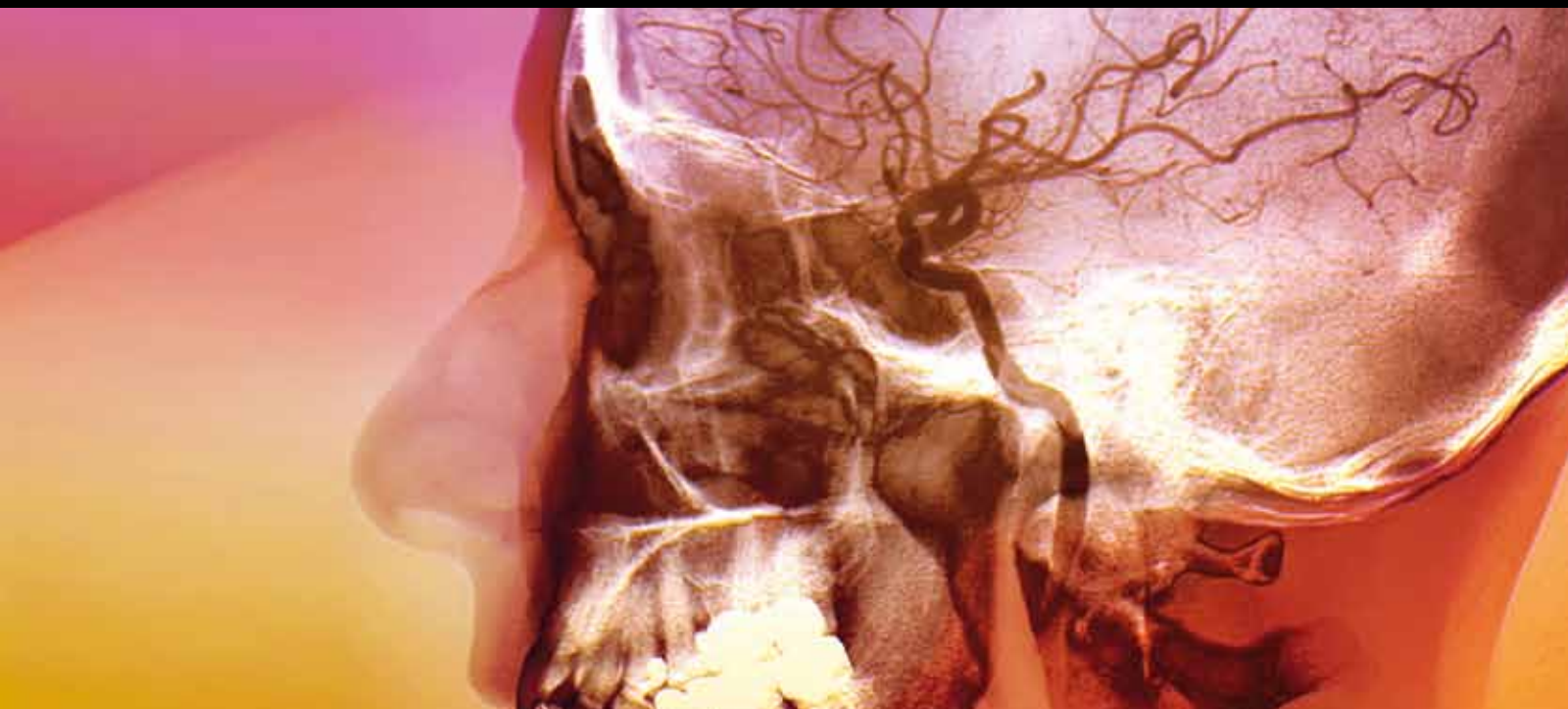# Brain Injury after Transient Global Cerebral Ischemia and Subarachnoid Hemorrhage

Guest Editors: Fatima A. Sehba, Ryszard M. Pluta, and R. Loch Macdonald

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Editorial Board

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Editorial

Brain Injury after Transient Global Cerebral Ischemia and Subarachnoid Hemorrhage

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Brain injury of diverse etiology is frequently encountered clinically. Management, thus, is diverse. Transient global ischemic (TGI) brain injury may result from cardiac arrest where cerebral perfusion diminishes to the point that blood supply can no longer meet the metabolic demand of the brain or from aneurysmal subarachnoid hemorrhage where hemorrhage from an intracranial aneurysm elevates the intracranial pressure above the blood pressure leading to momentary perfusion arrest.

Though there are commonalities and differences in the etiology and pathogenesis of these two brain injuries, whether they required differential management or they can be grouped together is not clear. Some risk factors of TGI and SAH are shared and common and others are unique to the injury. Shared risk factors include high blood pressure, smoking, alcohol abuse, and stress. The risk factors unique to TGI are clinical conditions such as cardiac arrest (major cause), shock that creates prolonged hypoxia or hypoglycemia, pathologically elevated cerebral metabolic rate, or decreased cerebral perfusion pressure. The risk factor unique to SAH is the presence of an intracranial aneurysm. Other contrasting risk factors are age and gender; whereas old age increases the risk of TGI, SAH occurs in a younger population with an average age of 52–55 years. Women harbor significantly more intracranial aneurysms than men and consequently are more frequently the victims of SAH. Although the average age of women with SAH is greater than men, the outcome is similar [1]. In contrast, more men than women are at risk of ischemic stroke and women with ischemic stroke are usually older and more likely to die of stroke than men [2].

Brain injury after TGI can be separated into an initial ischemic phase that lasts for the duration that the brain blood supply remains reduced (usually ≤10 minutes, otherwise death is inevitable) and the reperfusion phase that begins immediately after reconstitution of cerebral blood supply (>10 minutes). The initial phase of brain injury after SAH is more complex and lasts for 48 to 72 hours. A complex series of events occurs during this initial (early) phase, including blood-induced mechanical trauma, oxyhemoglobin (released upon degradation of blood) induced oxidative stress, inflammation, and ischemia [3]. A delayed phase of brain injury, unique to SAH, develops 3–7 days after SAH. This injury is characterized by angiographic vasospasm and delayed cerebral ischemia [4].

In order to help understand the management of brain injury after TGI and SAH, this special issue compares and contrasts the various mechanisms of brain injury after TGI and SAH. It presents two original research articles and 7 reviews. The research article by C. S. Jung et al. studies the correlation of serum and CSF injury markers with ischemic events in SAH patients and that by S. O. Eicker et al. compares neuroprotective qualities of vascular endothelial drive growth factor (VEGF) against stroke and cerebral vasospasm after SAH. F. A. Sehba and R. M. Pluta review the existing TGI and aSAH animal models and present a modified aSAH model which effectively mimics the disease and has the potential

of becoming a better resource for studying the brain injury mechanisms and developing a treatment. M. A. Kamp et al. review the mechanisms and clinical significance of the alteration in calcium and potassium channel after SAH and TGI. M. K. Tso and R. L. Macdonald review preclinical studies on microvascular changes and their therapeutic modification following SAH and TGI. N. Plesnila compares and contrasts pathophysiological events occurring in experimental models of SAH and TGI and evaluates the contribution and importance of global cerebral ischemia in the pathophysiology of SAH. M. Koide et al. summarize the current knowledge regarding the impact of SAH and global ischemia on neurovascular communication. J. A. Frontera reviews clinical trials in cardiac arrest and SAH and concludes that clinical trials in SAH assessing acute brain injury are conducted and that these trials may receive benefit from interventions identified successfully against brain injury following cardiac arrest.

We hope that the present special issue stimulates further research in this topic and brings to attention the information that would provide a better understanding of the management of TGI and SAH patients.

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Review Article

Aneurysmal Subarachnoid Hemorrhage Models: Do They Need a Fix?

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The discovery of tissue plasminogen activator to treat acute stroke is a success story of research on preventing brain injury following transient cerebral ischemia (TGI). That this discovery depended upon development of embolic animal model reiterates that proper stroke modeling is the key to develop new treatments. In contrast to TGI, despite extensive research, prevention or treatment of brain injury following aneurysmal subarachnoid hemorrhage (aSAH) has not been achieved. A lack of adequate aSAH disease model may have contributed to this failure. TGI is an important component of aSAH and shares mechanism of injury with it. We hypothesized that modifying aSAH model using experience acquired from TGI modeling may facilitate development of treatment for aSAH and its complications. This review focuses on similarities and dissimilarities between TGI and aSAH, discusses the existing TGI and aSAH animal models, and presents a modified aSAH model which effectively mimics the disease and has a potential of becoming a better resource for studying the brain injury mechanisms and developing a treatment.

1. Introduction

Stroke is the second major cause of death worldwide. According to the World Stroke Organization approximately 15 million people suffer from stroke each year. Five million people die from it, and another 5 million are left permanently disabled [1]. Ischemic stroke constitutes the most and hemorrhagic stroke 15 to 30% of the total annual stroke cases [2]. The 21-day to 1-month case fatality ranges from 13 to 23% for ischemic stroke, as compared to 25–35% for hemorrhage stroke [3]. The cost of survivor care and lost productivity (conservatively estimated to be more than 54 billion dollars annually) necessitates research to reduce stroke mortality and disability. An essential step in this direction is developing an experimental model that replicates the human condition. Numerous animal models addressing causes and pathophysiology of ischemic and hemorrhagic stroke have been developed. Whereas research using these models has clearly influenced the treatment of global ischemic stroke, it has made relatively small impact on treatment of hemorrhagic stroke.

2. Ischemic and Hemorrhagic Brain Injury

Ischemic stroke occurs when blood supply to the brain is reduced to a level that cerebral function and metabolism are no longer maintained. Cerebral ischemia could be focal or global and transient or permanent. A mix of any of them is also possible; for instance, after aneurysmal subarachnoid hemorrhage, a patient can develop a transient global ischemia (evoked by temporary increased ICP) followed by permanent focal ischemia because of a thrombosis or delayed vasospasm. Transient focal ischemia (TFI) affects a specific brain region, and transient global ischemia (TGI) affects the whole brain for a limited time; both are followed by reperfusion and/or hyperperfusion. In contrast to transient ischemia, in permanent ischemia blood flow is never reestablished to the part (local) or the whole (global) brain. Hemorrhagic stroke occurs when blood flow in the brain is reduced due to the intracranial bleeding. Aneurysmal subarachnoid hemorrhage (aSAH), a nontraumatic type of the intradural bleeding, constitutes 5% of all strokes and occurs when an intracranial aneurysm bursts and spews blood under high pressure into

the subarachnoid space. Such a violent flow of blood into a narrow, CSF-filled space results in a dramatic increase in intracranial pressure and decrease in the cerebral perfusion pressure (CPP) and cerebral blood flow (CBF) [4–6]. The ICP-dependent reduction in CBF after SAH is beneficial but also harmful, beneficial as it saves a patient's life by allowing a blood clot to seal the dome of the ruptured aneurysm and stop the bleeding and harmful as it limits blood flow to the whole brain for unpredictable time and may result, in the best-case scenario, in a transient local or global ischemic brain injury or, in the worst case, brain death. Thus, to best of our knowledge most of aSAH bleeding is associated with transient global hypoperfusion and/or ischemia. Though a role of TGI in aSAH outcome has been suspected since early 19th century, its true nature remains poorly defined and its importance largely unappreciated. As a consequence, the differences and similarities between TGI and aSAH are not determined, and knowledge that TGI researchers have accumulated over the years is not used to further understanding of the SAH-related injury to the brain. Three reasons of this oversight are: (1) events leading to a "spontaneous" TGI and TGI evoked by an aneurysmal SAH are different, (2) the sudden/abrupt nature of aSAH event makes association with TGI difficult to study [5, 7], and (3) until recently, most research on improving patient outcome has been on delayed cerebral vasospasm (AKA delayed neurological deficits) and not on events that occur early after aSAH [8]. Lately, limited improvement in patient outcome after more than half a century of research convinced many researchers to reevaluate the significance of early events and more importantly the influence of early TGI after aSAH on the outcome [9].

In recognition of this new trend we review animal models of TGI and aSAH, discuss why ischemic, but not aSAH models have proven successful in reducing the death and disability after stroke, and propose a modified aSAH model that incorporates features of TGI model and could be a better resource for studying the injury mechanisms and treatment of aSAH.

3. Animal Models of TGI and SAH

3.1. TGI Models (Table 1). Animal models of TGI induce either complete or incomplete global ischemia. In complete global ischemia blood flow is ceased completely, and in the incomplete global ischemia blood flow decreases to a degree that cellular metabolism and function can no longer be maintained [49, 50]. The injury and survival are proportional to the duration of global ischemic insult: greater when the insult is a short and resolvable; lasting 10 to 30 minutes and lower when the insult is longer or permanent. Thus, permanent TGI models work best for studying the mechanisms of injury, and the resolvable TGI models work best for studying therapeutic interventions. Below we describe the most extensively used TGI models. See Table 1 for a list of TGI models and animal species used.

3.1.1. Two-Vessel Occlusion (2-VO) Model. Ischemia in this model is created by a transient bilateral carotid occlusion.

Variations that allow investigator to control injury intensity are available. A mild-to-moderate injury is created by keeping arterial blood pressure normal during carotid occlusion [40, 51]. A severe injury is achieved by reducing arterial blood pressure to 40–50 mmHg during carotid occlusion. Blood pressure reduction is achieved by phlebotomy or by pharmacological manipulation [52].

The advantages of 2-VO model include one-stage surgical preparation, production of high-grade forebrain ischemia, ability to control ventilation to ensure normoxia and normocarbia, ease of reestablishing cerebral circulation, suitability for chronic studies, and a relatively low failure rate. The disadvantage is that pharmacologically induced hypotension may complicate the interpretation of results [78].

3.1.2. Four-Vessel Occlusion Model (4-VO). Ischemia in this model is created by almost simultaneous occlusion of four major cerebral vessels: bilateral both vertebral and common carotid arteries [43]. Usually, first, the vertebral arteries are electrocoagulated, and then the common carotid arteries are occluded by tightening the ligatures around them [78]. This model has been extensively studied to assure a high incidence of successful ischemia with acceptable mortality rate. Nevertheless, even in the best hands, animal survival rate following 4-VO is only 50% [43, 79]. A modification of 4-VO which combines a mild systemic hypotension (80–90 mmHg) with bilateral carotid occlusion creates less morbidity and more uniform brain injury [80, 81].

Both 2- and 4-VO models are frequently used to study TGI (see Table 1). 2-VO model is often preferred over 4-VO model as it requires less surgical manipulations; 4-VO requires two state surgical preparation and rarely achieves complete reversal of global ischemia [82].

3.1.3. Bihemispheric Forebrain Compression Ischemia (BFCI). This model was developed by Kramer and Tuynman in 1967 to define the duration of ischemia tolerated by the brain [28]. Ischemia here is induced by increasing intracranial pressure to the level of systolic blood pressure so that cerebral perfusion is disrupted. The increase in intracranial pressure is achieved by infusion of artificial cerebrospinal fluid (CSF) into the cisterna magna. Cushing's reflex evoked by increased ICP can be reduced by administration of the ganglion-blocking drug [83].

TGI produced by BFCI is consistent, reproducible and successfully created in several animal species. Though BFCI model is not as extensively used as the 2-VO or 4-VO model, it provides an excellent foundation for the modified aSAH model that we later propose in this review (see below).

3.2. SAH Models (Table 2). Brain injury evoked by aSAH consists of early and delayed events. Early events include rise in ICP, fall in CBF and CPP at the time of aSAH, and the delayed events are arterial vasospasm and delayed ischemic deficits that develop 3–7 days after the initial bleed. Due to unpredictable nature (not every aneurysm ruptures) of aSAH [5, 7], the information on ultra-early events is available only as the patient is admitted and monitored after the initial

TABLE 1: Experimental models of transient global ischemia.

(a) Complete TGI models

TGI method	Key features	Species	References
Cardiac arrest (i) KCl injection	Epinephrine injection, defibrillation, and CPR are used for resuscitation	Mouse, rat and monkey	[10–12]
(ii) Ventricular fibrillation	Can be used with CPR to study resuscitation	Cat, dog, pig and monkey	[13–16]
Aortic occlusion	Inhibits flow throughout the body	Rat, rabbit, cat and dog	[17–19]
Neck cuff/tourniquet with hypotension	Inhibition of blood flow to the head	Rat, cat, dog and monkey	[20–24]
Extracranial artery occlusion (i) Innominate and subclavian arteries	Inhibition of blood flow to the head	Cat	[25, 26]
(ii) Brachiocephalic and subclavian near aortic origin		Monkey	[27]

(b) Incomplete TGI models

TGI method	Key features	Species	References
Intracranial hypertension (i) Fluid infusion in cerebral cistern	A brain compression injury	Rabbit, cat, dog and monkey	[28–30]
(ii) Balloon inflation		Rat, cat, dog and monkey	[31–34]
Extracranial artery occlusion	Immediate ischemia and reperfusion allows possibility of permanent occlusion		
Bilateral common carotid (2-VO) (i) Without hypotension	Creates mild-to-moderate injury	Mouse, rat, gerbil, sheep and monkey	[35–39]
(ii) With hypotension		Rat, rabbit, cat and monkey	[40–42]
Bilateral common carotid + vertebral arteries (4-VO)	Creates severe injury	Rat, rabbit, cat, dog and monkey	[43–48]

TABLE 2: Experimental models of aSAH and/or vasospasm.

Species	SAH method			Phase studied			Reference
	Artery puncture	Blood injection		Clot	EBI	Vasospasm	
		Single	Double				
Mouse	+	+	+	−	+	?	[53–55]
Rat	+	+	+	−	+	+/−	[56–60]
Rabbit	+	+	+	−	+	+/−	[61–63]
Cat	+	+	+		+		[5, 64–66]
Pig		+	+	+	+	+	[67, 68]
Dog	+	+	+	+	+	+	[69–73]
Nonhuman primate	+	+	+	+	+/−	+	[74–77]

aneurysm rupture. Consequently, information obtained is already delayed, unless rebleed occurs, usually within hours after the initial bleed. However, because of the lingering effects of the initial bleed, the data obtained during rebleed cannot be directly extrapolated as a mimic of the first aSAH [5, 7]. Nevertheless, information obtained during the rebleed has been used to develop animal models of aSAH. These models are widely used to study early and delayed brain injury after aSAH and are accepted as mimics of clinical aSAH (see Table 2 for details) [84–86]. aSAH models can be broadly divided into three categories.

3.2.1. Blood or Hemolysate Injection or Infusion. Injection model involves introduction of autologous fresh blood [56, 67, 74, 87–89] into the cisterna magna, prechiasmatic cistern [90], or next to an intracranial [91, 92] or an extracranial artery [84, 93–97]. This model is quite extensively used to study early and delayed injury after aSAH. In several species (mouse, rat, and dog), a second blood injection 24 to 48 hours after the first is necessary for development of delayed vasospasm. Advantages of this model are that it produces reproducible injury and allows use of saline injected sham control. Disadvantage is a failure to reproduce the mechanical

trauma, the first insult felt by the cerebral vasculature upon aneurysm rupture (for review see [98] and references within).

3.2.2. Blood Clot Placement. In this model arterial blood is withdrawn and allowed to clot *ex vivo* and then surgically placed on the adventitial surface of an artery. Both intracranial (the middle cerebral artery [75]) and extracranial (femoral [96]) arteries have been used for clot placement. This model studies delayed vasospasm and not early injury. Advantages of this technique are the well-defined course of vasospasm and low animal mortality that permits pharmacological intervention. Disadvantages are lack of reproducing mechanical trauma (see above) and the high cost of experiment; this model is predominantly used in larger animals: dog, pig, and monkey.

3.2.3. Arterial Puncture. This aSAH model involves puncture of the intracranial artery adjacent to the skull base by an endovascular filament. The model is considered the best mimic of human aSAH as it replicates the mechanical trauma felt by cerebral vasculature upon aneurysm rupture, as well as the events observed during rebleed in aSAH patients: rapid fall in cerebral blood flow and blood accumulation into subarachnoid space [4–6, 98]. However, due to a number of reasons explained elsewhere this model provides a poor control of bleeding and high mortality (for review see [98–100]). Other disadvantages include complicated surgical procedure that requires a trained person and difficultly in adaptation to other, larger species. Nevertheless, arterial puncture is frequently used to study early injury after aSAH especially in rodents.

4. Success of Embolic Ischemia Model and Lesson Learnt about aSAH

The research focused on treatment of cerebral ischemia has been successful. It has provided us with recombinant tissue plasminogen activator (r-tPA) that, when used within 4.5 hours after ischemic episode, reduces brain injury and improves the outcome [101]. In contrast, despite extensive research, a therapy that could be translated to clinical SAH has not been found. Though several compounds have been found promising against SAH in animals, none succeed in clinical trials [98].

A proper disease modeling may have contributed to the success of TGI research. That varying degree and duration of CBF reduction produce varying effects on the neurovascular unit has been realized [102], and animal models that address a specific problem are accordingly developed. Focal ischemia models study injury following a thrombotic event, and global ischemia models study injury following cardiac arrest. Both models focus on developing a time-dependent intervention. Animal species used range from rodents to the AHA recommended primates [103, 104]. However, even this meticulous approach has not always worked. An example of failure is the free radical-trapping agent NXY-059 that showed promise as a neuroprotectant in rat and primate ischemic models but was ineffective in patients [105]. On the

other hand, a spectacular success was the development of thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) against acute transient focal and global ischemic stroke based on the results of studies using a rabbit model of embolic stroke [106, 107]. The success of a rabbit embolic model versus failure of favored ischemic primate model in development of successful treatment may indicate that an accurate model of a disease should provide results that are reproduced across species and successfully translated to clinic.

5. aSAH Models and Components of Injury

Mortality, neurological deficits, and diminished quality of life are the most important end points of a brain injury evoked by TGI and aSAH. However, some and not all of the mechanisms that TGI and aSAH evoke are shared (see Table 3). For example fall in CBF creating temporary global perfusion deficits occurs both in TGI and aSAH, but injury by a prolonged presence of blood in the subarachnoid space characterizes SAH only. Thus, in a new desired aSAH model all components of injury, the presence of controllable TGI, and an intracranial bleeding need to occur simultaneously. Unfortunately, the current animal models dissociate TGI from aSAH, replicate subarachnoid bleed but not a perfusion deficit that creates TGI, and thus these models only partially imitate injury produced by aSAH. This shortcoming may have contributed to a lack of clinical translation of therapies found successful in animals. A more inclusive model that incorporates all components of brain injury after aSAH is required to accelerate the development of adequate treatment for improving the patient's outcome.

6. A Modified aSAH Model

A number of different aSAH models are available for studying injury mechanism and treatment. Each carries its own advantages and disadvantages. One shortcoming common at all is the lack of requirement of CPP fall at SAH induction to a level that ensures TGI. As a result these models replicate some but not all of the components of injury that are present in human aSAH (discussed above). We here propose a modified aSAH model that reproduces all of the components of injury after aSAH and in addition requires limited surgical manipulation, carries low mortality, can be easily adapted to a number of species, and makes comparisons and interpretation of data from different laboratories possible.

After reviewing the existing aSAH models (above) we have formed an opinion that perhaps an *adaptation* of Kramer and Tuynman's TGI model (explained above), that uses autologous arterial blood instead of artificial CSF, provides the best foundation for the modified aSAH model [28].

Below we detail three features essential to this modified aSAH model. We discuss the reason we consider them essential and the techniques that can be used to attain them.

6.1. Blood Injection. As blood upon aneurysm rupture is released under high pressure and pools into subarachnoid

TABLE 3: Risk factors of TGI versus aneurysmal SAH.

Factor	TGI	aSAH
High blood pressure	Shared	Shared
Smoking	Shared	Shared
Alcohol abuse	Shared	Shared
Stress	Shared	Shared
Cardiac arrest or shock that creates prolonged hypoxia or hypoglycemia	Stroke only	
Pathologically elevated cerebral metabolic rate	Stroke only	
Decreased cerebral perfusion pressure	Stroke only	
Age (years)	≥65	≤56
Gender	Men prevalence	Women prevalence
Intracranial aneurysm	−/+	+

cisterns, a proper replication requires the same to occur in the animal model. The location, speed, and volume blood injection all are important considerations for consistent replication of aSAH injury in animals used within an experiment and across laboratories.

6.1.1. Blood Delivery Route. Technical details of each procedure can be found in adequate reference(s) in Table 2.

Several routes have been successfully used for intracranial, subarachnoid injection/infusion of blood. A brief description of these routes and techniques is presented below. Details can be found in the references in Table 2.

A Percutaneous Delivery of Blood. This route is often favored in small and large animals (rabbit, cat, dog, pig, and monkey). This technique requires good anatomical knowledge and reasonable but basic surgical skills. Briefly, after proper anesthesia and skin preparation (includes shaving the back of the head, between the ears, and the flexor surface of the neck), a short bevel 25-/27-gauge needle attached to an insulin syringe (size depends on the species used, can range from insulin to 10 cc) is introduced in the midline directly below the palpable edge of the cranium after significant head flexion. The needle is slowly advanced until an access to cisterna magna is confirmed with CSF presence in the syringe. At this moment a syringe is exchanged for the one that is filled with fresh, arterial, and preferably nonheparinized blood. Blood is then quickly (in less than 1 min) injected into subarachnoid space. The volume, time, and speed of injection are guided by the rise in ICP to mean arterial blood pressure rendering CPP zero or a drop in CBF below 10 mL/100 g/min. At this moment the needle is quickly removed, and steady compression is applied to the neck. The animal's head is then either returned to neutral or slightly extended position with the body of animal (if possible) tilted down for about 5–10 min to allow blood to flow toward the anterior cisterns. Monitoring of ICP, CPP, and CBF continues under anesthesia or animal is awaked after removal of monitoring devices.

A Direct Infusion into the Cisterna Magna. This is another frequently used route for blood delivery. It requires significantly more surgical experience but is still relatively easy (for

detailed description check references in Table 2). In short, an animal is anesthetized and placed in a prone positioning with the head tilted forward. The atlantooccipital membrane is exposed via a skin incision from the midline on the back of the neck and a delicate dissection of muscles from the occipital bone and C1-2 vertebral bodies. The atlantooccipital membrane is then punctured with a 27-gauge needle or PE-10 catheter that is attached to a syringe filled with fresh autologous blood. The injection follows the same parameters as the percutaneous infusion. The muscle is reapproached with sutures and wound closed and covered with antibiotic creams to speed the healing and prevent infection. Advantage of this approach is a possibility of sealing the hole by tissue glue as the needle is removed.

A Direct Anterior Intracisternal (Prechiasmatic) Blood Injection. This route was traditionally used in large animals (dog, monkey) [90, 108] and has been recently adapted to rodents [109–111]. The technique used requires advanced surgical skills, stereotactic apparatus, and access to radiological equipment. It can be achieved via intra(peri)orbital approach [110, 111] with or without enucleation [90] or through transparenchymal approach (Table 2) [109]. Approach used to access prechiasmatic cistern differs among species. In rodents a prechiasmatic cistern is usually approached by placing the animal in prone position and advancing a 27-gauge needle attached to a 1 mL syringe with nonheparin blood stereotactically until the tip reaches the base of the skull and a proper placement in a prechiasmatic cistern is confirmed by flow of CSF into the syringe [109]. The orbit and the optic foramen have also been used to access perichiasmatic cistern.

6.1.2. Blood Injection Parameters: Volume, Length, and Speed. The volume, length, and speed of blood infusion dictate the degree of ICP rise and CPP reduction and thus the intensity of SAH being created. To ensure similarity of SAH intensity within an experimental group these parameters need to be standardized and closely monitored. This however is not a simple task, as intracranial volume differs within and between species making an investigator's control on the injury intensity difficult. Consequently, in current practice a consensus on the injection parameters that work the best

in a particular species does not exist, and a wide array of volume, length, and speed options are available and used for injecting blood in a single species. A downfall of this is that since the intensity of SAH depends upon the volume, length, and speed of injection, variations in these parameters makes a comparison and interpretation of results from different laboratories difficult, if not impossible. For instance, SAH in rat has been induced by injecting 100 microL autologous blood over seconds [110], 0.2 mL of blood for more than 1 min [109] or 0.3 mL blood for 1 min [111].

On the Model. In a modified aSAH model injection parameters should be guided by the changes of ICP and CPP. The parameters that evoke dramatic but transient reduction in CPP to near zero should be selected and used for creating SAH. These parameters will of course differ between species and even within a species, but the selection criteria (transient reduction in the CPP to near zero) will remain same. This will facilitate comparison data obtained in different laboratories and among different species.

6.1.3. Factors Influencing Choice of Technique Used for SAH Induction. A number of factors need to be considered before a technique can be selected for creating aSAH. Some of these factors are as follows:

(i) *Simplicity and Reproducibility.* A technique that is simple and reproducible is increasingly attractive and has greater chances of becoming a favored method for studying a problem. A simple technique allows for a short training period and reduces the chances of surgeon's mistake. Reproducibility of injury decreases the cost of a project by reducing the number of animal required for an experiment. Above, we have examined simplicity and reproducibility of available aSAH techniques.

(ii) *Ease of Adaptation.* A technique allowing for adaptation in several animal species permits comparison of results. Several animal species have been used to study SAH. These range from smaller animals: mice, rats, rabbits, and gerbils, to larger animals: cats, dogs, pigs, and nonhuman primates. For animal species used for a particular SAH technique see Table 2 and associated references. Primates, due to their higher ranking in the evolution ladder, are considered the best choice for replication of human conditions. However, not every investigator and laboratory is equipped to use primates. Fortunately, the success of a rabbit embolic model versus failure of favored primate model proves that it is the disease modeling and not the closeness of species to human that translates into a successful treatment. Replication and cross-validation of results in more than one animal species are perhaps a stronger indication of future successful translation in clinical trial. Such option will only be available if the technique used to create SAH is applicable in other species with no or only minimal modifications.

(iii) *Low Mortality with Ethically Acceptable Morbidity.* Since computer simulation cannot be used to study mechanisms and test therapies, animal research remains to be the cornerstone of scientific research and drug development. However, respect for lives of all creatures is essential and is an important consideration in animal research. Reducing distress and suffering in animals is a crucial consideration in development of an animal model. A number of steps can be taken to prevent unnecessary animal suffering during experimentation. These steps include (1) use of perioperative and postoperative analgesia and anesthesia; (2) use of proper life support; (3) aseptic surgical technique; and (4) little amount of surgical manipulation etc.

Use of perioperative and postoperative analgesia and anesthesia during surgery reduce distress caused by the surgical manipulations for inducing SAH. The type and dose of anesthetic and analgesic depends upon the animal species being used. An investigator can refer to species-specific guideline on anesthesia and analgesia provided by their institution for agents that work best in the species used. The depth of anesthesia ensures that animal does not feel pain during surgery. A frequent check of corneal reflex and limb pinch as well as monitoring of heart rate is commonly employed to confirm anesthesia depth. Such as for rat Ketamine-Xylazine combination (50 mg/5 mg/Kg; intraperitoneal administration) is often used for reducing perioperative pain and buprenorphine (0.05 mg/Kg, subcutaneous administration) twice daily for reducing postoperative pain. In addition, inspired isoflurane (1% to 2% in oxygen-supplemented room air) is frequently used during surgery to maintain deep sedation in rats.

Proper life support during surgery reduces animal mortality. This support includes monitoring and regulation of breathing, body temperature, and a fluid intake. The increase in ICP upon blood infusion may increase pressure at the respiratory centers to the point that animal stops breathing. A respiratory support that ensures breathing such as intubation or placement of a nose cone ensures that animal does not expire. Similarly, unless a project is studying the effect of temperature on injury, body temperature of animal is maintained at 37°C (such as by a thermoblanket) from the start of anesthesia until the animal recovers. For proper hydration ringer lactate is administered as required.

Aseptic surgical technique protects against infection. As a minimum requirement, this includes sterilization of surgical equipments, applying antiseptics such as iodine to the wounds upon closing and if the project permits, administration of antibiotics to prevent infection from occurring and speed healing.

