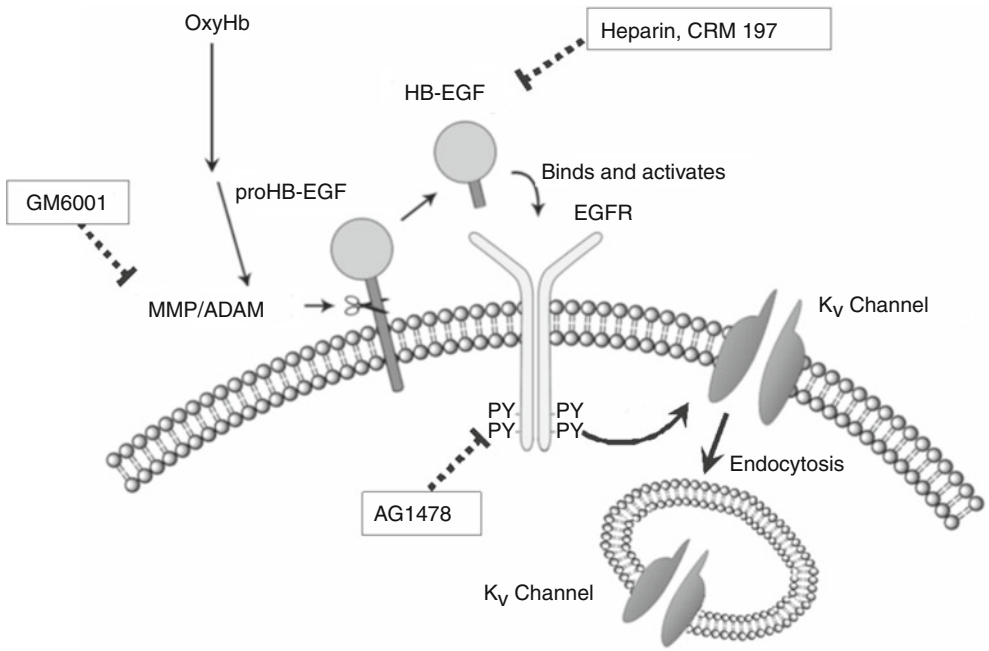

Fig. 33.2 Proposed signaling pathway of OxyHb-induced K_V current suppression involving HB-EGF and EGFR activation. Schematic diagram illustrates OxyHb-induced K_V current suppression via enhanced MMP activation and HB-EGF shedding. *OxyHb* oxyhemoglobin, *MMP* matrix metalloprotease, *ADAM* a disintegrin and metalloprotease, *HB-EGF* heparin-binding epidermal growth factor-like growth factor, *EGFR* epidermal growth factor receptor, *PY* phosphorylated tyrosine residue, *K_V channel* voltage-dependent potassium channel (Reproduced from Koide et al. [9])

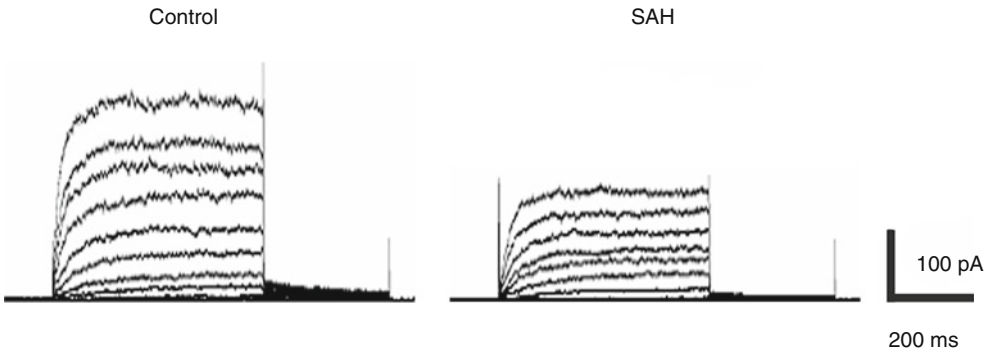

Fig. 33.3 Voltage-dependent K^+ channel currents are decreased in parenchymal arteriolar myocytes following subarachnoid hemorrhage (*SAH*). Representative traces of voltage-dependent K^+ channel currents recorded using the conventional whole-cell patch clamp technique from control (cell capacitance 8.3 pF) and SAH animals (cell capacitance 8.2 pF)

SAH model rats. Consistent with this possibility, using conventional whole-cell patch clamp electrophysiology, we have preliminary evidence indicating that outward voltage-dependent K^+ currents are indeed suppressed in parenchymal arteriolar myocytes freshly isolated from day 4 SAH model rats (Fig. 33.3). Consistent with our previous studies using myocytes isolated from pial arteries [3, 8, 9], we have found that 4-AP-sensitive K_V current density is dramatically decreased in freshly isolated parenchymal arteriolar myocytes from SAH animals. We have also observed that suppression of K_V currents by HB-EGF was reduced in parenchymal arteriolar myocytes from SAH animals. Further, we have found that MMP-2 activity, but not expression, is enhanced in homogenates of cerebral arteries obtained from SAH animals. These data are consistent with SAH-induced suppression of K_V currents in parenchymal arteriolar myocytes through a mechanism involving MMP and EGF receptor activation.

Conclusion

Parenchymal arterioles play a critical role and represent a potential bottleneck in the delivery of blood to brain cortex. Subarachnoid blood causes enhanced parenchymal arteriole constriction at physiological intravascular pressures. This enhanced vasoconstriction is due to K_V channel suppression leading to membrane potential depolarization and increased Ca^{2+} influx due to enhanced VDCC activity. SAH-induced parenchymal arteriolar constriction may contribute to decreased cerebral blood and the development of ischemic neuronal damage commonly observed in patients following cerebral aneurysm rupture.

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Conflicts of Interest We declare that we have no conflict of interest.

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SAH-Induced Suppression of Voltage-Gated K^+ (K_V) Channel Currents in Parenchymal Arteriolar Myocytes Involves Activation of the HB-EGF/EGFR Pathway

Masayo Koide and George C. Wellman

Abstract Potassium channels play an important role in the regulation of arterial tone, and decreased activity of these ion channels has been linked to pial artery vasospasm after subarachnoid hemorrhage (SAH). Our previous work has shown that acute application of a blood component, oxyhemoglobin, caused suppression of voltage-gated K^+ (K_V) channels through heparin-binding epidermal growth factor-like growth factor (HB-EGF)-mediated activation of epidermal growth factor receptor (EGFR). Using patch clamp electrophysiology, we have now examined whether this pathway of K_V channel suppression is activated in parenchymal arteriolar myocytes following long-term in vivo exposure to subarachnoid blood. We have found that K_V currents, but not large conductance Ca^{2+} activated or inwardly rectifying K^+ channel currents, were decreased in parenchymal arteriolar myocytes freshly isolated from day 5 SAH model rabbits. Interestingly, parenchymal arteriolar myocytes from control animals were more sensitive to exogenous HB-EGF (half-maximal inhibitory concentration [IC_{50}] 0.2 ± 0.4 ng/ml) compared to pial arterial myocytes (IC_{50} 2.4 ± 1.3 ng/ml). However, HB-EGF and oxyhemoglobin failed to decrease K_V currents in parenchymal arteriolar myocytes from SAH animals, consistent with EGFR activation and K_V current suppression by SAH. These data suggest that HB-EGF/EGFR pathway activation contributes to K_V current suppression and enhanced parenchymal arteriolar constriction after SAH.

Keywords K^+ channels • Heparin-binding EGF-like growth factor (HB-EGF) • Parenchymal arteriole • Patch clamp • Vascular smooth muscle • Vasospasm

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Introduction

Potassium-selective ion channels represent a family of ubiquitously expressed pore-forming proteins that have diverse physiological functions. Several types of K^+ channels, including voltage-gated K^+ (K_V) channels, large conductance Ca^{2+}-activated K^+ (BK) channels, inward rectifier K^+ (Kir) channels, and adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels are expressed in cerebral arterial myocytes and play an important role in the regulation of arterial tone [11]. In arterial myocytes, decreased K^+ channel activity promotes membrane potential depolarization, leading to activation of voltage-dependent Ca^{2+} channels, increased Ca^{2+} influx, and vasoconstriction [5].

Previous studies have demonstrated that decreased K_V channel currents contribute to enhanced arteriolar tone in pial cerebral arteries following subarachnoid hemorrhage (SAH) [2, 13, 14]. With respect to potential mechanisms of decreased K_V channel activity, our laboratory has demonstrated that acute exposure of cerebral artery myocytes to the blood component oxyhemoglobin caused K_V current suppression via a pathway involving activation of the tyrosine kinase epidermal growth factor receptor (EGFR) by its ligand heparin-binding epidermal growth factor-like growth factor (HB-EGF) [2, 8]. These studies provided evidence that oxyhemoglobin increased matrix metalloprotease activity in cerebral arteries, leading to the cleavage and release of HB-EGF from membrane-bound pro-HB-EGF. Binding of HB-EGF to EGFR may lead to K_V channel endocytosis and vasoconstriction [8]. Presently, it is unclear whether the HB-EGF/EGFR pathway is activated in the cerebral vasculature after SAH.

Parenchymal arterioles, located in brain parenchyma downstream of the Virchow-Robin space, play an important role in the regulation of both local and global cerebral blood flow. Compared to brain surface arteries, relatively little is currently known regarding the impact of SAH on parenchymal arteriolar function. Recently, Nystoriak et al. [12] demonstrated that parenchymal arterioles isolated from SAH model rats exhibit enhanced myogenic tone and vascular

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smooth muscle membrane potential depolarization. Here, we examine the hypothesis that decreased K_V currents contribute to enhanced tone in parenchymal arterioles isolated from SAH model rabbits via a pathway involving HB-EGF-induced activation of EGFR.

Materials and Methods

Rabbit SAH Model

This study used male New Zealand white rabbits (3.0–3.5 kg; Charles River) in accordance with the *Guidelines for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1996) and followed protocols approved by the Institutional Animal Use and Care Committee of the University of Vermont. Under isoflurane anesthesia, animals received two injections of unheparinized autologous arterial blood (2.5 ml) into the cisterna magna at an interval of 48 h [3, 6, 7, 10]. Immediately prior to each injection of blood, an equivalent volume of cerebral spinal fluid was removed. Buprenophine (0.01 mg/kg) was administered as an analgesic every 12 h for 36 h after each surgery. Five days after the initial surgery, animals were euthanized by exsanguination and decapitation under deep pentobarbital anesthesia (60 mg/kg, iv). Parenchymal arterioles from the middle cerebral artery territory were dissected in ice-cold physiological saline solution (in mM: 118.5 NaCl, 4.7 KCl, 24 $NaHCO_3$, 1.18 KH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 0.023 EDTA [ethylenediamine tetraacetic acid], and 11 glucose; pH 7.4) aerated with 20% O_2, 5% CO_2, 75% N_2.

Electrophysiology

Arteriolar Smooth Muscle Cell Isolation

Individual myocytes were enzymatically dissociated from parenchymal arterioles as described previously [4, 7, 8]. Briefly, parenchymal arterioles were incubated at 37°C for 17 min in glutamate-containing isolation solution (in mM: 55 NaCl, 5.6 KCl, 80 L-glutamic acid, 2.0 $MgCl_2$, 10 HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], and 10 glucose; pH 7.3) with 0.3 mg/ml papain and 0.7 mg/ml dithioerythritol. Arterioles were then transferred into isolation solution containing collagenase (0.7 mg/ml collagenase type F and 0.3 mg/ml collagenase type H) and 0.1 mM $CaCl_2$ for 10 min at 37°C. After incubation in isolation solution with 2 mM $CaCl_2$ on ice for 30 min, tissues were gently triturated using a small-bore fire-polished Pasteur pipette.

Whole-Cell Voltage-Dependent K^+ Channel Current Recordings

Whole-cell membrane K^+ currents were measured at room temperature using the conventional whole-cell configuration of the patch clamp technique. The bath solution consisted of (in mM): 134 NaCl, 6 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 10 HEPES (pH 7.4). Except when specifically mentioned, bath solution also contained 1.8 mM $CaCl_2$. Patch pipettes (8–10 MΩ) were filled with an (internal) solution containing (in mM) 87 K^+ aspartate, 20 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 EGTA [ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid], and 25 KOH (pH 7.2). Outward currents were elicited by a series of 500-ms depolarizing voltage steps from a holding potential of −70 to +50 mV, followed by a step to −40 mV for 300 ms. Voltage steps were made at 10 mV increments at intervals of 10 s.

Inward Rectifier K^+ Channel Current Measurement

Kir currents were measured using conventional whole-cell patch clamp electrophysiology. The bath solution contained (in mM) 140 KCl, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.4). Internal (pipette) solution contained (in mM) 87 K^+ aspartate, 20 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 EGTA, and 25 KOH (pH 7.2). From a holding potential of −70 mV, voltage was stepped to −100 mV for 100 ms, then ramped from −100 mV to +40 mV over a period of 500 ms. Kir currents were defined as membrane currents sensitive to 100 μM $BaCl_2$.

Statistical Analysis

Data are expressed as mean plus or minus SEM with n representing the number of cells per group and N representing the number of animals per group. Student's paired or unpaired t tests were used to determine statistical significance at the level of $p<0.05$ (*) or $p<0.01$ (**).

Results

Decreased K_V Currents in Parenchymal Arteriolar Myocytes After SAH

Whole-cell, voltage-dependent K^+ currents, representing a combination of BK and K_V channel activity, were measured in freshly isolated parenchymal arteriolar myocytes from control and SAH model animals. Whole-cell currents were significantly decreased in cells from SAH model animals (Fig. 1a,b). For example, current density at +50 mV was 30.0±2.8 pA/pF ($n=6$, $N=2$ animals) and 21.7±2.5 pA/pF ($n=5$, $N=2$) in

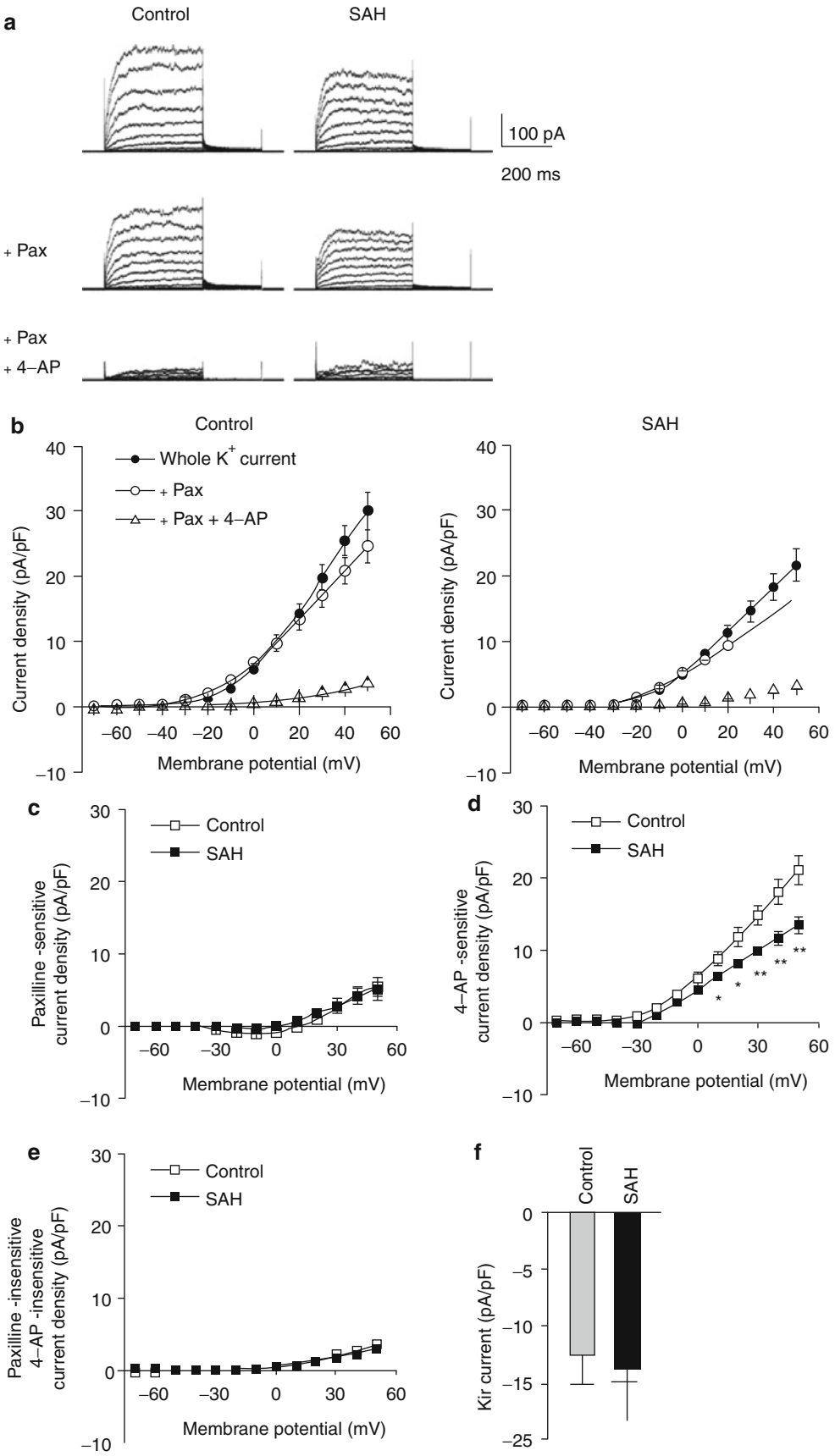

Fig. 1 Decreased voltage-gated K^+ (K_V) currents in rabbit parenchymal arteriolar myocytes following subarachnoid hemorrhage (*SAH*). (**a**) Representative traces of voltage-dependent K^+ channel currents obtained using conventional whole-cell patch clamp electrophysiology. Blockers of large conductance Ca^{2+}-activated K^+ (*BK*) channels (paxilline, *Pax*, 1 μM) and voltage-gated K^+ (K_V) channels (4-aminopyridine, *4-AP*, 10 mM) were cumulatively added to cells at an interval of 10 min. Cell capacitance in control and SAH myoctes were 11.1 and 10.9 pF, respectively. (**b**) Summary of current densities in the presence and absence of paxilline and 4-AP. Current density was calculated by dividing membrane currents by cell capacitance for each myocyte. Whole K^+ currents (in the absence of paxilline and 4-AP, *closed circles*) were significantly smaller in parenchymal arteriolar myocytes from SAH model animals. Control: $n=6$ cells from two animals; SAH: $n=5$ cells from two animals. (**c–e**) Summary of K^+ currents obtained in the presence of paxilline and 4-AP. 4-AP-sensitive (K_V) currents were decreased in myocytes obtained from SAH model animals (**d**). Paxilline-sensitive (**c**) and paxilline-insensitive/4-AP-insensitive (**e**) currents were similar between groups. (**f**) Inward rectifier K^+ (*Kir*) currents obtained at −100 mV were similar between control ($n=15$ cells from eight animals) and SAH ($n=12$ cells from five animals) myocytes.

myocytes from control and SAH animals, respectively. Cell capacitance was not different between groups (control 11.2 ± 1.4 pF, $n=6$; SAH 10.7 ± 0.8 pF, $n=5$). To examine BK channel currents, cells were treated with paxilline, a blocker of BK channels. Paxilline-sensitive currents were similar at +50 mV in myocytes from control (5.5 ± 1.3 pA/pF, $n=6$) and SAH (5.2 ± 1.5 pA/pF, $n=5$) animals, indicating that BK currents were unaltered after SAH (Fig. 1c). In contrast, currents sensitive to 4-aminopyridine (4-AP, 10 mM), a K_V channel blocker, were markedly decreased in parenchymal arteriolar myocytes from SAH model animals (Fig. 1d). At +50 mV, 4-AP-sensitive current density was 21.1 ± 2.0 pA/pF in myocytes from control animals ($n=6$) compared to 13.5 ± 1.1 pA/pF in myocytes from SAH animals ($n=5$). Outward K^+ currents insensitive to both paxilline and 4-AP were similar between groups (Fig. 1e), as were Kir currents (Fig. 1f). These data demonstrate that K_V currents are selectively decreased in parenchymal arteriolar myocytes from SAH model animals.

HB-EGF Causes K_V Current Suppression in Parenchymal Arteriolar Myocytes from Control Animals

We have previously shown that acute application of oxyhemoglobin suppresses K_V currents in pial artery myocytes from control animals via a pathway involving HB-EGF and EGFR activation [8]. We therefore hypothesized that activation of the HB-EGF/EGFR pathway contributes to K_V current suppression in parenchymal arteriolar myocytes after SAH. To examine this hypothesis, we first examined the ability of exogenous HB-EGF to suppress K_V currents in parenchymal arteriolar myocytes from control animals. In this experimental series, Ca^{2+} in the bath solution was lowered from 2 to 0.1 mM to minimize BK currents. Treatment of exogenous HB-EGF decreased K_V currents in a concentration-dependent manner in parenchymal arteriolar myocytes from control animals (Fig. 2a). Interestingly, control parenchymal arteriolar myocytes (IC_{50} 0.2 ± 0.4 ng/ml) were more sensitive to HB-EGF compared to pial artery myocytes (IC_{50} 2.4 ± 1.3 ng/ml). In control parenchymal arteriolar myocytes, 30 ng/ml HB-EGF suppressed whole-cell K^+ currents by approximately 45%, or 12.8 ± 1.9 pA/pF ($n=4$, $N=3$). In a second experimental series, the effect of HB-EGF on K_V current was also examined using bath solution containing 1.8 mM Ca^{2+} and the BK channel blocker paxilline (Fig. 2b). Using these conditions, HB-EGF (30 ng/ml)-induced current suppression was 12.4 ± 1.4 pA/pF, $n=5$, $N=3$, which was similar to that observed using 0.1 mM Ca^{2+} in the bath solution. These data demonstrate that HB-EGF can potently suppress K_V currents in parenchymal arteriolar myocytes in the absence of SAH.

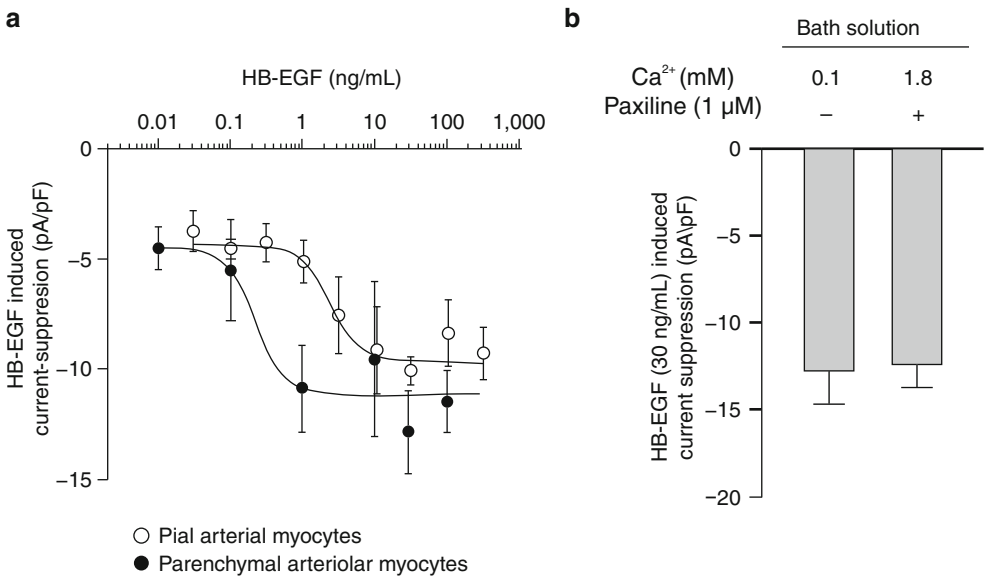

Fig. 2 Heparin-binding epidermal growth factor-like growth factor (*HB-EGF*)-induced K_V current suppression in parenchymal and cerebral arterial myocytes isolated from control rabbits. (**a**) Concentration-response curve of HB-EGF-induced K^+ current suppression. K^+ currents were obtained from rabbit parenchymal arteriolar (*closed circle*) and cerebral arterial myocytes (*open circle*) using 800-ms of voltage steps to +50 mV from a holding potential of −70 mV. To minimize large conductance Ca^{2+}-activated K^+ (*BK*) current, Ca^{2+} in the bath solution was lowered to 0.1 mM. Decreased current densities after 10 min HB-EGF treatment were plotted ($n=4$–6 cells) for each HB-EGF concentration. Data from pial arterial myocytes were modified from Koide et al. [8]. (**b**) Suppression of K_V currents by HB-EGF (30 ng/ml) in parenchymal arteriolar myocytes at +50 mV. Prior to treatment with HB-EGF, BK currents were minimized either by lowering Ca^{2+} in the extracellular solution from 1.8 to 0.1 mM or adding paxilline (1 μM), suggesting HB-EGF suppresses K_V current, not BK current ($n=4$–5)

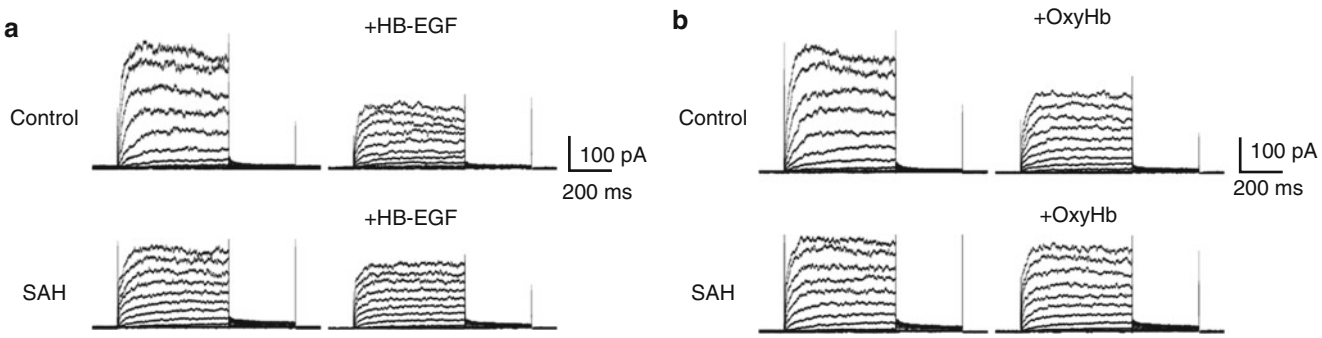

Fig. 3 Suppression of voltage-gated K^+ (K_V) currents by heparin-binding epidermal growth factor-like growth factor (*HB-EGF*) and oxyhemoglobin in parenchymal arteriolar myocytes is reduced in subarachnoid hemorrhage (*SAH*) compared to control animals. (**a**) Representative traces of the effects of HB-EGF treatment (30 ng/ml) on K_V currents in parenchymal arteriolar myocytes from control and SAH animals. Cell capacitance was 10.3 pF (*control*) and 9.7 pF (*SAH*). (**b**) Oxyhemoglobin (*OxyHb*, 10 μM) decreased K_V currents in parenchymal arteriolar myocytes from control but not SAH animals. Cell capacitance was 10.8 pF (*control*) and 11.1 pF (*SAH*). Recordings were obtained in the presence of the large conductance Ca^{2+}-activated K^+ (BK) channel blocker paxilline (1 μM)

HB-EGF and Oxyhemoglobin Do Not Cause K_V Current Suppression in Parenchymal Arteriolar Myocytes from SAH Model Animals

Considering that SAH may cause K_V current suppression via EGFR activation, it is conceivable that the actions of exogenous activators of EGFR may be diminished when applied to parenchymal arteriolar myocytes from SAH model animals. Consistent with this possibility, K_V current suppression induced by HB-EGF (Fig. 3a) and oxyhemoglobin (Fig. 3b) were markedly decreased in parenchymal arteriolar myocytes from SAH animals compared to similar cells from control animals. Although further study is needed, these data strongly suggest that activation of the HB-EGF/EGFR pathway contributes to K_V current suppression in parenchymal arteriolar myocytes after SAH.

Discussion

Here, we demonstrate that K_V currents are suppressed in parenchymal arteriolar myocytes after SAH. K_V current suppression would cause membrane potential depolarization [12], increased voltage-dependent Ca^{2+} channel activity [12], and enhanced arteriolar tone [3, 6, 12]. Our findings are in agreement with previous studies demonstrating SAH-induced K_V current suppression in pial arteries [2, 13, 14]. Further, we provide evidence of a novel mechanism linking SAH to K_V current suppression involving HB-EGF-mediated activation of EGFR. Consistent with activation of this pathway after SAH, suppression of K_V currents by HB-EGF and oxyhemoglobin in parenchymal arteriolar myocytes was reduced in SAH compared to control animals. Considering that the blood component oxyhemoglobin is able to activate the HB-EGF/EGFR pathway [8], release of oxyhemoglobin from subarachnoid blood may contribute to SAH-induced K_V current suppression in parenchymal arterioles. However, parenchymal arterioles are downstream of the Virchow-Robin space and are tightly encased by astrocyte end feet. Further research is required to determine whether blood components such as oxyhemoglobin directly interact with parenchymal arteriolar myocytes to cause K_V channel suppression.

Interestingly, we found that HB-EGF caused K_V current suppression at lower concentrations in parenchymal arteriolar myocytes compared to myocytes from pial arteries. This finding suggests that activation of the HB-EGF/EGFR pathway during pathological conditions (e.g., SAH) may have a greater impact on parenchymal arterioles than brain surface arteries. Further, the HB-EGF/EGFR pathway may play an important role in the physiological regulation of parenchymal arteriolar tone. Consistent with this possibility, the IC_{50} value determined for HB-EGF-induced K_V current suppression (approximately 0.2 ng/ml; Fig. 2) is close to serum levels of HB-EGF (0.05–0.15 ng/ml) reported in humans [9]. It is also possible the enhanced HB-EGF/EGFR-mediated K_V channel suppression may contribute to enhanced pressure-induced constrictions reported in parenchymal arterioles isolated from healthy animals [1, 12].

Conclusion

In summary, diminished K_V currents were observed in parenchymal arteriolar myocytes from SAH model rabbits. Suppression of K_V currents promoting enhanced arteriolar tone and decreased cerebral blood flow may contribute to the development of neurological deficits following SAH. Further, our findings suggest involvement of the HB-EGF/EGFR pathway in K_V current suppression following SAH. This

work describing a novel pathway of K_V current suppression should provide new insights for the development of treatments for patients after not only cerebral aneurysm rupture but also brain trauma, for which SAH is a common but understudied occurrence.

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

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Mechanisms of Microthrombosis and Microcirculatory Constriction After Experimental Subarachnoid Hemorrhage

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Abstract Microcirculatory dysfunction may contribute to delayed cerebral ischemia after subarachnoid hemorrhage (SAH). This study investigated structural changes in microvessels and their relationship to brain injury after SAH. We used 15 mice ($n=5$ for each group) to create sham, saline-injected (100 µl 0.9% NaCl) or SAH (100 µl autologous blood) model by injection into the prechiasmatic cistern. We sacrificed mice 2 days after surgery and examined the brains using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and immunohistochemical staining of fibrinogen. We assessed neuronal apoptosis by terminal deoxynucleotidyl transferase dUTP (deoxyuridine triphosphate) nick end labeling (TUNEL). Nitric oxide (NO) was measured with 4,5-diaminofluorescein-2-diacetate. TEM and SEM demonstrated that mice with SAH had significantly more of them arterioles with lesion characteristics consistent with microthrombi. Microthrombi number correlated with the number of apoptotic neurons and decreased NO in the brain. In conclusion, SAH causes microthrombosis and constriction of arterioles, which correlates with neuronal death and decreased NO. These data suggest NO depletion may contribute to the formation of microthrombosis and arteriolar constriction, which in turn results in neuronal cell death.

Keywords Subarachnoid hemorrhage • Microthrombosis • Scanning electron microscopy • Transmission electron microscopy • Nitric oxide • Mice

Introduction

Subarachnoid hemorrhage (SAH) accounts for about 6% of cases of stroke and afflicts more than 50,000 individuals annually in North America [20]. Cerebral angiographic vasospasm has been recognized as commonly associated with aneurysmal SAH for many years and has been regarded as potentially the most treatable prognostic factor for outcome [10]. However, recent studies showed that angiographic vasospasm does not predict the location of infarction in at least one fifth to one third of SAH patients [14]. Furthermore, some patients with SAH develop ischemia and cortical infarctions without any evidence of angiographic vasospasm [14, 22]. Other postulated secondary mechanisms after SAH include acute brain injury, microcirculatory dysfunction, microthromboembolism, and cortical spreading ischemia [10, 13]. Microthromboemboli were reported after fatal human SAH [18]. Transcranial Doppler ultrasound in humans with SAH detects microembolic signals, so it is not clear if these lesions are thrombi formed in situ or emboli from proximal vasospastic arteries [15]. If they are thrombi, do they form primarily due to endothelial injury, or are they secondary to vasoconstriction? The focus of this study was to determine whether experimental SAH is associated with microcirculatory constriction and thrombosis, what the nature of microthromboemboli (thrombi or emboli?) is, and whether these changes correlate with brain injury.

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Materials and Methods

Animal Model

Experimental protocols were approved by the Institutional Animal Care Committee. We used 15 male CD1 mice weighing 20–25 g in this study. We anesthetized mice with ketamine (10 mg/kg) and xylazine (4 mg/kg) by intraperitoneal injection. We maintained body temperature of the animals at 37.0 ± 0.5°C with a rectal temperature probe and a homeothermic heating pad (Harvard Apparatus, Holliston, MA). SAH was created by injection of autologous blood into the prechiasmatic cistern [17]. The mice were positioned in a stereotactic frame equipped with a mouse adapter (Stoelting Co., Wood Dale, IL). Relative cerebral blood flow (CBF) was measured using a laser Doppler flow probe (BLT21, Transonics Systems, New York City). We angled a drill 40° caudally at the site 4.5 mm anterior to the bregma and drilled a 0.9-mm hole slightly lateral to the midline of the skull to avoid the superior sagittal sinus. A 27-gauge spinal needle was advanced through the burr hole to the base of the skull and into the prechiasmatic cistern. We withdrew autologous blood (nonheparinized, 100 μl) from the ventral tail artery using a 25-gauge needle and injected it into the prechiasmatic cistern over 10 s ($n=5$). We used the same procedure to create saline-injected controls (100 μl 0.9% NaCl, $n=5$). For sham control animals, we did the same procedure but without injecting anything ($n=5$).

Scanning Electron Microscopy

Two days later, animals were anesthetized and perfused with 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer, pH 7.3, at physiological blood pressure. Brains were removed and immersed in this fixative for 24 h. Specimens were taken from coronal slices of the brain 2 mm from the bregma and 1–2 mm from the midline. Specimens were then washed, postfixed in 2% aqueous osmium tetroxide for 1 h, washed, and dehydrated in increasing concentrations of ethanol. They were critical point dried by placement in liquid CO_2 and then mounted on carbon tape and coated with gold-palladium. They were viewed in a scanning electron microscope (SEM; JEM-820, JEOL Corp., Peabody, MA) with images acquired using an image converter (IXRF Systems Inc., Houston, TX).

Transmission Electron Microscopy

Specimens of coronal brain slices 2 mm from the bregma and 1–2 mm from the midline were immersed in 4% formaldehyde and 1% glutaraldehyde in phosphate buffer, pH 7.3, for 24 h. They were postfixed in 1% osmium tetroxide for 1 h. They were dehydrated in a graded series of acetone solutions and then embedded in epon-araldite epoxy resin. A microwave oven was used for the processing steps from postfixation to polymerization of resin blocks (Pelco BioWave 34770, Pelco International, Clovis, CA). Ultrathin sections were cut with a diamond knife on a microtome (Reichert Ultracut E, Leica Inc., Vienna, Austria) and stained with uranyl acetate and lead citrate before examination in a transmission electron microscope (TEM; JEM-1011, JEOL Corp.). Digital TEM micrographs were acquired directly with a 1,024 × 1,024 pixel charge-coupled device camera (AMT Corp., Denver, CO).

Microvessel Vasoconstriction and Vascular Wall Assessment

Images of all visible vessels in both TEM and SEM slides were obtained using the following criteria: (1) vessels with a diameter that ranged from 10 to 20 μm, (2) vessels with at least one distinct endothelial cell visible on TEM, and (3) vessels with wall thickness greater than 0.5 μm in all directions. These criteria were thought to select for arterioles as opposed to venules. We assessed the lumen diameter, area, and perimeter using SEM images and vascular wall thickness using TEM. We averaged measurement of these parameters from all vessels for each animal. We measured microvessel lumen area using the freehand tool (Image J, National Institutes of Health, Bethesda, MD) to outline the artery lumen after calibration to the microscope-generated scale. Microvessel wall thickness was measured at four equally spaced points around the vessel circumference and averaged for each vessel. An observer blinded to the group the mouse was from performed all measurements.

Fibrinogen Staining and Measurement of Nitric Oxide

Coronal brain sections were deparaffinized and rehydrated. Antigen was retrieved by heating the sections for 20 min in 0.01 mmol/L sodium citrate (pH 6.0) at 96°C. Sections were permeabilized with 0.3% Triton X-100 for 10 min and then incubated with 10% normal goat serum for 60 min. Primary antibody used was rat polyclonal antifibrinogen (1:200, Abcam, Cambridge, MA), and secondary antibody was Alexa Fluor 568-conjugated antimouse for fibrinogen (1:1,000, Invitrogen). After incubating the sections with secondary antibody, they were washed and coverslipped with antifading mounting medium and sealed with nail polish.

Slides were viewed in a confocal microscope, and images were captured using consistent parameters (pinhole size, exposure time, and laser intensity).

The cell-permeable fluorophore 4,5-diaminofluorescein-2-diacetate (DAF-2DA; Alexis Biochemicals, Gruenberg, Germany) was used to detect nitric oxide (NO). Homogenized brain tissues were incubated with 10 μmol/L DAF-2DA for 30 min in transparent 96-well plates (Fisher, Ottawa, ON, Canada) at room temperature in the dark. DAF-2DA was excited at 495 nm and emission read at 515 nm in a spectrofluorometer (SpectraMAX-Gemini, Molecular Devices, Sunnyvale, CA). All experiments were repeated three times, with an average used for data analysis.

TUNEL and Fibrinogen Double Immunostaining

Apoptosis was detected using terminal deoxynucleotidyl transferase (deoxyuridine triphosphate) dUTP nick end labeling (TUNEL; DeadEnd Fluorometric kit, Promega, WI) according to the protocols from the manufacturer. We counterstained the slides with 4,6-diamidino-2-phenylindole (DAPI). Some slides were then stained with antifibrinogen antibodies (rat polyclonal antifibrinogen; 1:100, Abcam).

Statistical Analysis

All data are presented as means plus or minus standard deviation. Data were compared within groups over time and between groups at each time by analysis of variance (ANOVA) or Student *t* test for continuous variables and by χ^2 test for categorical variables; $p<0.05$ was considered significant. Correlations between variables were tested by linear regression.

Results

Scanning Electron Microscopy

Microvessel spasm was observed in all animals with SAH (Fig. 1). Microvessels from the brains of animals with SAH had significantly decreased lumen area, which was accompanied by thickened vessel wall (Fig. 1c). Mean microvessel lumen area was 289 ± 29 μm^2 for sham, 269 ± 39 μm^2 for saline-injected, and 115 ± 69 μm^2 for SAH animals ($p<0.01$, ANOVA; Fig. 1b). The interior of the microvessels from control animals was smooth. In contrast, the interior of microvessels from animals with SAH was corrugated and irregular with zigzagging structures (Fig. 1c). Some constricted microvessels contained intraluminal material that had an appearance consistent with thrombosis, rather than embolism, based on material arising from the endothelium and that was adherent to the endothelium. There were intact and crenated erythrocytes embedded in fibrin matrix that appeared to adhere to the vessel wall. There were constricted vessels without microthrombi, but no vessels were seen with microthrombi but no constriction.

Nitric Oxide

The decreased microvessel area was associated with decreased NO in the brain. The NO concentration was 44 ± 9 AU for sham and 46 ± 20 AU for saline-injected and 24 ± 11 AU for SAH animals ($p<0.05$, ANOVA).

Transmission Electron Microscopy

There was significantly increased microvessel wall thickness in animals with SAH as compared to sham or saline-injected controls (1.69 ± 0.35 μm for SAH, 0.83 ± 0.08 μm for sham, and 0.79 ± 0.04 μm for saline-injected; $p<0.01$, one-way ANOVA; Fig. 2b). Some of the constricted microvessels in animals with SAH contained thromboemboli, but this was not observed in sham or saline-injected controls, which showed smooth empty lumen (Fig. 2a). In microvessels from SAH animals, the lumen contained endothelial and perivascular cells that protruded into the lumen, and the cell membranes were corrugated, in contrast to the smooth membrane of endothelial and perivascular cells in the sham and saline-injected controls (Fig. 2a).

Correlation of Neuron Death with Microthromboemboli

To investigate whether vasoconstriction of microvessels and microthromboemboli were associated with brain injury, we stained brain slices with TUNEL to detect cell death. TUNEL-stained cells were found around fibrinogen-stained microthromboemboli in the brains of animals with SAH but not in sham or saline-injected controls (Fig. 3a). TUNEL-stained cells were obviously pyramidal neurons in the hippocampus and appeared to be neurons in the cortex. Quantification showed significantly more TUNEL-positive neurons and fibrinogen-positive microthromboemboli in

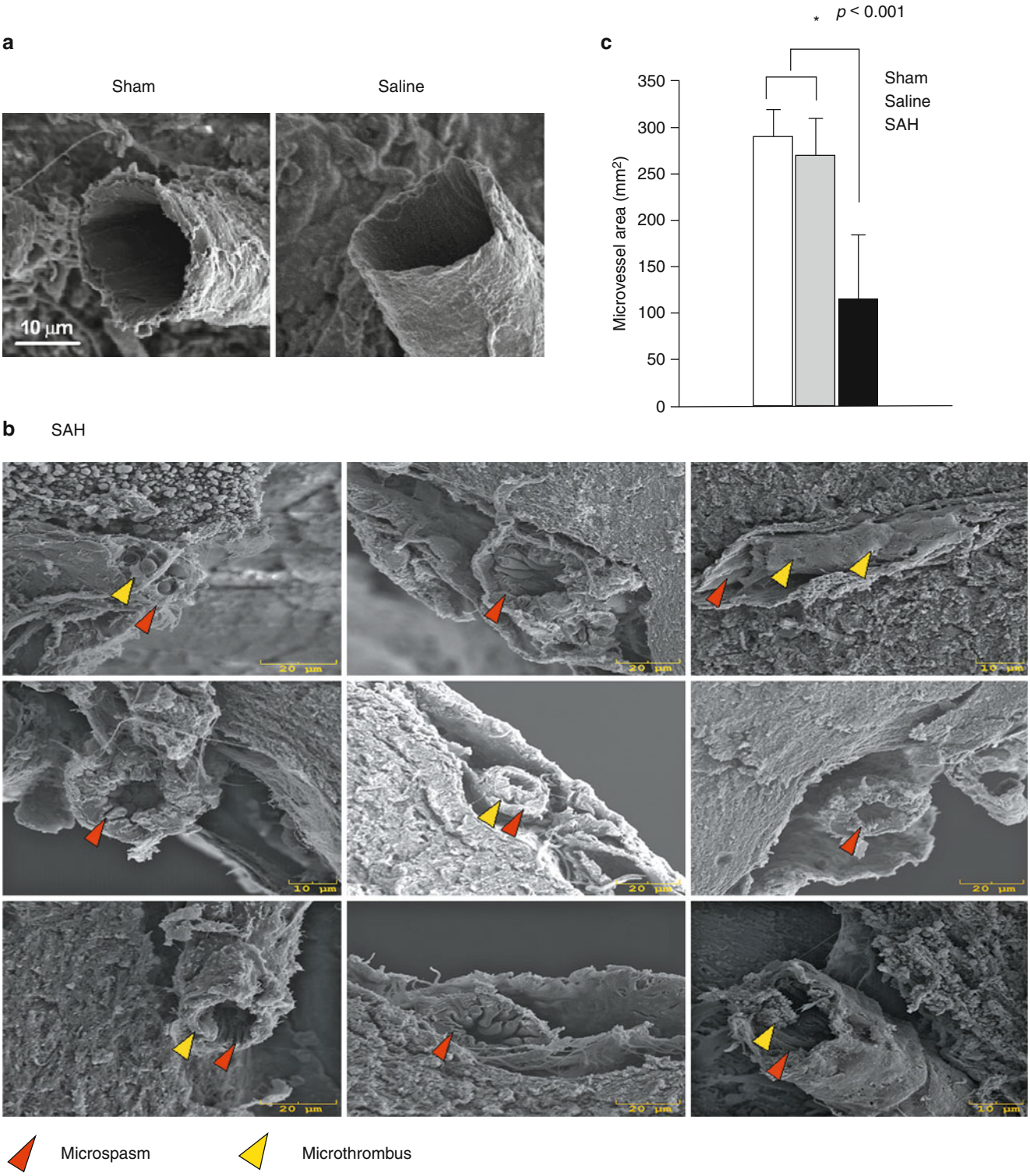
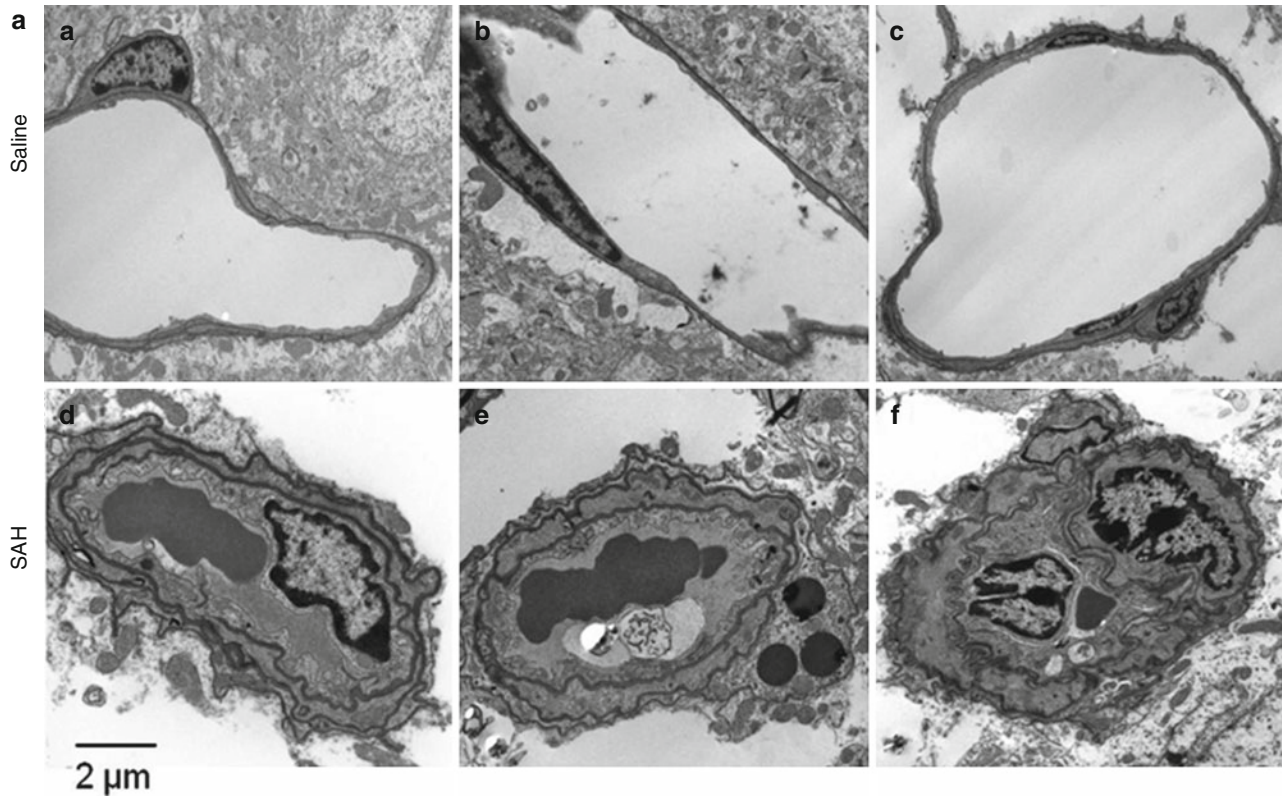


Fig. 1 Scanning electron microscopy (*SEM*) of microvessel constriction and microthrombi formation after subarachnoid hemorrhage (*SAH*). (**a**) and (**b**) Representative SEM images from sham and saline-injected (**a**) controls and SAH (**b**) showing smooth, empty microvessels in controls but constricted vessels, some filled with microthrombi, in SAH animals. (**c**) Quantification of microvessel constriction showing significantly decreased microvessel lumen area in animals with SAH compared to sham or saline-injected controls ($p < 0.01$, analysis of variance; data are means plus or minus standard deviations; $n = 5$ for all the groups)



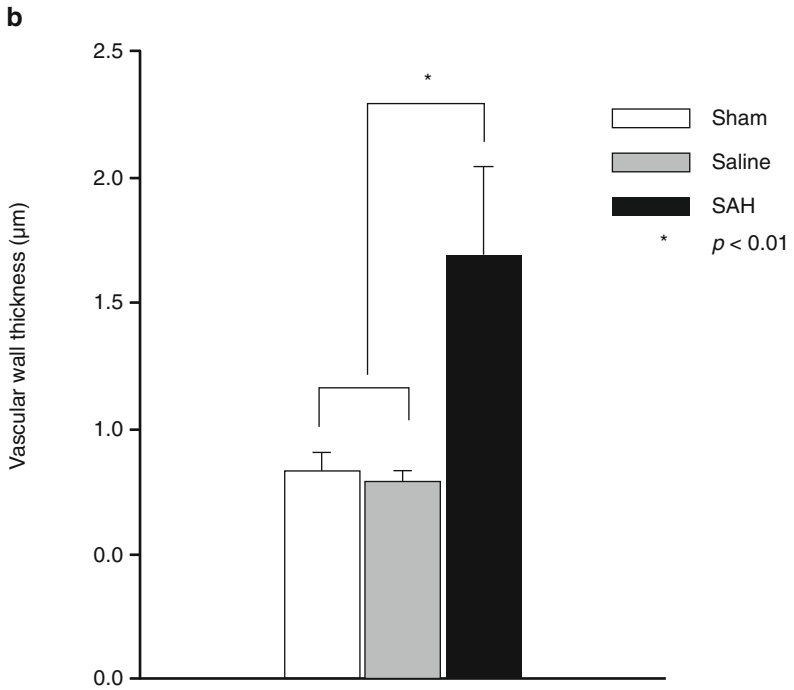


Fig. 2 Transmission electron microscopy (*TEM*) of microvessel vasoconstriction after subarachnoid hemorrhage (*SAH*). (**a**) *Upper panel* representative TEM images from saline-injected control animals showing thin-walled microvessels with smooth, empty lumen. *Lower panel* representative TEM images from SAH animals showing thickened microvessel walls and a thrombus in a constricted microvessel lumen. (**b**) Quantification of microvessel wall thickness showing significantly increased microvessel wall thickness in animals with SAH compared to sham or saline-injected controls ($p < 0.01$, analysis of variance; data are means plus or minus standard deviations; $n = 5$ for all the groups)

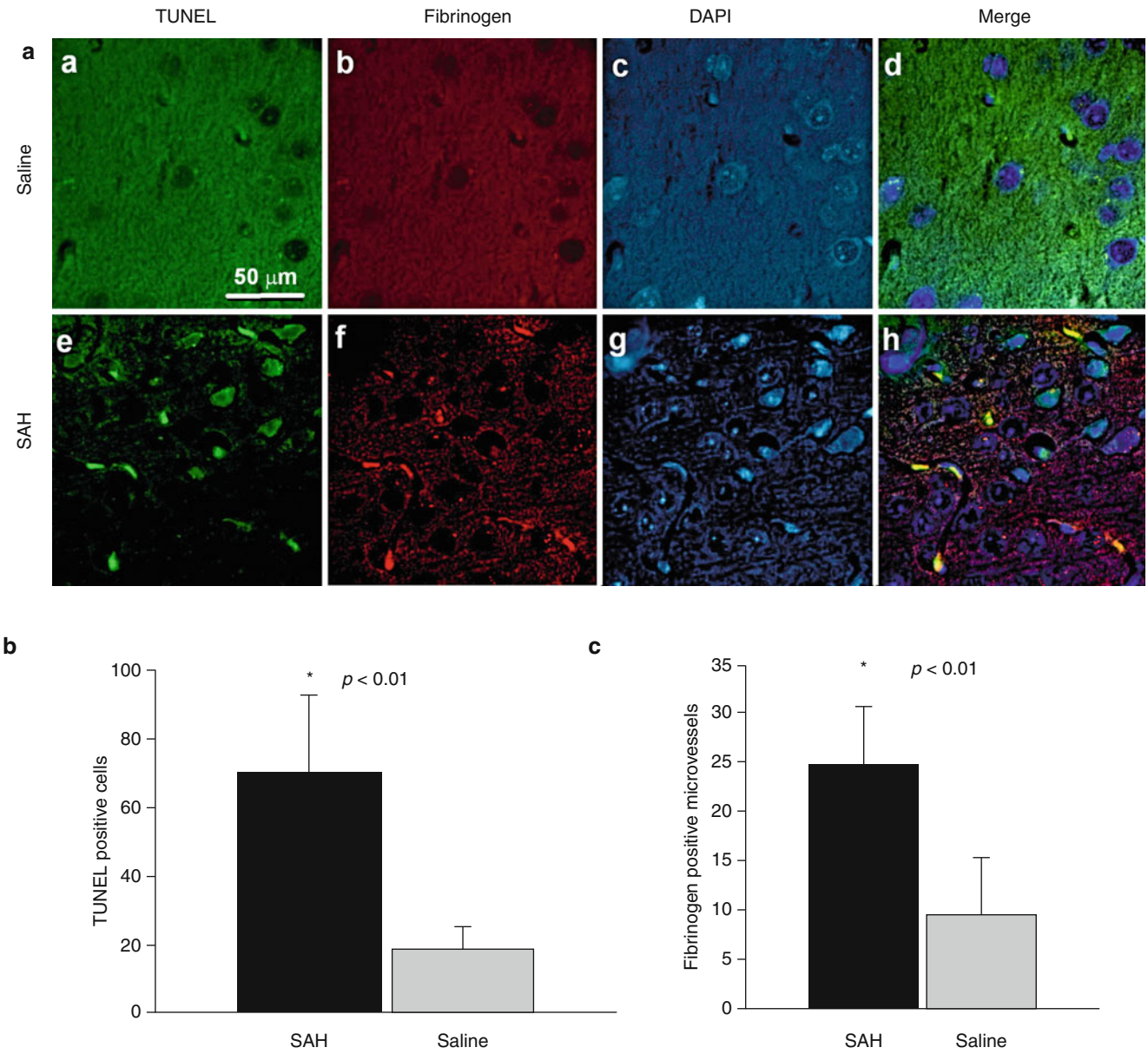


Fig. 3 Colocalization of cells that are terminal deoxynucleotidyl transferase dUTP nick end labeling (*TUNEL*) positive with fibrinogen-positive microthrombi. (**a**) Representative images showing colocalization of TUNEL and fibrinogen staining only in animals with subarachnoid hemorrhage (*SAH*) (*yellow* in *H*) but not in saline-injected control animals (*D*). (**b**) There is a significantly increased number of TUNEL-positive cells in SAH animals compared to saline-injected controls ($p<0.01$). (**c**) Marked increase of fibrinogen-positive microvessels are found in animals with SAH as compared to controls ($p<0.01$). There is a correlation of TUNEL positivity with fibrinogen positivity in the SAH group. All data in (**b**) and (**c**) are expressed as mean plus or minus standard deviation; *n* was 5 for all the groups

SAH as compared to saline-injected control animals (Fig. 3b, c). In animals with SAH, the TUNEL-positive cell count was 70 ± 23. This was 18 ± 7 for saline-injected controls (Fig. 3b; $p<0.01$, *t* test). Similar to the observations with TUNEL staining, in animals with SAH, fibrinogen-positive stained microthromboemboli counts were 25 ± 6. In contrast, the count was 10 ± 6 for saline-injected controls (Fig. 3c; $p<0.01$, *t* test). There was a linear correlation between TUNEL positivity and fibrinogen positivity.

Discussion

Controversy surrounds the etiology of delayed cerebral ischemia (DCI) and infarction in patients with SAH. Postulated mechanisms include angiographic vasospasm, cortical spreading ischemia, microcirculatory dysfunction, and microthromboembolism. We investigated microthromboembolism in an animal model. We used electron microscopy to visualize both microthromboembolism and microcirculatory

vasoconstriction in cortical and hippocampal arterioles after SAH. Few studies have determined if there is microthromboembolism. Electron microscopy was utilized in earlier studies to characterize ultrastructural differences in larger intradural arteries surrounding the region of hemorrhage [4, 19]. The changes observed included those associated with vasoconstriction, as well as pathological changes such as endothelial cell swelling, internal elastic lamina thickening, endothelial and smooth muscle cell vacuolation and necrosis, and loss of interendothelial cell tight junctions [5]. Adherence of platelets and potential embolic material was seen in some studies [1]. However, none of the studies reported ultrastructural differences in microvessels and parenchymal vessels after SAH.

In the current study, we demonstrated that microvessels in animals with SAH developed significant vasoconstriction with thickening of the endothelial and subendothelial layers. There were intimal convolutions and intraluminal thrombi in the majority of the constricted vessels. These vasoconstricted, thrombosed microvessels were spatially associated with TUNEL-stained cells that appeared to be neurons. Similar changes were seen on TEM, including thickening of vascular walls, intimal convolutions, and swelling of the endothelial cells with vacuolation. These findings are consistent with data showing that CBF is reduced days after SAH in animal models and in humans [3, 8, 9]. Prior studies also have examined larger penetrating brain arterioles by physiologic or anatomic methods and demonstrated vasoconstriction [12, 23] or, in some cases, vasodilation after SAH [6, 21]. Given that CBF is regulated mainly by the microcirculation, the present findings support the notion that microcirculatory changes contribute to brain injury after SAH.

We sought to determine if the intravascular lesions were thrombi or emboli. Other than directly visualizing the microcirculation in vivo after SAH, it is difficult to differentiate these possibilities, but several lines of evidence suggest the lesions are thrombi formed in situ. The e appearance was more consistent with thrombi than emboli [7]. Second, there were constricted microvessels but no thrombosed vessels that were not constricted, suggesting that constriction led to thrombosis, as opposed to emboli occluding microvessels, leading to secondary constriction. We previously demonstrated that in mice with SAH there is dysfunction of endothelial NO synthase that results in depletion of parenchymal NO and increased production of reactive oxygen species [16]. Decreased NO also was found after SAH in this study. We speculate that decreased NO may cause increased P-selectin. It has been shown that inhibition of NO synthesis results in an increase in P-selectin expression that is partially mediated by activation of protein kinase C in platelets [2, 11]. Inhibition of NO production also resulted in upregulation of P-selectin in endothelial cells, which may contribute to the formation of microthrombi in SAH animals.

Conclusion

In conclusion, this study found ultrastructural evidence of microthrombosis and vasoconstriction in smaller vessels after SAH. These correlated with apoptotic neurons in adjacent brain tissue. Both microvessel vasoconstriction and microthrombi were accompanied by decreased NO in the brain, both of which may contribute to reduced CBF and brain injury after SAH.

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

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Cerebral Hemodynamic and Metabolic Effects of Remote Ischemic Preconditioning in Patients with Subarachnoid Hemorrhage

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Abstract *Background*: Remote ischemic preconditioning (RIPC) is a form of endogenous neuroprotection induced by transient, subcritical ischemia in a distant tissue. RIPC effects on cerebral hemodynamics and metabolism have not been explored in humans. This study evaluates hemodynamic and metabolic changes induced by RIPC in patients with aneurysmal subarachnoid hemorrhage (SAH).

Methods: Patients underwent three or four RIPC sessions 2–12 days following SAH. Continuous vitals, intracranial pressure (ICP), and transcranial Doppler (TCD) data were collected. Brain microdialysis metabolic changes were monitored. ICP and TCD morphological clustering and analysis of intracranial pulse (MOCAIP) metrics were compared to positive and negative control groups for cerebral vasodilation.

Results: Seven ICP and six TCD recordings from four patients demonstrated an increase in mean ICP (8–14.57 mmHg, $p<0.05$). There was a reduction in middle cerebral artery (MCA) mean velocities (111–87 cm/s, $p=0.039$). ICP and TCD MOCAIP metrics demonstrated variances consistent with vasodilation that returned to baseline following the RIPC. Over the duration of the RIPC, microdialysis showed reduction in the lactate/pyruvate (L/P) ratio (42.37–33.77, $p=0.005$) and glycerol (174.04–126 μg/l, $p<0.005$), which persisted for 25–54 h after the last RIPC.

Conclusions: This study demonstrated cerebrovascular effects induced by RIPC consistent with transient vasodilation. Cerebral metabolic effects suggest protection from ischemia and cell membrane preservation lasting up to 2 days following RIPC.

Keywords Ischemic preconditioning • Cerebral ischemia • Stroke • Subarachnoid hemorrhage • Neuroprotection

Introduction

The concept of ischemic preconditioning was introduced in the late 1980s by Murry et al. [17]. In essence, preconditioning is an endogenous protection mechanism to increase tolerance against critical ischemia by inducing brief subcritical ischemic challenges to a tissue [7]. Numerous animal experiments and several human studies have shown that sublethal ischemic injuries can also confer a systemic protective effect to other organs distant to the one being preconditioned. This has been referred to as "remote ischemic preconditioning" (RIPC) [4, 19].

Early attempts to test RIPC in clinical settings for brain protection have not determined the appropriate timing and technique to induce the neuroprotective effect [13]. Furthermore, before RIPC can be successfully translated into clinical practice, there is a need to expand our understanding of the effects of RIPC on human cerebral metabolism and tolerance to ischemia.

The large majority of preclinical studies have centered on defining the mechanisms of preconditioning, but no investigation has addressed the direct effects that the RIPC may induce in the target organ vascular bed in humans and the durability of those effects. Moreover, the manifestations of

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increased tolerance to ischemia in human brain metabolic pathways induced by RIPC have not been examined.

The purpose of this study was to evaluate acute and delayed hemodynamic and metabolic changes induced in the intracranial circulation by the induction of RIPC in patients with aneurysmal subarachnoid hemorrhage (SAH).

Materials and Methods

Patients with continuous intracranial pressure (ICP), brain microdialysis, and transcranial Doppler (TCD) monitoring enrolled in an ongoing phase I clinical trial of RIPC in subarachnoid hemorrhage were included. The local institutional review board approved the study. Individuals considered for enrollment were 18–80 years of age, with SAH confirmed on computed tomographic (CT) scans or lumbar puncture with a single aneurysm protected by coiling or clipping. Patients with a history of peripheral vascular arterial or venous disease, peripheral nerve disease, or unprotected intracranial aneurysms were excluded.

RIPC Sessions

Patients underwent 3–4 RIPC sessions on nonconsecutive days during days 2–12 after aneurysm rupture. The RIPC consisted of 4 sessions of 5 min of inflation of an adult, large, lower-extremity blood pressure cuff to 30 mmHg above the systolic blood pressure baseline value for each patient. The absence of distal pulse was confirmed by Doppler evaluation of the dorsalis pedis pulse. If Doppler pulse signals were detected, the cuff was further inflated until they disappeared. After 5 min of ischemia time, the cuff was deflated, and distal pulse recovery was evaluated. After 5 min of reperfusion, the cuff was inflated again using the protocol described. A total of four inflations followed by reperfusion were performed in each session.

Patients were continuously monitored before, during, and after the RIPC sessions. Brain metabolic changes were monitored with microdialysis (CMA 600), and continuous ICP was monitored using the BedMaster™ system, which acquires data from the bedside monitors with a sampling rate of 240 Hz. TCD waveforms were measured continuously during the RIPC study, along with electrocardiogram (ECG), via PowerLab™ SP-16 acquisition system (ADInstruments, Colorado Springs, CO) at a sampling frequency of 400 Hz. The ICP and TCD continuous monitoring extended 120 min before the RIPC session and 120 min after the session was finished. Additional TCD monitoring of the internal carotid arteries (ICAs) before and after the sessions and daily complete TCD of all accessible vessels of the circle of Willis were performed. Systemic hemodynamic monitoring was conducted by recording systolic, diastolic, and mean arterial pressure, heart rate, and central venous pressure. Pain scales, temperature, and oxygen saturation were also recorded.