The amount of surgical manipulation can result into animal death. In general, the more the surgical steps, the more invasive the procedure becomes. In contrast, a simple procedure reduces unnecessary pain and suffering.

On the Model. The technique used for SAH induction should be simple, reproducible and allow adaption into different species.

6.2. Monitoring of SAH Physiology. Physiological monitoring is an essential feature of modified aSAH model as it confirms the intensity of SAH. This information can be used to ensure that all animals within and across an experimental group receive similar intensity and to interpret the results from different laboratories.

6.2.1. ICP and CPP Changes. Equilibrium between brain, and cranial vault volume via controlled intracranial blood and CSF flow is essential for maintenance of normal ICP. This equilibrium is disturbed by blood released upon aneurysm rupture. An ICP rise that occurs at aSAH reflects subarachnoid blood volume, status of brain and cerebrovascular disturbances. Furthermore, peak ICP value and the pattern of its decline associate with the intensity of injury after SAH [7, 112]. Hence, continuous and reliable ICP monitoring via a simple and easy technique is desired to determine and control the injury intensity and understand the underlying pathophysiologic events after aSAH.

ICP Measurement. Symptoms like headache, nausea, vomiting (particularly projecting), and the presence of papilledema strongly suggest an increased intracranial pressure; however, they do not allow for close monitoring of ICP changes. Fortunately, ICP can be assessed by a number of ways; however all these methods are invasive.

(i) *Intraventricular Catheter.* In this method a burr hole is drilled in the frontal region, and under either stereotactic or under radiographic guidance a catheter is introduced into the frontal horn or the lateral ventricle and secured to the skin. This method allows for continuous and accurate assessment of ICP and for eventual intervention if an ICP increase jeopardizes CBF.

(ii) *Intraparenchymal Probe.* The placement of an *intraparenchymal probe* with a pressure sensor or a fiberoptic catheter is an alternative to the ventricular catheter. However, this method is prone to a reference drift while recalibration is impossible after the probe is in place. Furthermore, the local changes of pressure evoked by metabolic changes related to disease or (a traumatic probe placement) can dramatically influence recordings.

(iii) *Subdural Bolt.* A burr hole is drilled, and a hollow screw is inserted through the dura, and pulsations of CSF in a subarachnoid space are recorded via a sensor.

(iv) *Epidural Sensor.* A burr hole is drilled, and an epidural sensor is inserted between the skull and the dura to register dural tension (pulsations).

The accuracy of measurements by subdural bolt or epidural sensor is lower than those by intraventricular catheter. Additional caveats are (1) ICP is not uniformly distributed through the brain, and (2) local pressure measurements made by an intraparenchymal probe may not match the intraventricular pressure [113].

On the Model. The intraventricular measurement, despite being technically demanding, seems to be a method of choice for the new aSAH model.

6.2.2. Blood Pressure and Heart Rate Changes ("Cushing's Reflex"). Cerebral perfusion pressure (CPP) is an important, if not crucial, clinical tool that provides information on perfusion of brain [113]. CPP falls as ICP increases. An ICP rise that is near or above systolic blood pressure leads to complete perfusion arrest; a reduction of CPP to zero. Recovery of CPP begins as ICP declines after reaching a peak. CPP is estimated as the difference between ICP and mean arterial blood pressure: CPP = MABP − ICP.

Furthermore, an increase in ICP at SAH evokes Cushing's reflex, a hypothalamic response to ischemia. During this reflex systolic blood pressure rises, heart rate decreases, and respiration becomes irregular (sympathetic stimulation); each either directly or indirectly influences CPP and CBF. Thus, monitoring of BP and heart rate changes is necessary to access CPP changes after SAH.

(1) Blood Pressure Measurement. Mean arterial pressure can be measured by invasive and noninvasive methods.

(i) *Invasive Method.* This surgical method is based on experiments conducted by Stephen Hales in 1733, that showed that blood pressure and heart beat can be observed by a glass tube inserted into an artery of horse who inserted a glass tube in artery of horse and observed changes in blood pressure with the heart beat [114]. Not much has changed since then, and to obtain reliable and long-lasting monitoring in surgical settings under anesthesia, a sterile catheter is placed into radial or femoral artery. This method is used mostly for acute experiments and/or in bigger animals but has been used to measure blood pressure in small animals: rabbit (ear) and rodents (tail artery).

(ii) *Noninvasive Method.* This method is further divided into auscultatory or oscillometric methods.

The *auscultatory* method is most commonly used for measuring blood pressure in clinics. It is based on Korotkoff's 1905 discovery of the auscultatory sounds [115]. This method uses a blood pressure cuff and stethoscope (or more recently a microphone), which are applied on the arm (monkey), leg, or tail (rodents) to register animal's pulse tones. It allows for single, serial, or continuous measurements but usually requires anesthesia, which may influence the results. Moreover, if the stethoscope is used, results can be inconsistent and operator dependent. However, the measurements of systolic and diastolic pressures allow for an easy and often automated assessment of mean arterial pressure.

The *oscillometric method* is widely used for blood pressure measurement in the experimental settings. It measures oscillations caused by blood flow (i.e., pulse) by means of a pressure cuff. This simple method does not require a skilled

operator and hence can be automated for blood pressure recording. However, it does have several, above-mentioned, limitations related to the use of a cuff.

(2) Heart Rate Monitoring. Sympathetic stimulation during Cushing reflex leads to reduction in heart rate (bradycardia) and significant increase of BP. The following techniques have been used for monitoring heart rate and other cardiac changes following SAH.

(i) *ECG Monitoring.* ECG changes are registered when the ICP increases toward the systolic arterial pressure.

(ii) *Transesophageal Echocardiography.* Can be used in large animals to assess wall motion changes and aortic and pulmonary flow velocities at SAH [116].

(iii) *Serum Markers of Myocardial Injury.* An increased serum creatine kinase-MB and cardiac troponin-1 (cTn-1) concentration is often used to diagnose acute myocardial injury after SAH. However, as CK-MB can be released from non-cardiac muscle damage, cTn-1 is a superior indicator of myocardial injury [117].

On the Model. In addition to the ICP measurements, BP monitoring is a required feature of a modified aSAH animal model. The technique used for monitoring BP and cardiac changes in the new aSAH animal model will depend upon nature of experiment and its requirements. If an animal survival is required, then noninvasive BP monitoring should be used. Similarly, if the effect of SAH on heart rate is of concern, then a simple ECG monitoring will work fine.

6.2.3. CBF Changes and Possibility of Repeated Arteriography or TCD for a Delayed Vasospasm Assessment. CBF monitoring and vasospasm assessment provide useful tools to examine potential therapeutic options. An animal model provides these assessments and, in addition, can help establish the influence of acute phase on the following subacute and delayed phases of brain injury after aSAH.

CBF Monitoring. CBF can be assessed quantitatively or qualitatively. ^{133}*xenon method* is a method for quantitative assessment of CBF, which was described by Kety-Schmidt [118]. CBF is calculated from data obtained from several detectors placed on the head surface after administration of radioactive xenon gas. This method is widely used in both clinical and experimental settings. However, it measures CBF mostly from cortical and subcortical structures of the middle cerebral artery, and the measurements obtained are not reproducible by other CBF measurement methods. In addition, this method is cumbersome, requires significant investment, knowledge, and experience.

(i) *Thermal Diffusion Method.* This method estimates cortical or interstitial blood flow from the temperature difference between the two gold plates at the tip of the probe placed on or in the brain through a burr hole [119–121]. This method provides continuous quantitative real-time CBF. However, measurements

are made from a limited (local) area only and may not represent the whole brain (global) CBF changes.

(ii) *Transcranial Doppler Method* (TCD). This is a noninvasive method that was introduced by Aaslid et al. in 1982 [122]. It measures blood flow velocity and not blood flow. The linear relationship between CBF and mean flow velocity under most of the experimental and many clinical conditions allows for accurate assessment of CBF by TCD method and permits real-time CBF measurements [121–123]. This method is easy to use, allows for continuous data collection over a long period of time, can be used repeatedly, and allows comparison with other experiments or data sets [113, 123]. The usefulness of TCD for assessment of CBF and arterial diameter has been confirmed by numerous experimental and clinical studies of SAH [121, 124–128]. In addition, TCD assesses vascular resistance and reactivity as well as status of autoregulation of CBF. This is of significant value since CBF is constant in the CPP range of about 50–150 mmHg because of autoregulation, which is frequently disturbed after aSAH. The limitations of TCD include indirect CBF measurement and interoperator variability.

(iii) *Jugular Oximetry.* As TCD, *jugular oximetry* does not measure CBF directly. Here, CBF is calculated from arteriojugular oxygen saturation difference (AJDO$_2$). The measurements assess CBF in relation to metabolic activity but are adequate only if coupling between CBF and metabolism is intact. Another limitation is that oximetry assesses oxygen content in a jugular bulb that may better represent hemispherical and not global CBF.

(iv) *Cerebral Angiography.* Spasm in large cerebral arteries sets in 3–7 days after SAH. Angiography is frequently used to assess the presence and severity of delayed arterial vasospasm. Though this technique is invasive it can be used repeatedly to follow the development and effects of pharmacological intervention on the delayed vasospasm [129].

(v) *EEG Monitoring.* EEG changes are registered when the ICP surpassed the systolic arterial pressure and the electrical silence results of arrest of the cerebral circulation.

On the Model. CBF measurement is crucial for a modified aSAH model and should be performed using a technique that is reliable, simple, easy, noninvasive, and allows repeated measurements. TCD fulfills this selection criterion.

6.3. Outcome Assessment. An animal outcome is an essential endpoint of an aSAH study. It confirms the importance of a pathway being studied in aSAH induced injury and helps decide whether modification of this pathway would be beneficial. It is also essential that outcome assessments studied in animals are relevant to the human condition so that

treatments found effective in animals can be translated to the patients [99].

In aSAH patient neurological and functional deficits develop early and/or after several days. In patients, status at admission and early deficits are assessed by the Hunt and Hess, the Glasgow coma (GCS), and the World Federation of Neurological Surgeons (WFNS) grading scales [130]. The long-term outcome in SAH patients is assessed by Katzman, Rankin, and/or Barthel scores. In animals, neurological injury is studied *indirectly* as diminished response to an external stimulus or reduced function or *directly* as death of brain cells by immunostaining or assays for apoptosis, autophagy, or neurodegeneration. The methods used for assessing neurological and functional deficits in SAH animals are less than perfect and often erroneously incorporate procedures intended for assessing focal ischemic injury. Furthermore, though a battery of exams for a number of species exists, a species-specific limitation for assessments is often not recognized (for review see [131, 132]). The review by Jeon and colleagues provides an excellent guide to the techniques used to assess outcome in rodents after aSAH [99].

On the Model. A new aSAH animal model should induce consistent and reproducible immediate-gradual and transient-permanent injury and deficits. Thus, it should use scales and exams for injury assessment that are similar or equivalent to the ones used in SAH patients. This strategy will increase the chances of successful translation of a therapy found beneficial in animals.

7. Modified aSAH Model

We applied quite a few restrictions to establish an improved aSAH model and and came up with several must have essentials and a spectrum of choices rather than a single, one-fits-all solution. An investigator of course will select the technique that suits the animal species and the phase of injury (acute versus delayed) being studied and permitted by the laboratory environment. The approach that in our opinion will work the best is as follows.

(1) Blood is injected, so that it pools in the subarachnoid space and elevates ICP to a level that CPP reduces to zero creating TGI.

(2) Physiological parameters that change after SAH and associate with the intensity of injury are monitored:

   (i) early ICP change via an intraventricular catheter;
   (ii) early BP change via an oscillometric method;
   (iii) early CBF change via TCD;
   (iv) delayed vasospasm via repeated arteriography or TCD.

(3) Outcome assessments are made using scale and exams that are equivalent to the ones used for assessing clinical outcome.

(4) Additional attributes are adaptable to other species (range from rodents to primates) with little modification and low mortality and morbidity.

8. Summary

Inadequate disease modeling may have contributed to the failure of improving outcome in aSAH patients. We presented here a proposal of a modified model of aSAH that incorporates all of the components and elements of injury after aSAH, which may provide a better resource for studying the injury mechanisms and developing a treatment.

Acknowledgment

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Review Article

Pathophysiological Role of Global Cerebral Ischemia following Subarachnoid Hemorrhage: The Current Experimental Evidence

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Subarachnoid hemorrhage (SAH) is the subtype of stroke with one of the highest mortality rates and the least well-understood pathophysiologies. One of the very early events which may occur after SAH is a significant decrease of cerebral perfusion pressure (CPP) caused by the excessive increase of intracranial pressure during the initial bleeding. A severely decreased CPP results in global cerebral ischemia, an event also occurring after cardiac arrest. The aim of the current paper is to review the pathophysiological events occurring in experimental models of SAH and global cerebral ischemia and to evaluate the contribution and the importance of global cerebral ischemia for the pathophysiology of SAH.

1. Introduction

Subarachnoid hemorrhage (SAH) is a relatively rare subtype of stroke (incidence: 10/100,000 person years; 5% of all first-ever strokes) which is characterized by the presence of blood in the subarachnoid space, the cerebrospinal fluid-filled space between the pia arachnoidea, a thin membrane which covers the brain parenchyma, and the dura mater [1–4]. The vast majority of SAHs (85%) is caused by the spontaneous rupture of a cerebral aneurysm located at the skull base. The consequence of blood being released into the subarachnoid space with a pressure almost equal to systolic blood pressure is that 20%–25% of patients die almost immediately after SAH [1]. From those patients reaching a hospital, 33% die within the first 30 days after hemorrhage and about 33% survive only with persisting neurological deficits making them dependent on daily care [2, 5]. The remaining 33% of patients were independent 18 months after SAH; however, only 1/3 of these patients reported no reduction in quality of life as compared to the premorbid state [6]. Accordingly, about 50% of SAHs are lethal and less than 8% of patients fully recover. Therefore, SAH is regarded as the subtype of stroke with the worst prognosis; due to the relatively young age at which SAH occurs, the loss of potential life before the age of 65 is comparable to that of ischemic stroke, a condition which is more than 20 times more frequent (incidence: 240/100,000 person years) [7].

Despite large technical and procedural achievements in the diagnosis of SAH, in the prevention of rebleedings, and in general intensive care over the past three decades, it is a matter of debate whether the outcome after SAH improved significantly [3, 5, 8, 9]. This disappointing situation may be the mere reflection of the severity of the disease, however, since most sequelae of SAH occur with a delay of several hours and even days, it is generally accepted that a deeper understanding of the pathophysiology of the secondary insults caused by the initial hemorrhage may be the key for the development of novel therapeutic strategies and, hence, for improving patient outcome.

The CNS-related pathophysiological events following SAH can be divided into an early component and a delayed component. The delayed component occurs later than four days after hemorrhage and is characterized by delayed spasms of large intracranial vessels and possibly cerebral microvessels leading to cerebral ischemia in distinct areas of the brain, that is, focal cerebral ischemia. Delayed large artery spam has been studied extensively over the past 20 years, and endothelin 1 receptors were found to be the molecular surrogate for posthemorrhagic vasospasm [10]. Unfortunately, recent clinical evidence suggests that although endothelin

receptor antagonists were able to reduce posthemorrhagic vasospasm, patient's outcome did not improve significantly [11]. Consequently, in recent years, research started to focus more on the early component of the pathophysiology of SAH [8, 12]. Another reason for this change of focus is certainly also that mortality during the first few days after SAH is four times higher than that during the late phase [1, 13, 14].

One of the main characteristics of early brain injury (EBI) following SAH is a severe reduction in cerebral blood flow in various regions of the brain [15] which may cause cortical spreading depolarization (CSD), spreading ischemia, and subsequent ischemic brain damage [16]. Interestingly, cerebral ischemia occurs under conditions of normal or almost normal cerebral perfusion pressure (CPP) suggesting that ischemia is caused by constriction of intracerebral vessels. Since neither clinical nor experimental evidence suggest that functionally relevant macrovasospasm is present at this early stage after SAH, the cerebral perfusion deficit has to be located on the level of the cerebral microcirculation. Indeed Uhl and colleagues and later Pennings and colleagues demonstrated already ten years ago that pial microvessels show pearl-string-like constrictions in SAH patients [17, 18]. Such microvasospasms were later also found in experimental studies using histological techniques [19] and *in vivo* imaging [20, 21].

Despite these interesting and clinically relevant findings explaining the occurrence of focal ischemic brain damage in the cortex and in the basal ganglia and the subsequent functional deficits observed after SAH, some other important features of the pathophysiology of SAH are still unclear. It is, for example, still unclear why SAH patients suffer from global brain edema [22, 23], why glutamate levels increase after SAH but decrease shortly thereafter [24], why in animal models of SAH neuronal injury is mainly observed in the hippocampus and not in the cerebral cortex [25], and why patients surviving SAH suffer from pronounced memory deficits [26]. These changes occur on top of early cortical ischemia and may be associated with global ischemia due to the exceedingly high increase in intracranial pressure and the resulting cessation of cerebral perfusion during and shortly after the initial vessel rupture as suggested by various authors already decades ago [27, 28]. Since the pathophysiological changes observed immediately, that is, within the first 60 minutes after SAH, cannot be investigated in patients, the aim of the current paper is to review the experimental literature and evaluate whether there is enough evidence to suggest that global cerebral ischemia is an important feature of the pathophysiology of SAH.

2. Pathophysiological Findings following Experimental SAH

A plethora of techniques and species were used during the past decades to study SAH under experimental conditions [29–32]. At the time when the emphasis of SAH research was mainly on delayed cerebral vasospasm, animal models able to reproduce this condition experimentally were predominantly developed [29–32]. The model of SAH and vasospasm most frequently used was the canine "two-hemorrhage" model, in
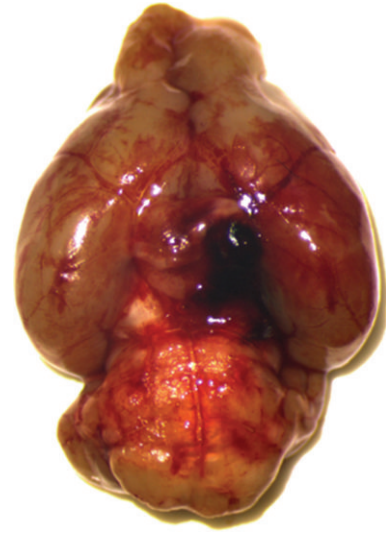

FIGURE 1: Perfused mouse brain three hours after experimental SAH (endovascular perforation model). A large clot formed at the perforation site (dotted white circle), and blood is distributed from the bleeding site into the subarachnoid space, preferentially along blood vessels.

which two injections of blood into the basal cistern were performed 48 hours apart. On the basis of its ability to accurately predict what occurs in human SAH, a primate model in which a blood clot is surgically placed around the large cerebral vessels at the base of the brain was used in dedicated centers [29]. After more recently scientists became also interested in early brain injury after SAH, animal models reproducing the early pathophysiology of SAH became more popular and more frequently used [30–32]. Among those models, the intravascular perforation model, where the Circle of Willis is perforated without craniotomy by an endovascular approach, seems to be the procedure which reproduces the early pathophysiology of SAH most adequately [30–33]. Therefore, most of the data currently reviewed derive from experiments performed with the filament perforation model.

When inducing SAH experimentally by endovascular perforation of the Circle of Willis, blood is released into the subarachnoid space at the skull base where it forms a large clot (Figure 1). Since the growing clot uses up a significant proportion of the intracranial volume, the intracranial pressure (ICP) starts to rise immediately after the hemorrhage to values around 100 mmHg (Figure 2(a)). The immediate increase in ICP triggers an increase of blood pressure, the so-called Cushing Reflex (Figure 2(b)), thereby aggravating the bleeding [34]. The intracranial hypertension results in a pathological decrease of cerebral perfusion pressure (CPP) for up to 5 minutes (Figure 2(c)). This CPP decrease results in a global suspension of cerebral blood flow for 2-3 minutes [24, 33, 35–37] which is equal to global cerebral ischemia.

The stop of cerebral circulation together with local vasoconstriction and activation of the coagulation cascade promote the formation of a blood clot at the bleeding site and, hence, cessation of hemorrhage as indicated by a gradual decrease of ICP over the next 2-3 minutes to values around
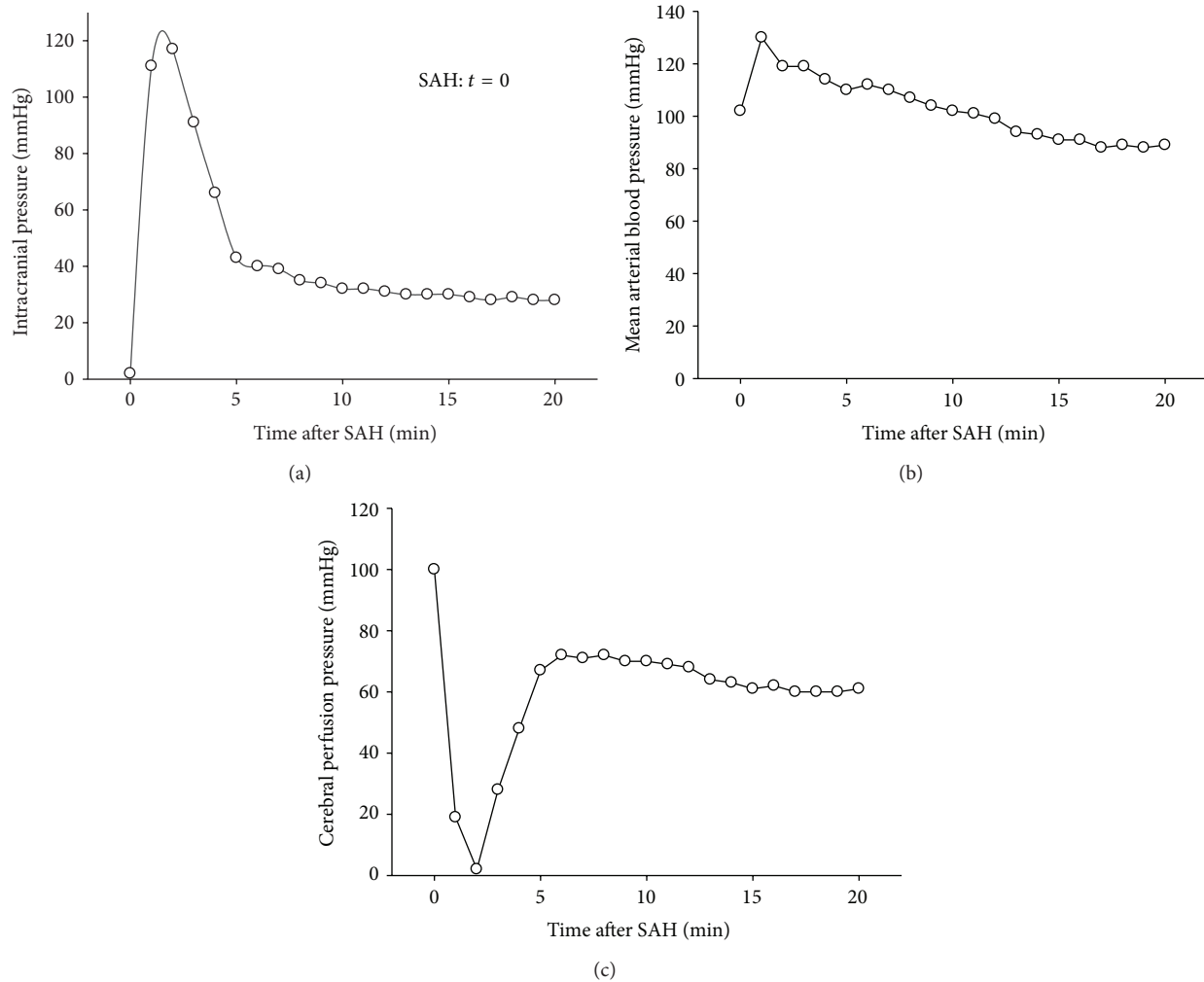
(a)

(b)

(c)

FIGURE 2: Intracranial pressure (ICP; (a)), mean arterial blood pressure (MAP; (b)) and cerebral perfusion pressure (CPP; (c)) after SAH in a mouse ($t = 0$; endovascular perforation model).

30 mmHg. Consequently, CPP recovers to near normal values of 60 mmHg or more (Figure 2). Interestingly, many groups report that despite the recovery of CPP, CBF does not necessarily recover and may stay at low levels in both hemispheres for up to 60 min after SAH [24, 33, 35, 36]. Acute vasoconstriction of large intracerebral arteries was made responsible for this phenomenon [33]; however, this early lack of CBF recovery after SAH is prevented when instead of anesthetics with a known blood pressure-lowering and Cushing Reflex-suppressive effect, that is, halothane or isoflurane, anesthetics are used which maintain systemic blood pressure [38]. Hence, it remains unclear whether the prolonged CPP-independent drop of CBF after SAH is a pure experimental phenomenon or indeed a component of the early pathophysiology of SAH.

No matter if CBF fully recovers or not, SAH results also in metabolic changes in the brain parenchyma as demonstrated by *in vivo* microdialysis [24, 33]. Glutamate increases up to sixfold already 30 min after SAH and gradually returns to near baseline values within the next 1.5 hours. This increase in glutamate is paralleled by an increase in the lactate/pyruvate ratio, an indicator of tissue ischemia [24]. Since microdialysis reflects the situation in the brain parenchyma only with a delay of up to 30 min (depending on sampling conditions), it is conceivable to conclude that the metabolic changes observed after SAH by microdialysis occur mainly immediately after the initial hemorrhage and are therefore a strong indicator for global cerebral ischemia [24].

Concomitant with the recovery of ICP, CPP, CBF, and tissue metabolism, the posthemorrhagic brain starts to display a slow but steady increase in brain water content from three to six hours until at least three days after SAH [25, 39, 40]. The delayed and slow development of brain edema suggests that the underlying pathophysiology may be linked to opening of the blood brain barrier (BBB) rather than to the initial posthemorrhagic global ischemia, since brain edema formation following global ischemia is caused by ischemic cell swelling and therefore disappears within minutes after reperfusion [41]. Indeed, injection of blood into the subarachnoid space of rats—a model devoid of all acute changes in ICP, CPP, and CBF described earlier—resulted in an increased vascular permeability brought about by focal disruption of endothelial tight junctions and the subsequent opening of the BBB

in the underlying cortex [42]. The molecular mechanisms responsible for this BBB opening have not been fully elucidated but involve activation of matrix metalloproteinase 9 and degradation of the microvascular basal lamina [37, 43]. If one takes into consideration that in SAH patients and in animals subjected to SAH by endovascular puncture blood is distributed in the whole subarachnoid space, it is conceivable that under these conditions vasogenic brain edema will develop in all cortical regions of the brain and may therefore make a "global" impression. When extrapolating to the human situation, it is, hence, very likely that the global edema observed in patients [22, 23, 44] is just the reflection of blood-induced microvascular leakage and has little or no pathophysiological link to the global ischemia observed immediately after the initial bleeding.

SAH in mice and rats is accompanied by a mortality of 35%–50% mainly between 24 and 72 hours after vessel perforation [25, 29, 30, 33, 37, 45], values well comparable to those observed in SAH patients [1, 2]. At least in experimental animals, the reason for this mortality is certainly not related to focal brain ischemia due to delayed vasospasm since rodents do not develop symptomatic large artery spasms neither at the time when mortality occurs nor later [31]. Accordingly, rebleedings or the sequels of early brain injury (EBI) have to be involved; however, the underlying mechanisms are by far not fully understood yet [8, 12, 46]. In any case, SAH-related mortality is certainly not related to hemorrhage-induced early global cerebral ischemia since the duration of global cerebral ischemia typically observed after SAH, that is, 2-3 minutes, does not cause any mortality in comparable experimental models of global cerebral ischemia; in these models, more than 8 minutes of three/four vessel occlusion are necessary to produce at least some mortality [47, 48]. In addition, mortality after experimental global ischemia typically occurs between day 3 and day 5 after the insult and not within the first three days like after experimental SAH. Accordingly, the mortality observed after SAH in rodents does not seem to be caused by global ischemia, but rather by later changes associated with blood-brain barrier opening, microcirculatory failure, and focal cerebral ischemia.

3. Pathophysiological Findings following Experimental Global Cerebral Ischemia

Cardiac arrest results in an immediate drop in systemic blood pressure and a subsequent cessation of cerebral blood flow resulting in global cerebral ischemia. The lack of cerebral blood flow results in anaerobic metabolism leading to tissue acidosis, anoxic depolarization of neuronal cells with release of glutamate and other neurotransmitters into the extracellular space, and in immediate swelling of glial cells. As a consequence, extracellular glutamate concentrations increase by several folds and cytotoxic edema develops [41, 49]. If the restoration of cardiac function occurs before the respiratory centers of the brain stem are permanently damaged, survival is possible [50]. Usually reperfusion of the brain is followed by a hyperemic response [51], and large as well as small cerebral vessels are fully perfused within a few minutes [52]. Despite

sufficient cerebral blood flow, usually neuronal cell death occurs with a delay of 3–5 days in the hippocampus [53] and in selective cortical areas resulting mainly in memory and executive function deficits [54–56]. These events are found in a very similar manner in experimental animals as well as in patients who suffered a cardiac arrest.

4. Similarities between Experimental Global Cerebral Ischemia and SAH

When comparing the pathophysiology observed after global cerebral ischemia and SAH, it becomes quite obvious that the early phase of SAH shows some phenomena which are very similar to those observed after global cerebral ischemia. In both conditions, cerebral blood flow may come to a complete stop or is at least reduced below the ischemic threshold of 20% of physiological cerebral blood flow [33, 35], and extracellular glutamate concentrations are increased significantly for at least 30–60 min [24, 33, 49]. These changes in blood flow trigger an acute activation of cerebrovascular endothelial cells and cause a delayed but transient interaction of inflammatory cells and platelets with cerebral vessels for only a few hours [52, 57]. The glutamate releases triggered by global cerebral ischemia and SAH result in excitotoxicity and delayed neuronal cell death selectively in the hippocampus and in subsequent memory and executive function deficits [25, 49].

Similarities between global cerebral ischemia and SAH are also found during the reperfusion phase which occurs after restoration of cerebral blood flow. Provided animals have a sufficiently high blood pressure [38, 51], reperfusion occurs within a few minutes and results in full restoration of flow in large and small cerebral vessels as well as on the level of the microcirculation [25, 48, 52, 57]. When blood pressure is not sufficiently high, both conditions result in slow or lacking reperfusion which results in low survival rates and exacerbation of delayed brain injury [33, 38, 45, 51].

5. Dissimilarities between Experimental Global Cerebral Ischemia and SAH

As soon as the acute phase of global cerebral ischemia and SAH is over, the pathophysiology of both conditions starts to show a growing number of dissimilarities. The main reason for this observation is the persisting triggering of further pathophysiological processes by the presence of blood in the subarachnoid space following SAH while in global cerebral ischemia the pathophysiology certainly proceeds, but no further pathophysiological events are additionally initiated [41, 51]. One important feature of SAH not found in global cerebral ischemia is the acute constriction of large intracranial vessels [19, 33] and the subacute occurrence of cerebral microvasospasm and microthrombosis [21, 58] in areas of subarachnoid blood deposition. These vascular changes may well prolong and/or exacerbate the perfusion deficits acutely caused by global ischemia and result in delayed focal cerebral ischemia and the formation of delayed brain edema as also observed in SAH patients [15, 22, 59].

6. Summary and Conclusion

The current literature as well as our own results suggests that the early pathophysiology of SAH consists of two phases: one related to the brief global ischemia caused by the initial bleeding and one linked to the vascular damage caused by the blood ensheathing the brain supplying arteries in the subarachnoid space. This concept is further supported by the fact that microvessels in the subarachnoid space adjacent to the cerebral cortex are functionally impaired, that is, do not react to CO_2 (unpublished data), show microvasospasms [19, 21], are prone to develop microthrombosis [21, 58], and show progressive opening of the blood-brain barrier [42]. Accordingly, events induced by global cerebral ischemia are well present after SAH and play an important pathophysiological role but represent only one out of many important components of the complex pathophysiology of SAH.

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Review Article

Acute Microvascular Changes after Subarachnoid Hemorrhage and Transient Global Cerebral Ischemia

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Subarachnoid hemorrhage and transient global cerebral ischemia result in similar pathophysiological changes in the cerebral microcirculation. These changes include microvascular constriction, increased leukocyte-endothelial interactions, blood brain barrier disruption, and microthrombus formation. This paper will look at various animal and preclinical studies that investigate these various microvascular changes, perhaps providing insight in how these microvessels can be a therapeutic target in both subarachnoid hemorrhage and transient global cerebral ischemia.

1. Introduction

Subarachnoid hemorrhage (SAH) is a type of hemorrhagic stroke, most commonly caused by a ruptured intracranial aneurysm. At the time of aneurysm rupture, blood pours into the subarachnoid space, and the intracranial pressure (ICP) inside the rigid calvarium increases sharply, causing a corresponding decrease in cerebral blood flow (CBF). The patient's clinical presentation on arrival to the hospital can depend on the degree and duration of this initial global cerebral ischemia.

Patients with aneurysmal SAH may develop angiographic vasospasm and delayed cerebral ischemia (DCI) with onset 3–12 days after the initial rupture [1]. DCI may or may not be accompanied by large artery vasospasm as seen with vascular imaging [2]. A multicenter randomized clinical trial has not shown improvement in neurologic outcome despite ameliorating the delayed large artery vasospasm [3]. Whether this is due to efficacy of rescue therapy in the placebo groups or drug toxicity abrogating beneficial effects in the clazosentan groups has not been resolved. Nevertheless, as a result of these results, research in SAH has also investigated early brain injury and acute microvascular changes [4]. Nimodipine, an L-type calcium channel antagonist, is

the only pharmacologic agent that has been shown to consistently improve neurologic outcomes in clinical trials of patients with SAH [5].

Similarly, cardiac arrest (CA) results in global cerebral ischemia that is transient in clinically relevant cases, since if cardiac function is not restored, the situation is of pathological interest only. Other causes of transient global cerebral ischemia (tGCI) include asphyxia, shock, and complex cardiac surgery [6]. The clinical presentation depends on the duration of cardiac arrest and time to initiating cardiopulmonary resuscitation. After global cerebral ischemia from SAH or tGCI, a cascade of molecular events occurs, resulting in variable degrees of brain injury and cerebrovascular changes.

Global cerebral ischemia in postcardiac arrest has also been studied extensively for many decades in various animal models. Other than early induced mild hypothermia [7, 8], clinical translation of neuroprotective strategies and therapeutics has largely been unsuccessful.

The study of the microcirculation after tGCI and SAH remains a difficult undertaking, but this strategy of study may reveal potential therapeutic targets and new insights into disease pathophysiology. The purpose of this paper is to look at relevant animal and preclinical studies investigating

acute microvascular changes (within the first 48 hours) occurring after either SAH or tGCI. Cerebral microvessels may be defined as vessels less than or equal to 100 micrometers in diameter [9]. Animal studies of focal ischemia or studies focused on the large cerebral vessels (i.e., circle of Willis arteries, basilar artery, etc.) are not included in this paper. While we acknowledge that tGCI may occur in a large heterogeneous group of disorders (i.e., traumatic brain injury, intracerebral hemorrhage, etc.), we have chosen to focus solely on tGCI secondary to cardiac arrest or mechanisms mimicking cardiac arrest, such as extracranial arterial occlusion. After providing an overview of various animal models and general trends in cerebral hemodynamics after SAH and tGCI, we provide an in-depth review of studies investigating specific microvascular changes that occur in these two conditions: (1) microvascular constriction; (2) increased leukocyte-endothelial cell interactions; (3) blood brain barrier (BBB) breakdown; and (4) platelet aggregation and microthrombosis.

2. Animal Models

There are numerous animal models that attempt to mimic the clinical conditions of SAH or tGCI. Large (nonhuman primates, cats, dogs, and pigs) and small animals (mice, rats, gerbils, and rabbits) may be used. It is important to take into consideration that experimental results may vary depending on the animal model used.

Techniques used to produce SAH include endovascular perforation, blood injection, artery avulsion or puncture, and clot placement. For example, the endovascular perforation model of SAH in the mouse may have more physiologic resemblance to the actual clinical scenario of a ruptured intracranial aneurysm, but the amount of blood in the subarachnoid space is quite unpredictable from animal to animal leading to increased variability in the results. The injection model of SAH (cisterna magna or prechiasmatic cistern) in the mouse provides the ability to control the amount of blood introduced into the subarachnoid space, but may not produce as dramatic rise in ICP compared to the endovascular perforation model, depending on the amount injected. As a result, the degree of global cerebral ischemia seen after SAH may not be as severe in the blood injection model as reflected by the overall lower mortality rate compared with the endovascular perforation model [10, 11]. A detailed review of various animal models of SAH has been published previously [12]. The type of SAH model utilized must be taken into account when interpreting experimental results.

Similarly, there are a large variety of animal models and techniques used to study tGCI. These techniques include cardiac arrest/asphyxia, thoracotomy with clamping of the aorta and great vessels, bilateral common carotid artery and vertebral artery (4 vessel) occlusion, and isolated bilateral common carotid artery occlusion. The severity of the ischemia depends on the technique used to produce ischemia, the type of animal, and even the strain of an animal species. For example, most gerbils are known to lack posterior communicating arteries that connect the forebrain and hindbrain circulations. Thus, bilateral common carotid artery occlusion produces very severe forebrain ischemia in gerbils [13]. However, in mice, the presence or absence of posterior communicating arteries varies depending on the strain used. BALB/C mice had larger infarct sizes and were more likely not to have posterior communicating arteries compared with BDF and CFW mice after concomitant ipsilateral common carotid artery and middle cerebral artery occlusions [14]. Also, the duration of ischemia and reperfusion can vary significantly between studies. A comprehensive review of available animal models of tGCI has been published [15]. Again, interpretation of study results must take into account the specific model of tGCI utilized.

3. Cerebral Hemodynamic Changes

After SAH, the ICP increases as a result of new subarachnoid blood occupying volume in the fixed intracranial space, with a corresponding decrease in cerebral perfusion pressure (CPP). There are no data on ICP during de novo aneurysm rupture in humans; but during rebleeding, the ICP frequently rises substantially [16]. The ICP may rise as high as the diastolic blood pressure and last for several minutes. Since not all patients go unconscious at the time of SAH, this only occurs in a subset of clinical cases. During this period, there may be a transient absence of forward CBF [17]. The mean arterial pressure (MAP) typically increases to partially compensate, but this change does not adequately restore CPP. The ICP then returns to normal or slightly supranormal levels over the course of less than an hour [17]. In a rat endovascular perforation model, CBF, which initially drops sharply to 20% of baseline flow, begins to slowly rise and then stabilizes at a level below the baseline [18]. The magnitudes of the initial drop in CBF and increase in ICP are related to the amount of subarachnoid blood [19]. If the ICP remains persistently high after SAH, then CBF does not recover and the animal dies [17].

In tGCI induced by either temporary cardiac arrest or four-vessel occlusion, there is negligible forward blood flow in the cerebral circulation. With temporary bilateral common carotid artery occlusion causing severe forebrain ischemia, the reduction in CBF is more variable depending on the intracranial collateral circulation, specifically the presence and patency of the posterior communicating arteries. Unlike in SAH, experimental models of tGCI do not produce a dramatic increase in ICP [20]. Upon reperfusion, there are two cerebrovascular response patterns seen. The first pattern is the "no-reflow phenomenon," which is characterized by decreased tissue perfusion upon subsequent intra-arterial injection of contrast or dye after an initial period of ischemia [21]. Although the no-reflow phenomenon is more commonly discussed in the context of coronary artery occlusion [22], the term was probably first used by Ames et al., in experiments involving the cerebral circulation in rabbits undergoing tGCI [23]. This phenomenon has been confirmed in other studies [24, 25]. The second pattern is postischemic reactive hyperemia followed by delayed hypoperfusion [21].

Experimental SAH and tGCI both result in impaired global CBF. However, in SAH, acute cerebral ischemia is secondary in part to high ICP, which is not present in tGCI, although other mechanisms may reduce CBF after ICP declines in SAH. Also, in tGCI, reperfusion involves restoring blood flow much like an "on" switch, whereas in SAH models, reperfusion is a much more gradual process as the ICP normalizes.

4. Microvascular Changes in Subarachnoid Hemorrhage

4.1. Microvascular Constriction. Although, earlier research focused more on delayed large vessel vasospasm in SAH, it is also known that acute microvessel constriction occurs. Topical application of blood onto the cortical surface of anesthetized guinea pigs revealed vasoconstriction of pial vessels [26]. Such constriction was reversed acutely by topical application of the alpha adrenergic blocker, phenoxybenzamine, and prevented by the beta-adrenergic blocker, propranolol [26]. It appears that acute vasoconstriction occurs predominantly in the arterioles and not the venules. In an endovascular perforation model of SAH in mice, pial surface microvessels observed with *in vivo* fluorescence microscopy demonstrated unchanged venular diameter but approximately 70% of arterioles constricted acutely (3–6 hours) and persisted even at 72 hours after SAH [27]. Smaller arterioles had more vasoconstriction than larger arterioles. Pial vessels constricted as early as 5 minutes after injection of hemolyzed erythrocytes into the cisterna magna of rats, and this persisted for at least 2 hours [28]. *In vivo* monitoring also revealed decreased blood flow in the arterioles as well as the venules. Erythrocytes take time to lyse after SAH, so the time course after injection of hemolyzed blood may not be the same as after actual SAH. Using a prechiasmatic SAH model in mice, Sabri et al. found an increased degree of vasoconstriction in the microvessels (10–20 micrometers in diameter) as well as increased overall wall thickness at 48 hours after SAH, as determined by electron microscopy [29]. In these experiments, the location of the microvessel constriction appeared to strongly correlate with regional distribution of brain injury and neuronal apoptosis [29].

In addition to constriction, arterioles also have been shown to demonstrate altered reactivity acutely after SAH and specifically to have impaired vasodilation. In an endovascular perforation model of SAH in rats, cortical surface pial arteriolar vasodilation in response to either topical adenosine or sodium nitroprusside was significantly impaired after SAH, but CO_2 reactivity was unaffected [30]. In addition, pial arteriolar vasodilation, which is typically seen in response to sciatic nerve stimulation, was attenuated during the first 3 days after SAH but returned to control levels by 4 days [30]. Cortical arterioles also demonstrated increased constriction in response to endothelin-1 20 minutes after injection of autologous blood into the cisterna magna injection of rats [31].

Ultrastructural changes in the walls of microvessels are also observed in experimental SAH. In an endovascular perforation model of SAH in rats, electron microscopy revealed partially collapsed capillaries with swollen astrocyte foot processes and small luminal protrusions emanating from the endothelial cells [32]. These changes occurred at least 1 hour after SAH. The significance of these luminal protrusions is unclear.

4.2. Leukocyte-Endothelial Interactions. Leukocyte adhesion to the microvessel wall may contribute to microvascular injury. In inflammatory conditions, the cerebral microvasculature increases the expression of endothelial adhesion molecules that attract and bind leukocytes, such as intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), P-selectin, and E-selectin [33]. With leukocytes rolling and then adhering to the microvessels, they can then traverse the luminal wall and enter the brain parenchyma by the process of diapedesis [34]. Neutrophils and macrophages may then cause direct neuronal injury [6].

After SAH induced by prechiasmatic blood injection in mice, there was a significant increase in endothelial cell membrane expression of P-selectin, but no difference in cytosolic P-selectin expression [29]. Although leukocyte adhesion was not specifically addressed in this study, the increase in P-selectin expression appeared to colocalize to regions with increased microthrombi burden [29]. Neutrophils appear to contribute to early microvascular injury after SAH. In an endovascular perforation model of SAH in rats, neutrophils were found to adhere to the cerebral microvasculature as soon as 10 minutes after SAH [35]. An inhibitor of neutrophil function, pyrrolidine dithiocarbamate (PDTC), decreased neutrophil accumulation in the parenchyma despite an increase in adherent neutrophils to the cerebral vasculature, meaning that neutrophils had impaired ability to undergo diapedesis [35]. In contrast, pharmacologic reduction of neutrophils (with vinblastine or antipolymorphonuclear serum) decreased both neutrophil adherence to cerebral microvessels and penetration into the brain parenchyma but increased subsequent bleeding. The treatments in this study also decreased collagenase activity and maintained the integrity of the BBB.

Intravital microscopy showed a progressive increase in the number of rolling and adherent leukocytes to venules at 30 minutes, 2 hours, and 8 hours after SAH induced by endovascular perforation in mice [36]. This was not seen after cisternal injection of blood, demonstrating the difference in results that can occur depending on the animal model used and suggesting a role for tGCI in the findings, since tGCI is more prominent in SAH induced by endovascular perforation compared to cisternal blood injection. Some mice were treated with a monoclonal antibody against P-selectin immediately after SAH, and this decreased leukocyte rolling and adhesion [36]. It is not clear based on preclinical SAH studies whether leukocyte plugging of microvessels as a result of increased adherence to the luminal wall is significant enough to cause ischemia in itself.

4.3. Blood Brain Barrier Disruption. Subarachnoid hemorrhage is believed to induce inflammatory states in the

brain. Inflammatory mediators (cytokines including IL-1β, IL-6, TNF-α, and oxidative damage from neutrophils and macrophages) may result in direct damage to the microvasculature, resulting in damage to the BBB [37]. The BBB maintains an exclusive intraparenchymal compartment for the brain, separate from the circulating blood. Unlike in the systemic microcirculation, the cerebral microvessels have endothelial cells with tight junctions to prevent passage of micro- and macromolecules from the blood into the brain interstitial environment [38]. There is a lack of fenestrations between cerebral endothelial cells, which means that molecules or cells that enter the parenchyma from the microvessel lumen must migrate through the polarized endothelial cell itself. There also may be reduced pinocytosis in cerebral endothelial cells. A basal lamina embedded in an extracellular matrix encircles the endothelial cells, and this is then covered by foot processes of local astrocytes. The cerebral endothelial cell, astrocyte, and neuron form the so-called neurovascular unit [39]. Damage to the integrity of the BBB can result in brain edema and brain injury [6].

There are several preclinical studies that suggest that there is disruption of the BBB after SAH. The time course of disruption, the magnitude, and to what molecules the BBB is disrupted to after SAH are not fully investigated. In an endovascular perforation model of SAH in rats, there was increased BBB permeability as determined by leakage of Evan's blue dye [40]. The BBB disruption was associated with an increase in brain edema, worse neurological deficit, and mortality. A pan-caspase inhibitor (z-VAD-FMK) administered 1 hour before and 6 hours after SAH prevented BBB disruption (measured by immunoglobulin extravasation) and decreased brain edema. Although SAH caused endothelial cell apoptosis in the basilar artery, endothelial cells of the microvasculature were not assessed. In a cortical SAH model in rats, significant impairment of the BBB as determined by Evan's blue dye extravasation was observed after SAH [41]. Furthermore, in spontaneously hypertensive rats with SAH, there was more BBB disruption compared with normotensive rats with SAH [42]. In a cisterna magna injection model of SAH in rats, the time course of BBB breakdown, assessed by Evan's Blue dye extravasation, was studied [43]. The BBB breakdown began 36 hours, peaked 48 hours, and resolved 3 days after SAH. In an intracisternal SAH model in cats, the authors did not observe BBB breakdown 30 minutes after SAH [44]. Cats subjected to arterial hypertension alone demonstrated regions of BBB breakdown, whereas animals subjected to arterial hypertension after SAH did not show BBB breakdown. This protective effect of hypertension conflicts with other studies [41].

Animal studies have investigated mechanisms by which SAH may compromise the BBB. Various matrix metalloproteinases (MMPs) are capable of breaking down the basal lamina and the associated extracellular matrix surrounding the endothelial layer [45]. This may lead to blood extravasation, associated edema, and brain injury. Sehba et al. studied the integrity of the microvasculature in an endovascular perforation model of SAH in rats [45]. There was decreased immunoreactivity to type IV collagen in the microvessel

basal lamina with corresponding increased levels of MMP-9 expression starting at 3 hours, peaking at 6 hours, and subsequently resolving by 48 hours after SAH. These changes were not observed at 10 minutes or 1 hour after SAH.

Extracellular matrix metalloproteinase inducer (EMM-PRIN, also known as collagenase stimulatory factor, basigin, CD147, or human leukocyte activation-associated M6 antigen), is a cell surface protein that can stimulate production of MMPs [46]. Inhibition of EMMPRIN with a monoclonal antibody against it decreased brain edema 24 hours after endovascular perforation SAH in rats [46]. Brain edema was maximal at 24 hours after SAH and declined thereafter in this model [46]. In another study, using the endovascular perforation model of SAH in rats, the tight-junction protein occludin in endothelial cells and collagen type IV in the basal lamina were decreased at 24 hour after SAH [47]. Electron microscopy confirmed disruption of the endothelial tight junctions and increased spaces between endothelial cells. The investigators found that p53 colocalized with the proinflammatory transcription factor nuclear factor κB (NF-κB) and MMP-9, which in turn could degrade occludin [47]. Because a selective p53 inhibitor decreased microvascular damage, the authors concluded that p53 is an important factor in BBB disruption.

The direct damage to the microvasculature after SAH may in part be due to reactive oxygen species produced by inflammatory cells. In a cisterna magna injection SAH model in rats, a hydroxyl free radical scavenger, when administered within 12 hours of SAH, decreased BBB permeability at 48 hours as determined by Evan's Blue dye extravasation [48].

4.4. Platelet Aggregation and Microthrombosis. In SAH, clot formation in the microcirculation could occur as a result of platelet aggregation and then embolization or propagation from the original bleeding site, which would be the rupture point in the intracranial aneurysm clinically. In experimental studies, this feature of active bleeding is a component of the endovascular perforation model but not the injection models. However, arterial injury and active bleeding do not seem to be the only initiator of platelet aggregation, since microthrombi are formed even in the injection animal model of SAH in which there is no vessel rupture [29]. Also, SAH predisposes to the formation of microthrombi, as rats undergoing a prechiasmatic injection model of SAH were found to be hypercoagulable [49].

Platelet aggregates are seen in the cerebral microvasculature as early as 10 minutes after SAH induced by endovascular perforation in rats [50]. The total microclot burden peaked at 24 hours, but fully resolved by 48 hours. In another study using the same model of SAH, platelet aggregates were associated with microvessels that were poorly perfused [51]. In addition, there was breakdown of the collagen IV component of the basal lamina [52]. Platelets, upon activation, can release proteases such as MMP-9 that can digest collagen IV in the basal lamina. In fact, platelets could be seen on the abluminal side of cerebral endothelial cells and in the local parenchyma by 10 minutes after SAH, with large numbers of platelets seen in the parenchyma by 24 hours

[52]. The investigators suggest that platelet aggregates may initiate or cause local endothelial cell injury, damage the BBB, and allow the extravascular escape of macromolecules and cells [51]. Sabri et al. found microthrombi throughout the mouse brain at 48 hours after the prechiasmatic injection of blood in mice [29]. These findings occurred later after SAH than demonstrated by some prior studies and in a model that has less global ischemia than the endovascular perforation model. The microclots appeared in about one-third of the constricted microvessels but in none of the normal microvessels. Also, the more severely constricted the vessel, the more numerous the microthrombi. There was a strong correlation between presence of microclots and regional brain injury.

The importance of the microthrombi to brain injury and outcome in experimental SAH was suggested in an endovascular perforation model of SAH in mice [53]. The number of microthrombi decreased upon administration of a mutant thrombin-activated urokinase-type plasminogen activator, and this correlated with decreased mortality. Platelet aggregates in SAH also adhered to leukocytes that were adherent to the walls of microvessels [36].

5. Microvascular Changes in Transient Global Cerebral Ischemia

5.1. Microvascular Constriction. In tGCI, the microvessels undergo significant changes in diameter during the global ischemia and then also during reperfusion; these changes affect CBF. However, reviews of the studies reveal inconsistent results. In a study by Pinard et al., a 4-vessel occlusion model of tGCI in rats was used to study *in vivo* changes of the surface pial microvessels [54]. During the 15 minutes of cerebral ischemia, arteriolar diameter transiently increased and then decreased. Cerebral autoregulation may explain this transient arteriolar vasodilation. Administration of 7-nitroindazole, a neuronal nitric oxide (NO) synthase inhibitor, reduced this transient vasodilation-implicating NO as an important participant in cerebral autoregulation. However, sustained vasodilation was not seen during the ischemic period, but this may be secondary to passive collapse of the microvessels due to slow perfusion and relatively low intravascular pressure. Despite occlusion of 4 vessels, there was residual forward flow during ischemia, which suggests that this animal model is one of incomplete global ischemia. Residual flow of plasma without erythrocytes could be seen *in vivo* in surface capillaries during the ischemia [54]. The transient arteriolar dilatation in response to tGCI was not seen in another study using a bilateral common carotid artery occlusion model in gerbils [55]. These investigators observed an initial mild arteriolar vasoconstriction in the first minute followed by a more extensive constriction beyond 1.5 minutes. These changes correlated with changes in cerebral metabolism.

Upon reperfusion in the study by Pinard et al., blood flow could be observed in the parenchymal arterioles with significant dilatation beginning 5 minutes after unclamping of the common carotid arteries, with return to baseline arteriolar diameter after 15 minutes [54]. Another study used 10 minutes of tGCI induced in cats by a 4-vessel occlusion and systemic hypotension protocol [20]. *In vivo* imaging through a cranial window revealed persistent dilated pial microvessels upon reperfusion although CBF was reduced [20]. Overall cerebrovascular resistance was unchanged, meaning that obstruction to flow must have been present distally in the penetrating arterioles or other vessels not seen on the cortical surface [20]. However, a contrasting result was found in a tGCI model of bilateral common carotid artery occlusion in gerbils, in which the investigators did not observe vasodilation but rather found decreased diameters in both surface precapillary arterioles and capillaries during reperfusion after 15 minutes of tGCI [56]. The authors concluded that the hypoperfusion that typically occurs in tGCI is a result of increased tone in precapillary arterioles, in contrast to any conclusion that could be drawn from other studies.

Endothelial protrusions can be seen in tGCI. In a 4-vessel occlusion model of tGCI in rats with 30 minutes of ischemia, cerebral endothelial microvilli projecting into the lumen could be identified throughout the brain, and this occurred in as little as 10 minutes after initiation of ischemia [57]. The frequency of microvilli increased with increasing duration of ischemia [57]. In another study, cerebral endothelial cell microvilli were also seen after tGCI was induced by occlusion of the cardiac vessel bundle, mimicking cardiac arrest in rats [58].

5.2. Leukocyte-Endothelial Interactions. The preclinical studies investigating leukocyte-endothelial interactions in tGCI have had mixed results. In a 4-vessel occlusion model of tGCI in rats, the investigators studied leukocyte-endothelial interactions in pial vessels via a closed cranial window and intravital microscopy [59]. At 2 hours after an ischemic period of 20 minutes, there was no significant increase in the number of rolling or adherent leukocytes in the microvessels when compared to the control group, despite evidence of neuronal injury on histology. In another study, 30 minutes of transient forebrain ischemia was induced in gerbils by bilateral carotid artery occlusion [60]. Gerbils were treated with cyclophosphamide to decrease neutrophil count (and as a side effect, slightly decreased platelets), but this did not affect the occurrence of the no-reflow phenomenon upon reperfusion, making leukocyte plugging of small microvessels less likely as a cause of postischemic hypoperfusion. Dirnagl et al. studied tGCI in rats with bilateral common carotid artery occlusion for 10 minutes followed by 4 hours of reperfusion and found that there was a trend toward increased leukocyte rolling and adherence to the endothelium during the postischemic period [61]. Very few microvessels were plugged with leukocytes and about half of the rats demonstrated leukocyte extravasation into the parenchyma during the post-ischemic period. The transition from hyperemia to post-ischemic hypoperfusion did not reveal any obvious change in leukocyte behavior, also suggesting that leukocyte plugging would not be a major contributor to hypoperfusion in the microvasculature. In contrast, other studies have demonstrated significant leukocyte adherence to the luminal walls of the microvasculature. Ritter and

colleagues found a significant increase in leukocyte rolling and adhesion in cerebral cortical venules at 30 minutes after reperfusion in a bilateral carotid artery occlusion model with induced hypotension in rats [62]. In a gerbil model of tGCI with bilateral common carotid artery occlusion for 15 minutes followed by reperfusion, there was an increase in leukocytes rolling or adhering to the venular endothelium within 3 hours of reperfusion, but no observed plugging of the capillaries, as determined by intravital fluorescence microscopy [63]. However, leukocyte-endothelial interactions had returned to baseline by 7 hours after ischemia and remained so at 12 hours and 4 days.

The conflicting results with regard to increased leukocyte-endothelial adherence after tGCI may be related to the diversity of animal models used, the variability in the duration of ischemia and reperfusion, as well as the varied resolution of the *in vivo* microscopy equipment.

5.3. Blood Brain Barrier Disruption. Transient global cerebral ischemia is also believed to induce an inflammatory state that results in BBB disruption. In a bilateral carotid artery occlusion model of global ischemia in gerbils, the BBB was disrupted, as determined by extravasation of Evan's blue dye and increased brain edema [64]. Brain edema was present immediately after reperfusion although Evan's blue dye leakage was not detected until 2 hours afterwards, and both were increased 3 hours after reperfusion, which was the latest time examined. In a 4-vessel occlusion model of global cerebral ischemia in rats, BBB breakdown, as determined by leakage of labeled albumin, was greater after longer ischemia time (60 minutes of global ischemia compared to 15 or 30 minutes) [65]. The degree of associated brain edema was also dependent on the duration of the initial ischemia. In a 4-vessel occlusion tGCI model in rats, BBB breakdown occurred during the ischemic insult, as demonstrated by leakage of fluorescein dye, beginning after as little as 8 minutes of ischemia and resolving by 30 minutes after reperfusion, after a preplanned total of 15 minutes of ischemia [54]. Similar to SAH, oxidative damage to the microvessels occurs with reperfusion after tGCI. Zheng et al. demonstrated decreased activities of superoxide dismutase and glutathione peroxidase in a bilateral common carotid artery occlusion mouse model of tGCI [66]. Loss of these enzymes that protect against oxidative damage resulted in cortical microvascular endothelial damage and mitochondrial injury. The authors also found that treatment with crocin, an antioxidant, inhibited this oxidative damage and attenuated MMP-9 expression.

5.4. Platelet Aggregation and Microthrombosis. In a circulatory arrest model of tGCI, aggregates of platelets were identified in the intraparenchymal vessels during reperfusion after 5 minutes of tGCI [67]. Platelet aggregates increased with increasing time of reperfusion. In a 4-vessel occlusion model of tGCI in rats, thrombi could be seen *in vivo*, temporarily obstructing cortical surface arterioles and venules during the hyperemic phase after reperfusion and causing turbulent blood flow [54]. In another study, tGCI was induced by occlusion of the cardiac vessel bundle in rats for 10

minutes followed by reperfusion [58]. Microthrombi were most prominent at 3 minutes to 6 hours after reperfusion and appeared to localize in regions of relative hypoperfusion [58]. The microthrombi were not seen 7 days after tGCI in this model.

Endothelial injury occurs in tGCI which causes breakdown of the BBB, exposing portions of the basal lamina to the cerebral circulation. This promotes platelet aggregation and thrombosis. Another potential initiator of microthrombi is the relative stasis of blood during the ischemia in both SAH and tGCI—resulting in *in situ* thrombosis, although this has not been confirmed experimentally.

6. Comparison of Microvascular Changes in SAH and tGCI

Although microvascular constriction is consistently demonstrated in SAH, such constriction is inconsistent during the ischemic and reperfusion phases of tGCI. This may be related to the heterogeneity in animal models utilized. However, endothelial luminal protrusions have been demonstrated in both SAH and tGCI, but the significance of this finding is unclear. Most studies that involve *in vivo* observations of microvessels typically focus on surface pial vessels, which are clearly more accessible and convenient to study. It is, however, much more difficult to assess penetrating parenchymal microvessels *in vivo*, but these vessels may be important in the pathophysiology of SAH and tGCI.

SAH and tGCI both are believed to induce inflammatory states in the brain. While less widely investigated, there does seem to be evidence that increased leukocyte adherence to the cerebral microvasculature occurs after SAH. Neutrophil adherence in tGCI has been inconsistently shown. Leukocyte rolling has also been inconsistently demonstrated in both SAH and tGCI. The no-reflow phenomenon after tGCI appears not to be directly caused by leukocyte plugging in the microvasculature.

The majority of studies investigating BBB integrity after SAH or tGCI do not use *in vivo* observation of the BBB. However, BBB disruption is consistently seen in all of these studies and appears to occur earlier after tGCI (as early as 8 minutes) compared with SAH (3 hours) [45, 54].

Platelet aggregation and presence of microthrombi in the microvessels occur after both SAH and tGCI. The models of SAH may induce some degree of tGCI, so it is difficult to determine how much of the pathophysiology after SAH is due to the subarachnoid blood itself.

7. Conclusions

Subarachnoid hemorrhage and tGCI share common pathophysiological changes in the microvasculature. This includes microvascular constriction during the ischemic phase, increased leukocyte-endothelial interactions, disruption of the BBB, and microvascular platelet aggregates and microthrombosis. The cerebral microvasculature may be an important target for treatments designed to reduce brain injury, although there are few such studies

and limited information about the importance of the pathophysiologic processes in humans. Due to similar pathological mechanisms between these two conditions, however, it may be that treatment strategies for SAH may be applicable to tGCI and vice versa.

Acknowledgments

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Research Article

The Impact of Experimental Preconditioning Using Vascular Endothelial Growth Factor in Stroke and Subarachnoid Hemorrhage

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Vascular endothelial growth factor (VEGF) stimulating angiogenesis was shown to be a potential novel therapeutic approach for the treatment of ischemic vascular diseases. The goal of the present study was to examine whether transfection of VEGF before occurrence of major stroke (part I) and cerebral vasospasm after experimental subarachnoid hemorrhage (SAH; part II) develops neuroprotective qualities. A total of 25 (part I) and 26 (part II) brains were analyzed, respectively. In part one, a significant reduction of infarct volume in the VEGF-treated stroke animals (43% reduction, $P < 0.05$) could be detected. In part two, significant vasospasm was induced in all hemorrhage groups ($P < 0.02$). Analyzing microperfusion, a significant higher amount of perfused vessels could be detected ($P < 0.01$), whereas no significant effect could be detected towards macroperfusion. Histologically, no infarctions were observed in the VEGF-treated SAH group and the sham-operated group. Minor infarction in terms of vasospasm-induced small lesions could be detected in the control vector transduced group ($P = 0.05$) and saline-treated group ($P = 0.09$). The present study demonstrates the preconditioning impact of systemic intramuscular VEGF injection in animals after major stroke and induced severe vasospasm after SAH.

1. Introduction

Cerebral vasospasm and delayed cerebral ischemia contribute the major part of secondary morbidity and mortality after severe subarachnoid hemorrhage (SAH) [1–5]. Despite current treatment strategies, the rate of related permanent disability is estimated at 10% to 20% [6–9].