Vascular Reactivity Analysis

To evaluate the vascular changes induced by the RIPC in the intracranial circulation, we utilized the morphological clustering and analysis of intracranial pulse (MOCAIP) algorithm developed by our group. This technique has been previously described in the literature [9]. In summary, the MOCAIP algorithm captures one to one the curves of every ICP and TCD cycle. The pulses are clustered, and nonartifactual ICP pulses are recognized. This permits reliable peak detection, which finally allows for optimal peak designation and analysis. One-hundred-and-twenty-eight metrics obtained by the analysis of these curves have been previously defined and characterized in prior publications [12]. For this study, a MOCAIP dominant pulse was calculated every 6 s for the duration of the baseline and RIPC procedure, yielding approximately 450 dominant pulses per study for both ICP and TCD. Following the calculation of the MOCAIP metrics, a linear model was fit to each trend (128 MOCAIP metrics); the sign of the slope and the respective p value were collected. If the p value was greater than 0.05, the metric was assigned zero. Therefore, a 7 by 128 array of 1, 0, or −1 represented the trending results of the 128 metrics for the seven studies. A metric was accepted as a consistently trending metric (either positive or negative) if there was no disagreement on sign (+1 or −1).

Control Groups

Two MOCAIP analysis control groups were used to correlate specific positive or negative metric trends with vasodilation: A positive control for vasodilation consisted of four non-SAH, uninjured individuals who underwent a CO_2 inhalation challenge in which acute cerebral vasodilation was produced by induced hypercapnia and confirmed by TCD; a negative control for vasodilation consisted of four non-SAH, uninjured individuals with normal-pressure hydrocephalus (NPH).

Statistical Analysis

Statistical analysis was performed with SPSS (version 19.0; SPSS, Chicago, IL) and SAS (version 9.1.3; SAS Institute, Cary, NC) statistical software packages. The SAS procedure Mixed was used for repeated measurements. Standard z score was calculated to compare the ICP and TCD MOCAIP metric

data. The standard score was calculated on an individual study basis; no statistical calculations were made for the study population. Vitals, ICP, and microdialysis data were compared with paired sample *t* test. Two-tailed significance levels are reported.

Results

We present the results of a total of seven ICP continuous pulse recordings and six TCD continuous recordings from four patients enrolled in the Remote Ischemic Preconditioning in Subarachnoid Hemorrhage Trial. We also present the results of the brain microdialysis during the RIPC sessions and during the complete duration of the patient's treatment. These patients were two males and two females with a mean age of 51.7 years (SD ± 8.6). Three patients were Hunt and Hess grade 4; one was a Hunt and Hess 2. All the patients had Fisher 4 CT scans at presentation (4 intraventricular hemorrhages and 1 intraparenchymal). The four individuals had external ventricular catheters placed and were managed in the intensive care unit (ICU) under our standard SAH protocol.

Systemic Hemodynamic Results

During the 40 min of the RIPC sessions, there were no significant changes ($p > 0.5$) in heart rate, blood pressure, temperature, respiratory rate, or central venous pressure in any session for any patient. Mean systolic blood pressure at baseline was 166.7 mmHg (SD 28.1) and 170 mmHg (SD 28.8) at the end of the sessions. Mean diastolic blood pressure at baseline was 86.5 mmHg (SD 17.2) and 89 mmHg (SD 18) at the end of the sessions. Baseline mean temperature was 37.2°C (SD 0.7°C) and at the end of the sessions was 37.3°C (SD 0.7°C). Mean respiratory rate at baseline was 17/min (SD 6.3) and 17/min (SD 5.4) at the end of the sessions. The mean heart rate was 74.8/min (SD 24.9) at baseline and 77.3 (SD 24) at the end of the sessions. The mean central venous pressure (CVP) at baseline was 10 mmHg (SD 2.1) and at the end of the sessions was 11 (SD 4.6). The analog pain scale score used in conscious patients showed no changes between the beginning and end scores for each patient (average 1/10).

ICP Changes

There was an increase in ICP in all but one of the RIPC sessions. The mean ICP changed from an average at baseline of 8–14.57 mmHg by the end of the session ($p < 0.001$). This change in ICP was not associated with pain in conscious patients or any other vitals change in conscious or unconscious individuals.

Cerebral Hemodynamic Changes

Transcranial Doppler Results

During the RIPC sessions, there was a tendency toward increased ICA velocities with a median ICA mean velocity of 34.5 cm/s at the beginning of the session versus 37 cm/s at the end. This difference was not significant ($p = 0.61$). On the other hand, there was a statistically significant reduction of the median middle cerebral artery (MCA) mean velocities from 111 to 87 cm/s by the end of the sessions ($p = 0.039$). This effect does not appear to be sustained beyond the period of the RIPC maneuvers as the MCA velocities did not follow any specific tendency in the 24–48 h after the sessions.

RIPC Session MOCAIP ICP Results

In the positive control group, 72 MOCAIP ICP metrics deviated during the CO_2 inhalation challenge. During the confirmed period of vasodilation in the CO_2 inhalation group, 50 metrics demonstrated a positive variance, while 22 had a negative variance. Based on the greatest relative time rate of change during hypercapnia and normal breathing post-test, the top 10 metrics (demonstrating the strongest correlation) indicating vasodilation were selected with positive and negative variances. In the negative control group, none of the ICP metrics demonstrated a consistent variation during the 40 min of continuous examination. In the RIPC group, 20 MOCAIP ICP metrics showed significant deviation from the baseline during the preconditioning session. Of these, 15 had a positive variance, all of which were included in the 50 metrics with positive variance in the CO_2 challenge group. Seven positively varying metrics were part of the top 10, or strong indicators of vasodilation, in the positive control group. A statistically significant increase was reached in 79.1% of the positively varying metrics. Five metrics in the RIPC group demonstrated a negative variance. Also, all of these metrics had exhibited negative variance in the CO_2 challenge group. Three of the five were also strong indicators of vasodilation. Statistically significant decrease was observed in 60% of the negatively varying metrics. No variance in the RIPC group was in a direction opposite to that observed in the CO_2 challenge group. These results indicate vasodilation of the cerebral vasculature during the RIPC sessions.

RIPC Session MOCAIP TCD Results

In the positive control group, 51 MOCAIP TCD metrics deviated during the CO_2 inhalation challenge. During the confirmed period of vasodilation in the CO_2 inhalation group, 28 metrics demonstrated a positive variance, while 23 had a negative variance. For MOCAIP TCD, 10 positively and

negatively varying metrics were also selected based on their greatest relative time rate. In the negative control group, none of the TCD metrics demonstrated consistent variance during the examination time. In the RIPC group, 3 MOCAIP TCD metrics showed significant deviation from the baseline during the preconditioning session. Of these, 2 had a positive variance, and both of them were part of the 28 metrics with positive variance in the CO_2 challenge group. One metric in the RIPC group demonstrated a negative variance. This singular metric had exhibited negative variance in the CO_2 challenge group as well. Once again in the MOCAIP TCD, no variance in the RIPC group was in a direction opposite to that observed in the CO_2 challenge group. These results also indicate vasodilation of the cerebral vasculature associated with RIPC.

Pre- and Post-RIPC Session MOCAIP Results

The MOCAIP analysis of the ICP and TCD curve metrics demonstrated no significant variance in a positive or negative direction immediately before or after the RIPC session, comparable with the results of the negative control group. This finding is consistent with the results observed in the daily TCD studies, which also showed no particular tendencies in the MCA velocities. These results indicate that the vasodilating effect of the RIPC session on the cerebral vasculature was limited to the duration of the procedure.

Brain Metabolic Results

The microdialysis data collected before and immediately after each RIPC session showed no significant variations in the glucose levels (0.91–0.81 mmol/l, $p=0.4$) or in the lactate levels (4.62–4.63 mmol/l, $p=0.87$). There was a tendency to a decrease in the lactate/pyruvate (L/P) ratio (27.25–24.39, $p=0.12$), which was also not significant. However, there was a significant reduction in the glycerol levels from 113.8 to 99.5 μg/l ($p=0.05$). This change occurred despite TCD and angiographic demonstration of vasospasm in two of the patients.

Over the complete duration of the RIPC sessions, from before the first session through the end of the last session, the microdialysis data showed a significant reduction in the L/P ratio from 42.37 to 33.77 ($p=0.005$) and in the glycerol levels from 174.04 to 126 μg/l ($p<0.005$). This reduction occurred despite the confirmed presence of vasospasm in two patients. The reduction of L/P ratio lasted between 26 and 50 h, and the reduction of glycerol lasted between 25 and 54 h after the last RIPC session. These results indicate that the metabolic shift induced by the RIPC session, with a reduction of cerebral L/P and glycerol, despite vasospasm, had an extended duration of 25–54 h after the RIPC session. In the individuals with vasospasm, after this period of ischemia protection there were peaks in both the glycerol and the L/P ratio that correlated with the clinical development of neurological deficit and, despite maximum medical therapy and endovascular intervention for vasospasm, the development of areas of stroke on follow-up MRI.

Discussion

An evaluation of the effects of RIPC on the human cerebral vascular bed and metabolism has not been published previously. The effects of preconditioning on cerebral blood flow have produced contradictory results in animal studies. Initially, the role of altered perfusion in preconditioning was largely discounted. This was based on early studies that used autoradiography [1, 3, 6], hydrogen clearance [15], or laser Doppler measurements [2] of cerebral blood flow that failed to demonstrate a difference between controls and preconditioned animals in the acute interval following the preconditioning stimulus. However, several recent experimental observations, such as the finding of progressive recovery of microvessel filling during ischemia in preconditioned rat brains [6] and the recovery from strokes even after complete occlusion without reperfusion in the hypertensive rat model of Barone et al. [2], strongly suggest a perfusion component in the protection mechanism. In a review of that model, Zhao and Nowak [23], using acute perfusion measurements, found a delayed but sustained increase of cerebral perfusion 3 h after vessel occlusion in animals treated with ischemic preconditioning. Furthermore, Woitzik et al. [21] have recently demonstrated increased vessel diameters of the leptomeningeal anastomoses in rats after hypoxic preconditioning by visualization of the brain angioarchitecture using a latex perfusion technique. Additional recent studies using laser Doppler flowmetry [16] and measurements of perfused microvessels [6] support these findings.

RIPC effects in the vascular bed of the target organ were indirectly investigated in the study of Kharbanda et al. [11], who demonstrated that RIPC prevented ischemia-reperfusion endothelial dysfunction in humans by assessing strain-gauge plethysmography measurements in healthy volunteers undergoing a 20-min cuff inflation in an arm after three cycles of 5 min of ischemia in the contralateral limb. In this study, the forearm responses to the endothelium-dependent dilator acetylcholine (ACh) were tested at three different doses in control and RIPC groups. The response to ACh after 15 min of reperfusion was actually increased in the RIPC group, while it was significantly blunted in the controls, demonstrating enhance vasodilation in the RIPC group.

In agreement with these later reports, our results suggest that RIPC induced a consistent vasodilation of the cerebral circulation in both TCD measurements and the more sensitive MOCAIP analysis. The variance of every metric reaching significance in both the ICP and TCD curves emulated the variance of patients with vasodilation in the CO_2 challenge. However, this effect was not sustained and dissipated immediately after the session. This vasodilation would also explain the elevation in ICP despite the absence of pain or other change in vitals, most likely indicating increased cerebral blood flow during the RIPC treatment.

Either metabolic depression or enhanced energy production of the target organ have been suggested to play a role in the mechanism of preconditioning. There is evidence that preconditioning preserves tissue levels of adenosine triphosphate (ATP) during periods of decreased perfusion. However, it is unclear whether this is due to decreased metabolic demand or increased energy production and mitochondrial protection. Supporting the second possibility, Janier et al. [10] used a rabbit cardiac model to show that 3 min of ischemic challenge led to less ischemic damage and higher ATP due to upregulation of compensatory anaerobic metabolism. In addition, Dave et al. [5] examined mitochondria extracted 24 h after a carotid occlusion challenge and found that mitochondria that had preconditioning challenges were able to retain normal metabolic function compared to controls without preconditioning. In support of this mechanism of brain ischemia protection, Yannopoulos et al. [22], using periods of limb ischemia induced by an inflatable blood pressure cuff in a porcine model, demonstrated cerebral protection when the animals underwent cardiopulmonary bypass. Using electroencephalographic (EEG) and brain microdialysis, they found increased recovery from burst suppression as well as lower lactate levels compared to the control group.

Several studies have indicated that bedside cerebral microdialysis provides valuable information in acute and delayed ischemic neurological deficits in subarachnoid hemorrhage patients [14, 20]. Lactate and glutamate are considered early markers of clinical vasospasm, followed by the lactate/pyruvate ratio and glycerol, during manifest vasospasm in patients with subarachnoid hemorrhage. Glycerol, which is an end product of phospholipid degradation, is a marker for membrane disintegration and has been associated with the degree of ischemic brain damage in experimental intracerebral vascular occlusion [8] and as a predictor of poor outcome or death [18]. Our results demonstrate a tendency to a reduction in the lactate/pyruvate brain microdialysis ratio during the RIPC sessions and a significant reduction during the complete period of the study, despite the presence of vasospasm. The glycerol levels were reduced during the sessions and during the complete period of study. These findings suggest a protective effect against ischemia and an increased membrane preservation that lasted between 25 and 54 h after the RIPC session.

Conclusion

In summary, our study demonstrated immediate cerebrovascular effects induced by the RIPC maneuvers consistent with transient vasodilation, which extinguished immediately after the session concluded. There were more durable metabolic effects suggestive of ischemia protection and cell membrane preservation that lasted 25–54 h after RIPC. This information is crucial for the planning of further pivotal trials of RIPC in SAH and can assist in defining the timing of the sessions, as well as provide a qualitative tool to evaluate potential neuroprotective effects induced by preconditioning.

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

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Cerebral Microvasculature Is an Early Target of Subarachnoid Hemorrhage

Fatima A. Sehba and Victor Friedrich

Abstract Most subarachnoid hemorrhage (SAH) patients exhibit clinical signs of cerebral ischemia at admission but no angiographic vasospasm. Consequently, the source of early cerebral ischemia is not understood. Parenchymal microvessels may contribute to early cerebral ischemia, but the low resolution of current imaging has prevented their analysis in SAH patients. Animal studies demonstrated that early after SAH structure and function of parenchymal vessels are compromised to the level that may very well contribute to early ischemia. We review these studies.

Keywords Subarachnoid hemorrhage • Cerebral vasospasm • Cerebral ischemia • Microcirculation

Introduction

Aneurysmal subarachnoid hemorrhage (SAH) accounts for 5% of all stroke cases and may occur in up to 30,000 North Americans each year [1]. Early brain injury is a major cause of early mortality (up to 70%) after SAH [2]. Cerebral microdialysis and autopsy studies in animals and humans indicate that early brain injury after SAH is ischemic in nature [3–5]. The source of early cerebral ischemia, however, is not clear.

In animals, intracranial pressure rises, cerebral perfusion pressure and cerebral blood flow fall, and large cerebral arteries constrict minutes after SAH. In humans, although a rise in intracranial pressure and a fall in cerebral perfusion pressure and cerebral blood flow are established after SAH, angiographic evidence of constriction of cerebral arteries is not found [6, 7]. Consequently early cerebral ischemia in humans is often attributed to the fall in cerebral perfusion pressure; however, studies supporting this claim are mostly performed during repeated hemorrhages, when brain compliance is already reduced [8, 9]. Experimental studies of first-time hemorrhages indicated that the drop in cerebral perfusion pressure is transient and not sufficient to cause perfusion arrest; hence, although it might contribute to early cerebral ischemia, it cannot be the primary cause [10].

In recent years, cerebral microvasculature has emerged as an important source and target of early cerebral ischemia after SAH. Given that these vessels are below the resolution of clinical imaging devices, they are mostly studied in experimental settings. However, recent observations made during surgery and during autopsy studies of SAH patients suggested changes in microvessels similar to those in animals [11, 12]. We here review the literature for structural damage and functional deficits in microvessels during the first 48 h after SAH and the mechanisms capable of causing them.

Structural Damage and Functional Deficits

Whereas large cerebral vessels bathe in the blood released on aneurysmal rupture, microvessels do not come in direct contact with that blood. Nevertheless, compared to the large cerebral vessels, microvessels experience a greater and more rapid compromise in structure and function after SAH [13]. Perhaps differences in structure make cerebral microvessels more vulnerable to damaging signal cascades and biochemical changes activated after SAH.

The cerebral microvessel contains three main units: the endothelial cell layer, the basal lamina, and the surrounding astrocyte end feet. In addition, pericytes are located at the abluminal side of the endothelium and are encased by basal

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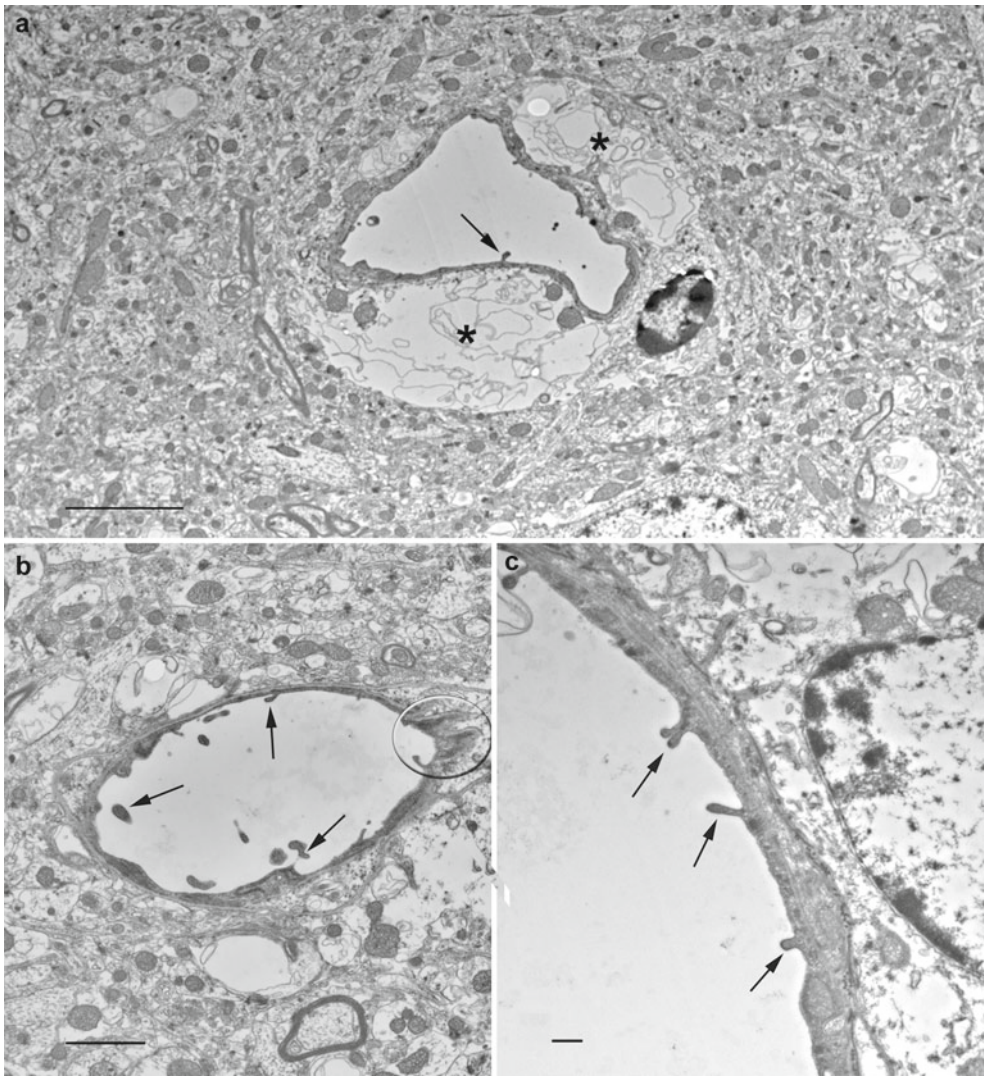

Fig. 1 Structural changes in cerebral microvessels 1 h after subarachnoid hemorrhage (*SAH*). (**a**) The vessel in the center of the field shows intact endothelium and endothelial basal lamina; however, the vessel is partially collapsed. Marked swelling of pervivascular astrocytic end feet (*asterisks*) has occurred, and luminal protrusions of the endothelium are visible (*arrows*). Scale bar: 5 µm. (**b**) The endothelium and basal lamina of this vessel are intact. Multiple luminal protrusions are visible (*arrows*). The basal lamina is crenellated at one point (*circle*), a possible effect of vasoconstriction. Scale bar: 2 µm. (**c**) Endothelial protrusions are viewed at higher magnification (*arrows*). Continuity of protrusions with the parent endothelial cell is clearly revealed. Scale bar: 500 nm

lamina that separates them from the endothelium and from the surrounding astrocytic end feet. Pericytes represent the contractile elements of central nervous system (CNS) microvessels [14]. The regulation of microcirculation depends on the integrity of each element of the microvascular unit and on constant communication between them. Endothelial cells, with astrocytes, provide a barrier that restricts passage of both cells and molecules across the vessel wall. Adhesion receptors (such as P- and E-selectin, vascular adhesion molecule 1 [VCAM-1], intercellular adhesion molecule 1 [ICAM-1], and integrin), expressed on endothelium, regulate events such as the activation and adhesion of platelets and of neutrophils in response to change in cellular environment. Substantial alterations in the microvascular unit, affecting its integrity and compromising microcirculation, are reported after SAH. These alterations appear within minutes and evolve with time; they include changes in endothelial cells themselves, in the endothelial basal lamina, and in perivascular astroctyic end feet.

Endothelial cytoplasmic flaps or microvilli appear and extend into the vessel lumen (Fig. 1a–c). These flaps are expansions of endothelial cell cytoplasm and are characteristic of cerebral ischemia. Their appearance is considered a cellular attempt to maintain normal tissue integrity as the cerebral oxygenation is altered [15]. At the capillary level, endothelial flaps are known to give rise to blebs that can obstruct the vessel lumen [16]. In many microvessels, focal areas appear at which the endothelium or basal lamina are fragmented and physically detached from basal lamina [17] (see Fig. 2). Such focal areas are present as early as 10 min after SAH and persist for 24 h. Some endothelial antigens disappear after SAH, likely because of focal endothelial damage [18–21]. These antigens, many of which have important cellular functions and others that signal endothelial integrity, include endothelial nitric oxide synthase (eNOS), endothelial barrier antigen (EBA), and rat endothelial cell antigen 1 (RECA-1). The state of adhesion receptors located on microvascular endothelium after SAH is not known, but studies of large arteries showed that of ICAM-1, which promotes neutrophil-endothelial adhesion, increases 3 h to 2 days after SAH [22, 23]. Remarkably, neutrophils accumulate in large number in microvessels 10 min after

SAH, suggesting a possible increase in ICAM expression [21]. As endothelial cells detach or withdraw from basal lamina, the proteins of basal lamina matrix become exposed directly to blood elements, making it possible for platelets to adhere to the collagen IV of basal lamina. Platelets interact with neutrophils and incorporate into growing vascular aggregates [24]. Collagenases capable of digesting collagen IV are present in platelets and neutrophils, and active collagenases, such as matrix metalloproteinase 9 (MMP-9) are found in microvessels after SAH. Minute holes appear in otherwise-continuous basal lamina, the likely product of collagenase activity [17, 18]. Agents that inhibit MMP-9 activity reduce edema and improve the outcome of animals after SAH [25, 26].

Astrocytes also respond early to SAH. Changes include swelling of perivascular end feet (Fig. 1) and increased glial fibrillary acidic protein (GFAP) immunostaining [27]. Degeneration of astrocytes is observed 2 days after SAH [28]. The exact role that reactive astrocytes play in the pathology of SAH is not known, but an increase in their size, process extension, and GFAP content is a characteristic response to brain injury [29]. Not much is known of pericyte response after SAH, but an early ultrastructural study by Dodson et al. found that their content increased after SAH [30]. Perhaps an indirect evidence of pericyte involvement in brain injury after SAH comes from the finding that Rho kinase inhibitors such as fasudil reduce brain injury in SAH animals. Rho kinase plays an important role in pericyte contractility and pericyte-mediated regulation of endothelial growth [31].

Each microvessel unit is important in maintaining microcirculation. Consequently, damage to these units after SAH leads to disruption in microcirculation, observed as widespread perfusion deficits and permeability increases [32, 33]. Endothelium keeps a check on the vessel tone and blood flow by releasing an array of vasoactive agents and by maintaining a delicate balance between them. These agents include powerful vasodilators such as nitric oxide (NO) and powerful vasoconstrictors such as endothelin 1. Injury to endothelium after SAH makes it dysfunctional, causing a decrease in NO at the arterial bed and loss of vasodilation by the agents that require a functional endothelium to elicit response [34, 35]. Meanwhile, the endothelin 1 level increases at the arterial bed, a consequence of excessive release by astrocytes during the period of initial ischemia [36]. A consequence of these events is a shift of the balance between vasodilator and constructive tone toward constriction. In animals, vessel constriction after SAH is widespread, is present in large and small vessels, and causes a reduction in cerebral blood flow [37–39]. Sabri and colleagues have reported that one of the mechanisms leading to decreased NO production by cerebral vessels post-SAH is uncoupling of eNOS (one of the enzymes responsible of arterial NO supply) so that it begins producing superoxide (O_2^-; a powerful free radical) instead of NO [40]. Whether the same occurs in microvessels is yet to be determined; however, the finding that an NO donor used within minutes after SAH reduces constriction in microvessels suggests that this may be the case [18]. Constriction in large and small (50 μm) vessels also involves protein kinase C, the delta isoform of which activates 1 h after SAH [41]. Constriction of microvessels in the absence of smooth muscle may involve compression by astrocyte swelling and edema and pericyte response to the change in cerebral environment (discussed previously). Another mechanism that would disrupt microcirculation is obstruction of the vessel lumen by platelet aggregates, neutrophils, and endothelium blebs present in the vascular lumen after SAH (as discussed).

Permeability of microvessels also increases after SAH. Vessels become leaky, leading to extravasation of blood components, including platelets, and proteins into the parenchyma, promoting edema and inflammation [17, 33]. Platelets themselves may promote inflammation by releasing leftover granule contents. In addition, platelets have neurotoxic effects and may promote neuronal death [42].

Mechanisms Contributing to Vascular Damage Post-SAH

A variety of mechanisms are implicated in microvascular damage after SAH. Three of these are discussed next.

Altered NO-NOS Pathway

NO plays an irreplaceable role in cerebral blood flow regulation. It does so by maintaining arterial diameter and inhibiting platelet adherence and aggregation and adhesion of leukocyte to the endothelium. A constant supply of NO is required for blood flow maintenance, and at the microvessel level is mainly maintained by its synthesis by eNOS located on the vascular endothelium. Animal studies demonstrated that cerebral NO level decreases within minutes after SAH. This decrease is present at 10 min post-SAH and may persist for 24 h. A number of mechanisms that activate early after SAH are capable of depleting cerebral NO, such as decreased synthesis, scavenging by hemoglobin or binding with oxygen radicals released by oxidative damage to the vessel wall or by macrophages during inflammation [35, 43]. A decreased in NO synthesis may result from uncoupling of eNOS or by damage to the endothelial lining, resulting in eNOS loss [17, 40]. Moreover, hemoglobin released on degradation of subarachnoid blood possesses high affinity for NO and can bind NO, creating its deficiency. Similarly, oxidative species released in the vessel lumen during an oxidative burst by neutrophils can bind NO, creating its deficiency. Irrespective of the cause, events that NO reduction would promote at microvascular

level—reduction in blood flow, constriction, aggregation of platelets in the vessel lumen, and adhesion of neutrophils to the vessel wall—appear minutes after SAH. Other evidence the NO-NOS pathway plays an important role in microvascular damage came from the study that demonstrated that recovering NO levels minutes after SAH (such as by an NO donor) recovers cerebral blood flow and decreases endothelial and collagen IV loss [18, 44].

Oxidative Stress

Oxidative stress is a phenomenon well known to cause damage to vessel walls and trigger astrocyte reactivity. Free radicals implicated in post-SAH oxidative injury include superoxide anion (O_2•), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2), nitric oxide (NO•), and peroxynitrate (ONOO−) (for review, see [45]). These free radicals are generated during autooxidation of hemoglobin on erythrocyte lysis in the subarachnoid space; during an oxidative burst of neutrophils, disruption of mitochondrial respiration, hypoxic conversion of endothelial xanthine dehydrogenase to xanthine oxidase, lipid peroxidation; by eNOS uncoupling (as mentioned); and upregulation of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase (for review, see [46]). As the activities of enzymatic and nonenzymatic antioxidant systems (defense systems against free radicals) saturate within 60 min [47, 48], little defense is available against oxidative stress-induced injury after SAH. At the microvascular level, oxidative stress can injure endothelium, disrupt the blood–brain barrier, and promote cell apoptosis by inducing proapoptotic enzymes. Signals promoting and marking cells for death, such as proapoptotic caspase-3, activate in endothelial cells at 10 min after SAH (Fig. 2), and cell death via apoptosis follows with time (Friedrich et al., unpublished; [49–53]). Consequently, antioxidants when used within 3 h after SAH prevent alteration in cerebral blood flow and cerebral perfusion pressure and protect microvascular endothelium and the blood–brain barrier [54–56].

Inflammation (Platelets and Neutrophils)

At least two components of inflammatory cascade, platelets and neutrophils, are activated within 10 min after SAH [21, 57], and soluble markers of inflammation are increased 24 h later. These include ICAM-1, VCAM-1, and E-selectin and cytokines [22, 58–60]. In humans, an increase in

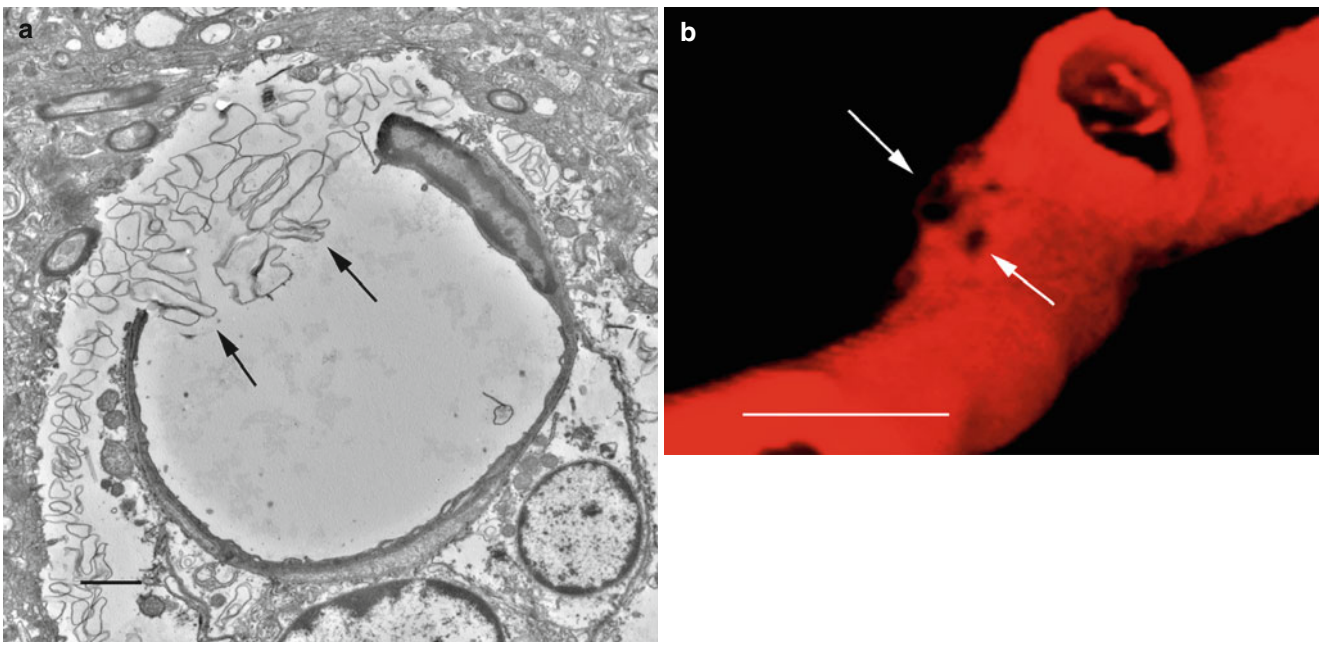

Fig. 2 Destruction of endothelium and basal lamina after subarachnoid hemorrhage (*SAH*). (**a**) Both basal lamina and endothelium are broken in this profile, leaving a gaping hole in the vessel. The surrounding astrocytic end feet are grossly swollen and disrupted. Scale bar: 2 µm. (**b**) Three-dimensional reconstruction of a vessel immunostained for the basal lamina component collagen IV and analyzed by confocal microscopy. The vessel appears as a continuous tube, with small holes visible in its basal lamina (*arrows*). The large opening represents the cut end of a branch, located at the section surface. Scale bar: 10 µm (Adapted from Friedrich et al. [33]). (**c**) Interruption of endothelium and basal lamina (*arrow*) viewed in cross section, computed from three-dimensional confocal immunofluorescence image data. *Pink* collagen IV; *green* rat endothelium cell antigen 1 (*RECA-1*; endothelial marker). Scale bar: 5 µm (Adapted from Friedrich et al. [33])

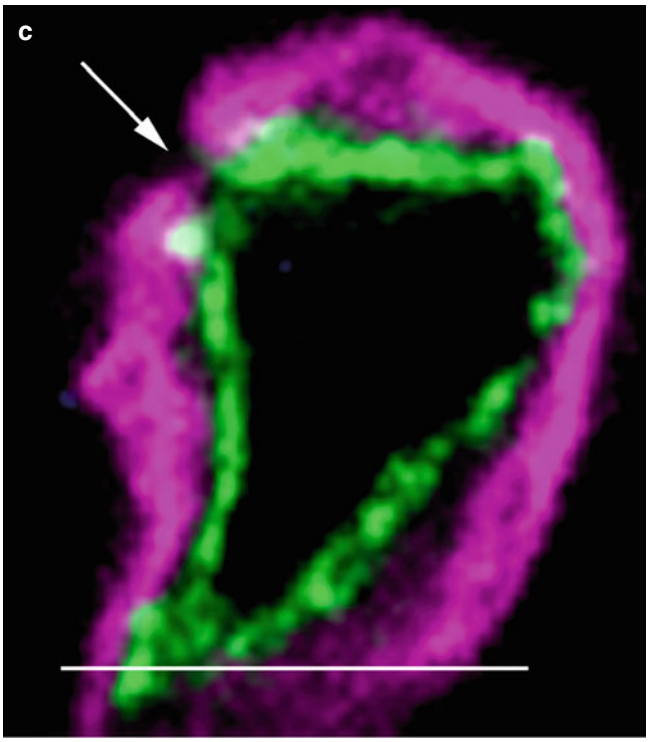

Fig. 2 (continued)

cerebrospinal fluid (CSF) levels of adhesion molecules is observed during the first 72 h after SAH [59]. Activated platelets and leukocytes can contribute to the endothelial injury after SAH. Activated platelets can disrupt and denude endothelium to make these sites attractive to passing emboli and promote further aggregation [61]. Moreover, platelet activation factor, which promotes platelet activation, accelerates transendothelial migration or diapedesis of leukocytes, forming microlesions in the endothelium [62]. Similarly, oxidative radicals produced during an oxidative burst by neutrophils may damage endothelium and reduce cerebral NO (as discussed). The importance of neutrophils in endothelium damage is evident in a recent study that demonstrated that their depletion prior to SAH reduced endothelium injury [21].

Conclusion

In conclusion, rapid damage in microvessel structure and deficits in function occur after SAH. In recent years, we are starting to comprehend the importance of post-SAH microvessel compromise in early ischemia and brain injury. The molecular signals and processes involved in microvessel injury are being studied, and compounds that protect microvessels against early injury are being identified. The majority of research in this field, however, remains experimental. Technological advances are required to allow its translation to SAH patients.

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

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The Roles of Early Brain Injury in Cerebral Vasospasm Following Subarachnoid Hemorrhage: From Clinical and Scientific Aspects

Shigeru Nishizawa

Abstract Cerebral vasospasm research has been focused on investigating the mechanisms of prolonged delayed vasoconstriction of cerebral arteries following subarachnoid hemorrhage (SAH). However, it has been clarified that induction of significant vasodilation of such arteries does not lead to better overall outcomes in SAH patients. On the other hand, early brain injury, such as cortical spreading depression, early cortical depolarization waves, and impairment of neurovascular coupling, is seen acutely after SAH and may play a significant role in early impairment of brain function following SAH. These results clearly indicate that it is time to reconsider what causes this early brain damage and dictates patient outcome following SAH; classical delayed cerebral vasospasm following SAH might be an epiphenomenon. It is of utmost importance to investigate whether early brain injury and delayed cerebral vasospasm correlate with each other following SAH or are independent. Recent results of cerebral vasospasm research indicates future directions, and such investigations would lead to better outcome for SAH patients.

Keywords Early brain injury • Subarachnoid hemorrhage • Cerebral vasospasm • Animal models

Introduction

It is well-known that delayed cerebral vasospasm (DCV) following subarachnoid hemorrhage (SAH) is a key factor contributing to outcome and prognosis of SAH patients. For this reason, many basic researchers and neurosurgeons have been investigating mechanisms of and approaches to DCV. Typically, when we refer to "delayed cerebral vasospasm following SAH," the target is vasoconstriction of major cerebral arteries. In experimental animal models, the major concerns are extent of constriction of major cerebral arteries and how this constriction can be inhibited by pharmacological intervention.

One experimental approach for cerebral vasospasm is to investigate the mechanism producing long-lasting cerebral arterial contraction. In this approach, the roles of the signal transduction mechanism of vascular smooth muscle cells have been highlighted [3, 7–11], that is, of protein kinase C (PKC) [7–11], protein tyrosine kinase (PTK) [3], mitogen-activated protein kinase (MAPK), among others. Another approach is to investigate molecules known to cause long-lasting cerebral arterial contraction, such as oxyhemoglobin (OxyHb) or endothelin 1 (ET-1). Candidates to inhibit those key mediators have been investigated, and some have successfully been shown to attenuate cerebral vasospasm in experimental animal models.

On the other hand, clinical neurosurgeons know well that initial patient neurological state following SAH, especially consciousness level, correlates very well with the final outcome. In other words, not only the DCV but also initial the consciousness state, which is known to reflect early brain injury accurately, are significantly important to indicate the prognosis of the patients.

Here, we discuss the past and recent research and possible future direction of research regarding cerebral vasospasm following SAH.

Results

Protein Kinase C

We have extensively investigated the roles of PKC in the DCV following SAH [3, 7–11]. Using a two-hemorrhage canine model (Fig. 1), the activity of PKC in smooth muscle cells of basilar arteries was measured by radioisotope study.

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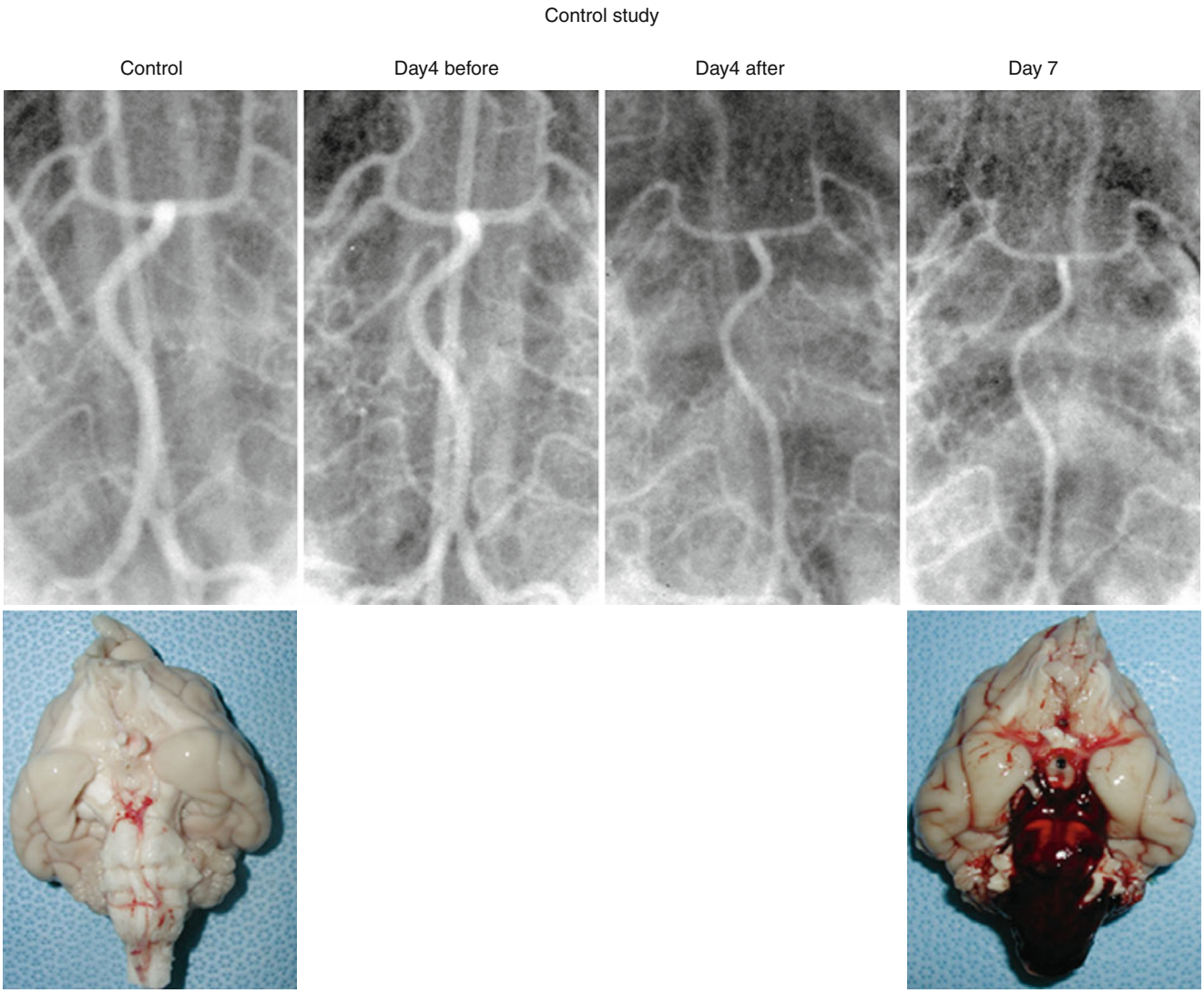

Fig. 1 The control study using a two-hemorrhage canine model. The cerebral vasospasm occurs after the second injection on day 4 up to 50% of the control diameter and continues until day 7

In vasospastic cerebral arteries, PKC is significantly enhanced [8], and the time courses of the progression of cerebral vasospasm and PKC activation are correlated well by an enzyme immunoassay study [10]. Based on these results, we concluded that PKC isoenzymes play a pivotal role in the mechanism of DCV after SAH.

Eleven isoforms of the PKC family have been identified. We examined which isoforms are important in the mechanism of DCV. In smooth muscle cells of a canine basilar artery, four PKC isoforms, PKCα, -δ, -ζ, and -η, were identified by Western blotting methods [9]. Among those isoforms, PKCδ was initially activated, followed by PKCα [9]. These results suggest us that PKCδ plays the role in the initiation and PKCα in the maintenance of DCV [9] (Fig. 2).

Using a high-throughput screening method, we investigated whether chemical agents to inhibit the determined PKC isoforms were available. In this study, we found one chemical product, M-62885, that shows significant inhibition of both PKCδ and PKCα. According to those results, we examined M-62885 in our two-hemorrhage canine model. Both PKCα and PKCδ activations were inhibited significantly, and angiographic DCVs were also completely inhibited (Fig. 3).

Our experimental results confirmed that both PKCδ and PKCα are significantly important for initiation and maintenance of DCV after SAH. A chemical agent such as M-62885 might be strongly beneficial. This experimental approach was also used in the clazosentan study described next.

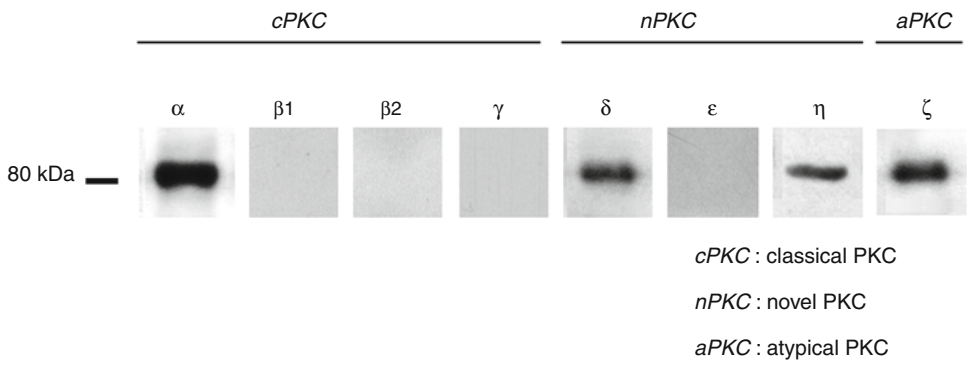

Fig. 2 Identification of protein kinase C (*PKC*) isoforms in canine basilar artery. Four PKC isoforms, PKCα, -δ, -η, and -ζ, were identified by western blotting. Only PKCα and PKCδ are activated during cerebral vasospasm following subarachnoid hemorrhage (SAH)

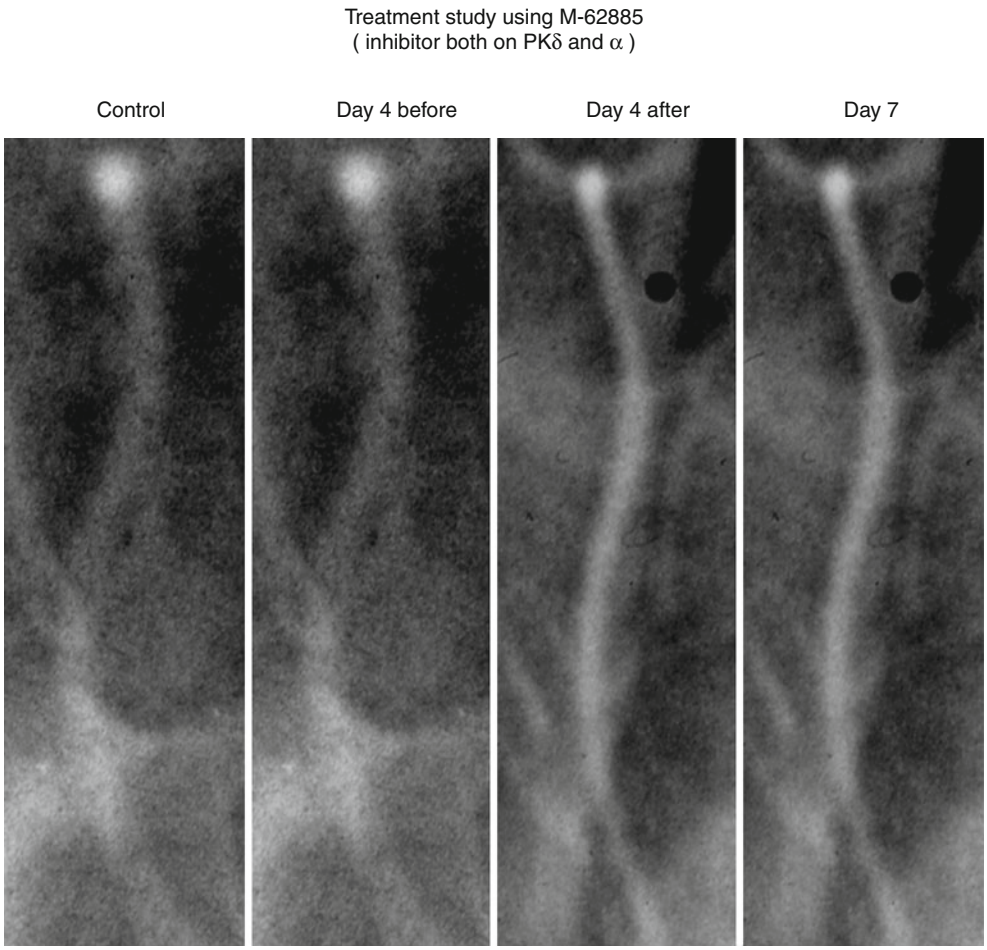

Fig. 3 Treatment study using M-62885, an inhibitor of both protein kinase C (*PKC*) α and PKCδ. Significant attenuation of cerebral vasospasm following subarachnoid hemorrhage (SAH) was obtained

Clazosentan

Clazosentan is a potent specific antagonist on ET-1 receptor, and it has been extensively tested in the two-hemorrhage canine model for inhibition of DCV following SAH. The results were very encouraging; complete inhibition of cerebral vasospasm was obtained in a dose-dependent manner [5, 13].

Based on those experimental results, multicenter clinical trials using clazosentan were initiated. As expected, clazosentan inhibited DCV after SAH in patients just as in the experimental animal study. However, despite these promising angiographic findings, the overall outcome of the patients treated with clazosentan was not significantly different from the control group. Moreover, mortality rate in the clazosentan treatment group was higher than the control group due to

adverse effects, such as pulmonary edema, renal dysfunction, and liver dysfunction [5, 6].

Nimodipine

Nimodipine is a calcium channel blocker, and it is widely used in North America and European countries for the prevention of neuronal deficits due to SAH. Nimodipine itself does not have a vasodilatory effect. However, a nimodipine-treated group of SAH patients was significantly better compared with the control. It was suggested that the effect of nimodipine on the better outcome of SAH patients might be due to a neuroprotective effect [1].

Discussion

What Do Those Studies Mean?

Full dilation of vasospastic cerebral major arteries does not lead to favorable patient outcome. Inhibition of the cerebral vasospasm of major cerebral arteries and the outcome of the patients are completely independent.

What does it mean? Many researchers have been focusing on the following issues for long time: What mechanisms contribute to produce long-lasting contractions of major cerebral arteries following SAH? What are the solutions to dilate major cerebral arteries for a better outcome for SAH patients? However, we now have to reconsider what the role of so-called delayed cerebral vasospasm is in the pathophysiology of SAH. In another words, we have to redefine the real role of DCV following SAH, and whether classical DCV is really the key factor to decide the final outcome of SAH patients.

Early Brain Injury

As mentioned, clinical neurosurgeons know the initial consciousness level of the SAH patient (assessed by various scales based on level of consciousness) is directly correlated with the patient's final outcome. The initial conscious level is definitely impacted by early brain injury due to SAH [1].

A number of articles for early brain injury have been published in both the clinical and the experimental fields. In the clinical articles, it has been reported that severity of brain damage after SAH is closely correlated with the final outcome [1]. However, these are observational studies, and no definite scientific mechanisms defining a relationship between early brain injury and the final outcome have yet been established.

In the research articles, mechanisms of early brain injury have been reported, such as cortical spreading depression, cortical depolarization wave [2, 4], and impairment of neurovascular coupling acutely following SAH. These data reinforce the significance of the role of early brain injury that causes overall poor patient outcome following SAH.

As mentioned, nimodipine does not have a vasodilatory effect but induces a better outcome for the SAH patient compared with the control. In another words, nimodipine shows its effect through a neuroprotective mechanism, likely by attenuation of early brain injury.

Conclusion

The results of the clazosentan study tell us a lot about the future direction of vasospasm research [12]. In clinical aspects, significant attenuation of spasm of the major cerebral arteries following SAH does not lead to improvement of patient outcomes. SAH patients are classified in clinical grades based on the clinical state, especially on the consciousness level; such grades, however, are directly correlated with the final outcome of SAH patients. The initial clinical grades of the SAH patients are strongly associated with early brain injury due to SAH. In that sense, it is significantly important to determine how to protect neural functions from early brain injury to improve the overall outcome of SAH patients. To investigate the mechanisms of early brain injury, such as cortical spreading depression, cortical spreading depolarization waves, and impairment of neurovascular coupling, is extremely important. Moreover, the relation between early brain injury and so-called classical DCV has to be identified. It is not clear that they are closely related or are completely independent events. DCV might be an epiphenomenon of the pathophysiology of SAH.

Prolonged cerebral arterial contraction is only seen in cerebral arteries following SAH. Investigation of such mechanisms is still important and interesting from the aspects of physiological, pharmacological, and biochemical research. However, as there appears to be little clinical correlation between vessel caliber and functional outcome, we should perhaps shift our research focus.

Even in experimental animal models, we have focused on how to produce irreversible delayed prolonged major cerebral arterial contraction following SAH. It is now time to reconsider whether classical SAH animal models themselves can be justified as effective models.

Conflicts of Interest Statement I declare that I have no conflict of interest.

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Matricellular Protein: A New Player in Cerebral Vasospasm Following Subarachnoid Hemorrhage

Hidenori Suzuki, Masato Shiba, Masashi Fujimoto, Kengo Kawamura, Mai Nanpei, Eriko Tekeuchi, Satoshi Matsushima, Kenji Kanamaru, Kyoko Imanaka-Yoshida, Toshimichi Yoshida, and Waro Taki

Abstract *Introduction*: Matricellular protein (MCP) is a class of nonstructural and secreted extracellular matrix proteins that exert diverse functions, but its role in vascular smooth muscle contraction has not been investigated.

Material and Methods: First, rat subarachnoid hemorrhage (SAH) models were produced by endovascular perforation and examined for tenascin-C (TNC) and osteopontin (OPN) induction (representatives of MCPs) in vasospastic cerebral arteries using immunostaining. Second, recombinant TNC (r-TNC), recombinant OPN (r-OPN), or both were injected into a cisterna magna in healthy rats, and the effects on the diameter of basilar arteries were determined using India ink angiography.

Results: In SAH rats, TNC immunoreactivity was markedly induced in the smooth muscle cell layers of spastic cerebral arteries on day 1 but not in control animals. The TNC immunoreactivity decreased on day 3 as vasospasm improved: OPN immunoreactivity, on the other hand, was more induced in the arterial wall on day 3. r-TNC injections caused prolonged contractions of rat basilar arteries, which were reversed by r-OPN, although r-OPN itself had no effect on the vessel diameter.

Conclusions: MCPs, including TNC and OPN, may contribute to the pathophysiology of cerebral vasospasm and provide a novel therapeutic approach against it.

Keywords Cerebral vasospasm • Extracellular matrix • Matricellular protein • Subarachnoid hemorrhage

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Introduction

The term *matricellular proteins* was introduced to explain the unusual diversity of functions that were beginning to be recognized in proteins such as thrombospondin 1 in 1995 [1], and the number of matricellular proteins is still increasing. Matricellular proteins are a class of secreted extracellular matrix (ECM) proteins that exert diverse functions through direct binding to cell surface receptors, other matrix proteins, and soluble extracellular factors such as growth factors and cytokines [4]. Matricellular proteins do not contribute directly to the structural organization of the ECM, but rather modulate cell-cell and cell-matrix interactions [4]. Their levels are generally low in steady-state condition in adult tissues, but they are readily upregulated in conditions that activate an inflammatory response [2]. Matricellular proteins are considered to be key mediators of some inflammatory diseases [2].

Osteopontin (OPN) and tenascin-C (TNC) are representative of matricellular proteins. We have already reported that recombinant OPN (r-OPN) induced mitogen-activated protein kinase (MAPK) phosphatase 1, an endogenous MAPK inhibitor, in the spastic cerebral arteries via binding to L-arginyl-glycyl-L-aspartate-dependent integrin receptors and prevented cerebral vasospasm after subarachnoid hemorrhage (SAH) in rats [9]. In a clinical setting, in addition, multivariate analyses demonstrated that higher TNC levels in the cerebrospinal fluid were an independent predictor of symptomatic cerebral vasospasm occurrence [11]. Here, we show some new findings, leading to one conclusion: that TNC and OPN are new players in cerebral vasospasm after SAH.

Materials and Methods

All procedures were approved by the Animal Ethics Review Committee of Mie University and were in accordance with the institution's Guidelines for Animal Experiments.

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SAH Model and Study Protocol

The endovascular perforation model of SAH was produced in male adult Sprague–Dawley rats (age 8–9 weeks, 270–320 g; SLC, Hamamatsu, Japan) as previously described [9]. Each animal was anesthetized by an intraperitoneal injection of 4% chloral hydrate (10 ml/kg). A sharpened 4-0 monofilament nylon suture was advanced rostrally into the left internal carotid artery from the external carotid artery stump to perforate the bifurcation of the left anterior and middle cerebral arteries. Blood pressure and blood gas were measured via the left femoral artery. Rectal temperature was kept at 37°C during surgery. Sham-operated rats underwent identical procedures except that the suture was withdrawn without puncture.

First, 24 rats were randomly divided into either SAH or sham groups, sacrificed after neurobehavioral tests and India ink angiography on days 1 and 3, and examined if TNC or OPN was induced in post-SAH cerebral arteries using immunostaining.

Second, phosphate-buffered saline (PBS) vehicle, recombinant TNC (r-TNC), r-OPN, or r-TNC plus r-OPN were injected into a cisterna magna in 66 healthy rats, and the effects on the diameter of basilar arteries were determined using India ink angiography following neurobehavioral tests on days 1–3.

Neurobehavioral Test

Neurological impairments were blindly evaluated using two methods. Neurological scores (2–18 points) were assessed by summing six test scores (spontaneous activity; spontaneous movement of four limbs; forepaw outstretching; climbing; body proprioception; and response to whisker stimulation) as previously described [9]. Beam balance tests investigated the animal's ability to walk on a narrow wooden beam (2.25-cm diameter and columnar) for 60 s: 4 points, walking more than 20 cm; 3 points, walking more than 10 cm but less than 20 cm; 2 points, walking more than 10 cm but falling; 1 point, walking less than 10 cm; and 0 points, falling with walking less than 10 cm [9]. The average score of three consecutive trials in a 5-min interval was calculated.

Intracisternal Infusion

Using a surgical microscope, the posterior cervical muscles were dissected through a suboccipital midline skin incision, and the atlanto-occipital membrane was exposed [12]. The membrane was penetrated by a 27-gauge needle. Sterile PBS vehicle (100 μl), mouse r-TNC (2 μg in 100 μl; R&D Systems, Minneapolis, MN); mouse r-OPN (3 μg in 100 μl; EMD Chemicals, La Jolla, CA); or 2 μg of r-TNC plus 1 or 3 μg of r-OPN in 100 μl of PBS were infused into a cisterna magna at a rate of 100 μl/min irrespective of the animal's body weight. The needle was removed 10 min after an infusion, and the pore was quickly plugged with oxidized cellulose.

India Ink Angiography

Gelatin-India ink solution was made by dissolving gelatin powder (7 g) in 100 ml PBS and mixing with 100 ml India ink (Kuretake Co., Nara, Japan) [9]. The ascending aorta was cannulated with a blunted 16-gauge needle attached to flexible plastic tubing, which was connected to a pressure transducer (Nihon Kohden Co., Tokyo, Japan) and a syringe on an automatic infusion pump. 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 [9]. The rat was refrigerated at 4°C for 24 h to allow gelatin solidification. The brains were harvested and high-resolution pictures of the circle of Willis and basilar arteries were taken with a scale before and after the removal of a subarachnoid clot. The brain was stored in 10% neutral buffered formalin for immunohistochemistry.

An experienced person who was unaware of the treatment groups measured the smallest lumen diameter within each vascular segment of intracranial cerebral arteries (sphenoidal segment of the middle cerebral artery, precommunicating segment of the anterior cerebral artery, intradural internal carotid artery, and basilar artery) three times using Image J software (National Institutes of Health, Bethesda, MD) and determined a mean value per segment.

Immunohistochemistry

Immunohistochemistry on formalin-fixed, paraffin-embedded sections was performed as described previously [13]. After dewaxing and rehydration, the sections were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activities, placed in 1 mmol ethylenediamine tetraacetic acid (pH 8.0), and heated in an autoclave at 121°C for 1 min. The sections were then blocked with 5% goat or horse serum and incubated overnight at 4°C with the mouse monoclonal anti-OPN (1:100, Santa Cruz Biotechnology, Santa Cruz, CA) and mouse monoclonal anti-TNC (1 μg/ml, Immuno-

Biological Laboratories, Takasaki, Japan) antibodies. They were subsequently incubated with biotinylated antimouse immunoglobulin (Vector Laboratories, Burlingame, CA) for 30 min and then with an avidin-biotin complex for 30 min at room temperature. Color reactions were developed in diaminobenzidine/hydrogen peroxide solution, and the sections were counterstained with hematoxylin solution for light microscopic examination. Negative controls consisted of serial sections incubated with buffer alone instead of the primary antibodies.

Statistics

Neurological and beam balance scores were expressed as median plus or minus the 25th–75th percentiles and were analyzed using Mann–Whitney *U* tests or Kruskal–Wallis tests, followed by Steel–Dwass multiple comparisons. Other values were expressed as mean plus or minus standard deviation, and unpaired *t* tests and one-way analysis of variance with Tukey-Kramer post hoc tests were used as appropriate. We considered $p < 0.05$ significant.

Results

Induction of TNC and OPN in Rat Cerebral Arteries After SAH

Three SAH rats and no sham-operated rats died before euthanasia. The final number of animals in the 1- and 3-day SAH groups therefore was 5 and 4, respectively, and that in the sham-operated rats was 6 in both 1- and 3-day groups. There were no significant differences in physiological parameters between SAH and sham-operated rats at any time point or within the groups over time. The sham-operated rats showed no vasospasm, while significant vasospasm occurred in all cerebral arteries, including the intradural internal carotid arteries in the SAH rats on day 1, associated with neurobehavioral impairments, and began to improve on day 3. On day 1, when vasospasm was the most prominent, marked induction of TNC immunoreactivity was noted, especially in the smooth muscle cell layers of cerebral arteries in the SAH rats (Fig. 1). The TNC immunoreactivity decreased on day 3 as vasospasm improved. On the other hand, OPN immunoreactivity was detectable in the adventitia but was less apparent in the smooth muscle cell layers of cerebral arteries in the SAH rats on day 1; OPN immunoreactivity was more markedly induced throughout the vascular wall on day 3, when vasospasm was improved (Fig. 1). Neither TNC nor OPN was induced in the sham-operated rats.

Effects of r-TNC on Basilar Arteries in Rats

A cisternal injection of r-TNC caused no mortality and neurobehavioral impairments, but induced significant contraction of basilar arteries (Fig. 2a). The contraction continued for 48 h compared with the PBS-injected rats (Fig. 2b).

Effects of r-OPN on r-TNC-Induced Contraction of Basilar Arteries in Rats

A cisternal injection of r-OPN had no effect on the diameter of basilar arteries (Fig. 3). However, r-OPN reversed r-TNC-induced contraction of basilar arteries in a dose-dependent manner (Fig. 3).