Vascular endothelial growth factor (VEGF) is involved in neurogenesis, inhibition of apoptosis, learning, and memory [10]. It can directly promote neuroprotection, but first of all VEGF is the main factor responsible for angiogenesis whereby an indirect neuroprotection is discussed. VEGF expression is increased during cerebral ischemia in humans and animals [11]. However, endogenous VEGF seems to be insufficient to protect the brain from ischemic injury completely. Interestingly, it could be shown that exogenous

administrated VEGF induces angiogenic changes that result in a reduction of cerebral ischemic injury [12, 13]. For this reason VEGF was adopted as a potential novel therapeutic approach for the treatment of ischemic vascular disease, particularly in ischemic stroke [14–18].

The aim of the present experimental study was to examine the effect of systemic overexpression of VEGF prior to stroke and SAH with regard to cerebral infarction, vasospasm, and perfusion.

2. Material and Methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The

experimental study was reviewed and approved by the local Committee for Animal Experimentation, Recklinghausen, Germany (approval no. 8.87-50.10.34.08.246). All invasive procedures were performed under general anesthesia with intraperitoneal application of xylazine hydrochloride and ketamine, and all efforts were made to minimize suffering. The animals were housed under a light/dark cycle with free access to food and water.

2.1. Construction of Vectors and Transduction.

DNA transduction was performed with a VEGF-containing expression vector. The initial vector (pcDNA3.1/His B/; Invitrogen, Karlsruhe, Germany) was first digested with EcoRI. A VEGF clone in EcoRI (descending from a pLEN/VEGF vector, kindly provided from Max-Planck-Institute, Planegg-Martinsried, Germany) was inserted. Control animals were injected with an empty expression vector (pcDNA3.1/His B; Invitrogen, Karlsruhe, Germany). Vector integrity was confirmed by sequence analysis. Large-scale preparation of plasmid DNA was performed with the EndoFree GigaPrep (Qiagen, Hilden, Germany). DNA was solved in 0.9% sterile saline and stored in aliquots at −20°C.

Weekly gene transfers into both anterior tibial muscles were performed thrice with 100 μg DNA per leg in a volume of 50 μL normal saline.

2.2. Transient Middle Cerebral Artery Occlusion.

Part one determined the effect of VEGF in stroke protection in order to verify the effect of VEGF in major stroke. Three groups of male Wistar rats weighting 250 to 275 g ($n = 32$) received intramuscular injections of VEGF vector, control vector, or saline for three times at intervals of seven days. Seven days after the last gene transfer, the rats underwent 45 minutes of transient middle cerebral artery occlusion (tMCAO) under continuous monitoring of laser Doppler flow (Moor Instruments, Axminster, UK) [19]. Body temperature was maintained at 37.0 ± 0.5°C. Reperfusion was performed by removing the filament. Animals were sacrificed after 24 h reperfusion time.

2.3. Rat Double SAH Model.

In part two a total of 80 male Wistar rats weighting 250 to 275 g were used. The animals were randomized to four groups: group (1) receiving intramuscular injections with a plasmid-containing VEGF, group (2) receiving a VEGF free plasmid, group (3) the saline group, and group (4) the sham-operated group.

In groups 1 and 2 the VEGF or VEGF free vector was injected 21, 14, and 7 days before the induction of vasospasm.

One week after the last gene transfer (VEGF vector, control vector, and sodium) vasospasm was induced by double blood injection into the cisterna magna as described previously [20–22]. Animals were positioned prone, and the atlantooccipital membrane was surgically exposed. The cisterna magna was punctured under microscopic view using a 27-gauge cannula, and 0.2 mL of cerebrospinal fluid was aspirated first and followed by injection of 0.2 mL of autologous blood. The animals were then placed head down for 10 minutes to avoid leakage of injected blood, and the operation wound was closed. The procedure was

repeated on day 2. In animals belonging to the sham-operated group, the atlantooccipital membrane was exposed; 0.2 mL of cerebrospinal fluid was aspirated and reinjected. The animals were positioned head down for 10 minutes, and the operation wound was closed. This procedure was repeated on day 2. During the procedure the body temperature was controlled and maintained at 37.0 ± 0.5°C.

The neurological condition was assessed daily according to a modified Bederson grading scale circling to one side [23].

2.4. Cerebral Angiography and Evaluation.

Angiography was performed on day five after double direct blood injection into the cisterna magna.

The angiographic studies were performed under intraperitoneal general anesthesia as described above. After positioning the animals supine, a cervical midline incision was made to expose the common carotid artery bilaterally. As described previously the artery was tapped using a small cannula attached to a microcatheter (27-gauge needle, Prowler 14 microcatheter; Cordis Endovascular, Miami Lakes, FL, USA), and the angiography was performed under automated, controlled injection of a total of 0.1 mL of contrast agent (Ultravist 300; Schering AG, Berlin, Germany; Integris Allura; Philips Medical Systems, Best, The Netherlands) [20]. The angiography was repeated up to four times to achieve best quality. On the one hand digital imaging was measured, software based, to evaluate the reduction of large vessel diameter as described before [24]. On the other hand a visualization of low-density structures in given regions of interest (RIO) was determined in order to obtain information concerning perfusion by the use of minimum intensity projection (MinIP) [25]. A MinIP in time direction from the beginning arterial phase to the parenchyma phase was carried out. The algorithm uses all the data by projecting the volume of interest into a viewing plane. Before contrast agent arrived, an individual baseline image was determined and filtered to reduce background information (OsiriX v. 3.8.1, http://www.osirix-viewer.com/). Major ROIs (including angiographically visible major vessels, e.g., A. carotis interna) and smaller cortical ROIs (without angiographically visible major vessels, e.g., area between A. carotis interna beneath the junction of A. cerebri media) were defined. The resulting bidimensional image represents the contrast-perfused vessels/tissue. Measurement of average grey levels in the above-defined ROIs represents a higher or lower perfusion. These values of minimal intense projection are inversely correlated with the amount of perfused vessels.

The animals were sacrificed after the angiography by intraperitoneal injection of a lethal dose of sodium pentobarbital (200 mg/kg body weight, Sanofi-Aventis, Frankfurt, Germany).

2.5. Histology.

A 12 μm microtome was used for cresyl violet staining and hematoxylin and eosin as well as for TUNEL analysis. Coronal sections of the frontal, parietal, and occipital brain were taken to detect morphological alterations in terms of ischemic lesions. In part one recording of all sections was obtained by a digital camera. Infarct area and total area of the brain were outlined manually
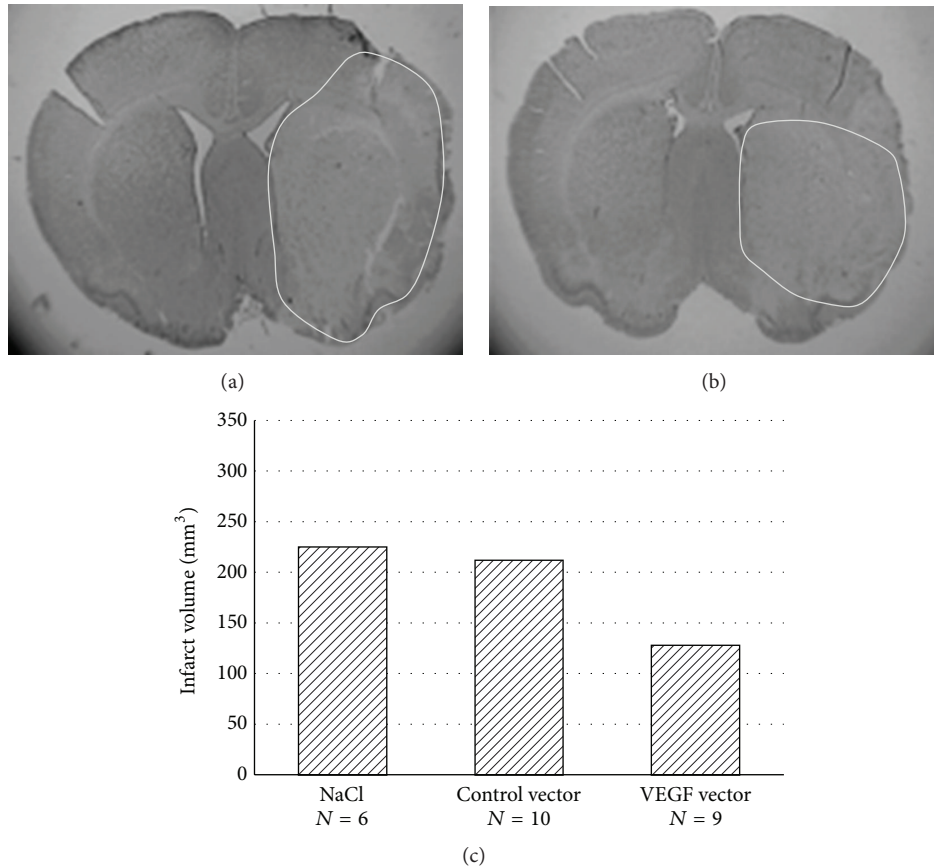
(a)

(b)

(c)

FIGURE 1: Cresyl violet staining after 45 minutes of tMCAO and 24 h reperfusion time. (a) Distinct ischemic brain injury after i.m. injection with saline. (b) Attenuated ischemic brain injury after i.m. injection with VEGF. (c) Infarct volume in the three different groups in mm^3.

and volume calculated, software based, in mm^3 (Leica QWin, Leica, Germany). In part two the infarctions in each section were assessed and divided into three groups as previously published: (1) no infarction, (2) minor infarction, and (3) territorial infarction [26]. For detection of apoptotic cells in part one, an in situ cell death detection kit (Roche Diagnostics, Mannheim, Germany) based on terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) technique was used.

3. Results

3.1. Part One: Attenuated Ischemic Brain Injury after Transient Focal Cerebral Ischemia. In part one VEGF in major stroke showed a significant reduction of infarct volume in the VEGF-treated animals (43% reduction, $P < 0.05$, Mann-Whitney U test, Figure 1). Five animals passed away due to subarachnoid hemorrhage and two as the result of stroke. 25 brains were analyzed (9 in the gene-transferred group, 10 in the control group, and 6 in the saline group). TUNEL staining showed no significant differences in the three groups.

3.2. Part Two. A total of 80 animals were examined. 24 animals died immediately after the double hemorrhage injection

and 12 during the course of the experiment. The clinical evaluation revealed no delayed neurological deficit over the 5-day observation period in terms of hemiparesis. All animals were sacrificed on day 5 after the initial bleeding.

3.2.1. Angiographic Analysis of Macroperfusion. Overall, 176 angiographic examinations were performed in 44 animals belonging to the 4 experimental groups. Angiographic evaluation was not possible in 18 animals as a result of technical problems or mortality from the angiography itself. 96 series in 26 animals were technically sufficient. Of these, the angiogram with the highest contrast of each animal was chosen for further evaluation by an independent observer (SM) blinded to the groups.

Statistically significant reduction of arterial diameter was induced with the double hemorrhage model (SAH groups compared with sham group, $P < 0.02$, Figure 2). Among the three SAH groups, there were no statistically significant differences of relative intracranial filling intensity defined as macroperfusion (SAH/VEGF compared with SAH/control, $P = 0.56$; SAH/VEGF compared with SAH/NaCl, $P = 0.51$, t-test).

3.2.2. Angiographic Analysis of Microperfusion. In 26 animals, a complete arterial to venous phase (circulation time) was
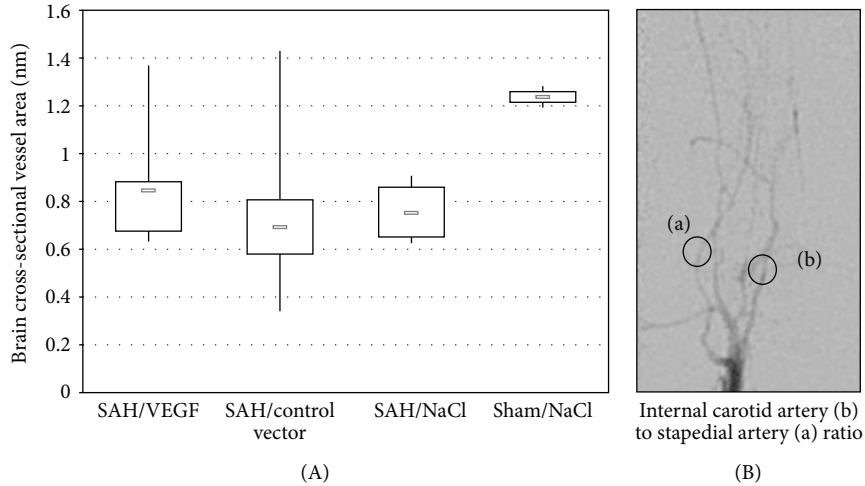
FIGURE 2: (A) The brain cross-sectional vessel area in mm in the three SAH groups and the sham group. Displayed are the lowest, highest, and median values of brain cross-sectional vessel area (in mm), with upper (75%) and lower (25%) quartiles. (B) Digital subtraction angiography of rat number 11 demonstrating the internal carotid artery (b) to stapedial artery (a) ratio.
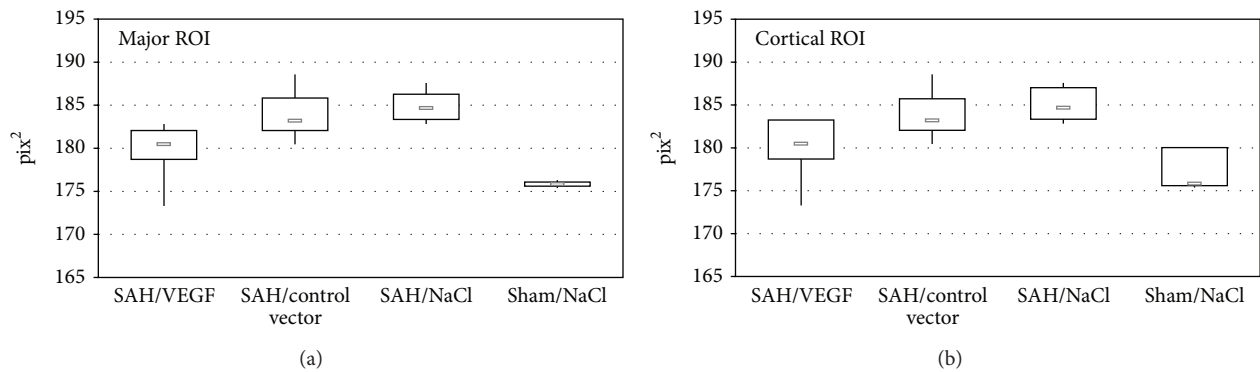

FIGURE 3: Minimum intense projection of cortical (without angiographic visible vessels) and major ROIs (with angiographic visible vessels): values of minimal intense projection are inversely correlated with the amount of perfused vessels. Displayed are the lowest, highest, and median pix^2 values, with upper (75%) and lower (25%) quartiles.

carried out (Figure 3). Comparing the VEGF-treated SAH group (group 1) with the control vector treated SAH group (group 2) and the NaCl-injected SAH group a significant difference was detected (major ROI: $P < 0.01$ and $P < 0.01$; cortical ROI: $P < 0.02$ and $P < 0.01$). Differences between the VEGF-treated and NaCl groups without SAH were not statistically significant (major ROI: $P = 0.94$; cortical ROI: $P = 0.63$).

3.2.3. Morphological Examination. Macroscopic pathological evaluation revealed clear residuals of SAH in the basal cisterns in 26 animals belonging to the SAH groups and in none of the sham-operated group. Histologically, no infarctions were observed in the VEGF-treated group and the sham-operated group. Minor infarction in terms of vasospasm-induced small lesions could be detected in the control vector transduced group ($P = 0.05$) and saline-treated group ($P = 0.09$) (Figure 4).

4. Discussion

4.1. Part One. Analyzing therapeutic effect in experimental cerebral ischemia usually a tMCAO model is used [27, 28]. Therefore the developed VEGF DNA was initially tested in this experiment under well-defined conditions with clearly expected ischemic lesions. Different VEGF levels and accordingly immunohistochemical analysis of VEGF were not carried out, but a distinct effect on the reduction of infarct volume in the VEGF-treated animals could be shown (43% reduction). Based on these results it is assumed that intramuscular injection of VEGF has a neuroprotective effect in cerebral ischemia animal model.

4.2. Part Two

4.2.1. SAH Model. The double hemorrhage model was used because of the higher vasospastic impact in comparison to other SAH models as described [29]. As previously published,
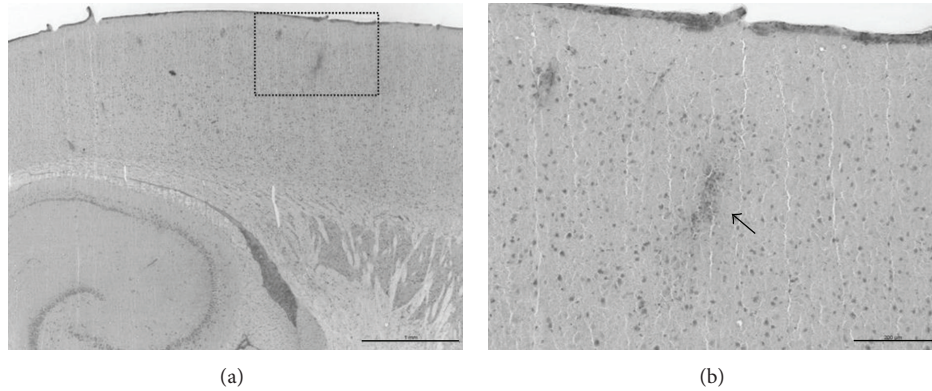
(a)

(b)

FIGURE 4: H&E stained section with cortical microinfarct in the cortical layers in a nontreated animal (a). At higher magnification mononuclear infiltration can be distinguished (b, arrow). Scale bar corresponds to 1000 μm (a) and 200 μm (b), respectively.

the highest level of measurable vasoconstriction was induced on day 5 after induction of SAH [20, 30]. Thus, day 5 was chosen for angiographic studies. Mortality rate in the present trial was 30%, which is in line with previously published studies using the double hemorrhage model in rats [20, 31, 32].

4.2.2. Efficacy on Macro- and Microperfusion. Angiographic evaluation of small vessels in rats is complex, and thus different techniques have been reported [33–35]. In the present study, a previously described software-based measurement tool for analysis of small cerebral vessels was used to detect vasospasm [24]. Using this technique, a significant induction of vasospasm in all SAH groups could be detected, which is in line with previously published studies [20].

In the VEGF-treated group, a significant difference of cerebral vessel caliber (cerebral macroperfusion) in relation to the other SAH groups was not measurable. In contrast, the analysis of cerebral microperfusion using the vascular density technique as previously described revealed a significant increase in the VEGF-treated group [25]. One explanation could be the induction of neoangiogenesis due to VEGF. This observation is in line with results after the experimental cerebral ischemia model in animals [35]. Two major limitations have to be mentioned. First, the blood pressure during DSA was not measured and therefore may have affected time to peak and cerebral blood flow values. However, to detect real hyperperfusion or hypoperfusion a perfusion imaging in terms of perfusion CT or perfusion MRI is needed. Second, limitation is the infusion time of contrast agent. Automated injection facilitated a standardized condition in our setting.

4.2.3. Morphological Effects. One issue with experimental models of SAH in small animals is the lack of clear morphological ischemic damage wherefore part one was performed in order to evaluate the effect in major stroke [22]. Similarly, in the present study, only a few ischemic areas in the nontreated SAH groups could be detected.

4.3. Efficiency of Intramuscular VEGF and Limitation of the Trial. Hypoxia itself induces an increase of VEGF expression in ischemic areas of the brain, but this endogenous VEGF secretion is inadequate to entirely protect the brain injury [36]. Based on the significant reduction of infarct volume in part one it is assumed that intramuscular injection of VEGF has a neuroprotective effect in cerebral ischemia animal model.

Short half-life and poor penetration over the blood brain barrier appear to have a lower impact in this model than frequently described [13, 27]. These findings were corroborated by other investigators who verified that high dose of intravenous infusion of VEGF after cerebral embolic ischemia induces leakage of blood brain barrier in the animal model [37]. Controversially, reduction of edema formation after VEGF application in a stroke model despite the leakage of blood brain barrier has been described [38]. The detailed activity of VEGF, besides the known neoangiogenesis and mitogenic activity in stroke and particularly in SAH, remains therefore mostly unknown.

Although VEGF administration appears promising, several disadvantages have to be mentioned. VEGF protein is not stable in vivo, and it has a short half-life. Direct protein implantation into ischemic lesions via sustained release delivery systems or focal virus mediated overexpression might protect the immediately surrounded neuronal tissue, and, therefore, direct implantation seems to be a good approach for defined hypoxic-ischemic brain injuries [27, 39]. In SAH, delayed cerebral ischemia and related infarction are known to occur diffusely. Therefore, recommendation of treatment and the target of therapy should address the whole brain. In consequence, a systemic increase of VEGF using a DNA gene transfer could be discussed as a potential beneficial approach.

Within the chronic phase of vasospasm, the cerebral circulation adapts to hypoxia with angiogenesis and dilatation of microvessels [15]. Nevertheless, it is suggested that the relation between vasoconstrictive factors (free hemoglobin, activated endothelin-1, and free oxygen radicals) and vasodilatory substances (NO) is disturbed in favor of constrictors, and neoangiogenesis potentially starts too late. In this study no influence of VEGF on the vasoconstrictive or vasodilatory

elements could be detected directly. On the opposite, a significant increase of perfusion could be detected. However, a nonsignificant trend to a lesser extend of ischemic lesions in histological examinations could be identified, and a significant increase of microperfusion was detected via an angiographic approach despite proven vasospasm. Against this background the indirect neuroprotection by dint of angiogenesis seems to have a high importance.

The upregulation of VEGF can trigger this angiogenesis in the early period of vasospasm. This means preconditioning the cerebrum to forthcoming ischemic event by improvement of blood supply.

The present study demonstrates the preconditioning impact of systemic VEGF injection on cerebral microperfusion and ischemic lesions in animals after induced severe vasospasm. These results justify further investigations in particular with regard to modus of application, dose of VEGF injection, time interval of preconditioning, immunohistochemical examination (VEGF, CD 34), and detailed measurement of behavior changes.

Disclosure

D. Hänggi is a Scientific Officer of Edge Therapeutics and a Consultant for Codman. The authors have no financial or personal interests in the materials and devices described.

Conflict of Interests

For none of the authors a conflict of interests does exist.

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Review Article

Subarachnoid Hemorrhage, Spreading Depolarizations and Impaired Neurovascular Coupling

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Aneurysmal subarachnoid hemorrhage (SAH) has devastating consequences on brain function including profound effects on communication between neurons and the vasculature leading to cerebral ischemia. Physiologically, neurovascular coupling represents a focal increase in cerebral blood flow to meet increased metabolic demand of neurons within active regions of the brain. Neurovascular coupling is an ongoing process involving coordinated activity of the neurovascular unit—neurons, astrocytes, and parenchymal arterioles. Neuronal activity can also influence cerebral blood flow on a larger scale. Spreading depolarizations (SD) are self-propagating waves of neuronal depolarization and are observed during migraine, traumatic brain injury, and stroke. Typically, SD is associated with increased cerebral blood flow. Emerging evidence indicates that SAH causes inversion of neurovascular communication on both the local and global level. In contrast to other events causing SD, SAH-induced SD decreases rather than increases cerebral blood flow. Further, at the level of the neurovascular unit, SAH causes an inversion of neurovascular coupling from vasodilation to vasoconstriction. Global ischemia can also adversely affect the neurovascular response. Here, we summarize current knowledge regarding the impact of SAH and global ischemia on neurovascular communication. A mechanistic understanding of these events should provide novel strategies to treat these neurovascular disorders.

1. Pathophysiology of Subarachnoid Hemorrhage

Aneurysmal subarachnoid hemorrhage (SAH) is associated with high morbidity and mortality with limited therapeutic options [1]. The major contributor to poor outcome of patients surviving the initial surge in intracranial pressure is delayed cerebral ischemia (DCI) manifesting 4–10 days after aneurysm rupture as new and otherwise unexplained neurological deficits and/or ischemic lesions within the brain [2]. Despite decades of study, mechanisms contributing to SAH-induced DCI remain controversial. For many years, a delayed and prolonged vasospasm of large conduit arteries was thought to be the major contributor to DCI and the ensuing death and disability observed in SAH patients [3, 4]. Recent data, however, challenge this view [5–7] and strongly suggest that additional mechanisms contribute to poor outcomes

after SAH, including early brain injury suffered at the time of bleed [6, 8–10], blood-brain barrier disruption [11, 12], inflammation [13–15], and impaired microcirculatory function [16–19]. Evidence suggests that a pathological inversion of neurovascular coupling may play an important role in SAH pathology both in the context of spreading depolarization waves [20] and at the level of the neurovascular unit in response to focal neuronal activity [21].

2. Spreading Depression and Injury Depolarizations

Spreading depression (SD) is the historical term used to describe intense neuronal and glial depolarization events that propagate within cortical or subcortical grey matter at a rate of 2–4 mm/min regardless of functional divisions or arterial

boundaries [22]. Initially implicated in migraine aura, SD-like depolarization waves also occur in stroke and traumatic brain injury [23, 24]. The pivotal event during SD is a massive K^+ efflux that increases extracellular K^+ concentration to >40 mM. Massive influx of Ca^{2+}, Na^+, and water accompanies the K^+ efflux and triggers uncontrolled release of neurotransmitters, most importantly the excitatory amino acid glutamate. Released K^+ and glutamate are believed to depolarize other neurons in the vicinity, and SD slowly propagates in grey matter by way of contiguity. Therefore, extracellular medium, including the perivascular space, is flooded with K^+ and neurotransmitters that are vasoactive. Because complete membrane depolarization precludes action potentials and synaptic transmission, SD is associated with suppression of all spontaneous or evoked electrical activity. Consequently, the normal neuronal influence on the vasculature is absent at least until the ability of neurons to generate action potentials returns, which can take several minutes. Moreover, there is ample evidence suggesting that physiological neurovascular coupling is impaired not only during the depolarization but for hours after the SD event [25–28].

SD is triggered when a minimum critical volume of brain tissue is simultaneously depolarized. Therefore, cerebral ischemia, anoxia, and other forms of brain injury can all trigger SD. Both animal models and clinical studies have clearly demonstrated the occurrence of SD waves associated with traumatic brain injury, cerebral ischemia, and subarachnoid hemorrhage [24, 29–33]. With respect to the emergence of SD after SAH, a number of potentially interacting factors have been implicated. These SD promoting factors and influences from subarachnoid blood include increased extracellular K^+ combined with decreased nitric oxide bioavailability [30, 34, 35], oxyhemoglobin [34, 36, 37], and endothelin-1 [37–40]. Such spreading injury depolarizations occur repetitively over hours and days and propagate throughout the unhealthy, but not yet depolarized or necrotic tissue (e.g., ischemic penumbra). Indeed, such injury depolarizations are indistinguishable from SD when they often propagate into noninjured tissue. The existence of injury depolarizations has been recognized for decades, and their detrimental effect on tissue outcome has been attributed to their profound metabolic impact. More recently, however, an additional mechanism was discovered that exacerbates the energy supply-demand mismatch in injured brain. This novel mechanism, termed inverse or vasoconstrictive neurovascular coupling, leads to a reduction in tissue perfusion instead of the usual hyperemia SD causes in normal brain tissue.

3. Influence of Spreading Depolarizations on Cerebral Blood Flow

In most species and studies, and under normal physiological conditions, SD is typically associated with a profound hyperemic response that starts shortly after the onset of depolarization and outlasts it by a few minutes [41–46]. As SD has a profound metabolic impact on brain tissue [47], this increase in the flow of nutrients enables neurons to recover from the massive ion and water imbalance occurring during SD events. However, the vasomotor impact of SD can also

be complex. For example, a brief hypoperfusion occasionally precedes the hyperemia, its onset coinciding with the onset of depolarization. This initial hypoperfusion is augmented by nitric oxide (NO) inhibition, particularly when extracellular potassium ($[K^+]_e$) is artificially elevated [34, 48–51]. In mice, the initial vasoconstriction is much more pronounced and hyperemia is completely absent [41]. Vascular response also appears to vary depending on vessel caliber and/or cortical depth. Larger pial surface arterioles respond to SD with a small initial constriction followed by dilation, whereas smaller parenchymal arterioles mainly constrict [52]. In general, vasoconstrictive tone develops during the depolarization, followed by a vasodilator tone during repolarization, and then a second vasoconstrictive phase that can last up to an hour [53]. The magnitude and time course of these opposing vasomotor components vary depending on species and experimental conditions, can be modulated physiologically and pharmacologically, and determine the final morphology of the hemodynamic response [53]. Altogether, these observations suggest that SD exerts multiple opposing vasomotor effects on blood vessels, with vasodilation predominating in healthy tissue.

Pathological circumstances such as ischemic stroke or subarachnoid hemorrhage modulate the magnitude and timing of the vasomotor components. Under such conditions, the vascular response becomes predominantly vasoconstrictive, that is, inverted [46, 54, 55]. This likely represents a shift in the balance of vasomotor influences to vasoconstriction. As a result, injury depolarizations cause hypoperfusion rather than hyperemia that could potentially lead to a downward spiral of increased brain injury [33, 56, 57]. In ischemic penumbra, the more ischemic the tissue is (i.e., closer to the core), the more severe the vasoconstrictive component becomes [33, 56, 58–60]. Such conditions can be recreated to transform the CBF response. For example, in the presence of extravascular hemoglobin and elevated $[K^+]_e$ or low glucose, mimicking subarachnoid hemorrhage, SD is associated with severe vasoconstriction [34]. Induced hypoxia and hypotension independently augment the hypoperfusion component of the hemodynamic response to SD and significantly diminish the hyperemia [61]. Although hypotension appears to be more potent than hypoxia in this regard, combined hypoxia and hypotension, most closely mimicking ischemic penumbra, transforms the predominantly dilator response into a biphasic one. Neither induced hyperoxia nor hyperglycemia restores the CBF response [55, 62], suggesting that cerebral perfusion pressure affects SD-mediated vascular responses by a mechanism unrelated to tissue energy status.

Despite the fact that SD in normal cortex is not damaging, this severe vasoconstrictive response can lead to injury and cell death, even in the absence of any preexisting energy depletion [36]. Indeed, injury depolarizations worsen tissue and neurological outcome in focal cerebral ischemia and other brain injury states including aneurysmal SAH [20, 29, 31, 57, 63]. Conversely, drugs that are known to inhibit cortical spreading depression, such as NMDA receptor antagonists MK-801, diminish the severity of episodic hypoperfusions and prevent the expansion of severely hypoperfused cortex, eventually reducing the infarct size [20, 29, 31, 33, 63].

However, in vivo studies have shown the efficacy of MK-801 to prevent SD was greatly diminished when extracellular K^+ was elevated [64]. Topical application of vasodilator agents such as nitric oxide and the L-type voltage-dependent Ca^{2+} channel blocker nimodipine reverts the vasoconstrictive response to vasodilation [34, 54, 65]. Therefore, mechanisms transforming the CBF response from hyperemia to hypoperfusion during injury depolarizations may be targeted to interrupt the vicious cycle and improve tissue outcome. Further, recent evidence suggests SAH can have a profound impact on the individual neurovascular unit leading to inversion of neurovascular coupling in the absence of SD.

4. Functional Hyperemia at the Level of the Neurovascular Unit

Functional hyperemia and neurovascular coupling are terms often used interchangeably to describe increased cerebral blood flow (CBF) in brain regions with enhanced neuronal activity, which forms the basis of functional magnetic resonance imaging (fMRI) [66]. This localized vasodilation to meet activity-dependent metabolic demand involves interplay of cells comprising the neurovascular unit—neurons, astrocytes and intracerebral (parenchymal) arterioles [67–69]. Astrocytes act as key intermediaries in the neurovascular response, structurally having close "synapse-like" associations with neurons as well as processes (astrocytic endfeet) that completely encase parenchymal arterioles. Over the past decade, numerous investigators primarily using cortical brain slices have provided evidence linking increased neuronal activity and nerve-mediated glutamate release to the activation of astrocytic metabotropic glutamate receptors (mGluRs), inositol triphosphate-(IP_3-) mediated increase in astrocyte Ca^{2+} and Ca^{2+}-dependent release of vasodilator influences from astrocytic endfeet [68, 70–75]. Excitatory and inhibitory interneurons may also modulate the neurovascular coupling process via an influence on astrocyte Ca^{2+} or through direct effects on parenchymal arterioles [76–78]. Multiple vasodilator mechanisms have been proposed to contribute neurovascular coupling. Elevations in astrocytic endfoot Ca^{2+} have been linked to increased Ca^{2+}-dependent phospholipase A_2 (PLA_2) activity and release of vasodilatory arachidonic acid metabolites. These include prostaglandin E_2 (PGE_2) produced by cyclooxygenase-1, and epoxyeicosatrienoic acids (EETs) produced by the cytochrome P450 epoxygenase, CYP 2C11 [70, 71, 79–81]. In addition, large conductance Ca^{2+}-activated K^+ (BK) channels are localized to astrocytic endfeet [82] and play a key role in neurovascular coupling [69, 83, 84]. Endfoot BK channel activation by moderate increases in astrocytic Ca^{2+} causes localized increases in K^+ in the perivascular space that stimulate inwardly rectifying K^+ (K_{ir}) channels located on the smooth muscle of parenchymal arterioles leading to membrane potential hyperpolarization and vasodilation [69, 71, 75, 83–85]. In sum, increased endfoot Ca^{2+} is a critical step linking local neuronal activity to parenchymal arteriolar dilation.

5. Neurovascular Coupling Can Also Lead to Pathological Vasoconstriction

In vitro studies have reported that under certain conditions, neuronal activation can also lead to parenchymal arteriolar constriction [84, 86–88]. Neurally evoked vasoconstriction likely represents a pathological phenomenon promoting a decrease, rather than an increase in blood flow to metabolically active brain tissue. Mulligan and MacVicar [88] were the first to report this phenomenon in brain slices using the neurotransmitter norepinephrine or the release of caged Ca^{2+} to increase Ca^{2+} levels in the astrocyte soma. These constrictions were abolished by blockers of Ca^{2+}-sensitive PLA_2 activity and the CYP4a-mediated metabolism of arachidonic acid to the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE). Both neurally mediated vasodilation and vasoconstriction have been observed in the retina [87]. In the retina, the balance between constriction and dilation was dependent upon nitric oxide (NO) levels, with 20-HETE synthesis contributing to constriction. Work by Girouard et al. [84] demonstrated that the level of astrocytic endfoot Ca^{2+} and endfoot BK channel activity dictate the polarity of the diameter changes caused by neuronal stimulation in cortical brain slices. These investigators observed that modest increases in endfoot Ca^{2+} (<500 nM) and endfoot BK channel activity lead to enhanced arteriolar K_{ir} activity, membrane potential hyperpolarization, and vasodilation. However, more robust elevations in endfoot Ca^{2+} (>500 nM) lead to sufficient BK channel-mediated K^+ efflux from endfeet causing arteriolar smooth muscle membrane potential depolarization and constriction. Further, modest elevation of bulk extracellular K^+ also caused inversion of neurovascular coupling from vasodilation to vasoconstriction. Thus, several factors including astrocyte endfoot Ca^{2+} levels, extracellular K^+ concentration and endfoot BK channel activity can influence the polarity and amplitude of the neurovascular response.

6. Inversion of Neurovascular Coupling from Vasodilation to Vasoconstriction after Subarachnoid Hemorrhage

To examine the impact of experimental SAH on neurovascular coupling, our laboratory has used a combination of multiphoton confocal imaging and infrared-differential interference contrast (IR-DIC) microscopy to simultaneously measure astrocytic endfoot Ca^{2+} and parenchymal arteriolar diameter in cortical brain slices from SAH model rats [21]. Neurovascular responses were evoked using electrical field stimulation (EFS) of neurons using parameters that did not directly affect astrocytes or parenchymal arterioles. In brain slices from control and sham-operated animals, neuronal activation caused the anticipated increase in astrocytic endfoot Ca^{2+} and vasodilation. This vasodilation was greatly diminished by paxilline, a BK channel blocker, consistent with involvement of endfoot BK channels [69, 83, 84]. In marked contrast, a similar level of neuronal activation and elevation in endfoot Ca^{2+} caused vasoconstriction rather

than vasodilation in brain slices from SAH model animals (Figure 1). This SAH-induced shift in neurovascular coupling from vasodilation to vasoconstriction likely represents a pathological response that could locally limit blood flow to cortical regions and was not due to increased 20-HETE or prostaglandin production. However, neurally evoked vaso-constriction after SAH was abolished by block of endfoot BK channels. Our evidence suggests the inversion of neurovascular coupling after SAH is due to increased basal endfoot BK channel activity and increased K^+ in the restricted perivascular space between astrocytic endfeet and parenchymal arteriolar smooth muscle. This abnormal elevation of basal perivascular K^+ combined with "normal" BK channel-mediated K^+ efflux stimulated by neuronal activity elevates K^+ above the dilation/constriction threshold, switching the polarity of arteriolar responses to vasoconstriction. Consistent with this interpretation, increasing concentrations of extracellular K^+ elicit a bimodal response in isolated parenchymal arterioles [21, 83, 84]. Modest increases in K^+ (<20 mM) induce smooth muscle hyperpolarization and arteriolar dilation through activation of K_{ir} channels expressed on arteriolar myocytes [89]. However, K^+ increases greater than ~20 mM cause a depolarizing shift in the K^+ equilibrium potential (E_K) sufficient to increase the activity of voltage-dependent Ca^{2+} channels leading to enhanced Ca^{2+} influx and vasoconstriction. Although the vascular responses are inverted after SAH, both neurovascular responses (i.e., vasodilation in control animals and vasoconstriction in SAH animals) involve the same mechanistic elements: elevated astrocytic endfoot Ca^{2+} and K^+ efflux mediated by endfoot BK channels with the polarity of the vascular response dictated by basal perivascular K^+ levels.

Our data also indicate fundamental changes in the resting activity of astrocyte Ca^{2+} signaling underlying SAH-induced elevation in basal perivascular $[K^+]$, leading to inversion of neurovascular coupling. In addition to responding to neurally released signals, astrocytes exhibit spontaneous Ca^{2+} oscillations [90]. These Ca^{2+} oscillations occur in both soma and endfeet and have been observed in isolated brain slices [90, 91] and in vivo [92–94]. This spontaneous activity occurs in the presence of Na^+ channel blocker tetrodotoxin to inhibit neuronal action potentials and represent intracellular Ca^{2+} release events from the endoplasmic reticulum [91]. An increase in the frequency of spontaneous astrocytic Ca^{2+} events in mouse models of Alzheimer's disease has been linked to vascular instability in vivo [94]. In brain slices from SAH model animals, we observed a marked increase in the amplitude of these events [21] (Figure 2). After SAH, the mean peak amplitude of spontaneous Ca^{2+} oscillations in astrocyte endfeet was ~490 nM compared to a mean peak amplitude ~320 nM in brain slices from control animals. In comparison, neurally-evoked increases in astrocytic Ca^{2+} were ~350 nM in both control and SAH animals. Considering that EFS-induced increases in astrocytic endfeet Ca^{2+} have been shown to induce K^+ efflux through endfoot BK channels, spontaneous Ca^{2+} events are also likely capable of activating endfoot BK channels. Based on these observations, it is conceivable that higher amplitude spontaneous Ca^{2+} events following

SAH enhance BK channel activity contributing to increased basal K^+ in restricted perivascular space (Figure 3). Factors leading to higher amplitude spontaneous Ca^{2+} events after SAH are not currently known; however, determining their identity will provide valuable new information in the search for finding new therapeutic strategies to help SAH patients.

7. Impact of Global Ischemia on Neurovascular Coupling

Global cerebral ischemia represents a generalized reduction in brain blood flow caused by, for example, cardiac arrest, shock, asphyxia, and strokes including SAH. The impact of global ischemia on brain function can range from relatively mild and temporary cognitive impairment to brain death, depending on the severity and length of the ischemic insult. Multiple mechanisms have been implicated in neuronal injury caused by global cerebral ischemia including neurotransmitter (e.g., glutamate) toxicity, cortical spreading depression, inflammation, and apoptosis [95]. Emerging evidence indicates that global ischemia may also influence neurovascular coupling. In rats, moderate, temporary forebrain ischemia can be achieved by a combination of bilateral carotid artery occlusion and controlled hypotension via the withdrawal of blood. Using this approach, Zhou et al. [96] examined the impact of 15 minutes of ischemia and reperfusion on the ability of whisker stimulation to increase relative cerebral blood flow (rCBF) to the somatosensory cortex using laser speckle imaging. Prior to the ischemic insult, rCBF to the somatosensory cortex increased ~10% in response to whisker stimulation. Following ischemia and 20 minutes of reperfusion, increased rCBF to whisker stimulation was slightly diminished and response time increased; responses returned to preischemic levels within two hours. Recently, Baker et al. examined varying levels of global forebrain ischemia on the ability of forepaw stimulation to increase cerebral blood flow in the somatosensory cortex of rats [97]. Neurovascular coupling was attenuated with increasing levels of ischemia, with severe ischemia (60% reduction in global cerebral blood flow) causing greater than a 90% reduction in the neurovascular response. The attenuation of neurovascular coupling associated with severe global ischemia lasts for several days following reperfusion [98]. Currently, little information is available regarding the cellular mechanisms contributing to decreased neurovascular coupling associated with global ischemia. However, it is likely that ischemia may impact more than one component of the neurovascular unit. For example, ischemia has been shown to impair cerebral artery function that may limit vasodilation [99, 100]. Further, global ischemia has been shown to alter expression of K^+-selective ion channels and TRPV4 nonselective cation channels in astrocytes from rat hippocampus [101, 102].

Global cerebral ischemia may also contribute to brain pathologies associated with SAH. Immediately following cerebral aneurysm rupture, increased intracranial pressure caused by the release of blood into the subarachnoid space can lead to transient global ischemia and contribute to a cascade of events referred to as "early brain injury" [6, 10].
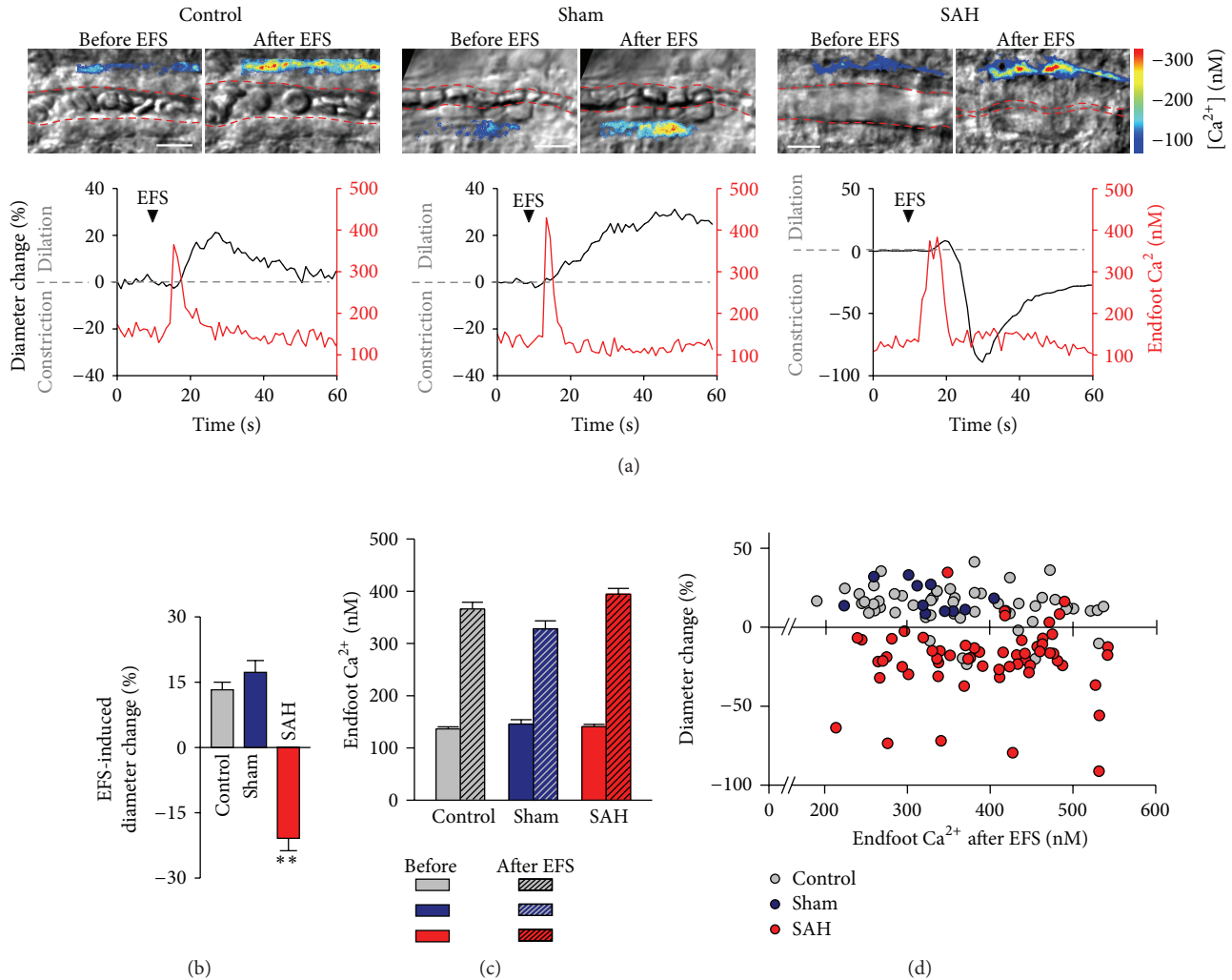
FIGURE 1: Inversion of neurovascular coupling in cortical brain slices from SAH model animals. (a) (Upper) Infrared-differential interference contrast images from brain slices of control, sham-operated, and SAH model rats before/after electrical field stimulation (EFS). Parenchymal arterioles were preconstricted with U46619 (100 nM). Dashed lines in red display the intraluminal diameter of parenchymal arterioles. Overlapping pseudocolor-mapped Ca^{2+} levels in astrocyte endfeet were obtained by simultaneous imaging using the fluorescent Ca^{2+} indicator fluo-4 and two-photon microscopy. Scale bar: 10 μm. (Lower) Simultaneous recordings of EFS-induced changes in diameter and estimated endfoot Ca^{2+} concentrations obtained from brain slices depicted in upper images. (b)–(d) Summary data of EFS-evoked changes in arteriolar diameter and astrocytic endfoot Ca^{2+} obtained from control ($n = 53$), sham-operated ($n = 11$), and SAH model ($n = 59$) animals. Diameter changes were expressed as percentage of the diameter in the same point before EFS as 100%. $^{**}P < 0.01$ by one-way ANOVA followed by host hoc comparison of means using the Tukey test (modified from Koide et al. [21]).

Further, delayed blood-induced vasospasm of brain surface conduit arteries [103] and enhanced constriction of resistance-size cerebral arteries and arterioles [18, 104, 105] may also reduce blood flow to ischemic levels, contributing to the development of delayed ischemic neuronal deficits. Data presented above indicate that both SAH and global ischemia can lead to decreased neurovascular coupling. However, a marked difference regarding the influence of SAH and global ischemia on neurovascular coupling is apparent; SAH causes inversion of the neurovascular response from vasodilation to vasoconstriction whereas global ischemia causes a decrease in the magnitude of the dilation to evoked neuronal activity.

8. Conclusions

Subarachnoid hemorrhage is a multifaceted pathology exhibiting both acute and long-term injury to the brain. It is now clear that SAH profoundly impacts neuronal influences on the vasculature leading to decreased cerebral blood flow that can exacerbate the extent of brain damage. One type of SAH-induced impaired neurovascular signaling arises in the context of SD that can impact large areas of cortical and subcortical grey matter. In the absence of SAH, SD is most frequently associated with a hyperemic response, that is, an increase in cerebral blood flow. However, SAH causes an

(a)

(b)

(c)
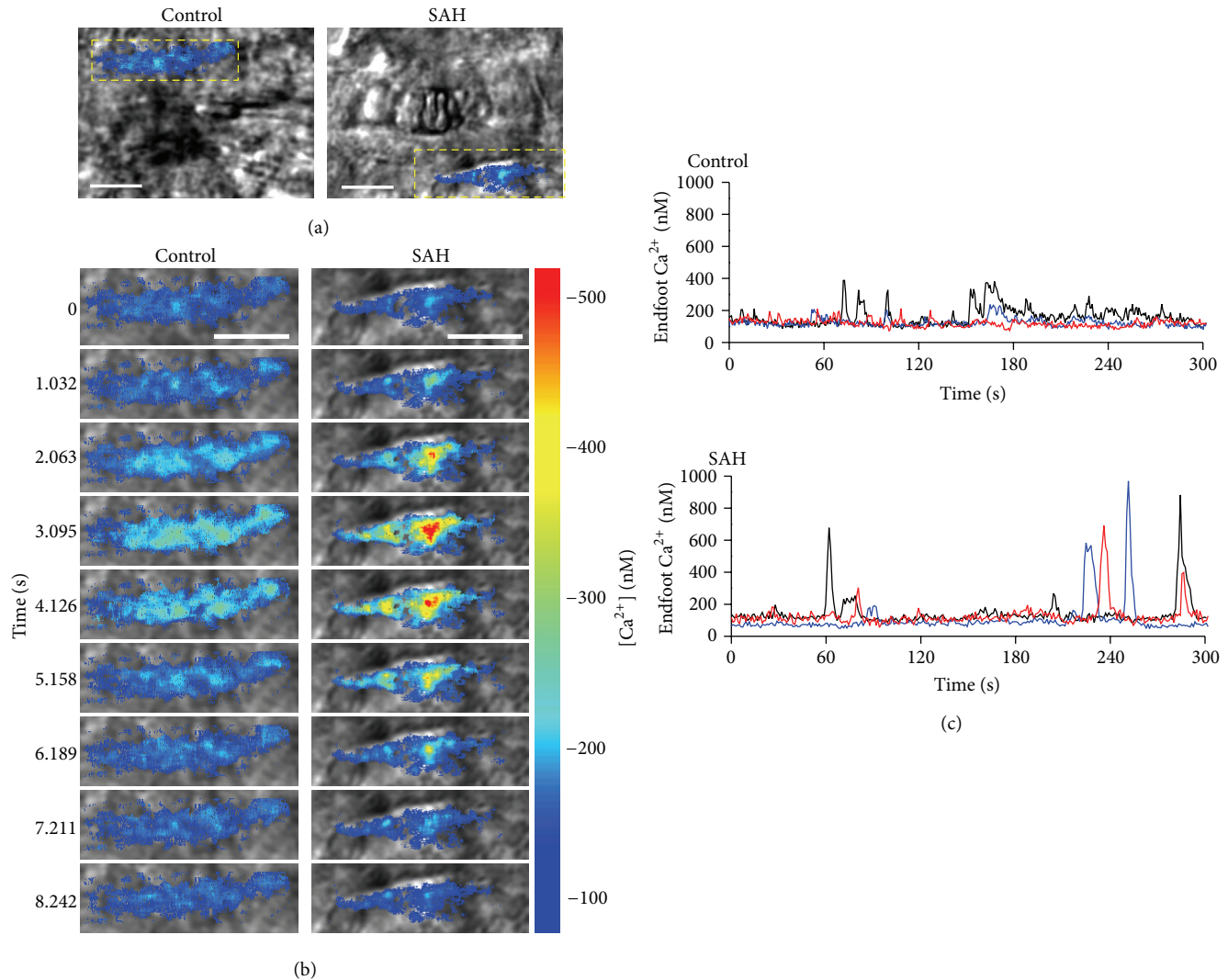
FIGURE 2: Increased amplitude of spontaneous Ca^{2+} oscillations in astrocyte endfeet following SAH. (a)-(b) Representative images of spontaneous Ca^{2+} oscillation in astrocyte endfeet in brain slices from control and SAH model animals. (b) Time laps images from the area within the yellow dotted box in Figure 2(a). Scale bar: 10 μm. (c) Spontaneous Ca^{2+} oscillations in a brain slice from control (upper) and SAH model (lower) animals. Traces were obtained from 1.2×1.2-μm regions of interest placed on distinct astrocyte endfeet in 5 min recordings without stimulation (modified from Koide et al. [21]).

inversion of the SD-induced neurovascular response leading to vasoconstriction and decreased blood flow to tissue during a time of high metabolic demand. Recently, it has also been shown that SAH can cause inversion of neurovascular coupling at the level of the individual neurovascular unit. Physiologically, coordinated activity of neurons, astrocytes, and parenchymal arterioles ensures increase local blood flow to active neurons in specific regions of the brain engaged in task-dependent processes. After SAH the neurovascular response to neuronal activation switches from vasodilation to vasoconstriction; this also promotes a pathological decrease in the flow of oxygen and nutrients to metabolically active neurons. Evidence suggests that elevated perivascular K^+ due to the enhanced amplitude of spontaneous Ca^{2+} signaling events in astrocytic endfeet may underlie this inversion of neurovascular coupling, consistent with a bimodal effect of extracellular K^+ to cause vasodilation at concentrations below 20 mM and constriction when this threshold of 20 mM is exceeded. Presently mechanisms associated with inversion of the neurovascular response caused by SAH-induced SD have not completely been resolved. However, inversion of SD-induced neurovascular response likely reflects a combination of increased extracellular K^+ and the impact of SAH on the relative balance of vasoconstrictor and vasodilator influences. Development of agents and approaches to prevent SAH-induced inversion of neurovascular coupling may provide a much needed additional therapeutic option for SAH patients.

Acknowledgments
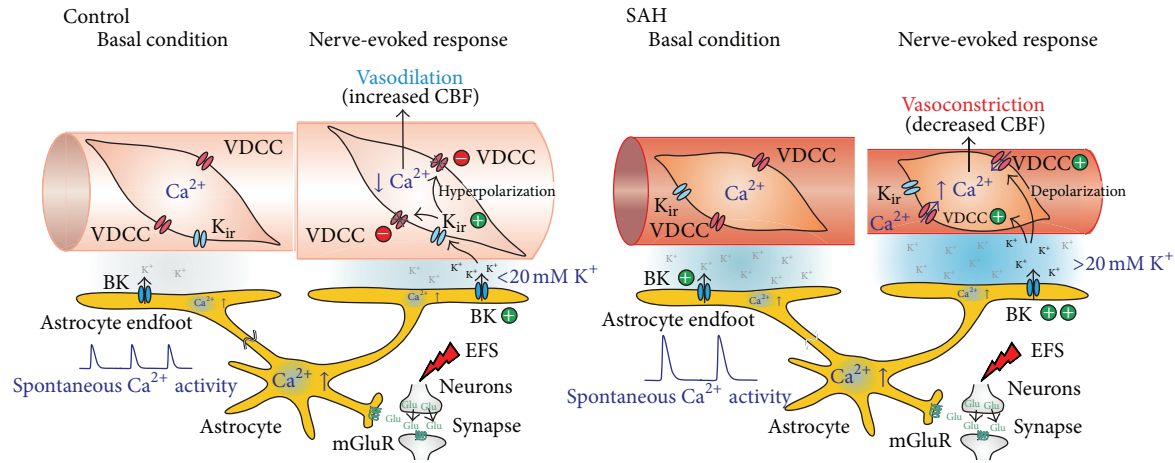
FIGURE 3: Schematic model liking SAH to inversion of neurovascular coupling. In control animals, EFS causes elevated cytoplasmic Ca^{2+} in astrocytes leading to increased BK channel activity and modest (<20 mM) increases in perivascular K^+, promoting vasodilation. SAH increases the magnitude of spontaneous astrocytic Ca^{2+} oscillations and basal activity of BK channels, elevating K^+ in restricted perivascular space. The summation of increased basal perivascular K^+ and "normal" nerve-evoked astrocyte BK channel activity results in extracellular K^+ concentrations that exceed the dilation-constriction threshold (~20 mM), inducing vasoconstriction. BK: large conductance Ca^{2+}-activated K^+ channel, CBF: cerebral blood flow, EFS: electrical field stimulation, Glu: glutamate, K_{ir}: inward rectifier K^+ channel, mGluR: metabotropic glutamate receptor, VDCC: voltage-dependent Ca^{2+} channel (modified from Koide et al. [21]).

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Review Article

Clinical Trials in Cardiac Arrest and Subarachnoid Hemorrhage: Lessons from the Past and Ideas for the Future

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Introduction. Elevated intracranial pressure that occurs at the time of cerebral aneurysm rupture can lead to inadequate cerebral blood flow, which may mimic the brain injury cascade that occurs after cardiac arrest. Insights from clinical trials in cardiac arrest may provide direction for future early brain injury research after subarachnoid hemorrhage (SAH). *Methods*. A search of PubMed from 1980 to 2012 and clinicaltrials.gov was conducted to identify published and ongoing randomized clinical trials in aneurysmal SAH and cardiac arrest patients. Only English, adult, human studies with primary or secondary mortality or neurological outcomes were included. *Results*. A total of 142 trials (82 SAH, 60 cardiac arrest) met the review criteria (103 published, 39 ongoing). The majority of both published and ongoing SAH trials focus on delayed secondary insults after SAH (70%), while 100% of cardiac arrest trials tested interventions within the first few hours of ictus. No SAH trials addressing treatment of early brain injury were identified. Twenty-nine percent of SAH and 13% of cardiac arrest trials showed outcome benefit, though there is no overlap mechanistically. *Conclusions*. Clinical trials in SAH assessing acute brain injury are warranted and successful interventions identified by the cardiac arrest literature may be reasonable targets of the study.

1. Introduction

For decades, research efforts in subarachnoid hemorrhage (SAH) have focused on vasospasm and delayed ischemic neurological deficits. However, brain injury at the time of aneurysm rupture is a significant predictor of functional outcome. Indeed, poor admission neurological status (Hunt-Hess or World Federation of Neurological Surgeons Score), which reflects acute brain injury, is a larger contributor to death or severe disability than delayed cerebral ischemia [1, 2]. However, the mechanism of early brain injury after aneurysm rupture remains elusive and no current therapies are available.

One possible mechanism of acute injury was described in a small case series of 6 patients with observed recurrent aneurysm rupture either during transcranial Doppler (TCD) or during craniotomy with open skull but intact dura. The investigators report a spike in intracranial pressure (ICP) that developed over 1 minute and then declined over several minutes. This abrupt increase in ICP approached levels near mean arterial pressure and led to a concomitant drop in cerebral

blood flow resulting in circulatory arrest, as documented by TCD [61]. This study examined aneurysm rebleeding and does not provide direct evidence that intracranial circulatory arrest occurs with de novo aneurysm rupture. However, inadequate cerebral blood flow is frequently evidenced clinically by the transient loss of consciousness that occurs at SAH ictus. This mechanism of global transient circulatory arrest has been described in animal models of SAH at the time of initial hemorrhage [62, 63] and mimics the anoxic/hypoxic ischemic mechanism incurred by cardiac arrest.

In this paper, published and ongoing clinical trials in cardiac arrest are compared to those in aneurysmal SAH to identify overlapping or complementary approaches to treatment as well as new avenues for potential research.