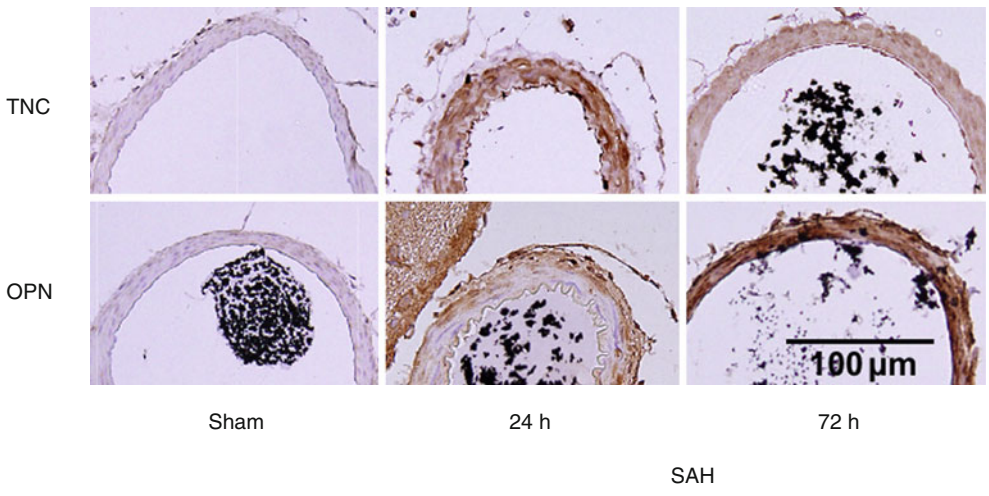

Fig. 1 Immunohistochemistry for tenascin-C (*TNC*) and osteopontin (*OPN*) in the intradural internal carotid artery at 24 and 72 h after subarachnoid hemorrhage (*SAH*) in rats. *Sham* sham-operated rats. *Black stains* depict India ink because specimens were harvested after India ink angiography

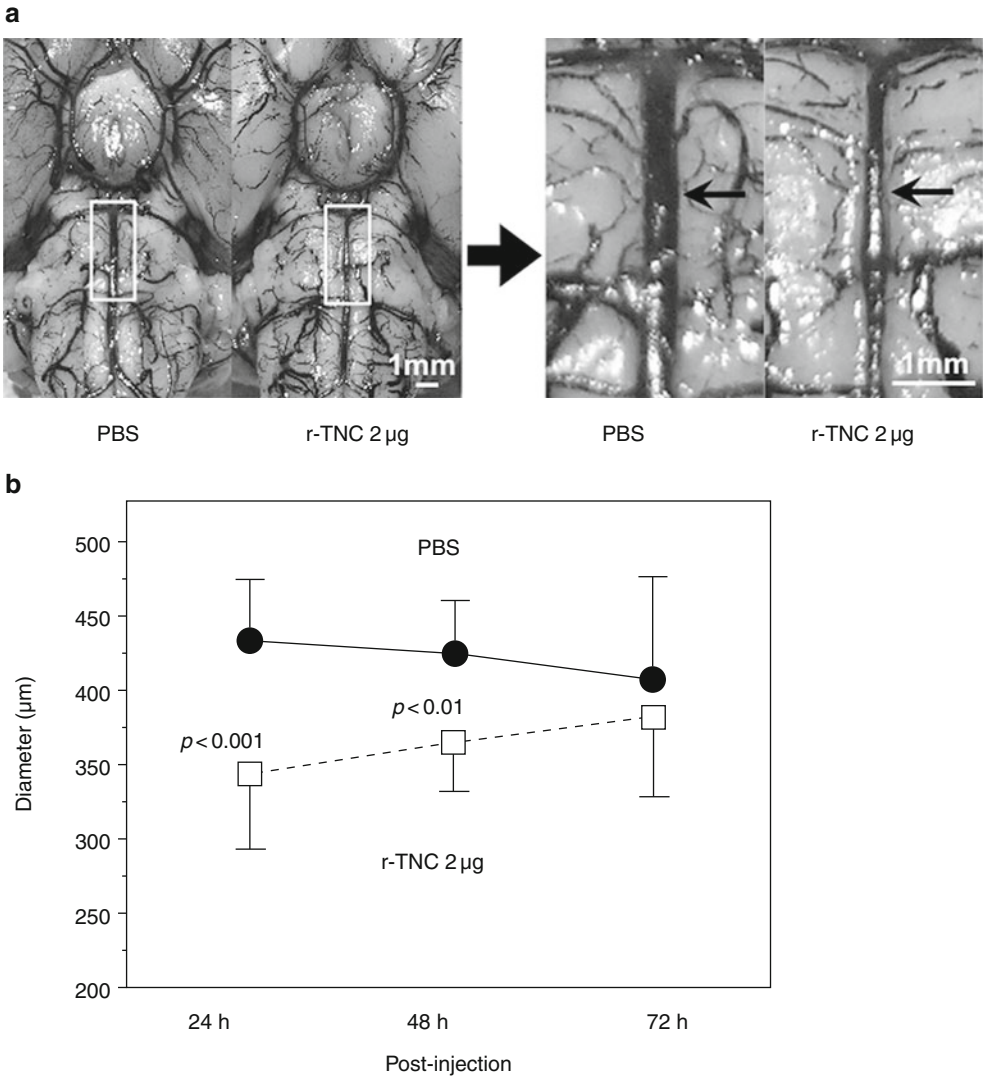

Fig. 2 Effects of a cisternal injection of 2 µg of recombinant tenascin-C (*r-TNC*) on the diameter of basilar arteries in rats. (**a**) Representative India ink angiograms; (**b**) vessel diameter of basilar arteries at 24–72 h after a cisternal injection of r-TNC or phosphate-buffered saline (*PBS*) vehicle. *Arrow* basilar artery; $n=6$ per group; data, mean ± standard deviation; p values, unpaired t tests between r-TNC- and PBS-injected rats

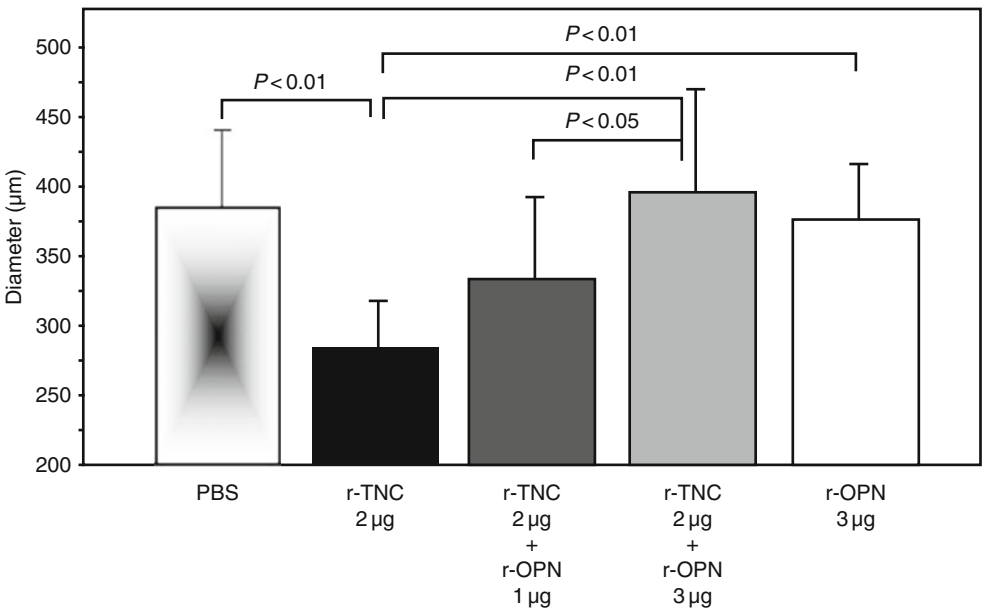

Fig. 3 Effects of a cisternal injection of recombinant osteopontin (*r-OPN*; 1 or 3 µg) on recombinant tenascin-C (*r-TNC*)-induced contraction of rat basilar arteries at 24 h postinjection. *PBS* phosphate-buffered saline vehicle; $n=6$ per group; data, mean ± standard deviation; p values, one-way analysis of variance with Tukey-Kramer post hoc tests

Discussion

The components of ECM include basic structural proteins such as collagen and elastin and specialized proteins such as fibronectin, proteoglycans, and matricellular proteins. A few studies have focused on the relationships between basic structural ECM proteins and vasospasm [6], but matricellular proteins have not been investigated in the context of cerebral vasospasm. The biological functions of matricellular proteins are highly variable and often seemingly contradictory, depending on the biological scenario surrounding its induction [8]. However, in our recent studies, r-OPN has consistently been found to have protective effects on post-SAH early brain injury and vasospasm [8, 9]. On the other hand, clinical studies demonstrated that TNC, another matricellular protein, was induced in SAH patients with symptomatic vasospasm [11]. This study furthermore demonstrated that SAH induced TNC in the cerebral arterial wall associated with the development of vasospasm, while OPN was induced in the artery wall associated with the resolution of vasospasm. A cisternal injection of r-TNC caused prolonged contractions of rat basilar arteries, while r-OPN prevented r-TNC-induced arterial contraction. These findings suggest that TNC as well as OPN are important players in the pathophysiology of post-SAH vasospasm.

A previous study reported that r-OPN inactivated MAPK, a potential final common pathway for the signaling transduction during cerebral vasospasm [10], and therefore prevented vasospasm after SAH, although OPN can also inhibit proinflammatory reactions and protein kinase C activation, other potential mediators of vasospasm [9]. In regard to TNC, however, the mechanisms to induce vasospasm have not been investigated. Therefore, the mechanisms of how OPN antagonizes TNC's effect also remain unclear; one possibility is that OPN may inhibit TNC's binding to its receptor competitively because they share some receptors [5, 14]. Reportedly, TNC can activate phospholipase C, protein kinase C, calcium/calmodulin kinase, MAPK, and some growth factor receptors; upregulate proinflammatory cytokines and endothelin receptor type A; and induce cell apoptosis [5], all of which potentially cause cerebral vasospasm [10]. As TNC activates MAPK-dependent and RhoA-dependent pathways, both of which also induce vasospasm, and is induced via either pathway at the transcriptional level in smooth muscle cells [3], TNC signaling may positively feed back on upregulation of TNC itself, leading to more activation of the signaling transduction and the development of cerebral vasospasm. TNC and OPN may be efficient modulators of cerebral vasospasm at several different levels.

There are many other matricellular proteins known [2, 4], and they are also potentially involved in the pathophysiology of cerebral vasospasm because most matricellular proteins are known to affect potential mediators of vasospasm. For example, thrombospondin 1 inhibits nitric oxide pathways at multiple levels to control acute vascular tone and blood flow and activates a Src kinase in vascular cells [15]; secreted protein, acid and rich in cysteine (SPARC; also known as osteonectin) has anti-inflammatory properties [2]; and connective tissue growth factor (CTGF/CCN2) induces MAPK and inflammation and activates some growth factor signaling [7].

Conclusion

Future studies will determine whether matricellular proteins other than TNC and OPN are involved in the pathophysiology of post-SAH vasospasm. In addition, further studies are necessary to understand the mechanism of how each matricellular protein orchestrates various phases of vasospasm and to define the therapeutic potential of these matricellular proteins in post-SAH vasospasm.

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

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Role of Platelet-Derived Growth Factor in Cerebral Vasospasm After Subarachnoid Hemorrhage in Rats

Masato Shiba, Hidenori Suzuki, Masashi Fujimoto, Naoshi Shimojo, Kyoko Imanaka-Yoshida, Toshimichi Yoshida, Kenji Kanamaru, Satoshi Matsushima, and Waro Taki

Abstract *Background and purpose*: The role of platelet-derived growth factor (PDGF) remains unknown in cerebral vasospasm after subarachnoid hemorrhage (SAH). In this study, we examined the effects of PDGF receptor (PDGFR) inactivation on cerebral vasospasm in the endovascular perforation model of SAH in rats.

Methods: Rats were assigned to sham, SAH plus vehicle, and SAH plus imatinib mesylate (imatinib) groups ($n=4$ per group). Imatinib (50 mg/kg body weight), an inhibitor of the tyrosine kinases of PDGFR, or vehicle was administered intraperitoneally 30 min post-SAH. Vasospasm was evaluated in the left (perforation-sided) internal carotid artery by means of neurobehavioral tests, India ink angiography, and immunohistochemistry at 24 h after SAH.

Results: Imatinib significantly inhibited post-SAH PDGFR activation in the left internal carotid artery, in which vasospasm was significantly prevented. Animal's neurobehavior also showed a tendency to improve by imatinib treatment.

Conclusions: PDGF may play an important role in the pathogenesis of vasospasm after SAH.

Keywords Imatinib • Cerebral vasospasm • Subarachnoid hemorrhage • Platelet-derived growth factor

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Introduction

Although the pathogenesis of vasospasm is multifactorial, it remains unknown if platelet-derived growth factor (PDGF) is involved in the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage (SAH). Some studies have suggested the linkage between post-SAH PDGF production and vasospasm occurrence [3, 5, 14, 15], while others have negatively reported it [4, 6, 13]. Thus far, only one in vivo study tested if PDGF caused vasospasm using a PDGF receptor (PDGFR)-β antagonist, trapidil, in a single cisternal autologous blood injection model of SAH in rabbits [15]. In this study, we selected imatinib mesylate (imatinib), a selective inhibitor of the tyrosine kinases of PDGFR-α and -β, as a PDGF inhibitor because it is clinically available [7] and examined whether imatinib prevents cerebral vasospasm to determine the role of PDGF in the development of vasospasm in the endovascular perforation model of SAH in rats, which shows a high mortality and acute metabolic changes similar to clinical findings [11].

Materials and Methods

All procedures were approved by the Animal Ethics Review Committee of Mie University and were in accordance with the institution's Guidelines for Animal Experiments.

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SAH Model and Study Protocol

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Twenty-two rats underwent the endovascular perforation SAH or sham operation. After 30 min, 12 surviving rats were randomly assigned to 3 groups (sham + vehicle, SAH + vehicle, and SAH + imatinib groups), and 50 mg/kg body weight of imatinib (Novartis, Basel, Switzerland) or vehicle (phosphate-buffered saline [PBS], 10 ml/kg) were administered intraperitoneally. Neurological scores were evaluated prior to and at 24 h after the operation. Cerebral vasospasm was evaluated by India ink angiography at 24 h post-SAH, followed by the assessment of severity of SAH and immunohistochemistry.

Neurological Scoring

Neurological scores were evaluated with the scoring system in a blinded fashion [8]. 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.

Severity of SAH

The severity of SAH was assessed in a blinded fashion using high-resolution photographs as described previously [8]. 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.

India Ink Angiography

Gelatin-India ink solution was made by dissolving gelatin powder (7 g) in 100 ml PBS and mixing with 100 ml India ink (Kuretake Co., Nara, Japan) [9]. The ascending aorta was cannulated with a blunted 16-gauge needle attached to flexible plastic tubing, which was connected to a pressure transducer (Nihon Kohden Co., Tokyo, Japan) and a syringe on an automatic infusion pump. 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 [9]. The rat was refrigerated at 4°C for 24 h to allow gelatin solidification. The brains were harvested, and high-resolution pictures of the circle of Willis were taken with a scale before and after the removal of a subarachnoid clot. The brain was stored in 10% neutral buffered formalin for immunohistochemistry.

An experienced person who was unaware of the treatment groups measured the smallest lumen diameter in the left ICAs three times using Image J software (National Institutes of Health, Bethesda, MD) and determined a mean value.

Immunohistochemistry

Immunohistochemistry on formalin-fixed, paraffin-embedded sections was performed as described previously [12]. After dewaxing and rehydration, the sections were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, placed in 1 mmol ethylenediamine tetraacetic acid (pH 8.0) and heated in an autoclave at 121°C for 1 min. The sections were then blocked with 5% goat serum and incubated overnight at 4°C with the rabbit anti-PDGFR-β (1:200; Cell Signaling Technology, Danvers, MA) and rabbit polyclonal antiphosphorylated PDGFR (α: Y572/574, β: Y579/581; 1:200; Biosource, Camarillo, CA) antibodies. They were subsequently incubated with biotinylated antirabbit immunoglobulin (Vector Laboratories, Burlingame, CA) for 30 min and then with an avidin-biotin complex for 30 min at room temperature. Color reactions were developed in diaminobenzidine/hydrogen peroxide solution and the sections were counterstained with hematoxylin solution for light microscopic examination. Negative controls consisted of serial sections incubated with buffer alone instead of the primary antibodies.

Statistics

Neurological scores were expressed as median plus or minus 25th–75th percentiles and were analyzed using Kruskal-Wallis tests, followed by Steel-Dwass multiple comparisons. Other values were expressed as mean plus or minus standard error of the mean (SEM). Comparisons were assessed using chi-square, unpaired *t* tests, or one-way analysis of variance (ANOVA) with Tukey-Kramer post hoc tests as appropriate. We considered $P<0.05$ significant.

Results

Comparisons of physiological parameters, severity of SAH, and mortality were not significantly different between the vehicle- and imatinib-treated SAH rats. None of the sham-operated rats died.

Neurological scores were impaired at 24 h after SAH in comparison with the sham group, and post-SAH administration of imatinib improved neurological scores, but these differences were not significant (Fig. 1). However, significant vasospasm occurred in the left ICA at 24 h post-SAH, which was significantly attenuated by imatinib treatment (Fig. 2).

Immunohistochemistry showed that PDGFR-β and phosphorylated PDGFR were increased mainly in the smooth muscle layer of the spastic ICAs in the SAH-vehicle rats. These immunoreactivities were attenuated by imatinib treatment (Fig. 3).

Discussion

This study showed that SAH activated PDGFR associated with upregulation of PDGFR-β in the spastic cerebral artery, and that inhibition of the tyrosine kinases of PDGFRs by imatinib suppressed the activation of PDGFR and the upregulation of PDGFR-β, leading to improvement of vasospasm.

After SAH, coagulated clot around cerebral arteries triggers thrombin activation, which induces the expression of PDGF in arterial smooth muscle cells [3, 13, 14]. However, the functional significance of PDGF upregulation has been controversial in cerebral vasospasm. In a clinical setting, SAH patients with symptomatic vasospasm were reported to have significantly higher cerebrospinal fluid levels of the BB [3] or AB [2] isoform of PDGF (PDGF-BB or PDGF-AB), while Lad et al. [4] considered that PDGF was not an important biomarker for cerebral vasospasm. In experimental studies as well, a cisternal injection of recombinant PDGF-BB caused long-lasting vasoconstriction of basilar arteries in rabbits, resembling post-SAH vasospasm [14, 15], while PDGF-AB alone caused no significant vasoconstriction in the same model [6]. Maeda et al. [5] reported that PDGF-BB caused only slight vasoconstriction of basilar arteries in rabbits, but the contractile response of basilar artery to PDGF-BB was enhanced after SAH in rabbits [5]. Zhang et al. reported that the blockage of PDGF-BB production by two kinds of thrombin inhibitors [14] or PDGF-BB inactivation by a PDGFR-β antagonist trapidil [15] prevented vasospasm after SAH in rabbits. This study for the first time demonstrated that SAH upregulated PDGFR-β in the spastic cerebral artery, and that inhibition of the tyrosine kinases of PDGFRs

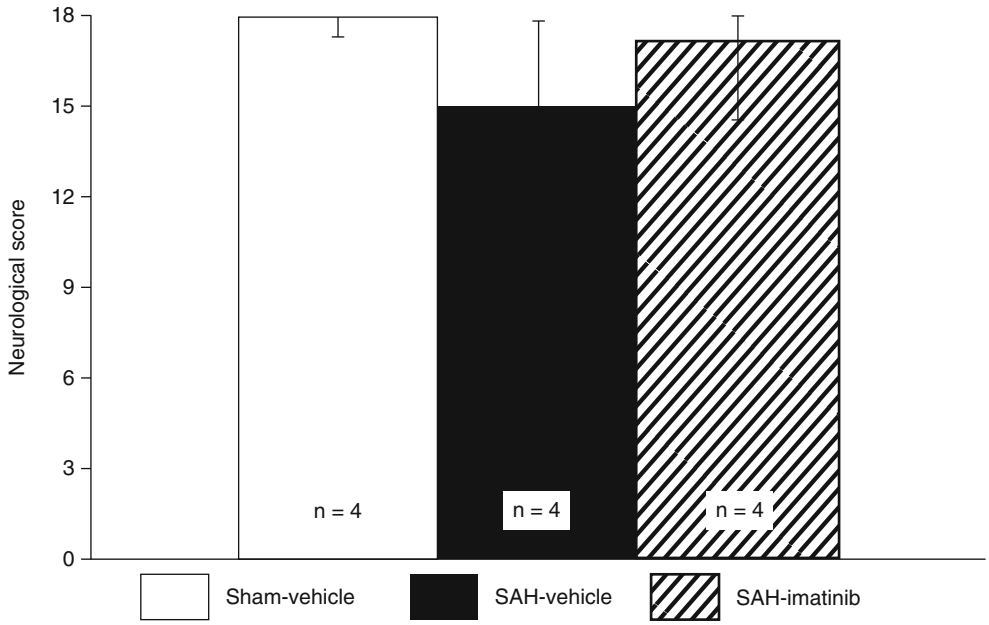

Fig. 1 Effects of imatinib treatment after subarachnoid hemorrhage (*SAH*) on neurological score at 24 h. Data are expressed as median plus or minus 25th–75th percentiles

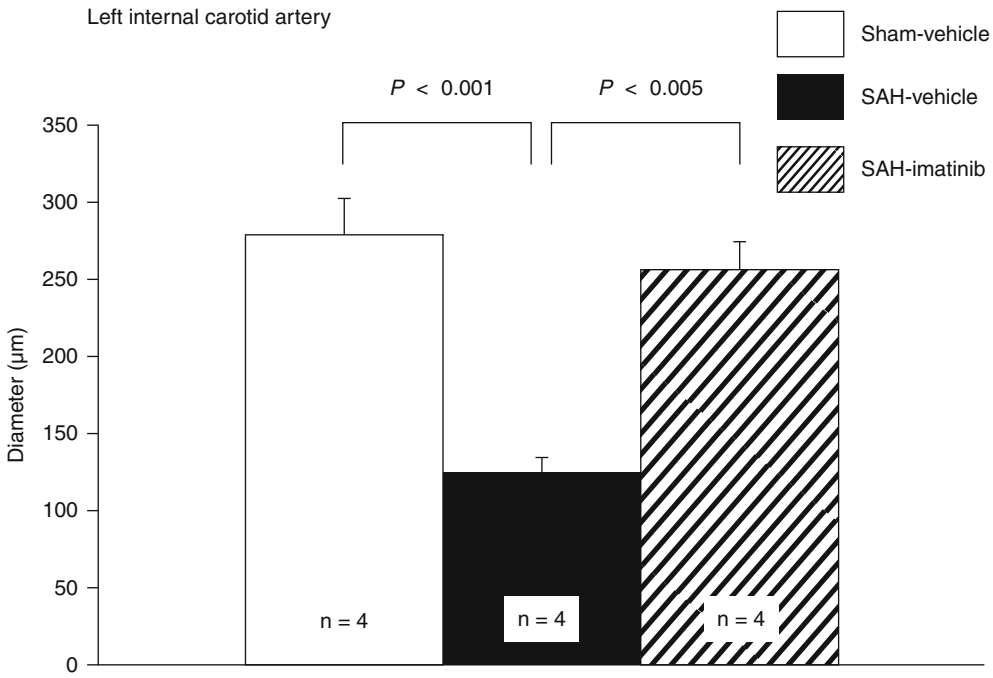

Fig. 2 Effects of imatinib treatment after subarachnoid hemorrhage (*SAH*) on vasospasm. Vessel diameter of left internal cerebral artery at 24 h postsurgery. Data, mean plus or minus standard error of the mean (*SEM*); *P* value, analysis of variance (*ANOVA*)

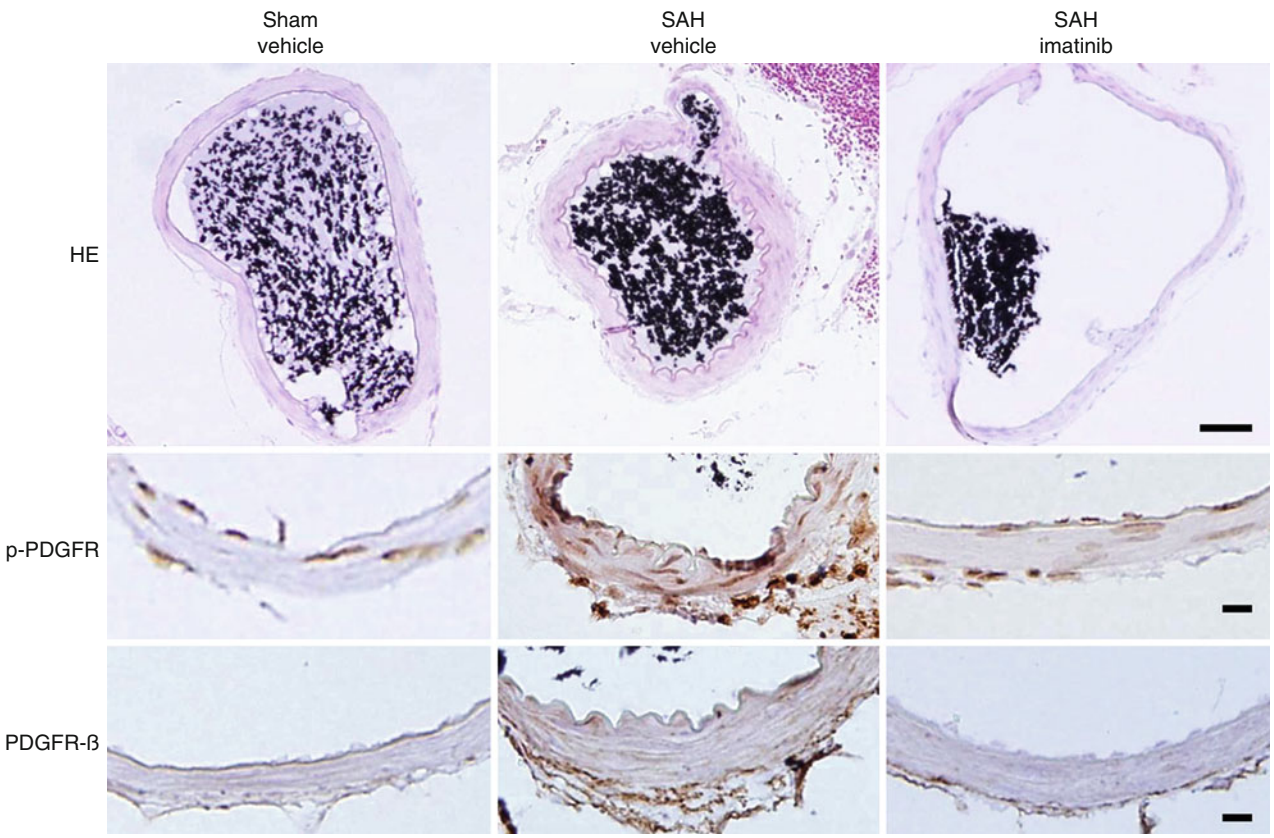

Fig. 3 Hematoxylin and eosin (*HE*) and immunohistochemical stainings of the intracranial internal carotid artery at 24 h after subarachnoid hemorrhage (*SAH*). *PDGFR* platelet-derived growth factor receptor. Bar = 20 μm

by imatinib suppressed PDGFR activation, PDGFR-β upregulation, and vasospasm in the endovascular perforation model of SAH in rats. Taken together, it is possible that not only PDGF-BB production but also PDGFR-β upregulation contribute to enhanced contractility of post-SAH cerebral artery and therefore the development of vasospasm.

PDGF-induced arterial contraction may occur via Ca^{2+}-dependent myosin-light chain kinase [5], tyrosine kinase [5], RhoA/Rho-associated kinase [15], and mitogen-activated protein kinase pathways [1, 10]. Maeda et al. [5] demonstrated that Ca^{2+}-dependent myosin-light chain kinase and tyrosine kinase were involved in the mechanism of post-SAH vasospasm development in rabbits. However, the involvement of other pathways remains undetermined in vasospasm.

Conclusion

This study positively supported the association between PDGF and cerebral vasospasm, but the detailed mechanism was not explored. In this regard, further investigations are needed.

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

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Relevance of Animal Models of Subarachnoid Hemorrhage for Examining Neurobehavioral Changes

Ryszard M. Pluta, Boris Skopets, and Jerald D. Kralik

Abstract *Background and purpose*: For many years survival and neurological functionality of patients were the main outcome measures after treatment of intracranial aneurysms. But, the variable outcomes of patients operated on in a delayed fashion or before the aneurysm rupture indicate that more precise measures are needed for assessment of not only the neurological but also the neuropsychological outcome. However, development and testing of such new tools requires better understanding of pathomechanisms of neurobehavioral changes evoked by aneurysmal subarachnoid hemorrhage (aSAH), which can be achieved using animal models.

Methods: We reviewed and selected (1) animal models developed to investigate delayed cerebral vasospasm that could be useful for examining effects of brain injury evoked by aSAH and (2) a battery of neurobehavioral animal testing that can be used for assessment of patients after aSAH.

Results: For every species used as an aSAH model, a battery of neurobehavioral test exists.

Conclusion: Albeit some limitations must be recognized, research using animal models of SAH should continue to play a critical role in assessment of cognitive and behavioral functions after aSAH.

Keywords SAH • Vasospasm • Animal models • Neurobehavior

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Introduction

Mortality and morbidity after treatment of intracranial aneurysms have declined significantly as early clipping and coiling become a standard treatment of aneurysmal subarachnoid hemorrhage (aSAH) [66]. But, methods assessing the outcome of these treatments have remained unchanged despite the growing body of evidence that the Glasgow Outcome Scale (GOS) and the Quality of Life (QOL) scale inadequately assess the impact of aSAH on a patient's life [26, 32]. Thus, it becomes imperative to reevaluate the "classic" outcome measures assessing the outcome of surgical and endovascular treatments, delayed cerebral vasospasm, and delayed ischemic neurological deficits (DINDs) after aSAH.

Sources of Neurobehavioral Changes After aSAH

The pathomechanism of poor outcome of patients after aSAH remains unclear. For many years, a delayed cerebral vasospasm was recognized as the single most important cause of clinical deterioration and poor outcome after aSAH after successful treatment of aneurysm [17, 66]. But recently, several ultraearly, early, delayed, and late mechanisms of brain injury by aSAH have been recognized and linked to the clinical and neurobehavioral outcome. Also, surgical trauma or cerebral blood flow changes evoked by the endovascular intervention [66, 74] may add yet another insult to the brain [36]. Unfortunately, the list of events affecting the outcome of aSAH does not end here as adjunct treatments (cerebrospinal fluid [CSF] diversion, administration of nimodipine, or triple H [hypertension, hypervolemia, and hemodilution] therapy [14, 59, 61, 72]) may also contribute to development of DINDs and neurobehavioral changes. Furthermore, the increased number of treated unruptured aneurysms [30] raises expectations for a good postoperative outcome among

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patients, their families, and health providers. As an effect of this, a mere lack of neurological deficits becomes unsatisfying, and treatment outcome is expected to be "a state of complete physical, mental, and social well-being" [66, 74]. Thus, neurobehavioral effects of aSAH and their treatment are becoming increasingly investigated.

Neurobehavioral Changes After Aneurismal SAH

Several small, retrospective studies (Table 1) have addressed neurobehavioral changes after aSAH. Unfortunately, no comprehensive and reliable conclusions can be drawn about the role of acute, delayed, or chronic changes evoked by aSAH on neurobehavior because these studies were focused only on delayed vasospasm [18, 49, 58, 65, 70]. Nevertheless, despite remaining controversial [26, 34, 68], results of these studies revealed that 30–50% of patients after aSAH suffered some attention and memory deficits [25]. In addition, these studies established several new factors beyond vasospasm to be associated with the neurobehavioral deficits (Table 2) and showed that the results of neuropsychological testing predicted the patient's well-being and ability to resume work [45]. However, time of the functional recovery remains unknown [25]; there is a significant range in neuropsychological status that receives the same score on the current outcome scales, such that patients with a Glasgow Outcome Scale (GOS) score of 5 can still have compromised function; and, somewhat surprisingly, there is a lack of difference in neurobehavioral deficits between patients who had surgery for aSAH compared with those without surgery [25]. The practical consequence of these reports is that even a patient whose clinical status is assessed at GOS 5 can be significantly socially or economically disabled and may benefit from neuropsychological diagnostics and rehabilitation. Thus, establishing causes and pathophysiology of neurobehavioral changes after aSAH and eventual ways to prevent them should advance diagnostics and enrich treatment options. Proper animal models should facilitate better insight into causes, pathophysiology, and pathomechanisms of neurobehavioral deficits, thereby providing a necessary tool to develop targeted treatments.

Models of aSAH

We have developed excellent experimental models of SAH, but all of them were focused on pathophysiology or treatment of delayed cerebral vasospasm because vasospasm was considered the single most important cause of poor outcome [17, 44, 66]. Recently, this dogma has been questioned [40, 54], and research has focused on acute, delayed, and chronic mechanisms that seem to be as important as vasospasm in evoking neurological and neurobehavioral consequences [40, 54].

There are numerous animal models mimicking the aSAH (Table 3). A detailed description of these models can be found in two recently published excellent reviews on this topic [29, 71]. But, all these models have been developed to investigate vasospasm. So, it remains unclear whether they can be adopted to examine neurobehavioral changes evoked by aSAH (Table 4).

Animal Neurobehavioral Tasks

There are numerous animal neurobehavioral tasks that could be used to examine the aSAH-related phenomena (Table 5). These tasks have proven to be applicable to clinical situations (e.g., http://www.cantab.com), particularly if there were many confounding factors that obscured or even prevented separation of their influence on disease progression,

Table 1 Clinical trials devoted to assessment of neurobehavioral changes in patients with aneurysmal subarachnoid hemorrhage (aSAH) and vasospasm

Reference	Leading cognitive dysfunction	Limitations
[58]	Moderate deficit in free recall	Retrospective, small
[34]	Free recall and short memory tests affected AcoA and vasospasm associated	Retrospective, 2–14 years after SAH
[18]	31% of amnesia in the acute phase	Retrospective, small, 23% of patient had the aneurysm trapped
[65]	Cognitive defects and frontal lobe syndrome	Retrospective, small, 1–7 years after aSAH and one-third with hydrocephalus
[49]	The only prospective study; mild- to-moderate cognitive and memory dysfunction at GOS 5	Neurobehavioral dysfunctions unrelated to vasospasm, ischemia or hydrocephalus
[70]	65% of the patients were impaired in at least one cognitive domain	Retrospective, small

SAH subarachnoid hemorrhage, *aSAH* aneurismal subarachnoid hemorrhage, *GOS* Glasgow Outcome Scale

Table 2 Prognostic factors of neurobehavioral changes after aneurysmal subarachnoid hemorrhage (aSAH)

Factor	References
Initial Hunt and Hess grade	[26, 45]
Severity of bleeding (Fisher's scale)	[26, 45]
Intraperenchymal/intraventricular bleeding	[49]
Persistent neurological deficits	[75]
Age at the time of aSAH	[26]

Table 3 Experimental models of aneurysmal subarachnoid hemorrhage (aSAH) or vasospasm

	SAH method			Effect			
		Single	Double				
Species	Artery	Puncture	Intrathecal injection	Clot	SAH	Vasospasm	Reference
Mouse	+	+	+	−	+	?	[52]
Rat	+	+	+	−	+	±	[56]
Rabbit	+	+	+	−	+	±	[39]
Cat	+	+	+		+		[73]
Pig		+	+	+	+	+	[42]
Dog	+	+	+	+	+	+	[79]
Nonhuman primate	+	+	+	+	±	+	[15]

Table 4 Modified neuropsychological and psychopathological sequelae of aneurysmal subarachnoid hemorrhage (aSAH)

Visual field defects
Neglect
Defects of visual and spatial constructive capacities
Distortions of face and object recognition
Memory disorders
Language disturbances (aphasias)
Deficits in problem solving
Apraxias
Attention deficits
Mental rigidity
Dementia
Deficits of concentration capacity
Personality changes/emotional disorders
Behavioral disorders
Organic personality disorder
Apathy and loss of interest
Impairment of social judgment
Aggressiveness or rage
Affect labiality, disinhibition, or syndrome (depression)
Organic psychosocial maladjustment

Source: From [25]

its treatment, or outcome. In aSAH, such factors are (1) location of subarachnoid blood; (2) the amount of blood in subarachnoid cisterns and ventricles; (3) the effect of surgical or endovascular manipulations; (4) the presence of increased intracranial pressure or (5) hydrocephalus; and (6) other secondary complications of aSAH (e.g., cerebral edema). The use of aSAH animal models allowed for separation and in-depth examination of pathophysiological effects of these factors and application of the findings to clinical settings.

Neurobehavioral Tasks and a Nonhuman Primate Model of aSAH

It is widely accepted that a clot model of SAH mimics faithfully a clinical course of delayed cerebral vasospasm [44]. However, it rarely evokes neurological deficits [29] despite producing cortical injury [62]. There are several reasons why DINDs in this model are not present, including (1) a limited distribution of clot in the subarachnoid space; (2) a collateral blood flow; (3) a small area of the brain exposed to blood clot; and (4) lack of the "early" SAH-related events [29, 54]. The modification of this model by a wider exposure of cortex to clot after removal of the arachnoid over the "eloquent" parts of the brain not only may result in cortical changes, as has been recently shown [62], but also should allow examination of the pathophysiological, neurological, and neurobehavioral changes evoked by a clot placed on the exposed surface of the brain.

There are numerous standard behavioral tasks (Table 6) that can be conducted to examine and characterize potential

Table 5 Neurobehavioral tasks for small animal models of aneurysmal subarachnoid hemorrhage (aSAH)

		Animal species					
Defects	Task	Mouse	Rat	Dog	Cat	Rabbit	Pig
Visual defects	No task available						
Neglect	Task for neglect		[57]		[53]		
Visual spatial capacities	No task available						
Distortions of face and object recognition	Matching/non matching task	See memory models					
Memory	Morris water maze	[69]	[46]				[63]
	Radial arm maze	[6]	[60]				[21]
	Radial arm water maze	[6]	[29]				
	Barnes maze	[67]	[46]				
	Y- or V-maze	[6]	[46]				
	T-maze	[6, 12]	[46]		[37]		[48]
	Other types of mazes	[3]	[46]				[28]
	Passive-avoidance learning	[3, 6]	[29, 46]				
	Active avoidance	[6]	[29]	[11]	[9]		[22]
	Matching /non matching task	[10, 12]	[29]	[24]	[50]		[48]
	Displacement			[13, 47]	[13]		
	Obstacle memory				[13, 43]		
Language disturbances (aphasias)	No task available						
Deficits of problem solving	Matching/nonmatching task	See memory models					
	Go-trial reaction time (GoRT) task and stop-signal reaction time (SSRT) task	See attention deficits					
	Attention set shifting	See attention deficits					
	Other types of mazes	See memory models					
	String-pulling task			[51]			
Apraxia	No task available						
Attention deficits	Five-choice serial reaction time task	[10]	[7]				
	Attentional set shifting	[4]	[5, 33]				
	Go-trial reaction time (GoRT) task and stop-signal reaction time (SSRT) task		[5]				
Mental rigidity	No task available						
Dementia	See memory models						
Deficits of concentration capacity	No task available						
Personality changes/emotional disorders/behavioral disorders	Elevated-plus maze	[3]	[33]				[1]
	Open field task	[3, 6]	[29]	[2]	[41]	[81]	[1, 63]
	Light–dark exploration task	[3]	[38]				[1]
	Social interaction task	[3]	[33]				
	Shock probe defensive burying task	[16]	[33]				
	Novelty-induced hypophagia	[3]	[64]				
	Attention set shifting	See attention deficits					
Affect labiality, disinhibition, or syndrome (depression)	Forced swimming task	[8]	[8]				
	Tail suspension task	[8]					
Organic personality disorder	No task available						
Apathy and loss of interest	No task available						
Impairment of social judgment	No task available						
Aggressiveness or rage	Social interaction task	See personality changes/emotional disorders/behavioral disorders					
Organic psychosocial maladjustment	No task available						

Table 6 Behavioral tasks assessing signs and deficits in a nonhuman primate model of subarachnoid hemorrhage

Neurobehavioral defects	Test	References
Visual defects	Visual discrimination tasks	[19]
Neglect	Test for neglect	[19]
Visual spatial capacities	Discrimination based on object location task; spatial recognition task	[19, 77]
Distortions of face and object recognition	Object and face recognition tasks using match/nonmatch to sample	[19, 77]
Memory	Delayed response task	[19]
	Delayed nonmatch to sample	[19, 77]
	Paired-associates task	[77]
	Memory versus attention task	[35]
Language disturbances (aphasias)	N/A	
Deficits of problem solving	Wisconsin card-sorting task analogs	[19, 77]
	Strategy task	[20]
	Reverse-reward task	[31]
	Rule-learning task	[76]
	Decision-making task	[23]
Apraxia	Reaction time task; motor coordination bimanual task; motor-sequencing tasks; conditional motor-learning tasks	[19, 77]
Attention deficits	Cuing tasks	[19, 77]
	Visual search	[19, 77]
	Intra-/extradimensional shift	[77]
Mental rigidity	Object reversal learning; extinction; Wisconsin card-sorting task analogs; reverse-reward tasks	[19, 31, 77]
Dementia	No current tests	
Deficits of concentration capacity	Fixation tasks	[19, 77]
Personality changes/emotional disorders/ behavioral disorders	Classical conditioning; stimulus-reward association	[19, 27]
	Snake fear task	[27]
	Intruder task	[27]
	Affective go/no go task	[77]
Affect lability, disinhibition, or syndrome (depression)	Gambling task	[77]
	Stop signal task	[19, 77]
Organic personality disorder	No available task	
Apathy and loss of interest	Progressive ratio task; gambling task; reward evaluation	[77]
Impairment of social judgment	Intruder test; social images choice task	[27]
Aggressiveness or rage	Direct facial expression and behavioral assessment	[19]
Organic psychosocial maladjustment	No available task	

damage to particular regions of cerebral cortex of the monkey to address neurobehavioral changes reported in patients after aSAH.

It seems that a slightly modified, by the additional removal of arachnoid from cortical surface, nonhuman primate model of cerebral vasospasm offers not only a possibility to apply a variety of sophisticated tasks to characterize specific cortical damage but also perhaps an advantage over other animal models by allowing clinical examination of important areas of the cortex that are shared with humans [55, 80] as well as the aSAH impact on the higher-order cognitive functions [78].

Conclusion and Study Limitations

For years, neurosurgeons have been focused on the neurological functionality of patients as a main measure of their success after treatment of intracranial aneurysms. But, for many patients these measures have been inadequate in the face of everyday life challenges. Several clinical studies and an individual experience have suggested that more precise and in-depth assessments of neurobehavioral changes are necessary [26]. A better understanding of the pathomechanism(s) of neurobehavioral changes should precede development of such new tools. To address this issue,

we need to expand aSAH-related research beyond delayed vasospasm [40, 54] and include neurobehavioral testing to facilitate development of proper diagnostic and therapeutic tools, to separate and examine components of psychobehavioral changes evoked by aSAH, and to objectify neurobehavioral complaints of patients. Neurobehavioral testing of nonhuman primates after aSAH can provide the precision to distinguish between aSAH- and vasospasm-related neurobehavioral changes and become a reliable translational tool to develop appropriate treatments. However, several limitations of translating the experimental results to the patient population have to be recognized early and carefully addressed. These include the difficulty in separating early versus delayed effects of aSAH and the effects on vasospasm of surgical versus endovascular intervention. Although these limitations must be kept firmly in mind, research that uses animal models should continue to play a critical role in determining the effects aSAH has on the cognitive and behavioral functions of patients.

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

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Inflammation in Subarachnoid Hemorrhage and Delayed Deterioration Associated with Vasospasm: A Review

J. Javier Provencio

Abstract Delayed deterioration associated with vasospasm (DDAV) after subarachnoid hemorrhage (SAH), (often called vasospasm) continues to be both a difficult entity to treat and a leading cause of morbidity in patients. Until recently, attention was focused on alleviating the vascular spasm. Recent evidence shows that vascular spasm may not account for all the morbidity of DDAV. There is renewed interest in looking for other potential targets for therapy. Inflammation has become a promising area of research for new treatments. This review explores the evidence that inflammation is a driver of DDAV by asking three questions: (1) If inflammation is important in the pathogenesis of the disease, what part or parts of the inflammatory response are involved? (2) When does inflammation occur in SAH? (3) In what compartment of the skull does the inflammation occur, the cerebrospinal fluid and meninges, the cerebral arteries, or the brain itself?

Keywords Inflammation • Delayed cerebral vasospasm • Subarachnoid hemorrhage • Neutrophils

Introduction

Delayed neurological deterioration after subarachnoid hemorrhage (SAH) was initially described before the advent of cerebral angiography. After the development of cerebral angiography, the association with cerebral vasospasm became apparent, prompting researchers to focus on reversal of the vascular spasm. Results of trials using the calcium channel blocker nicardipine and the endothelin-1 antagonist clazosentan revealed an unexpected finding: The cerebral vasculature can be dilated without improvement in patient outcomes [17, 28]. Recently, there have been a number of investigators who have questioned whether vascular constriction is associated with *all* the morbidity of the syndrome [10, 20, 39, 52]. Over the last 15 years, a number of laboratories have investigated the inflammatory underpinnings of both vascular constriction and the brain damage associated with SAH.

The names associated with delayed neurological deterioration after SAH have been a source of confusion. Over the years, the terms delayed cerebral vasospasm (DCV), delayed ischemic neurological deficits (DIND), and delayed cerebral ischemia (DCI) have all been employed to describe the syndrome. Unfortunately, all of these names assume that the cause of damage is due to vasospasm or ischemia. The studies of nicardipine and clazosentan would suggest that other mechanisms might be at play [17, 28]. The ideal name should both appreciate the association with vasospasm and not make assumptions about the cause of damage. I favor the name *delayed deterioration associated with vasospasm* (*DDAV*) as it meets both criteria and keeps open the possibility that forces other than ischemia, such as inflammation, may play a role. In this review, I use *DDAV* to describe the syndrome of delayed deterioration and *vasospasm* to denote specifically changes in vessel caliber.

This review asks a series of questions about the possible role of inflammation in DDAV. I describe the research to date that supports the answers to the specific questions.

What Type of Inflammation Is Associated with DDAV?

The study of inflammation in SAH dates back more than 30 years with the finding that early elevations in white blood cell (WBC) cell counts are associated with the later development of vasospasm [29, 31, 35, 37]. A number of studies have investigated inflammation by examining cytokine levels

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in blood and cerebrospinal fluid (CSF) in patients with SAH. In addition, there have been a number of interesting animal studies that have suggested the involvement of the inflammatory system in DDAV.

We must first define what inflammation is in the context of SAH. The term *inflammation* very broadly describes a local response to tissue injury that is marked by a number of specific events: increased capillary permeability and fluid extravasation into the tissue, leukocyte infiltration, and the four cardinal signs described by Celsus over 2,000 years ago of tumor, calor, rubor, and dolor. Inflammation is the final common pathway of processes directed by the immune system.

The immune system is a complex and tightly regulated system of effectors against external pathogens *and* endogenous tissue damage. It can be broadly divided into two component parts, the innate immune system and the adaptive immune system. It is out of the scope of this review to discuss the intricacies of the two systems, but a good review was published in 2000 [8]. Although the immune system functions both to attack external pathogens and to mitigate internal cell malfunctions, the role of immunity against external pathogens has been better studied.

The innate immune system is the first line of defense against external pathogens that includes processes from the skin (preventing entry of foreign pathogens) to marrow-derived innate immune cells that form the first responder corps against bacteria and viruses. In infection, the innate cell response, which consists mainly of neutrophils (also macrophages, natural killer cells, and monocytes) enters an infected tissue and nonspecifically releases tissue-destroying enzymes and reactive oxygen species. The signals to which innate immune cells respond were originally thought to be due to recognition of self versus nonself. This theory has been largely replaced by a newer theory that is supported by evidence that neutrophils respond to specific *danger signals* called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that can be either from pathogens or from release of intracellular contents when a cell is damaged or cancerous [1]. When presented these signals, innate cells destroy the tissue and send signals to the adaptive immune system to develop a more specific response.

The adaptive immune system is a well-regulated set of processes that has as its end product a very specific response to a specific organism (or tissue). T cells and B cells are able to develop a specific response to cellular elements of an external pathogen by selection and clonal expansion. This response takes longer to develop than the innate system but has long-lasting effects, including immunity against pathogens years after initial exposure. The process of regulation of adaptive immunity is based on communication between antigen-presenting cells in the lymph nodes and naïve lymphocytes with signaling from numerous cytokines.

Given the time that it takes DDAV to develop, one would suspect that the actor in inflammation would be the adaptive immune system (Fig. 1). Interestingly, there is mounting evidence in other critical illness-associated injuries that suggests that in a noninfected environment, the innate immune system can have actions that are longer lasting than in infection [48, 51]. Research from our laboratory suggested that neutrophils are associated with vasospasm in patients with SAH [46]. This is consistent with studies done in animal models that suggested that interfering with the mechanism of neutrophil signaling or availability ameliorated vasospasm. Studies from the laboratory of Tamargo and colleagues have shown that blocking the CD11b/CD18 complex (the cell surface ligand on myeloid cells that pairs with intercellular adhesion molecule 1 [ICAM-1] for trafficking out of the bloodstream) prevents vasospasm [5]. Ma and colleagues have shown that there is evidence of signaling of the largely neutrophil-associated Toll-like receptor 4 in the brain after SAH [27]. Minami and colleagues also found evidence of neutrophil interaction with basilar artery interstitium after SAH associated with vasospasm [33]. The laboratory of Zhang and colleagues has been investigating early inflammation after SAH and its implications for vasospasm. They have found that administration of L-arginine decreases early inflammation after SAH [57]. L-Arginine is the substrate for NADPH oxidase, a neutrophil enzyme. This is evidence that innate mechanisms are active early after SAH.

There is little evidence from sampled CSF in patients with SAH that adaptive immune system cells (T cells and B cells) are associated with DDAV, although definitive investigation of this is lacking (Provencio, unpublished data). There is some conflicting data in the field that decrease our certainty that innate immunity has a definite role. A study from patients with SAH who had surgical clipping showed that levels of interleukin (IL) 1β but not tumor necrosis factor (TNF) α were increased and correlated with the later development of vasospasm [34]. If the innate immune system were the driver, one would expect that both IL-1β and TNF-α would correlate to vasospasm. A study by Oruckaptan and colleagues in Turkey found no role for neutrophils or myeloperoxidase (MPO, a neutrophil-derived effector enzyme [38]). Although there were methodological issues that call into question the results of this study, replication of this study has not been published to my knowledge. These few contradictory studies do not dissuade my enthusiasm for inflammation as a reasonable area of research in SAH.

When Does Inflammation Occur That Leads to Delayed Deterioration Associated with Vasospasm?

One of the most tantalizing prospects for treatment of DDAV is its occurrence often a week after the onset of SAH. With so much time between ictus and onset of symptoms, it is hoped that treatments can be implemented in this window. The

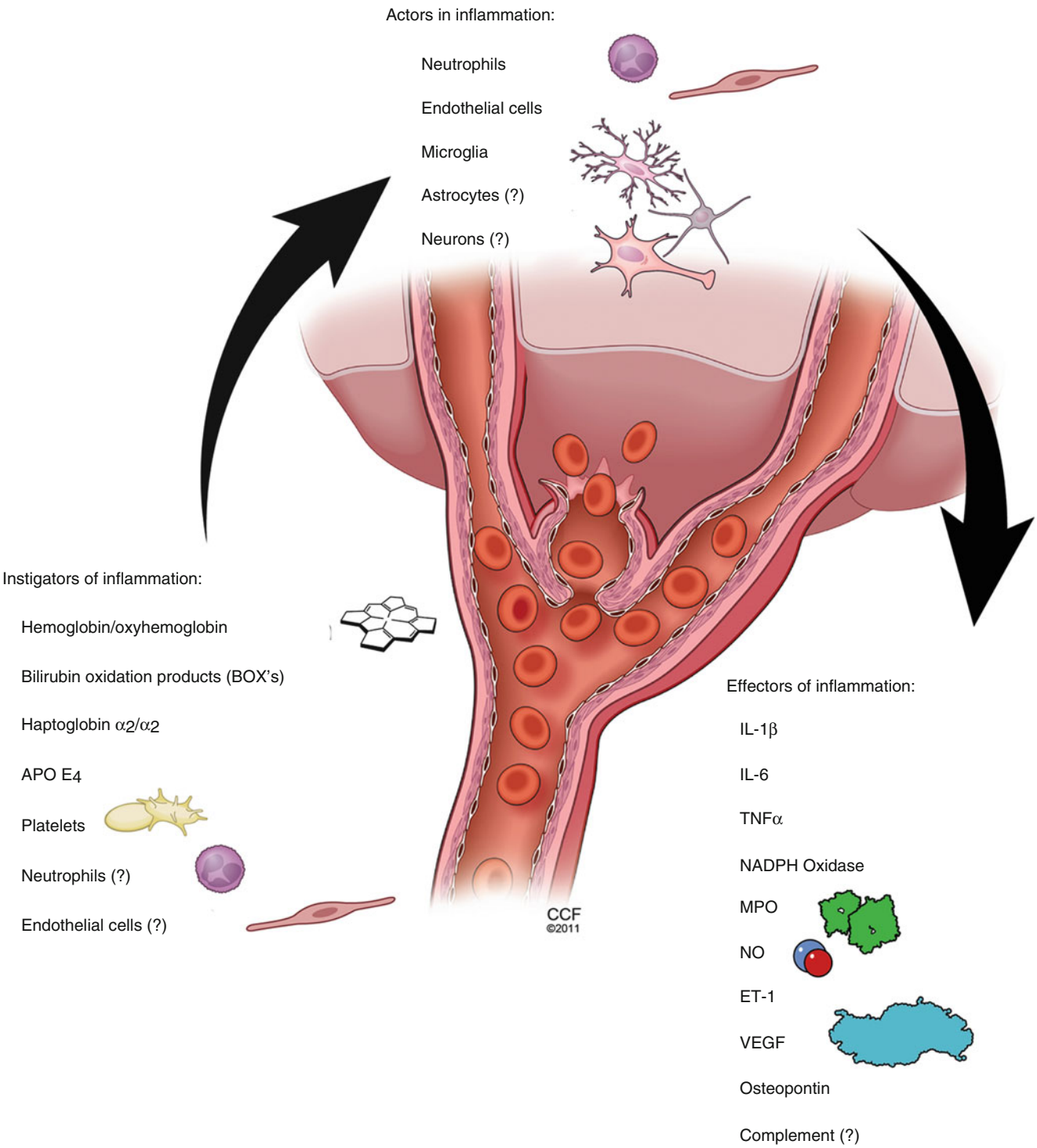

Fig. 1 Depiction of possible instigators, actors, and effectors of inflammation that could contribute to both vascular changes and poor outcomes in patients with delayed deterioration associated with vasospasm (DDAV) after aneurysmal SAH

important question that arises is whether the processes that lead to DDAV and cerebral vasospasm develop at the time of clinical presentation or are the end product of a process that begins at the time of SAH and takes time to develop clinically.

Evidence from studies of early inflammation after SAH suggests that inflammatory processes begin early. Zhang and colleagues have shown that vascular endothelial growth factor (VEGF) and mitogen-activated protein kinase (MAPK) are upregulated early after experimental SAH [9]. Both are associated with innate inflammatory responses. Likewise, McGirt, Laskowitz, and colleagues found that in patients with SAH, early, elevated levels of matrix metalloproteinase 9 (MMP-9) and VEGF were associated with vasospasm [30].

An interesting study by Clatterbuck and colleagues showed that inhibition of lymphocyte function-associated antigen 1 (LFA-1) within 3 h of the hemorrhage prevents vasospasm of isolated femoral arteries, suggesting that the process of inflammation occurs very early in the course of SAH [6]. In our laboratory, we have found that neutrophils are elevated in the first 3 days after SAH, days before the onset of clinical symptoms or evidence of vasospasm [46]. In a mouse model of vasospasm, depletion of myeloid cells (neutrophils, monocytes, and macrophages) prior to SAH prevents both the vascular manifestations of DDAV and the behavioral deficits [45].

Where Does the Inflammation Occur?

There are three possible sites of inflammation after SAH as it pertains to DDAV: the meninges and CSF space, the brain, and the cerebral arteries. It is possible that more than one compartment is affected in the process. Studies investigating the different compartments are lacking. One of the strongest criticisms of inflammation as a driver for vasospasm and DDAV is that there is not as robust a meningeal reaction to spilled blood in the CSF space as in bacterial meningitis, which is less associated with this syndrome. It is important to reiterate the caution that innate inflammatory responses in infected tissues appear to differ from those responses in sterile environments.

Work in our laboratory and others have suggested that inflammation does occur in the CSF of patients with SAH, although not to the extent seen in meningitis [13, 21, 25, 36, 43, 46, 54]. Whether the inflammatory cells cause permanent cerebral damage is still unclear.

Damage to the blood vessels is the most studied of the inflammatory pathways. There is evidence of inflammation in blood vessels after SAH associated with vasospasm [55, 57]. Inflammatory cell infiltration of vascular intima has been described [7, 22, 42]. Tamargo and colleagues described inflammation in blood vessels in conceptual terms as the "leukocyte-endothelial cell interaction" [14]. This was supported by a number of studies that reported endothelial dysfunction in animal models of vasospasm [18, 23, 26].

Inflammation of the brain directly causing both the vascular syndrome of vasospasm and the clinical syndrome of delayed deterioration is possible based on the data but is a sobering finding because the process seems to occur extremely early after SAH, obliterating the window-of-treatment theory that is integral to our optimism for a cure (see the work of Sehba, Pluta, and Zhang for an informative review [50]). A number of studies suggested that early inflammation leads to poor outcome after SAH [55, 56, 58]. The innate brain inflammatory cell most likely to participate in early brain inflammation is the microglial cell. Results from our laboratory showed that microglial activation in the first day of SAH in a murine model histologically correlated well with the presence of later vasospasm and behavioral deficits [45]. It is still unclear if microglia direct the inflammation from the brain or respond to outside influences. In brain trauma, microglia seem to play an important and direct role in the inflammation [11, 12, 16].

What Are the Drivers of Inflammation?

It is well recognized that where there is cerebral ischemia, there is brain inflammation. The question at the root of DDAV is whether inflammation is the mechanism of cell injury in SAH or whether other factors directly or indirectly (through vasoconstriction and stroke) lead to poor outcomes in patients. There are a number of putative agents that have been proposed to cause vasospasm and (by assumption) DDAV because most studies have not looked at long-term outcome from DDAV. The studies of clazosentan and nicardipine warned that the assumption that vasospasm and outcome are tightly linked may be faulty [17, 28].

Early studies of vasospasm focused on hemoglobin and oxyhemoglobin as the causative agents [41]. Two correlates of this theory are that blood breakdown produces either (1) bilirubin oxidation products (BOXs) that lead to damage or (2) free hemoglobin, which is a sump for nitric oxide, which leads to spasm based on relative overexpression of endothelin-1 [3, 4, 40, 41, 44, 47, 49]. There are number of genetic allele variants that seem to be important in the risk for the development of vasospasm in humans. They include the apolipoprotein E4 (ApoE4) and the haptoglobin α2/α2 genotype and are thought to code for products that alter the risk of vasopasm [2, 15, 24, 32]. Finally, other blood cell components, such as platelets, have been postulated to lead to direct damage to blood vessels and the cause of vasospasm [19, 53].

It is possible that all of these mediators are actually early triggers for inflammation in the brain, the blood vessels, or the meninges. It has been postulated for a number of the mentioned entities that inflammation may be the mechanism of injury.

Conclusion

A great deal of work is left to resolve whether inflammation is a driver of DDAV or a consequence of the ischemia that results from vasospasm. The failure of vasodilators to improve patient outcome despite improving vascular constriction suggests that investigating other possible entities is warranted.

Inflammation seems the most probable nonvascular research avenue to pursue. In the next few years, I look forward to new studies and mechanistic evaluations of inflammation in SAH based on the three questions discussed here. When we know what cells, when, and in what compartment of the cranium inflammation acts, it is hoped we will be able to develop rational treatment strategies.

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Conflicts of Interest Statement I declare that I have no conflict of interest.

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Attenuation of Cerebral Vasospasm Following Experimental Subarachnoid Hemorrhage by the Bronchodilator KMUP-3

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Abstract Delayed cerebral vasospasm is a main cause of morbidity and mortality as well as poor outcome in patients following aneurysmal subarachnoid hemorrhage (SAH). In this study, the effect of the bronchodilator KMUP-3 (7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine) on basilar artery narrowing, neurological outcome, and expression of rhoA/rho kinase II (ROCKII), rhoA, and protein kinase C (PKC) γ proteins were evaluated in a rat model of SAH. SAH was induced by double injection of autologous blood into the cistern magna on days 0 and 3. KMUP-3 was administered (0.3 mg/kg/day) by osmotic minipumps implanted subcutaneously (beginning day −3 in pretreatment group and at 1 h after the initiation of the first autologous blood injection in the treatment group). Neurological outcome was assessed by ambulation and placing/stepping reflex responses at 48 h after the second injection of autologous blood. Tissue morphology and protein expression were conducted on day 7 post-day 0 injection. Both KMUP-3 treatment regimens significantly improved neurological outcome and completely attenuated basilar artery narrowing as well as reduced the enhancement of ROCKII, rhoA, and PKCγ protein expression in rats subjected to SAH, compared with normal and untreated SAH rats. These results suggest that KMUP-3 may be a novel agent for the treatment of cerebral vasospasm following SAH.

Keywords KMUP-3 • Bronchodilator • Cerebral vasospasm • Experimental subarachnoid hemorrhage

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Introduction

Delayed cerebral vasospasm has been recognized as the main cause of morbidity and mortality as well as poor outcome in patients following aneurysmal subarachnoid hemorrhage (SAH) [6, 14]. Despite advancement in various technologies and treatments used for the management of this devastating condition, current therapies are still considered inadequate [1, 13], and extensive preclinical studies have been conducted to search for new mechanisms/medications for more effective control of the problem. These efforts have led to the identification of key factors involved in the pathogenesis of delayed arterial narrowing, exemplified by excessive productions of oxyhemoglobin as well as the vasoconstrictor endothelin-1 (ET-1) and defective generation of the vasodilator nitric oxide (NO) [15]. Consequently, compounds such as ET-1 receptor antagonists, endothelin-converting enzyme inhibitors, and agents that potentiate the levels of NO have all been shown to have beneficial effects for limiting delayed cerebral vasospasm in preclinical animal models and humans [3, 8, 10, 18].

It has been found that oxyhemoglobin-induced contraction of cerebrovascular smooth muscle is modulated by

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ET-1 via the protein kinase C (PKC) and rhoA/rho kinase (ROCK) pathways [9]. RhoA belongs to a family of small G proteins that play an important role in intracellular signaling [2, 16]. On stimulation by vasoactive agents, rhoA interacts with its downstream effectors such as ROCK to elicit vasoconstrictive effects. The involvement of ROCK in cerebral vasospasm following SAH has been supported by several studies [4, 17]. KMUP-3 (7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine) is a bronchodilator, and it inhibits the tumor necrosis factor-α-induced tracheal contraction in the rat by increasing the endogenous NO release and activating the cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) pathway [11]. The purpose of the present study was to evaluate the effect of KMUP-3 on cerebral vasospasm induced in a rat model of double SAH. Hemodynamic parameters, morphology of the basilar artery, and neurological outcome after treatment with KMUP-3 were assessed. Furthermore, the effect of KMUP-3 on the expression of ROCKII, rhoA, and PKCγ proteins in the basilar artery of rats subjected to SAH were also examined.

Materials and Methods

Materials

Male Sprague–Dawley rats weighing 250–300 g were purchased from National Animal Center, Taiwan. Antimouse ROCKII, antimouse rhoA, antimouse PKCγ, and horseradish peroxidase-labeled goat antimouse immunoglobulin G (IgG) antibodies were products of Upstate Biotech (Lake Placid, NY, USA), Santa Cruz Biotech (Santa Cruz, CA, USA), BD Biosciences (San Jose, CA, USA), and Chemicon International (Temecula, CA, USA), respectively.

Animal Procedures

All animal procedures were approved by the Kaohsiung Medical University Hospital animal research committee. After arriving at the Kaohsiung Medical University Hospital vivarium, the rats were acclimated for at least 1 week before being used in the experiment. They were housed in a room on a 12-h light/dark cycle under controlled temperature and relative humidity of 22.1°C and 55%, respectively, and were provided with normal chow and water ad libitum. Thirty rats used in this study were divided into the following five groups ($n = 6$/group): group 1, normal; group 2, animals subjected to SAH without treatment; group 3, SAH rats treated with vehicle (5% glucose water); group 4, SAH animals pretreated with KMUP-3 (pretreatment group); and group 5, SAH rats treated with KMUP-3 (treatment group). SAH was induced by double injection of autologous blood into the cistern magna. Briefly, rats were anesthetized with a mixture of KetaVed (55 mg/kg) and xylazine (9 mg/kg) intraperitoneally (ip), and fresh blood (1 ml/kg) was drawn from the central tail artery and injected into the cistern magna on day 0. This procedure was repeated on day 3. KMUP-3 was administered at a dose of 0.3 mg/kg/day by osmotic minipumps implanted subcutaneously, starting on day −3 in the pretreatment group (group 4) and at 1 h after the initiation of the first autologous blood injection in the treatment group (group 5).

Behavioral Assessment

Behavioral changes in the SAH rats were assessed by an investigator blinded to the experiment at 48 h after the second injection of autologous blood. Assessment of ambulation (walking with lower extremities) and placing/stepping reflex (dragging the dorsum of hind paw over the edge of a surface) responses was used as an index of motor function according to a scoring system published previously and shown in Table 1A [5, 12]. The sum of scores from ambulation and placing/stepping reflex responses is referred to as the motor deficit index (MDI).

Hemodynamic Measurements

Heart rate and blood pressure were monitored by a tail-cuff method before the injection of the first autologous blood and again prior to sacrifice on day 7.

Tissue Morphometry

On day 7 postinjection of the first autologous blood, animals were anesthetized by chloral hydrate (0.3 mg/kg ip), and perfusion-fixation was conducted according to a published method [7]. Basilar arteries were then harvested from the brain stems. The middle third of each artery was dissected for morphometrical analysis by an investigator blinded to the experiment; the rest of tissue was frozen in liquid N_2 and stored at −70°C until used for measurements of levels of ROCKII, rhoA, and PKCγ protein expression. Five sections of the basilar artery were randomly selected for measurement of the cross-sectional area, and the average of these five numbers was used as a single value for each rat.

Table 1 Behavioral changes induced by experimental SAH in the rat

A. Scoring system used for motor function assessment [5, 12]

Motor function	Behavior	Score
Ambulation	Walking with lower extremities	0
	Normal (symmetric and coordinated ambulation)	
	Toes flat under the body while walking with ataxia	1
	Knuckle walking	2
	Movement in lower extremities but unable to knuckle walk	3
	No movement, dragging lower extremities	4
Placing/stepping reflex	Dragging the dorsum of hind paw over the edge of a surface	0
	Normal (coordinated lifting and placing response)	
	Weak	1
	No stepping	2

B. Effect of MKUP-3 on behavioral changes induced by experimental SAH in the rat

Group	Ambulation	Placing/stepping reflex	Motor deficit index (MDI)
Normal	0***	0***	0***
SAH	1.28 ± 0.18	1.42 ± 0.17	2.11 ± 0.10
SAH + vehicle	1.19±0.21	1.34 ± 0.32	2.42 ± 0.28
SAH + KMUP-3 (T)	1.13 ± 0.04	1.29 ± 0.14	2.31 ± 0.19
SAH + KMUP-3 (P)	0.7 ± 0.05*	0.88 ± 0.04*	1.51 ± 0.17*

Subarachnoid hemorrhage (SAH) was induced in rats by injecting autologous blood into the cisterna magna on day 0 and again on day 3. KMUP-3 (7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine) was administered at a dose of 0.3 mg/kg/day by osmotic minipumps implanted subcutaneously, starting on day −3 (pretreatment group, P) or at 1 h after the initiation of the first autologous blood injection (treatment group, T). The motor function was assessed using the scoring system shown in Table 1A and was performed on day 5 after the first SAH injection. MDI is the sum of scores from ambulation and placing/stepping reflex

* $p < 0.05$ versus the SAH group

*** $p < 0.001$ versus the SAH group

Protein Expression

Basilar arteries were homogenized in a buffer containing C, N, and M for extraction of cytoplasmic, nuclear, and membrane-bound proteins, respectively, according to the instructions of the manufacturer. Expression of ROCKII, rhoA, and PKCγ was determined by western blot analysis using specific antibodies according to the instructions of the respective manufacturers. The intensity of the protein expression was quantified by densitometry, and the results were normalized by β-actin expression.

Statistical Analysis

All results were expressed as mean plus or minus SEM ($n = 6$). An analysis of variance (ANOVA) was performed, followed by Mann-Whitney's test to determine statistical significance between two experimental groups. A p value of less than 0.05 was considered statistically significant.

Results and Discussion

Hemodynamic Measurements and General Observations

No statistically significant differences in blood pressure or heart rate were noted among the five groups before the initiation of SAH, and there were not any significant differences in these parameters on day 7 after the injection of the first autologous blood (results not shown). On removal of the brain, visual inspection confirmed the formation of subarachnoid clots that covered the basilar artery in all rats subjected to SAH.

Behavioral Assessment

As expected, normal animals had no neurological deficit and had a score of zero in both the ambulation and placing/stepping reflex responses. In contrast, the scores in both the SAH

and SAH plus vehicle groups were significantly higher (Table 1B). The values were 1.28 ± 0.18 (mean ± SEM, $n = 6$) and 1.32 ± 0.17 for the ambulation and placing/stepping responses, respectively, in the SAH group, while the respective values were 1.19 ± 0.21 and 1.34 ± 0.32 for the SAH plus vehicle group (all $p < 0.05$ compared with corresponding value in the normal group) (Table 1B). The values of MDI in the SAH and SAH plus vehicle groups were 2.51 ± 0.14 and 2.42 ± 0.28, respectively, compared with a score of 0 in the normal rats. Treatment with KMUP-3 at 0.3 mg/kg/day beginning at 1 h before the induction of SAH did not show significant improvement in MDI when compared with the SAH or SAH plus vehicle groups (Table 1B). However, pretreatment with KMUP-3 significantly improved the ambulation and placing/stepping reflex scores to 0.7 ± 0.05 and 0.88 ± 0.04, respectively ($p < 0.05$ for both compared with the SAH plus vehicle group). The MDI value for the pretreatment group (1.51 ± 0.17) also decreased significantly compared with the SAH plus vehicle group (Table 1B).

Tissue Morphometry

Compared with the internal elastic lamina in the basilar artery of normal animals, those of the SAH and SAH plus vehicle groups showed substantial corrugation (Fig. 1 micrographs). Corrugation was significantly less prominent in both the KMUP-3 treatment and pretreatment groups. The cross-sectional areas of basilar artery in the five groups of animals are shown in Fig. 1 (lower panel). The basilar artery in the normal animals had an average cross-sectional area of 0.24 ± 0.01 mm^2 (mean ± SEM, $n = 6$). In contrast, the cross-sectional areas from the SAH or SAH plus vehicle groups were decreased by approximately 60% compared with that of the normal group. Treatment or pretreatment with KMUP-3 attenuated the decrease in the basilar artery cross-sectional areas to levels not significantly different from that obtained in the normal animals (Fig. 1, lower panel).

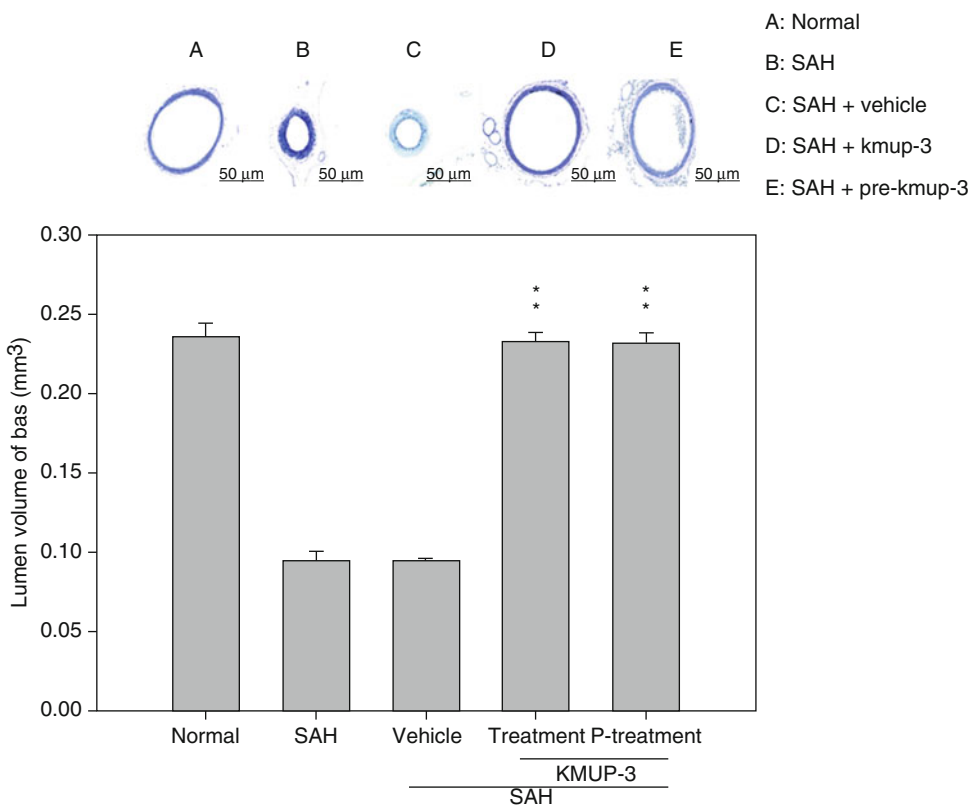

Fig. 1 Effect of 7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine (*KMUP-3*) on morphology and the cross-sectional area of basilar artery in rats subjected to subarachnoid hemorrhage (*SAH*). SAH was induced by double injection of autologous blood into the cistern magna on day 0 and again on day 3. KMUP-3 was administered at a dose of 0.3 mg/kg/day by osmotic minipumps implanted subcutaneously, starting on day −3 in the pretreatment group and at 1 h after the initiation of the first autologous blood injection in the treatment group. The SAH and SAH plus vehicle groups showed substantial corrugation of the internal elastic lamina compared with that of the normal rats (*upper panel*). Corrugation was significantly less prominent in both the KMUP-3 treatment and pretreatment groups. The cross-sectional areas from the SAH or SAH plus vehicle groups were substantially decreased, while treatment or pretreatment with KMUP-3 attenuated the decrease in the basilar artery cross-sectional areas to levels not significantly different from that obtained in the normal animals (*lower panel*). *$p < 0.05$ compared with the SAH+vehicle group

ROCKII, rhoA, and PKCγ Protein Expression in the Basilar Artery

Western blot analysis revealed that ROCKII protein expression in the basilar artery of the SAH and SAH plus vehicle groups was significantly higher when compared with that obtained from the normal rats (Fig. 2, upper panel). Treatment with KMUP-3 prevented the increase in ROCKII expression to levels not significantly different from that obtained in the normal animals (Fig. 2, lower panel). A similar effect was also achieved in the KMUP-3 pretreatment group.

The effect of KMUP-3 on rhoA protein expression in the

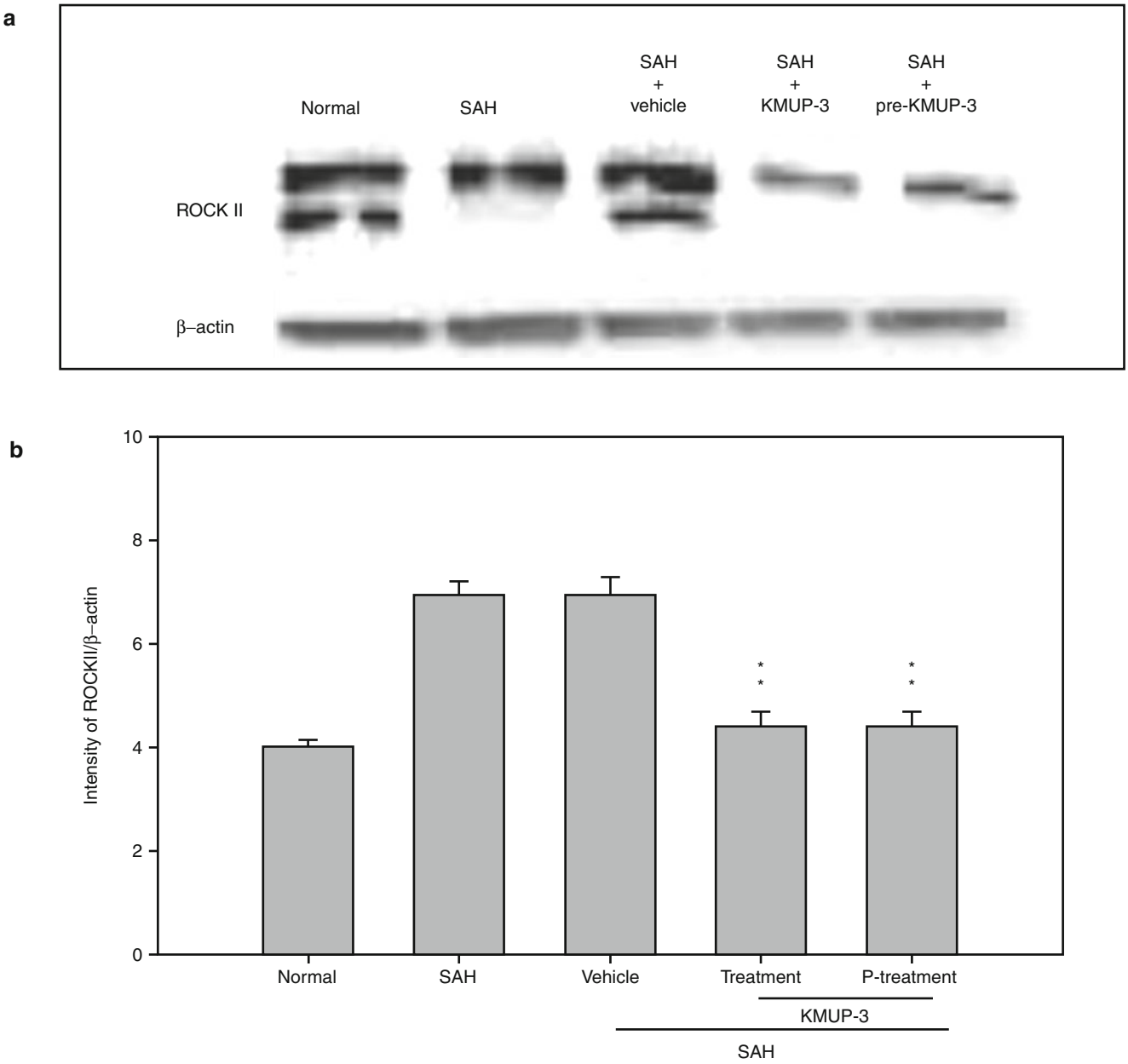

Fig. 2 Effect of 7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine (*KMUP-3*) on rhoA/rho kinase II (*ROCKII*) protein expression in the basilar artery of rats subjected to subarachnoid hemorrhage (*SAH*). The conditions for induction of SAH and administration of KMUP-3 are stated in the legend of Fig. 1. A representative Western blot analysis of ROCKII expression in the basilar artery of five groups of animals is shown in the (*upper panel*). The intensity of the protein expression was quantified by densitometry and normalized by β-actin expression (mean ± SEM, $n = 6$) (*lower panel*). ROCKII protein expression in the basilar artery of the SAH and SAH plus vehicle groups were significantly higher when compared with that obtained from the normal rats. Treatment or pretreatment with KMUP-3 prevented the increase in ROCKII expression to levels not significantly different from that obtained in the normal animals. $^{**}p < 0.01$ compared with the SAH+vehicle group

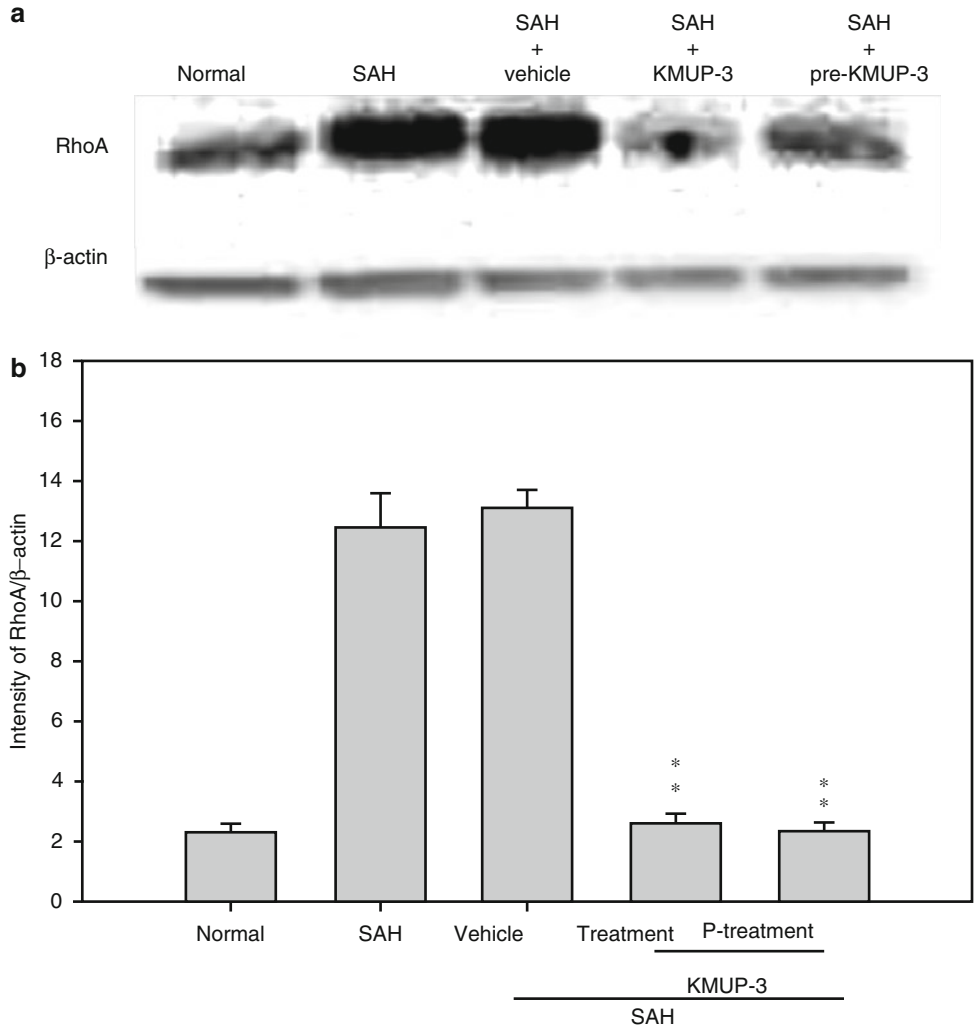

Fig. 3 Effect of 7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine (*KMUP-3*) on rhoA protein expression in the basilar artery of rats subjected to subarachnoid hemorrhage (*SAH*). The conditions for induction of SAH and administration of KMUP-3 are stated in the legend of Fig. 1. A representative western blot analysis of rhoA expression in the basilar artery of five groups of animals is shown in the (*upper panel*). The intensity of the protein expression was quantified by densitometry and normalized by β-actin expression (*lower panel*). RhoA protein expression in the basilar artery of the SAH and SAH plus vehicle groups were significantly higher when compared with that obtained from the normal rats. Treatment or pretreatment with KMUP-3 prevented the increase in rhoA expression to levels not significantly different from that obtained in the normal animals. ***$p < 0.001$ compared with the SAH+vehicle group

basilar artery resembled that seen with the ROCKII results, except that rhoA protein expression was drastically increased (greater than fivefold) in the basilar artery of rats subjected to SAH compared to the normal group (Fig. 3, lower panel). Again, both of the KMUP-3 treatment groups attenuated the increase in rhoA expression to levels not significantly differently from that obtained from the normal animals (Fig. 3, lower panel).

Likewise, western blot analysis showed that the levels of PKCγ protein expression in the basilar artery of the SAH rats were significantly increased when compared with normal animals (Fig. 4, upper panel). Interestingly, treatment with KMUP-3 reduced the expression of PKCγ to levels lower than that of the normal rats (Fig. 4, lower panel).

Conclusion

In summary, this study showed that treatment with KMUP-3 significantly improved neurological outcome and completely attenuated basilar artery narrowing as well as reduced the enhancement of ROCKII, rhoA, and PKCγ protein expression in rats subjected to SAH. These results suggest that KMUP-3 may be a novel agent for the treatment of cerebral vasospasm following SAH. The beneficial effect is at least partially due to inhibition of the PKC and rhoA/ROCKII pathways involved in this devastating condition. However, further clinical studies are needed to substantiate these findings.

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

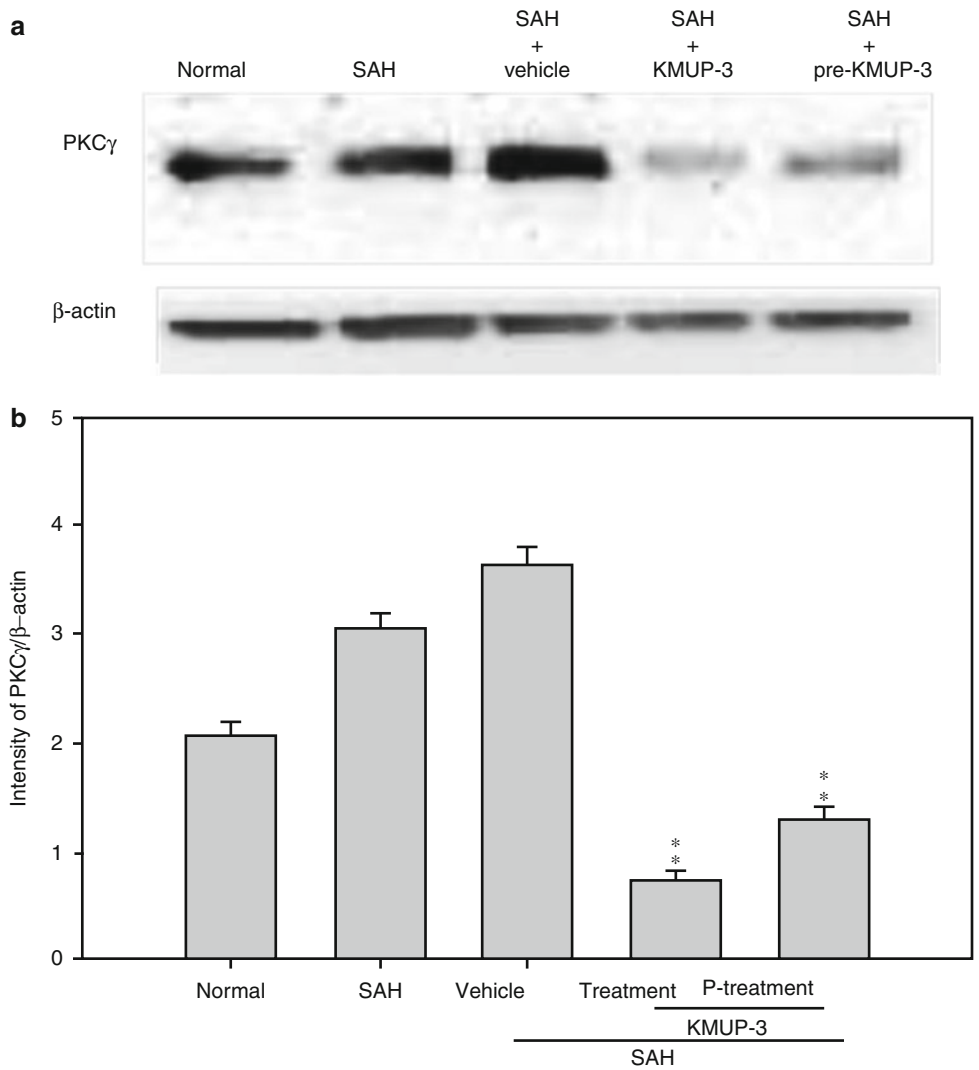

Fig. 4 Effect of 7-[2-[4-(4-nitrobenzene)piperazinyl]ethyl]-1,3-dimethylxanthine (*KMUP-3*) on protein kinase Cγ (*PKCγ*) protein expression in the basilar artery of rats subjected to subarachnoid hemorrhage (*SAH*). The conditions for induction of SAH and administration of KMUP-3 are stated in the legend of Fig. 1. A representative western blot analysis of PKCγ expression in the basilar artery of five groups of animals is shown in the (*upper panel*). The intensity of the protein expression was quantified by densitometry, and the results were normalized by β-actin expression (*lower panel*). RhoA protein expression in the basilar artery of the SAH and SAH plus vehicle groups were significantly higher when compared with that obtained from the normal rats. Treatment or pretreatment with KMUP-3 prevented the increase in rhoA expression to levels below that obtained in the normal animals. ***$p < 0.001$ compared with the SAH+vehicle group

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Pharmacologic Reduction of Angiographic Vasospasm in Experimental Subarachnoid Hemorrhage: Systematic Review

Tommaso Zoerle, Don Ilodigwe, Hoyee Wan, Katarina Lakovic, Mohammed Sabri, Jinglu Ai, and R. Loch Macdonald

Abstract Animal models have been developed to simulate angiographic vasospasm secondary to subarachnoid hemorrhage (SAH) and to test pharmacologic treatments. Our aim was to evaluate the effect of pharmacologic treatments that have been tested in humans and in preclinical studies to determine if animal models inform results reported in humans. A systematic review and meta-analysis of SAH studies was performed. We investigated predictors of translation from animals to humans with multivariate logistic regression. Pharmacologic reduction of vasospasm was effective in mice, rats, rabbits, dogs, nonhuman primates, and humans. Animal studies were generally of poor methodologic quality, and there was evidence of publication bias. Fresh blood injection to simulate SAH (vs. clot placement) and evaluation of vasospasm more than 3 days after SAH were independently associated with successful translation. We conclude that reduction of vasospasm is effective in animals and humans, and that injection of fresh blood and evaluation of vasospasm more than 3 days after SAH may be preferable for preclinical models.

Keywords Subarachnoid hemorrhage • Cerebral vasospasm Animal models • Vasospasm therapy

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Introduction

Many drugs have been tested in animal models for reducing angiographic vasospasm, but few have proven effective in high-quality clinical trials. There are many possible reasons for lack of translation of reducing angiographic vasospasm in animal models to improving outcome in humans, including clinical trial design, whether the animal model reproduces the disease and methodological flaws in experimental studies [1]. Preclinical subarachnoid hemorrhage (SAH) studies for pharmaceutical reduction of vasospasm differ in terms of animal used, induction of SAH, time course and severity of angiographic vasospasm [2], as well as quality of design (i.e., randomization, blinding). All these characteristics could have an impact on the results of experimental treatment and perhaps the chance of translation, but to our knowledge there is no evidence that one model of SAH is better than any other for the purposes of informing human clinical trials.

A meta-analysis of human clinical trials of drugs to decrease vasospasm showed that some, but obviously not all, drugs tested did reduce vasospasm in randomized clinical trials [3]. The goal of this work was therefore to determine the effect of pharmacologic treatments tested in SAH clinical trials on angiographic vasospasm in preclinical studies. The questions were whether there is evidence that any animal model can inform clinical trials, and whether any specific model or study characteristics more accurately reflect results reported in humans.

Materials and Methods

Search Methods for Identification of Studies

PubMed and Embase were searched using the following terms: (subarachnoid hemorrhage) AND [(statin) OR (tissue plasminogen activator) OR (ticlopidine) OR (erythropoietin) OR (clazosentan) OR (nimodipine) OR (nicardipine) OR

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(fasudil) OR (tirilazad)] AND (animal OR experimental). The same search was performed for magnesium, endothelin-converting enzyme and endothelin receptor antagonist.

Criteria for Included Animal Studies

We included preclinical studies in vivo in which SAH was simulated by endovascular puncture of a cerebral artery, subarachnoid clot placement, or injection of blood into the subarachnoid space. We included studies of treatment of cerebral vasospasm with any drug tested in both experimental and clinical trials for the same purpose. Intervention was defined as treatment for the purposes of this study if drug administration was started before the development of angiographic vasospasm. For selecting drugs we considered a systematic review of drug treatments for humans with SAH and included all drugs tested in randomized, blinded clinical trials [3]. The primary outcome was cerebral vasospasm evaluated by catheter, computed tomographic (CT), magnetic resonance angiographic, or histological methods.

Data Collection

For every included study, we extracted data on the species and sex of animals, method of induction of SAH, drug tested, timing and method of delivery of the drug, time and method for outcome assessment, randomization, blinding, and physiological variables that were measured. Data on the number of animals and degree of vasospasm in treatment and control groups were extracted. If in the same study different doses or delivery manners were used, data on the effect of the intervention were extracted for every group, and the most effective regimen was used for the analysis. If in the same study different drugs were tested, data on the effect of each drug were extracted and analyzed separately. Vasospasm was expressed as the percentage reduction in cerebral artery diameter from baseline or control values. Methodologic quality of the animal studies was assessed by two reviewers by extracting data on study design, allocation concealment, blinding, number of animals randomized, age of animals, recording of physiologic variables, and inclusion of controls. To assess the effect of each drug in human trials, the data were obtained from Etminan et al. [3].

Statistical Analysis

To test which species may reflect more accurately the effect of drug treatment on reducing vasospasm in humans, we analyzed the concordance between the drug effect in human and in the different animals. The drug effect in humans was defined as reported [3]. The effects were defined as concordant if the drug showed evidence of positive effect in humans as well as in animals or no evidence of effect in humans as well as in animals. Drug effect was defined as discordant if the drug showed evidence of efficacy in animals but not in humans or vice versa.

A random effect weighted meta-analysis was used to assess the effect of pharmacologic reduction of vasospasm in animal studies. Effect sizes were expressed as pooled standard mean difference. Subgroup analyses by drug and animal species were performed. Publication bias was investigated using Begg's and Egger's tests.

For human studies, effect sizes were expressed in pooled risk ratio estimates. To assess predictors of translation from animal to human, univariable and multivariable logistic regressions were used with the dependent variable being concordance/discordance of the drug. Variables entered in the regressions were species and sex of animals; method of induction of SAH; drug tested; route of drug delivery; treatment start time (before or after SAH); time and method of outcome assessment; randomization; blinding (for outcome assessment and whether animals belonged to treatment or control groups before surgery); monitoring of physiological variables (temperature, blood pressure, heart rate, oxygenation, intracranial pressure); and monitoring of CO_2 (Table 1). We considered $p<0.05$ significant.

Results

A total of 453 studies were identified. Three hundred nineteen studies were excluded after review of the title and abstract showed the study was a review article, clinical study with no single treatment, in vitro study, or test of a treatment to reverse established angiographic vasospasm. An additional 64 studies were excluded after reading the full text because they did not completely fulfill the inclusion criteria, or they reported preliminary or incomplete data. Data from 70 papers were then extracted. There were 556 animals assigned to treatment groups and 664 to control groups. Characteristics of the studies are reported in Table 1.

The pooled effect size expressed as standard mean difference showed that pharmacologic treatments significantly reduced cerebral vasospasm in the experimental studies (standard mean difference of −1.74; 95% confidence interval [CI] −2.04 to −1.44). Substantial and significant heterogeneity was detected (heterogeneity χ^2 statistic = 340.60 [degrees of freedom = 73], $p = 0.0001$) (Fig. 1). Subgroup analysis by drug showed that every drug except magnesium was

Table 1 Characteristics of animal studies

Characteristic	Number (%)
Species	
Monkey	16 (22)
Dog	23 (31)
Rabbit	24 (32)
Rat	9 (12)
Mouse	2 (2)
Sex	
Male	27 (36)
Female	11 (14)
Male/female	36 (49)
Method of induction of SAH	
Clot placement	17 (23)
Two injections cisterna magna	29 (39)
One injection cisterna magna	21 (28)
Endovascular perforation	4 (5)
Other[a]	3 (4)
Severity of vasospasm in control group	
Mild	16 (22)
Moderate	55 (74)
Severe	3 (4)
Evaluation of vasospasm	
Angiography	52 (70)
Histology	22 (30)
Day of evaluation of vasospasm	
≤3	21 (28)
>3	53 (72)
Drug	
Tirilazad	8 (11)
Calcium antagonist	14 (19)
Erythropoietin	4 (5)
Statins	7 (9)
Endothelin antagonist/endothelin-converting enzyme inhibitor	26 (35)
Fasudil	4 (5)
Tissue plasminogen activator	9 (12)
Magnesium	1 (1.5)
Other[b]	1 (1.5)
Route of drug delivery	
Intracranial	30 (40)
Systemic	44 (60)
Pretreatment	
Before induction of SAH	7 (9)
After induction of SAH	67 (91)
Blinding of outcome	
No	29 (40)
Yes	45 (60)
Blinding to induction of SAH versus saline/sham surgery	
No	59 (80)
Yes	15 (20)
Inclusion and exclusion criteria	
No	65 (88)
Yes	9 (12)
Randomization	
No	36 (49)
Yes	38 (51)
Monitoring of physiological variables	
No	15 (20)
Yes	59 (80)
Monitoring CO_2	
No	16 (22)
Yes	58 (78)

SAH subarachnoid hemorrhage
[a]Modified cisterna magna injection and prechiasmatic cistern injection
[b]Tissue plasminogen activator plus endothelin antagonist

effective for reducing vasospasm (Fig. 2). Heterogeneity was detected for every subgroup except for fasudil. Subgroup analysis by species showed that pharmacologic treatment was effective in all species. Again, heterogeneity was detected. Both the Begg's (z score continuity-corrected 6.68, $p=0.000$) and Egger's test (bias coefficient −4.83; 95% CI −5.96 to −3.69; $p=0.000$) returned strong evidence of publication bias.

We next considered data in a systematic review of the effect of pharmaceutical reduction of radiographic vasospasm with different drugs in human clinical trials [3]. Drug effects were classified as concordant or discordant as defined in the methods. Fasudil, endothelin antagonists (clazosentan), and tissue plasminogen activator showed a beneficial effect on angiographic vasospasm in humans, while calcium antagonists (nimodipine and nicardipine), erythropoietin, statins, and tirilizad showed no significant effect (data not shown). Since no data were available on the effects of magnesium and tissue plasminogen activator plus endothelin antagonists, experiments using these treatments were excluded from further analysis.

Considering animal studies individually, 55% were concordant with human results. In multivariate logistic regression, significant predictors of translation were the type of SAH model (classified into fresh blood vs. blood clot placement, unadjusted odds ratio [95% CI] 0.20 [0.06–0.70], $p=0.013$) and the day of assessment of vasospasm (≤ vs. >3 days, unadjusted odds ratio [95% CI] 11.2 (3.13–40.1),

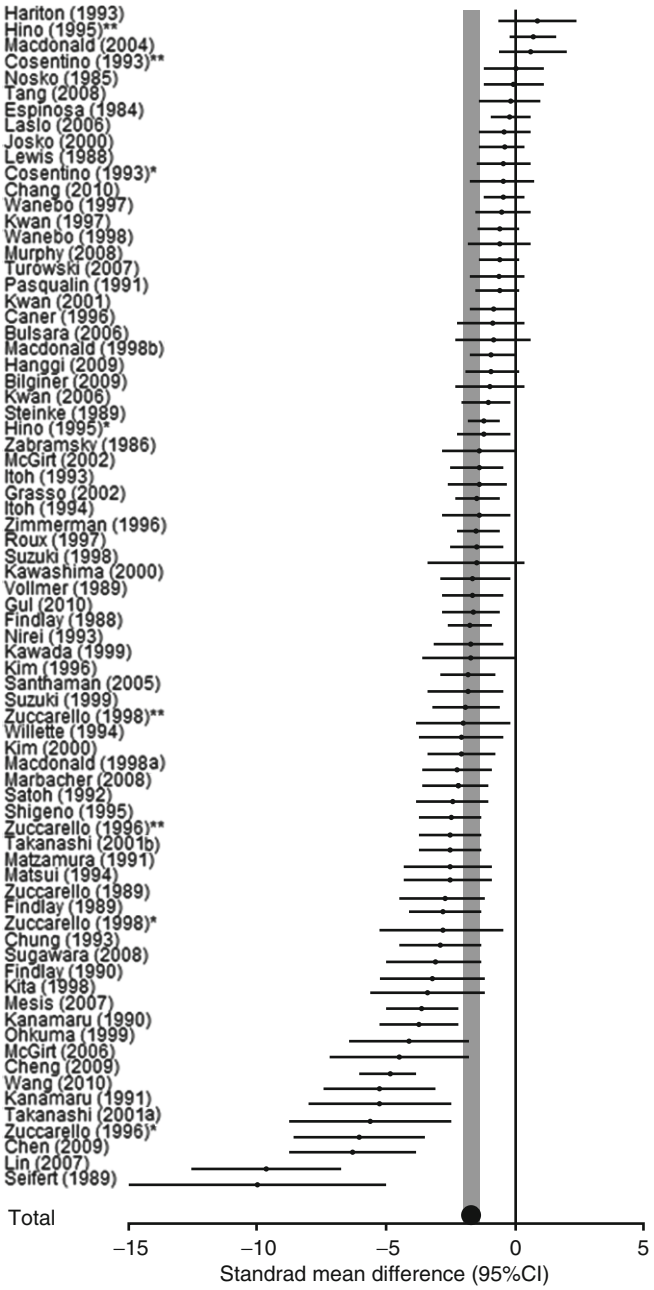

Fig. 1 Effect size of 74 animal studies of pharmacologic reduction of vasospasm included in meta-analysis. *Gray* band represents overall effect with 95% confidence intervals (* and ** are effect of different drugs in the same publication)

$p = 0.0002$). Studies in which fresh blood is used to simulate SAH and studies in which vasospasm was evaluated after day 3 from SAH had results more concordant with human trials than others.

Discussion

This systematic review found that pharmacologic treatments that are effective in animal models of SAH and vasospasm are also usually effective in humans. However, publication bias was detected, suggesting that some studies remain unpublished, and that our data probably overestimate the true effect of the drugs. On the other hand, methodologic quality of many of the studies was poor and would limit the interpretation of experimental studies showing treatment benefits, as well as of those showing no benefit.

In multivariate logistic regression, only fresh blood (injection and endovascular puncture), as compared to clot placement, and the day of evaluation of vasospasm (more than 3 days after SAH) were correlated with successful translation of results to humans. These observations have practical implications for study design and model selection, and it may be that in further preclinical experiments long-term drug effect evaluation and fresh blood models should be preferred.

There are a number of limitations to this analysis. Most instances of concordance were positive in that drugs worked in animals and humans. However, additional important confirmatory data would be studies showing that drugs that were not effective in humans also were not in animals. Only 15% of the studies used rats or mice, and almost all recent studies use them. In human trials, angiographic vasospasm frequently was considered as a dichotomous variable (none/mild or moderate/severe), while in animal studies it is expressed as a continuous variable. For this reason, it was not possible to compare exactly the effects in animals and humans. Most models of SAH differ from human SAH in that animals do not develop cerebral infarctions or other delayed processes that are proposed to be important in humans, such as cortical spreading ischemia and microthromboembolism. The endpoints in human clinical trials are usually clinical outcome and delayed cerebral ischemia, but neurological and functional examination after experimental SAH is still uncommonly used.

Conclusion

We conclude that reduction of vasospasm is effective in animals and humans, and that injection of fresh blood and evaluation of vasospasm greater than 3 days after SAH may be preferable for preclinical models.

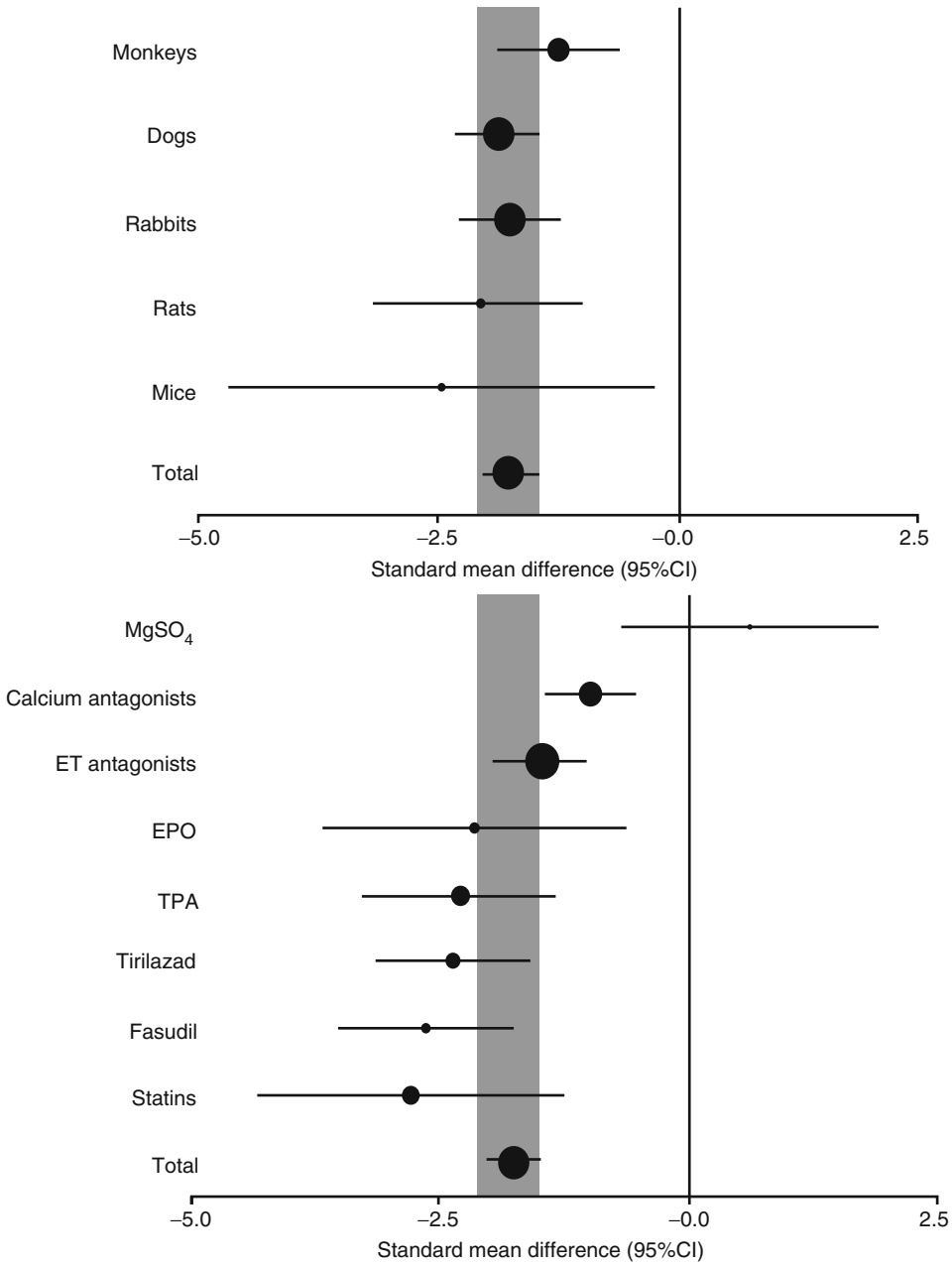

Fig. 2 Effect size of the 74 included studies grouped by animal and drug. *Gray* band represents overall effect with 95% confidence interval. The size of the circle reflects the number of animals included in the different subgroups. *ET antagonist* endothelin antagonist and endothelin-converting enzyme inhibitor, $MgSO_4$ magnesium, *EPO* erythropoietin, *TPA* tissue plasminogen activator

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

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Low-Dose Lipopolysaccharide Injection Prior to Subarachnoid Hemorrhage Modulates Delayed Deterioration Associated with Vasospasm in Subarachnoid Hemorrhage

Saksith Smithason, Shari K. Moore, and J. Javier Provencio

Abstract There is increasing evidence that inflammation plays a role in the development of Delayed Deterioration associated with vasospasm (DDAV) after subarachnoid hemorrhage (SAH). Lipopolysaccharide (LPS) is an activator of the innate inflammatory system that causes DDAV in animal models. The effect of low-dose LPS has been shown to be protective in stroke models but has not been investigated in SAH. Two treatments were studied: (1) a single intraperitoneal dose of 0.6 mg/kg injected 24 h prior to SAH and (2) four daily doses administered prior to SAH. DDAV was determined by India ink angiography at day 6; behavioral testing was done in a different cohort of animals, and analysis of brain chemokine levels was accomplished by dot blot. Vessel caliber was improved compared to the SAH group in the single-injection group (ldLPS ×1) ($p<0.05$). In the multiple-injection group (ldLPS ×4), the vessel caliber was similar to SAH ($p<0.05$). ldLPS ×1 improved performance on the Barnes maze test, whereas the ldLPS ×4 was worse ($p<0.001$). Brain levels of the inflammatory chemokine KC (keratinocyte-derived chemokine) were decreased in the ldLPS ×1 and increased in the ldLPS ×4 group. Single-injection low-dose LPS preconditioning was protective for delayed deterioration associated with vasospasm (DDAV), whereas the multiple-injection course exacerbated DDAV. This further supports that inflammation plays an important role in the development of DDAV, and that modulating the inflammatory system may be a potential target for future therapies in SAH.

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Keywords Inflammation • Lipopolysaccharide • Tolerance
Delayed cerebral vasospasm • Subarachnoid hemorrhage

Introduction

Delayed deterioration associated with vasospasm (DDAV) in aneurysmal subarachnoid hemorrhage (SAH) is the major cause of morbidity and mortality in patients who survive the initial bleeding episode [1]. This vexing disease presents with spasm of proximal arteries and delayed neurological deficits 4–12 days after the aneurysm rupture. Although the terms delayed cerebral ischemia (DCI) and delayed neurological ischemic deficits (DINDs) have been used to describe the proposed cause of the injury, recent evidence suggests that mechanisms other than ischemia may be at play [2–4]. A more appropriate term to describe the phenomenon may be DDAV to denote the uncertainty in the cause of the brain injury.

Our laboratory has focused on the role of early innate inflammation in both vascular and cerebral manifestations of DDAV. Lipopolysaccharide (LPS) A from E. *coli* is a known signaling molecule of the innate immune system mediated through the TLR4 receptor on the neutrophil and endothelial cell surface. We have previously shown that the neutrophil percentage in the cerebrospinal fluid (CSF) early in the course of SAH can predict who will later develop DDAV [5]. We have also shown that myeloid cell depletion in a mouse model of DDAV ameliorates both the vascular and the behavioral effects [6]. Previous animal work has shown that early administration of modulators of innate inflammation can alter the course of the disease [7–10]. Direct administration of LPS into the CSF without SAH causes vasospasm [9], and systemic administration of LPS worsens DDAV after SAH in a myeloid cell-dependent manner [11].

Data from stroke animal models showed that low-dose LPS injection prevents inflammation and is neuroprotective [12–14]. The mechanism of this protection has been

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proposed to be due to overexpression of genes coding for proteins in the TLR signaling pathway [15]. In this study, we found that systemic administration of low-dose LPS 1 day prior to SAH protected against DDAV, but four daily sequential doses did not.

Materials and Methods

All experiments were conduct under the supervision of the Cleveland Clinic Institutional Animal Care and Use Committee (IACUC). Animals were randomized into six groups for the vasospasm study and four groups for the behavioral, immunohistochemistry, and chemokine studies: (1) sham surgery (sham), (2) SAH (SAH), (3) low-dose LPS administration 1 day prior to SAH (ldLPS ×1), and (4) low-dose LPS administration daily for 4 days prior to SAH (ldLPS ×4) for all the studies and the two control groups low-dose LPS administration 1 day prior to sham surgery (sham ldLPS ×1) and low-dose LPS administration 4 days prior to sham surgery (sham ldLPS ×4) in the vessel diameter study. All surgeries were done by one investigator (S.S.), who randomly assigned animals to each of the three treatment groups. Analysis of the perfusion experiments and all behavioral tests were done by a different investigator (S.K.M.) blinded to the surgical assignments.

Experimental SAH

We studied male C57BL6 mice (Jackson Labs, Maine) weighing 20–32 g, 10–12 weeks of age. Our murine model of SAH has been described [16]. Briefly, mice were anesthetized and placed in a prone position. An incision was made in the midline of the neck, the atlanto-occipital membrane was punctured, and a subarachnoid vein was transected. The bleeding was allowed to stop spontaneously, after which the incision was closed. Saline injection sham surgery involved the same procedure except that the atlanto-occipital membrane was entered with a 30-gauge needle, and 50 µl of saline were instilled. All animals that had surgery survived all the posthemorrhage testing.

LPS Administration

We injected 0.6 mg/kg of LPS in 150 µl diluent (Sigma Aldrich, Saint Louis, MO) into the peritoneal cavity of experimental animals either 24 h prior SAH (ldLPS ×1) or daily for 4 days prior to SAH (ldLPS ×4). This dosage was increased threefold from previous studies of low-dose LPS-induced tolerance in stroke [12, 15] due to the 1-day protocol in our study (unlike 2 days prior to stroke in published protocols).

India Ink Assessment of Vessel Caliber

Animals were anesthetized with pentobarbital (6 mg/100 g ip); transcardiac perfusion was performed with 20 ml 4% paraformaldehyde followed by 10 ml of warmed 5% India ink in gelatin. Animals were decapitated, and their brains were removed carefully, preserving the vasculature. The circle of Willis vasculature was examined under the surgical microscope and relevant pictures captured (Leica, Wetzler, Germany) and analyzed with Adobe Photoshop CS2 (San Jose, CA). The diameter of the middle cerebral artery (MCA) segment was measured 1 mm from the posterior wall of the carotid artery by a member of the research team not involved in the surgery and blinded to the intervention. All measurements were made on the sixth day after the hemorrhage based on previous work that showed that delayed vasospasm occurs around the sixth posthemorrhage day in our model [6]. Ten mice were used for each of the four conditions (sham, SAH, ldLPS ×1, and ldLPS ×4).

Behavioral Testing

Animals in the behavioral study underwent three batteries of tests in groups of 10 mice. All tests were done at the same time of the day by a single handler (S.K.M.) who was blinded to the treatment allocation. Groups of 5–10 animals randomized for treatment and blinded to the handler were tested together. This was repeated until 10 animals from each group were tested. On day 2 after the hemorrhage, the mice underwent rotarod testing to evaluate motor function and coordination. The third day posthemorrhage, the mice underwent Y-maze testing to evaluate spatial working (immediate) memory. On days 2 and 3, the animals also had training for the Barnes maze. On days 4 through 9, the mice were tested in a Barnes maze to test spatial learning and short-term and long-term memory.

In the rotarod test, time on the rod and maximum speed of rotation (rpm) were measured. Animals were placed in one of four separated chambers with the spinning rod and a landing base. After a habituation for 30 s at low speed, the mice were tested with a continuously increasing rate of speed for 5 min or until the subject fell off the rod.

In the Y maze, animals were placed in a random orientation in the center of a high-walled maze that had three

equal-length passages in a Y configuration. The mice were recorded by video monitoring during 5 min of exploration and scored later. Percentage alterations were calculated as the ratio of the number of times the animals chose to enter the three arms of the maze in succession to the total number of unique arms the animal chose to enter.

In the Barnes maze, the mice were placed on a round table with multiple open holes in the periphery, one of which contains an undermounted box that represents a safe haven for the mice. There are visual cues on the wall around the table for the animal to orient itself. Each animal had 2 days of training during which it was gently guided to the goal box. On subsequent days, animals were placed in the center of the table in a covered enclosure until ready for the trial. The cover was lifted, and mice were allowed to find the goal box. The movements were recorded by video monitoring and scored later. The time it took to find the goal box (latency) was recorded.

Immunohistochemistry

The four groups of animals were tested using monoclonal antimicroglial cell antibody (Iba1) (generous gift from Dr. Bruce Trapp) and fluorochrome-conjugated monoclonal antibody against ICAM-1 (intercellular adhesion molecule 1; BioLegend, San Diego, CA) (which tests for endothelial activation). We incubated 30-μm brain slices in primary antibody for 24 h using a free-floating technique. Briefly, slices were microwaved for 2 min in citrate buffer followed by incubation in 500 μL of 3% goat serum and primary antibody at 20°C overnight in a 24-well plate. Iba1 antibodies were incubated with a horseradish peroxidase (HRP)-conjugated, isotype-specific secondary antibody and resolved with 3,3′-diaminobenzidine (DAB).

Chemokine Determination

Brain inflammatory chemokine levels were determined by dot-blot analysis (R&D Systems, Minneapolis, MN) 24 h after surgery. Animals were anesthetized with pentobarbital (6 mg/100 g ip); transcardiac perfusion was performed with 20 ml 1% phosphate-buffered saline (PBS). Animals were decapitated, and their brains were removed carefully and homogenized. After tissue protein extraction, the supernatant was incubated on the dot-blot membrane. Three brain samples were pooled to increase concentrations of chemokines. Dots were visualized by radiography. Dot intensity was measured and normalized to a positive control and analyzed with Image J (NIH, Bethesda, MD).

Statistics

Statistical analysis was performed with the aid of Graphpad Prism 5.0 (Graphpad Software, Inc., San Diego, CA). Multiple comparisons were assessed using one-way analysis of variance (ANOVA). Comparisons between individual groups in experiments in which one-way ANOVA was significantly different were assessed using Tukey's multiple comparison test. Two-way ANOVA was used to evaluate the effects of SAH over time. A p value of 0.05 was considered significant in all comparisons.

Results

Vasospasm and Behavioral Assessment After SAH

Comparison of all four groups in the study for the vascular constriction showed that the groups were significantly different [ANOVA, $F(5, 30) = 10.56$, $p < 0.0001$]. SAH animals had significant vasospasm at day 6 compared to sham control animals (Tukey's multiple comparison test, $p < 0.05$) (Fig. 1a). On Barnes maze behavioral testing, the four groups were significantly different with respect to time to find the goal box [two-way ANOVA, $F(3,15) = 47.76$, $p < 0.0001$]. SAH animals took a significantly longer time to find the goal box than the sham control animals [two-way ANOVA, $F(1,5) = 60.05$, $p < 0.0001$] (Fig. 1b). The differences between the SAH and sham animals in both vasospasm and behavioral assessment confirmed an earlier finding from our laboratory [6]. There were no significant differences between the groups with regard to rotorod testing or Y-maze testing.

Single-Dose Preconditioning

Single-dose preconditioning with LPS ameliorated both the vasospasm and the behavioral effects of SAH, while multiple-dose preconditioning did not. Animals in the ldLPS ×1 group did not show significant vasospasm (Tukey's multiple comparison test, ldLPS ×1 to SAH, $p < 0.05$) or behavioral changes on Barnes maze [two-way ANOVA, ldLPS ×1 to SAH, $F(1,5) = 28.36$, $p < 0.0001$] when compared to sham animals (Fig. 1a, b). Conversely, animals in the ldLPS ×4 group had similar vasospasm (Tukey's multiple comparison test, ldLPS ×4 to SAH, not significant) to animals with SAH. Interestingly, the behavioral changes on Barnes maze were more severe than for the SAH animals [two-way ANOVA, ldLPS ×4 to SAH, $F(1,5) = 7.898$, $p = 0.0064$]. These results show that a single dose of LPS prior to SAH is protective of

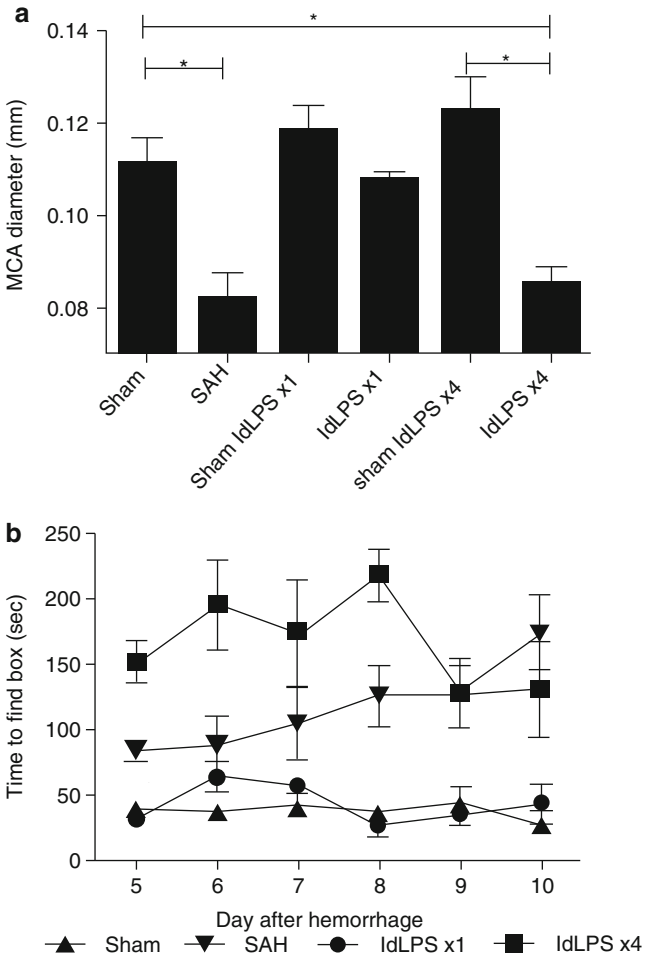

Fig. 1 Single-dose versus multiple-dose low-dose lipopolysaccharide (*LPS*) schedule in subarachnoid hemorrhage (*SAH*). (**a**) Middle cerebral artery (*MCA*) diameter 6 days after SAH shows that SAH and low-dose LPS given 4 days prior to SAH (ldLPS ×4) have significantly smaller diameters, indicating vasospasm [analysis of variance (*ANOVA*), $F(5,30)=10.56$, $p<0.0001$). The low-dose LPS administered once did not exhibit vasospasm compared to sham ldLPS ×1 (Tukey's multiple comparisons test, $p=$NS). *$p<0.05$ on Tukey's multiple comparisons test for comparisons shown by bars. (**b**) On Barnes maze test, animals in the ldLPS ×4 group and SAH had worse times to find the goal box than those in the sham or ldLPS ×1 group [two-way ANOVA, $F(3,15)=47.76$, $p<0.0001$]. Together, these data show that a single dose of LPS prior to SAH prevented both the vasospasm and behavioral effects, whereas the four-dose regimen prior to SAH was not protective

both vasospasm and the behavioral consequences, whereas multiple doses appeared to not be protective and made the behavioral manifestations worse.

Immunohistochemical Changes

Immunohistochemical changes suggest that ldLPS ×1 decreases brain and vascular inflammation, whereas ldLPS ×4 exacerbated inflammation. Immunohistochemical analysis of brain slices showed that animals in the ldLPS ×1 has decreased staining for ICAM-1 on endothelial cells of the blood vessels, suggesting that despite SAH the endothelial cells were not activated, and ramified morphology of the microglia, suggesting decreased microglial activation. By contrast, animals in the ldLPS ×2 group showed the opposite, with increased staining of ICAM-1 and ameboid morphology of the microglia suggesting inflammation (Fig. 2). For animals in the sham and SAH groups, the ICAM-1 staining and morphology of microglia predictably showed signs of inflammation in the SAH group and no inflammation in the sham group.

Expression of Keratinocyte-Derived Chemokine

The keratinocyte-derived chemokine (KC) was suppressed in ldLPS ×1 and increased in ldLPS ×4 animals after SAH. Mice do not express homologs to the human CXCL1 (Groα) and CXCL8 (interleukin 8 [IL-8]) chemokines. Instead, they express the analogous KC chemokine, which stimulates the CXCR2 receptor on neutrophils. We tested whole-brain levels of KC in mice in the ldLPS ×1 and ldLPS ×4 groups (Fig. 3). To develop adequate signal, three brains were pooled for each experiment. Each experiment represents pooled samples of three different animals. We found that compared to sham and SAH animals, animals in the ldLPS ×1 group had lower whole-brain KC levels and the ldLPS ×4 group had higher KC levels at both day 1 and day 6 after SAH. This supports the findings in the vasospasm, behavioral assessment, and immunohistochemical analysis that a single dose of LPS decreased inflammation, whereas the multiple-dose course increased inflammation. This further suggests that part of the mechanism of inflammation suppression is due to decreased signaling for neutrophils.

Discussion

Our study shows that a single dose of low-dose LPS given prior to SAH prevents both the vascular and behavioral sequelae of DDAV. Interestingly, the same dose of LPS given four consecutive days prior to SAH had no effect on vasospasm and opposite effects on behavioral ability on Barnes maze tests. Immunohistochemical staining and chemokine analysis suggested that single-dose LPS silenced the inflammatory response to the blood, whereas multiple doses increased the inflammatory signaling in the brain.

The mechanism by which low-dose LPS pretreatment induces "tolerance" in stroke has been investigated. In the case of LPS-induced tolerance, the mechanism revolves around induction of IRF3 activation in the brain [15, 17]. It is still not clear if systemic administration of LPS directly

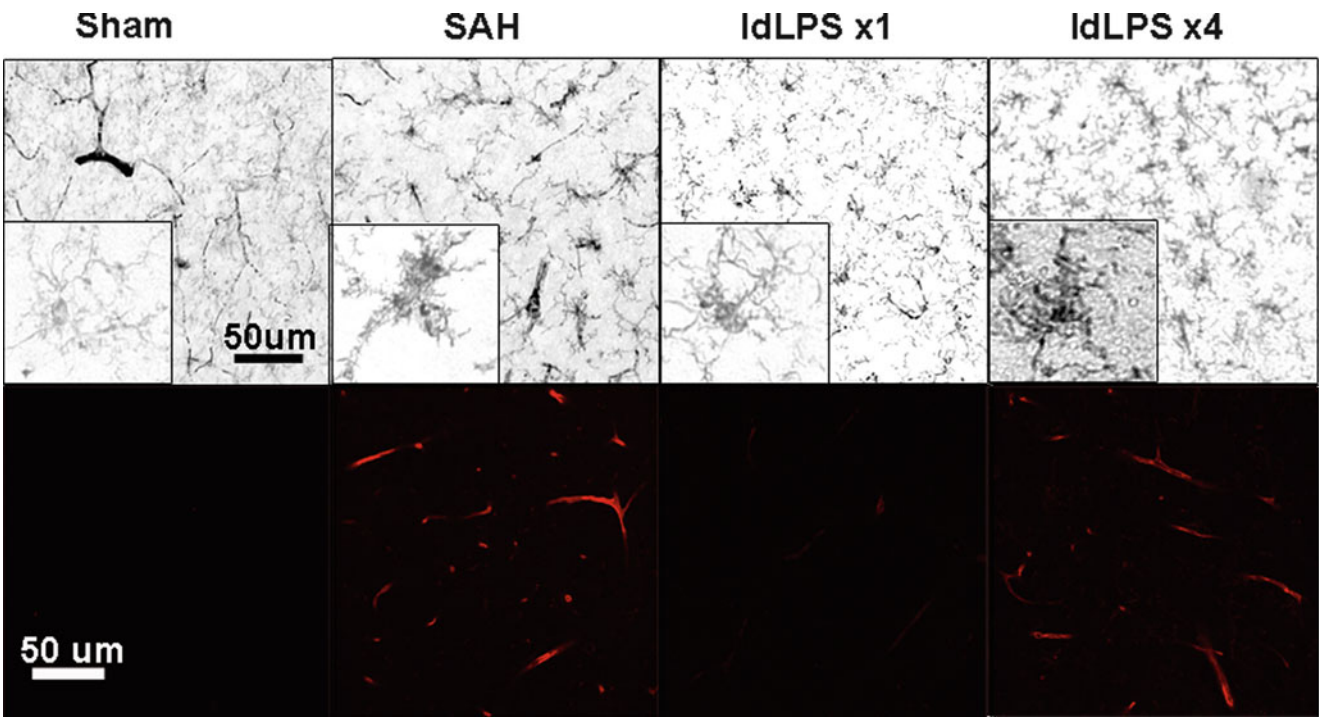

Fig. 2 Immunohistochemical analysis of brain slices. Sham animals show ramified morphology of microglia (by Iba1 staining resolved with 3,3 -diaminobenzidine [*DAB*]) (*top*) and no vascular staining for intercellular adhesion molecule 1 (*ICAM-1*) (*bottom*, confocal microscopy), suggesting no inflammation. Subarachnoid hemorrhage (*SAH*) animals show ameboid morphology of microglia and increased vascular staining for ICAM-1. Animals in the single-dose lipopolysaccharide (*LPS*) course (ldLPS ×1) show a pattern similar to sham animals. Animals in the multiple-dose course (ldLPS ×4) show a pattern similar to SAH animals. Nonmicroglial staining on Iba1-stained sections represents nonspecific blood vessel staining

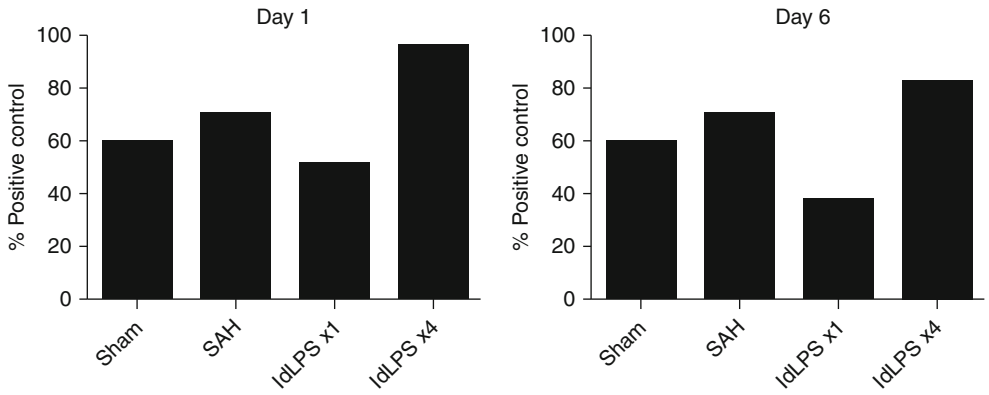

Fig. 3 Level of keratinocyte-derived chemokine (KC) in pooled brain samples. In both the day 1 samples (*left*) and day 6 samples (*right*), low-dose lipopolysaccharide (*LPS*) administered one time prior to subarachnoid hemorrhage (*SAH*) (ldLPS ×1) decreased the production of the neutrophil attractant chemokine KC, whereas the four-dose regimen prior to SAH increased KC production

affects the brain parenchyma or works through mediators in the endothelium.

In our study, we found differential effects of LPS depending on the administration schedule. This suggests two possible mechanisms require further research: (1) The endothelium mediates depression of brain inflammatory responses and has a summated effect based on LPS doses. That is to say, a single dose of LPS decreases endothelial propensity to signal inflammation and multiple doses summate to release inflammatory signaling molecules. Or (2), LPS crosses the blood–brain barrier intact and signals inflammatory cells in the brain (microglia, astrocytes, etc.) to develop an inflammatory response.