2. Methods

A search of PubMed was conducted in 11/2012 to identify randomized, controlled trials of aneurysmal SAH and cardiac arrest. Only human studies of adults (≥18 years of age), which

TABLE 1: Randomized controlled trials assessing neurologic outcomes after aneursymal Subarachnoid hemorrhage—completed trials.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
			Calcium channel blockers—nimodipine			
Cerebral Arterial Spasm—a controlled Trial of Nimodipine in Patients with Subarachnoid Hemorrhage	Randomized, placebo-controlled, double-blind, multicenter prospective study of Hunt Hess grade I-II SAH patients	Nimodipine 0.7 mg/kg PO bolus, then 0.35 mg/kg q 4 × 21 days. Starting within 96h of SAH ($N = 58$)	Placebo ($N = 63$)	Primary outcome: neurological deficit from arterial spasm and severity of neurologic deficit at 21 days	Nimodipine significantly reduced death or severe deficits from spasm at 21 days (2% versus 13% with placebo, $P = 0.03$)	Allen et al., NEJM 1983 [3]
Nimodipine treatment in poor-grade aneurysm patients. Results of a multicenter double-blind placebo-controlled trial	Randomized, multicenter, double-blind, placebo-controlled trial	Nimodipine 90 mg PO q 4 h × 21 d ($N = 91$)	Placebo ($N = 97$)	Primary outcome: 3-month GOS Secondary outcomes: delayed ischemic deficits, angiographic vasospasm	Better 3-month GOS in treatment group (29% versus 9% of treatment group, $P < 0.001$). Significantly less delayed cerebral ischemia in treatment group, no difference in angiographic vasospasm	Petruk et al., J Neurosurg 1988 [4]
Controlled study of nimodipine in aneurysm patients treated early after subarachnoid hemorrhage	Randomized, double-blind, placebo-controlled trial of all Hunt-Hess grades within 96 hours of SAH	Nimodipine 60 mg q 4 h PO × 21 days + Nimodipine 200 mcg IV intraoperatively into basal cistern ($N = 38$)	Placebo ($N = 37$)	Primary outcome: mortality, cerebral blood flow measured by Xenon CT Secondary outcomes: 3-month intellectual or neurological deficit	Mortality was lower in the nimodipine group (4% versus 24% with placebo, $P < 0.05$). Nimodipine did not significantly increase cerebral blood flow	Mee et al., Neurosurgery 1988 [5]
Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid hemorrhage: British aneurysm nimodipine trial	Randomized, double-blind, placebo-controlled, multicenter trial within 96h of SAH	Nimodipine 60 mg q 4 PO × 21 d ($N = 276$)	Placebo ($N = 278$)	Primary outcome: 3-month cerebral infarction Secondary outcome: 3-month GOS	Significantly less cerebral infarction in the nimodipine group (22% compared to 33% in placebo, $P = 0.014$). Poor GOS outcomes significantly reduced in nimodipine group at 3-months	Pickard et al., BMJ 1989 [6]
Early aneurysm surgery and preventive Therapy with intravenously administered nimodipine: A multicenter, double-blind, dose-comparison study	Randomized, double-blind, dose-comparison, multicenter study	Nimodipine 2 mg/h IV for 9–15 days ($N = 101$)	Nimodipine 3 mg/h IV for 9–15 days ($N = 103$)	Primary outcome: delayed neurological deficits, adverse drug reactions	No difference in delayed neurological deficits between the two groups	Gilsbach et al., Neurosurgery 1990 [7]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Long-term effects of nimodipine on cerebral infarcts and outcome after aneurysmal subarachnoid hemorrhage and surgery	Randomized, double-blind, placebo-controlled of Hunt-Hess I–III SAH patients	Nimodipine IV 0.5 mcg/kg/min × 7–10 days followed by 60 mg q 4 h PO × 21 days total (N = 104)	Placebo (N = 109)	Primary outcome: delayed ischemic deterioration and CT infarcts Secondary outcomes: GOS at 1–3 years	Significantly fewer deaths caused by delayed cerebral ischemia in nimodipine group (P = 0.01) and fewer cerebral infarcts on CT (P = 0.05). No differences in 1–3 year GOS or CT scan	Ohman et al., J Neurosurg 1991 [8]
A randomized outcome study of enteral versus intravenous nimodipine in 171 patients after acute aneurysmal subarachnoid hemorrhage	Randomized, single-center study	Nimodipine 2 mg/h IV × 10 days then changed to PO × 6 d (N = 87)	Nimodipine 60 mg PO q 4 × 16 days (N = 84)	Primary outcome: delayed ischemic neurological deficit Secondary outcomes: 12 month GOS, mRS, Karnofsky, MRI infarcts, HRQoL	No difference in delayed ischemic neurological deficits (20% in enteral versus 16% in IV group, P = 0.61), no difference in 12-month clinical outcomes	Soppi et al., World Neurosurgery 2012 [9]
Calcium channel blockers—nicardipine						
A randomized controlled trial of high-dose intravenous nicardipine in aneurysmal subarachnoid hemorrhage. A report of the cooperative aneurysm study	Randomized, double-blind, placebo-controlled, multicenter study	Nicardipine IV 0.15 mg/kg/h (N = 449)	Placebo (N = 457)	Primary outcome: 3-month GOS Secondary outcomes: angiographic vasospasm, TCD vasospasm, mortality, disability from vasospasm, symptomatic vasospasm, CT infarction, NIHSS	No difference in 3-month GOS. Less symptomatic vasospasm in treatment group (32% versus 46% in placebo group, P < 0.001) and less angiographic vasospasm in treatment group (33% versus 51% of placebo, P < 0.01) and less TCD vasospasm (23% versus 49% of placebo, P < 0.001)	Haley et al., J Neurosurg 1993 [10, 11]
A randomized trial of two doses of nicardipine in aneurysmal subarachnoid hemorrhage. A report of the cooperative aneurysm study	Randomized, double-blind, multicenter study	Nicardipine IV 0.15 mg/kg/h × 14 days (N = 184)	Nicardipine IV 0.075 mg/kg/h × 14 days (N = 181)	Primary outcome: symptomatic vasospasm, adverse drug events Secondary outcomes: 3-month GOS and NIHSS, mortality, disability due to vasospasm, CT infarction	No difference in symptomatic vasospasm or 3-month outcome. More adverse effects in high-dose nicardipine group	Haley et al., J Neurosurg 1994 [12]
Effect of nicardipine prolonged-release implants on cerebral vasospasm and clinical outcome after severe aneurysmal subarachnoid hemorrhage. A prospective, randomized, double-blind phase IIa Study	Randomized, prospective double-blind phase IIa study in clipped SAH patients	Nicardipine prolonged-release implants (10 × 4 mg prolonged release rod shaped polymers) placed in basal cisterns (N = 16)	Control-basal cisterns opened and washed out (N = 16)	Primary outcome: angiographic vasospasm Secondary outcome: delayed ischemic lesion on HCT, 1-year mRS and NIHSS	Angiographic vasospasm significantly reduced in treatment group (7% versus 73% in controls, P < 0.05). No significant difference in CT infarct. Decreased mortality in treatment group (6% versus 38% in control group, P = 0.042) and better 1-year mRS and NIHSS (P = 0.0001)	Barth et al., Stroke 2007 [13]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
			Antifibrinolytics			
Antifibrinolysis with tranexamic acid in aneurysmal subarachnoid hemorrhage: A consecutive controlled clinical trial	Randomized, placebo-controlled, study	Tranexamic acid 1 g q 4 h IV × 1 week then 1 g q 6 h IV × 1 week then 1.5 g q 6 h PO × 1 week (N = 30)	Placebo (N = 29)	Primary outcome: recurrent hemorrhage diagnosed by LP, HCT, echoencephalogram or autopsy. Secondary outcome: angiographic vasospasm, delayed cerebral ischemia, death	Tranexamic acid protected against rebleeding during the first 2 weeks of treatment but also resulted in cerebral ischemic complications	Fodstad et al., Neurosurgery 1981 [14]
Comparative clinical trial of epsilon amino-caproic acid and tranexamic acid in the prevention of early recurrence of subarachnoid hemorrhage	Randomized trial	Epsilon amino-caproic acid 6 g q 6 h IV continued until surgery or discharge (N = 90)	Tranexamic acid 1 g q 6 h IV continued until surgery or discharge (N = 61)	Primary outcome: recurrent hemorrhage diagnosed clinically by HCT, LP, or autopsy Secondary outcome: delayed ischemic deficit diagnosed by clinical deterioration, angiographic vasospasm, and infarct on HCT	Rebleeding occurred in 8% of aminocaproic-acid-treated patients and 10% of tranexamic acid treated patients. Delayed ischemic deficits occurred in 7% of aminocaproic acid patients and 5% of tranexamic acid patients. Mortality was 11% in each group. P = NS for all outcomes	Chowdhary and Sayed, JNNP 1981 [15]
Antifibrinolytic treatment in subarachnoid hemorrhage	Randomized, double-blind, placebo-controlled, multicenter study	Tranexamic acid 1 g q 4 h IV × 1 week then 1 g q 6 h IV × 3 weeks (N = 241)	Placebo (N = 238)	Primary outcome: 3-month GOS Secondary outcome: neurological deterioration, rebleeding, infarction, hydrocephalus, edema, epilepsy	Rebleeding reduced from 24% in control group to 9% in treatment group (P < 0.001), but with concurrent increase in ischemic complications (24% in treatment group versus 15% in placebo, P < 0.01). No difference in 3 month GOS	Vermeulen et al., NEJM 1984 [16]
Antifibrinolytic treatment in subarachnoid hemorrhage: a randomized placebo-controlled trial (STAR)	Prospective, double-blind, placebo-controlled, multicenter, randomized trial within 96 hours of SAH onset in whom aneurysm repair was delayed beyond 48 hours	Tranexamic acid 1 g IV q 4 h × 1 week then 1.5 g PO q 6 h × 2 weeks (N = 229)	Placebo (N = 233)	Primary outcome: 3-month GOS Secondary outcomes: rebleeding, delayed cerebral ischemia, hydrocephalus, postoperative ischemia	No difference in 3-month GOS. Significant decrease in rebleeding from 33% in placebo group to 19% in treatment group. No difference in delayed cerebral ischemia, hydrocephalus, or postoperative ischemia	Roos, Neurology 2000 [17]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Immediate administration of tranexamic acid and reduced incidence of early rebleeding after aneurysmal subarachnoid hemorrhage: a prospective randomized study	Randomized, placebo-controlled trial	Tranexamic acid 1 g IV bolus, then 1 g IV q 6 hours until aneurysm repair or 72 hours post ictus. ($N = 254$)	Placebo ($N = 251$)	Primary outcome: Rebleeding by HCT Secondary outcome: 6-month GOS, clinical vasospasm/delayed ischemic neurological deficit, TCD spasm	Treatment group had reduced rebleeding rate of 2.4% compared to 10.8% in the placebo group ($P < 0.01$). More favorable outcome in the treatment group (74.8% compared to 70.5% in the control group, P = NS). No increased risk of ischemia	Hillman et al., J Neurosurg 2002 [18]
Neuroprotectives drugs						
A double-blind clinical evaluation of the effect of nizofenone on delayed ischemic neurological deficits following aneurysmal rupture	Randomized, placebo controlled trail	Nizofenone for 5–10 days ($N = 42$)	Placebo ($N = 48$)	Primary outcome: delayed ischemic neurological deficits with angiographically confirmed vasospasm. Secondary outcomes: one-month disability index, motor, and speech function	No difference in delayed ischemic events between treatment groups. Among patients with vasospasm, those who received nizofenone had better one-month functional outcomes ($P < 0.05$)	Saito et al., Neurol Res 1983 [19]
Nizofenone administration in the acute stage following subarachnoid hemorrhage. Results of a multicenter controlled double-blind clinical study	Randomized, double-blind, placebo-controlled, multicenter study of Hunt Hess grade I–IV	Nizofenone 5 mg × 2 weeks ($N = 102$)	Placebo ($N = 106$)	Primary outcome: neurological exam at 1-month and discharge	Significantly improved one-month or discharge functional outcome in treatment group compared to placebo ($P < 0.05$). No difference in mortality	Ohta et al., J Neurosurg 1986 [20]
Effect of a free radical scavenger, edaravone, in the treatment of patients with aneurysmal subarachnoid hemorrhage	Randomized, controlled, single-center study	Edaravone 30 mg IV BID × 14 days ($N = 49$)	Control (usual treatment) ($N = 42$)	Primary outcome: delayed ischemic neurological deficits Secondary outcomes: cerebral infarction due to vasospasm, 3-month GOS	No difference in delayed ischemic neurological deficits between treatment and control groups. Less cerebral infarction in treatment group (0% versus 66%, $P = 0.028$). Poor outcome caused by vasospasm 0% in treatment group and 71% in control group ($P = 0.046$)	Munakata et al., Neurosurgery 2009 [21]
Eicosapentaenoic Acid Cerebral Vasospasm Therapy Study (EVAS)	Randomized, controlled, open label, multicenter, efficacy study of surgically clipped SAH patients	Eicosapentaenoic acid (omega 3 fatty acid) 900 mg TID × 30 days ($N = 81$)	Control (usual treatment) ($N = 81$)	Primary outcome: symptomatic vasospasm or infarct on HCT Secondary outcome: 1-month GOS	Symptomatic vasospasm occurred significantly less in the treatment group (15% versus 30% in controls, $P = 0.022$) as did infarction from vasospasm (7% versus 21% in controls, $P = 0.012$)	Yoneda et al., World Neurosurg 2012 [22]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Safety and efficacy of NA-1 in patients with iatrogenic stroke after endovascular aneurysm repair (ENACT): a phase 2, randomized, double-blind, placebo-controlled trial	Randomized, double-blind, placebo-controlled study of ruptured (WFNS 1–3) and unruptured aneurysms undergoing endovascular repair	NA-1 2.6 mg/kg infusion over 10 minutes ($N = 92$)	Placebo ($N = 93$)	Primary outcome: safety, number and volume of ischemic strokes on MRI DWI and FLAIR 12–96 hours after infusion Secondary outcome: 30-day mRS, NIHSS, neurocognitive outcome	No difference in MRI lesion volume, but fewer ischemic lesions in NA-1 group compared to placebo ($P = 0.012$). In the SAH subgroup (20% of cohort) their MRI number and ischemic volume was significantly less in the treatment group. No difference in 30 day NIHSS or mRS between groups	Hill et al., Lancet Neurol 2012 [23]

Statins

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Simvastatin reduces vasospasm After aneurysmal subarachnoid hemorrhage: results of a pilot randomized clinical trial	Randomized, placebo-controlled pilot trial	Simvastatin 80 mg qd for 14 days ($N = 19$)	Placebo ($N = 20$)	Primary outcome: delayed ischemic neurological deficit confirmed by TCD or angiography. Secondary outcomes: liver transaminases, CK, von Willebrand factor, S100	Vasospasm occurred in 26% of treatment group compared to 60% of placebo group ($P < 0.05$). No differences in transaminitis or myositis. VWF and S100 were significantly lower in the treatment group ($P < 0.05$)	Lynch et al., Stroke 2005 [24]
Effects of acute treatment with pravastatin on cerebral vasospasm, autoregulation, and delayed Ischemic deficits after aneurysmal subarachnoid hemorrhage. A phase II randomized placebo-controlled trial	Randomized placebo-controlled, phase II Trial	Pravastatin 40 mg PO qd × 14 d ($N = 40$)	Placebo ($N = 40$)	Primary outcome: incidence, severity, and duration of vasospasm on TCD, duration of impaired autoregulation measured by transient hyperemic response on TCD Secondary outcome: vasospasm-related delayed ischemic deficits, disability at discharge	TCD vasospasm and severe vasospasm were reduced in the treatment group ($P = 0.006$ and $P = 0.044$, resp.). Duration of impaired autoregulation shortened in treatment group ($P < 0.01$). Vasospasm-related delayed ischemic deficits was reduced ($P < 0.001$) and mortality was reduced ($P = 0.037$)	Tseng et al., Stroke 2005 [25]
A randomized, double-blind, placebo-controlled pilot study of simvastatin in aneurysmal subarachnoid hemorrhage	Randomized, double-blind, placebo-controlled pilot study	Simvastatin 80 mg qd in statin naïve Fisher 3 SAH until discharge or 21 days ($N = 19$)	Placebo ($N = 20$)	Primary outcome: death and drug morbidity (elevated CK, transaminases) Secondary outcomes: TCD, angiographic or clinical vasospasm, vasospasm-related infarcts, clinical outcomes at discharge, cardiac, and infectious morbidities	Mortality in 0% treatment group and 15% placebo group. Angiographically confirmed vasospasm in 26% treatment group and 25% placebo group. Vasospasm infarcts in 11% treatment group and 25% placebo group. All differences $P = $ NS	Chou et al., Stroke 2008 [26]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Biological effects of simvastatin in patients with aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled randomized trial	Double-blind, placebo-controlled randomized trial	Simvastatin 80 mg PO × 15 days ($N = 16$)	Placebo ($N = 16$)	Primary outcome: effect of simvastatin on laboratory parameters of endothelial function, fibrinolysis, coagulation, inflammation and cholesterol Secondary outcomes: TCD vasospasm, clinical signs of DCI, 3- and 6-month GOS	Simvastatin group had significantly lower total cholesterol and LDL, but no differences in coagulation, fibrinolysis, endothelium function, or inflammation. No differences in TCD vasospasm, clinical DCI, or poor outcome	Vergouwen et al., J Cereb Blood Flow Metab 2009 [27]
			Aneurysm repair			
Timing of operation for ruptured supratentorial aneurysms: a prospective randomized study	Randomized, prospective study of Hunt Hess grade I–III SAH patients	Acute surgery (day 0–3 after SAH) ($N = 71$)	Intermediate surgery (day 4–7 after SAH) ($N = 70$) Late surgery (day 8 or later after SAH) ($N = 70$)	Primary outcome: 3-month dead, dependent or independent. Secondary outcomes: Neurological deficit from direct effect of initial bleed, complication of surgery, confirmed rebleeding, delayed ischemic deterioration, hydrocephalus, extracranial complications	Acute surgery patients were more often independent at 3-months (92% versus 79% in intermediate timing and 80% in the late timing group, $P <$ 0.01). Mortality was 6% in the early surgery group versus 13% in the late surgery group ($P =$ NS)	Ohman and Heiskanen, J Neurosurg 1989 [28]
International subarachnoid aneurysm trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomized trial	Randomized, unblinded trial of SAH patients with an aneurysm judged technically suitable for either clipping or coiling and clinical equipoise	Surgical clipping ($N = 1070$)	Endovascular treatment by detachable platinum coils ($N = 1073$)	Primary outcome: 1-year mRS 3–6 versus 1-2 Secondary outcomes: rebleeding, quality of life at 1 year (euroQol), frequency of epilepsy, cost effectiveness, neuropsychological outcomes	Dependent or dead at 1 year: 23.7% endovascular versus 30.6% clipping ($P = 0.0019$).	Molyneux et al., ISAT Collaborative Group, Lancet 2002 [29]
International subarachnoid aneurysm trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomized comparison of effects on survival, dependency, seizures, rebleeding, subgroups, and aneurysm occlusion	Randomized, unblinded trial of SAH patients with an aneurysm judged technically suitable for either clipping or coiling and clinical equipoise	Surgical clipping ($N = 1070$)	Endovascular treatment by detachable platinum coils ($N = 1073$)	Primary outcome: 1-year mRS 3–6 versus 1-2 Secondary outcomes: rebleeding, quality of life at 1-year (Euroqol), frequency of epilepsy, cost effectiveness, neuropsychological outcomes	Dead or dependent at 1-year: 23.5% of endovascular group versus 30.9% of clipping group. ARR 7.4%. Early survival advantage of coiling maintained up to 7 years ($P =$ 0.03). Lower risk of epilepsy in coiled group but higher late rebleeding risk in coiled group	Molyneux et al., Lancet 2005 [30]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
The barrow ruptured aneurysm trial	Randomized, open-label, prospective, single-center study	Surgical clipping (N = 238)	Endovascular coiling (N = 233)	Primary outcome: 1-year mRS > 2	Poor outcome in 33.7% of clipped and 23.2% of coiled patients (P = 0.02)	McDougall et al., J Neurosurg 2012 [31]

Lipid peroxidation inhibitor

Randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand	Double-blind, randomized, vehicle-controlled study in men and women with aneurysmal SAH	Tirilazad 0.6 mg/kg/ (N = 257) Tirilazad 2 mg/kg/d (N = 249) Tirilazad 6 mg/kg/d (N = 256) up to 11 days	Placebo containing citrate vehicle (N = 253)	Primary outcome: Symptomatic vasospasm Secondary outcome: 3-month GOS, NIHSS, infarct volume on head CT	The subgroup 6 mg/kg treatment arm had reduced mortality (P = NS) and better 3-month GOS (P = NS) compared to placebo. Less symptomatic vasospasm in 6 mg/kg group, but not significant. Men showed more benefit than women. No significant improvement with lower dosing groups	Kassell et al., J Neurosurg 1996 [32]
A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in North America	Double-blind, randomized, vehicle-controlled study in men and women with aneurysmal SAH	Tirilazad 2 mg/kg/d (N = 298) Tirilazad 6 mg/kg/d (N = 299) up to 11 days	Placebo containing citrate vehicle (N = 300)	Primary outcome: mortality at 76 days Secondary outcome: 3-month GOS and NIHSS, infarct volume on head CT symptomatic vasospasm, incidence, and severity of angiographic vasospasm	No difference in mortality, favorable GOS outcome, or employment between groups. No differences in symptomatic or angiographic vasospasm	Haley et al., J Neurosurg 1997 [33]
Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part I a cooperative study in Europe, Australia, New Zealand, and South Africa	Double-blind, randomized, vehicle-controlled study in women with aneurysmal SAH	Tirilazad mesylate 15 mg/kg/d IV hours for 11 days (N = 405)	Placebo containing citrate vehicle (N = 414)	Primary outcome: 91-day mortality Secondary outcome: 3-month GOS, use of clinical vasospasm, use of hypervolemic hypertensive therapy, neurological worsening from vasospasm, cerebral infarction, use of angioplasty, safety endpoints	Mortality rates and 3-month GOS not different between groups. Lower symptomatic vasospasm in tirilazad group (24.8% versus 33.7% in placebo group, P = 0.005). Cerebral infarction 8% in treatment group versus 13% in placebo group (P < 0.04)	Lanzino et al., J Neurosurg 1999 [34]
Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part II a cooperative study in North America	Double-blind, randomized, vehicle-controlled study in women with aneurysmal SAH	Tirilazad mesylate 15 mg/kg/d IV up to 11 days (N = 410)	Placebo containing citrate vehicle (N = 413)	Primary outcome: mortality at 91 days in WFNS grade IV-V patients Secondary outcomes: 3 month GOS or clinical vasospasm 1–14 days from dosing, use of hypervolemic hypertensive therapy, neurological worsening from vasospasm, cerebral infarction, use of angioplasty, safety endpoints	No differences in mortality when analyzing the entire population. No difference in GOS, symptomatic vasospasm, vasospasm severity. In WFNS grades IV-V, lower mortality in treatment group (24.6% versus 43.4% in placebo, P = 0.016). In WFNS I–III improved GOS in placebo group (83.3% versus 76.7% in treatment group, P = 0.04)	Lanzino and Kassell, J Neurosurg 1999 [35]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Thrombolytics						
Prevention of delayed ischemic deficits after aneurysmal subarachnoid hemorrhage by intrathecal bolus injection of tissue plasminogen activator (rTPA)	Prospective, controlled trial of Fisher III clipped SAH patients	rTPA 10 mg IV intracisternal immediately following aneurysm clipping ± 5–10 mg IV TPA intraventricularly in patients with IVH (N = 52)	No TPA instillation (N = 68)	Primary outcome: clinical delayed ischemic deficits attributed to vasospasm Secondary outcome: 3-month GOS	Significantly less transient and permanent delayed ischemic deficits and better GOS in rTPA group	Seifert et al., Acta Neurochir 1994 [36]
A randomized trial of intraoperative, intracisternal tissue plasminogen activator for the prevention of vasospasm	Randomized, double-blinded, placebo-controlled, multicenter study	rTPA 10 mg intracisternal at the time of aneurysm clipping (N = 51)	Placebo vehicle (N = 49)	Primary outcome: angiographic vasospasm Secondary outcome: mortality, 3-month GOS, symptomatic vasospasm, clot clearance on CT, TCD velocities, use of HHH on angioplasty to treat vasospasm	No difference in angiographic vasospasm, vasospasm treatment, TCD velocities, mortality, or 3-month GOS	Findlay, Neurosurgery 1995 [37]
Efficacy of low-dose tissue-plasminogen activator intracisternal administration for the prevention of cerebral vasospasm after subarachnoid hemorrhage	Randomized, controlled trial	Intermittent Tisokinase 960,000 IU via cisternal drain (N = 20) Continuous infusion Tisokinase 1920 IU/h × 48 h via cisternal drain (N = 20)	Control (standard treatment) (N = 20)	Primary outcome: clearance of subarachnoid clots by HCT Secondary outcome: delayed cerebral ischemia, 3-month mRS and GOS	Subarachnoid clot by HCT and delayed cerebral ischemia were significantly less in the treatment groups compared to control (P < 0.05). The intermittently treated group had better neurological outcomes than the control group (P < 0.05)	Yamamoto et al., World Neurosurgery 2010 [38]
Anti-platelets						
Dipyridamole and postoperative ischemic deficits in aneurysmal subarachnoid hemorrhage	Randomized, placebo-controlled, single-blind controlled trial	Dipyridamole 100 mg PO qd or 10 mg/day IV × 3-months (N = 336)	Placebo (N = 314)	Primary outcome: 3-month GOS Secondary outcome: neurological deterioration following aneurysm repair	No differences in 3-month GOS or delayed neurological deterioration	Shaw et al., J Neurosurg 1985 [39]
Randomized controlled trial of acetylsalicylic Acid in aneurysmal subarachnoid hemorrhage: the MASH study	Randomized controlled pilot study; factorial design (magnesium versus placebo and ASA versus placebo, separated a priori)	Aspirin 100 mg PR qd × 14 days within 12 hours of aneurysm occlusion. (N = 87)	Placebo (N = 74)	Primary outcome: delayed ischemic neurological deficits within 3-months of SAH consisting of HCT infarcts plus clinical decline Secondary outcome: new CT infarcts of any cause, postop hemorrhage, mRS ≥ 4, mRS ≥ 1	No difference in delayed ischemic events, CT infarction, or 3-month outcomes	Van den Bergh, Stroke 2006 [40]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Cilostazol improves outcome after subarachnoid hemorrhage: a preliminary report	Randomized, single-blind, prospective, multicenter study	Cilostazol 100 mg PO BID (N = 49)	Control (usual care) (N = 51)	Primary outcome: symptomatic vasospasm and cerebral infarction, discharge mRS	No difference in symptomatic vasospasm or cerebral infarction. mRS at discharge better in treatment group (1.5 versus 2.6 in controls, $P = 0.041$)	Suzuki et al., Cerebrovasc Dis 2011 [41]
Steroids						
Effect of fludrocortisone acetate in patients with subarachnoid hemorrhage	Randomized, placebo controlled, multicenter trial	Fludrocortisone 400 mcg/day BID × 12 days PO or IV (N = 46)	Placebo (N = 45)	Primary outcome: plasma volume change, fluid balance, sodium balance. Secondary outcome: delayed cerebral ischemia within 28 days and 28-day GOS	Treatment reduced negative sodium balance ($P = 0.014$) but did not affect plasma volume. No significant difference in cerebral ischemia (22% versus 31% in controls, $P = 0.349$). Similar outcome in each group	Hasan et al., Stroke 1989 [42]
A randomized controlled trial of hydrocortisone against hyponatremia in patients with aneurysmal subarachnoid hemorrhage	Randomized, placebo-controlled study	Hydrocortisone 300 mg q 6 h × 10 d then taper over 4 d (N = 35)	Placebo (N = 36)	Primary outcome: hyponatremia < 140 mmol/L. Secondary outcome: 30-day mRS, symptomatic vasospasm	Less sodium excretion and urine volume in treatment group ($P = 0.04$). No significant differences in vasospasm or mRS	Katayama et al., Stroke 2007 [43]
Randomized, double-blind, placebo-controlled, pilot trial of high-dose methylprednisolone in aneurysmal subarachnoid hemorrhage	Randomized, double-blind, placebo-controlled, single center study	Methylprednisolone 16 mg/kg IV qd × 3 days (N = 49)	Placebo (N = 46)	Primary outcome: symptomatic vasospasm and infarct on HCT. Secondary Outcomes: 1 year GOS, functional outcome scale, and severity of delayed ischemic deficits	No significant difference in symptomatic vasospasm or infarct on HCT. No difference in 1-year GOS or delayed ischemic deficits at 3-months. Poor outcome by functional outcome scale was reduced in treatment group ($P = 0.02$)	Gomis et al., J Neurosurg 2010 [44]
Transfusion/erythropoietin/albumin						
Acute systemic erythropoietin therapy to reduce delayed ischemic deficits following aneurysmal subarachnoid hemorrhage: a phase II randomized, double-blind, placebo-controlled trial	Phase II randomized, double-blind, placebo-controlled trial	Erythropoietin IV (30,000 u) every 48 h for a total of 90,000 U (N = 40)	Placebo (N = 40)	Primary outcome: incidence, duration and severity of TCD vasospasm; duration of impaired autoregulation by TCD. Secondary outcome: incidence of delayed ischemic deficits, mRS, GOS, and NIHSS at discharge and 6-months	No differences in incidence of TCD vasospasm or adverse events. Treatment group had less severe TCD vasospasm ($P = -0.037$), reduced delayed ischemic deficits/delayed cerebral infarcts ($P = 0.001$), and shortened duration of impaired autoregulation ($P < 0.001$) and more favorable discharge outcome ($P = 0.039$)	Tseng et al., J Neurosurg 2009 [45]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Prospective, randomized trial of higher goal hemoglobin after SAH	Prospective, randomized pilot safety, and feasibility study	Packed RBC transfusion to goal Hgb 11.5 g/dL ($N = 21$)	Packed RBC transfusion to goal Hgb 10 g/dL ($N = 23$)	Primary outcomes: days of core temp > 100.4 F; ventilator-free days, hemoglobin level Secondary outcomes: NIHSS, mRS, and MRI at 14 days, mRS at 28 days and 3-months	Higher target Hgb resulted in more transfusions. No difference in safety endpoints. Number of MRI infarcts, NIHSS, and mRS similar between both groups at all timepoints	Naidech et al., Neurocrit Care 2010 [46]
The Albumin in Subarachnoid Hemorrhage multicenter pilot clinical trial: safety and neurologic outcomes (ALISAH)	Open label, dose escalation study	Albumin in 3 tier doses: 0.625 g/kg/d ($N = 20$), 1.25 g/kg/d ($N = 20$), 1.875 g/kg/d ($N = 7$) × 7 days ($N = 47$ total)	NA	Primary outcomes: severe to life threatening heart failure, anaphylaxis Secondary outcomes: functional outcome at 3-months	Doses up to 1.25 g/kg/d × 7 days tolerated without dose-limiting complications. Trend toward better outcomes in 1.25 g/kg/d dose compared to 0.625 g/kg/d	Suarez et al., Stroke 2012 [47]
		Vasodilators—CRGP and endothelin receptor antagonist				
Effect of calcitonin-gene-related peptide in patients with delayed postoperative cerebral ischemia after aneurysmal subarachnoid hemorrhage	Randomized, single-blind, controlled, multicenter study	Calcitonin-related gene peptide (0.6mcg/min) × 10 days ($N = 62$)	Standard medical therapy ($N = 55$)	Primary outcome: 3-month GOS	No difference in 3-month GOS. Hypotension common in treatment group.	Bell, European CGRP in subarachnoid Hemorrhage study group, Lancet 1992 [48]
Clazosentan, an endothelin receptor antagonist, in patients with aneurysmal SAh undergoing surgical clipping: a randomized, double-blind, placebo-controlled phase 3 trial (CONSCIOUS 2)	Phase 3 randomized placebo-controlled double-blinded	Clazosentan (5 mg/h IV up to 14 days) ($N = 748$)	Placebo ($N = 389$)	Primary outcomes: cerebral vasospasm-related morbidity (DCI/DIND/vasospasm therapy), all-cause mortality at 6 weeks Secondary outcomes: 12-week GOSE	No effect on primary endpoint (21% in clazosentan group and 25% in placebo group $P = $ NS). Poor outcome (GOSE) in 29% clazosentan and 25% placebo group	MacDonald et al., Lancet Neuol 2011 [49]
Randomized trial of clazosentan in patients with aneurysmal subarachnoid hemorrhage undergoing endovascular coiling (CONSCIOUS 3)	Phase 3 randomized placebo-controlled double-blinded; terminated early for futility (planned $N = 1500$)	Clazosentan (5 or 15 mg/h IV up to 14 days) ($N = 194$)	Placebo ($N = 189$)	Primary outcomes: cerebral vasospasm related morbidity (DCI/DIND/vasospasm therapy), all-cause mortality at 6 weeks Secondary outcomes: 12-week GOSE	Clazosentan 15 mg/h significantly reduced vasospasm-related morbidity/all-cause mortality at 6 weeks but did not improve long-term outcome Primary outcome: 24% in clazosentan 5 mg/h and 27% in placebo group $P = $ NS and 15% in clazosentan 15 mg/h $P = 0.007$ Poor outcome in 25% of clazosentan 5 mg/h, 28% clazosentan 15 mg/h, and 24% of placebo group $P = $ NS.	MacDonald et al., Stroke 2012 [50]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
				Hypertensive, hypervolemic therapy (prophylactic)		
Effect of hypervolemic therapy on cerebral blood flow after subarachnoid hemorrhage: A randomized controlled trial	Randomized, controlled, single-center study	High-volume management (with colloid and crystalloid) to target PADP ≥ 14 mmHg or CVP ≥ 8 mmHg ($N = 41$)	Normal volume management (with colloid and crystalloid) to target PADP ≥ 7 mmHg or CVP ≥ 5 mmHg ($N = 41$)	Primary outcome: CBF by Xenon CT and blood volume by tagged RBC Secondary outcomes: symptomatic vasospasm, medical complications, GOS and 3, 6, and 12 months	High-volume management patients received significantly more fluid but there was no effect on net fluid balance or blood volume. No difference in CBF or vasospasm	Lennihan et al., Stroke 2000 [51]
Prophylactic hyperdynamic postoperative fluid therapy after aneurysmal subarachnoid hemorrhage: a clinical, prospective, randomized, controlled trial	Randomized, controlled Prospective, trial of Hunt Hess I–III patients	Hypertensive (MAP 20 mmHg greater than pre-op), hypervolemic (CVP 8–12 mmHg) and hemodilutional (Hct 30–35%) therapy ($N = 16$)	Normovolemic crystalloid fluid therapy until day 12 ($N = 16$)	Primary outcome: TCD vasospasm, CBF by SPECT on day 12 Secondary outcomes: 1 year GOS, neuropsych outcomes, and SPECT	No differences in TCD vasospasm or SPECT CBF. No difference in 1-year GOS, SPECT, or neuropsych outcomes	Egge et al., Neurosurgery 2001 [52]
			Magnesium			
Intravenous magnesium sulfate for aneurysmal subarachnoid hemorrhage (IMASH): a randomized double-blinded, placebo-controlled, multicenter phase III trial	Randomized double-blinded, placebo-controlled, multicenter phase III trial.	MgSO$_4$ IV infusion to 2x baseline value (20 mmol over 30 minutes then continuous infusion of 80 mmol/d $\times$ 14 days; maximum allowed serum Mg of 2.5 mmol/L ($N = 169$)	Equivalent volume of normal saline infusion. Occasional changes in infusion rates to maintain blinding. ($N = 158$)	Primary outcome: 6-month GOSE 5–8 Secondary outcome: clinical vasospasm during initial 2 weeks, 6-month mRS, Barthel Index, and Short Form 36	Favorable 6-month GOSE (5–8) 64% of Mg group and 63% placebo ($P = $ NS) No difference in mRS, Barthel, Short Form 36, or clinical vasospasm. No subgroup differences	Wong et al., Stroke 2010 [53]
Magnesium for aneurysmal subarachnoid hemorrhage (MASH-2): a randomized placebo-controlled trial	Randomized, double-blind, placebo controlled, multicenter, phase III trial	MgSO$_4$ IV 64 mmol/day ($N = 606$)	Placebo ($N = 507$)	Primary outcome: 3-month mRS 4–6	No difference in poor outcome in the MgSO$_4$ group (26.2% versus 25.3% in placebo group)	Mees et al., Lancet 2012 [54]