The immunohistochemical studies presented here suggest that the endothelium is the gatekeeper of inflammation, and that the brain follows the lead of the endothelium. This affords welcome opportunities to interact with the endothelial lining without having to devise drug or biological therapies that cross the blood–brain barrier. We did not investigate activation of IRF3 or other TLR4 pathway mediators.

There are a number of limitations of our study. First, the severity of DDAV elicited by our model was mild compared to other models. It is possible that the mechanisms are different for mild versus severe DDAV. We feel that this mild form of DDAV was similar to what we see clinically in patients with good-to-medium grade SAH (Hunt and Hess 1–3).

Second, our study did not address directly the molecular mechanism of tolerance. We felt that it was more important to determine the cells involved before entertaining specific molecular or cellular mechanisms.

The development of DDAV appears to be dependent on inflammation after SAH. How early this inflammation develops is still unknown but of critical importance for the development of meaningful therapies. In stroke models, increased inflammatory mediator levels are seen as early as 3 h after the infarction [15, 18, 19]. In stroke in humans, inflammation and edema develop over the span of 3–5 days. In SAH patients, DDAV begins later. It is possible that this is due to later activation of the inflammatory system, making first-day therapies realistic.

Conclusion

This study added to our understanding of how inflammation in the brain of patients with SAH may lead to DDAV. It focuses attention on the acute innate inflammatory response and confirms earlier findings that neutrophils are important in this process. Further work will need to focus on the molecular pathway that initiates this inflammatory response. This is the best hope for a viable treatment or preventive.

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

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Statin-Induced T-Lymphocyte Modulation and Neuroprotection Following Experimental Subarachnoid Hemorrhage

Robert E. Ayer, Robert P. Ostrowski, Takashi Sugawara, Qingy Ma, Nazanin Jafarian, Jiping Tang, and John H. Zhang

Abstract *Introduction*: Statins influence immune system activities through mechanisms independent of their lipid-lowering properties. T cells can be subdivided based on cytokine secretion patterns into two subsets: T-helper cells type 1 (Th1) and type 2 (Th2). Independent laboratory studies have shown statins to be potent inducers of a Th2 switch in immune cell response and be neuroprotective in several models of central nervous system (CNS) disease. This study was the first to evaluate the immune modulating effects of statins in subarachnoid hemorrhage (SAH).

Methods: Simvastatin was administered to rats intraperitoneally in two dosages (1 and 20 mg/kg) 30 min after the induction of SAH using endovascular perforation. Neurological scores were assessed 24 h later. Animals were then sacrificed, and samples of cortex and brain stem were tested for expression of the T-regulatory cell cytokine transforming growth factor (TGF) β1, as well as interleukin (IL) 1β, a proinflammatory cytokine associated with Th1 immune responses. The presence of TGF-β1 secreting T cells was evaluated with the use of brain slices.

Results: SAH significantly impaired neurological function in all SAH groups (treated and untreated) versus sham. Animals treated with high-dose simvastatin had less neurological impairment than both untreated and low-dose groups. Cortical and brain-stem levels of TGF-β1 were significantly elevated following SAH in the high-dose group. IL-1β was significantly elevated following the induction of SAH but was inhibited by high-dose simvastatin. Double-labeled fluorescent immunohistochemical data demonstrated the presence of lymphocytes in the subarachnoid and perivascular spaces following SAH. Expression of TGF-β1 by lymphocytes was markedly increased following treatment with high-dose simvastatin.

Conclusion: The present study elucidated the potential role of a Th2 immune switch in statin provided neuroprotection following SAH.

Keywords Endovascular perforation model • Subarachnoid hemorrhage • Simvastatin • Early brain injury • TGF-β1 • Th2 lymphocytes • Th3 lymphocytes

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Introduction

Aneurysmal subarachnoid hemorrhage (SAH), accounting for roughly 3% of all strokes, is a devastating neurological event that has been the focus of scientific investigation for decades [13, 38]. Significant advances in the surgical treatment and intensive care for these patients have resulted in

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significantly improved mortality and functional outcome [20]. However, the disease still has mortality as high as 40%, and a majority of the survivors still have significant disability [34]. An inflammatory response occurs within the central nervous system (CNS) following SAH and likely plays a significant role in the early brain injury following SAH [4]. Whether the infiltration of lymphocytes into the CNS following injury has a neuroprotective and regenerative role versus being a response that is detrimental to recovery is still being intensively investigated [19]. Resolution appears to lie with understanding the roles of subpopulations of cells, particularly T-helper cells type 1 (Th1), type 2 (Th2), and type 3 (Th3) subsets. Th1-dominated immune responses are associated with significant CNS injury [7, 8]. These responses are exacerbated or increased in frequency under certain conditions, such as systemic infection in conjunction with CNS injury [7]. The ability to inhibit Th1 immune responses has been found to reduce infarct size and improve outcomes following ischemic stroke [8]. In addition, the augmentation of Th2 and Th3 immune responses has been associated with neuroprotection and may augment neuroregeneration [15, 19]. Statins have been described as being able to elicit a Th2 immune response, and they been found to promote neuronal recovery in models of spinal cord injury and traumatic brain injury [2, 18]. The immunomodulating properties of statins may help explain their reported efficacy in treating SAH patients [25, 28, 32, 42]. In this experiment, we hypothesized that statins would provide neuroprotection against the neurological injury following experimental SAH that is associated with a Th2 immune switch in the CNS characterized by the infiltration of leukocytes producing the immunosuppressive cytokine transforming growth factor (TGF) β1. We also predicted that this immune modulation would result in the suppression of proinflammatory cytokines, such as interleukin (IL) 1β.

Materials and Methods

Experimental Animals and Groups

One hundred fifteen adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing between 250 and 350 g were divided randomly into four weight-matched groups: sham-operated treated with vehicle (sham, $n=24$), SAH treated with vehicle (SAH, $n=32$), SAH treated with low-dosage simvastatin (Calbiochem, CA, USA) (1 mg/kg, S-1, $n=33$) or high-dosage simvastatin (20 mg/kg, S-20, $n=26$). Our group's previous experiments have shown that 20 mg/kg simvastatin had no effect on cerebral physiology in sham animals [39]; therefore, a sham group treated with 1 mg/kg or 20 mg/kg simvastatin was not evaluated in this experiment.

Animals experiencing mild SAH (grades 0–7) were excluded from the study as per the SAH grading system criteria because mild SAH does induce detectable neurological deficits in experimental studies [40]. In brief, the SAH grading system is as follows: The basal cistern is divided into six segments, and each segment is allotted a grade from 0 to 3 depending on the amount of subarachnoid blood clot in the segment: grade 0, no subarachnoid blood; 1, minimal subarachnoid blood; 2, moderate blood clot with recognizable arteries; 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. Mild SAH was categorized as all animals that received a total score of five or less. The SAH grading was done in a blinded fashion.

Induction of SAH

All procedures and experiments were approved by the Institutional Animal Care and Use Committee of Loma Linda University. The endovascular perforation model of SAH in rats was used for this study as previously described [9, 30]. Briefly, general anesthesia was induced with ketamine (100 mg/kg ip) and xylazine hydrochloride (10 mg/kg ip) followed by atropine (0.1 mg/kg sc). After intubation, the animals were ventilated with an animal ventilator (Harvard Apparatus). A heating pad and a heating lamp were used to maintain the rectal temperature at 36.0±0.5°C. SAH was induced by endovascular perforation of the internal carotid artery (ICA) bifurcation with a sharpened 4-0 nylon suture. After exposing the left common carotid artery (CCA), external carotid artery (ECA), and ICA through a midline skin incision, the ECA was ligated, cut, and shaped into a 3-mm stump. The suture was advanced rostrally into the ICA from the ECA stump until resistance was felt (~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. Operative procedures were exactly same for the sham group, except that the suture was removed once resistance was felt without puncture. The incision was then closed, and rats were housed individually following their recovery from anesthesia. All rats received 3 ml normal saline intraperitoneally to prevent dehydration, and animals had free access to food and water until euthanization.

Drug Administration

Thirty minutes after the procedure, treatment groups received either a high (20 mg/kg) or low (1 mg/kg) dose of simvastatin via an intraperitoneal injection. High-dose simvastatin (20 mg/kg) was selected on guidance from previous literature [29], whereas the low dosage (1 mg/kg) is comparable to that used

in current clinical settings. All simvastatin dosages were dissolved in ethanol and adjusted to a final concentration of 10%, with a total volume of 1.5 ml when they were administered. Sham and SAH groups received vehicle (1.5 ml of 10% ethanol in normal saline). This concentration of ethanol was not likely to affect any of the physiologic parameters assessed during this experiment [3, 5, 10].

Neurological Scoring

Neurological scores were evaluated in a blinded fashion 24 h after SAH with a modification of the scoring system reported by Garcia et al. [14]. An 18-point scoring system was used to evaluate the sensorimotor deficits.

Western Blotting Analysis

A standard western blotting protocol using the following antibodies: anti-TGF-β1 (Cell Signaling, cat. 3711) and IL-1β (Santa Cruz Biotechnology, sc-74138) was performed on brain tissue collected 24 h after SAH [22]. Briefly, animals were euthanized under anesthesia (5% isoflurane anesthetic in 70% medical air with 30% oxygen), and brains were immediately removed and stored at −80°C until analysis ($n=6$ for each group). Protein extraction from the brain obtained by gently homogenizing in RIPA (Radioimmunoprecipitation Lysis Buffer) (Santa Cruz Biotechnology, sc-24948) and further centrifuged at 14,000 *g* at 4°C for 30 min. The supernatant was used as whole-cell protein extract, and the protein concentration was determined by using a detergent-compatible assay (Bio-Rad, Dc protein assay). Equal amounts of protein (50 μg) were loaded on a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel. Protein was electrophoresed and transferred to a nitrocellulose membrane; the membrane was then blocked and incubated with one of two primary antibodies, anti-TGFβ1 (Cell Signaling, cat. 3711) or IL-1β (Santa Cruz Biotechnology, sc-74138) overnight at 4°C. Nitrocellulose membranes were then incubated with secondary antibodies (Santa Cruz Biotechnology) for 1 h at room temperature. Immunoblots were then probed with an ECL Plus chemiluminescence reagent kit (Amersham Biosciences, Arlington Heights, IL) and exposed to films. The data were analyzed by the software ImageJ 1.41 (National Institutes of Health).

Fluorescence Immunohistochemical Staining

Double-fluorescence labeling for TGF-β1 and T cells in the brain slices mounted on histological glass slides was carried out as previously described [12]. Four 10-μm thick sections per brain, from the central portion of the subarachnoid blood clot, were used in each group ($n=4$). Brain slices were immersed in the citrate buffer and boiled in a microwave oven for 10 min for antigen retrieval, then washed three times with phosphate-buffered saline (PBS) as described elsewhere [41]. This was followed by incubation with 5% donkey serum for 1 h at room temperature (RT). After removing excess blocking serum, primary antibodies were applied overnight at 4°C followed by secondary antibodies for 2 h at RT. Histological preparations were coverslipped with antifade reagent (Millipore) and observed under an Olympus BX51 epifluorescent microscope. The primary antibodies used were rabbit anti-TGF-β1 (Cell Signaling) and mouse anti-T-cell marker (Santa Cruz), each diluted 1:100. The respective secondary antibodies (Jackson Immunoresearch Labs) were diluted 1:200. All other histological regents were obtained from Fisher Scientific Company. Photomicrographs were captured with a computer-assisted camera and merged with aid of Magna Fire Software (Systat Co.) [11].

Statistical Analysis

The data are expressed as mean plus or minus SEM. Statistical differences between the various groups were assessed with a one-way analysis of variance (ANOVA) with Holm-Sidak post hoc analysis. Statistical differences between two groups were assessed with Student *t* test. Mortality was analyzed using a chi-square test. A value of $p<0.05$ was considered statistically significant.

Results

Physiological Data

Physiological parameters were monitored before, during, and after surgery. No statistical differences were observed between the SAH group ($n=6$) and the S-20 group ($n=6$) with regard to mean arterial blood pressure, arterial blood gases, paO_2 and $paCO_2$, pH levels, and glucose levels before, immediately after puncture, and 30 min after SAH (data not published).

Mortality and Exclusion

Twelve rats were excluded from further evaluation following the grading of their SAH at 24 h. Eleven were due to mild grade SAH (0–7), and one was due to the presence of

an acute subdural hematoma. This resulted in the following number of animals in each experimental group: Sham $n=24$, SAH $n=29$, S-1 $n=27$, S-20 $n=25$. Nine rats died before intended sacrifice time and were excluded from assessment of vasospasm and neurological deficits at 24 h. However, these rats were included in mortality statistics. The 24-h mortality rates in each group were as follows: sham, 0.0% (0/24); SAH, 17.2% (5/29); S-1, 11.1% (3/27); S-20, 4.0% (1/25). This resulted in 24 animals being in each experimental group at 24 h. The SAH groups (both treated and untreated) did not differ significantly in their mortality rates ($p>0.05$, ANOVA).

High-Dose Simvastatin

High-dose simvastatin provided neuroprotection following SAH. SAH created significant neurological injury in all groups (Fig. 1). Animals treated with 20 mg/kg of simvastatin had improved neurological scores over vehicle or groups treated with 1 mg/kg simvastatin 24 h after SAH (Fig. 1).

High-dose simvastatin administration was associated with increased expression of the anti-inflammatory cytokine TGF-β and inhibition of the proinflammatory cytokine IL-1β. Cortical and brain-stem samples demonstrated a differential expression of IL-1β and TGF-β1 following SAH that was affected by simvastatin treatment. TGF-β1 levels were not significantly increased by the induction of experimental SAH, and low-dose simvastatin (1 mg/kg) did not have a significant effect on TGF-β1 levels following SAH (Fig. 2a, c). High-dose simvastatin (20 mg/kg) resulted in a significant increase in the expression of TGF-β1 following SAH (Fig. 2a, c). Following SAH, cortical brain samples showed a significant increase in the expression of IL-1β versus sham (Fig. 2b). Treatment with simvastatin appeared to reduce IL-1β expression following SAH in a dose-dependent manner, with significant reduction seen at a dose of 20 mg/kg (Fig. 2b). Similar results were found in brain-stem samples from the same animals (Fig. 2d).

Fluorescence Immunohistochemical Evidence of Th2 Switch

Representative photos of double-fluorescence labeling for TGF-β1 and T cells in the brain slices mounted on histological glass slides demonstrated that lymphocytes were not visible in the subarachnoid space of the basal cisterns following sham surgery (Fig. 3). Lymphocytes were present following SAH, and their number did not appear to be affected by the administration of simvastatin (Fig. 3). Cells expressing TGF-β1 were present in the subarachnoid space in the statin-treated groups (Fig. 3). Evaluation of the merged images demonstrated that these cells were lymphocytes (Fig. 3). Comparison of the SAH and S-20 groups clearly demonstrated that leukocytes were only expressing TGF-β1 in the simvastatin-treated animals (Fig. 3).

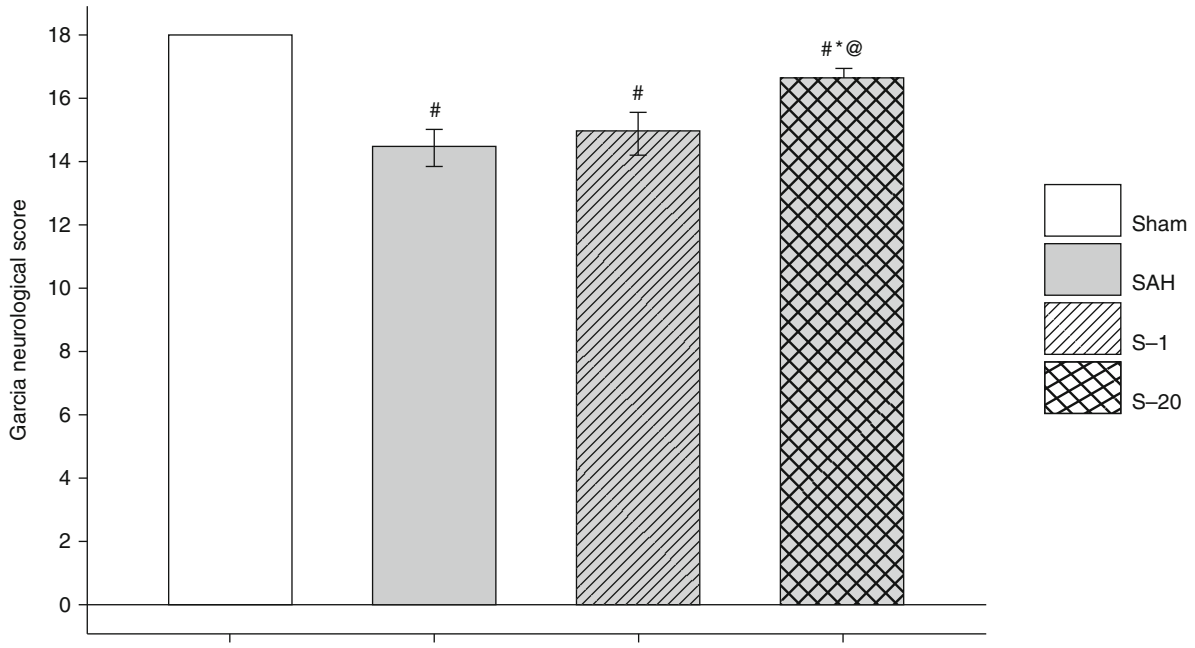

Fig. 1 Effects of statin treatment on neurological score 24 h following subarachnoid hemorrhage (*SAH*). A modified Garcia Neurological Score was used, which ranges from 0 to 18. Eighteen represents normal neurological function. $n=24$ per group; error bar, SE. #$p<0.05$ versus sham; *$p<0.05$ versus vehicle; @$p<0.05$ versus S-1; analysis of variance (*ANOVA*). *LPS* lipopolysaccharide, *MCA* middle cerebral artery

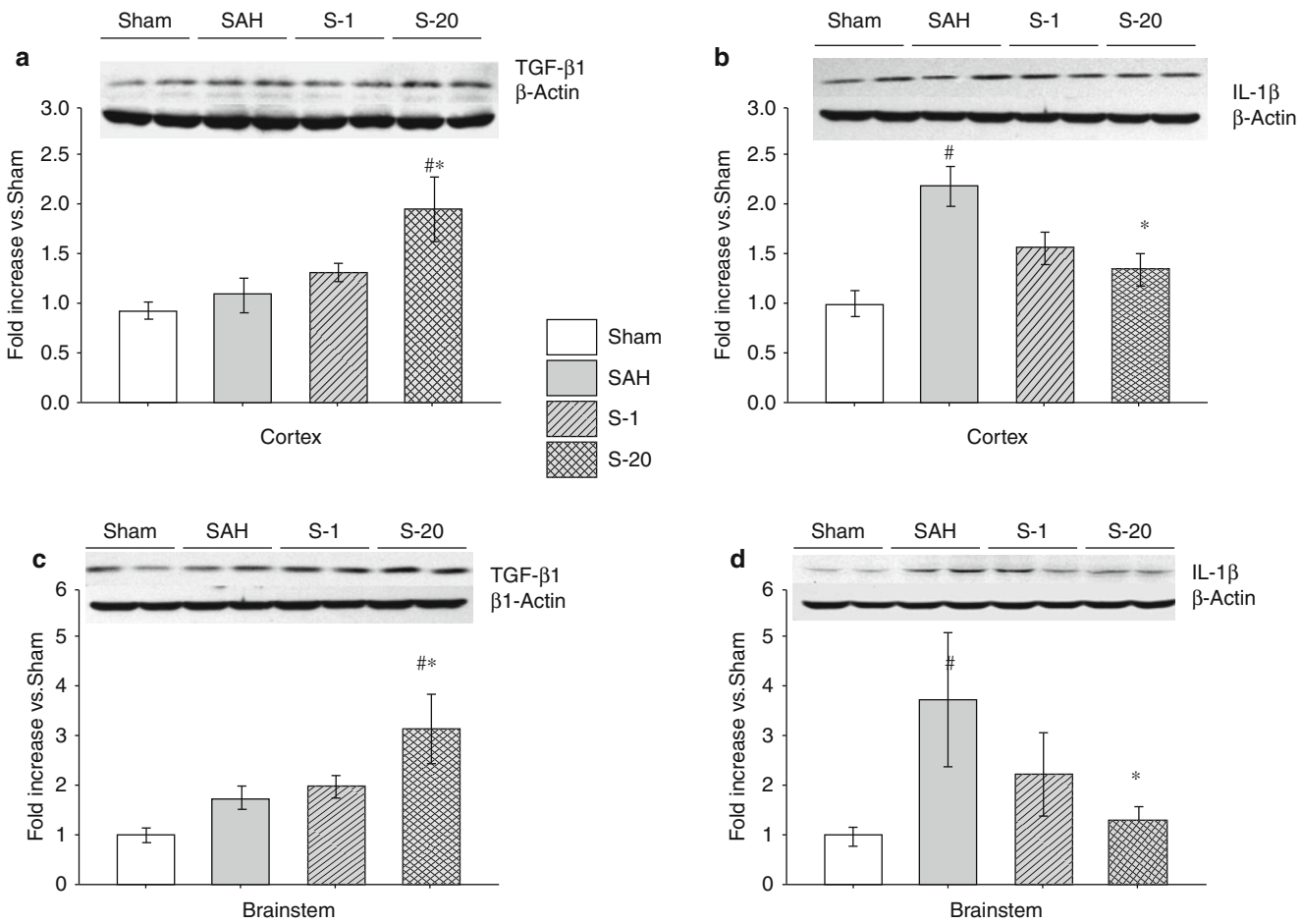

Fig. 2 Effects of statin treatment on transforming growth factor (*TGF*) β1 and interleukin (*IL*) 1β on expression in the cortex and brain stem 24 h after subarachnoid hemorrhage (*SAH*). Western blots for cortex TGF-β1 (**a**) and IL-1β (**b**), as well as brain stem TGF-β1 (**c**) and IL-1β (**d**). Expression levels of each protein in western blot are expressed as a ratio of β-actin levels for normalization. $n=6$ rats per groups; error bar, SE. #$p<0.05$ versus sham; *$p<0.05$ versus vehicle; analysis of variance (*ANOVA*)

Discussion

Subarachnoid hemorrhage, like nearly all CNS injuries, results in an inflammatory reaction. Several investigators have characterized the timeline and population of cells infiltrating the CNS following subarachnoid hemorrhage, indicating that SAH may elicit its own characteristic inflammatory reaction [23, 27]. Kubot et al. looked at several populations of leukocytes, including macrophages and lymphocytes, and found that populations of these cells peaked between 24 and 48 h [23]. Lymphocyte subpopulations, such as Th1, Th2, and Th3, have been found to play critical roles in determining the extent of injury in ischemic stroke and other experimental CNS injuries [7, 9, 19]. Circumstances such as concordant systemic infection or history of previous CNS antigen exposure, can determine which type of lymphocyte population dominates a CNS inflammatory reaction [7, 9, 19]. Typically, Th1-dominant responses are associated with greater degrees of neuronal apoptosis and CNS injury, while Th2- or Th3-dominant responses are associated with neuroregeneration and attenuated detrimental inflammatory reactions [15, 19]. Manipulation of the immune system following CNS injury to produce Th2 and Th3 lymphocyte-dominated immune responses, which are characterized by the secretion of immunomodulatory cytokines such as IL-4, IL-10, and TGF-β1, has been sought after by investigators with the goal of providing neuroprotection [15, 19]. Wolf et al. showed that Th2 cells support neuronal survival better than Th1 cells in vitro [44]. Gimsa et al. demonstrated the Th2 cells mediated suppression of inflammatory signals in brain slices [16]. In addition, vaccination for the treatment of experimental CNS injury showed that Th2-inducing adjuvants promote axon regeneration better than the Th1-inducing adjuvant [21, 35]. Becker et al. demonstrated that a Th1 immune response following stroke resulted in larger infarctions in ischemic stroke models, while smaller infarctions were observed when Th2 immune responses were elicited [7, 8].

Statins, the drugs widely recognized as 3-hydroxy-3-methylglutaryl-coenzyme A–coenzyme A (HMG-CoA)

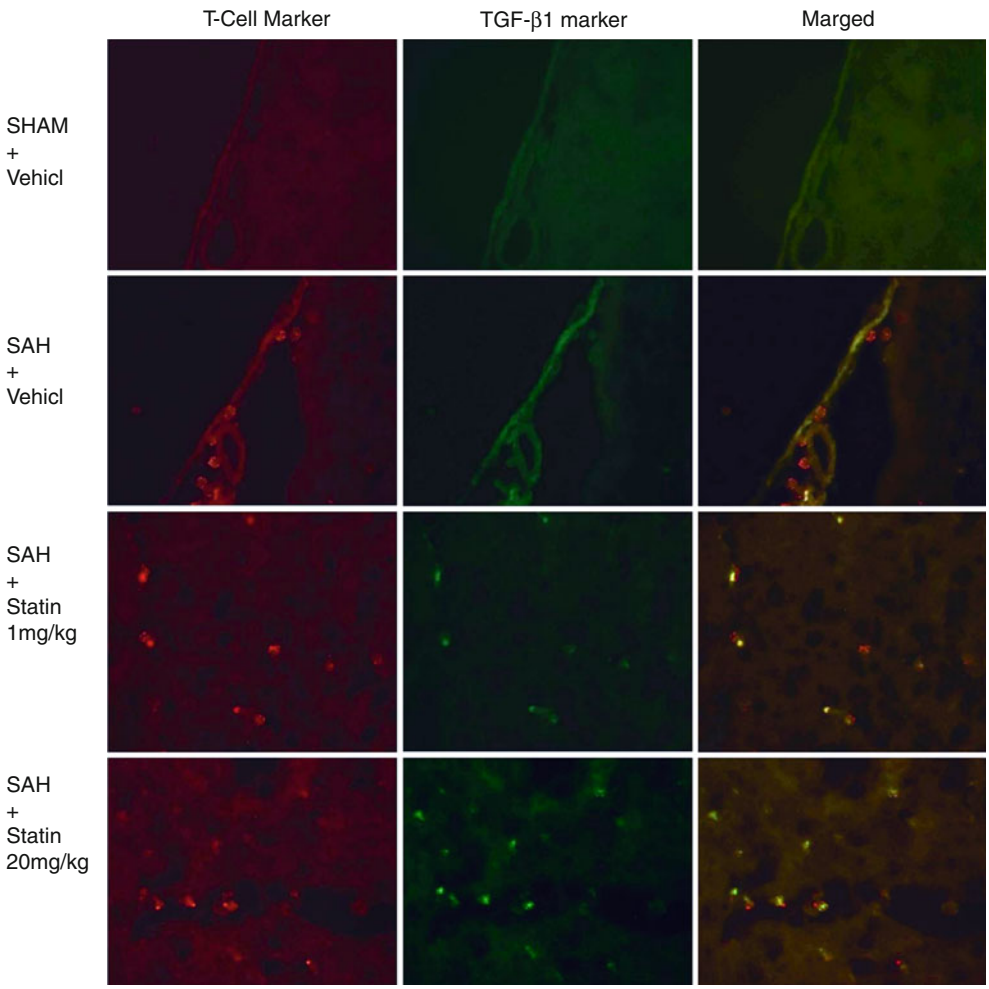

Fig. 3 Representative photographs of double-fluorescence labeling for transforming growth factor (*TGF*) β1 and T cells in the brain slices mounted on histological glass slides. The mouse anti-T-cell maker indicated T cells are not visualized in the subarachnoid space following sham surgery but become present following subarachnoid hemorrhage (*SAH*). The TGF-β1 expression by leukocytes was detected following treatment with simvastatin with the use of rabbit anti-TGFβ-1 antibodies. Samples collected 24 h after SAH

reductase inhibitors and cholesterol-lowering agents, have been shown to be able to promote Th2- and Th3-dominated immune responses in various experimental conditions [1, 2, 18, 45]. For example, Youssef et al. showed that atorvastatin induced a Th2 switch characterized by TGF-β upregulation, interleukin downregulation, and reversed paralysis in experimental autoimmune encephalomyelitis [45]. Other investigators have found statins to be neuroprotective in several CNS diseases, including Alzheimer's disease, multiple sclerosis (MS), stroke, and traumatic brain injury [7, 8, 24, 31, 33, 36]. Pannu et al. found that statin treatment reduced IL-1β expression in the spinal cord while preventing neuronal apoptosis [31]. We have previously demonstrated that simvastatin reverses vasospasm following experimental SAH in rats, and that this treatment is associated with less-severe neurological injury [39]. However, many authors have previously noted that vasospasm does not induce neurological deficits in rats due to their abundant collateral circulation and neuronal physiology [17]. Therefore, we sought to investigate the potential mechanisms of this statin-mediated neuroprotection. In this study, we have shown that simvastatin in a dosage of 20 mg/kg results in a significant reduction in the neurological injury induced by SAH. In addition, high-dose simvastatin increased the cortical and brain-stem expression of TGF-β1 following SAH. TGF-β1 is a cytokine that is expressed in the normal adult brain by parenchymal microglial cells, exerting a trophic anti-inflammatory effect [26]. It has been shown to promote the survival of neurons and inhibit microglial and astrocyte proliferation in the setting of CNS inflammatory reactions [27]. TGF-β1 is a potent inhibitor of cell-mediated immunity and a key marker for Th2 lymphocyte-dominated inflammatory responses [43]. Our results also showed that simvastatin reduces the expression of IL-1β. IL-1β is known to be markedly elevated following CNS trauma, infection, and stroke and is also a marker of Th1-mediated inflammatory reactions [6, 37].

Our fluorescence immunohistochemical staining from cortex and brain-stem samples helped to provide a potential explanation for the marked elevation of TGF-β1. Lymphocytes were observed to accumulate in the subarachnoid space following SAH (Fig. 3). In untreated SAH animals, infiltrating lymphocytes did not demonstrate TGF-β1 expression. However, animals treated with high-dose simvastatin following SAH showed marked expression of TGF-β1 by

lymphocytes in the subarachnoid space and brain parenchyma (Figs. 2 and 3). These observations help to support the hypothesis that simvastatin induces a Th2 immune switch in these animals, and that infiltrating Th2 lymphocytes are responsible for the measured increase in TGF-β1 expression in the brain following treatment.

Conclusion

The present study provided evidence for the neuroprotective role of statins following SAH and suggested that statins induce the presence of regulatory T lymphocytes in the subarachnoid space following SAH. The use of immune modulation through the use of statins or other inducers of Th2 switches has the potential to provide better outcomes for patients who suffer aneurysmal SAH.

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

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Role of Bilirubin Oxidation Products in the Pathophysiology of DIND Following SAH

Gail J. Pyne-Geithman, Sunil G. Nair, Danielle N. Caudell Stamper, and Joseph F. Clark

Abstract Despite intensive research efforts, by our own team and many others, the molecules responsible for acute neurological damage following subarachnoid hemorrhage (SAH) and contributing to delayed ischemic neurological deficit (DIND) have not yet been elucidated. While there are a number of candidate mechanisms, including nitric oxide (NO) scavenging, endothelin-1, protein kinase C (PKC) activation, and rho kinase activation, to name but a few, that have been investigated using animal models and human trials, we are, it seems, no closer to discovering the true nature of this complex and enigmatic pathology. Efforts in our laboratory have focused on the chemical milieu present in hemorrhagic cerebrospinal fluid (CSF) following SAH and the interaction of the environment with the molecules generated by SAH and subsequent events, including NO scavenging, immune response, and clot breakdown. We have identified and characterized a group of molecules formed by the oxidative degradation of bilirubin (a clot breakdown product) and known as BOXes (bilirubin oxidation products). We present a synopsis of the characterization of BOXes as found in human SAH patients' CSF and the multiple signaling pathways by which BOXes act. In summary, BOXes are likely to play an essential role in the etiology of acute brain injury following SAH, as well as DIND.

Keywords Subarachnoid hemorrhage • Delayed ischemic neurological deficit • Bilirubin • Vascular smooth muscle

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Introduction

Subarachnoid hemorrhage (SAH) resulting from a ruptured aneurysm afflicts approximately 30,000 Americans yearly. Of those who survive the initial insult, 30–40% will subsequently suffer from delayed cerebral vasospasm (CV). This is a serious complication, leading to further stroke with higher mortality, increased morbidity, and significant neurological deficit [1], resulting in increased need for extensive rehabilitation and long-term health care. The pathological constriction of cerebral vessels (large conductance [2, 3] and microvessels [4]) that occurs during CV may lead to ischemia, infarction, or death; however, the 3- to 10-day hiatus between the initial hemorrhage and onset of CV potentially affords the clinician a therapeutic window [5]. Unfortunately, despite considerable research efforts, the etiology of CV is still unknown and appears to be a complex combination of events [4, 6]. Until we understand more clearly the molecular events contributing to delayed ischemic neurological deficit (DIND) and acute brain injury after SAH, developing intelligent and effective prognostic, diagnostic, and therapeutic approaches will be a real challenge. Understanding the perturbations that occur in the cerebral microvasculature after SAH, including cortical spreading depolarization (CSD) and conversion of hyperemia to microvessel spasm in the presence of blood products [7], is a vitally important step toward identification, development, and validation of an effective prophylactic therapy against DIND.

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Based on extensive studies, from our own and others' laboratories, showing that bilirubin oxidation products (BOXes) affect large-vessel contractility [8], signaling [9], and metabolic function [10] and that they are present only in SAH patients with vasospasm [11], we posit that BOXes play a significant role in perturbations of microvessel reactivity and conversion of CSD-induced hyperemia into hypoperfusion [12] via constriction.

Discovery and Chemical Characterization of BOXes

The literature records a bewildering array of molecules that may be implicated in the etiology of CV after SAH. They include endothelin [13], hemoglobin [14, 15], bilirubin [16–19], arachidonic acid and its peroxidized metabolites [20–24], and immune system molecules [25]. However, almost all of these molecules are present in cerebrospinal fluid (CSF) from SAH patients that develop and do not develop DIND, so they cannot be the only determinant of vasospasm. There is also the question of time course: These agents are present acutely and probably play a role in short-term vasoconstriction to minimize hemorrhage and perhaps brain injury, but DIND does not occur until 3–10 days after the SAH. Bilirubin, however, is produced in this time frame. The formation of bilirubin occurs as a result of the following reactions: Heme is converted into biliverdin by the inducible enzyme heme oxygenase 1 (HO1, also known as heat shock protein [HSP] 32). HO1 is induced in the presence of heme, or trauma. It is found in the microglia of the brain and the choroid plexus and arachnoid cells of the ventricular system [26]. Biliverdin is then converted into bilirubin by the enzyme biliverdin reductase, which is found principally in the liver, but in low levels in all tissues. Biliverdin reductase is not rate limiting as it is constitutively active and ubiquitous [27]. These reactions are illustrated in Fig. 1.

BOXes can be synthesized in the laboratory from bilirubin [17, 28]. We have found that their formation requires molecular oxygen [29], and that enzymatic processes can also produce BOXes in vitro [30–32].

BOXes and Their Properties in Human Patients

We have found that BOXes are present in measurable quantities in CSF of SAH patients, but that there are significantly higher concentrations of BOXes in CSF from patients who later developed DIND compared with those who did not; this was independent of hemoglobin concentration in the CSF [11]. By replicating as closely as possible the

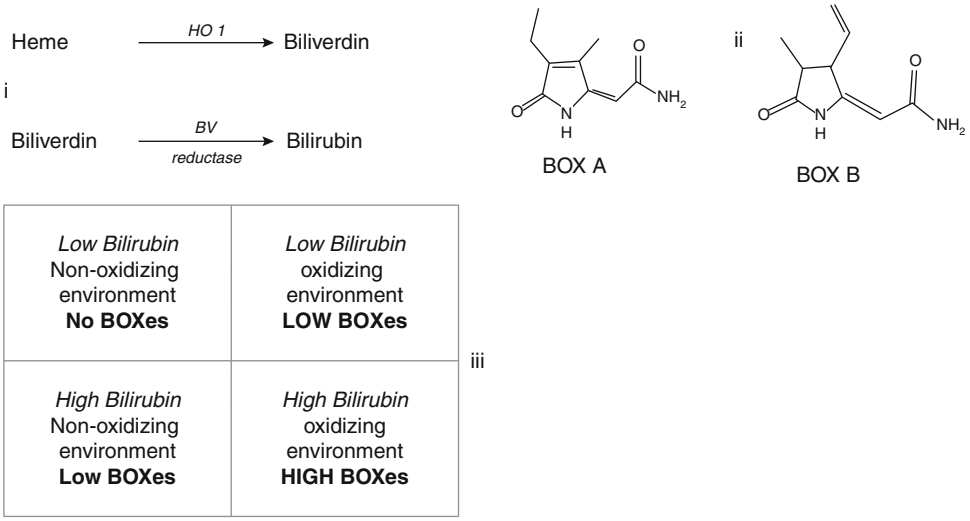

Fig. 1 Formation of BOXes in vivo. (*i*) The conversion of heme to bilirubin involves two enzymes: heme oxygenase 1 (1.14.99.3) and biliverdin reductase (1.3.1.24). These reactions occur in the cerebrospinal fluid (*CSF*); the enzymes are found in brain tissue. (*ii*) Structures of bilirubin oxidation products (*BOXes*) that are found in CSF from patients with subarachnoid hemorrhage (*SAH*) with delayed ischemic neurological deficit (*DIND*) (but not SAH patients without DIND). They are formed in the presence of bilirubin and an oxidizing environment (see *iii*). These molecules are regularly synthesized in our laboratories. (*iii*) Matrix illustrating the four conditions that can be found in SAH patient CSF and the likelihood of BOXes being found in pathophysiological concentrations under that condition

conditions likely to exist in post-SAH CSF, we were able to synthesize BOXes in vitro and purify them to examine their effects on smooth muscle and brain tissue [17, 33]. We found that in vivo conditions favorable to the formation of BOXes were quite specific; an oxidative environment and high levels of bilirubin were required, and these seemed to be independent of initial hemorrhage volume [11]. Figure 1b diagrammatically represents these observations. Bilirubin appears in SAH CSF after around 12 h posthemorrhage, but the levels do not peak until days later. We have shown that bilirubin levels are in fact higher in CSF from DIND SAH patients, and that this bilirubin production peaks at 3–8 days [11, 19]. The oxidizing environment required for the formation of BOXes is also present in CSF from DIND SAH patients, as reported by us and others. An oxidizing environment may result from macrophage infiltration [34], which leads to elevated peroxide concentrations and nonspecific peroxidation of CSF components, as well as induction of cyclooxygenase [33], glutathione peroxidase [35], and lipooxygenase enzymes that produce hydroxyeicosatetranoic acid (HETE) molecules from arachidonic acid [22, 24]. These molecules are particularly implicated in resistance vessel spasm [36], and they are known to have actions on potassium channels [37], which are associated with CSDs [7]. Recently, we found that BOXes also affect smooth muscle potassium channel function [38]. BOXes synthesized in the laboratory (chemically identical to those isolated from SAH patient CSF [17, 28]) have been shown to be vasoactive both in vivo and in vitro, whereas unoxidized bilirubin had no effect [8, 10, 18].

Signaling Pathways Implicated in DIND Where BOXes May Act

The balance of basal tone in the cerebral vasculature is regulated by the ratio of endothelin/nitric oxide (NO) [39–41]. The disturbance of this balance has been reported to be due to hemoglobin scavenging of NO [42, 43], inhibition of nitric oxide synthase (NOS) [41, 44, 45], or upregulation of endothelin receptor expression [13]. Recent research showed that NO affects smooth muscle relaxation in two main ways: the multiple mechanisms by which intracellular calcium concentration is reduced [46–48] and a recently discovered role [49]. Kitazawa et al. reported that NO also effects vascular relaxation via decreased phosphorylation, and thus deactivation, of the intrinsic myosin light-chain phosphatase (MLCP) inhibitor CPI-17.

Protein kinase C (PKC) has been shown to play a role in the sensitization of the smooth muscle contractile apparatus to calcium [50–53]. More than one isoform of PKC has been implicated in the etiology of CV after SAH [54]. These studies, as well as work from our laboratory, showed that PKCδ is translocated to the cell membrane during the initiation of contraction in the basilar artery, and PKCα is translocated during the maintenance phase [8, 55]. However, this translocation does not appear to result in activation of the PKC enzymes (S.G. Nair, unpublished data). PKC, then, may play a role in initial contraction and sensitization of smooth muscle contractile apparatus, but the sustained pathological constriction seen in CV also involves a pathological failure to relax.

Phosphorylation of Myosin Light Chains (MLC20) activates the intrinsic adenosine triphosphatase (ATPase) activity of the myosin heavy chain and thus allows cross-bridge interaction and contraction. For the energy-efficient "latch" state to be entered, or for relaxation to be effected, MLC20 must be dephosphorylated by MLCP, a type 1 protein phosphatase (PP1) [56, 57]. The latch state is a physiological event that enables smooth muscles to maintain tension at low energy cost, and it responds to physiological vasodilatory signals [58]. Dephosphorylation must also occur to allow relaxation. Vasospasm is not energy efficient and does not respond to physiological or pharmacological dilatory signals [59, 60]. Phosphorylation events control the contractile activity, the calcium sensitivity of smooth muscle contraction, and perturbation of these events and their control is likely to cause vascular dysfunction and pathology [6, 60, 61]; inhibition of MLCP leads to a failure to relax [62]. Increased myosin ATPase phosphorylation has been reported in the canine double-hemorrhage model [15, 63–65]. These increases are reported to be due to mobilization of Rho, which activates rho kinase (ROCK), which in turn phosphorylates and inactivates MLCP [66–68]. Rho is activated by a number of agonists, including thromboxane A_2 and other lipid-signaling molecules [69]. Investigations in our laboratory have shown that BOXes induce expression and activation of rho A and ROCK in both intact tissue [8] and cells in culture [70]. Rho activation by BOXes leads to potentiation of smooth muscle contraction (vessel rings) in response to phenylephrine [8] and formation of stress fibers (in cultured cells) [70], a hallmark of rho-mediated perturbations [71, 72]. Figure 2 illustrates these data.

Conclusion

Given the widespread effects of BOXes, it is likely that they are a significant contributor not only to vascular pathophysiology but also to neuronal damage (Crutcher K,

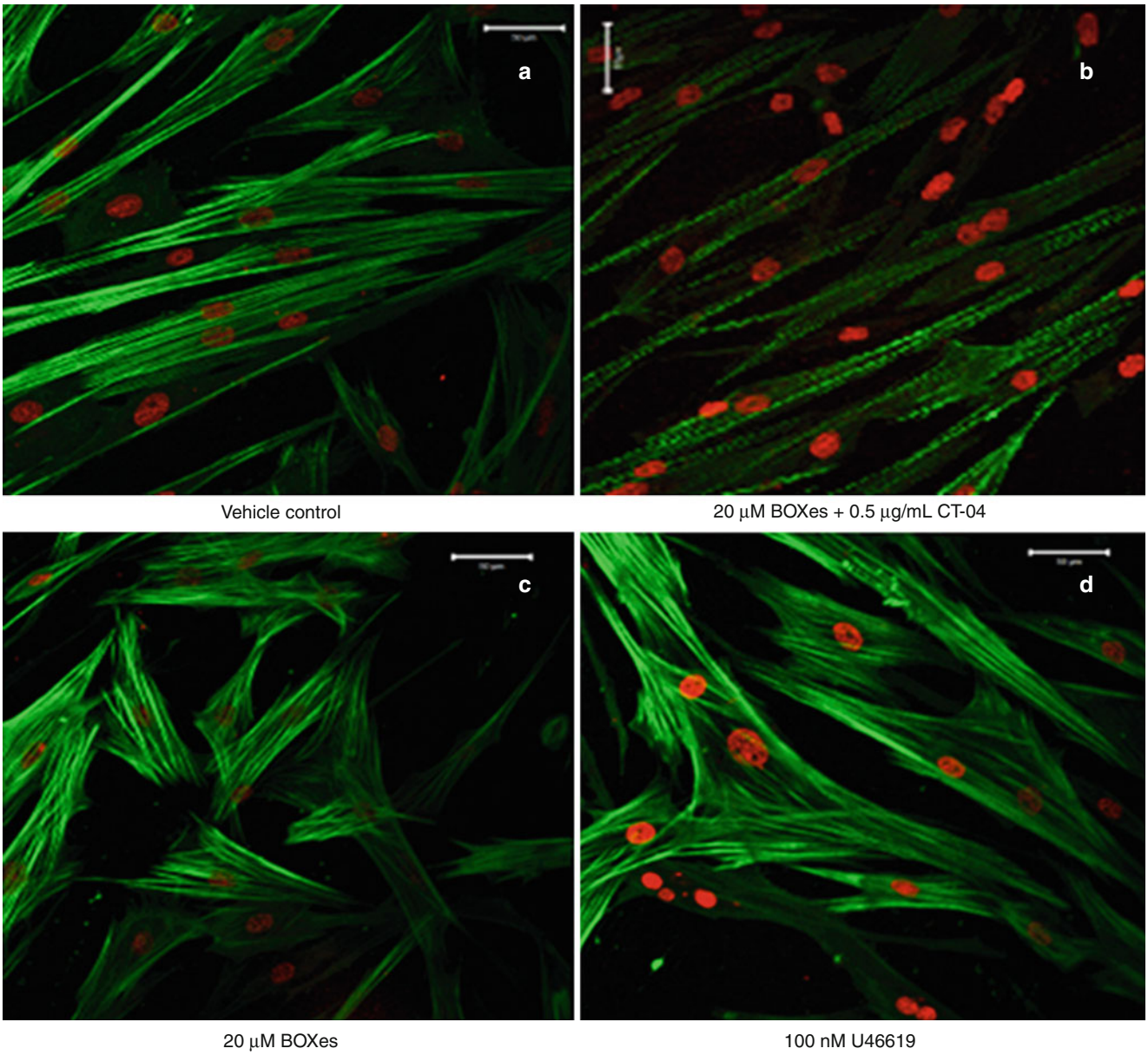

Fig. 2 Rho-dependent stress fiber formation in primary smooth muscle cell culture. Representative images of smooth muscle cells in culture under various conditions. Cells were cultured from porcine carotid arteries for 24 h in serum-depleted media. Cells were exposed to treatment for 30 min, then fixed in 3% paraformaldehyde. Cells were then stained for actin using smooth muscle actin αSMA-mAb (1:50). Alexafluor-488 (*green*) goat antimouse immunoglobulin (Ig) G (1:400) (Molecular Probes) was used as secondary antibody, and Topro-3 (*red*) (1:500) (Molecular Probes) was used to counterstain nuclei. (**a**) Vehicle control-treated cells (0.9% NaCl) show typical smooth muscle morphology and actin distribution. (**b**) Cells treated with bilirubin oxidation products (*BOXes*) at pathophysiological levels in the presence of a rho inhibitor (CT-04) exhibit similar typical morphology. (**c**) BOXes at levels seen in delayed ischemic neurological deficit (*DIND*) subarachnoid hemorrhage (*SAH*) patient cerebrospinal fluid (*CSF*) (20 μM) elicited stress fiber formation. Cells are retracted, and actin filaments are no longer arranged along the axis of the cells. (**d**) Remarkably similar morphology is seen in cells treated with U46619 at 100 nM (a thromboxane A_2 receptor agonist, which is known to act through rho). Scale bar represents 50 μm

Pyne-Geithman GJ, unpublished data). Figure 3 shows a schematic including (many, but not all) known and postulated pathways implicated in the vascular etiology of DIND following SAH. BOXes have been found to have effects in many of these pathways, as indicated in the schematic. Although much of our work has, to date, been focused on large-vessel physiology and biochemistry, many of our findings are applicable to cerebral microvessels. Given the increasing evidence for the role of microvessel integrity and signaling and CSDs in DIND following SAH, we are now pursuing these avenues with respect to the role of BOXes.

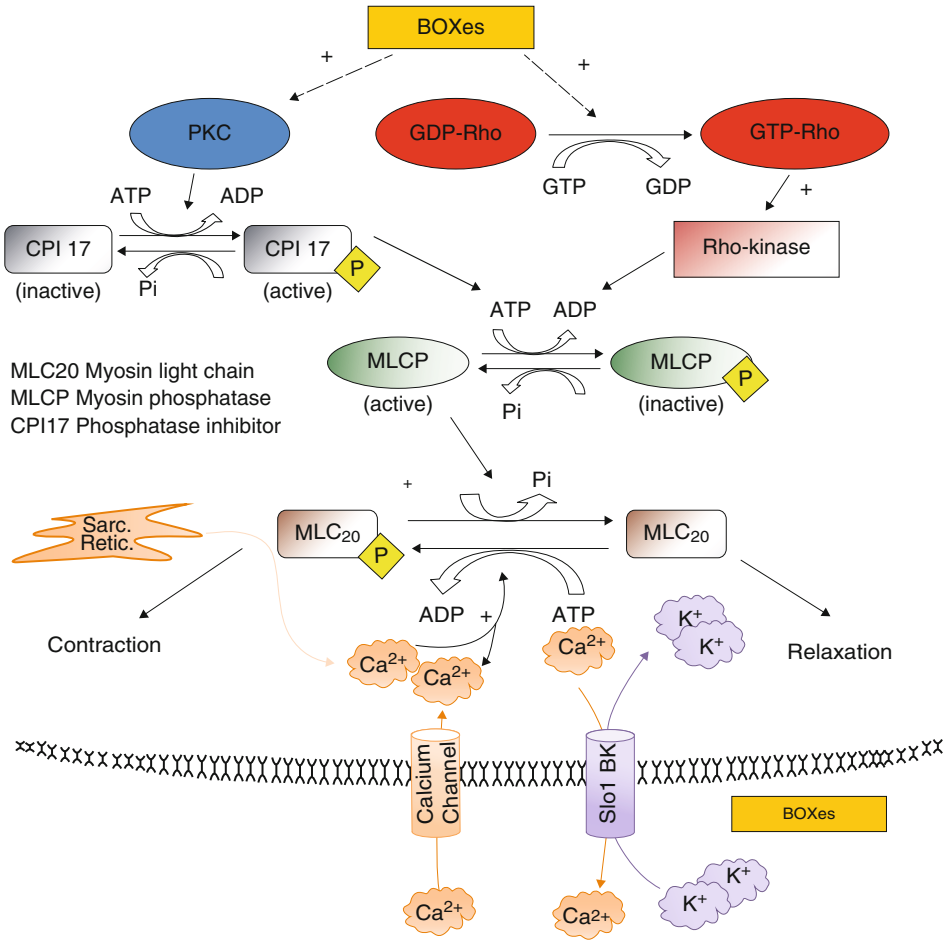

Fig. 3 Schematic showing the vascular signaling pathways implicated in the etiology of delayed ischemic neurological deficit (*DIND*) and the role of bilirubin oxidation products (*BOXes*) based on our research. From our investigations, BOXes appear to inhibit relaxation pathways in two ways: rho-mediated inhibition of myosin light-chain phosphatase (*MLCP*) and protein kinase C (*PKC*)-mediated phosphorylation and activation of CPI-17, an endogenous MLCP inhibitor. In addition, BOXes stabilize the closed state of Slo1 BK channels without compromising calcium sensitivity of the channels, leading to intracellular accumulation of calcium and hyperexcitability in human cerebral microvessel smooth muscle cells. These pathways were largely investigated in preparations derived from arterial tissue (ex vivo or in vitro); however, similar pathways exist in cerebral microvessels and may implicate BOXes in cortical spreading depolarization and microvessel spasm, resulting in DIND after subarachnoid hemorrhage (*SAH*)

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

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Association of Morphologic and Demographic Features of Intracranial Aneurysms with Their Rupture: A Retrospective Analysis

Sayantani Ghosh, Saugat Dey, Stavropoula Tjoumakaris, Fernando Gonzalez, Robert Rosenwasser, Jabbour Pascal, and Jack Jallo

Abstract *Background*: In spite of its common occurrence, the factors predictive of the rupture of intracranial aneurysms (IAs) remain poorly defined.

Method: A retrospective analysis of patients admitted with a primary diagnosis of cerebral aneurysm in a single institution was done. The factors studied were age, sex, size, site, side, multiplicity, neck type, aspect ratio, positive family history, smoking and drinking habits, and hypertension. The morphological parameters were evaluated for a total of 5,138 aneurysms obtained from the 2,347 patients. Factors found significant on univariate analysis were further tested on a multivariate model.

Findings: We found 1,088 patients (46.36%) had at least a single aneurysmal rupture. Among the morphologic factors, size greater than 10 mm, right sidedness, aspect ratio greater than 1.6, deviated neck type, and multiplicity were found to be associated with higher incidences of rupture. Aneurysms on posterior communicating and middle cerebral arteries were found to be more prone to rupture. The demographic factors that were more linked with the ruptured aneurysms were positive family history, smoking, and hypertension.

Conclusions: Relevant cases should be started on intensive lifestyle modification, and extensive screening of those with a positive family history is highly warranted. All "at-risk" patients should be evaluated for early surgical intervention.

Keywords Cerebral aneurysms • Risk factors • Familial cerebral aneurysms • Hypertension • Smoking

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Introduction

Intracranial aneurysms (IAs) affect 2–5% of the entire population [18]. But, despite their expected common occurrence, only 1% of all IAs actually rupture [20]. Ruptured IAs classically cause subarachnoid hemorrhage (SAH) but may cause intraventricular hemorrhage and subdural blood [8]. About 65% of patients die of the first SAH, and a further 20–25% experience complications [22]. Hence, understanding of the pattern of rupture in IAs is helpful in predicting the rupture risk in patients and would result in earlier and better management of this disease.

Although there have been clinical studies in the past concerning the various factors leading to aneurysmal rupture, including those involving absolute size [12, 18, 24]; various angles, indices, and size ratios [6, 21]; fluid dynamic studies [3, 9, 19]; location of the aneurysm [4, 15]; age and sex [5]; family history [14]; hypertension [16]; and smoking and alcohol consumption [11], there has been little large-scale research taking all the factors together. Hence, we took the 12 most controversial yet vital factors and compared them for ruptured and unruptured cases to discover their actual significance.

Methods and Materials

Retrospective review was made of all patients admitted in the cerebrovascular facility of Philadelphia's Thomas Jefferson University from March 2006 to January 2010, irrespective of when or where their aneurysms were operated on; this made us include many people having their surgeries prior to 2006. Only patients with a radiographically confirmed diagnosis of cerebral aneurysm by computed tomographic angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA) were included. There were 2,347 patients who met the criteria, among which 1,088 had rupture of at least a single IA. There were 884 patients with multiple aneurysms, so a total of 5,138 IA s were obtained from the 2,347 cases.

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Information on age, sex, familial preponderance, hypertension (>140/90 mmHg), aneurysm size, aspect ratio, location, multiplicity, and history of smoking and alcohol consumption was retrieved from the database. Former smokers who had quit smoking more than 2 years ago and sporadic alcohol consumers were excluded from the smoking and alcohol analysis. The information about the size (the maximum perpendicular height), neck type, and the aspect ratio (the ratio of the maximum perpendicular height to the average neck diameter) was either noted from the operative and discharge notes or measured from angiographic images obtained from iPhilips. Approval for the collection and review of data was obtained from the institutional review board at Thomas Jefferson University.

Statistical Analysis

All data were analyzed using JMP 7.0.2 (SAS Institute, Cary, NC). Initial univariate analysis was done to assess the statistical significance of the observed difference between the ruptured and unruptured groups for each parameter. A chi-square test was performed for rate versus rate, and logistic regression was used for rate versus continuous variable. The p values and 95% confidence intervals were calculated and reported. Effect of age was also assessed by analysis of variance (ANOVA) with age as the dependent variable and rupture, sex, and family history as the main effects. All parameters that were found to be significant ($p<0.05$) in the univariate analysis were further analyzed using multivariate regression to identify those parameters that retained significance while accounting for all relevant variables.

Results

There were a total of 1,555 females and 792 males in the study, so the ratio of females to males was 1.96:1. The rupture rate [(number of persons having a rupture/total number of persons)×100] was higher in males (49.24%) than in females (44.89%) ($p=0.045$), with 38.26% of the total number of males and 37.36% of the total number of females having more than one IA ($p=0.67$). The rupture rate among the 884 cases with multiple aneurysms was 65.38%, while it was 34.86% for those with a single aneurysm ($p<0.0001$). Mean age of rupture in males was 4.04 years more than that in females. Increasing age, when considered alone, was associated with less chance of rupture (odds ratio 0.98; 95% confidence interval 0.97–0.99).

Of those with a history of aneurysm in a first-degree relative, 72.21% had a rupture against 38.50% of those who did not have a single family member having an IA ($p<0.0001$). Also, people who did have a positive family history had more chance of having multiple aneurysms ($p<0.0001$) and had a rupture 3.22 years (95% confidence interval 1.83–4.62) before those who did not ($p<0.0001$).

Among the other demographic factors, smoking and hypertension seemed to have a role in rupture. Of current smokers or ex-smokers who had smoked for at least 10 pack years and have quit smoking less than 2 years ago, 67.94% had a rupture compared to 40.20% of nonsmokers ($p<0.0001$). Of hypertensive people, 58.21% had a rupture compared to 40.32% of those with a normal blood pressure ($p<0.0001$). Smoking and hypertension were also associated with increased incidences of multiple aneurysms ($p=0.00078$ and $p=0.032$, for smoking and hypertension respectively).

Aneurysm size was found to have the most significant effect among all factors. Of IAs greater than 10 mm in size, 58.32% ruptured, while only 18.97% of those with a size less than 10 mm did rupture ($p<0.0001$). Other morphologic factors like aspect ratio also had a major role to play: 52.5% of IAs with aspect ratio greater than 1.6 ruptured, while only 19.72% of those with aspect ratio less than 1.6 ($p<0.0001$) did rupture. As expected, IAs with deviated neck ruptured more (35.77%) than those with classical necks (28.43%) ($p=0.0003$). Of patients having a deviated neck aneurysm greater than 10 mm size and greater than 1.6 aspect ratio, 69.21% did rupture ($p<0.0001$). Although most of the aneurysms were left sided (69.85%), right-sided ones had a higher rupture rate of 39.38% compared to 32.96% for the left-sided ones ($p<0.0001$).

Most of the IAs (71.7%) were in the anterior circulation, with most of them in the anterior communicating artery. The posterior circulation harbored a higher fraction of the smaller-size IAs ($p=0.004$) than the anterior circulation, with most of them in the posterior communicating artery. The rupture rates for the posterior circulation IAs were also higher (37.55%) than for the anterior circulation ones (33.85%) ($p=0.0121$). The highest rupture rate was in the posterior communicating artery, but the maximum number of rupture cases occurred in the middle cerebral artery.

Discussion

Our study revealed that aneurysm size greater than 10 mm was the most imperative factor for rupture, which is contrary to recent large-scale studies, such as the International Study of Unruptured Intracranial Aneurysms [10, 24], which predicted extremely low rupture rates based on absolute IA size. Numerous other studies have also disputed the role of size as a rupture predictor by illustrating incidences of rupture from small aneurysms [1, 2].

Family history of ruptured IA in even one first-degree relative proved to increase the rupture risk manyfold and

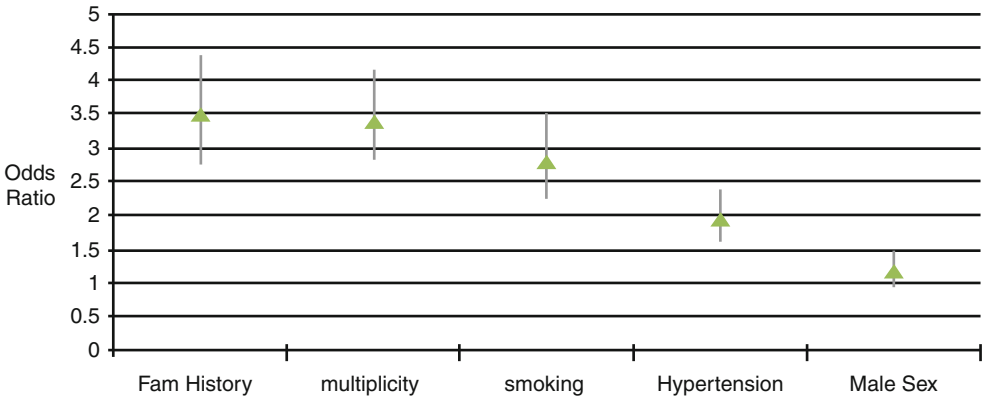

Fig. 1 Odds ratio (with 95% confidence interval) for rupture for the different demographic parameters in the multivariate model

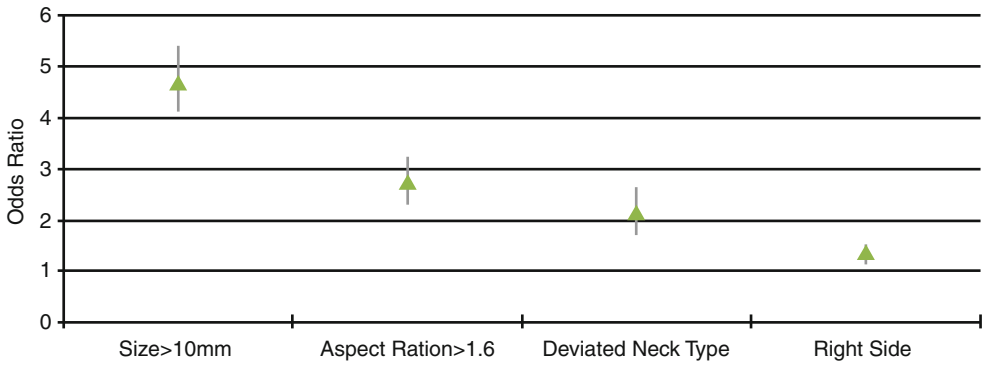

Fig. 2 Odds ratio (with 95% confidence interval) for rupture for the different morphologic parameters in the multivariate model

reduce the age at rupture. Family history of aneurysm also increased the risk for multiple aneurysms. This should persuade early and regular screening for IAs in the family members of patients with a ruptured IA. Smoking and hypertension also increased the chance of IA multiplicity. Having multiple aneurysms alone can also amplify the rupture risk.

Current smoking and resting blood pressure above 140/90 also seemed to be independent risk factors for aneurysmal SAH. Alcohol intake had no definite relationship with rupture, whereas increasing age reduced the chances of rupture. IAs were more widespread in females, but males were found to have slightly higher chances of rupture, although the difference lost its significance on multivariate analysis ($p = 0.08$). Figure 1 illustrates the odds ratio for rupture for the different demographic parameters in the multivariate model.

Higher aspect ratio also accounted for higher rupture rates, which is in sync with the previous studies [21, 23]. Presence of deviated neck (i.e., side-wall aneurysms), although it was associated with higher rupture risk but was not as important as the other factors, corroborating a recent volumetric study that parent vessel geometry has more role to play than type of aneurysm [19]. Right-sided aneurysms, although less common, had higher chances of rupture. Figure 2 illustrates the odds ratio for rupture for the different morphologic parameters in the multivariate model.

Previous studies based on the location of IAs showed that certain vessels, such as the posterior communicating artery and the anterior communicating artery, have a higher incidence of ruptured aneurysms when compared with other locations, such as the internal carotid artery [1, 2, 7, 13, 22]. In our study, we obtained the highest rupture rates in the posterior communicating artery, followed by the middle cerebral artery and anterior communicating artery. As a whole, we had higher rupture rates in the posterior circulation. Also, we had smaller aneurysms rupturing in the posterior circulation, with most of them in the posterior communicating artery; previous studies showed a high percentage of small ruptured IAs in the anterior communicating artery [13, 17].

Conclusion

Our study reignited the debate on the implication of greater aneurysmal size in the rupture of IA again by demonstrating a considerable proportion of larger aneurysms rupturing. This should pilot more large-scale prospective trials in this regard. The effects of aspect ratio and aneurysm neck type on rupture were distinguished, although not that remarkably. Right-sided aneurysms also seemed to be related to the rupture process. Family history of ruptured IAs, aneurysm multiplicity, smoking, and hypertension also were observed to have an enormous effect on the rupture process.

Hence, periodic screening for IAs in the family members of patients with ruptured IA may be done from an early age. Large or multiple aneurysms discovered on angiograms should be referred to immediate surgical interventions. All at-risk patients should be motivated to quit smoking and adopt lifestyle modifications to protect against cardiovascular diseases.

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

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Location, Interaction, and Anticipation of Aneurysm Formation

Daniel Woo

Abstract It is well established that family members of those individuals with intracranial aneurysm are at high risk of intracranial aneurysms. In the past several years, more information on the heritability of location, familial susceptibility to cigarette smoking, and whether "anticipation" of aneurysm formation has been published. We review these three topics in detail and summarize what we gathered from this information.

Keywords Genetics • Stroke • Intracranial aneurysm • Family history

Introduction

The familial aggregation of intracranial aneurysm (IA) and aneurysmal subarachnoid hemorrhage (SAH) has been extensively documented in the literature. Such familial aggregation suggests that heritable factors may contribute to aneurysm formation, but other factors also aggregate within families, such as the risk factors of smoking and hypertension. In addition, prior reports have suggested that "anticipation" of aneurysm formation occurs. We examine three phenomena to better understand the heritability of IA.

Location of Aneurysm

There are case reports in the literature of identical twins having IAs in the same locations [4]. In addition, some studies suggested that "shear stress" in particular anatomic locations lends those territories to greater susceptibility to aneurysm formation [5]. If we take these two facts together, it could reasonably be hypothesized that anatomic vulnerability may be heritable.

If true, then we would expect that if a proband in a family has an aneurysm in a particular location, that person's first-degree relatives, who share 50% of the person's genetic variation, would be more likely to have an aneurysm in the same territory compared to someone in a different family (who would share less than 50% of the genetic variation). To test this hypothesis, Mackey et al. examined families in the Familial Intracranial Aneurysm study to determine if aneurysmal territory concordance within families was greater than that between families [2].

For that analysis, affected subjects with a definite or probable aneurysm were used, and the proband was defined as the affected subject within each family pedigree that had the most affected first-degree relatives. To summarize the results, within-family concordance occurred more frequently than between-family concordance with some territories demonstrating greater concordance than others. This finding strongly supports the hypothesis that a genetic predisposition for aneurysms in the same arterial territory exists.

Gene-Environment Interactions

Both smoking and family history of aneurysmal SAH are independent risk factors for aneurysmal SAH [1]. Smoking also tends to aggregate within families, and although family history is an independent risk factor, it is also possible that an inherited factor increases the susceptibility to the risk factor of smoking. Woo et al. examined this hypothesis using a population-based case–control study of hemorrhagic stroke [7]. Using a total of 339 cases and 1,016 controls, the study confirmed that current smoking (odds ratio [OR] 3.1, 95% confidence interval [CI] 2.2–4.4) and family history of SAH (OR 2.5, 95% CI 1.0–6.9) were independent risk factors but further identified that the combination of both family history

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and current smoking (OR 6.4, 95% CI 3.1–13.2) markedly increased the risk, and that this increase was more than additive.

This finding suggests that an inherited risk factor for susceptibility to smoking leading to IA formation exists. This may affect management in that family members of individuals with aneurysmal SAH who smoke should be advised to quit smoking with the further information of their marked increase in risk.

Anticipation of Intracranial Aneurysm

Not all genetic variants are the same. A type of variant called a microsatellite consists of multiple repeats of the same sequence, such as trinucleotide repeats. These repeats are unstable and may increase in size over generations, thus leading to more severe or early disease called *anticipation*. In Huntington's disease, increase in CAG repeats from one generation to the next has been documented along with earlier and more severe presentation. If anticipation occurs for IA, one may expect to see aneurysm formation at a younger age in subsequent generations, and this may inform us regarding the type of variant that may be causative.

However, anticipation studies have to be considered carefully for design flaws of follow-up period [3]. For example, if a proband is aged 40 years and one of the person's offspring has an aneurysm documented at 20 years of age, this may appear to be a case of anticipation. However, if none of their offspring has actually reached the age of 40 or greater, there was no chance for them to have developed an aneurysm at an "older" age than the proband. For example, if another offspring has an aneurysm form and rupture at age 60 years, the average between the two offspring would be 40 years, and no anticipation may occur. A study may require 40 years of additional follow-up to find such an occurrence.

To address this issue, Woo et al. performed a Kaplan-Meier curve to determine the age of aneurysm identification from one generation to the next [6]. In their report, they found that the subsequent generation, once correcting for the age reached, did not have an earlier presentation of aneurysm and may actually have been delayed (perhaps due to earlier identification and treatment).

This finding suggests that the type of variant to be sought is not a microsatellite that may lead to anticipation, but that more typical variants such as polymorphisms, insertions, or deletions may be responsible.

Conclusion

To summarize, recent studies have provided strong support that aneurysm formation has a genetic component, and that there may be an anatomic vulnerability that is heritable and that a risk factor that increases the susceptibility to smoking behavior may be responsible. In addition, anticipation is unlikely to occur for aneurysm formation consistent with the type of inherited variant being of a type other than microsatellites.

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Conflicts of Interest Statement I declare that I have no conflict of interest.

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Symptomatic Vasospasm in Elderly Patients with Aneurysmal Subarachnoid Hemorrhage: Comparison with Nonelderly Patients

Mizuho Inoue, Tatsuya Sasaki, Hiroki Takazawa, Takahiro Morita, Ayumi Narisawa, Atsushi Saito, Hiroshi Midorikawa, and Michiharu Nishijima

Abstract *Objective*: To clarify the influence of age on the occurrence of symptomatic vasospasm (SVS), we retrospectively compared 34 elderly (over 70 years) and 71 nonelderly patients with aneurysmal subarachnoid hemorrhage (SAH).

Methods: Between 2008 and 2010, at our hospital 105 patients (Hunt and Kosnik grades I–IV) underwent aneurysm surgery within 72 h of the insult. They were divided into four groups based on their age (younger/older than 70 years) and treatment (aneurysmal clipping or coiling). In all patients, we used the same protocol, which included the delivery of intrathecal urokinase and intravenous fasudil chloride; in patients with angiographic evidence of vasospasm, we also injected fasudil chloride intra-arterially.

Results: Among the elderly patients, 4.3% of those treated by clipping and 9.1% of those treated by coiling experienced SVS; the comparative incidence in younger patients was 6.5% and 4.0%, respectively. The differences were not statistically significant ($p=0.40$). The ratio of ventriculo peritoneal (VP) shunts was higher in the elderly patients ($p=0.00007$). The incidence of favorable treatment outcomes was significantly lower in elderly patients ($p=0.00004$).

Conclusion: Under our treatment protocol, patient age did not affect the incidence of SVS. Our protocol may be effective for the prevention of SVS after aneurysmal SAH regardless of patient age.

Keywords Subarachnoid hemorrhage • Cerebral vasospasm • Urokinase • Fasudil chloride

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Introduction

As vasospasm is the major cause of morbidity and mortality after aneurysmal subarachnoid hemorrhage (SAH), it is important to predict the risk for vasospasm, especially symptomatic vasospasm (SVS), for early diagnosis and intervention. While patient age is thought to be an independent predictor of vasospasm, this issue remains controversial. We routinely deliver urokinase intrathecally and fasudil chloride intravenously to patients with aneurysmal SAH treated by clipping or coiling. Patients with angiographic evidence of vasospasm are also injected intra-arterially with fasudil chloride. We compared the incidence of SVS among elderly and nonelderly patients treated under this protocol.

Materials and Methods

Between 2008 and 2010 at our hospital, 105 patients (Hunt and Kosnik [H&K] grade I–IV) underwent aneurysm surgery within 72 h of the insult. All were treated under the same protocol after aneurysmal clipping or coiling. They were divided into four groups. Group 1 ($n=23$) was comprised of elderly patients (older than 70 years) treated by clipping, group 2 ($n=11$) had elderly patients subjected to coiling, group 3 ($n=46$) consisted of nonelderly patients treated by clipping, and group 4 ($n=25$) was nonelderly patients subjected to aneurysmal coiling. The male:female ratio was 1:2.7; the mean age was 61.3 years.

Our treatment protocol is shown in Fig. 1. All patients treated by clipping ($n=69$) underwent intraoperative cisternal irrigation with urokinase (30 IU/ml); the intrathecal delivery of urokinase (20,000 IU/day) via cisternal drainage was added for 3 days after clipping. In patients treated by aneurysmal coiling ($n=36$), we also administered urokinase (20,000 IU/day for 3 days postcoiling) via spinal drainage. All 105 patients underwent the intravenous administration of fasudil chloride (90 mg/day) for 14 days after surgery. Digital

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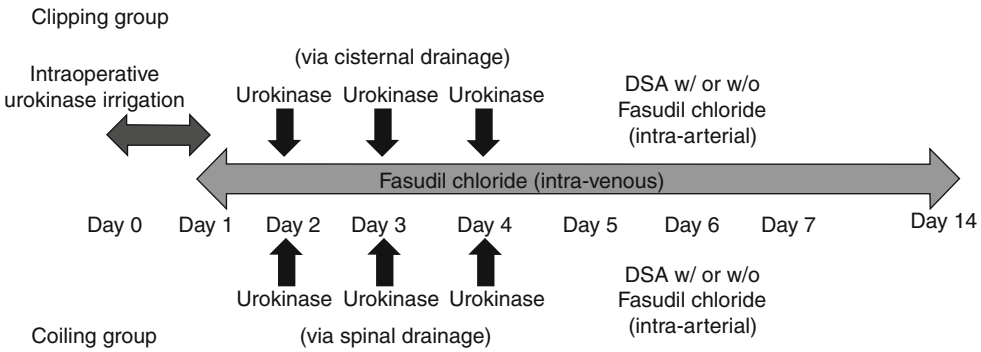

Fig. 1 Treatment protocol after clipping or coiling

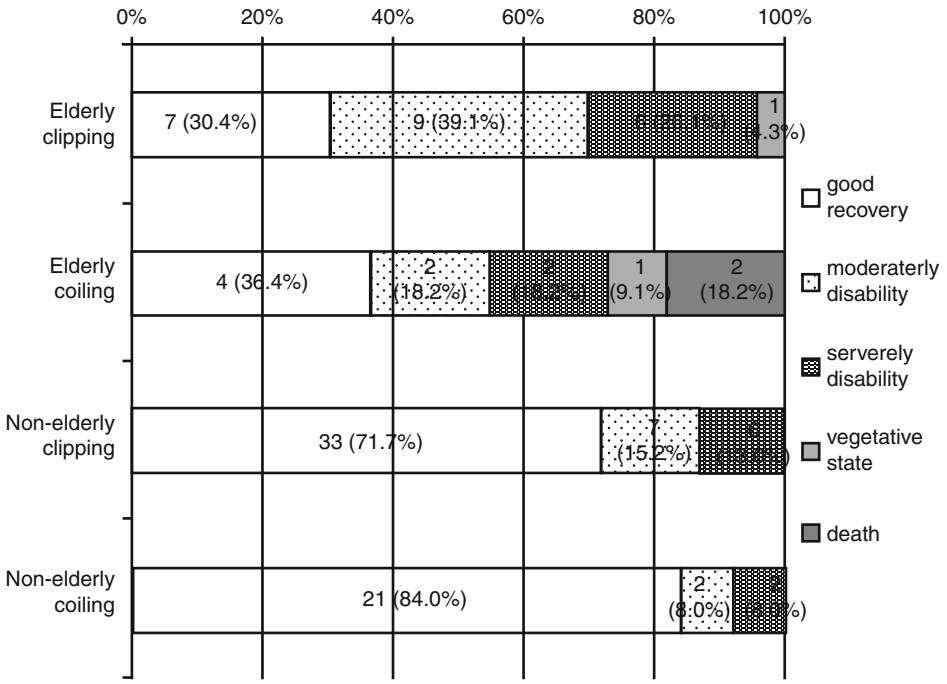

Fig. 2 Glasgow Outcome Scale of each group

subtraction angiography was performed on postoperative days 5–7. Patients with angiographic evidence of arterial narrowing were also treated with 30 mg fasudil chloride injected intra-arterially.

Treatment outcomes were evaluated based on the Glasgow Outcome Scale (GOS) at discharge, the incidence of SVS, the ratio of patients with ventriculo peritoneal (VP) shunts, and the length of hospitalization. Differences of $p<0.05$ by chi-square analysis were considered statistically significant.

Results

The H&K grade on admission was more severe in elderly patients. Among them, 21.7% of those subjected to clipping and 27.3% of patients treated by coiling manifested H&K grade IV; in nonelderly patients, the comparative incidence was 10.9% and 8.0%, respectively (Table 1). The aneurysm site was not significantly different between elderly and nonelderly patients. Middle cerebral artery (MCA) aneurysms prevailed among patients treated by clipping (clipping $n=26$, coiling $n=2$), while the aneurysms in the posterior circulation tended to be subjected to coiling (clipping $n=1$, coiling $n=7$).

The GOS at discharge was worse in the elderly irrespective of coiling or clipping. Among the elderly, 35.3% had unfavorable outcomes severely disabled, vegetative state and death; this was true in 11.3% of nonelderly patients ($p=0.00004$) (Fig. 2). Elderly patients subjected to coiling tended to have unfavorable treatment outcomes compared to patients treated by clipping; however, the difference was not statistically significant ($p=0.058$). Based on the GOS, patients with worse H&K grades at admission tended to have poorer treatment outcomes. The average length of hospitalization was 45.7 days for elderly and 35.3 days for nonelderly patients; the difference was not statistically significant

Table 1 Patient characteristics and outcomes

		Elderly clipping (n = 23, 21.9%)	Elderly coiling (n = 11, 10.5%)	Non-elderly clipping (n = 46, 43.8%)	Non-elderly coiling (n = 25, 23.8%)
Hunt &Kosnik grade	Grade 1 &2	7 (30.4%)	3 (27.3%)	22 (47.8%)	17 (68.0%)
	Grade 3	11 (47.8%)	5 (45.5%)	19 (41.3%)	6 (24.0%)
	Grade 4	5 (21.7%)	3 (27.3%)	5 (10.9%)	2 (8.0%)
Location of aneurysm	ICA	3 (13.0%)	4 (36.4%)	8 (17.4%)	10 (40.0%)
	MCA	10 (43.5%)	–	16 (34.8%)	2 (8.0%)
	AComA	7 (30.4%)	5 (45.5%)	14 (30.4%)	7 (28.0%)
	V-B	–	1 (9.1%)	1 (2.2%)	6 (24.0%)
	Others	3 (13.0%)	1 (9.1%)	7 (15.2%)	–
Incidence of vasospasm	Angiographic	12 (52.2%)	7 (63.6%)	22 (47.8%)	5 (20.0%)
	Symptomatic	1 (4.3%)	1 (9.1%)	3 (6.5%)	1 (4.0%)
Ventriculo-peritoneal shunt		9 (39.1%)	5 (45.5%)	8 (17.4%)	3 (12.0%)
Duration of hospitalization (days)		47.8	41.2	36.6	32.8

(p=0.67). Patients with higher H&K grades required longer hospitalization.

Among the elderly patients, 4.3% of those treated by clipping and 9.1% of patients subjected to aneurysm coiling experienced SVS; the comparative incidence in younger patients was 6.5% and 4.0%, respectively, and the differences were not statistically significant (p=0.40). The rate of angiographically confirmed vasospasm was lowest in nonelderly patients treated by coiling.

The incidence of VP shunt was significantly higher (41.2%) in elderly than nonelderly patients (15.5%) (p=0.00007) and was not affected by treatment via clipping or coiling.

Discussion

It remains controversial whether age is an independent predictor for the risk of SVS. Some studies showed that its incidence was lower in the elderly [1, 9, 10], while in others it was not significantly different [2, 6] or higher than in younger patients [4]. A lower incidence of SVS may be explicable by the presence of age-related atherosclerosis, which impairs the contractility and elasticity of the muscle wall of small arteries and arterioles [2, 9].

Although there was selection bias in our study, we found that the incidence of SVS was not significantly different for elderly and nonelderly patients (5.9% vs. 5.6%). In our series, the incidence of SVS was lower in both age groups compared to earlier reports. This suggests that our treatment protocol effectively prevented SVS in both elderly and nonelderly patients.

Our treatment protocol includes the intrathecal administration of urokinase to wash out SAH and the intravenous administration of fasudil chloride to prevent vasoconstriction. If arterial narrowing is observed on angiographs obtained 5–7 days after the operation, we inject fasudil chloride intra-arterially.

Cisternal irrigation therapy with urokinase is performed in many institutions. Kodama et al. [3] combined urokinase with ascorbic acid and lowered the occurrence of SVS in their patients to 2.8%. Sasaki et al. [7] demonstrated the efficacy and safety of the irrigation therapy with urokinase, and Moriyama et al. [5] delivered a bolus injection of urokinase via cisternal drainage to reduce the risk for SVS. Although a bolus injection may be less effective for washing out spasmogenic substances than continuous irrigation as it is easier to perform and lowers the risk for infectious complications, we chose this method in our patients.

Fasudil chloride is a rho kinase inhibitor that prevents myosin phosphorylation; its use is currently permitted only in Japan. Shibuya et al. [8] demonstrated that it effectively reduces the incidence of both angiographic and symptomatic vasospasm. To the best of our knowledge, ours is the first study to assess the effectiveness of combined therapy with intrathecal urokinase and intravenous fasudil chloride.

Conclusion

Multiple studies, as did ours, showed that advanced age is a predictor of a poor outcome after SAH [4]. Although the incidence of SVS was not significantly different in our age groups, the poorer outcomes in elderly patients may be explained by their worse clinical status at admission and a high incidence of hydrocephalus.

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

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Appendix-Select Abstracts

1. Repeate Intra-arterial Infusions of Fasudil Hydrochloride (IAF) and a Single Intra-arterial Infusion of Nicardipine (IAN) for Cerebral Vasospasm: A Case Report

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We report a case of repeated intra-arterial infusions of fasudil hydrochloride (IAF) and a single intra-arterial infusion of nicardipine (IAN) for cerebral vasospasm. A 67-year-old woman suffering from ruptured aneurysm was admitted to our hospital on day 12 from its onset of subarachnoid hemorrhage (SAH). Computed tomography (CT) showed minimal subarachnoid hemorrhage with subacute hydrocephalus. Cerebral angiography showed an aneurysm on the right internal carotid-anterior choroidal artery (IC-AchA), and severe and diffuse cerebral vasospasm was observed in the bilateral middle cerebral artery (MCA) area. We diagnosed her condition as SAH (Hunt and Kosnik grade 2, Fisher group 1) on day 12. Cerebral vasospasm was so severe it precluded performing coil embolization; first IAN (1 mg) was performed in the right M1 to dilate the proximal artery. By using IAN, we expected not only therapeutic effect for prolonged vasospasm but also a preventive effect for mechanical vasospasm. Then, the aneurysm was successfully embolized with platinum coils. Spastic vessels were partially dilated by IAN, and angiography showed improvement of their blood flow to some extent after IAN and embolization. Because the vasodilative effects of IAN was mild and temporary, an additional treatment, 25 mg fasudil hydrochloride were injected into the bilateral spastic M1 artery selectively. Cerebral vasospasm was almost recovered after IAF. However, on the next day after embolization, the patient suddenly developed severe left motor weakness. Repeated angiography revealed recurrence of moderate cerebral vasospasm in the right M1, M2 segments. IAF was performed, and left motor weakness improved gradually. She had no neurological deficits on the day of discharge. Nicardipine is a calcium channel antagonist, and fasudil is a protein kinase inhibitor. Because of the different mechanism of vasodilative effects, IAF and IAN may be good combination therapy for treatment of vasospasm following SAH.

2. Comparison of Surgical- or Endovascular-Treated Aneurysmal SAH Patients with a Special Emphasis on Cerebral Vasospasm

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Introduction: We evaluated the incidence of delayed cerebral ischemia (DCI) and outcome in 139 consecutive subarachnoid hemorrhage (SAH) patients with a special reference to the treatment modality who were admitted to our hospital in the past 3 years for surgical or endovascular procedures to treat cerebral aneurysm. *Materials and Methods*: Patients were evaluated according to demographic data, severity of illness on admission according to Fisher and WFNS (World Federation of Neurosurgical Societies) grade, incidence of DCI, outcome, and choice of treatment. Statistical evaluations were made by *t* test for parametric and Fisher's exact test for nonparametric values. *Results*: All patients' demographic data were as shown in Table 1. Clinical vasospasm diagnosis was made by the existence of three signs: headache, neurological deficit, and worsening of Glasgow Coma Scale (GCS) score. Due to this evaluation, 42 of the 139 patients demonstrated DCI. While 26 of them were in clipping group, the other 16 were in the coiling group. Hypertensive (H) therapy and cerebrospinal fluid (CSF) drainage with lumber puncture started more liberally, to 88 and 57 patients, respectively, due to at least two of the following findings or clinicians' decision: headache, agitation, elevated leukocyte level (without infection), new motor deficit, and worsening in GCS score. The severity of cases on admission in the clipping and coiling groups was similar for both (APACHE-II [Acute Physiology and Chronic Health Evaluation II]: 3.8 ± 2.8 to 6.3 ± 5.8).

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Although mortality and morbidity rates were higher in the coiling group, there was no statistical difference in both groups (Table). *Conclusion*: This study demonstrated that although mortality, morbidity rates, and DCI incidence were lower in the surgical group, there was no statistical significance.

3. Magnesium Use on Prophylaxis of Vasospasm Morbidity and Mortality Rate in Subarachnoid Hemorrhage (SAH)

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Introduction: Cerebral aneurysms are an important cause of morbidity and mortality. We propose this study in order to reach two endpoints: (1) its clinical incidence, confirmed by computed tomography (CT) and (2) the mortality of these patients in 28 days. It shows a comparison of a group of patients who received magnesium (intervention group 1) from those who did not use it (control group 2). *Method*: After institutional approval and informed consent, a prospective, randomized, single-blind study was conducted between February 2008 and March 2009. The study evaluated the magnesium use on patients from the 1 to 4 beds and control group from 5 to 8 beds. The serum measure of magnesium was made by colorimetry to reach a measurement between 2.5 and 3.5 mg/dl using a solution of 2% magnesium (5% specific gravity, 400 ml + 10% $MgSO_4$ 100 ml/24 h) during the first 14 days after an event (aneurysm rupture). *Admission Criteria*: Patients diagnosed with subarachnoid hemorrhage (SAH) confirmed by CT or cerebral angiography and Î"t less than 96 h. *Exclusion Criteria*: We excluded patients with SAH and Î"T > 96 h; patients who presented a vasospasm episode in less than 24 h of Mg solution infusion; multiple organic failure; previous hepatic failure documented or total bilirubin (TB) >1.2. *Results*: In a previous study, we analyzed a total of 94 patients with $n=46$ in group 1 and $n=48$ in group 2 (Tables 1 and 2). The main results were as follows: Group 1 vasospasm frequency 19.6%, confidence interval (CI) 9.4–33.9%; and mortality 17.4% in 28 days, CI 7.8–31.4%; group 2 vasospasm frequency 54.2%, CI 39.2–68.6%, and mortality 22.9% in 28 days, CI 12.0–37.3%. The analysis for the vasospasm showed an odds ratio (OR) of 0.20, CI 0.08–0.51%, and $p=0.0011$; the mortality had an OR of 0.70, CI 0.25–1.95%, and p value = 0.6818. *Conclusion*: Group 1 obtained greater protection on the vasospasm incidence in comparison to group 2 but showed no difference in mortality. The p value was significant for vasospasm but still not significant for mortality.

4. Nitroprusside Sodium Intrathecal for Prophylaxis and Treatment of Cerebral Vasospasm Associated with Subarachnoid Hemorrhage

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Introduction: Therapy using sodium nitroprusside (SNP) intrathecal aims for a more effective approach for prophylaxis and treatment of cerebral vasospasm associated with subarachnoid hemorrhage (SAH). *Results*: There were two patients; the first was a female 62 years old with aneurysm rupture of the left posterior communicating artery, SAH Fisher III, Hunt and Hess 2. The second was a male 46 years old with artery rupture of the middle cerebral artery, SAH Fisher III, Hunt and Hess 2. Both submitted to embolization, leading to acute hydrocephalus, in which external ventricular drainage (EVD) was established. Through the EVD, a prophylactic intrathecal protocol was instituted (2 ml SNP solution with 10.5 ml normal saline 0.9%, applying 2 ml SNP through the EVD each 12 h for 1 h by infusion pump). Patients evolved well with no neurologic or motor sequelae; with removal of the EVD and insertion of a ventricle-peritoneal shunt, patients were sent to a ward, then discharged without complications with a modified Rankin scale of 0. A third patient was male, 37 years old, with aneurysm rupture of the anterior communicating artery, SAH Fisher III, Hunt and Hess 4; severe vasospasm per operative in the left middle cerebral artery was treated by angioplasty with a balloon. Starting by lumbar catheter, the treatment protocol for the cerebral vasospasm was 50 mg (2 ml) SNP in solution with 4 ml SNP at 6 ml of normal saline 0.9%, applying 4 ml through the lumbar catheter every 12 h for 1 h by infusion pump. The patient progressed without complications with modified Rankin scale of 1. *Conclusion*: The use of intraventricular SNP may be a viable therapeutic option in preventing and treating cerebral vasospasms and cerebral ischemia. We noted that the cost for prophylactic therapy for 14 days was U$627.86; if the patient developed clinical vasospasm, the cost for a 14-day treatment would be an average of U$15,287.80, having a great impact on the reduction of morbidity, mortality, and cost of hospital stay.

5. Simvastatin Improves Outcomes in Subarachnoid Hemorrhage with Heavy Blood Load: Results from a Single-Centre Audit

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Introduction: Do statins reduce the risk of vasospasm, delayed cerebral ischemia, and death after aneurysmal subarachnoid hemorrhage (SAH)? Two recent meta-analyses [1, 2] reached opposite conclusions. The current analysis looked at outcomes associated with different prescribing practices by two neurovascular surgeons within our neurosurgical department. *Materials and Methods*: We analyzed 150 patients; 80 patients received simvastatin, and 70 patients did not. Primary outcome measures were modified Rankin score (mRS) and

Extended Glasgow Outcome Scale (EGOS) score. Secondary outcome measure was infarction on computed tomography (CT). The interaction between Fisher grade and outcome was also assessed. *Results*: In the statin group at 8 months follow-up, the mRS was better (1.1 vs. 2.5), as was the EGOS (6.8 vs. 5.5). The incidence of infarction was also less in the statin group (5% vs. 7%). Fisher's exact test was used to assess the interaction between functional outcome and Fisher grade. Patients were dichotomized into those with Fisher grade 3 (F3) or those who fulfilled both Fisher 3 criteria and had intraparenchymal/intraventricular blood (F3+F4). In these subsets, the improvement in functional outcome was magnified (mRS 1.0 vs. 3.0 and EGOS 7.0 vs. 5.3, $p<0.05$). Incidence of infarction was also significantly reduced in patients who had Fisher 3 or Fisher 3 plus 4 hemorrhages (3% vs. 9% for Fisher 3 and 3% vs. 8% for Fisher 3 plus 4, $p<0.05$). *Conclusion*: There is a trend towards better outcomes in patients who received simvastatin. The trend towards better functional outcomes and reduced incidence of infarction was magnified in those patients with Fisher 3 and Fisher 3 plus 4 subarachnoid hemorrhages. The results of the ongoing STASH trial and other large randomized controlled trials will provide further insight into this potentially important therapeutic intervention.

Aneurysm Formation, Characterization, Treatment

6. Anatomical Classification of Posterior Communicating Artery Aneurysms

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This article aims to review literature on the classification of aneurysms of the posterior communicating artery. Scientific research on aneurysms of the posterior communicating artery is a practical necessity for clinical neurology and neurosurgery as they represent 0.1–2.8% of all aneurysms. The classification of aneurysms of the posterior communicating artery is important in that it favors a firm diagnosis to facilitate surgical technique. The results showed that there are marked differences in the results of surveys conducted among the authors studied, yet all believed that the classification of aneurysms of the posterior communicating artery facilitates the surgical technique and can eliminate possible complications during surgery.

7. Lenticulostriate Brain Aneurysms

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Institution: Santa Paula Hospital, São Paulo, Brazil

Objective: Lenticulostriate artery (LSA) aneurysms are rare. We present two cases of lenticulostriate artery aneurysm, their clinical presentation, diagnosis, surgical treatment, and postoperative follow-up, with a review about the topic. *Case Reports*: We report two cases of LSA aneurysms among 194 surgically treated aneurysms in Santa Paula and São Camilo Hospital. The first one is a 48-year-old whose aneurysms were detected incidentally in an angiographic study performed due to a cavernous sinus thrombosis. Both cerebral angiogram and magnetic angioresonance were performed and demonstrated a left lenticulostriate aneurysm and two parassylvian aneurysms. The other case is a 62-year-old Japanese hypertensive patient who presented with subarachnoid hemorrhage (SAH), Fisher scale 2, and Hunt and Hess classification 2. Her angiogram demonstrated right LSA, left middle cerebral artery, and anterior communicating artery aneurysms. No other pathology or infectious etiology was noted. Both patients were treated by opening of the sylvian fissure, allowing the visualization of lenticulostriate vessels and aneurysm clipping. Postoperative angiographies were performed in both cases. During 2-year follow-up, the younger patient remained with distal right arm paresis and Rankin scale score 1; the older patient developed normal-pressure hydrocephalus and needed peritoneal ventricular drainage. *Conclusion*: LSA aneurysms are uncommon. The most common clinical presentation is intraparenchymal hemorrhage. Microsurgical treatment is often the chosen modality of intervention. Elderly people are more likely to develop postoperative complications before and after hospital discharge. They are under higher risk of more severe vasospasm and risk of hydrocephalus development in cases of ruptured aneurysms.

8. Biological Mechanisms of Adaptive Remodeling in Flow-Loaded Cerebral Arteries: Potential Significance to the Pathogenesis of Cerebral Aneurysms in a Rat Model

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Cerebral aneurysms (CAs) are the product of an interaction between hemodynamic factors in a permissive biological and anatomical milieu; in animal models, flow loading is a necessary hemodynamic factor. The spectrum of promotional biological factors likely includes variant forms of the adaptive response to flow loading. Using a rat model, this study characterizes the molecular and anatomical events comprising the cerebral arterial response to flow loading and reveals their significance in relation to the CA phenotype. Rats were subjected to bilateral common carotid artery ligation or sham carotid surgery. Tail cuff blood pressure was monitored at baseline and postoperatively. Nineteen days after surgery, the vertebrobasilar arterial complex was harvested from each rat and processed for analysis of messenger RNA (mRNA) and protein expression. Each basilar artery terminus was

preserved and processed for histological examination. Flow-induced changes in mRNA expression, protein expression, and mural structure/histology were determined by comparing carotid ligated to sham surgery rats. Flow-induced changes in mRNA and protein expression were assessed. Blood pressure was not significantly different between sham and carotid-ligated animals. Over 1,500 significantly ($p<0.05$) altered gene expressions (more than twofold) were identified. These included Cbp/p300-interacting transactivator, a vascular endothelial growth factor (VEGF)-responsive element active during vascular remodeling; interferon gamma receptor 1, known to be activated in vascular/endothelial repair after injury; abselon helper integration site 1, involved in actin filament generation; alpha-2 laminin, a blood-brain barrier component; and ectonucleotide pyrophosphatase/phosphodiesterase, which has been shown to be upregulated in vascular calcification and plaque formation. Thus, our preliminary findings suggested several interconnected processes implicated in the pathogenesis of CA.

Animal Models of SAH/Vasospasm

9. Intracisternal Magnesium Injection Therapy (Experimental Data for Its Clinical Application)

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Introduction: The temporal profile of the vasodilatory effect and optimal cerebrospinal fluid (CSF) Mg^{2+} concentration after intracisternal magnesium sulfate ($MgSO_4$) solution were investigated in the canine subarachnoid hemorrhage (SAH) model (part 1). We also evaluated $MgSO_4$ therapy via a microcatheter for the treatment of cerebral vasospasm in the clinical setting of endovascular treatment of ruptured aneurysm (part 2). *Materials and Methods*: Cerebral vasospasm was induced in 33 dogs. Part 1: In 26 dogs, on day 7, 0.5 ml/kg of 15, 10, 5, or 0 mmol/l $MgSO_4$ in Ringer was injected into the cisterna magna. Angiography was performed on day 1 and before and 1, 3, and 6 h after the intracisternal injection on day 7 to measure arterial diameters. Part 2: In 7 dogs, angiography was repeated on day 1 and on day 7 before and 1.5 h after injection of 0.5 ml/kg of 15 mmol/l $MgSO_4$ via the tip of a microcatheter placed in the cisterna magna from the lumbar spine. *Results*: Part 1: The arterial diameter ratios (ratio of arterial diameter after $MgSO_4$ injection to diameter before injection on day 7) at 1 and 3 h after intracisternal injection were positively correlated with CSF Mg^{2+} concentration. Arterial diameter ratios exceeded 1 if the CSF Mg^{2+} concentration was more than 3 mEq/l. Animals with CSF Mg^{2+} concentration more than 3 mEq/l showed significantly increased arterial diameters 3–6 h after injection compared with before injection. Part 2: After intracisternal injection of $MgSO_4$ via a microcatheter, the spastic arterial diameters significantly increased (Fig. 1). *Conclusion*: The reversible effect of intracisternal $MgSO_4$ therapy requires a CSF Mg^{2+} concentration of more than 3 mEq/l. The vasodilatory effect continues for 3–6 h after injection; therefore, continuous or intermittent injection is needed to constantly ameliorate vasospasm. Intracisternal $MgSO_4$ therapy using a microcatheter can be effective against vasospasm in the clinical setting of endovascular treatment of ruptured aneurysm.

10. Dissociation of Vasospasm and Secondary Effects of Experimental Subarachnoid Hemorrhage by Clazosentan

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Introduction: A common complication of subarachnoid hemorrhage (SAH) is cerebral vasospasm of the large arteries at the base of the brain. However, a recent clinical trial of clazosentan demonstrated a 65% relative risk reduction in angiographic vasospasm but had no effect on clinical outcome. We used clazosentan to gain insight into the pathophysiology of SAH by determining if decreasing vasospasm is associated with alleviation of other secondary complications of SAH, such as oxidative stress, endothelial nitric oxide synthase (eNOS) dysfunction, microthromboembolism, loss of long-term potentiation (LTP), and neuronal injury. *Materials and Methods*: Mice or rats were subjected to SAH by injection of blood into the chiasmatic cistern. For rats, 10 mg/kg clazosentan bolus was administered intravenously 1 h after SAH, followed by a continuous infusion of 1 mg/kg through an osmotic pump with a catheter inserted into the jugular vein. For mice, 1 mg/kg bolus was given intraperitoneally 1 h after SAH and infused through an osmotic pump implanted into the peritoneum. Infusions were for 48 h (mice) or 7 days (rats). Middle cerebral artery vasospasm, microthromboemboli, cerebral blood flow, neuronal injury, and mortality were assessed for both mice and rats. Mice were also assessed for eNOS uncoupling, superoxide anion radical, and peroxynitrite in the brain. Rats were also tested for LTP presence. *Results*: Clazosentan preserved cerebral blood flow, alleviated vasospasm, and decreased mortality but did not affect superoxide anion radical, peroxynitrite, and microthromboemboli or prevent endothelial NOS uncoupling and neuronal injury in mice. In rats, clazosentan reduced vasospasm and mortality but did not reverse the loss of LTP, microthromboemboli or neuronal cell death. *Conclusion*: This study suggested that large-artery vasospasm is pathophysiologically independent of some other effects of SAH. The findings have implications for development of treatments for this disease.

11. Predeterminative Effect of Pterygopalatine Ganglion Neuron Density on Choroid Plexus Degeneration in Subarachnoid Hemorrhage-Induced Choroidal Artery Vasospasm: Experimental Study

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Introduction: The choroid plexuses (CPs) have important roles in cerebral nutrition, detoxification, immunity, endocrine, secretory, repository, and regulation of cerebrospinal fluid pH areas [1]. CP degeneration may be possible due to anterior choroidal artery (AChA) vasospasm induced by subarachnoid hemorrhage (SAH) [2]. We examined whether there is a relationship among CP degeneration, severity of AChA vasospasm, and vasodilatory pterygopalatine ganglion (PPG) [3] neuron density in SAH. *Materials and Methods*: This study was conducted on 24 rabbits that had formerly been used in experiments. Five of them were normal, 5 of them from sham, and 14 were from animals with SAH created by injecting autologous blood into their cisterna magna and followed up 20 days. Their CPs, AChAs stained with TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling), and PPGs stained with masson's trichrome (MTC) were examined stereologically. Wall ring surface/lumen surface values ($R2–r2/r2$) were accepted as the vasospasm index (VSI). Densities of CP cells and PPG neurons were estimated stereologically. Densities of apoptotic CP cells and VSI values were compared with PPG neuron density. The data analysis consisted of the Kruskal-Wallis and Mann-Whitney *U* test. *Results*: The mean VSI was 0.375 ± 0.032, the mean CP cells density was $10{,}320 \pm 1{,}890/mm^3$, the mean apoptotic CP cells number was $110 \pm 14/mm^3$, and the mean neuronal density of the PPGs was $12{,}310 \pm 1{,}590/mm^3$ in all animals ($n=24$). There were no important differences in the sham ($n=5$; $p<0.5$) and in animals that developed less vasospasm animals ($n=6$; $p<0.05$). The mean VSIs were estimated as 1.380 ± 0.150, apoptotic CP cell density was $6{,}342 \pm 957/mm^3$, and the mean neuronal density of the PPGs was $8{,}960 \pm 990/mm^3$ in animals that developed severe vasospasm ($n=8$; $p<0.005$) (Fig. 1: a, b : PPG; c: ChA; d: CP). *Conclusion*: The high neuron density of PPGs may play significant roles on the prevention of AChA vasospasm. Ischemic CP degeneration may play significant roles on the development of various neurodegenerative diseases.

12. Preconditioning Atorvastatins Attenuate Endothelins in Chronic Vasospasm

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Statins have been reported to provide a beneficial effect in cardiovascular disease as well as neurodegenerative disease. Evidence of clinical and experimental study also revealed these compounds have the potential to induce apoptosis in neurons and astroglia. Thus, it was of interest to examine the effect of statins on endothelin-1 (ET-1), which is generated partly from glia cells, and vasospasm in experimental subarachnoid hemorrhage (SAH). A rodent SAH model was employed. Animals were assigned to four groups (sham, vehicle, 10 mg/day atorvastatin preconditioning, 10 mg/day atorvastatin). Basilar artery cross-sectional area was measured to evaluate vasospasm. Cerebrospinal fluid ET-1 was measured using enzyme-linked immunosorbent assay (ELISA). Significant vasospasm was noted in the vehicle group (lumen potency 63.4%, compared with the sham group), but neither in the atorvastatin preconditioning and treatment group (lumen potency, 87.3% and 70.2%, respectively). In addition, preconditioning atorvastatin effectively decreased release of ET-1 in cerebrospinal fluid ($p=0.204$) when compared with the sham group and avoided experimental vasospasm, while the same condition was not found in the atorvastatin treatment group. This study supported that preconditioning with atorvastatin attenuates endothelin production in vasospasm and suggested that astroglia might play a role in chronic vasospasm.

13. Thalidomide, a Glutamic Acid Derivative, Attenuates TNF-α-Mediated Adhesive Molecules in Experimental Vasospasm

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Increased adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin, are observed in the serum of patients following aneurysmal subarachnoid hemorrhage (SAH). Thalidomide proved to be effective in halting arterial narrowing in a rodent SAH model. This present study was to examine the effect of thalidomide on adhesion molecules/tumor necrosis factor α (TNF-α) in this animal model. *Methods*: A rodent SAH model was employed. Animals were each injected with autologous blood into the cisterna magna, and oral administration with thalidomide (1 mg/kg) was initiated 1 h before (prevention) or later (reversal). The compound was subsequently administered at 24-h intervals post-SAH. Serum samples were gathered at 72 h post-SAH to determine TNF-α, ICAM-1, VCAM-1, and E-selectin levels (via enzyme-linked immunosorbent assay, ELISA). The basilar arteries (BAs) were harvested and sliced to measure their cross-sectional areas. Furthermore, TNF-α (1 ng/ml) was administered to test the downregulation of thalidomide on TNF-α-mediated adhesion molecules. *Results*: Morphologically, convoluted internal elastic lamina, deformed endothelial walls, and necrotic smooth muscle

were well perceived in the SAH groups that were not in the thalidomide plus SAH groups or the healthy controls. The levels of ICAM-1, VCAM-1, and E-selectin were increased in all animals subject to SAH (SAH-only and SAH plus vehicle groups) compared with the healthy controls (no SAH), but not in the thalidomide group (SAH plus thalidomide reversal and prevention). Likewise, the levels of TNF-α in the SAH-only and SAH plus vehicle groups were significantly elevated ($p<0.01$), and pretreatment and treatment with thalidomide reduced TNF-α to control levels. *Conclusion*: These results showed that TNF-α may play a role in mediating SAH-induced vasospasm, and a reduction of both adhesive molecules and TNF-α after SAH may contribute to the antispastic effect of thalidomide.

14. Intracranial-Pressure-Controlled Rabbit Subarachnoid Hemorrhage Model for the Study of Early Brain Injury

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Introduction: Cerebral vasospasm is no longer believed to be solely responsible for unfavorable outcome following subarachnoid hemorrhage (SAH). Pathophysiological derangements at the time of bleeding and within the first few days are made responsible for significant brain damage. This phenomenon of early brain injury (EBI) after SAH has moved into the center of current research activities. To this day, a rabbit model that reflects early events after SAH is missing. *Methods*: Experimental SAH was initiated by opening of an extra-intracranial (EC/IC) shunt from the subclavian artery into the cerebromedullar cistern in eight rabbits. Standard cardiovascular monitoring (arterial blood pressure, heart rate, electrocardiogram [ECG], end-tidal carbon dioxide partial pressure), intracranial pressure (ICP), cerebral perfusion pressure (CPP), and bilateral regional cerebral blood flow (rCBF) were continuously measured. Apoptosis was detected 24 h post-SAH using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Neurodegeneration secondary to ischemia was determined using Fluoro-jade B (FJB) in bilateral basal cortex and hypocampal regions (CA1 and CA3). *Results*: After initiation of the EC/IC blood shunt, ICP rose to diastolic blood pressure within 1–2 min and returned to a steady state in 3–5 min. Increase of ICP caused decrease of CPP to almost zero (8.2 ± 4.9 mmHg) and drop of left and right rCBF to 23% ± 8% and 19% ± 9% of its baseline value. TUNEL- and FJB-stained sections revealed significant apoptosis and neurodegeneration in both cortex and hypocampal regions when compared to sham-operated animals ($p<0.05$). *Conclusion*: The presented experimental bleeding technique closely simulated human pathophysiological sequelae of aneurysm rupture and reflected early pathophysiological derangements after SAH. The detection of marked neuronal cell death and neurodegeneration warrants the study of EBI in the EC/IC blood shunt-induced SAH model in rabbits.

15. Effect of Intrathecal Injection of Lipocalin-Type Prostaglandin D Synthase on Cerebral Vasospasm After Subarachnoid Hemorrhage and Outcome in Primates

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Lipocalin-type prostaglandin (PG) D synthase (L-PGDS) is one of the major proteins in human cerebrospinal fluid (CSF) and acts as both a PGD2-producing enzyme and as an extracellular transporter for lipophilic ligands. We have already reported that the L-PGDS concentration increased in the CSF of patients with aneurysmal subarachnoid hemorrhage (SAH) and combined with biliverdin, which was one of vasoconstrictors [1, 2]. In this paper, we present a newly developed, less-invasive SAH model with delayed cerebral vasospasm using a primate and investigate the effect of intrathecal administration of L-PGDS on cerebral vasospasm and cognitive outcome. Under general anesthesia, a polyethylene tube was inserted into the subarachnoid space by a lumbar puncture, and the tip of the tube was placed in the prepontine cistern of a cynomolgus monkey. SAH was induced by 2 ml of arterial blood injection through the tube twice with a 2-day interval. L-PGDS (2 mg/kg/day) (L-PGDS group, $n=5$) or artificial CSF (control group, $n=6$) was administrated through the tube for 7 days after SAH. Serial cerebral angiography, magnetic resonance imaging (MRI) (perfusion study), and food retrieval test [3] (to estimate spatial memory) were performed for 1 month. All monkeys showed cerebral vasospasm on angiogram 7 days after SAH. Diameter of the basilar artery of the control group (68.82% ± 5.03%) was smaller than that of the L-PGDS group (79.00% ± 8.07%), but there was no statistical significance. MRI perfusion study showed that mean transit time (MTT) tended to be prolonged in both groups on days 7–14. Spatial memory function of L-PGDS group in the chronic state was significantly better than that of the control group. We developed a less-invasive and reproducible SAH model with delayed cerebral vasospasm using a primate. Intrathecal administration of L-PGDS improved chronic cognitive outcome after SAH and would become a new treatment for SAH sequelae.

16. L-Citrulline Therapy Prevents Basilar Artery Vasospasm in Haptoglobin 2-2 Transgenic Mice After Induced Subarachnoid Hemorrhage

Authors: Pradilla, Gustavo; Garzon-Muvdi, Tomas; Ruzevick, Jacob J.; Bender, Matthew; Edwards, Lindsay; Momin, Eric; Thompson, Reid C.; Tamargo, Rafael J. (Presenting)

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Introduction: Depletion of nitric oxide (NO) and periadventitial inflammation contribute to the pathophysiology of posthemorrhagic cerebral vasospasm. L-Citrulline is an amino acid that participates in the urea cycle and increases L-arginine levels, resulting in elevated NO synthesis. Transgenic C57Bl6 mice with a haptoglobin (Hp) 2-2 genotype appear to develop more severe vasospasm, with higher concomitant inflammatory cell penetration than wild type (Hp1-1) mice after subarachnoid hemorrhage (SAH). In the present study, the toxicity of systemic L-citrulline, its effect on basilar artery (BA) vasospasm, neurobehavioral scores, and inducible nitric oxide synthase/endothelial nitric oxide synthase (iNOS/eNOS) expression after SAH were evaluated in Hp2-2 mice. *Materials and Methods*: Hp2-2 genotypes were confirmed by reverse transcriptase polymerase chain reaction (RT-PCR). L-Citrulline toxicity was assessed with escalating doses. To test efficacy, Hp1-1 and Hp2-2 mice ($n=64$) were divided into four groups ($n=32$/genotype): sham surgery ($n=8$), SAH no treatment ($n=8$), SAH plus vehicle ($n=8$), and SAH plus L-citrulline (200 mg/kg every 8 h ip, $n=8$). Post-SAH neurobehavioral scores were recorded at 24 h, animals were perfused, and BAs were processed for analysis. Expression of iNOS and eNOS was determined by RT-PCR. *Results*: The administration of L-citrulline resulted in higher BA lumen patencies in both genotypes (Hp1-1: SAH plus vehicle 77.8% ± 3.2% vs. SAH plus L-citrulline 91.8% ± 5.9% [mean ± SEM, $p<0.05$]; Hp2-2, SAH plus vehicle 67.1% ± 2.0% vs. SAH plus L-citrulline 86.9% ± 2.2%, $p<0.0001$). Neurobehavioral scores were higher in Hp2-2 mice treated with L-citrulline (SAH plus vehicle 1.2 ± 0.2 vs. SAH plus L-citrulline 2.4 ± 0.2, $p<0.01$). Expression of iNOS and eNOS increased in Hp2-2 mice after L-citrulline treatment, but limited sample sizes prevented further statistical analysis. L-Citrulline was not toxic even at the highest dose. *Conclusion*: L-Citrulline was safe, increased BA patency, neurobehavioral scores, and NOS expression in Hp 2-2 mice post-SAH, and was a potential agent for treatment of vasospasm after SAH.

17. Administration of S-4-CPG (S-4-Carboxyphenylgicine) Decreases Vasospasm in Haptoglobin 2-2 Mice by Inhibition of Metabotropic Glutamate Receptors

Authors: Garzon-Muvdi, Tomas (Presenting); Pradilla, Gustavo; Ruzevick, Jacob; Bender, Matthew; Edwards, Lindsay; Tamargo, Rafael

Institution: Johns Hopkins School of Medicine, Baltimore, MD, USA

Introduction: Cerebral vasospasm is an important cause of stroke after aneurysmal subarachnoid hemorrhage (SAH) [1, 2]. Infiltrating immune cells such as neutrophils secrete glutamate [3]. S-4-CPG (S-4-carboxyphenylgicine) is a selective antagonist of metabotropic glutamate receptors (mGluR) 1 and 5, which are expressed in endothelial cells. Vasodilator-stimulated phosphoprotein (VASP) is a protein that regulates endothelial function by modulating actin polymerization and tight junctions and nitric oxide/cGMP pathway effects. Hp2-2 genotype in humans predicts a higher risk of presenting vasospasm after SAH. We tested the efficacy of S-4-CPG in the treatment of vasospasm after induction of SAH in Hp2-2 and Hp1-1 mice. *Methods*: The penetration of S-4-CPG through the blood-brain barrier (BBB) in vivo after systemic injection was studied. Hp1-1 and Hp2-2 mice were randomized to four groups to measure basilar artery lumen patency: (1) sham surgery (sham, $n=5$), (2) SAH only ($n=5$), (3) SAH plus vehicle ($n=8$), and (4) SAH plus S-4-CPG ($n=8$). We evaluated the presence of neutrophils surrounding the basilar artery after experimental SAH. VASP phosphorylation status in response to S-4-CPG and glutamate was assessed through immunoblotting. *Results*: S-4-CPG was able to cross the BBB with a concentration of 4.02 ± 0.7 μg/ml at 1.5 h ($t½ = 2.76 ± 1.8$ h). S-4-CPG was not toxic to wild-type mice. Treatment of mice with S-4-CPG after SAH significantly decreased vasospasm after 24 h. Exposure of human brain microvascular endothelial cells to glutamate decreased the phospho-VASP. S-4-CPG maintained phospho-VASP in the presence of glutamate. *Conclusion*: S-4-CPG is a potential therapeutic agent to prevent vasospasm after aneurysmal SAH. It crosses the BBB and prevents vasospasm after SAH. Maintenance of phospho-VASP suggests a possible mechanism for this effect through the maintenance of the integrity of the BBB and possibly maintenance of the nitric oxide/cGMP signaling pathway.

18. Continuous Intravenous Sodium Nitrite Can Maintain Reversal of Cerebral Vasospasm After Subarachnoid Hemorrhage in Primates

Authors: Fathi, Ali R. (Presenting); Pluta, Ryszard M.; Bakhtian, Kamran D.; Lonser, Russell R.

Institution: Kantonsspital Aarau, Aarau, Switzerland

Introduction: Subarachnoid hemorrhage (SAH)-induced vasospasm is a significant cause of morbidity and mortality. While long-term intravenous sodium nitrite ($NaNO_2$) infusion has been shown to prevent cerebral vasospasm after SAH in primates, the purpose of this study was to determine if intravenous $NaNO_2$ can reverse established SAH-induced vasospasm in primates and whether this effect can be maintained with continuous infusion. *Methods*: SAH-induced vasospasm was created in 14 cynomolgus macaques. Animals were randomized to either control (saline infusion, $n=5$) or treatment groups (intravenous $NaNO_2$ infusion for 3 h [$n=7$] or 8 h [$n=2$] at 300 μg/kg/h). Arteriographic vessel diameter was blindly analyzed to determine the degree of vasospasm. Nitric oxide metabolites (NO_2, NO_3, and S-nitrosothiols) were measured in whole blood and cerebrospinal fluid (CSF). *Results*: Moderate-to-severe vasospasm was present in all animals before treatment (control 36% ± 8.3% [SD]; treatment 45% ± 12.5%; $p=0.9$). While saline infusion did not

reduce vasospasm, $NaNO_2$ infusion significantly reduced the degree of vasospasm (27% ± 7.6%; $p = 0.008$). Two hours after cessation of $NaNO_2$ infusion, the reduction in vasospasm persisted (mean increase vessel diameter, 17.8% ± 10.9%; $p < 0.05$). The $NaNO_2$-induced vasodilation lasted 4 h after infusion cessation. At 6 and 8 h after infusion cessation, vessels returned to spasm (mean reduction artery diameter, 38.4% ± 8.3%). Continuous $NaNO_2$ infusion over 8 h maintained a significantly reduced degree of vasospasm (32.3% ± 5.3% compared to 56.8% ± 23.1% after SAH; mean increased vessel diameter 24.5% ± 17.8%). Nitrite (peak value 3.7 ± 2.1 μmol/l), nitrate (18.2 ± 5.3 μmol/l), and S-nitrosothiols (33.4 ± 11.4 nmol/l) increased significantly in whole blood, and nitrite increased significantly in CSF. *Conclusion*: These findings suggest that intravenous infusion of $NaNO_2$ can reverse SAH-induced vasospasm in primates and that this effect can be maintained with prolonged infusions.

Blood and CSF Biomarkers

19. The Value of Serial Plasma/CSF Nuclear and Mitochondrial DNA Levels in Aneurysmal Subarachnoid Hemorrhage

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Institution: Chang Gung Memorial Hospital, Kaohsiung, Taiwan

Introduction: Increased levels of plasma nuclear and mitochondrial DNA have been reported in critically ill patients, and extracellular DNA may originate from damaged tissues to peripheral blood. We tested the hypothesis that nuclear and mitochondrial DNA levels in cerebrospinal fluid (CSF) and plasma are substantially increased in acute spontaneous aneurysmal subarachnoid hemorrhage (SAH) patients and decrease thereafter, and that nuclear and mitochondrial DNA levels can predict treatment outcomes. *Materials and Methods*: We examined serial nuclear and mitochondrial DNA levels in CSF and plasma from 21 adult spontaneous aneurysmal SAH patients. The nuclear and mitochondrial DNA levels in CSF and plasma were also evaluated from 39 volunteer subjects who received myelography examinations during the study period. *Results*: Our data showed that circulating plasma nuclear DNA concentrations and both nuclear and mitochondrial DNA levels in CSF significantly increased in aneurysmal SAH patients at admission compared with the volunteer group. The levels of nuclear and mitochondrial DNA in both CSF and plasma were significantly increased initially and substantially decreased thereafter. Both CSF nuclear DNA and CSF mitochondrial DNA levels on admission were significantly negatively correlated with the Barthel Index (average) at 6 months after discharge (average) ($r = -0.668$, $p = 0.013$, and $r = -0.713$, $p = 0.006$, respectively) in this study. Both higher CSF nuclear (cutoff value of >85.1 ng/ml) and mitochondrial DNA levels (cutoff value of >31.4 ng/ml) at presentation were associated with worse outcome in aneurysmal SAH patients. *Conclusion*: Based on our results, the higher CSF DNA levels, rather than plasma DNA levels, at presentation were associated with a worse outcome. Therefore, we look forward to more prospective multicenter investigations specifically confirming the predictive value of CSF and plasma DNA levels in outcome prediction.

20. Serial Expression of SOCS3 in CSF After Subarachnoid Hemorrhage

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Institution: Aichi Medical University, Nagakute, Aichi, Japan

Introduction: We have previously reported that elevation of the inflammatory cytokine interleukin 6 (IL-6) activates the janus kinase signal transducer and activator of transcription 3 (JAK-STAT3) signaling pathway in the rat single subarachnoid hemorrhage (SAH) model (Fig. 1) [1]. This pathway might play an important role in cerebral vasospasm. In this study, we explored the expression of suppressor of cytokine signaling 3 (SOCS3), which regulates the JAK-STAT3 signaling pathway in CSF after SAH. *Materials and Methods*: Eight patients who underwent clipping surgery within 24 h after the onset of SAH (Fisher Group 2–3) were included in this study. Cerebrospinal fluids (CSFs) were collected during surgery (day 0) and on days 1, 3, 5, 7, 10 after surgery through the cisternal drainage tube. CSF samples from unruptured aneurysms were used as controls. Concentrations of IL-6 were measured using Enzyme-linked immunosorbent assay (ELISA) kits. SOCS3 immunoprecipitated from CSF with a SOCS3 antibody. The resulting immunocomplexes were subjected to Western blot analysis with SOCS3 antibody. *Results*: Concentrations of IL-6 in CSF were increased transiently on day 1 compared with day 0. Those of IL-6 decreased gradually thereafter. Expressions of SOCS3 were detected on days 1 and 3, which decreased thereafter. *Conclusion*: JAK-STAT3 signaling pathway activated by IL-6 is strictly regulated by multiple factors. SOCS3 is a well-known inhibitor of the JAK-STAT3 signaling pathway that attaches to JAK molecules and inhibits the activity of JAK. From our data, expression of SOCS3 was immediately induced in CSF and might regulate the signaling pathway of JAKSTAT3 after SAH.

21. Do NSE and S100 in Serum as Well as Excitatory and Inhibitory Amino Acids in CSF Indicate Cerebral Vasospasm or Ischemia After Subarachnoid Hemorrhage?

Author: Jung, Carla (Presenting)

Institution: University of Heidelberg, Heidelberg, Germany

Delayed cerebral vasospasm after aneurysmal subarachnoid hemorrhage (SAH) and delayed ischemic neurological

deficits are still feared complications after SAH. Although microdialysis has been demonstrated to be a useful method for detection of brain ischemia, it remains a local indicator for intracerebral events. Therefore, we sought to determine if alterations in neuron specific enolase (NSE) and S100 in serum or cerebrospinal fluid (CSF) levels of free amino acids (AAs), including inhibitory and exitatory AAs, may be associated with cerebral vasospasm or ischemic lesions in patients after SAH. Levels of free AAs (including glutamate, aspartate, glycine, and gamma aminobutyric acid (GABA)) in CSF were analyzed by high-performance liquid chromatography (HPLC) in patients after SAH. Cerebral arteriograms were performed to assess cerebral vasospasm, and follow-up CCT scans were performed to assess ischemic brain lesions. Glutamate increased after SAH. S100 in serum indicated ischemic events, and glycine was associated with arteriograpic cerebral vasospasm. Further studies with a larger number of cases are needed to validate these results.

Clinical Trials (Ongoing, Challenges, Design)

22. Effect of Intrathecal Urokinase, Intravenous Fasudil, and Additional Intra-arterial Fasudil for Prevention of Cerebral Vasospasm: Comparison Between Clipping and Coiling

Authors: Takazawa, Hiroki (Presenting); Sasaki, Tatsuya; Morita, Takahiro; Narisawa, Ayumi; Saito, Atsushi; Arai, Masayuki; Koide, Kenichiro; Harada, Jun; Hasegawa, Takeshi; Nishijima, Michiharu

Institution: Koseiren Takaoka Hospital, Takaoka, Toyama, Japan

Object: To prevent symptomatic vasospasm (SVS), study of intrathecal urokinase (UK), intravenous fasudil chloride (FC), and additional intra-arterial FC has been performed in patients who underwent acute surgery for aneurysmal subarachnoid hemorrhage (SAH). The effect of our protocol in preventing SVS was evaluated separately in clipping and coiling groups. *Materials and Methods*: From January 2008 to January 2011, we had 132 patients with aneurysmal SAH (Hunt and Kosnic grades I–IV). These patients were divided into two groups: clipping group (77 patients) and coiling group (55 patients). In the clipping group, intraoperative cisternal irrigation of UK occurred, and intrathecal UK via cisternal drainage was performed for 3 days after clipping. In the coiling group, intrathecal UK via spinal drainage was performed for 3 days after coiling. Intravenous administration of FC (90 mg) was combined in all cases for 14 days after surgery. Digital subtraction angiography (DSA) was performed at days 5–7, and intra-arterial administration of FC (30 mg) was added if the arterial narrowing was observed. *Results*: Intra-arterial FC was performed in 58 in the clipping group (75.3%) and in 34 patients in the coiling group (61.8%). Symptoms due to vasospasm remained in 3 of the clipping group (3.9%) and in 5 patients of the coiling group (9.1%). No adverse effect of these therapies was observed. *Conclusion*: Intrathecal UK and intravenous FC and additional intra-arterial FC may be safe and effective for prevention of SVS after aneurysmal SAH. There were fewer SVSs in the clipping group than in the coiling group. This result might reflect the effect of intraoperative UK irrigation.

23. A Prospective, Randomized, and Controlled Trial Investigating the Use of Lumbar Cerebrospinal Fluid Drainage Following Aneurysmal Subarachnoid Hemorrhage

Authors: Al-Tamimi, Yahia [Award Finalist] (Presenting); Bhargava, Deepti; Hall, Greg; Feltbower, Richard; Goddard, Anthony; Quinn, Audrey; Ross, Stuart

Institution: Leeds Teaching Hospital NHS Trust, Leeds, West Yorkshire, UK

Introduction: A single-center prospective, randomized, and controlled trial has been conducted to test the hypothesis that lumbar drainage of cerebrospinal fluid (CSF) following aneurysmal subarachnoid hemorrhage (aSAH) reduces the prevalence of delayed ischemic neurological deficit (DIND) and improves outcome. *Materials and Methods*: Patients with World Federation of Neurosurgical Societies (WFNS) grades 1–3 aSAH and Fisher grades 2–4 were randomized to either the study group of standard therapy plus insertion of a lumbar drain or the control group of standard therapy alone. The primary outcome measure was the prevalence of delayed ischemic neurological deficit. *Results*: We recruited 210 patients with aSAH (166 females, 44 males; median age 54 years, interquartile range 45–62 years) between October 2006 and July 2010 into the control ($n=105$) and study ($n=105$) groups of the trial. WFNS grades were as follows: 1, $n=139$; 2, $n=60$; and 3, $n=11$. Fisher grades were 2, $n=87$; 3, $n=85$; and 4, $n=38$. There was no significant difference in patient characteristics between the two groups. The prevalence of DIND was 35.2% in the control group and 21.0% in the study group. This was statistically significant ($p=0.021$). The prevalence of a modified Rankin score of 4, 5, or 6 at day 10 postictus was 62.5% in the control group and 44.8% in the study group ($p=0.009$). At 6 months, this was 18.6% in the control group and 19.8% in the study group (not significant, NS). There were 22 and 15 patients with a radiologically proven infarct at discharge in the control and study groups, respectively (NS). The prevalence of permanent CSF shunting was 7.6% in the control group and 5.7% in the study group (NS). There were two cases of meningitis associated with lumbar drain use and one case of a superficial lumbar drain exit site infection. All were treated successfully with antibiotics. *Conclusion*: There was a significant reduction in the prevalence of DIND and improvement in early outcome with the use of lumbar CSF drainage following aSAH.

24. Safety and Feasibility of Long-Term Intravenous Sodium Nitrite Infusion in Healthy Volunteers

Author: Pluta, Ryszard (Presenting)

Institution: NIH/JAMA, Bethesda, MD, USA

Introduction: Infusion of sodium nitrite could provide sustained therapeutic concentrations of nitric oxide (NO) for the treatment of a variety of vascular disorders. The study was developed to determine the safety and feasibility of prolonged sodium nitrite infusion. *Materials and Methods*: Healthy volunteers, aged 21–60 years old, were candidates for the study performed at the National Institutes of Health (NIH; protocol 05-N-0075) between July 2007 and August 2008. All subjects provided written consent to participate. Twelve subjects (5 males, 7 females; mean age 38.8 ± 9.2 years [range 27–56 years]) were intravenously infused with increasing doses of sodium nitrite for 48 h (starting dose at 4.2 μg/kg/h; maximal dose of 533.8 μg/kg/h). Clinical, physiologic, and laboratory data before, during, and after infusion were analyzed. *Results*: The maximal tolerated dose for intravenous infusion of sodium nitrite was 267 μg/kg/h. Dose-limiting toxicity occurred at 446 μg/kg/h. Toxicity included a transient asymptomatic decrease of mean arterial blood pressure (more than 15 mmHg) or an asymptomatic increase of methemoglobin level above 5%. Nitrite, nitrate, and S-nitrosothiol concentrations in plasma and whole blood increased in all subjects and returned to preinfusion baseline values within 12 h after cessation of the infusion. The mean half-life of nitrite estimated at maximal tolerated dose was 45.3 min for plasma and 51.4 min for whole blood. *Conclusion*: Sodium nitrite can be safely infused intravenously at defined concentrations for prolonged intervals. These results should be valuable for developing studies to investigate new NO treatment paradigms for a variety of clinical disorders, including cerebral vasospasm after subarachnoid hemorrhage, and ischemia of the heart, liver, kidney, and brain, as well as organ transplants, blood-brain barrier modulation, and pulmonary hypertension.

25. Erythropoietin for the Treatment of Vasospasm: A New Age May Be Close

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Institution: University of Palermo, Palermo, Italy

Subarachnoid hemorrhage (SAH) associated with ruptured cerebral aneurysm is a devastating clinical syndrome constituting 3% of all strokes. Although several drugs have been developed with the aim to prevent the arterial narrowing and limit the delayed ischemic neurological deficit (DNID) following the initial hemorrhage, no pharmacological agent has been shown conclusively to improve the outcome in clinical practice. Neuroprotection has been advocated as the ultimate goal in the treatment of acute neurological conditions, maintaining the highest possible integrity of cellular interactions in the brain and protection of neural function. Among all the neuroprotective agents so far proposed, a large body of preclinical and clinical studies has pointed out the high beneficial effect of recombinant human erythropoietin (rHuEPO) in reducing neuronal injury. Systemic EPO therapy acts via EPO receptors on cerebrovascular endothelia and ischemic neurons, inhibiting neuronal apoptosis, favoring production of antioxidant enzymes in neurons and neoangiogenesis, with the ultimate effect of reversing impaired autoregulation, reducing vasospasm and DNID. Besides the encouraging results provided by the use of rHuEPO in experimental SAH, the recent clinical trials have not been conclusive on EPO efficacy in this setting. The clinical side effects related to rHuEPO administration, such as hypertension, hypertensive encephalopathy, atherosclerosis, seizures, and thrombotic events, may be overcame using the new EPO-deriving drugs, which have been demonstrated to present the same neuroprotective effect without erythropoietic action. Further clinical investigations that consider the optimal tolerated dosage, therapeutic time window, and duration of therapy are encouraged to assess safety and efficacy of this promising therapeutic agent.