Table 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Adrenergic blockade						
Beneficial effects of adrenergic blockade in patients with subarachnoid hemorrhage	Randomized controlled trial	Phentolamine 20 mg q 3 h + propranolol 80 mg q 8 × 3 weeks ($N = 68$)	Placebo ($N = 66$)	Primary outcome: neurological deficit at 28 days	Trend toward less neurological deficit in the treated group ($P = 0.053$)	Walter et al., BMJ 1982 [55]
Endovascular therapy						
Effect of prophylactic transluminal balloon angioplasty on cerebral vasospasm and outcome in patients with Fisher grade III subarachnoid hemorrhage: results of a phase II multicenter, randomized clinical trial	Unblinded, randomized phase II trial of Fisher III and Fisher III + IV SAH patients after clipping or coiling within 96 h of rupture	Balloon angioplasty of bilateral A1, M1, P1, basilar, intradural vertebral artery, and supraclinoid ICA. Protocol later revised to exclude A1 and P1 ($N = 85$)	No prophylactic balloon angioplasty ($N = 85$)	Primary outcome: 3-month GOS Secondary outcome: delayed ischemic neurological deficit, TCD vasospasm, ICU, and hospital length of stay	Nonsignificant difference in delayed ischemic neurological deficits but less therapeutic angioplasty required in treatment group ($P = 0.03$). No significant difference in GOS outcomes. LOS similar. Four patients had procedure related vessel perforation, three of whom died	Zwienenberg-Lee et al., Stroke 2008 [56]
Rho kinase inhibitor—fasudil						
Effect of AT877 on cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Results of a prospective placebo-controlled double-blind trial	Randomized, placebo controlled, double-blind, multicenter study in Hunt Hess I–IV clipped SAH patients	Fasudil (AT877) 30 mg IV over 30 minutes, TID × 14 days ($N = 131$)	Placebo ($N = 136$)	Primary outcome: reduction of incidence or severity of angiographic vasospasm, reduction of incidence and size of low-density CT lesions due to vasospasm, reduction of incidence of symptomatic vasospasm, poor outcome (1-month GOS) due to vasospasm	Fasudil significantly reduced angiographic vasospasm (38% in treatment group versus 61% in placebo group, $P = 0.0023$), infarcts reduced (16% in treatment versus 38% in placebo group, $P = 0.0013$) and symptomatic vasospasm reduced (35% in treatment versus 50% in placebo, $P = 0.0247$). Poor outcome (GOS 1–4) attributable to vasospasm occurred in 12% of treatment group and 26% of placebo group ($P = 0.0152$). No serious adverse events in fasudil group	Shibuya et al., J Neurosurg 1992 [57]
Efficacy and safety of fasudil in patients with subarachnoid hemorrhage: final results of a randomized trial of fasudil versus nimodipine	Randomized, open label, multicenter study of SAH Hunt-Hess grade I–IV clipped patients	Fasudil 30 mg IV TID × 14 days ($N = 63$)	Nimodipine 1-2 mg/h × 14 days ($N = 66$)	Primary outcome: symptomatic vasospasm or infarct on HCT Secondary outcome: 1-month GOS	No difference in symptomatic vasospasm or HCT infarcts. Improved GOS outcomes in fasudil group (good outcome in 74.5% versus 61.7% in nimodipine group, $P = 0.040$)	Zhao et al., Neurol Med Chir (Tokyo) 2011 [58]

TABLE 1: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
			Intensive insulin therapy			
The effect of intensive insulin therapy on infection rate, vasospasm, neurologic outcome and mortality in neurointensive care unit after intracranial aneurysm clipping in patients with acute subarachnoid hemorrhage: a randomized prospective Pilot trial	Randomized, controlled study	Intensive Insulin Infusion (80–120 mg/dL) × 14 d ($N = 40$)	Conventional insulin infusion (glucose 80–220 mg/dL) × 14 d ($N = 38$)	Primary outcome: infection Secondary outcomes: vasospasm, 6-month mortality, and mRS	Higher infection rate in the conventional group (42% versus 27% in intensive group, $P < 0.001$). Similar vasospasm, mortality, and mRS at 6 months	Bilotta et al., J Neurosurg Anesthesiol 2007 [59]
			Hypothermia			
Mild intraoperative hypothermia during surgery for intracranial aneurysm (IHAST)	Randomized, prospective, partially blinded, controlled, multicenter trial of WFNS grade I–III SAH patients	Intraoperative hypothermia (target 33°C with surface cooling) ($N = 499$)	Intraoperative normothermia (target 36.5°C) ($N = 501$)	Primary outcome: GOS at 90 days Secondary outcomes: 90-day mRS, Barthel Index, NIHSS, neuropsych testing, adverse events	No difference in 90 day GOS. Good GOS in 66% of hypothermia versus 63% of control patients ($P = $ NS). No differences in death, length of stay, or discharge disposition. Postoperative bacteremia more common in the hypothermia group (5% versus 3%, $P = 0.05$)	Todd et al., NEJM 2005 [60]

TABLE 2: Randomized controlled trials assessing neurologic outcomes after cardiac arrest—completed trials.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Calcium channel blockers						
Effects of nimodipine on cerebral blood flow and cerebrospinal fluid pressure after cardiac arrest: correlation with neurologic outcome	Randomized, double-blind study	Nimodipine IV 0.25 mcg/kg/min (N = 25)	Placebo (N = 26)	Primary outcome: CBF measured by Xenon CT Secondary outcomes: ICP, neurological disability	Higher CBF in nimodipine group in first 4 hours after arrest ($P < 0.05$) but no difference at 24 hours. No difference in neurological outcomes	Forsman et al., Anesth Analg 1989 [64]
Neuropsychological sequelae of cardiac arrest	Randomized, double-blind, placebo-controlled, study of out-of-hospital ventricular fibrillation	Nimodipine 10 mcg/kg IV then 0.5 mcg/kg/min × 24 hours (N = 35)	Placebo (N = 33)	Primary outcome: 3- and 12-month neuropsychological and cognitive batteries	No difference in neuropsychological or cognitive outcome between groups	Roine et al., JAMA 1992 [65]
A randomized clinical study of a calcium-entry blocker (lidoflazine) in the treatment of comatose survivors of cardiac arrest	Randomized, double-blind, placebo-controlled, multicenter study	Lidoflazine 1 mg/kg loading dose then 0.25 mg/kg at 8 and 16 hours after resuscitation (N = 259)	Placebo (N = 257)	Primary outcome: Pittsburgh Cerebral Performance Scale at 6 months Secondary outcomes: mortality, complications	No difference in 6-month neurological outcome or mortality between groups	Brain Resuscitation Clinical Trial II Study Group, NEJM 1991 [66]
Nimodipine after resuscitation from out-of-hospital ventricular fibrillation: a placebo-controlled, double-blind, randomized trial	Randomized, double-blind, placebo-controlled, study of out-of-hospital ventricular fibrillation	Nimodipine 10 mcg/kg IV then 0.5 mcg/kg/min × 24 hours (N = 75)	Placebo (N = 80)	Primary outcome: survival, 1-year GOS Secondary outcomes: death related to anoxic encephalopathy, GCS at 24 hours and 1 week, 3- and 12-month mini-mental state exam, activities of daily living, Barthel index, neurological exam, seizure, SPECT, myocardial infarction, arrhythmias	No difference in the survival rate, GOS at 3 or 12 months. No difference in minimental state exam, activities of daily living, or seizures	Roine et al., JAMA 1990 [67]
Neuroprotective						
Coenzyme Q10 combined with mild hypothermia after cardiac arrest: a preliminary study	Randomized, placebo-controlled, double-blind, single-center study of out-of-hospital cardiac arrest	Hypothermia 35-36°C × 24 hours + Coenzyme Q 10 250 mg PO × 1 then 150 mg PO TID (N = 25)	Hypothermia 35-36°C × 24 hours + Placebo (N = 24)	Primary outcome: survival to ICU discharge Secondary outcomes: 3-month survival, 3-month GOS, S100 levels	3-month survival was 68% in the treatment group and 29% in the control group ($P = 0.0413$). There was no significant difference in survival until discharge or GOS outcome	Damian et al., Circulation 2004 [68]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Thrombolytics						
A pilot randomized trial of thrombolysis in cardiac arrest (the TICA trial)	Randomized, double-blind, placebo controlled, single-center, feasibility trial for out-of-hospital cardiac arrest	Tenecteplase 50 mg IV × 1 (N = 19)	Placebo (N = 16)	Primary outcome: ROSC Secondary outcomes: survival to ED, ICU and hospital discharge	ROSC in 42% of tenecteplase and 6% of placebo group. No difference in survival to hospital discharge	Fatovich et al., Resuscitation 2004 [69]
Thrombolysis during resuscitation for out-of-hospital cardiac arrest	Randomized, double-blind, controlled, multicenter study of out-of-hospital cardiac arrest	Tenecteplase 0.5 mg/kg IV (N = 525)	Placebo (N = 525)	Primary outcome: survival at 30 days Secondary outcomes: survival to admission, ROSC, 24-hour survival, survival to discharge, cerebral performance score at discharge	No difference in 30-day survival, hospital admission, ROSC, 24-hour survival, discharge, or neurologic outcome. More intracranial hemorrhages in treatment group	Böttiger et al., NEJM 2008 [70]
Steroids and pressors						
Vasopressin, epinephrine, and corticosteroids for In-hospital cardiac arrest	Randomized, double-blind, placebo-controlled, single-center study	Vasopressin 20 IU IV + epinephrine 1 mg IV + methylprednisolone 40 mg IV followed by hydrocortisone (N = 48)	Epinephrine + placebo (N = 52)	Primary outcome: ROSC, survival to discharge Secondary outcomes: blood pressure after CPR, organ failure-free days, discharge Glasgow-Pittsburg cerebral performance scale	More ROSC in treatment group (81% versus 52%, $P = 0.003$) and more survival to discharge (19% versus 4%, $P = 0.02$)	Mentzelopoulos et al., Arch Intern Med 2009 [71]
Pressors						
A comparison of standard-dose and high-dose epinephrine in cardiac arrest outside the hospital	Randomized, double-blind, prospective, multi-center study	Epinephrine 0.2 mg/kg IV (N = 648)	Epinephrine 0.02 mg/kg IV (N = 632)	Primary outcome: return of spontaneous circulation (ROSC), admission to the hospital Secondary outcome: cerebral performance scale at admission, and discharge	No difference in ROSC rates, admission, survival, or discharge neurological status	Brown et al., NEJM 1992 [72]
Standard doses versus repeated high doses of epinephrine in cardiac arrest outside the hospital	Randomized, double-blind, prospective, single-center study	Repeated epinephrine 5 mg IV (N = 271)	Repeated epinephrine 1 mg IV (N = 265)	Primary outcome: ROSC Secondary outcomes: admission to the hospital, discharge, cerebral performance category at discharge and 6 months	No difference in ROSC, admission, discharge, or 6-month neurological outcomes	Choux et al., Resuscitation 1995 [73]

Table 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
A randomized, double-blind comparison of methoxamine and epinephrine in human cardiopulmonary arrest	Randomized, double-blind, single-center study	Methoxamine 40 mg bolus IV then 40 mg 4 minutes later ($N = 77$)	Epinephrine 2 mg bolus then 2 mg IV q 4 min ($N = 68$)	Primary outcome: Mortality and Glasgow-Pittsburgh coma score Secondary outcomes: ROSC, successful resuscitation	No difference in ROSC or neurologic outcome, initial resuscitation, or survival to discharge	Patrick et al., Am J Respir Crit Care Med 1995 [74]
Randomised comparison of epinephrine and vasopressin in patients with out-of-hospital ventricular fibrillation	Randomized, double-blind, single-center, controlled study of out-of-hospital ventricular fibrillation patients who failed defibrillation	Vasopressin 40 IU IV ($N = 20$)	Epinephrine 1 mg IV ($N = 20$)	Primary outcome: survival to admission Secondary outcome: 24-hour survival, survival to discharge, GCS at discharge	No significant difference in survival to admission but more vasopressin patients survived 24 hours (60% versus 20%, $P = 0.02$). No difference in survival to discharge or GCS at discharge	Lindner et al., Lancet 1997 [75]
High-dose versus standard-dose epinephrine treatment of cardiac arrest after failure of standard therapy	Randomized, controlled, single-blind, multicenter study of patients who had failed on standard dose of epinephrine 0.5–1.0 mg IV	Epinephrine 0.1 mg/kg IV up to 4 doses ($N = 78$)	Epinephrine 0.01 mg/kg IV up to 4 doses ($N = 62$)	Primary outcome: improvement in cardiac rhythm or ROSC Secondary outcomes: GCS at 6, 24, and 72 hours	No differences in ROSC, survival, or neurologic function between groups	Sherman et al., Pharmacotherapy 1997 [76]
A comparison of repeated high doses and repeated standard doses of epinephrine for cardiac arrest outside the hospital	Randomized, controlled, prospective multicenter study	Epinephrine 5 mg IV up to 15 doses at 3-minute intervals ($M = 1677$)	Epinephrine 1 mg IV up to 15 doses at 3-minute intervals ($M = 1650$)	Primary outcome: ROSC, admission to the hospital, number of admissions after a single dose of epinephrine, hospital discharge Secondary outcomes: survival, neurological outcome by GCS and cerebral performance scale	Significantly more ROSC in high dose group (40% versus 36% of control group, $P = 0.02$) and more survival to admission (26.5% versus 23.6% of controls, $P = 0.05$). No difference in survival to discharge or neurological status	Gueugniaud et al., NEJM 1998 [77]
Vasopressin versus epinephrine for in-hospital cardiac arrest: a randomized controlled trial	Randomized, controlled, triple-blind, multicenter study of in-hospital cardiac arrest for asystole, PEA, or refractory ventricular fibrillation	Vasopressin 40 IU IV (first pressor) ($N = 104$)	Epinephrine 1 mg IV (first pressor) ($N = 96$)	Primary outcome: survival for 1 hour Secondary outcomes: survival to hospital discharge, modified mini-mental state exam at discharge, cerebral performance score at discharge, ROSC, adverse events	No difference in survival at 1 hour or survival to hospital discharge. No difference in mini-mental state exam scores or cerebral performance scores	Stiell et al., Lancet 2001 [78]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
A comparison of vasopressin and epinephrine for out-of-hospital cardiopulmonary resuscitation	Randomized, controlled, multicenter study of out-of-hospital cardiac arrest with ventricular fibrillation failing defibrillation, PEA, or asystole	Vasopressin 40 IU IV × 2 doses maximum ($N = 589$)	Epinephrine 1 mg IV × 2 doses maximum ($N = 597$)	Primary outcome: survival to hospital admission Secondary outcomes: survival to hospital discharge, cerebral performance score in survivors	No difference in survival to admission among patients with ventricular fibrillation or PEA. Higher rates of hospital admission for asystole in vasopressin group (29% versus 20%, $P = 0.02$) and hospital discharge (4.7% versus 1.5% with epinephrine, $P = 0.04$). Patients who received rescue epinephrine after vasopressin had better survival to admission and discharge than the epinephrine alone group. No difference in cerebral performance	Wenzel et al., NEJM 2004 [79]
Vasopressin and epinephrine versus epinephrine alone in cardiopulmonary resuscitation	Randomized, controlled, multicenter trial	Epinephrine 1 mg IV + Vasopressin 40 IU IV ($N = 1442$)	Epinephrine 1 mg IV + Placebo ($N = 1452$)	Primary outcome: Survival to hospital admission Secondary outcomes: ROSC, survival to hospital discharge, good neurological recovery by cerebral performance scale, and GCS, 1-year survival	No differences in survival to admission, ROSC, survival to discharge, 1-year survival, or good neurologic recovery	Gueugniaud et al., NEJM 2008 [80]
Magnesium						
Randomised trial of magnesium in in-hospital cardiac arrest (MAGIC trial)	Randomized, placebo-controlled, single-center study of cardiac arrest in the ICU or general ward	Magnesium 2 g IV bolus then 8 g over 24 hours ($N = 76$)	Placebo ($N = 80$)	Primary outcome: ROSC Secondary outcomes: 24-hour survival, survival to hospital discharge, GCS, and discharge Karnofsky	No difference in ROSC, 24-hour survival, survival to discharge, or GCS	Thel et al., Lancet 1997 [81]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Magnesium in cardiac arrest (the MAGIC trial)	Randomized, double-blind, placebo-controlled, single-center study of out-of-hospital cardiac arrest	MgSO$_4$ 5 g IV × 1 ($N = 31$)	Placebo ($N = 36$)	Primary outcome: ECG rhythm 2 minutes after drug, ROSC Secondary outcomes: survival to ED, ICU, and hospital discharge	No differences in ROSC or survival	Fatovich et al., Resuscitation 1997 [82]
Magnesium sulfate in the treatment of refractory ventricular fibrillation in the prehospital setting	Randomized, double-blind, placebo-controlled, multicenter study of prehospital ventricular fibrillation refractory to 3 shocks	MgSO$_4$ 2 g IV × 1 ($N = 58$)	Placebo ($N = 58$)	Primary outcome: ROSC Secondary outcomes: admission to hospital, hospital discharge	No difference in ROSC, survival to admission or discharge	Allegra et al., Resuscitation 2001 [83]
A randomized trial to investigate the efficacy of magnesium sulphate for refractory ventricular fibrillation	Randomized, double-blind, placebo-controlled trial of ventricular fibrillation refractory to 3 shocks	MgSO$_4$ 2–4 g IV × 1 ($N = 52$)	Placebo ($N = 53$)	Primary outcome: ROSC Secondary outcome: hospital discharge	No differences in ROSC or survival to discharge	Hassan et al., Emerg Med J 2002 [84]
Randomized clinical trial of magnesium, diazepam, or both after out-of-hospital cardiac arrest	Randomized, double-blind, placebo-controlled factorial design study	Tier 1: magnesium 2 g IV + placebo ($N = 75$) Tier 2: diazepam 10 mg IV + placebo ($N = 75$) Tier 3: magnesium 2 g IV + Diazepam 10 g IV ($N = 75$)	Placebo only ($N = 75$)	Primary outcome: awakening at 3 months (comprehensible speech and command following) Secondary outcome: days to awakening, days to death, independent at 3 months	No difference in neurological outcome between the 3 groups	Longstreth et al., Neurology 2002 [85]
			Insulin			
Intravenous glucose after out-of-hospital cardiopulmonary arrest: a community-based randomized trial	Randomized, single-center controlled study	5% dextrose (D5W) infusion ($N = 374$)	0.45 saline infusion ($N = 374$)	Primary outcome: command following or comprehensible speech Secondary outcomes: survival to hospital admission and discharge	No difference in neurological outcomes, or survival to admission or discharge	Longstreth et al., Neurology 1993 [86]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Strict versus moderate glucose control after resuscitation from ventricular fibrillation	Randomized, controlled, multicenter study of out-of-hospital ventricular fibrillation cardiac arrest	Strict glucose control (4–6 mmol/L) with insulin infusion × 48 hours ($N = 39$)	Moderate glucose control (6–8 mmol/L) with insulin infusion × 48 hours ($N = 51$)	Primary outcome: 30-day all-cause mortality after ROSC Secondary outcomes: neuron-specific enolase levels at 24 and 48 hours	No difference in 30-day mortality	Oksanen et al., Intensive Care Med 2007 [87]
			Hypothermia			
Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia	Randomized, controlled, single-blind, prospective study	Hypothermia 33°C × 12 h ($N = 43$)	Normothermia 37°C ($N = 34$)	Primary outcome: discharge disposition Secondary outcomes: adverse events, hemodynamic parameters	Good discharge disposition in 49% of treatment group compared to 26% of normothermia group ($P = 0.046$). No difference in adverse events	Bernard et al., NEJM 2002 [88]
Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest	Randomized, controlled, single-blind, multicenter, prospective study	Hypothermia 32–34°C × 24 h ($N = 137$)	Normothermia 37°C ($N = 138$)	Primary outcome: 6 month neurologic outcome using Pittsburgh cerebral performance scale Secondary outcome: 6-month mortality, complications at 7 days	Hypothermia group had more favorable neurological outcome at 6 months (55% versus 39% of normothermia group, $P = 0.009$). Less death in hypothermia group (41% versus 55% in normothermia group, $P = 0.02$). No difference in complication rates	The hypothermia after cardiac arrest study group, NEJM 2002 [89]
Pilot randomized clinical trial of prehospital induction of mild hypothermia in out-of-hospital cardiac arrest patients with a rapid infusion of 4 degrees C normal saline	Randomized, controlled, safety and feasibility study of out-of-hospital cardiac arrest	2 L 4°C normal saline infusion ($N = 63$)	Standard care ($N = 62$)	Primary outcome: esophageal temperature, adverse events Secondary outcomes: awakening, hospital discharge	Significant differences in temperature between groups ($P < 0.001$). No difference in awakening or hospital discharge	Kim et al., Circulation 2007 [90]
Prehospital therapeutic hypothermia for comatose survivors of cardiac arrest: a randomized controlled trial	Randomized controlled trail of out-of-hospital cardiac arrest	4°C Ringers solution 30 mL/kg to target temperature 33°C ($N = 19$)	Conventional fluid therapy ($N = 18$)	Primary outcome: nasopharyngeal temperature Secondary outcomes: hospital mortality and cerebral performance scale	Lower core temperature in the treatment group ($P < 0.001$). No difference in safety, mortality, or neurologic outcome	KÄmÄrÄinen et al., Acta Anaesthesiol Scand 2009 [91]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Intra-arrest transnasal evaporative cooling: a randomized, prehospital, multicenter study (PRINCE: pre-rosc intranasal cooling effectiveness)	Randomized, controlled, prospective, single-blind, multicenter study for out-of-hospital arrest	Intra-arrest intranasal cooling with RhinoChill device + cooling at hospital arrival to 34°C (N = 93)	Standard care with cooling at hospital arrival to 34°C (N = 101)	Primary Outcome: adverse events, length of stay, mechanical ventilation days, ROSC, survival to discharge, discharge Pittsburgh cerebral performance scale.	Time to target temperature was shorter in the intranasal cooling group (P = 0.03). No difference in ROSC, survival of admitted patients, or neurologic outcome at discharge	Castrén et al., Circulation 2010 [92]
Chest compressions						
A comparison of active compression-decompression cardiopulmonary resuscitation with standard cardiopulmonary resuscitation for cardiac arrests occurring in the hospital	Randomized, controlled, single center study	CPR using suction device (Ambu CardioPump) (N = 29)	Standard CPR (N = 33)	Primary outcome: ROSC Secondary outcomes: 24-hour survival, hospital discharge, GCS at 24 hours	ROSC occurred in 62% of treatment group versus 30% of control group (P < 0.03) and 45% of treatment group survived 24 hours compared to 9% of control group (P < 0.004). GCS at 24 hours was better in the treatment group (P < 0.02)	Cohen et al., NEJM 1993 [93]
The Ontario Trial of Active Compression-Decompression Cardiopulmonary Resuscitation for In-Hospital and Prehospital Cardiac Arrest	Randomized, single-blind, multicenter controlled trial of prehospital and in-hospital cardiac arrest	Active compression-decompression CPR using a suction device (N = 906)	Standard CPR (N = 878)	Primary outcome: survival for 1 hour Secondary outcome: survival to hospital discharge, modified mini-mental state exam, cerebral performance scale	No differences in survival at 1 hour, survival until hospital discharge or mini-mental state exam for either prehospital or in-hospital arrest	Stiell et al., JAMA 1996 [94]
Cardiopulmonary resuscitation by chest compression alone or with mouth-to-mouth ventilation	Randomized controlled study of out-of-hospital cardiac arrest	Bystander chest compression plus mouth to mouth resuscitation (N = 279)	Bystander chest compressions alone (N = 241)	Primary outcome: survival to hospital discharge Secondary outcomes: admission to the hospital, neurological status	Similar outcome with bystander chest compressions alone versus chest compressions with mouth to mouth	Hallstrom et al., NEJM 2000 [95]
Constant flow insufflations of oxygen as the sole mode of ventilation during out-of-hospital cardiac arrest	Randomized, controlled study of out-of-hospital cardiac arrest	Constant flow insufflations of oxygen (N = 487)	Standard endotracheal intubation and mechanical ventilation (N = 457)	Primary outcome: survival to ICU discharge Secondary outcomes: ROSC, survival to hospital admission, spO_2 > 70%	No difference in ROSC, hospital admission or ICU discharge. Higher O_2 sats in continuous flow insufflation group	Bertrand et al., Intensive Care Med 2006 [96]
Compression-only CPR or standard CPR in out-of-hospital cardiac arrest	Randomized, controlled, multicenter study of out-of-hospital cardiac arrest	Compression only CPR (N = 620)	Standard CPR (N = 656)	Primary outcome: 30 day survival Secondary outcomes: 1 day survival, ROSC, survival to hospital discharge	Similar 30-day survival, 1-day survival and survival to hospital discharge	Svensson et al., NEJM 2010 [97]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
CPR with chest compression alone or with rescue breathing	Randomized, controlled, multicenter study of out-of-hospital cardiac arrest	Chest compressions alone (N = 981)	Chest Compressions + Rescue breathing (2 breaths to 15 chest compressions) (N = 960)	Primary outcome: survival to hospital discharge Secondary outcomes: discharge cerebral performance Score, ROSC	No difference in survival to hospital discharge or in neurologic outcome. Trend toward improved survival at discharge in those with cardiac cause of arrest and shockable rhythm ($P = 0.09$)	Rea et al., NEJM 2010 [98]
A trial of an impedance threshold device in out-of-hospital cardiac arrest	Randomized, controlled, double-blinded, multicenter study of out-of-hospital cardiac arrest	Impedance threshold device (ITD) which increasing negative intrathoracic pressure and improves cardiac output (N = 4373)	Sham ITD (N = 4345)	Primary outcome: survival to hospital discharge with mRS 0–3 Secondary outcomes: survival to ED admission, hospital admission and hospital discharge, adverse events	No difference in survival with good mRS	Aufderheide et al., NEJM 2011 [99]
Adenosine antagonist						
Aminophylline in bradyasystolic cardiac arrest: a randomized placebo-controlled trial	Randomized, placebo-controlled, double-blind, multicenter study of asystole and PEA arrest unresponsive to epinephrine and atropine	Aminophylline 250 mg (N = 486)	Placebo (N = 485)	Primary outcome: ROSC Secondary outcomes: duration of ROSC, survival to admission, survival to discharge, length of stay, 24-hour tachyarrhythmias, 24-hour seizures, 1-year neurologic outcome by GCS, Glasgow-Pittsburgh cerebral and overall performance scales, modified mini-mental state exam, functional status questionnaire	No difference in ROSC. More tachyarrhythmias in the treatment group. Survival to hospital admission and survival to discharge were not different	Abu-Laban et al., Lancet 2006 [100]
Fluid management						
Capillary leakage in postcardiac arrest survivors during therapeutic hypothermia: a prospective, randomized study	Randomized, controlled study	72% hypertonic saline with 6% poly starch solution × 24 hours (N = 10)	Standard Fluid (Ringer's acetate) × 24 hours (N = 9)	Primary outcome: amount of fluid administered in 24 hours Secondary outcome: MRI vasogenic edema	The treatment group required significantly less fluid than the control group. There was no difference in MRI brain edema	Heradstveit et al., Scand J Trauma Resusc Emerg Med 2010 [101]

Table 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
			Barbiturate			
Randomized clinical study of thiopental loading in comatose survivors of cardiac arrest	Randomized, controlled, multicenter study	Thiopental 30 mg/kg IV load (N = 131)	Standard therapy (N = 131)	Primary outcome: Pittsburgh cerebral Performance scale at 6 and 12 months Secondary outcomes: best neurological performance ever obtained during followup, time to recovery	No difference in neurological outcome or mortality between groups	Brain Resuscitation Clinical Trial I Study Group. NEJM 1986 [102]
			Calcium			
Calcium chloride: reassessment of use in asystole	Randomized, double-blind, placebo-controlled study in prehospital asystolic cardiac arrest refractory to epinephrine, bicarbonate, and atropine	Calcium chloride (N = 18)	Placebo (N = 14)	Primary outcome: ROSC Secondary outcome: hospital discharge	No difference in ROSC	Stueven et al., Ann Emerg Med 1984 [103]
The effectiveness of calcium chloride in refractory electromechanical dissociation	Randomized, blinded, placebo-controlled study of prehospital PEA arrest refractory to epinephrine and bicarbonate	Calcium chloride (N = 48)	Placebo (N = 42)	Primary outcome: ROSC Secondary outcomes: survival to hospital discharge	No difference in ROSC but subgroup of patients with widened QRS did have more ROSC in calcium group (P = 0.028)	Stueven et al., Ann Emerg Med 1985 [104]
Lack of effectiveness of calcium chloride in refractory asystole	Randomized, blinded, placebo controlled study of prehospital asystolic cardiac arrest refractory to epinephrine, bicarbonate, and atropine	Calcium chloride (N = 39)	Placebo (N = 34)	Primary outcome: ROSC Secondary outcomes: survival to hospital discharge	No difference in ROSC or hospital discharge	Stueven et al., Ann Emerg Med 1985 [105]
			Sodium bicarbonate			
Buffer therapy during out-of-hospital cardiopulmonary resuscitation	Randomized, double-blind, placebo-controlled study of out-of-hospital asystole or ventricular fibrillation refractory to first defibrillation attempt	Sodium bicarbonate 250 mL IV × 1 (N = 245)	Placebo (N = 257)	Primary outcome: survival to ICU admission, survival to hospital discharge	No difference in survival to ICU admission or hospital discharge	Dybvik et al., Resuscitation 1995 [106]

TABLE 2: Continued.

Trial name	Study design	Treatment group	Control group	Outcome measure	Results	Reference
Sodium bicarbonate improves outcome in prolonged prehospital cardiac arrest	Randomized, double-blind, placebo-controlled trial of prehospital cardiac arrest	Sodium bicarbonate (1 meq/kg) IV × 1 ($N = 175$)	Placebo ($N = 155$)	Primary outcome: survival to ED Secondary outcomes: ROSC	No difference in survival to ED admission or ROSC. Better survival with bicarbonate in the prolonged (>15 minute) arrest group ($P = 0.007$)	Vukmir and Katz, Am J Emerg Med 2006 [107]
			Hemofiltration			
High-volume hemofiltration after out-of-hospital cardiac arrest: a randomized study	Randomized, controlled trial of out-of-hospital ventricular fibrillation or asystole cardiac arrest	Tier 1: hemofiltration (200 mL/kg/h) over 8 hours ($N = 20$) Tier 2: hemofiltration plus hypothermia to $32°C × 24$ hours ($N = 22$)	Standard care ($N = 19$)	Primary outcome: survival at 6 months Secondary outcome: intractable shock, Pittsburgh cerebral performance scale	Significantly better survival compared to control in hemofiltration group ($P = 0.026$) and hemofiltration plus hypothermia group ($P = 0.018$). No difference in 6-month neurologic outcome	Laurent et al., J Am Coll Cardiol 2005 [108]
			Rhythm analysis			
Early versus later rhythm analysis in patients with out-of-hospital cardiac arrest	Cluster randomized, controlled, multicenter study of out-of-hospital cardiac arrest	Early rhythm analysis: 30–60 seconds of EMS CPR followed by ECG analysis ($N = 5290$)	Later rhythm analysis: 180 seconds of EMS CPR followed by ECG analysis ($N = 4643$)	Primary outcome: survival to hospital discharge with mRS 0–3 Secondary outcomes: survival to discharge, survival to hospital admission, ROSC	No difference in outcome between a brief and longer period of CPR before ECG analysis of rhythm	Stiell et al., NEJM 2011 [109]

tested an intervention published in English between 1980 and 2012, were included. Only trials examining mortality or neurologic outcome as a primary or secondary endpoint were reviewed. Only trials specific to SAH (not trials that included other neurocritical diagnoses or brain injury diagnoses) were included. Cardiac arrest trials included both out-of-hospital and in-hospital arrest and all arrest rhythms were included. Post hoc analyses of preexisting trials were not reviewed. If phase III results of a trial were available, earlier phases of the same trial were not included in analysis unless the patient population or methodology differed substantially.