26. Prospective Randomized Phase II Trial on Concomitant Intraventricular Thrombolysis and Low-Frequency Head Motion After Severe Subarachnoid Hemorrhage: Analysis of Effect on Clot Clearance Rate, Radiological Vasospasm, Delayed Cerebral Ischemia, and Functional Outcome

Authors: Etminan, Nima (Presenting); Eicker, Sven; Beseoglu, Kerim; Perrin, Jason; Steiger, Hans-Jakob; Haenggi, Daniel

Institution: Heinrich-Heine University, Düsseldorf, NRW, Germany

Previous pilot studies demonstrated a positive effect on delayed cerebral ischemia (DCI) and functional outcome in patients suffering from aneurysmal subarachnoid hemorrhage (SAH) using a combination of intracerebral thrombolysis and kinetic therapy. The goal of this prospective, randomized phase II study was to investigate the effect of concomitant low-frequency head motion therapy and intraventricular thrombolysis on clot clearance rate, radiological vasospasm, clinical features of DCI, and clinical outcome in patients suffering from severe SAH. We included 50 patients suffering from severe SAH (World Federation of Neurosurgical Societies [WFNS] III–V) were included in the study. Experimental therapy in the study group consisted of intraventricular application of recombinant tissue plasminogen activator (rt-PA) and lateral rotational therapy (RotoRest®) for 48 h after admission, and aneurysm treatment was compared to best medical treatment (control group). Clot clearance rate was evaluated based on computerized tomography (CT). For these patients, radiological vasospasm, clinical features of DCI, and functional outcome, as measured by modified Rankin Scale (mRS), were observed during the course of treatment. There were no severe adverse events in the study group. Clot clearance rate was higher in the study

group than in the control group ($p=0.003$). The incidence of radiological vasospasm did not differ between the two groups ($p=0.713$). The incidence of clinical features of DCI in awake patients was significantly decreased in the study group ($p=0.016$). A distinct trend of mRS improvement ($p=0.06$) was noted in the study group at 1 month follow-up. The preliminary data of our phase II study suggested a beneficial effect of concomitant intraventricular thrombolysis and lateral rotational therapy regarding clot clearance rate and clinical features of DCI in the absence of severe adverse events. The effect on cerebral perfusion, cerebral infarction, and functional outcome at 3 months remains to be analyzed.

27. Interim Analysis of a Prospective Randomized Controlled Trial to Investigate the Efficacy of Endovascular Treatment in Cerebral Vasospasm After Subarachnoid Hemorrhage

Authors: Platz, Johannes (Presenting); Berkefeld, Joachim; Güresir, Erdem; du Mesnil de Rochemont, Richard; Mayer, Thomas E.; König, Ralph W.; Seifert, Volker; Vatter, Hartmut

Institution: Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Introduction: Endovascular treatment (EVT), like transluminal balloon angioplasty (TBA) or intra-arterial nimodipine (IAN), represents rescue therapy for cerebral vasospasm (CVS) after subarachnoid hemorrhage (SAH). Even though improvement of vessel diameter and cerebral blood flow was shown, its efficacy to prevent delayed cerebral infarction (DCI) is missing. The aim of the present study was therefore to investigate if delayed cerebral infarction can be reduced by repeated EVT. *Methods*: The design of the trial was prospective, controlled, and multicentric. Patients with CVS proven by magnetic resonance (MR)-based perfusion (PWI)/diffusion weighted imaging (DWI) mismatch (baseline MR) were randomized into an invasive or conventional treatment arm. All patients were treated with induced hypertension. In the invasive arm, additional digital subtraction angiography (DSA) was performed after the MR imaging. CVS is then treated by TBA or IAN. At 48 ± 12 h after treatment, follow-up MR imaging was acquired to assess the treatment success. If CVS persisted, DSA and EVT are repeated. The primary endpoint was the development of DCI, defined as new DWI lesions on a final MRI. For analysis, the brain was partitioned into 19 segments of comparable volume. Major infarct (MI) was defined as a lesion 50% or greater of one segment. *Results*: This interim analysis was based on the first 18 (of 92 scheduled) patients of the trial. We randomized 7 into the invasive and 11 into the conventional arms. In the interventional arm, 1 patient died before the final MR imaging. DCI was observed in 7 (64%) patients in the conventional and in 3 (50%) in the interventional arm. MI occurred in 4 (36%) and in 2 patients (33%), respectively. *Conclusion*: This interim analysis suggests that there are patients who may benefit from EVT. However, DCI was also observed in spite of supposedly successful EVT. It remains, therefore, to be seen if the efficacy of endovascular therapy is significantly higher than the periprocedural risk, including transport of these patients.

CNS Pathology: Acute and Delayed Deficits

28. Microglia (MG) Activation After Acute Subarachnoid Hemorrhage (aSAH): An Intraparenchymal Reaction to an Extraparenchymal Disease

Authors: Schneider, Ulf (Presenting) [Winner, Young Investigator Award]; Radon, Anja-Maria; Brandenburg, Susan; Brück, Wolfgang; Heppner, Frank; Vajkoczy, Peter

Institution: Charité-Universitätsmedizin Berlin, Berlin, Germany

Objective: Recently, inflammatory reactions have been discussed as a contributor to brain damage after aneurysmal subarachnoid hemorrhage (aSAH). To characterize the intraparenchymal reaction to the extraparenchymal noxa of aSAH, we evaluated time course and cytokine expression of microglia (MG) activation after experimental and clinical aSAH. *Methods*: (1) Experimental aSAH was induced in mice. On days 4, 14, and 28, brain slices were stained for ionized calcium-binding adaptor molecule 1 (Iba-1) and amyloid precursor protein (APP). (2) CD11b-positive MG cells were isolated from murine brains after aSAH on days 4, 14, and 28. Polymerase chain reaction (PCR) was performed for tumor necrosis factor alpha (TNF-α), interleukin (IL) 1a, -b, and 6 and their corresponding receptors. (3) Human brain sections of patients who had died from aSAH were stained for Iba-1 and APP. *Results*: In murine as well as human brain sections after aSAH, an intraparenchymal accumulation of Iba-1-positive cells was documented that started around day 4 and peaked around day 14. Furthermore, axonal damage could be recorded in murine and human brain slices according to intense APP expression, following the same time course and expression pattern. (human day 4/day 14/day 28: Iba-1 (cells/hpf): 13.5 ± 8.7/73.4 ± 36.8/15.7 ± 4.6; APP activation (arbitrary scale): 0.3/1.8/1.0; murine day 4/day 14/day 28: Iba-1 (area in mm^2): 0.23 ± 0.04/4.13 ± 1.22/3.15 ± 2.77; APP (area in mm^2): 0.19 ± 0.01/3.36 ± 1.01/0.27 ± 0.02). Cytokine levels of IL1a, -b, and 6 and TNF as well as the corresponding receptors in the isolated CD11b-positive cells were significantly increased on day 14 (IL1a 2.5, IL1b 5.2, TNF 4.1, IL1R2 3.2, TNFR1 2.4, TNFR2 4.1–fold vs. control). *Conclusion*: A significant intraparenchymal accumulation of MG cells was documented after aSAH. Time course, expression pattern, and upregulated transcription of inflammatory cytokines corresponded well with signs of axonal damage. For the first time, MG activation as an intraparenchymal inflammatory reaction to aSAH was

characterized, and a hint of the elucidating the underlying mechanisms could be achieved.

29. The Influence of Parenchymal Damage and Hemorrhage on Cortical Spreading Depression in Patients with Subarachnoid Hemorrhage

Authors: Eriksen, Nina (Presenting); Pakkenberg, Bente; Rostrup, Egill; Lauritzen, Martin, J.; Fabricius, Martin E.; Woitzik, Johannes; Scheel, Michael; Strong, Anthony J.; Dreier, Jens P.

Institution: Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

Introduction: Acute brain injury is often followed by serious complications. The initial brain damage is the most important factor for outcome; hence, we are focusing on the early phase after subarachnoid hemorrhage. In ischemic brain injury, a phase of delayed deterioration sometimes develops between 2 and 5 days postictus and is associated with severe and refractory brain swelling and poor outcome. Experimental investigations have shown that spreading depolarization of gray matter may lead to progressive deterioration in border zones of ischemic foci. This project is a part of COSBID, the CoOperative Study on Brain Injury Depolarizations, and the aim is to focus on volume estimation of injured regions as a prognostic tool. *Materials and Methods*: Quantitative measurements, such as regional volumes under various conditions, are essential for understanding both structural and functional changes in the brain and assessing prognosis. The affected brain tissue in patients suffering from acute brain injury is very inhomogeneous; hence, traditional methods are not always applicable, and automatic methods may not be able to match the individual observer. Stereological techniques are alternative tools for quantification of damage to the human brain. In the current project, we used the Cavalieri method to obtain the volume of injured regions and hemorrhage in patients during the acute and subacute stage on computed tomographic (CT) scans. The volumes were correlated to events of spreading depolarizations. *Results*: Our preliminary data in 20 patients showed a positive correlation between the number of spreading depolarizations versus volume of parenchymal damage and hemorrhage. *Conclusion*: This may suggest that increased parenchymal damage and blood in the subarachnoid space favor the occurrence of spreading depolarization in acute brain injury. However, we will need to analyze more patients to fully understand the relationship between parenchymal damage, hemorrhage, and spreading depolarization.

30. Early CT Perfusion Measurement in 50 Patients with Aneurysmal Subarachnoid Hemorrhage: A Tool to Detect Primary Damage Determining Clinical Course and Outcome

Authors: Heiroth, Hi-Jae (Presenting) [Award Finalist]; Kamp, Marcel A.; Turowski, Bernd; Steiger, Hans-Jakob; Hänggi, Daniel

Institution: University of Düsseldorf, Düsseldorf, NRW, Germany

Introduction: The clinical outcome of patients suffering from aneurysmal subarachnoid hemorrhage (SAH) seems to be influenced by the primary perfusion damage as well as by delayed cerebral ischemia. The goal of the present study was to investigate the predictive impact of early computed tomographic (CT) perfusion measurement in SAH patients on the clinical course and outcome. *Materials and Methods*: We included 50 patients with aneurysmal SAH in the prospective setting of this study. An early CT-based perfusion measurement within the first 12 h after initial bleeding was performed. The mean transit time (MTT) and the time to peak (TTP) were recorded. The results were correlated with the World Federation of Neurosurgical Societies (WFNS) score and the Glasgow Outcome Scale (GOS). *Results*: The initial TTP correlated significantly with the WFNS scale ($p=0.004$; correlation coefficient $r=0.405$; Spearman correlation [Sc] with significance level [sl] of 0.01); that is, the higher the TTP, the higher the WFNS score was. The initial MTT correlated negatively with the GOS ($p=0.016$; $r=-0.339$; Sc, sl: 0.05), as did the initial TTP ($p=0.018$; $r=-0.333$; Sc, sl: 0.05); that is, the higher the MTT or TTP was, the lower the GOS score was. There were 36 patients (i.e., 72%) who required external ventricular drainage (EVD). Patients with EVD showed a significantly higher MTT than those without EVD ($p=0.001$; $r=0.472$; Sc, sl: 0.01). The occurrence of vasospasm in the clinical course correlated highly with initial TTP ($p=0.008$; $r=0.370$; Sc, sl: 0.01); that is, the higher the initial TTP was, the more likely was the occurrence of vasospasm in the clinical course. *Conclusion*: The results of the present study revealed that (1) early CT perfusion measurement correlated highly significantly with the initial clinical grade and (2) contained a highly predictive impact for determining the clinical course and outcome in patients suffering from aneurysmal SAH. Based on the present results, delayed cerebral ischemia as the major secondary morbidity after SAH could be estimated as a sequel of the primary decreases in perfusion status.

31. Early Activation of Cell Death After Subarachnoid Hemorrhage

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Introduction: Cell death in brain by apoptosis and necrosis is present at 24 h after experimental subarachnoid hemorrhage (SAH). It is not known how soon after SAH cell death begins. We studied apoptosis and necrosis in the brain during the first 24 h after SAH. *Methods*: Animals (rats) were sacrificed at 10 min to 24 h after SAH or sham surgery ($n=5$ per group per time studied). Apoptosis in brain sections was studied by cleaved caspase-3 immunostaining and necrosis by fluorojade staining. Vascular and parenchymal origin of caspase-3-positive cells was studied by rat endothelial cell antigen (RECA-1; endothelial marker) and DAPI staining. Brain sections were photographed, and the number of

caspase-3- and fluorojade-positive cells was determined (IP lab). Brain areas examined included cerebral cortex, caudate putamen, and hippocampus. *Results*: Caspase-3 staining was present in endothelial and parenchymal cells at 10 min after SAH and was significantly greater than time-matched shams ($p < 0.05$). Caspase-3 staining increased further at 24 h after SAH and was similar in all brain regions and across hemisphere. Fluorojade-positive neurons were present in the caudate putamen at 6 h after SAH. By 24 h, fluorojade staining had spread to cortex, hippocampus, and caudate putamen and was significantly greater in the right compared to the left hemisphere. No fluorojade staining was present in sham animals. *Conclusion*: Our data established an early activation of endothelial and parenchymal cell apoptosis and neuronal necrosis after SAH and identified endpoints that can be targeted to reduce early brain injury after SAH.

Definition and Diagnosis of Vasospasm

32. Evaluation of Cerebral Vasospasm with CT Angiography and Perfusion CT

Authors: Kasuya, Hidetoshi (Presenting); Tanaka, Noriko; Hagiwara, Shinji; Tani, Shigeru; Akiyama, Mami; Koseki, Hirokazu; Hana, Taijun; Yoshimura, Chika

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Introduction: We investigated the usefulness of three-dimensional computed tomographic angiography (CTA) and perfusion CT (pCT) for the diagnosis and management of cerebral vasospasm (CV). *Materials and Methods*: We retrospectively evaluated 41 consecutive patients suffering from aneurysmal subarachnoid hemorrhage (SAH). CTA and pCT were performed on the day of admission (day 0) and at least once between day 5 and day 14. Data were analyzed by comparing clinical characteristics and findings with conventional CT and digital subtraction angiography (DSA). *Results*: Eighteen patients were more than 60 years old; 24 were female; 22 were World Federation of Neurosurgical Societies (WFNS) grades 4 and 5; 32 were in Fisher group 3; 35 of the ruptured aneurysms were located in the anterior circulation, and 18 were treated by craniotomy. Sensitivity and specificity for detecting severe CV on DSA was 100% and 76%, respectively. Four patients received intra-arterial vasodilator treatment according to the results found in CTA and pCT. False-positive findings were caused by coil artifacts, parenchymal hematoma, and effects related to the surgical and endovascular procedures. CV was well characterized with a combination of mean transit time, cerebral blood flow, and cerebral blood volume. *Conclusion*: CTA/pCT can detect critical vasospasm and be used as an indicator for invasive treatment.

33. Application of Noninvasive Continuous Monitoring System, INVOS® for Detecting Cerebral Vasospasm

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Institution: Okayama University Graduate School of Medicine, Dentistry, and Sciences, Okayama, Japan

Introduction: Detection of cerebral vasospasm after subarachnoid hemorrhage is somehow difficult because, so far, there are no absolute tools for timely and accurate detection. Although transcranial Doppler monitoring, single-photon emission computed tomography (SPECT), three-dimensional computed tomography (3D-CT), or angiography are all excellent monitoring tools for vasospasm, these are not continuous noninvasive monitoring systems. The INVOS monitor shows continuous regional saturation data in the brain cortex through the skin and skull, and it reveals site-specific insights on perfusion of brain cortex. Also, it is not only a noninvasive detachable monitor but also a small and portable one. We here report whether it can be useful for detection of cerebral vasospasm and analyze its advantages and disadvantages for vasospasm detection. *Methods and Results*: Patients showing Hunt and Kosnik grade between 2 and 4 were included from 2005 to 2011 in Okayama University Hospital. The INVOS monitoring system was used for detecting vasospasm from day 3 to day 14. Monitoring probes were usually attached to the regions predicted for vasospasm. When INVOS values decreased, immediate angiography was routinely performed, and we checked a degree of vascular narrowing; then, intra-arterial fasudil infusion was carried out if necessary. In 38 patients, the INVOS was applied, and saturation was analyzed for detecting vasospasm. Except for 2 patients, vasospasm was well detected in a timely and accurate manner. Vasospasm could be treated very well by intra-arterial fasudil injection in the early vasospastic period. However, local vasospasm or vasospasm in the very peripheral lesions could not be detected well in 2 patients. *Discussion and Conclusion*: The INVOS monitor can detect cerebral vasospasm in a timely and accurate fashion. It may be a good monitoring system for detecting vasospasm. In the meantime, we need some ingenuity for detecting local vasospasm or vasospasm in the very peripheral regions.

Endovascular Approaches

34. Intra-arterial Verapamil-Induced Seizures: An Underrecognized Side Effect or the Result of Rapid Reperfusion?

Authors: Rahme, Ralph (Presenting); Abruzzo, Todd; Zuccarello, Mario; Khan, Usman; Ringer, Andrew

Institution: University of Cincinnati/Mayfield Clinic, Cincinnati, OH, USA

Introduction: Seizures induced by intra-arterial infusion of vasodilators in patients with posthemorrhagic vasospasm are exceptionally reported and thought to result from a direct

effect of the drug on metabolically challenged neurons. We present evidence that challenges this concept. *Materials and Methods*: A 27-year-old female patient suffered rupture of a left posterior communicating artery aneurysm, resulting in massive subarachnoid hemorrhage. Despite stent-assisted coiling, the aneurysm exhibited early recanalization and rerupture within a few weeks, necessitating additional coiling. Five days later, the patient became obtunded with a dense left hemiparesis. Cerebral angiography demonstrated severe vasospasm of the right internal carotid and middle cerebral arteries. The patient was treated by direct intra-arterial infusion of verapamil (20 mg) into the right internal carotid artery. *Results*: Immediately after verapamil infusion, the patient complained of severe headache and became agitated. This was quickly followed by left hemibody clonic convulsions and a self-limited secondary generalized tonic-clonic seizure. Repeat angiography documented immediate resolution of vasospasm and enhanced right hemispheric perfusion. Following a transient postictal state, the patient regained normal consciousness and left hemibody motor strength. Over the next few days, the patient had recurrence of severe symptomatic vasospasm twice. She was treated with intra-arterial verapamil and exhibited each time headache, agitation, and a secondarily generalized tonic-clonic seizure followed by neurological improvement. *Conclusion*: Intra-arterial verapamil-induced seizures do not necessarily represent an adverse event. In the absence of an angiographically demonstrable complication such as intraprocedural aneurysm rupture, seizures may be caused by rapid drug-induced reperfusion of the ischemic brain and may be followed by neurological improvement.

35. Continuous Intra-arterial Nimodipine Infusion for the Treatment of Cerebral Vasospasm

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Institution: Friedrich-Schiller-University, Jena, Germany

Background: To treat cerebral vasospasm, balloon angioplasty has been shown to be effective and permanent in selected cases. But, since it is technically challenging and insufficient, if more distal segments are involved, it has not proven to be clinically effective. Intra-arterial infusion of vasodilators is known to reverse vasospasm. There is indication that calcium channel blockers have longer-lasting effects, including the also systemically applied nimodipine, which has been used intra-arterially by neurointerventional groups for subarachnoid hemorrhage and catheter-induced vasospasm. Nevertheless, infarctions occurs in patients treated by intra-arterial short-time infusion in up to three sessions with nimodipine. Therefore, we investigated long-term intra-arterial infusion. *Methods*: In case of clinical, transcranial Doppler (TCD), cerebral blood flow (CBF), or angiographic signs of vasospasm, patients were included in the study protocol. Patients were treated by one to four microcatheters implanted for 12–288 h in the internal carotid arteries (ICAs) or vertebral arteries (VAs). Nimodipine was infused through the microcatheters with a dosage of 0.5–3 mg/h per vessel. All patients received at least single antiplatelet therapy. *Results*: So far, in the first 25 patients there were two major complications, with one internal carotid artery dissection and one embolism. In the first patient, the guiding catheter was left in place, not exchanged for a microcatheter. The other patient only received heparin for anticoagulation but not antiplatelet therapy. Only one patient did not respond to an intra-arterial dosage of 1 mg/h, but the following day, the dosage was increased to 2 mg/h nimodipine in one ICA, and this led to normalization of the vessel diameters. In all other patients, complete or almost-complete recanalization occurred. Clinical evaluation at this time is not completed. *Conclusion*: Continuous intra-arterial nimodipine infusion for the treatment of cerebral vasospasm has the potential to recanalize all patients until the end of the vasospastic phase. Randomized clinical studies are demanded.

36. Transluminal Balloon Angioplasty for Symptomatic Distal Vasospasm Refractory to Medical Therapy in Patients with Aneurysmal Subarachnoid Hemorrhage

Authors: Santillan, Alejandro (Presenting) [Award Finalist]; Knopman, Jared; Zink, Walter; Patsalides, Athos; Gobin, Y. Pierre

Institution: New York Presbyterian Hospital/Weill Cornell Medical Center, New York, NY, USA

Introduction: Cerebral vasospasm (VS) is a major cause of morbidity and mortality associated with subarachnoid hemorrhage (SAH). The current endovascular paradigm for vasospasm refractory to medical therapy is to perform angioplasty for proximal vessel VS and vasodilator infusion for distal vessel VS. We report our experience of a large series of balloon angioplasty for distal VS refractory to medical therapy in patients with aneurysmal SAH. *Materials and Methods*: We studied a retrospective series of 32 patients with SAH and symptomatic VS refractory to medical therapy who were treated with balloon angioplasty for distal vessel VS. Immediate angiographic results, procedure-related complications, and clinical outcomes were assessed. *Results*: From September 2001 to January 2010, there were 32 patients with symptomatic vasospasm refractory to medical therapy who underwent angioplasty for distal arterial vasospasm. There were 26 women (81.3%), aged from 29 to 67 years. A total of 175 vessels were angioplastied (95 proximal and 80 distal). The only complication was due to rupture of an incompletely clipped aneurysm that was treated by immediate coiling and did not result in any clinical worsening. Repeated treatment was needed for 6 arteries (6/80 = 7.5%). There was no procedure-related symptomatic complication. Good outcomes (modified Rankin Scale [mRS] < 2) were observed in 23/28 (82.1%) patients with follow-up. *Conclusion*: Balloon angioplasty for distal VS was safe and

effective and decreased the need for repeated intra-arterial treatments seen with infusion of vasodilator.

37. Comparison of the Clinical Outcome of Cerebral Vasospasm Post-Aneurysmal Subarachnoid Hemorrhage in Patients Undergoing Two Types of Angioplasty: Mechanical and Chemical

Authors: Aburto-Murrieta, Yolanda (Presenting); Marquez, Juan Manuel; López-de-Santiago Iván, Iván Tomás; Hernández-Curiel, Bernardo; Bonifacio-Delgadillo, Dulce

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Introduction: Cerebral vasospasm (CV) causes significant morbidity and mortality in patients with aneurysmal subarachnoid hemorrhage (aSAH). Angiographic vasospasm (AV) is seen in 30–70% of patients post-aSAH, and delayed ischemic neurologic deficit (DIND) has been associated with this. Prevention of rebleeding with exclusion of ruptured aneurysm is followed by a combination of interventional procedures, such as mechanical and chemical angioplasty and medical therapy. *Methods*: In a retrospective clinical trial of 30 patients post-aSAH (anterior circulation) with AV were treated with intra-arterial nimodipine therapy or mechanical angioplasty for CV and fulfilled Eskridge criteria. At admission, Hunt and Hess (H&H) and Fisher Scale were registered. Transcranial Doppler was performed before and after intra arterial nimodipine. Clinical follow-up was recorded every 3 months for 1 year. AV was classified as none/mild, moderate, or severe (0–33%, 34–66%, >67%, respectively), and infarctions were categorized as secondary to AV, other, or unknown causes. *Results*: We performed 22 intra-arterial nimodipine and 8 mechanical angioplasties in 30 patients (18 female) aged 18–74 years. At admission, the patients were HH scale I: 20%, II: 30%, III: 37%, and IV: 13% and Fisher III: 33%, IV: 20%. No differences in both scales were found. Intracranial Doppler velocities postprocedure diminished at least 45%. Clinical outcome was defined as good when a modified Rankin Scale (mRS) score of 0–2 was present at 12 months. Good outcome was present in 37% of patients. Good outcome between the two groups was similar, $p = 0.36$. Mortality was 20%. *Conclusion*: We did not find differences in the clinical outcome despite the modality of endovascular strategy and adequate angiographic and ultrasound response.

38. Treatment of RCVS with Intra-arterial Verapamil: A Novel Treatment Paradigm?

Authors: Khan, Usman (Presenting); Rahme, Ralph; Abruzzo, Todd; Zuccarello, Mario; Ringer, Andrew

Institution: University of Cincinnati/Mayfield Clinic, Cincinnati, OH, USA

Introduction: reversible cerebral vasoconstriction syndrome (RCVS) is a poorly understood clinical entity, and the precise underlying pathophysiology is unknown. Both primary or idiopathic and secondary types have been described. The diagnosis is usually made clinically with sparse angiographic evaluation depending on institutional preference and the index of suspicion for underlying vasculitis. Other than avoiding the triggers and withdrawal of secondary causes, no treatment has gained therapeutic currency. *Methods*: Four patients at this institution were treated with intra-arterial (IA) verapamil after an angiographic diagnosis of RCVS was made. All patients had a clinical history suggestive of the diagnosis, prompting the angiographic evaluation. Three patients were females, and 1 was male. Age range was 19–58 years. Clinical presentation encompassed thunderclap headache in 3 patients, subarachnoid hemorrhage in 1 patient, cerebral ischemia/infarction in 2 patients, and reversible parieto-occipital T2 signal hyperintensities in 1 patient. None of the patients had disabling neurological deficits. The mean dose of IA verapamil used was 10–20 mg per vessel. *Results*: Complete resolution of angiographic abnormalities with dramatic improvement in arterial luminal caliber was demonstrated 15 min after verapamil infusion in all cases. There were no adverse neurological sequelae. The improvement in vascular luminal caliber was the same in anterior and posterior circulations. There were no procedure-related complications in any of these cases. No angiographic follow-up was done as there was no recurrence of symptoms. *Conclusion*: IA verapamil may have a role in proving the angiographic confirmation of the RCVS diagnosis and could be a therapeutic modality available in the acute phase of the disease to prevent symptomatic ischemia in severely affected patients.

Human Imaging Studies

39. Quantification of Subarachnoid Hemorrhage by 3D-CT Part 1: Comparison of Hematoma Volume Between 3D-CT and 2D-CT

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Institution: Fukushima Medical University, Fukushima, Japan

Object: Estimation of hematoma volume of subarachnoid hemorrhage (SAH) on computed tomography (CT) has been subjective and not quantitative, although the relation between hematoma volume and development of delayed ischemic neurological deficit has been confirmed. Attempts to quantify the SAH with a novel software-based technique and two-dimensional CT (2D-CT) encountered problems posed by the partial volume effect and the absence of SAH in the convexity or posterior fossa. We compared hematoma volume between our new method by three-dimensional CT

(3D-CT) and the conventional one by 2D-CT. *Methods*: We used the width of CT number 40–80 Hounsfield units (HU), which was the reported CT number of SAH ranges. We examined correlation of hematoma volume by actual measurement and by 3D-CT using experimental hematomas and compared SAH volumes by 3D-CT with those by 2D-CT. Experimental hematomas were made with blood obtained from ten volunteers. Clinical materials were 50 patients with aneurysmal SAH. *Results*: The experimental hematoma volume was determined by actual measurements and by 3D-CT on days 1, 4, 7, 11, and 14. The coefficients on days 1 and 4 were relatively high, and the correlation between measured and estimated volumes was significant on days 7, 11, and 14. These results suggest that a CT number of 40–80 may be reliable for estimating the hematoma volume by 3D-CT. The SAH volume, including the volume of normal structures, was automatically calculated (V1). The volume of normal structures (V2) was calculated in another 50 patients without intracranial lesions as 12 ml. The total SAH volume was defined as V1 minus mean V2. The mean SAH volumes by 3D-CT and by 2D-CT were 44 ml and 34 ml, respectively, on day 0. The SAH volume was significantly larger by 3D-CT than by 2D-CT ($p<0.05$). *Conclusion*: The SAH volume by 3D-CT may be reliable for estimating the hematoma volume. This method can rapidly measure SAH volume based on 3D-CT, and chronological changes in the SAH volume can be monitored easily.

Markers, Monitoring, and Point of Care

40. Paradoxical Increases in Near-Infrared (NIR) Cerebral Oximetry in Subarachnoid Hemorrhage Suggest Vasospasm

Authors: Corry, Jesse (Presenting); Varelas, Panayiotis; Abdelhak, Tamer; Bartscher, James; Wellwood, Jody

Institution: Henry Ford Hospital, Detroit, MI, USA

Introduction: The combination of hypertensive and vasodilatory (HVD) therapy via intravenous nicardipine demonstrated faster reductions in mean middle cerebral artery (MCA) velocities following subarachnoid hemorrhage (SAH). Recently, we reported reductions in brain oxygenation, measured by near-infrared spectrophotometry (NIRS)-determined regional cerebral oxygen saturation, with this therapy. We report findings in a patient that potentially explain this finding. *Methods*: A report of a patient treated with HVD. Data included daily transcranial Doppler (TCD) mean velocities of the middle cerebral and basilar arteries and NIRS oximetry. NIRS is a noninvasive, optically based technique to monitor brain oxygenation by determining the cerebral tissue oxygen saturation. Light from the NIRS forehead sensor passes through extracerebral and brain tissues, the latter containing oxy- and deoxyhemoglobin within cerebral arterioles, capillaries, and venules. NIRS oximetry is a mixed-vascular oxygen saturation parameter. *Results*: Of the patients, 5 of 7 demonstrated reduced TCD velocities correlating to nicardipine administration. Six had NIRS performed. These values significantly ($p<0.05$) fell 8–10% in 5 of 6 patients correlating with nicardipine administration. NIRS reductions correlated to neurologic exam improvements. One patient required intra-arterial therapy for vasospasm. The patient's TCD velocities and NIRS readings increased in the days before angiography. Angiography demonstrated external-carotid-artery-to-internal-carotid-artery (ECA-to-ICA) collateral flow via branches of the internal maxillary artery. After angioplasty and intra-arterial nicardipine, the NIRS values decreased 4–8%, continued to decrease 5–8% over 8 h, then rose to preangiography levels. *Conclusion*: In this small series of HVD therapy, we found reductions in both TCD velocities and NIRS values in the majority of patients treated. These findings suggest reductions in NIRS values were the result of reduced ECA arterial contribution. Increases in NIRS values may suggest increased ECA-to-ICA cross flow in vasospasm.

41. Early Platelet Activation After Subarachnoid Hemorrhage Is Related to Poor Admission Hunt-and-Hess Grade

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Introduction: Brain injury that occurs at the time of aneurysm rupture is the most important predictor of long-term outcome, yet little is known about the mechanism of injury. Animal models of subarachnoid hemorrhage (SAH) suggest that early platelet activation and microthrombosis may play a role. *Methods*: Spontaneous SAH patients were prospectively enrolled within 72 h of ictus, excluding those with baseline coagulation abnormalities. Jugular and peripheral venous blood was sampled at admission prior to angiography or aneurysm repair and then every 12 h for 72 h and assessed by thromboelastography (TEG) and standard measures of coagulation. TEG studies were compared between good- and poor-grade patients (Hunt and Hess [H&H] 1–3 vs. 4–5) using repeated measures analysis. Logistic regression analysis was used to compare TEG and standard coagulation values with admission demographic features and neurological status. *Results*: A total of 15 SAH patients were studied. The median HH score was 3 (range 1–5), and 27% of patients were HH 4–5. Abnormally high TEG measures of platelet activation occurred in 88% of patients. Jugular venous TEG markers of platelet activation were significantly higher in poor-grade compared to good-grade patients. This difference in platelet activation was evident earlier in venous jugular blood (beginning at 24 h) and was more pronounced ($p=0.008$) as compared to peripheral venous blood (36 h; $p=0.033$). There was

a trend toward increasing TEG measures of platelet activation with each increase in HH grade (odds ratio [OR] 4.6, 95% confidence interval [CI] 0.8–26.0, $p = 0.083$). D-Dimer levels were elevated in 90% of patients (median 2.0, range 0.3–18.5), as were fibrinogen levels (median 523, range 264–617), but neither was related to clinical status. *Conclusion*: Platelet activation, as measured by TEG, was increased acutely in poor-grade SAH patients compared to good-grade patients and may play a role in the mechanism of early brain injury.

42. Is Routine Renal Screening in Aneurysmal Subarachnoid Hemorrhage Patients Rational?

Authors: Ghosh, Sayantani; Dey, Saugat (Presenting); Maltenfort, Mitchell; Tjoumakaris, Stavropoula; Gonzalez, L. Fernando; Jabbour , Pascal; Rosenwasser, Robert; Jallo, Jack

Institution: Thomas Jefferson University Hospital, Philadelphia, PA, USA

Introduction: Acute kidney injury is a problem of paramount importance in the majority of critically ill patients; hence, a routine renal screening is warranted. But, there is little known regarding the burden of acute kidney injury in aneurysmal subarachnoid hemorrhage (aSAH); therefore, we have studied the effects of levels of serum BUN (blood urea nitrogen), creatinine, and BUN:creatinine ratio in such patients to investigate the extent of kidney damage. *Materials and Methods*: From the records of Thomas Jefferson University Hospital, we retrospectively reviewed 1,000 cases of aSAH with no prior history of kidney disease for their levels of serum BUN, creatinine, and their ratio on the day of admission and on the fourth day of hospitalization. Outcome of the patients on discharge was measured via extended Glasgow Outcome Sale (GOSE) score. Parameters were initially analyzed by Student *t* test and were further scrutinized by multivariate regression analysis. *Results*: Kruskal-Wallis (nonparametric) comparisons across outcomes were significant for BUN, creatinine, and BUN:creatinine ratio ($p < 0.0001$); however, in a multivariate regression model, serum creatinine was not found to have any effect on the outcome. Odds ratio for a poor outcome with per unit increase of the BUN:creatinine ratio, in a multivariate regression, was 1.39 (95% confidence interval [CI] 1.22–1.68) for the fourth day and 1.22 (95% CI 1.14–1.31), while a ratio of less than 10:1 on the day of admission was also related to poor prognosis ($p < 0.0001$). Inverse prediction provided a 50% chance of good outcome at the maximum BUN:creatinine ratio of 22.2:1 (95% CI 21.2:1–23.5:1). *Conclusion*: It was concluded that serum BUN imbalances affecting the short-term prognosis of aSAH patients are mainly following the cardiovascular or neurologic damage and are less likely as a result of direct kidney injury; hence, routine renal screening in all such patients is questionable.

43. Copeptin as Possible Prognostic Biomarker for Disease Severity and Detector for Cerebral Vasospasm in Patients with Aneurysmal Subarachnoid Hemorrhage

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Introduction: Aneurysmal subarachnoid hemorrhage (SAH) is known to be a severe disease with high morbidity and mortality rates. Early prediction of possible outcome is consequential for optimized care and treatment decision. Copeptin, a stable and sensitive surrogate for vasopressin release, has recently emerged as a valid prognostic biomarker in a variety of severe diseases. Its prognostic value for spontaneous SAH is yet unknown. *Materials and Methods*: Copeptin levels were measured with a line immunoassay in ten consecutive SAH patients on day of admission. Further measurements were conducted in synchronization with performed computed tomographic (CT) perfusion scans also to evaluate a possible correlation between copeptin levels and changes of the mean transit time. Initial disease severity was assessed by Glasgow Coma Scale (GCS) and World Federation of Neurosurgical Societies (WFNS) grade on admission, and recovery by Glasgow Outcome Score (GOS) on discharge. GCS smaller than 10 and WFNS larger than 3 was considered severe; a favorable outcome was defined as a GOS of 4 and above. *Results*: Preliminary results demonstrated that there was a positive correlation between copeptin level on admission and disease severity. Higher copeptin levels were detectable in patients with GCS 10 or lower. Copeptin levels also correlated positively with changes of the mean transit time measured by repeated CT perfusion scans ($r \gg 0.6$). Copeptin levels were notably higher in patients during cerebral vasospasm. Statistical significance could not yet be reached due to the present low number of patients. *Conclusion*: Copeptin is a promising prognostic biomarker for mortality and outcome in a variety of diseases associated with cerebral damage. Its predictive value for the disease severity of aneurysmal SAH patients seems promising. Furthermore, copeptin could be considered as a possible supportive detector of cerebral vasospasm. Larger studies with close consideration of comorbidities have to be conducted for further evaluation.

Neurocritical Care Monitoring

44. Multicenter Controlled Trial About the Reexamination of Triple H Therapy After Subarachnoid Hemorrhage: Preliminary Report

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Institution: Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Introduction: Volume management is crucial in intensive care; however, in some patients, it is hard to achieve optimal

water balance. A subarachnoid hemorrhage (SAH) patient is a representative example. Cardiopulmonary complications are common after SAH, including neurogenic pulmonary edema, cardiac failure, and so on. Triple H (hypertensive, hypervolemic, and hemodilution) therapy is a standard management after SAH, but it also has adverse effects: pulmonary edema, increased intracranial pressure, hyponatremia, sepsis, and so on. We have started a multicenter controlled trial about cardiopulmonary function after SAH. We describe here a trial of minimally invasive PiCCO Plus monitoring of cardiopulmonary function to reexamine the effect of triple H therapy after SAH. *Materials and Methods*: This multicenter controlled trial analyzed the cardiopulmonary functions of 87 patients after SAH by PiCCO Plus monitoring over a period of 2 weeks. *Results*: Output, contractility, and afterload were essentially normal after SAH. However, slightly elevated intrathoracic blood volume led to fluid redistribution that caused hydrostatic fluid retention in the lung tissues. Triple H therapy had no additional cardiopulmonary features except for the elevated plasma brain natriuretic peptide (BNP) levels. Persistent catecholamine release and altered sensitivity of blood vessels to catecholamines caused the blood volume redistribution and hydrostatic pulmonary edema. Cardiac preload due to catecholamine release led to (BNP) release, resulting in natriuresis. This appeared to be the underlying mechanism of cerebral salt-wasting syndrome. *Conclusion*: We found that hydrostatic pulmonary fluid retention occurred after SAH. Triple H therapy gave no additional benefits on the systemic circulation after SAH.

45. Continuous Regional Cerebral Oxygenation Monitoring by Multichannel Near-Infrared Spectroscopy to Assist Intra-arterial Fasudil Therapy for Vasospasm After Subarachnoid Hemorrhage

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Introduction: Cerebral vasospasm following aneurysmal subarachnoid hemorrhage (SAH) that has been refractory to maximal medical management can be treated with intra-arterial administration of vasodilators. However, it remains unclear regarding the effectiveness on regional cerebral circulation to assume the treatment response of distal/diffuse vasospasm. *Methods*: We describe a 63-year-old male with SAH and intracerebral hematoma due to ruptured right middle cerebral artery aneurysm; he developed aphasia and right-side weakness on day 9 after SAH onset, which were highly suspected of delayed cerebral ischemia attributable to diffuse vasospasm in the distal territory of the left anterior and middle cerebral arteries. The patient was refractory to hyperdynamic therapy but successfully treated with intra-arterial infusions of fasudil hydrochloride assisted by continuous monitoring with regional cerebral oxygen saturation (rSO_2) with 4-channel flexible near-infrared spectroscopy sensors and cardiac output. *Results*: Decreased and fluctuating rSO_2 in angiographically documented vasospasm territories elevated immediately after intra-arterial fasudil infusion in accordance with the relief of vasospasm that correlated with neurological improvements. The procedure was repeated on day 11 since the effect was transient, and vasospasm-related neurological deterioration recurred. The symptoms gradually resolved accompanied by maintenance of stable rSO_2 values, resulting in favorable functional outcome. *Conclusion*: Our clinical experience suggests that rSO_2 with multichannel near-infrared spectroscopy may provide noninvasive, real-time, clinically relevant information to assist intra-arterial fasudil therapy for detecting and treating distal/diffuse vasospasm.

46. Mechanism for Dobutamine-Induced Hyperdynamic Therapy for Reversing Focal Cerebral Ischemia Affected by Vasospasm After Subarachnoid Hemorrhage

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Introduction: Therapeutic hemodynamic augmentation by increasing cardiac output (CO) with dobutamine (DOB) is a valuable method of maintaining regional cerebral blood flow (rCBF) and oxygenation in the dysautoregulated vascular territories by vasospasm following aneurysmal subarachnoid hemorrhage (SAH). We aimed to determine the effect of DOB-induced hyperdynamic therapy on CO and regional cerebral oxygenation (rSO_2) for reversing clinical deterioration attributable to vaospasm. *Materials and Methods*: Fifty-five consecutive patients with SAH treated surgically within 24 h of ictus and diagnosed to have symptomatic vasospasm between days 4 and 14 were investigated. For medical treatment, DOB was administered at an initial dose of 3 μg/kg/min and then increased in 3-μg/kg/min increments until resolution of the symptoms. CO and rSO_2 changes during the therapy in conjunction with the assessment of neurological improvements were analyzed. *Results*: A total of 225 DOB challenges were performed with a maximum dose of 11 ± 3 μg/kg/min. In spasm-affected territories, decreased or fluctuating rSO_2 was detected compared with recordings in other brain regions. Patients who exhibited rapid elevation of CO by DOB challenges had subsequent uptake and stabilization of rSO_2 followed by improvement of the symptoms. A fairly strong relationship was found between peak CO slope and rSO_2 elevation during each DOB challenge ($r = 0.79$, $p < 0.0001$), while a poor correlation was found between peak CO change and rSO_2 ($r = 0.33$, $p = 0.09$). The area under the ROC curve to discriminate neurological responders to DOB was higher for peak CO slope (0.86 ± 0.08) than for peak CO (0.65 ± 0.12) ($p < 0.05$). Values of average

peak CO slope of 0.007 predicted neurological improvement with DOB therapy with 83% specificity and 70% sensitivity. *Conclusion*: Maximal hemodynamic acceleration rather than the peak CO values plays a key role of DOB hyperdynamic therapy in relieving focal cerebral ischemia in patients suffering from vasospasm after SAH.

47. Cerebral Hemodynamic Changes After Wartime Traumatic Brain Injury

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Introduction: Traumatic brain injury (TBI) is associated with the severest casualties from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). From October 1, 2008, the army medicine (AMEDD) TBI program initiated transcranial Doppler (TCD) ultrasound service for TBI patients who were presented for care at the National Naval Medical Center and at the Walter Reed Army Medical Center. *Materials and Methods*: Seventy-seven patients (3 females) aged 18–40 years (mean 25.9 years) who had suffered wartime TBI injuries (with Glasgow Coma Scale scores ranging from 3 to 15) were investigated with daily TCD studies. A total of 483 TCD recordings (mean 6.5 tests per patient, range 1–30) were made after admission. There were 28 (36.4%) patients after explosive blast injury, 18 patients (23.4%) after gunshot wound (GSW), and 31 (40.2%) after other causes of TBI (closed, penetrating, motor vehicle accident [MVA], falls, etc.). A comprehensive TCD protocol and well-published diagnostic criteria for vasospasm and abnormally high intracranial pressure (ICP) applied in all cases. *Results*: The TCD signs of mild, moderate, and severe vasospasm were observed in 28 (36.4%), 16 (20.7%), and 9 (11.6%) of patients, respectively. The TCD signs of intracranial hypertension were recorded in 51 (66.2%) patients. Abnormally high cerebral blood flow velocities (CBFVs) without TCD signs of vasospasm and abnormally low CBFVs were recorded in 7 (9%) and 12 (15.5%) of all patients, respectively. Four patients (5.1%) underwent transluminal angioplasty for post-traumatic vasospasm treatment. *Conclusion*: These findings demonstrate that delayed cerebral arterial spasm is a frequent complication of combat TBI, and that the severity of spasm is comparable to that seen in aneurysmal SAH. In addition, TCD provided valuable information about the presence of abnormally high ICP. Because vasospasm and intracranial hypertension represent significant events in a high proportion of patients after wartime TBI, close daily TCD monitoring is recommended for the management of such patients.

48. Illustrative Case: Failure to Detect Severe Cerebral Vasospasm Following Subarachnoid Hemorrhage Despite Multiparameter Neuromonitoring

Authors: Sikorski, Christopher (Presenting); Dent, Wolfgang; Wyss, Sabine; Farokhzad, Faraneh; Keller, Emanuela

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Introduction: The detection of delayed ischemic neurological deficits (DINDs) in sedated or comatose patients after aneurysmal subarachnoid hemorrhage (SAH) remains an unsolved challenge in neurocritical care. *Methods*: An illustrative case of a patient is presented with multiple delayed ischemic infarctions with late onset at day 16 not detected by multimodality neuromonitoring. Specific limitations of actual available neuromonitoring modalities are discussed. *Results*: A 43-year-old patient was admitted to the neurointensive care unit, University Hospital of Zurich, suffering from poor-grade SAH (Hunt and Hess [H&H] grade 4, World Federation of Neurosurgical Societies [WFNS] grade 5, Fisher grade 3). Ruptured aneurysm of the basilar artery was successfully coiled within 24 h after admission. Further treatment included insertion of an external ventricular drainage, intravenous administered nimodipine, high-dose magnesium sulfate and pravastatin. As the patient remained comatose, multiparameter neuromonitoring was established, including bilateral probes for brain tissue oxygenation and cerebral microdialysis supplemented by oximetry from the right-sided jugular bulb and daily transcranial Doppler (TCD) blood flow measurements. TCD showed increased mean blood flow velocities in both middle cerebral arteries (MCAs) in the early stage after SAH onset up to a maximum of 198 cm/s; however, this was without corresponding pathological findings in repeated CT perfusion scans. No clear signs for ischemia or impaired cerebral metabolism were detected by invasive neuromonitoring until a routine CT scan on day 16 demonstrated bihemispheric MCA infarction due to angiographically confirmed diffuse vasospam. *Conclusion*: In this case, brain-specific neuromonitoring failed to indicate severe vasospasm, underlining the limitations of the various techniques. Neuromonitoring data should be carefully interpreted, and additional strategies in vasospasm screening are needed, focusing on real-time, global parameters.

49. Prognostic Role of Basic CSF Analysis in Aneurysmal Subarachnoid Hemorrhage Patients

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Introduction: Basic cerebrospinal fluid (CSF) analysis is routinely performed in aneurysmal subarachnoid hemorrhage (aSAH) patients but is seldom studied. We have considered the effect of the CSF picture on the patient outcome and

whether it can serve as a prognostic indicator for such patients. *Materials and Methods*: A retrospective review of 661 cases of aSAH with no preexisting systemic infection was done from the records of Thomas Jefferson University Hospital. The levels of CSF glucose, protein, leukocyte (white blood cell, WBC) count and culture on the day of the admission and tenth day postadmission were compared with the patient outcome at discharge, as measured via Extended Glasgow Outcome Score (GOSE). Parameters were analyzed by Student *t* test. *Results*: CSF glucose greater than 80 mg/dl ($p<0.0001$), CSF protein greater than 90 mg/dl ($p<0.0001$) on admission, CSF glucose greater than 75 mg/dl ($p=0.0013$), and CSF protein greater than 85 mg/dl ($p<0.0001$) on the tenth day worsened the chances of a good outcome, although CSF glucose less than 40 mg/dl ($p<0.0001$) and CSF protein less than 15 mg/dl ($p<0.0001$) at admission were also associated with higher mortality. A 1-mg/dl rise of CSF glucose had an odds ratio of 1.087 (95% confidence interval [CI] 1.078–1.096) for a poor outcome and 1.048 (95% CI 1.041–1.055) for death; a 1-mg/dl rise in CSF protein had an odds ratio of 1.93 (95% CI 1.84–2.1) for a poor outcome and 1.55 (95% CI 1.37–1.73) for death. CSF WBC count of 3–5/dl was associated with the best outcome ($p=0.0014$), more so on the tenth post admission day ($p<0.0001$). Culture-negative cases had a better prognosis than culture-positive ones on both days. *Conclusion*: All CSF parameters, both on admission and at follow-up, gave their best prognostic results in their median values. Hence, basic CSF analysis can give an insight to the prognosis of aSAH patients; however, more elaborate and prospective studies could offer us a clearer picture.

50. Improved Cerebral Oxygenation After Intrathecal Nicardipine for Vasospasm: Case Report

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Introduction: The effects of intrathecal nicardipine are described by pre- and post-transcranial Doppler (TCD) ultrasound. We describe a patient with improvement in multimodal monitoring variables of alpha delta ratio (ADR) monitoring by electroencephalogram (EEG), noninvasive near-infrared spectroscopy (NIRS), cerebral oximetry, and intracranial pressure/cerebral perfusion pressure (ICP/CPP) values after intrathecal nicardipine for vasospasm. *Methods and Results*: A case report is given of a 60-year-old Asian female with a modified Fisher 4 subarachnoid hemorrhage with right frontal intraparenchymal hematoma from rupture of a right anterior cerebral artery pericallosal aneurysm. The patient arrived comatose, Glasgow Coma Scale 7 (E1M5V1T), with intact brain-stem reflexes. She underwent right frontal craniotomy and clipping of her aneurysm along with placement of an external ventricular drain (EVD). Thick temporal bone revented TCD vasospasm monitoring. The patient developed severe vasospasm of the bilateral anterior cerebral artery (ACA) A1, middle cerebral artery (MCA) M1s, and mild bilateral posterior cerebral artery (PCA) P1 and basilar arteries on computed tomographic (CT) angiogram on day 13. Intrathecal nicardipine 4 mg was administered and transiently elevated EEG ADR from 0.4 over the left hemisphere to 0.6 and from 0.6 on the right to 0.8 about 15 min after the procedure, lasting up to 1 h before returning to baseline values. NIRS oximetry rose from a baseline of 82 over the right frontal head region to a peak of 99, whereas the left rose from 78 to 84, and CPP rose from 87 to 122 before returning to baseline. ICP went from 14 to 11 mmHg during the same time frame. The patient did not sustain cerebral infarction as measured clinically or by noncontrast CT scan by hospital discharge. *Conclusion*: Intrathecal nicardipine elevated cerebral oximetry by NIRS, increased CPP, and elevated the ADR transiently but was short lived. Larger studies are needed for intrathecal nicardipine concerning optimal dosing, frequency, and its effects on vasospasm, cerebral blood flow, CPP, and delayed cerebral infarction.

Nitric Oxide and Oxidative Stress

51. Simvastatin Recouples Dysfunctional Endothelial Nitric Oxide Synthase in Experimental Subarachnoid Hemorrhage

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Introduction: Reduced endothelial nitric oxide synthase (eNOS) function has been linked to secondary complications of subarachnoid hemorrhage (SAH). We previously found that there is increased eNOS function after SAH, but that it is uncoupled, leading to secondary complications such as vasospasm, microthromboembolism, and neuronal apoptosis. Here, we test the hypothesis that recoupling eNOS with simvastatin can prevent these complications. *Materials and Methods*: Anterior circulation SAH was created in mice that were treated with vehicle or simvastatin starting 2 weeks before or 30 min after SAH. Animals were then divided into multiple groups for either immunohistochemical detection of nitrotyrosine (oxidative stress), caspase-3/TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) (apoptosis), and fibrinogen (microthromboemboli) or western blot detection of eNOS-PSer1177 and truncated nitric oxide synthase (TeNOS), iNOS (inducible nitric oxide synthase), and neuronal nitric oxide synthase (nNOS). Furthermore, fresh homogenates were used for the biochemical detection of superoxide radicals and nitric oxide NO using chemiluminescent and fluorescence detection methods, respectively. *Results*: SAH increased phosphorylated eNOS, which was

prevented by pre- or post-treatment with simvastatin. Simvastatin pretreatment also prevented the increase in eNOS monomer formation that was associated with SAH, decreased superoxide anion radical production, and increased NO. These changes were associated with decreased vasospasm, microthromboemboli, and neuronal injury. *Conclusion*: The data suggest that simvastatin recouples eNOS after SAH, leading to decreased secondary complications such as vasospasm, microthromboemboli, and neuronal injury.

52. Estrogen Induces Nitric Oxide Production via Nitric Oxide Synthase Activation in Endothelial Cells

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Introduction: 17b-Estradiol (E2) has been found to induce vasodilation in the cardiovascular system and, at physiological levels, to prevent cerebral vasospasm following SAH in animal models. The goal of this study was to analyze the cellular mechanism of nitric oxide (NO) production in vitro and specifically compare its effects on brain endothelial cells with those of peripheral endothelial cells. *Methods*: To evaluate the impact of E2 on endothelial nitric oxide synthase (eNOS) activity and consequently NO production, human umbilical endothelial cells (HUVECs) as well as brain endothelial cells (bENDs) were treated with three different concentrations of E2 (0.1, 10, and 1,000 nmol), and supernatant was collected after 2 h for nitrite (NO_2) measurements. Cells were also treated for 2 h with E2 in the presence of 1,400 W, a potent eNOS inhibitor. To evaluate whether the E2-induced NO release is mediated via estradiol receptors (ERs), we used ICI, an antagonist of ERs. Western blot analyses were performed to verify the presence of eNOS in the cells and to assess the effects of E2 on eNOS expression. *Results*: E2 significantly increased NO_2 levels irrespective of its concentration in both cell lines by 35% and 42% ($p<0.05$). The addition of an E2 antagonist, ICI (10 μmol), prevented the E2-induced increases in NO_2 levels (11%, $p>0.05$). The combination of E2 (10 nmol) and a NOS inhibitor (1,400 W, 5 μmol) inhibited NO_2 increase also (4%, $p>0.05$). E2 also induced increases in eNOS protein levels at concentrations of 10 and 1,000 nmol. *Conclusion*: This study indicated that E2 induces increased NO levels in cerebral and peripheral endothelial cells in vitro via eNOS activation and through E2-receptor-mediated mechanisms. Further in vivo studies are warranted to evaluate the therapeutic value of estrogen for the treatment of SAH-induced vasospasm.

NSICU Care of SAH Patients

53. Neurogenic Pulmonary Edema, a Complication of Aneurysmal Subarachnoid Hemorrhage: A Single-Center Experience

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Institution: University Hospital Zurich, Zurich, Switzerland

Introduction: Neurogenic pulmonary edema (NPE) can lead to acute cardiopulmonary failure with global hypoperfusion and hypoxia. These circumstances might cause severe secondary ischemic brain damage in patients with subarachnoid hemorrhage (SAH) as their brain is especially vulnerable. We aimed to assess clinical presentation and risk factor for the development of NPE and to report on our experience. *Patients and Methods*: The database contained prospectively collected data from 477 patients in an 8-year period. Baseline characteristics, clinical and radiologic severity of the bleeding, localization of the ruptured aneurysm, and clinical outcome of patients with NPE were compared with those of patients without NPE. Further, in patients with NPE, intracranial pressure, serum cardiac biomarkers, and hemodynamic parameters during the acute phase were evaluated retrospectively. *Results*: The incidence of NPE was 8% (39 of 477 patients). Most patients with NPE were severely impaired, and all of them presented with radiologically severe hemorrhage. The incidence of NPE was significantly higher in patients with ruptured aneurysm in the posterior circulation. Elevated intracranial pressure was found in 67% and pathologically high cardiac biomarkers in up to 83% of patients with NPE. However, no patient suffered from persistent cardiac dysfunction. Compared with patients without NPE, patients with NPE showed poor neurologic outcome. *Conclusion*: Patients with clinically and radiologically severe SAH, ruptured aneurysm in the posterior region, and elevated intracranial pressure during the acute phase had a higher risk for developing NPE. Morbidity and mortality due to cardiopulmonary failure might be reduced by appropriate recognition and treatment. However, patients with NPE have a high mortality rate more likely due to the severity grade of their bleeding.

Prevention of DIND, Including Neuroprotection

54. Cerebral Vasospasm After Aneurysmal Subarachnoid Hemorrhage in Elderly Patients

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Introduction: Cerebral vasospasm is known to occur in 20–30% of patients with aneurysmal subarachnoid hemorrhage (SAH) and to contribute to morbidity and mortality. The cause of the vasospasm is multifactorial, and age is reported to be one of the risk factors. However, it is unclear

whether treatment modality (clipping or coiling) affects the occurrence. We compared the incidence of vasospasm after surgical clipping and endovascular coiling in elderly patients with SAH. *Methods*: The medical chart was retrospectively reviewed in consecutive 64 elderly (>75 years old) patients with aneurysmal SAH who underwent surgical repair (clipping 34, coiling 30) during a 36-month period (April 2007 to March 2010) in our stroke center. Treatment modality was mainly decided based on aneurysmal topography, independent from age or patient status. Cisternal irrigation was performed in the clipping group, while spinal drainage was placed in the coiling group after surgical repair. *Results*: Of 34 patients treated by surgical clipping (mean age 79.9, mean Hunt and Kosnik grade 3.29), 3 (8.8%) were found to have vasospasm followed by cerebral infarction. On the other hand, 6 patients (20%) were found to have vasospasm in the coiling group (mean age 82.6, mean Hunt and Kosnik grade 2.8). A modified Rankin Scale score on discharge was 4.12 and 3.4, respectively. *Conclusion*: No statistical significance was found regarding occurrence of vasospasm between surgical clipping and endovascular coiling. The clipping group tended to have a low incidence of vasospasm. Clot removal by cisternal irrigation may have contributed to the results.

55. Effectiveness of Continuous Cisternal Irrigation with Mock CSF Containing Ascorbic Acid and Mg^{++} for Prevention of Symptomatic Vasospasm

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Introduction: Symptomatic vasospasm (SVS) is still a major cause of unfavorable outcome in patients suffering subarachnoid hemorrhage. By using postoperative continuous cisternal irrigation with mock cerebrospinal fluid (CSF) containing ascorbic acid (ASA) and Mg^{++}, we could have achieved an excellent result regarding prevention of SVS in patients who underwent surgical intervention for ruptured intracranial aneurysm at acute stage. The result was better with a Mg^{++} concentration of 4 mEq/l than with that of 3 mEq/l. *Methods*: Mock CSF comprises 500 ml of lactated Ringer's solution, 4 mg/l of ASA, and 3 or 4 mEq/l of Mg^{++} and is adjusted to pH 7.4 with sodium bicarbonate. One hundred and seven consecutive cases were treated with mock CSF containing 3 mEq/l of Mg^{++} (3M) and 35 cases with 4 mEq/l. Statistical analysis was done by Student *t*, chi-square, or Mann-Whitney test. *Results*: In the 3M and 4M groups, age (62 vs. 59), male-to-female ratio (0.52 vs. 0.33), and incidence of Fisher group 3 (78% vs. 78%) were not different. There was no difference regarding grade at admission (grades 1 and 2: 34% vs. 42%; grade 3: 39% vs. 36%; grade 4: 19% vs. 11%; grade 5: 8% vs. 11%). Incidence of SVS in which permanent neurological deficits or low-density areas on computed tomography (CT) remained was 7.5% in the 3M group and 0% in the 4M group. Overall outcome was significantly better in the 4M group than in the 3M group ($p < 0.02$). *Conclusion*: Continuous cisternal irrigation with ASA and Mg^{++} was exceedingly effective in preventing postoperative SVS, especially when Mg^{++} concentration was 4 mEq/l.

56. Magnesium Therapy to Prevent Delayed Ischemic Neurological Deficits (DINDs) in aSAH Patients: Pharmacokinetic Considerations

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Institution: University Hospital Zurich, Zurich, Switzerland

Introduction: Several clinical trials showed promising results for high-dose $MgSO_4$ therapy as a prophylactic treatment option in aneurysmal subarachnoid hemorrhage (aSAH). However, a recent phase III study questioned the favorable effect of $MgSO_4$ [1]. As some investigators used $MgSO_4$ regimens with fixed dosages, the aim of the present study was to establish a clue about the pharmacokinetics of applied $MgSO_4$ and, furthermore, if a fixed-Mg regimen can guarantee the drug's assigned properties. *Materials and Methods*: Plasma Mg profiles of 47 aSAH patients treated with a standardized intravenous $MgSO_4$ schedule (1.5–2.0 mmol/l target plasma Mg level) were retrospectively analyzed. Plasma Mg levels were measured every 6 h, and Mg dosage was adapted according to the protocol. Special attention was given to (A) the affordable Mg doses and duration until therapeutic Mg levels were reached, (B) the Mg rate that was necessary to maintain therapeutic Mg levels, and (C) the changes of Mg dosage needed over time. *Results*: The mean Mg application period to reach therapeutic plasma levels was 37 h and 50 min(SD ± 21.5; range 12–104 h). Therefore, a mean amount of 89.7 mmol/l Mg (area under the curve; SD ± 60.1; range 27.0–296.5 mmol/l) was necessary. While Mg reached therapeutic plasma levels, current Mg dose was 66.8 mmol/24 h on average (SD ± 21.4; range 44–124 mmol/24 h). To hold therapeutic Mg levels, a subsequent steady elevation of Mg dosage was necessary. *Conclusion*: To keep Mg in the aspired therapeutic range, we found considerable differences of Mg dosages required for our aSAH patients. Moreover, to maintain constant therapeutic plasma Mg levels, a steady increase of the Mg was necessary. This means that Mg regimens without adaption of Mg to plasma levels might inevitably result in a significant Mg over- and underdosage. In turn, the real capability of Mg therapy in preventing DINDs cannot be estimated based on the phase III study recently published.

57. Proposed New Grading System for Delayed Vasospasm Following Aneurysmal Subarachnoid Hemorrhage: Value of Cisternal Irrigation with Ascorbic Acid and Mg^{++}

Authors: Ogura, Takeshi (Presenting); Akira, Satoh; Hidetoshi, Ooigawa; Tatsuya, Sugiyama; Ririko, Takeda;

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Introduction: Even with application of multimodal therapies, symptomatic vasospasm (SVS) continues to result in morbidity and mortality in patients with aneurysmal subarachnoid hemorrhage (SAH). We propose a new grading system for SVS with investigation of the effectiveness of cisternal irrigation with mock cerebrospinal fluid (CSF) containing ascorbic acid and Mg^{++}(CCI) for prophylaxis. *Materials and Methods*: The medical charts of 173 consecutive patients with aneurysmal SAH who underwent surgical repair during a 5-year period were retrospectively reviewed to access the incidence of SVS. There were 133 patients (76.9%) treated with CCI for SVS prophylaxis. Severity of SVS was graded as follows: grade 1: without any SVS symptom; grade 2: transient symptom requiring no treatment; grade 3: transient symptom requiring additional treatment such as hyperdynamic or interventional therapy; grade 4: permanent ischemic symptom or development of cerebral infarction. *Results*: The occurrence of SVS was significantly lower($p<0.01$) in the CCI group (15%) than in the non-CCI group (25%). SVS in the CCI group was found to be more mild form (grades 3 and 4; 5.6%), compared to non CCI group (20%) *Conclusion*: Analysis of the results suggests that cisternal irrigation with ascorbic acid and Mg^{++} has a role in cerebral vasospasm prophylaxis.

58. Combined Intravenous Magnesium and Nimodipine Injection for Prevention of Symptomatic Cerebral Vasospasm After Aneurysmal Subarachnoid Hemorrhage: A Prospective Study

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Objective: Symptomatic cerebral vasospasm is a still major problem after aneurysmal subarachnoid hemorrhage. Intravenous magnesium injection is controversial for prevention of symptomatic cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Several drugs were used for prevention of vasospasm, but the rate of cerebral vasospasm is still significantly high. The purpose of this pilot study was to evaluate the efficacy and safety of combined intravenous magnesium and nimodipine to prevent symptomatic cerebral vasospasm after aneurysmal subarachnoid hemorrhage. *Methods*: One hundred and forty-eight patients were treated in our hospital from March 2007 to December 2010. Early in the study, 92 patients were treated with only intravenous magnesium until November 2009. Later, 56 patients were treated with combined magnesium and nimodipine. There was no complication using intravenous treatment. *Results*: Fifty-six patients were enrolled in this prospective study. In the combined group ($n=56$), 8 patients (14%) experienced symptomatic vasospasm, which was confirmed with cerebral angiography. In the nimodipine-only group ($n=92$), 28 patients (30%) experienced symptomatic vasospasm. *Conclusion*: Combined intravenous magnesium and nimodipine injection was an effective and safe method for prevention of symptomatic cerebral vasospasm after aneurysmal subarachnoid hemorrhage.

59. Evaluation of Endovascular Treatment of Cerebral Vasospasm After Subarachnoid Hemorrhage: Establishment of Inclusion Criteria and Assessment of Its Efficacy by MR Perfusion/Diffusion Mismatch

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Institution: Johann Wolfgang Goethe-University, Frankfurt am Main, Germany

Introduction: Endovascular treatment, like transluminal balloon angioplasty or intra-arterial nimodipine, represents a rescue therapy for cerebral vasospasm (CVS) after aneurysmal subarachnoid hemorrhage (SAH). However, the selection of patients who might benefit from this treatment is difficult, and the efficacy of the therapy is inconsistent. We therefore evaluated (1) the usefulness of magnetic resonance imaging (MRI) perfusion- (PWI)/diffusion-weighted imaging (DWI) for patient selection and (2) the efficacy of the endovascular therapy based on MR PWI/DWI mismatch. *Materials and Methods*: In case of suspected CVS, MRI was performed. For quantitative evaluation, the brain was partitioned into 19 arbitrary segments of comparable volume. A segment was defined "at risk" (SAR) when a significant PWI/DWI mismatch was detected. In these cases, MRI was followed by digital subtraction angiography (DSA), including endovascular treatment. Follow-up MRI was acquired 48 ± 12 h after treatment; in case of new or persisting SAR, endovascular treatment was repeated. Treatment efficacy was classified by the improvement of the proximal vessel diameter in DSA after the treatment. *Results*: In 25 patients, 48 treatment cycles, each consisting of MRI, DSA, and follow-up MRI, were completed. Overall, 95 SAR were identified. Delayed infarction was significantly higher in SAR (37%) compared to segments without risk (4%). The risk of infarction in SAR was significantly reduced if endovascular treatment could improve severe or moderate CVS to mild proximal CVS only. In case of persisting severe CVS, infarcts occurred in all SAR. *Conclusion*: Our results suggest that the development of delayed infarction can be predicted by a PWI/DWI mismatch. The risk of infarction was lower if proximal CVS was sufficiently reduced by endovascular treatment.

60. The Effect of Surgical Treatment on Delayed Ischemic Neurological Deficit (DIND) in Patients with Aneurysmal Subarachnoid Hemorrhage

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Introduction: The role of aggressive surgical manipulation with clot evacuation, arachnoid dissection, and papaverine-guided adventitial dissection during aneurysm surgery after subarachnoid hemorrhage (aSAH) in reducing clinically significant vasospasm is controversial. Here, we describe a single-institution experience with and without aggressive surgical manipulation and compare it to patients undergoing coil embolization. *Materials and Methods*: Retrospective analysis of all patients older than 18 years that presented with aSAH of anterior circulation aneurysms between 1996 and 2010 at the University of New Mexico Hospital. Vasospasm was characterized on days 3–14 post-SAH based on (1) angiographic evidence of vascular narrowing, (2) whether vasospasm required intervention during angiography, and (3) development of a delayed ischemic neurological deficit (DIND). *Results*: A total of 188 patients were included. Of those, 33 (17.5%) underwent coiling; 154 (82%) were clipped. Of the surgical group, 111 (72%) had aggressive vascular manipulation, and 43 (28%) had limited manipulation. Over 60% of all patients presented with a Hunt and Hess score of 3 or greater and Fisher grade of 4. All patients were similar in their demographic characteristics. There was a statistically significant decrease in the incidence of DIND in all surgical patients (23.4%) compared to the nonsurgical group (42.4%, $p=0.02$). Similar data were observed with aggressive surgery (18.9%) when compared with limited surgery (34.9% vs. 18.9%). There was a nonsignificant ($p=0.09$) reduction of 14% of ipsilateral radiographic vasospasm (30.6% vs. 44.2% for aggressive vs. limited surgery). A nonsignificant trend was observed in the need for angiographic intervention (12.6% vs. 16.3% for aggressive vs. limited surgery, $p=0.6$). *Conclusion*: We conclude that (1) surgical manipulation resulted in a lower incidence of clinically significant vasospasm in patients treated for aSAH; (2) the more aggressive this surgical manipulation was, the lower this incidence became.

Resistance Vessel Pathology After SAH

61. Delayed Cerebral Ischemia Associated with Spreading Depolarization Can Occur Despite Absence of Proximal Vasospasm After Aneurysmal Subarachnoid Hemorrhage

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It has been hypothesized that proximal vasospasm is the prime mechanism of delayed cerebral ischemia (DCI) after aneurysmal subarachnoid hemorrhage (aSAH). Recently, it was found that clusters of spreading depolarizations (SDs) are associated with DCI. SDs could mediate lesion progression or be involved in repair mechanisms under the ischemic condition induced by proximal vasospasm. Surgical placement of nicardipine prolonged-release implants (NPRIs) was shown previously to strongly attenuate proximal vasospasm. In the present study, we tested in 13 patients with major aSAH whether DCI and SDs are abolished when proximal vasospasm is reduced or abolished by NPRIs. After clipping of the ruptured aneurysm, 10 NPRIs were placed next to the proximal intracranial vessels. SDs were recorded using a subdural six-contact electrode strip. The degree of proximal vasospasm was assessed by digital subtraction angiography (DSA). DCI was assessed by repeated neurological exams and serial computed tomography (CT) or magnetic resonance imaging (MRI) scans. We recorded 534 SDs in 10 of 13 patients (77%). DSA revealed no vasospasm in 8 of 13 patients (62%) and only mild or moderate vasospasm in the remaining patients. Five patients developed DCI associated with clusters of SD, while proximal vasospasm was absent in 3 of those patients. There was no correlation between the degree of proximal vasospasm and the occurrence of DCI. In contrast, the number of SDs and the total duration of the electrocorticographic depression periods correlated significantly with the development of DCI. In conclusion, SDs occurred abundantly after aSAH, although proximal vasospasm was strongly attenuated. Occurrence of SDs must have been the consequence of other mechanisms that may explain why reduction of proximal vasospasm alone has not been sufficient to improve outcome in clinical studies.

62. Matrix Metalloproteinase and Epidermal Growth Factor Receptor Activation Cause Suppression of Voltage-Gated Potassium (KV) Channels to Enhance Constriction of Rat Parenchymal Arterioles After Subarachnoid Hemorrhage

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Introduction: Constriction of the cerebral microcirculation may contribute to neuronal deficits in subarachnoid hemorrhage (SAH) patients. We have previously shown that oxyhemoglobin can acutely suppress voltage-gated potassium (KV) currents in pial artery myocytes via activation of matrix metalloproteinases (MMPs) and epidermal growth factor receptors (EGFRs) [1]. Here, our goal was to determine the role of MMPs, EGFRs, and KV channels in the enhanced constriction of parenchymal arterioles following SAH. *Methods*: The conventional whole-cell patch clamp technique was used to measure KV currents of parenchymal arteriolar myocytes from control and SAH model rats. To assess

arteriolar function, diameter measurements were obtained from isolated parenchymal arterioles. Reverse transcriptase polymerase chain reaction (RT-PCR) was used to examine KV subtype expression. *Results*: Parenchymal arteriolar tone was elevated and KV current density was decreased by more than 40% in myocytes from SAH model animals. Although SAH decreased KV currents, messenger RNA levels of KV subunits were similar between groups. Consistent with SAH-induced suppression of KV currents, 4-aminopyridine-induced constriction was reduced in parenchymal arterioles from SAH animals. The EGFR ligand, HB-EGF, suppressed KV currents in myocytes from control, but not SAH, animals, suggesting EGFR activity may be upregulated following SAH. Compounds that specifically interfere with either HB-EGF signaling (CRM197) or MMP activity (GM6001) increased KV current density in myocytes isolated from SAH, but not control, animals. Further, vasodilation induced by CRM197 and GM6001 was significantly enhanced after SAH. *Conclusion*: These data suggest increased MMP activity causing HB-EGF shedding and enhanced EGFR activity leads to KV channel suppression in parenchymal arterioles from SAH animals. We propose that EGFR-mediated KV suppression contributes to enhanced parenchymal arteriolar constriction after SAH. Supported by National Institutes of Health P01HL095488, R01HL078983, and Totman Medical Research Trust.

Smooth Muscle Signaling

63. The Combination of Argatroban and Vitamin C Normalizes the Increased Vascular Contractile Response After Subarachnoid Hemorrhage (SAH)

Authors: Katsuharu, Kameda (Presenting); Yuichiro, Kikkawa; Satoshi, Matsuo; Akira, Nakamizo; Tomio, Sasaki

Institution: Kyushu University, Fukuoka, Japan

Introduction: Increased vascular reactivity plays a key role in the pathogenesis of cerebral vasospasm in subarachnoid hemorrhage (SAH). We investigated the role of thrombin and its receptor PAR_1 (proteinase-activated receptor 1) in the increased vascular contractile response in SAH and examined the preventive effects of the thrombin inhibitor argatroban and vitamin C on contractility. *Materials and Methods*: We investigated the role of thrombin and its receptor PAR_1 in the increased vascular contractility utilizing a rabbit double-SAH model. The contractile response of the isolated basilar artery and the level of oxidative stress of brain tissues were evaluated. *Results*: In the control basilar arteries, 1 U ml^{-1} thrombin and 100 μmol PAR_1-activating peptide (PAR1-AP) induced a small and transient contractile response, while they induced an enhanced and sustained contractile response in SAH. When the arteries were consecutively stimulated with PAR1-AP, the reactivity to the second stimulation was markedly attenuated in the control, while it was maintained in SAH. Intrathecal treatment with 1 μg argatroban/kg $weight^{-1}$ per injection (ARG) attenuated the response to thrombin and PAR1-AP in SAH. However, the contraction was sustained, and the tachyphylactic attenuation of the contraction was impaired. The combination of ARG and 0.6 mg vitamin C/kg $weight^{-1}$ per injection (VC) normalized the vascular contractility to PAR_1 agonists. Oxidative stress was increased in SAH. Treatment with ARG, VC, or their combination normalized the level of oxidative stress. *Conclusion*: The contractility of the basilar artery to thrombin was enhanced and prolonged after SAH. The combination of ARG and VC is suggested to normalize the increased vascular contractility in SAH.

64. Potentiation of Endothelin-1-Induced Myofilament Ca^{2+} Sensitization Following Subarachnoid Hemorrhage

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Introduction: Increased vascular contractility in response to endothelin-1 (ET-1) plays an important role in the development of cerebral vasospasm. We elucidated some mechanisms of the increased vascular contractility to ET-1 using the basilar artery in a rabbit subarachnoid hemorrhage (SAH) model. *Materials and Methods*: The contractile response and the expression of regulatory protein of the isolated basilar artery were evaluated. *Results*: ET-1 induced greater contraction than other agonists or high-K^+ depolarization for the extent of $[Ca^{2+}]i$ elevation, suggesting that myofilament Ca^{2+} sensitivity is a greater contributor to ET-1-induced contraction than other contractions. ET-1-induced contraction of α-toxin-permeabilized strips was significantly enhanced and sustained in SAH compared to control, suggesting that the ET-1-induced myofilament Ca^{2+} sensitization was enhanced after SAH. Therefore, we investigated the intracellular signaling pathway involving rho kinase (ROCK) and protein kinase C (PKC), which are two major signaling molecules that contribute to Ca^{2+} sensitization. ET-1-induced contraction of α-toxin-permeabilized control strips was blocked by inhibitors to ROCK and PKC in a concentration-dependent manner, whereas the concentration-response curve shifted to the right in SAH, suggesting that ROCK and PKC signaling was activated in SAH. Immunoblotting showed that the expression of PKCα, ROCK2, CPI-17, and MYPT-1 was significantly upregulated after SAH. Phosphorylation of MYPT-1 at T853 and CPI-17 at T38 was significantly increased in SAH, but the phosphorylation of MYPT-1 at T696 remained unchanged. However, in response to ET-1 stimulation, the phosphorylation of T696 was significantly increased in SAH, whereas the phosphorylation of T853 and T38 was increased in both control and SAH. *Conclusion*:

ET-1-induced myofilament Ca^{2+} sensitization is potentiated after SAH, leading to enhancement of vascular contractility in response to ET-1.

Spreading Depolarizations

65. The Role of Spreading Depolarization, Spreading Depression, and Spreading Ischemia in Patients with Aneurysmal Subarachnoid Hemorrhage

Authors: Dreier, Jens (Presenting); Isele, Thomas

Institution: Charité University Medicine Berlin, Berlin, Germany

The term *spreading depolarization* describes a wave in the brain's gray matter characterized by near-complete breakdown of ion homeostasis, a large change of the slow electrical (or direct current [DC]) potential, swelling of neurons, and distortion of dendritic spines. The DC change is a direct extracellular index of spreading depolarization (Canals et al., J Neurophysiol 94:943–951, 2005), and it shows a wide spectrum in the human brain, ranging from short- to very long-lasting events (Dreier, 2011, Nat Med, in press). Spreading depolarization can be associated with three different depression patterns of brain activity: (1) non-spreading depression, (2) spreading depression, and (3) persistent depression of activity. The depression pattern is observed in a higher-frequency range of the electrocorticogram than the DC change. Hence, spreading depolarization and the depression pattern are observed as two clearly distinct signals. The near-complete breakdown of the electrochemical gradients leads to a dramatic loss of Gibbs free energy. Based on the intra-/extracellular cation changes and the resulting changes in mixing entropy, we here calculated that the loss in Gibbs free energy during spreading depolarization exceeds that during epileptic seizure activity seven times. To restore ion homeostasis and the normal tissue level of Gibbs free energy, additional chemical energy (adenosine triphosphate, ATP) is required to fuel the sodium pump. Therefore, resistance vessels respond to spreading depolarization with tone alterations, causing transient hyperperfusion (physiological hemodynamic response) in healthy tissue. After aneurysmal subarachnoid hemorrhage (aSAH), this neurovascular coupling can be disturbed in such a way that spreading depolarization induces severe vasoconstriction and hypoperfusion (inverse hemodynamic response or spreading ischemia). Hence, in a moment of maximal metabolic demand, energy supply is reduced, a mechanism that may be involved in delayed ischemic stroke after aSAH.

66. Subarachnoid Hemorrhage Increases the Susceptibility to Ischemic Stroke But Not to Peri-Infarct Depolarizations After Middle Cerebral Artery Occlusion

Authors: Ulrike, Hoffmann (Presenting); Lee, Jeong-Hyun; Park Hopson, Kristen; Yzawa, Izumi; Qin, Tao; Nozari, Ala; Ayata, Cenk

Institution: Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Introduction: Peri-infarct depolarizations (PIDs) akin to spreading depression (SD) occur frequently after ischemic stroke and subarachnoid hemorrhage (SAH) and worsen the outcome of compromised tissue by exacerbating the blood flow-metabolism mismatch. SAH is often associated with ischemic brain injury presumed to be due to vasospasm. We tested whether SAH increases the sensitivity of brain tissue to focal arterial occlusion and whether the mechanism involves increased susceptibility to SD or PIDs. *Materials and Methods*: Mice (C57BL/6, male) and rats (Sprague-Dawley, male) were subjected to single or double intracisternal autologous blood or saline injection. Twelve or 24 h later, middle cerebral artery was occluded (MCAO) for 1 h using an intraluminal filament to assess infarct volume, neurological deficits, and PIDs. SD susceptibility was assessed in a separate group using KCl or electrical stimulation. To investigate vascular mechanisms, vasoreactivity was assessed using isolated pressurized posterior cerebral arteries (PCAs), and cerebral blood flow (CBF) deficit was mapped using laser speckle flowmetry with high spatiotemporal resolution during distal MCAO. In all applicable experiments, physiologic parameters were monitored continuously. *Results*: SAH increased infarct volumes by approximately 30% after both filament and distal MCAO, when ischemia was induced 12 but not 24 h after SAH. Contrary to our hypothesis, however, both SD susceptibility and PID frequency were decreased after SAH. Isolated PCAs showed smaller resting diameters and higher myogenic tone, suggesting vasospasm. Consistent with this, resting CBF was lower and CBF deficits during dMCAO were worse in the SAH group to explain the larger infarct volumes. *Conclusion*: Our data suggest that SAH worsens the outcome of focal ischemia by causing vascular dysfunction and worsening the perfusion deficit, and that cortical susceptibility to SD or PIDs is not increased after SAH.

67. Impact of Body Temperature on Occurrence of Cortical Spreading Depolarizations in Subarachnoid Hemorrhage

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From animal models, it is known that elevated temperature can abet or trigger the generation of cortical spreading depolarizations (CSDs). CSDs were demonstrated to occur in patients suffering from aneurysmal subarachnoid hemorrhage (aSAH), and it is assumed that they are a negative prognostic factor for outcome of these patients. To investigate the impact of normothermia and hyperthermia on the

occurrence of CSD, 21 patients with acute aSAH where monitored for 38–266 h after onset of the initial SAH. Temperature was determined every 30 min, leading to a total of 9,739 episodes of temperature measurement in all patients. A cortical electrode strip to record the electrocorticogram (ECoG) was implanted within 24 h after SAH onset in the course of the aneurysm clipping. The post hoc analysis of the ECoG showed a total of 460 CSDs in 17 patients. Four patients did not show evidence for CSD. For every CSD, the temperature at the time point of occurrence was determined and divided into three temperature ranges (normothermia 36.0–36.9°C, mild hyperthermia 37.0–37.9°C, moderate hyperthermia 38.0–38.9°C). Of the 9,739 monitored temperature episodes, 1,580 (=790 h) showed normothermia, and 16 CSDs were recorded within this temperature range, resulting in an average of 0.02 CSD per hour. During 6,037 (=3,018.5 h) temperature episodes with mild hyperthermia, 120 CSDs occurred (0.04 CSD per hour average), and during 1,678 (=839 h) temperature episodes of moderate hyperthermia, 92 CSDs were recorded (0.11 CSD per hour average). For the comparison of body temperature and the occurrence of CSD within a temperature range from 36.0–38.9°C, we calculated a correlation coefficient of 0.76. During moderate hyperthermia, the probability for a CSD was more than fivefold higher than during normothermia. Our data suggest an association between higher temperature and CSD. It would be interesting to investigate whether reduction of temperature would decrease the incidence of CSD.

68. Spreading Depolarization-Induced Injury to Neurons and Astrocytes

Authors: Kirov, Sergei (Presenting); Risher, W. Chris; Sword, Jeremy; Croom, Deborah; Yuan, Jianghe

Institution: Georgia Health Sciences University, Augusta, GA, USA

Introduction: Two-photon laser scanning microscopy (2PLSM) enables real-time visualization of cells expressing green fluorescent protein (GFP) deep within living neocortex in vivo. Using 2PLSM, we have previously shown that spreading depolarizations (SDs) cause acute damage to neurons in the ischemic penumbra. We also have evidence that cardiac arrest-induced global ischemia elicits astroglial swelling, indicating acute damage. *Materials and Methods*: 2PLSM was used to monitor astroglial changes concurrently with neuronal injury in two different models of ischemic stroke and mild traumatic brain injury (TBI). The first method was transient bilateral common carotid artery occlusion, which allowed for the induction of global ischemia and subsequent reperfusion. The second method was modified photothrombotic occlusion, in which a square-shaped ischemic lesion was made to surround a penumbra-like "area at risk" with SDs recurring for several hours following photothrombosis. TBI was induced by a nonpenetrating localized deformation of the cortex with a controlled cortical impact device. *Results*: In stroke models, SD wave coincides with astroglial swelling alongside dendritic beading. Although rapid neuronal recovery was seen in both models, astroglial swelling persisted long after the occurrence of SD, with no structural recovery seen for the duration of the acute imaging period. In the TBI model without spontaneous SDs, dendritic injury in the pericontusional area was developing relatively slowly, but injury was greatly facilitated by SDs evoked by injecting KCl with a micropipette. Astroglial swelling persisted in the pericontusional area for the duration of acute imaging. *Conclusion*: Dendrites rapidly bead in concert with propagating SDs, recovering when there is sufficient local capillary flow. The accumulating stress of repeated SDs eventually results in "terminal" injury. Astrocytes swell during recurring spontaneous SDs, with no recovery seen in acute experiments. Early astroglial swelling may exacerbate functional outcome as astrocytes fail to provide neuronal support.

69. Trauma Trumps Stroke: Why Is Our Higher Brain Inept at Dealing with Blocked Blood Flow?

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While poorly documented, at the moment of traumatic brain injury (TBI), neurons in the impacted cortex completely depolarize as a consequence of mechanical sheer/stretch. Within seconds, this "traumatic depolarization" (TD) propagates to adjacent gray matter, driving neurons beyond the point of discharge, thereby silencing the contused region while inducing regional vasoconstriction. TD promotes a "lie-low" survival strategy that immobilizes the subject for minutes or more, as well as counteracting hemorrhage. We propose that evolutionary pressure to develop this trauma defense has created a deadly catch-22: Our higher brain is inept at dealing with the TD-like ischemic depolarizaton (ID) triggered by global ischemia or occlusive stroke. Head trauma was a common way to die throughout our brutal vertebrate evolution, so there was strong selective pressure to cope with it. At the same time, our ancestors' lives were usually brief, rigorous, and nutrient inadequate, so few died from clogged brain arteries. Without evolutionary selection for mechanisms to deal with occlusive stroke, sudden loss of blood flow in cortical gray matter evokes ID and vasoconstriction (inverse neurovascular coupling) as though the region is hemorrhaging from TBI. In support of ID being a rapid, but inappropriate, shutdown defense, we also contend the following: (a) To maintain vital functions, internal brain structures (hypothalamus, brain stem) do not generate potent ID with resultant rapid shutdown as do more exposed gray regions in telencephalon, cerebellum, and spinal cord [1, 2]. This helps explain why the brain stem survives global ischemia better. (b) Cortical ID is generated by all vertebrates despite they do not naturally suffer stroke. (c) Migraine cortical spreading depolarization (CSD) is a residual ID response

to a misperceived loss of blood flow, evoking headache as part of the lie-low strategy of dealing with TBI. (d) Anoxia induces ID and immobilization in insects [3], which lack arterioles/capillaries. Neuronal type dictates if a region shuts down.

70. Intracortical Electroencephalography Monitoring for Detection of Cortical Spreading Depolarisations in Three Patients After Traumatic Brain Injury

Authors: Zakrzewska, Agnieszka (Presenting); Walsh, Daniel; Tolias, Christos; Strong, Anthony John

Institution: King's College Hospital, London, UK

Introduction: Cortical spreading depolarizations (CSDs) occur in 50–60% of patients after traumatic brain injury (TBI) in areas of cortex adjacent to contusions in the injured human brain, in 70–90% of subarachnoid hemorrhage (SAH) World Federation of Neurosurgical Societies (WFNS) values 3–5, and in all patients with malignant hemisphere stroke. These events are believed to be an important pathophysiological mechanism of secondary brain injury, but currently can only be detected with a subdural strip inserted when craniotomy has been required. *Aims*: We aimed to test CSD detection sensitivity of a Spencer depth electrode as an alternative to subdural strip. *Methods*: Electroencephalography (EEG) was recorded continuously in 3 patients undergoing craniotomy for TBI: A subdural strip electrode was placed on cortex exposed by the craniotomy, and a depth electrode was implanted intraparenchymally close to the strip. *Results*: In the first patient, a CSD event was recorded on the depth electrode and on the subdural strip simultaneously. In the first two patients, several (3 in patient 1 and 12 in patient 2) slow potential change (SPC) events occurring synchronously across channels were detected on both strip and depth electrodes. In the third patient, no CSDs and SPC events were observed on both strip and depth electrodes. *Conclusion*: There is preliminary evidence that the depth electrode can record SPC events that also appear on the surface electrode. Based on these observations, we propose that continuous monitoring from a depth electrode inserted into cortex via an access bolt will allow less-invasive monitoring for depolarizations.

71. Vascular, Electrophysiologic, and Metabolic Consequences of Cortical Spreading Depression in a Mouse Model of Simulated Neurosurgical Conditions

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Institution: University of New Mexico, Albuquerque, NM, USA

Introduction: Cortical spreading depression (CSD) is a metabolically taxing wave of cellular depolarization. CSD occurs frequently in humans after brain injury and is associated with worse outcomes [1]. Less is known about possible contributions of CSD to injury following standard neurosurgical procedures. The current work evaluated CSD in a mouse model of simulated intraoperative conditions. *Materials and Methods*: Mice were intubated and ventilated; an arterial line was placed. Normothermia and normocapnia were maintained and neuroanesthesia simulated with fentanyl, propofol, and isofluorane. Craniotomies were made to record responses to cortical coagulation with bipolar cautery. Separate sets of experiments (3 animals each) examined electrocorticographic (ECoG) activity, optical measures of blood volume and vascular diameters (540-nm absorbance), or autofluorescence attributed to NADH (750 nm, 2-photon excitation). *Results*: Ipsilateral cauterizations invariably resulted in a robust propagating CSD wave, identified by slow direct current (DC) potential shifts (2.8 ± 0.2 mm/min, $n = 6$) and suppression of ECoG activity (range 0.5–7.3 min, $n = 10$). Each evoked CSD was associated with an initial pronounced arteriolar constriction, followed by a longer-lasting vasodilation. These changes led to similar tissue blood volume responses. Tissue oxygenation, assessed indirectly by NADH imaging, was consistent with sustained demand on oxidative metabolism. Furthermore, repetitive SDs resulted in progressive loss of tissue autofluorescence, suggestive of tissue compromise. *Conclusion*: CSD is consistently elicited by simulated neurosurgical stimuli under simulated intraoperative conditions in mice. These events caused prolonged ECoG depression, transient vasoconstriction, and significant metabolic demand that propagated from the manipulation site. It is possible that CSD events contribute to metabolic challenge at locations distant from sites of surgical manipulation.

72. Detection of Spreading Depression by Surface EEG

Authors: Fabricius, Martin (Presenting); Lauritzen, Martin

Institution: Glostrup University Hospital, Glostrup, Denmark

Introduction: Cortical spreading depression/depolarization (CSD) of the acutely injured human brain may be readily detected by electrocorticogram (ECoG). CSD, supposedly the mechanism of migraine aura, spreads at a speed of 2–3 mm/min and recovers within 6 min in the normal brain; thus, depression of the electroencephalogram (EEG) occurs in a narrow strip of cortex, around 2 cm wide. Noninvasive surface EEG recordings have failed to demonstrate EEG depressions during migraine aura. In brain injury, CSD duration may be prolonged, thus expanding the strip of depressed activity. This might increase the likelihood of detecting changes in surface EEG. *Materials and Methods*: Six patients suffering from aneurysmal subarachnoid hemorrhage (aSAH) ($n = 4$) or intracerebral hematoma ($n = 2$) underwent craniotomy on clinical indication, and a 6-electrode platinum strip was placed subdurally. Postoperatively, intradermal EEG electrodes were placed. EEG and ECoG were recorded on two separate recording systems. Surface EEG trends (e.g., Brain Symmetry Index, BSI) were inspected for episodes suggesting transient (10–30 min) periods of regional loss of EEG power. The ECoG was scored independently for

episodes of CSD. *Results*: During 250 h of recording, 51 CSDs were recorded on ECoG. On surface EEG, 17 transient reductions in BSI occurred in the same period as a CSD. There were 34 CSDs without any obvious changes in EEG trends; 18 transient changes in EEG trends occurred without any CSD being recorded simultaneously. *Conclusion*: Episodes of CSD may be reflected in surface EEG trends, particularly BSI. In this limited study, material sensitivity was 33% and specificity about 50%. Some false-positive changes in surface EEG may in fact represent episodes of CSD occurring in regions remote from the subdural strip, and some CSDs recorded from a dural strip placed below the frontal lobe may never reach the convexity of the brain. Further refinement of the EEG analysis may lead to increased sensitivity and pave the way for noninvasive detection of CSD.

73. On the Role of External Patient Movements that Can Trigger Spreading Depolarizations in Subarachnoid Hemorrhage Patients

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Institution: University of Heidelberg, Heidelberg, Baden Württemberg, Germany

Introduction: It has recently been reported that spreading depolarizations (SDs) are spontaneously produced in patients suffering from brain injury. In animals, SDs can be induced even in healthy brains using electrical, mechanical, and K^+ stimuli. Since patients in the neurocritical care environment undergo various nursing maneuvers and are rarely kept in one position, we hypothesized that even these minute movements could trigger SD "mechanically." *Methods*: To study the relationship between SDs and movement, we retrospectively analyzed 18 SAH patients, enrolled in the COSBID (CoOperative Study on Brain Injury Depolarisations) study, who presented SDs. We compared the overall occurrence of SDs to those time periods when usually fewer nursing maneuvers occur (i.e., changes of shift). In 4 consecutive patients, a movement sensor was placed on the skin of the frontal or parietal area of the head to study these events prospectively. The time relationship between SDs and movement was assessed. *Results*: A total of 435 SDs were summed in a histogram according to the hours of the day. There were a mean of 18.1 ± 6.7 SDs per hour. Occurrence of SD was not evenly distributed. During the first scheduled break during the morning report, there were 11 SDs per hour. During the second scheduled break at noon, there were 6 SDs per hour. From the prospectively analyzed patients, one presented 36 SDs, from which 24 were associated with previous movement (3–7 min before the SDs). The second patient presented 3 SDs that were preceded by movement in all the cases (7–10 min). The third patient presented 2 SDs, but they were not preceded by movement. The fourth patient did not show SDs. *Conclusion*: SDs that are associated with external patient movements may be found in SAH patients. The triggering mechanism remains speculative. Movement of the patients should be documented in the studies because it could be a confounding factor for the occurrence of SDs, and it might have a negative effect on outcome.

74. Toward Use of Near-Infrared Spectroscopy (NIRS) to Detect Cortical Spreading Depolarizations (CSDs) Noninvasively

Authors: Zakrzewska, Agnieszka (Presenting); Blasi, Anna; Everdell, Nick; Mifsud, Victoria; Walsh, Daniel; Pahl, Clemens; Strong, Anthony John

Institution: King's College Hospital, London, UK

Introduction: Near-infrared spectroscopy (NIRS) detects changes in the concentration of oxygenated, deoxygenated, and total hemoglobin by measuring changes in the absorbance of light in the NIR region of the spectrum over the illuminated tissue. Brain activity is closely related to regional changes in cerebral blood flow (CBF) and oxygenation. The method is based on the assumption that change in neuronal activity is reflected by a change in CBF and blood volume that affects the mean local oxygenation. Previous experimental studies have shown that cortical spreading depolarizations (CSDs) produce hyperemia in healthy brain; in the injured human or experimental brain, perilesion depolarizations often induce vasoconstriction and spreading ischemia, all of which may result in tissue oxygenation changes measurable with NIRS. *Aims*: We wanted to develop a noninvasive method for detection of CSDs in acutely brain injured patients. *Methods*: We recorded 23 sessions of continuous wave (CW) NIRS of mean duration 2 h 4 min 47 s in 17 patients after head injury, subarachnoid or intracerebral hemorrhage, or stroke. Monitoring was performed using a model of the university college london (UCL) NIRS system with an array of 6 sources and 4 detectors, providing a total of 10 channels placed over the brain area of interest in patients either after surgery or treated conservatively. *Results*: A total of 47 h 50 min 19 s of data were recorded. Eight subjects showed transient changes in the concentrations of the three chromophores that could potentially be related to CBF alterations caused by CSDs. A further validation against electrocorticography monitoring will enable distinction between local oxygenation variations induced by CSD and those caused by systemic hemodynamic changes such as transient hypotension. *Conclusion*: Relative changes in the concentrations of oxy-, deoxy-, and total hemoglobin measured by CW-type instruments may be sufficient to detect CSDd, constituting a less-invasive method than currently used, with the advantage that it can be used on both unconscious and conscious individuals.

75. Analgesics and Sedative Drugs Have an Impact on Frequency of Spreading Depolarizations in the Injured Human Brain

Authors: Hertle, Daniel (Presenting); Hartings, Jed A.; Woitzik, Johannes; Dreier, Jens P.; Vidgeon, Steven; Strong, Anthony J.; Kowoll, Christina; Dohmen, Christian; Diedler, Jennifer; Veltkamp, Roland; Unterberg, Andreas W.; Sakowitz, Oliver W.

Institution: University of Heidelberg, Heidelberg, Germany

Spreading depolarizations (SDs) are known to occur in humans after brain injury. These patients are in critical medical condition and therefore often treated with analgesics and sedatives. Such drugs influence brain activity and are possible modulators for the frequency of spreading depressions. We therefore undertook a study to analyze drug effects on SDs, ieri-infarct depolarizations (PIDs), and clusters of SDs. We included 115 patients with acute brain injury (subarachnoid hemorrhage, trauma, ischemic stroke) from the CoOperative Study on Brain Injury Depolarisations (COSBID) study. Midazolam, flunitrazepam, thiopental, fentanyl, sufentanil, remifentanil, ketamine, propofol, gamma-hydroxybutyric acid (GHB), morphine, and clonidine were included in our analysis. Recorded hours with and without SDs under the influence of each drug were counted. Combined data of several drugs as indicated were used to calculate the odds ratio (OR). In this preliminary analysis, we found marked effects of midazolam and fentanyl increasing the probability for occurrence of SDs with an OR of 1.2, 95% confidence interval (95% CI) 1.1–1.4. In contrast, propofol, sufentanyl, ketamine, and morphine seemed to reduce the number of hours with SDs in the combined analysis (OR 0.76, 95% CI 0.7–0.8). The result of this preliminary report suggests a possible impact of anesthetic drugs on the occurrence of SDs. A careful evaluation of potential modulators is necessary. This might affect the choice and use of anesthetic drugs after acute brain injury.

76. Spatiotemporal Patterns of Cerebral Blood Flow and Hemoglobin Oxygenation During the Propagation of Spreading Depolarisations Following Middle Cerebral Artery Occlusion

Authors: Takagaki, Masatoshi; Gramer, Markus; Feuerstein, Delphine; Backes, Heiko; Kohl-Bareis, Matthias; Graf, Rudolf (Presenting)

Institution: MPI for Neurological Research, NRW, Germany

Objectives: In the surrounding of focal ischemia, spreading depolarizations (SDs) are thought to cause secondary tissue damage. However, the underlying mechanisms are obscure. We combined here laser speckle flowmetry (LSF) to track the SD waves and hemodynamic responses to them with RGB reflectometry (RGBR) to measure tissue concentrations in oxyhemoglobin (HbO_2) and deoxyhemoglobin (HHb). *Methods*: The temporoparietal cortex of Wistar rats was exposed to laser illumination (for LSF) and high-power white light LEDs (light-emitting diodes; for RGBR) through thinned skull. Reflected signals were spectrally separated and acquired by two CCD chips within a single camera [1]. After baseline measurement, the middle cerebral artery was occluded (MCAO) by slow intracarotid injection of TiO_2 macrospheres [2]. Simultaneous LSF and RGBR imaging was continued for 3 h. *Results*: Immediately following MCAO, a gradient of CBF levels developed, differentiating ischemic core from border zones [3]. Subsequently, a primary, concentric wave of CBF, HbO_2, and HHb changes originated from the primary ischemic territory. CBF and oxygenation waves were almost synchronous and congruent in space. Subsequently, multiple waves appeared over the observation period. They mostly propagated circumferentially around the ischemic core. A first analysis suggests a very close synchronization and congruence of these LSF and RGBR waves as well. CBF declines were typically accompanied by HbO_2 decrease and HHb increase. First observations also suggest a gradual and steady decrease in HbO_2 and increase in HHb as the SD waves repeat. *Conclusion*: SD waves were tracked as concomitant and congruent waves of both CBF and O_2 alteration. Our results suggest that the fast, almost-immediate change in CBF after MCAO is accompanied by a slower, gradual aggravation of tissue oxygenation with time and with repeating SDs.

77. Coupling of Cerebral Blood Flow and Glucose Metabolism During Spreading Depolarisations: A Multimodal Study

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Institution: MPI for Neurological Research, Cologne, Germany

Objectives: Recent clinical studies suggested that multiple spreading depolarizations (SDs) in human injured brain cause failure of metabolic coupling [1, 2]. We have characterized this "uncoupling" using an in vivo multimodal approach. Laser speckle flowmetry (LSF) tracked SDs and corresponding blood flow responses, positron emission tomography (PET) of ^{18}F-2-fluoro-2-deoxy-D-glucose (FDG) measured glucose metabolism, and rapid-sampling microdialysis (rsMD) measured glucose and lactate. *Methods*: In Wistar rats, the temporoparietal cortex was exposed to laser illumination through thinned skull. A microdialysis probe was inserted to assess glucose and lactate using rsMD [3]. Within the PET scanner, the baseline of rsMD and LSF was measured; subsequently, FDG was injected, and PET images were acquired for 90 min. SDs were induced 20 min after FDG injection by either a needle prick (single-SD group) or by epidural potassium chloride (multiple-SD group). *Results*: The passage of a single SD wave ($n = 10$) caused a moderate fall in glucose and a sharp increase in lactate by −15% and +35%, respectively. FDG uptake increased compared to the contralateral hemisphere. Both rsMD levels and FDG uptake returned to normal by 20 min post-SD. Following multiple

SDs ($n=10$), FDG preferentially accumulated in regions with a high occurrence of SDs. The amplitude of increased FDG uptake correlated with the frequency of SDs ($r=0.729$, $p=0.002$). rsMD glucose steadily decreased, while lactate remained elevated. *Conclusion*: The passage of an SD wave causes a local transient increase in energy utilization. Normalization of FDG uptake and rsMD levels at 20 min post-SD suggests a resting phase of reduced energy consumption. However, when SDs repeat frequently, the increased energy demand leads to irreversible changes in FDG uptake, depletion of glucose, and elevation of lactate. This energy "crisis" during multiple SDs would be amplified in already-compromised tissue after brain injury.

Surgical Management and Intervention

78. Neck Clipping of Paraclinoid Small Aneurysms

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Introduction: The International Study of Unruptured Intracranial Aneurysms reported that, in the absence of a history of previous rupture, the risk of rupture for small (<7 mm) anterior circulation aneurysms was low, at only 0.1% per year [1]. However, many experienced neurosurgeons and endovascular therapists reported that, in practice, most ruptured aneurysms encountered are small [2, 3]. According to other reports, the extent of SAH after the rupture of small aneurysms is often greater than after the rupture of larger aneurysms [3, 4]. Despite the advent of sophisticated endovascular materials, embolization of very small aneurysms is associated with relatively high rates of intraprocedural rupture, especially intraoperative rupture [5]. From a surgical point of view, blood blisterlike aneurysms arising from the anterior wall of the internal carotid artery [ICA] may be devastating once they rupture [6]. In the present study, we demonstrated ten cases with small aneurysms in the paraclinoid segment of the ICA, and all of those were successfully clipped. *Materials and Methods*: The patients included 6 women and 4 men, 41–66 years of age (mean 56 years). Key points of surgical procedures were as follows: (1) frontotemporal craniotomy with removal of sphenoid ridge; (2) open the sylvian fissure as much as possible; (3) removal of anterior clinoid process and falciforme ligament to create sufficient space for clip application; (4) dural adherence to aneurysm left intact for protection of thin aneurysm dome; (5) avoid touching the dome of aneurysm before neck clipping; (6) angled clip is suitable for anterior wall aneurysms and a straight one is for ophthalmic artery aneurysms; (7) additional wrapping and coating may be necessary for anterior wall aneurysms if parent artery is affected entirely. *Results*: There were no perioperative complications. Glasgow Outcome Scale after 3 months demonstrated excellent results in all patients.

79. Comparison of Clearance of Clots in Patients with Subarachnoid Hemorrhage Between Surgical Clipping and GDC Embolization

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Institution: Yamaguchi University School of Medicine, Ube, Yamaguchi, Japan

Objective: Subarachnoid clots play an important role in the development of delayed vasospasm after subarachnoid hemorrhage (SAH). The purpose of this study was to compare clearance of subarachnoid clot and the incidence of symptomatic vasospasm between surgical clipping and Guglielmi detachable coil (GDC) embolization for aneurysmal SAH. *Methods*: The subjects were 115 patients with Fisher group 3 aneurysmal SAH on a computerized tomographic (CT) scan at admission, in whom the aneurysm was treated by surgical clipping (clip group, $n=86$) or GDC embolization (coil group, $n=29$) within 72 h of ictus. Software-based volumetric quantification of the subarachnoid clot was performed. The amount of hemoglobin (Hb) in drained cerebrospinal fluid was also measured by biochemical assay. *Results*: Clearance of the subarachnoid clot on the CT scan was rapid in the clip group until the day after the operation but slow in the coil group (58.9% removed vs. 27.8% removed, $p=0.008$). However, postoperative clearance of the clot occurred more rapidly in the coil group. Difference in the reduction of clot until days 3–5 was not significant between the two groups (72.9% removed vs. 75.2% removed). The amount of Hb in the clip group was greater than 0.8 g/day until day 3 and then gradually decreased ($n=15$), but Hb in the coil group remained at more than 0.8 g/day until day 5 ($n=17$). The incidence of symptomatic vasospasm did not differ between both groups. *Conclusion*: Subarachnoid clot can be directly removed during surgical clipping, which is not possible for endovascular treatment. However, the percentage reduction of the clot on days 3–5 did not differ between the two groups.

Other

80. Supply or Demand?

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Introduction: There is an evolving understanding that delayed ischemic neurological deficit (DIND) is a result of various interdependent pathologic processes like vasospasm,

microthrombosis, inflammation, cortical spreading ischemia, and effects of early brain injury. All these processes are believed to reduce cerebral blood flow (CBF), that is, the supply and cause of cellular ischemia. However, clinical features of DIND have been observed above the ischemic threshold of CBF. With real-time neuromonitoring, we explored cerebral pathophysiology in aneurysmal subarachnoid hemorrhage patients before and during DIND and with institution of triple H (hypertensive, hypervolemic, and hemodilution) therapy. *Methods*: Patients with Fisher grade 3/4 were recruited. Probes to monitor tissue oxygenation, regional CBF, and microdialysis were placed at the time of clipping/coiling. Normoxia and normovolemia were maintained afterward; no prophylactic triple H therapy was used. If the patient developed symptoms of DIND, the patient was treated with standardized triple H therapy. *Results*: We recruited 16 patients; 2 patients died early, and 2 were sedated throughout monitoring. Of the remaining 12, 7 developed DIND. Six of 14 had reduced CBF; of these, 2 were symptomatic (DIND). Interestingly, 6/7 patients with DIND had parallel elevation of lactate and pyruvate at time of symptoms; 4/5 asymptomatic patients did not (odds ratio [OR] = 24:1). CBF and metabolism did not seem to bear any direct association. Triple H therapy increased CBF; symptoms improved. *Conclusion*: We present evidence that DIND might be an issue of demand as well as supply. Triple H therapy may restore balance.

81. Early Brain Injury Linearly Correlates with Reduction in Cerebral Perfusion Pressure During the Hyperacute Phase After Subarachnoid Hemorrhage

Authors: Marbacher, Serge (Presenting) [Runner-up, Bench-to-Bedside-and-Back]; Neuschmelting, Volker; Andereggen, Lukas; Widmer, HansRudolf; Takala, Jukka; Jakob, Stephan M.; Fandino, Javier

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Introduction: Early brain injury (EBI) after aneurysmal subarachnoid hemorrhage (SAH) emerged as a recent concept that embraces complex pathophysiological mechanisms that are linked to the initial bleed. The triggers and consequences of EBI are still poorly understood. The aim of the present study was to investigate whether hyperacute depletion of cerebral perfusion pressure is correlated with an increase in neuronal injury following experimental SAH. *Methods*: Various degrees of SAH in terms of intracranial pressure (ICP) increase were initiated and controlled using a shunt from the subclavian artery to the cerebromedullar cistern in 14 rabbits. Standard cardiovascular monitoring, ICP, cerebral perfusion pressure (CPP), and bilateral regional cerebral blood flow (rCBF) were continuously measured. Apoptosis was detected using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Neurodegeneration secondary to ischemia was determined 24 h after SAH using fluorojade B (FJB) in bilateral basal cortex and hippocampal regions (CA1 and CA3). *Results*: rCBF was significantly correlated to reduction in CPP during the initial 15 min after SAH in a linear regression pattern (regression coefficient $r = 0.82$, $r^2 = 0.68$, $p < 0.001$). The mean amounts of TUNEL- and FJB-labeled cells were linearly correlated to reduction in CPP during the first 3 min of hemorrhage in the hippocampal regions CA1 and CA3 (regression coefficient $r = 0.71$, $r^2 = 0.50$, $p < 0.01$) and the basal cortex (regression coefficient $r = 0.76$, $r^2 = 0.54$, $p < 0.01$). *Conclusion*: The degree of EBI in terms of neuronal cell degeneration in both basal cortex and hippocampal regions linearly correlated with the first few minutes of reduced CPP and impeded CBF following SAH. However, it remains unknown whether global ischemia itself or subsequent events are responsible for the detected cell death and neurodegeneration.

82. Addition of New Criteria to the SOFA (Sequential Organ Failure Assessment) for Patients with Subarachnoid Hemorrhage

Authors: Macedo, Sergio Kiffer; Silva, Andre; Bastos, Nathalia; Paz, Juliano; Pessamilio, Thiago; Castro, Rodolfo; Lacerda, Mauricio; Teixeira De Oliveira, Henrique Nuss (Presenting)

Institution: Sao Jose do Avai Hospital, Itaperuna, Rio de Janeiro, Brazil

Introduction: The sequential organ failure assessment (SOFA) was originally created for sepsis, but its quality is now used in other medical conditions. Therefore, we added some criteria to the index to assess patients with subarachnoid hemorrhage. *Purpose*: New criteria for the SOFA were glycemia, arterial lactate, magnesium, calcium, sodium, hourly diuresis, and axillary temperature for predictions (death or survival) in patients with subarachnoid hemorrhage in view of the importance of these criteria in the clinical course of that group of patients. *Method*: We used informed consent for each patient/family; APACHE II (criteria for admission) and SOFA weekly, serum glucose, lactate, calcium, sodium, and magnesium and measurement of axillary temperature and hourly diuresis as the additional prognostic index SOFA. The study enrolled 103 patients diagnosed with SAH. The conduct in relation to the approach to these new criteria was the same in all patients evaluated. These patients were divided into two groups according to their development in the intensive care unit (ICU): group 1, patients who had good evolution (out of ICU); and group 2, patients who progressed to death in the ICU. *Results*: Among 103 patients, 74 (71.84%) were female, and 29 (28.16%) were male. The APACHE II for admission varied from 2 to 34, with an average of 15.5. The maximum time spent in the ICU was 49 days (2 patients). Group 1 had 64 patients (62.14%) and the 39 remaining patients (37.86%) were classified as group 2. *Conclusion*: The group of patients with SAH were predominantly female (74:29). For the APACHE II, group 1 was 10.9, while group 2 was 17.9. The only criteria that showed statistical significance in the prediction of death was the serum sodium ($p = 0.002$). It is necessary to have a new complementary study to

standardize these additional criteria to SOFA as an assessing method of prognosis of patients with SAH, but we can conclude that the change in serum sodium has fundamental importance in the evolution of this group of patients.

83. CSD-Monitor: Concept and Demonstration of a Software for Online Bedside Analysis and Simultaneous Visualization of ECoG Data from Brain-Injured Patients for Care and Research

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Introduction: Spreading depolarizations (SDs) are a potential cause of secondary brain injury following traumatic, hemorrhagic, or ischemic incidents to the cerebrum. Although SDs can be quite easily discerned by an experienced investigator in electrocorticographic (ECoG) recordings, this has to be carried out off line and involves large datasets, which are cumbersome to handle. A bedside monitoring tool could provide additional data not only during off-line analysis, but also in real time and thereby help to improve the outcome of brain-injured patients. *Features*: Implemented features include those for routine inspection of ECoG data and filtering algorithms to emphasize SD-specific signal parts. Fast automatic detection of artifacts is implemented to inform about problems in the recording setup. Specific modules for the analysis of SDs include the projection of ECoG data onto a three-dimensional (3D) model of the electrodes on the cortex. Furthermore, automatic detection and visualization of clusters of SDs with similar patterns will also be implemented. Automatically inserted events like SDs or artifacts can be approved manually in a comfortable user interface to ensure data quality for off-line analysis. *Methods*: Our implementation (C++, GCC-compiler, Qt 4.7) ensures platform independence. A modular plug-in concept allows for easy extension of functionality with plug-ins written even in different programming languages like Matlab or Java. An advanced user interface enables authorized users with different qualifications to adjust configurations and parameter settings. *Conclusion*: Off-line analysis and annotation functionality have been tested with ECoG data from different patients of the large dataset acquired within the COSBID (CoOperative Study on Brain Injury Depolarisations) study. Results of the analysis algorithms will be discussed and compared with the already-established signal analysis in the COSBID group. Interested members of the COSBID group can contact us to work together in use and development of the software.

84. Epidemiological Analysis of Patients with Cerebral Aneurysms Admitted for an Embolization at Sao José do Avaí Hospital

Authors: Macedo, Sergio Kiffer (Presenting); Siqueira, Carlos Mauricio; Siqueira, Savio; Oliveira; Dias, Lucas; Da Matta, Nayara; Almeida, Erica; Brito, Lara; Bastos, Nathalia; Carvalho, A.S.

Institution: Sao Jose do Avai Hospital, Itaperuna, Rio de Janeiro, Brazil

Introduction: The treatment of intracranial aneurysms evolved since the introduction of endovascular neurosurgery by the Guglielmi detachable coils (GDCs) because of the safety and feasibility of this method. *Method*: This study was conducted from the database of patients submitted for an ablation in the neurosurgery department in the period 2006–2009. *Results*: We studied 1,504 patients who had ablation. Of these, 1,120 were females (74.46%) and 384 males (25.84%). The average age was 52 years (variable 10–91). There were 176 (34.4%) patients who required hospitalization in the intensive care unit (ICU) was 176, staying on average 6.1 days (odds ratio 5.0). Hunt-Hess scale prevalence was as follows 1, 68.48%; 2. 16.35%; 3, 8.04%; 4, 4.78%; 5, 2.32%. Fisher (tomography scale) values were 1. 62.58%; 2, 7.91%; 3, 23.5%,; 4, 7.58%; and incidental, 4.85%. The main risk factors involved in cerebral vascular accident were systemic arterial hypertension ($n=608$, 40.4%) and smoking ($n=463$, 30.8%). The arteries more commonly involved were posterior communicating=725 (25.33%), median cerebral=562 (19.62%), anterior communicating=469 (19.38%), ophthalmic=124 (4.34%), posterior inferior cerebellar artery (PICA)=52 (1.81%), and pericallosa=82 (2.6%). Included among the 218 events that occurred were coil into the vascular lumen in 96 cases (6.38%), bleeding in 58 (3.85%), and others. The materials used were 334 balloons (71.06%) and 136 stents (28.94%). Angiographic vasospasm occurred in 178 patients. *Conclusion*: We noted in the occurrence of cerebral vascular aneurysmal accident the predominance of females, the average age of 52 years, and systemic hypertension and smoking are associated. The arteries of the previous segment were those that had a higher incidence of aneurysms. More than half of the patients did not have complications during the procedure; however, when they did, coil in the lumen and angiographic vasospasm were most frequent. Embolization of cerebral aneurysms was revealed to be the method with lowest lethality.

85. Frequency/Prevalence Analysis of Risk Factors on Aneurysmal Subarachnoid Hemorrhage

Authors: Macedo, Sergio Kiffer (Presenting); Siqueira, Carlos Mauricio; Siqueira, Savio; Nuss, Rodrigo; Dias, Joana; Carvalho, Robson; Guarçoni, Angelo; Fiorot, Jessica

Institution: Sao Jose do Avai Hospital, Itaperuna, Rio de Janeiro, Brazil

Introduction: Subarachnoid hemorrhage (SAH) is a catastrophic clinical event in which two-thirds of spontaneous SAH are characterized by rupture and bleeding of a cerebral aneurysm. *Methods*: After institutional approval and informed consent, this prospective observational study took place from April 2008 to November 2009 and involved all adult patients with spontaneous SAH admitted to the intensive care unit (ICU). We evaluated the frequency/prevalence of some factors: gender, age, skin color, arterial hypertension (AH), smoking habit, diabetes mellitus (DM), alcoholism,

dyslipidemia, sedentary, and use of oral contraceptive method. *Results*: There were a total of 128 patients (*n*), average age of 55.3 years. We obtained the following main results for frequency with respective confidence intervals (CIs): Gender: female 73.4% (94), CI 64.9–80.9; male 26.6% (34), CI 19.1–35.1. Skin color: white 46.2% (54), CI 36.9–55.6; black 24.8%(29), CI 17.3–33.6; brown 29.1% (34), CI 21.0–38.2. AH: yes 72.7% (93), CI 64.1–80.5; no 27.3% (35), CI 19.8–35.9. Smoking habit: yes 44.5% (57), CI 35.7–53.6; no 55.5% (71), CI 46.4–64.3. DM: yes 14.8% (19), CI 9.2–22.2; no 85.2% (109), CI 77.8–90.8. Alcoholism: yes 12.6% (16), CI 7.4–19.7; no 87.4% (111), CI 87.4–92.6. Dyslipidemia: yes 10.9% (14), CI 6.1–17.7; no 89.1% (114), IC, 82.3–93.9. Sedentary: yes 25.8% (33), CI 18.5–34.3; no 74.2% (95), CI 65.6–81.5. Obesity: yes 11.7% (15), CI 6.7–18.6; no 88.3% (113), CI 81.4–93.3. Oral contraceptive method (obtained only for female population): yes 5.3% (5), CI 1.7–12.0; no 94.7% (89), CI 88.0–98.3. *Conclusion*: We can show that, among all risk factors, female gender, AH, and smoking habit had the greatest prevalence index; the factor skin color had wide distribution from its variants; other risk factors (RF) (DM, alcoholism, dyslipidemia, sedentary, and use of oral contraceptives) did not obtain significant prevalence for patients with aneurysmal SAH.

86. Effects of Simvastatin in Prevention of Vasospasm in Nontraumatic Subarachnoid Hemorrhage (Preliminary Data)

Authors: Macedo, Sergio Kiffer (Presenting); Siqueira, Carlos Mauricio; Siqueira, Savio; Campeao, Andre; Dias, Lucas

Institution: Sao Jose do Avai Hospital, Itaperuna, Rio de Janeiro, Brazil

Introduction: Vasospasm is the main cause of death and cognitive deficits in patients with subarachnoid hemorrhage after rupture of the aneurysm (aSAH). Some trials have shown that statins in the acute phase of aSAH reduce the incidence, morbidity, and mortality of cerebral vasospasm. *Methods*: We made a prospective, randomized, nonblind study for 21 days between January and December 2008 using 80 mg of simvastatin (SVT; at night) in the first 72 h of bleeding; the control group did not use SVT. Informed consent was obtained for all patients. Computed tomographic (CT) scans were performed as a control, and another CT scan was done for patients with altered neurological signals. In the presence of changes suggestive of vasospasm or correlation in clinical and CT scans, the patients were given a cerebral arteriography exam followed by angioplasty if necessary. Liver and renal function and low-density lipoprotein (LDL) cholesterol were evaluated weekly, and total creatine kinase (CK) was evaluated every 3 days. Exclusion criteria were liver and renal disease, pregnant, elevation of serum transaminases (three times the normal value), creatinine 2.5 or greater, rhabdomyolysis or CK total of 10,00 U/l or greater. *Results*: We excluded 2 patients with bleeding more than 72 h. There was no significant change in the levels of total CK or renal or liver function. We included 21 patients, 11 in the SVT group and 9 in the control group. The mortality was 8 patients (38%), 6 patients in the control group and 2 of the SVT group. Vasospasm was confirmed by cerebral arteriography exam in 4 patients in the control group and 1 patient in the SVT group. All the patients who died were ranked as scale Fisher scale IV. *Conclusion*: The SVT at a dose of 80 mg was effective in reducing the mortality (18.1% vs. 66%)compared to the group that did not use SVT and decreased the incidence of cerebral vasospasm despite the higher APACHE II level in the group that used SVT (14.3 vs. 10.7). Less morbidity occurred in the SVT group, with an average of 3.25 versus 2.1 on the Glasgow scale.

87. Outer Skull Landmark-Based Coordinates for Measurement of Cerebral Blood Flow and Intracranial Pressure in Rabbits

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Institution: Bern University Hospital and University of Bern, Bern, Switzerland

Objectives: Despite the increased use of intracranial neuromonitoring for the evaluation of acute pathophysiological derangements during experimental subarachnoid hemorrhage (SAH), reliable stereotactic coordinates for the placement of monitoring probes in rabbits are missing. The aim of the present study was to determine and evaluate the safety and reliability of various locations of intraparenchymal intracranial pressure (ICP) and cerebral blood flow (CBF) probes according to outer skull landmarks. *Methods*: Experimental SAH was performed in 22 rabbits using an arterial shunt cisterna magna model. Intraparenchymal recordings from ICP probes placed in the frontal lobe were compared to measurements recorded from the olfactory bulb. CBF probes were placed on various locations in the frontal cortex anterior to the coronary suture. Insertion depth, relation to the ventricular system, and ideal placement location were determined on postmortem gross total dissections and histological examination. *Results*: There were no significant differences in ICP recordings obtained from the right frontal lobe (8.8 ± 1.9 mm anterior to the bregma and 1.5 ± 0.5 parasagittal) compared to the right olfactory bulb (midpupillary line anterior to the bregma and 2 mm parasagittal). Ideal coordinates for intraparenchymal CBF probes in the left and right frontal lobe were found to be located 4.6 ± 0.9 and 4.5 ± 1.2 anterior to the bregma, 4.7 ± 0.7 mm and 4.7 ± 0.5 mm parasagittal, and at a depth of 4 ± 0.5 mm and 3.9 ± 0.5 mm, respectively. *Conclusion*: This study demonstrated that coordinates in relation to cranial landmarks allow feasible and fast hitting of intraparenchymal locations for ICP and CBF probes. Practical accuracy of these coordinates can be warranted for safe and reproducible

neuromonitoring in the rabbits' brain without the use of a stereotactic frame.

88. Angiopoietin-1 Is Associated with Cerebral Vasospasm and Delayed Cerebral Ischemia in Subarachnoid Hemorrhage

Authors: Fischer, Marlene; Broessner, Gregor; Dietmann, Anelia; Beer, Ronny; Helbok, Raimund; Pfausler, Bettina; Chemelli, Andreas; Schmutzhard, Erich; Lackner, Peter

Institution: Innsbruck Medical University, Innsbruck, Austria

Background: Angiopoietin-1 (Ang-1) and -2 (Ang-2) are key players in the regulation of endothelial homeostasis and vascular proliferation. Angiopoietins may play an important role in the pathophysiology of cerebral vasospasm (CVS). Ang-1 and Ang-2 have not been investigated in this regard so far. *Methods*: Twenty patients with subarachnoid hemorrhage (SAH) and 20 healthy controls (HCs) were included in this prospective study. Blood samples were collected from days 1–7 and every other day thereafter. Ang-1 and Ang-2 were measured in serum samples using a commercially available enzyme-linked immunosorbent assay. Transcranial Doppler sonography was performed to monitor the occurrence of cerebral vasospasm. *Results*: SAH patients showed a significant drop of Ang-1 levels on days 2 and 3 post-SAH compared to baseline and HCs. Patients who developed Doppler sonographic CVS showed significantly lower levels of Ang-1 with a sustained decrease, in contrast to patients without Doppler sonographic CVS, whose Ang-1 levels recovered in the later course of the disease. In patients developing cerebral ischemia attributable to vasospasm, significantly lower Ang-1 levels had already been observed on the day of admission. Differences of Ang-2 between SAH patients and HCs or patients with and without Doppler sonographic CVS were not statistically significant. *Conclusion*: Ang-1, but not Ang-2, was significantly altered in patients suffering from SAH, especially in those experiencing CVS and cerebral ischemia. The loss of vascular integrity, regulated by Ang-1, might be in part responsible for the development of cerebral vasospasm and subsequent cerebral ischemia.

89. Cellular Microparticles as a Marker for Cerebral Vasospasm in Spontaneous Subarachnoid Hemorrhage

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Institution: Innsbruck Medical University, Innsbruck, Austria

Background: Spontaneous subarachnoid hemorrhage (SAH) still carries a high risk for poor outcome, which is frequently attributable to symptomatic cerebral vasospasm (CVS). We hypothesized that cellular microparticles (MPs) play a role in the pathogenesis of CVS and may serve as biomarkers for CVS. *Methods*: In 20 consecutive SAH patients, endothelial, leukocyte, platelet, and erythrocyte MPs were measured during 15 days after ictus. CVS was detected by transcranial Doppler (TCD) sonography. Twenty matched volunteers served as healthy controls (HCs). *Results*: Endothelial, leukocyte, and erythrocyte MPs were elevated in SAH patients compared to HCs. $CD105^+$ and $CD62e^+$ endothelial MPs (EMPs) were significantly higher in SAH patients with Doppler sonographic CVS (dCVS). Especially, $CD105^+$ EMPs were increased on the days of dCVS onset. In patients experiencing cerebral infarction due to vasospasm (CIV), $CD41^+$ platelet MP (PMPs) were elevated in addition to EMPs. $CD41^+$ PMPs were significantly higher in patients with any level of disability (modified Rankin Scale [mRS] 1) compared to those who made a full recovery (mRS = 0) on discharge from the hospital. *Conclusion*: EMPs were elevated in patients with SAH. This elevation coincided with the occurrence of dCVS and could therefore be a novel biomarker for CVS. PMPs might be involved in the pathogenesis of CIV, resulting in neurological morbidity.

90. Screening for Vasospasm Significant Biomarkers in Cerebral Spinal Fluid Using Multidimensional Separation Techniques with MALDI-TOF/TOF-MS and ESI-LTQ-MS

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Worldwide, subarachnoid hemorrhage (SAH) and its sequela cerebrovasospasm (CV) kill or seriously debilitate an estimated 1.2 million people annually. One of the barriers to research progress in prevention or reversal of CV after SAH is the ability to predict which SAH patients will develop vasospasm and elucidation of the signaling pathways that contribute to cerebral vasospasm. Approximately half of all people who survive SAH suffer from CV 3–10 days following the initial stroke. Currently, transcranial Doppler flow determinations performed every 24–48 h can help screen for the onset of vasospasm [1]. This delayed onset gives researchers and clinicians a window to treat SAH patients to prevent vasospasm. Investigations involving traditional biological techniques into the etiology of CV have led to promising advances [2–5]. These studies have found that excessive oxidative environments [2], platelets [3], glutathione peroxidase [5], and even bilirubin [4] could contribute to the onset of CV. This study focused on using multidimensional separation techniques by combining isoelectric focusing and strong cation exchange chromatography with liquid chromatography matrix-assisted laser desorption ionization-time of flight/time of flight mass spectrometry (MALDI-TOF/TOF-MS) and liquid chromatography electrospray ionization-linear trap quadrupole mass spectrometry (LC-ESI-LTQ-MS) to explore potential biomarkers in three different cerebral spinal fluid (CSF) samples: control (normal, healthy, CSF control); SAH stroke patients (no vasospasm, CSF C); and SAH CV patients (CSF V). A number of potentially significant proteins were identified, and continuing studies are currently under way for further investigations of these proteins.

91. Complications Associated with the Endovascular Management of Vasospasm with Unsecured Intracranial Vascular Lesions

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Introduction: The management of cerebral ischemia due to vasospasm involves increasing cerebral perfusion with hypervolemic, hemodilutional, hypertensive (HHH) therapy, intra-arterial vasodilators, or angioplasty. All therapies are usually undertaken with secured vascular lesions. Patients may rarely present with vasospasm with unidentifiable pathology or a lesion that cannot be secured prior to treating vasospasm. This unique circumstance requires cautious application of medical and endovascular treatment to balance the risk of stroke from vasospasm with rerupture of the vascular lesion. *Methods*: We retrospectively reviewed our endovascular database of patients treated from 2002 to 2011. We searched the database for the keywords vasospasm, verapamil, papaverine, and angioplasty to identify subarachnoid hemorrhage (SAH) patients with ruptured unsecured intracranial vascular lesions receiving intra-arterial vasodilators or angioplasty. We excluded patients with secured lesions or those who only had diagnostic studies. Charts were reviewed for complications associated with endovascular management. *Results*: Our search yielded 100 possible patients who met our initial search terms. Of these, 70 had secured lesions, 12 had missing records, and 9 had only diagnostic studies, leaving 9 patients with SAH with unsecured pathology receiving endovascular therapy. Of 13 treatments in these 9 patients, 2 (15%) resulted in brain death within 24 h of treatment. One patient had a giant vertebrobasilar aneurysm and had a brain-stem stroke after angioplasty of a vertebral artery, and the other patient had intraoperative rerupture of a vertebral artery dissection during verapamil administration and coil occlusion. *Conclusion*: Patients with unsecured intracranial aneurysms and dissections have a high major complication rate when using endovascular means to treat vasospasm and should be approached cautiously. It may be safer to treat angiogram-negative SAH patients with endovascular means than patients with known pathology.

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