A PubMed search of the term "subarachnoid hemorrhage" and "neurologic outcome" with the limits of human, age > 18, English and randomized, controlled trial yielded 23 results. A PubMed search of the term "subarachnoid hemorrhage" and "mortality" with the limits of human, age > 18, English and randomized, controlled trial yielded 78 results. An additional review of articles identified by a broader search of "subarachnoid hemorrhage" with the limits of human, age > 18, English and randomized, controlled trial yielded 244 results. Review of these studies yielded 57 aneurysmal SAH trials that met inclusion criteria and were analyzed. A pubmed search of the terms "cardiac arrest" and "neurologic outcome" with the limits of human, English, age > 18 and randomized, controlled trial yielded 21 results. A PubMed search of the terms "cardiac arrest" and "mortality" with the limits of human, English, age > 18 and randomized, controlled trial yielded 197 results. Review of these studies yielded 46 cardiac arrest trials that met inclusion criteria and were analyzed.

Clinicaltrials.gov was searched for ongoing interventional trials in cardiac arrest and aneurysmal subarachnoid hemorrhage. Only ongoing studies that were open and recruiting or preparing to recruit were included. Terminated studies were excluded from review. A search of ongoing studies on clinicaltrials.gov for the term "subarachnoid hemorrhage", limited to interventional studies of adults ≥18 years old, produced 86 results and a search for the term "cardiac arrest" limited to interventional studies of adults with neurologic outcomes produced 46 results. Of these, 25 ongoing SAH trials and 14 cardiac arrest trials met the criteria for review.

3. Results

3.1. Trials Analyzed. A total of 142 trials (82 SAH, 60 cardiac arrest) met review criteria. Of these, 103 were published in peer-reviewed journals and 39 were ongoing studies. Fifty-seven published randomized, controlled studies were identified in the SAH population and 46 in the cardiac arrest population. These studies are reviewed in detail in Tables 1 and 2. Additionally, 25 ongoing SAH trials and 14 ongoing cardiac trials were reviewed (Tables 3 and 4).

3.2. Interventions Studied. The main hypothetical mechanisms of intervention tested in published SAH trials were related to treating or preventing delayed cerebral ischemia ($N = 40$, 70%), preventing aneurysm rebleeding ($N = 5$, 9%), improving aneurysm repair technique ($N = 5$, 9%),

improving fluid balance ($N = 2$, 4%), and others ($N = 3$, 5%). Among ongoing SAH trials, mechanisms of study include treating or preventing delayed cerebral ischemia ($N = 19$, 76%), limiting rebleeding ($N = 1$, 4%), improving aneurysm repair ($N = 1$, 4%), seizure control ($N = 2$, 8%), and other ($N = 2$, 8%). There are no published or ongoing SAH clinical trials that focus on treating acute brain injury after aneurysm rupture.

Conversely, the main mechanisms of intervention studied in *published* cardiac arrest trials focused on acute intervention to treat and limit early brain injury. All 46 (100%) published cardiac arrest trials focused on the acute time frame (first few hours) after cardiac arrest. Interventions studied included decreasing cerebral metabolic demand with hypothermia or barbiturate ($N = 6$, 13%), high-quality chest compressions or pressor use to return cerebral blood flow ($N = 16$, 35%), electrolyte/metabolic optimization with calcium, magnesium, sodium bicarbonate or insulin administration ($N = 12$, 26%), neuroprotective drugs including calcium channel blockers ($N = 5$, 11%), thrombolysis to treat the underlying cause of cardiac arrest ($N = 2$, 4%) and other ($N = 5$, 11%). Among *ongoing* cardiac arrest trials, mechanisms of study include decreasing cerebral metabolic demand with hypothermia ($N = 9$, 64%), high-quality chest compressions to return cerebral blood flow ($N = 2$, 14%) electrolyte/metabolic optimization with magnesium ($N = 1$, 7%), neuroprotective drugs ($N = 1$, 7%), and monitoring cerebral oxygenation ($N = 1$, 7%). A detailed list of interventions from published and ongoing studies in both the SAH and cardiac arrest population are listed in Table 5.

3.3. Outcome Measures. The most common neurological outcomes assessed in the SAH trials were delayed cerebral ischemia ($N = 24$, 42%), functional outcome (Glasgow outcome scale, modified Rankin scale or functional outcome scale, $N = 24$, 42%), angiographic or transcranial Doppler vasospasm ($N = 6$, 11%), and death ($N = 4$, 7%). Among cardiac arrest trials, the most often assessed neurological outcomes were the Pittsburgh cerebral performance score ($N = 18$, 40%), Glasgow outcome score or modified Rankin Score ($N = 4$, 9%), Glasgow coma score ($N = 4$, 9%), awakening and command following ($N = 3$, 7%), cognitive or neuropsychological testing ($N = 1$, 2%), "disability" ($N = 1$, 2%), death ($N = 13$, 30%), discharge disposition ($N = 1$, 2%) and others ($N = 1$, 2%).

3.4. Trial Results. Of the clinical trials reviewed for SAH, 30% (17/57) showed that the intervention tested had a statistically significant impact on neurological outcome or mortality. These include studies of nimodipine [4–6, 8, 110], phase II data for nicardipine implants during aneurysm clipping [13], the neuroprotectants edavarone [21] and nizofenone [20], pravastatin [25], early aneurysm surgery [28], endovascular coiling [29–31], cilostazol [41], methylprednisolone [44], erythropoietin [45], and fasudil [57, 58]. Similarly, 30% (17/57) of studies showed a positive impact on delayed cerebral ischemia, infarction, angiographic or TCD vasospasm, though there was incomplete overlap with the

TABLE 3: Randomized controlled trials assessing neurologic outcomes after aneurysmal Subarachnoid hemorrhage—ongoing trials.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
			Statins				
Statins and cerebral blood flow in SAH	Randomized, double-blind efficacy study	Simvastatin 80 mg/d for 21 days	Placebo	60	Primary outcome: resting CBF and autoregulation 7–10 days after SAH Secondary outcomes: OEF and $CMRO_2$ 7–10 days after SAH	Michael Diringer, NCT00795288	Uses PET to understand the mechanism of statin use in vasospasm
The role of statins in preventing cerebral vasospasm secondary to subarachnoid hemorrhage	Randomized, double-blind, parallel assignment	Simvastatin 80 mg PO qd × 21 days	Placebo	80	Primary outcome: 6-month clinical outcome	Eberval Figueiredo, NCT01346748	—
Use of simvastatin for the prevention of vasospasm in aneurysmal subarachnoid hemorrhage	Randomized, double-blind, parallel assignment efficacy trial	Tier 1: Simvastatin 40 mg × 21 d or Tier 2: Simvastatin 80 mg × 21 d	Placebo	150	Primary outcome: 21-day GOS, mRS, and Barthel Index Secondary outcome: clinical vasospasm	Ben Roitberg, NCT00487461	—
High-dose simvastatin for aneurysmal subarachnoid hemorrhage (HDS-SAH)	Randomized, parallel assignment, double-blind efficacy study	Simvastatin 80 mg PO × 21 days	Simvastatin 40 mg PO × 21 days	240	Primary outcome: delayed ischemic neurological deficit Secondary Outcomes: LFTs, rhabdomyolysis, 3-month mRS, cost effectiveness	George Wong, NCT01077206	There may be a biochemical and neuroprotective dose-related relationship between simvastatin and delayed ischemic neurological deficits.
Simvastatin in aneurysmal subarachnoid hemorrhage (STASH): a multicentre randomised controlled clinical trial	Randomized, placebo-controlled, double-blind phase III trial	Simvastatin 40 mg PO qd × 21 days	Placebo	1600	Primary outcome: 6-month mRS Secondary outcome: need and intensity of delayed ischemic deficit rescue therapy, incidence and duration of delayed ischemic deficits, incidence and severity of sepsis, length of stay, discharge disposition	Peter Kirkpatrick, NCT00731627	Simvastatin may improve CBF and inflammation following SAH

Table 3: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Aneurysm repair							
International subarachnoid aneurysm trial II comparing clinical outcomes of Surgical clipping and endovascular coiling for ruptured intracranial aneurysms not included in the original ISAT study (ISAT II)	Randomized, open label, safety/efficacy study of WFNS I–IV	Surgical Clipping	Endovascular Coiling	1724	Primary outcome: 12-month mRS > 2 Secondary outcomes: ICH following treatment, failure of aneurysm occlusion, all cause morbidity and mortality, aneurysm recurrence, hospitalization > 20 days or discharge other than home, aneurysm rebleed	Tim Darsaut, Max Findlay, and Jean Raymond, NCT01668563	ISAT included primarily small anterior circulation aneurysms. The optimal treatment of other locations and sizes of aneurysms remains unclear and coiling may not be as durable as clipping
Lipid peroxidation inhibitor							
Acetaminophen in aSAH to inhibit lipid peroxidation and cerebral vasospasm	Randomized, double-blind, placebo-controlled, safety/efficacy trial	Group1: Acetaminophen 1 g q 6 Group 2: NAC IV 0.5 g/h Group 3: Acetaminophen 1 g q6 + NAC 0.5 g/h Group 4: Acetaminophen 1.5 g q 6 + NAC 0.5 g/h	Placebo	120	Primary outcome: F2-IsoP biomarkers for lipid peroxidation. Secondary outcome: vasospasm and brain ischemia as assessed by CTA/CTP or MRI DWI	John Oates, NCT0058559	Hemoglobin released from lysed RBCs oxidizes and generates protein radicals that induce lipid peroxidation. Metabolites of peroxidations (F2-isoprostanes) are potent vasoconstrictors. Acetaminophen can inhibit these metabolites and NAC can inhibit lipid peroxidation
Neuroprotective drugs							
Effects of tiopronin on 3-aminopropanal level and neurologic outcome after aneurysmal SAH	Randomized, double-blind, phase 2 bioavailability	Tiopronin	Placebo	60	Primary outcome: serum and CSF 3AP levels Secondary outcomes: 12 month mRS, Barthel, Lawton, NIHSS, TICS adverse events	E Sander Connolly, NCT01095731	3AP is toxic metabolite produced during cerebral ischemia. It is neutralized by tiopronin
Lycopene following aneurysmal subarachnoid haemorrhage (LASH)	Randomized, double-blind, placebo-controlled, efficacy study	Lycopene 30 mg PO qd × 21 days	Placebo	124	Primary outcome: TCD vasospasm, duration of impaired autoregulation measured by TCD Secondary Outcomes: LDL, oxy-LDL, CRP, circulating endothelial cells, endothelial progenitor cells	Karol Budohoski, NCT00905931	Lycopene is a natural antioxidant that may reduce vascular injury and inflammation and limit vasospasm

Table 3: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Thrombolytics							
Intraventricular tPA in the management of aneurysmal subarachnoid hemorrhage	Randomized, placebo-controlled, double-blind safety trail	tPA intraventricular q 12 h × 5 doses	Placebo administered q 12 h × 5 doses	12	Primary outcome: HCT rate and variance of ventricular and cisternal clot clearance. Secondary outcome: hemorrhagic complications, ventriculostomy-related infections, TCD vasospasm, CT angio vasospasm, symptomatic vasospasm, CSF cytokines and coagulation measurements, ICP, fever burden, volume of CSF drainage, 6-month GOSE and EuroQOL	Andreas Kramer, NCT01098890	Intraventricular TPA may accelerate clearance of IVH ameliorating vasospasm, hydrocephalus, and ICP
Transfusion							
Effect of red blood cell transfusion on brain metabolism in patients with SAH	Open label safety/efficacy study in SAH patients with Hgb < 12.5 g/dL and DCI, high risk for vasospasm or angiographic vasospasm	Transfusion of 1 unit of packed RBC over 1 hour	NA	48	Primary outcome: percent of brain regions with low oxygen delivery before and 1 hour after transfusion. Secondary outcomes: relationship of oxygen delivery and angiographic vasospasm	Michael Diringer, NCT00968227	Uses PET to assess the relationship between Hct and oxygen delivery in SAH patients
Vasodilators							
Sildenafil for prevention of cerebral vasospasm (SIPCEVA)	Randomized, double-blind, placebo-controlled, safety and efficacy study	Tier 1: sildenafil 25 mg PO TID day 3–14 after SAH Tier 2: sildenafil 50 mg PO TID day 3–14 after SAH	Placebo	18	Primary outcome: New neurological deficit due to vasospasm up to 14 days after SAH. Secondary outcomes: TCD spasm, mortality, adverse drug effects, length of stay, discharge mRS	Andre Cerutti Franciscatto, NCT01091870	—
Safety study of dantrolene in SAH	Randomized, double-blind safety study	Dantrolene	Placebo	30	Primary outcome: tolerability, hyponatremia. Secondary outcomes: liver toxicity, hemodynamics, ICP, TCD, angiographic vasospasm treatments, 90-day GOS, mRS, and Barthel	Susanne Muehlschlegel, NCT01024972	Dantrolene is a muscle relaxant that may ameliorate vascular muscle tone and limit vasospasm

Table 3: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Safety and pharmacokinetic evaluation of nitrite for prevention of cerebral vasospasm	Randomized, single-blind, parallel assignment safety study	Tier 1: sodium nitrite 32 nmol/kg/min Tier 2: sodium nitrite 48 nmol/kg/min Tier 3: sodium nitrite 64 nmol/kg/min	Placebo vehicle	18	Primary outcome: pharmacokinetics of 14-day sodium nitrite infusion Secondary outcomes: safety and efficacy	Edward Oldfield, NCT00873015	—
Effects of prostacyclin infusion on cerebral vessels and metabolism in patients with subarachnoid hemorrhage	Randomized, placebo controlled, double-blind, parallel assignment pharmacodynamics study	Tier 1: prostacyclin 1 ng/kg/min day 5–10 after SAH Tier 2: prostacyclin 2 ng/kg/min day 5–10 after SAH	Placebo, IV infusion day 5–10 after SAH	90	Primary outcome: vasospasm measured by CT perfusion Secondary outcomes: cerebral metabolism measured by microdialysis, 3-month GOS, clinical vasospasm, brain tissue oxygen, CT angio vasospasm, MAP, serum S100b	Rune Rasmussen, NCT01447095	Prostacyclin may cause vasodilation and ameliorate vasospasm
Hypertensive, hypervolemic therapy							
Induced hypertension for treatment of delayed cerebral ischemia after aneurysmal SAH HIMALAIA	Randomized, Single blind safety/efficacy study of patients with SAH and DCI (clinically defined)	Induced hypertension with vasopressors and fluids for 48 hours	No induced hypertension	240	Primary outcome: mRS at 3 months Secondary outcomes: proportion of treated patients who did not have clinical improvement of DCI symptoms within 24 hours, 30-day mortality, 3-month Barthel, SSQoL, hospital anxiety and depression scale, cognitive failures questionnaire, hospital complications, CTP results, medical costs	Arjen Slooter and Walter van den Bergh, NCT01613235	CBF measured in all patients using CTP at enrollment and 24–36 hours
Intensive management of pressure and volume expansion in patients with subarachnoid hemorrhage (IMPROVES)	Randomized, single-blind, factorial assignment	Tier 1: hypervolemia + conventional blood pressure Tier 2: normovolemia + hypertension Tier 3: hypervolemia + hypertension	Normal volume, normal blood pressure	20	Primary outcome: achievement of hemodynamic goals in each group	Miriam Treggiari, NCT01414894	Though triple H is a common therapy, its safety and efficacy have not been well quantified

TABLE 3: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
			CSF diversion				
EARLYDRAIN: outcome after early lumbar CSF: drainage in aneurysmal subarachnoid hemorrhage	Randomized, 2-arm controlled trial	Continuous lumbar CSF drainage of 120 mg qd × 7 d	Standard NICU care	300	Primary outcome: 6-month mRS Secondary outcomes: 6-month mortality, angiographic vasospasm, TCD vasospasm, shunt insertion rate at 6 months	Bardutzky J, NCT01258257	Lumbar drainage to remove blood from the basal cisterns may limit delayed cerebral ischemia
Cerebrospinal fluid (CSF) drainage study	Randomized, open label, parallel assignment study of SAH patients requiring external ventricular drainage (EVD)	High volume CSF diversion (EVD at 5 mmHg) × 10 days	Conventional CSF diversion (EVD at 15 mm Hg), weaned at physician discretion	20	Primary outcome: 90-day mRS Secondary Outcome: radiologic infarction, TCD or angiographic vasospasm, shunt placement, ventriculitis, discharge mRS, 90 day mini-mental status exam, length of stay	Giuseppe Lanzino, NCT01420978	More aggressive CSF drainage may improve brain microcirculation and perfusion and lead to better neurological outcomes
			Antiepileptics				
Comparison of short duration levetiracetam to extended course for seizure prophylaxis after subarachnoid hemorrhage	Randomized, prospective, open label, parallel assignment, phase III, safety/efficacy study	Levetiracetam 1000 mg BID × 3 days	Levetiracetam 1000 mg BID × hospital stay	460	Primary outcome: In hospital seizures Secondary outcome: Incidence of seizure after hospital discharge, adverse drug reactions, length of stay, cognitive and functional outcomes	Rajat Dhar, NCT01137110	Antiepileptics can have long-term cognitive side effects. A short course may be just as efficacious as prolonged use
Antiepileptic drugs and vascular risk markers	Randomized, open label, parallel assignment study	Tier 1: phenytoin 5 mg/kg/d divided in 2 doses Tier 2: valproate 15 mg/kg/d divided in 3 doses Tier 3: levetiracetam 1000-1500 mg/d divided in 2 doses	No drug intervention	200	Primary outcome: serum cholesterol, non-HDL cholesterol, HDL, lipoprotein a, CRP Secondary outcome: acute seizures, late seizures, mRS at 8 and 16 weeks	Prema Kishna and Scott Mintzer, NCT00774306	Certain seizure medications may raise cholesterol levels and increase the risk of heart attack and stroke

TABLE 3: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
			Sedation				
Effects of dexmedetomidine on inflammatory cytokines in patients with aneurysmal subarachnoid hemorrhage	Randomized, open label, parallel assignment efficacy study	Dexmedetomidine 0.2–1.5 mcg/kg/h	Propofol 5–80 mcg/kg/min	10	Primary outcome: serum and CSF cytokines over 48 hours Secondary outcomes: sedative and analgesic requirements, RASS and CAM-ICU scores, length of stay, delayed cerebral ischemia, GOSE at discharge	Shaun Keegan and Brittany Woolf, NCT01565590	Dexmedetomidine may cause less inflammation over time than propofol
			Rehabilitation				
Rehabilitation of patients after subarachnoid hemorrhage	Nonrandomized, open label, parallel assignment	Early multidisciplinary rehab and mobilization	No intervention	160	Primary outcome: 10-week GOS Secondary outcome: 3–6 month and 12-month GOSE, functional independence measure, coma recovery scale, disability rating scale, High-level Mobility Assessment tool, pain score	Tanja Karic and Angelika Sorteberg, NCT01656317	Early rehab may reduce complications and improve physical and cognitive function after SAH
			Blood pressure control				
Safety and Efficacy Study of Clevidipine to Control Hypertension in Patients Admitted with Aneurysmal Subarachnoid Hemorrhage (CLASH)	Open label, safety, efficacy study, single group assignment (Phase 2)	Clevidipine IV 2–32 mg/h for 24–48 hours	NA	20	Primary: Blood pressure within target range	Panayiotis Varelas, NCT00978822	To assess how rapidly and safely Clevidipine can be used to control blood pressure in SAH patients.
			Other				
Cervical spinal cord stimulation for the prevention of cerebral vasospasm	Nonrandomized, open label	Spinal cord stimulation using MTS Trial System 3510	NA	12	Primary outcome: cerebral vasospasm Secondary outcome: adverse events	Konstantin Slavin, NCT00766844	—

Table 4: Randomized controlled trials assessing neurologic outcomes after cardiac arrest—ongoing trials.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Neuroprotective drugs							
Selenium to Improve Neurological Outcome after Cardiac Arrest (SCPR)	Randomized, double-blind, placebo-controlled, single-center, phase 2a efficacy study	Sodium-selenite infusion × 7 days	Placebo	52	Primary outcome: neuron-specific enolase Secondary outcomes: inflammation and oxidative stress markers, NIHSS and Glasgow Pittsburgh performance score at 6 months, selenium blood levels, glutathione peroxidase plasma levels	Vanessa Stadlbauer and Karlheinz Smolle, NCT01390506	Selenium can reduce oxidative stress after cardiac arrest and reduce inflammation
Magnesium							
Clinical Study of the LRS ThermoSuit System in Post Arrest Patients with Intravenous Infusion of Magnesium Sulfate	Randomized, double-blind, parallel assignment, safety/efficacy study of any rhythm	Thermosuit to target 34°C plus magnesium sulfate IV (30 mg/kg over 15 minutes)	ThermoSuit to target 34°C plus placebo (normal saline)	14	Primary outcome: cooling rate Secondary outcomes: time to target temperature, percentage of time in target temperature range, shivering, length of stay, neurologic status at discharge and 6 months, adverse events, survival at 24 hours, discharge and 30 days	Michael Holzer and Andreas Janata, NCT00593164	Tests new device to achieve therapeutic hypothermia and the impact of magnesium on cooling performance and hemodynamics
Hypothermia							
Target Temperature Management after Cardiac Arrest (TTM)	Randomized, double-blind, parallel assignment, multicenter, safety/efficacy trial for out-of-hospital cardiac arrest	Target temperature 36°C × 24 h	Target temperature 33°C × 24 h	850	Primary outcome: All cause mortality Secondary outcomes: 6-month composite all cause mortality and poor neurological outcome by cerebral performance scale, bleeding, 6-month neurological status and quality of life, mRS, Cerebral performance scale, mini-mental test, IQCODE, SF-36, adverse events	Niklas Nielsen and Hans, Friberg NCT01020916	Attempts to identify optimal hypothermia target temperature

Table 4: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Hypothermia After in-Hospital Cardiac Arrest (HACAinhospital)	Randomized, single-blind, parallel assignment, single-center, safety/efficacy study for in-hospital arrests of any rhythm	Mild therapeutic hypothermia 32–34°C × 24 hours.	Standard care, no hypothermia	440	Primary outcome: all cause mortality at 6 months Secondary outcomes: 6 month Glasgow-Pittsburgh cerebral performance scale, in-hospital all-cause mortality	Sebastian Wolfrum and Volkhard Kurowski, NCT00457431	Tests whether hypothermia treatment will improve outcome after in-hospital arrest of any rhythm
Intra-arrest Therapeutic Hypothermia in Prehospital Cardiac Arrest (HITUPPAC-BIO)	Randomized, open label, parallel assignment, efficacy trial	Hypothermia induction prehospital	Hypothermia induction at hospital arrival	250	Primary outcomes: brain injury biomarkers at 72 h Secondary outcomes: ROSC, survival 72 h, GCS at 48 hours, cerebral performance scale at 28 days	Guillaume Debaty Jean Francois Timsit, NCT00886184	Assess utility of early hypothermia prehospital
Induction of Mild Hypothermia Following Out-of-hospital Cardiac Arrest	Randomized, open label, single group assignment, efficacy study of any rhythm out of hospital arrest	Rapid infusion of 2 L of 4°C normal saline prior to ED arrival	Standard therapy	1364	Primary outcome: awake and command following at hospital discharge Secondary outcomes: days to awakening, days to death, 3 month neurological outcome	Francis Kim, NCT00391469	Tests whether rapid induction of hypothermia with cold saline infusion is efficacious
Comparing Therapeutic Hypothermia Using External and Internal Cooling for Post-Cardiac Arrest Patients	Randomized, open label, parallel assignment, efficacy trial	External device (Arctic Sun) induced hypothermia	Internal device (Alsius) induced hypothermia	51	Primary outcome: Survival to hospital discharge Secondary outcome: 1 year neurological status	Marcus Ong, NCT00827957	Identifying the most efficient method of cooling may improve outcome after cardiac arrest
Hypothermia + ECMO							
Refractory Out-of-Hospital Cardiac Arrest Treated with Mechanical CPR, Hypothermia, ECMO and Early Reperfusion (CHEER)	Nonrandized, single group, open label, safety/efficacy trail for patients who fail standard resuscitation	Automated CPR, ECMO, coronary angiography, therapeutic hypothermia	NA	24	Primary outcome: survival to hospital discharge Secondary outcomes: cerebral performance Scale, time to ECMO insertion, neurologic biomarkers, cardiac recovery	Stephen Bernard and Dion Stub, NCT0II86614	Aggressive resuscitation may improve outcome in patients who fail standard resuscitation

TABLE 4: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
Hyperinvasive approach to out-of-hospital cardiac arrest using mechanical chest compression device, prehospital intraarrest cooling, extracorporeal Life support and early Invasive assessment compared to standard of care: Prague OHCA Study	Randomized, open-label, parallel group, safety/efficacy study	Prehospital mechanical compression device, intraarrest cooling and in hospital ECLS (compression device, Rhinochill, PLS ECMO)	Standard care	170	Primary outcome: composite endpoint of survival with good neurological outcome (cerebral performance scale) Secondary outcome: 30 day cerebral performance scale, 30 day cardiac recovery	Jan Belohlavek and Ondrej Smid, NCT01511666	Aggressive, early intervention may improve cerebral outcomes
Emergency Preservation and Resuscitation (EPR) for Cardiac Arrest from Trauma (EPR-CAT)	Nonrandomized, open label, parallel assignment, safety/efficacy study	Profound hypothermia <10°C with cold saline infusion into aorta followed by resuscitation/rewarming with cardiopulmonary bypass	Standard treatment	20	Primary outcome: survival to hospital discharge without major disability by GOSE Secondary outcomes: achieving target temperature in 1 hour, 28 day survival, 6 month neurological function, multiple organ system dysfunction	Samuel Tisherman, NCT01042015	Resuscitation technique for trauma patients that have arrested from exsanguination
HypotherMia + xenon							
Effect of xenon and therapeutic hypothermia on brain and on neurological outcome following brain ischemia in cardiac arrest patients nonperfusing ventricular tachycardia (Xe-hypotheca)	Randomzied, open label, parallel assignment, phase 2 safety/efficacy trial for ventricular fibrillation and nonperfusing ventricular tachycardia	Hypothermia 33°C × 24 h and Xenon inhalation × 24 hours target end tidal 40%	Hypothermia 33°C × 24 h	110	Primary outcome: PET and MRI ischemia at 24 hours and 10 days Secondary outcomes: neurological outcome at 6 months, TTE	Timo Laitio, NCT00879892	Xenon may be synergistically neuroprotective in combination with hypothermia post arrest by limiting cerebral hypoxia, neuronal loss, and mitochondrial dysfunction
Chest compresions							
Continuous chest compressions	Randomized, open label, multicenter, crossover assignment study of out-of-hospital cardiac arrest of any rhythm	Continuous chest compressions	Interrupted chest compressions with ventilation 30:2	23600	Primary outcome: survival to hospital discharge Secondary outcomes: mRS at discharge, adverse events	Myron Weisfeldt, NCT01372748	Continuous CPR without interruption for ventilation may be superior to interrupted compression with ventilation ratio of 30:2

TABLE 4: Continued.

Trial name	Study design	Treatment group	Control group	Target enrollment	Outcome measure	PI	Comments
LUCAS chest compressor versus manual chest compression in out-of-hospital sudden cardiac arrest: LUCAT trial	Randomized, open label, parallel assignment, efficacy study	Mechanical continuous chest compressions performed by LUCAS device	Manual chest compressions	400	Primary outcome: survival to hospital admission, survival to discharge with good neurological state by cerebral performance Scale Secondary outcomes: ROSC, end tidal CO_2, SOFA scale, length of stay, metabolic and inflammatory markers, LV function	Francesc Carmona Jimenez, Rosa-Maria Lidon, NCT01521208	Mechanical chest compression may be superior to manual chest compression
			Cerebral oxygenation				
Cerebral Oxygenation in Cardiac Arrest and Hypothermia	Open label, safety and efficacy study	Near-infrared monitoring	Standard therapy, no monitoring	70	Primary outcome: survival to discharge Secondary outcomes: cerebral performance scale at discharge and 12 months	Christian Storm, NCT01531426	Near-infrared spectroscopy (NIRS) could be a new-noninvasive marker for outcome after cardiac arrest. Low NIRS may correlate with poor outcome

TABLE 5: Number of randomized, controlled trials published and ongoing for aneurysmal subarachnoid hemorrhage and cardiac arrest.

Intervention	SAH		Cardiac arrest	
	Published N (%)	Ongoing N (%)	Published N (%)	Ongoing N (%)
Calcium channel blockers	10 (18)	0	4 (9)	0
Antifibrinolytics	5 (9)	0	0	0
Neuroprotective drugs	5 (9)	2 (8)	1 (2)	1 (7)
Statins	4 (7)	5 (20)	0	0
Aneurysm clip or coil	4 (7)	1 (4)	NA	NA
Lipid peroxidation inhibitor	4 (7)	1 (4)	0	0
Thrombolytics	3 (5)	1 (4)	2 (4)	0
Antiplatelets	3 (5)	0	0	0
Steroids	3 (5)	0	1 (2)	0
Transfusion/blood products/erythropoietin	3 (5)	1 (4)	0	0
Vasodilators	3 (5)	4 (16)	0	0
Pressors or HHH	2 (4)	2 (8)	9 (19.5)	0
Magnesium	2 (4)	0	5 (11)	1 (7)
Rho-kinase inhibitor (fasudil)	2 (4)	0	0	0
Adrenergic blockade	1 (2)	0	0	0
Endovascular therapy	1 (2)	0	NA	NA
Insulin/glucose control	1 (2)	0	2 (4)	0
Hypothermia	1 (2)	0	5 (11)	9 (64)
CSF diversion	0	2 (8)	0	0
Antiepileptics	0	2 (8)	0	0
Sedation	0	1 (4)	0	0
Rehabilitation	0	1 (4)	0	0
Blood pressure	0	1 (4)	0	0
Other	0	1 (4)	0	0
Chest compressions	NA	NA	7 (15)	2 (14)
Adenosine antagonist	0	0	1 (2)	0
Fluid management	0	0	1 (2)	0
Barbiturate	0	0	1 (2)	0
Cerebral oxygenation	0	0	0	1 (7)
Calcium chloride	0	0	3 (7)	0
Sodium bicarbonate	0	0	2 (4)	0
Hemofiltration	0	0	1 (2)	0
Rhythm analysis	0	0	1 (2)	0
Total	57	25	46	14

above studies that showed outcome benefit. Eight studies found both a significant improvement in delayed cerebral ischemia/vasospasm/infarction and outcome including studies of nimodipine [4, 6, 8], nicardipine implants in the basal cistern [13], edavarone [21], pravastatin [25], fasudil [57], and erythropoietin [45]. Nine studies found a benefit for decreasing delayed cerebral ischemia/vasospasm/infarction but no neurologic outcome benefit including studies of IV nicardipine [10, 11], eicosapentaenoic acid (omega-3 fatty acid) [22], the neuroprotectant NA-1 [23], simvastatin [24], tirilazad [34], intracisternal rTPA [36, 38], and clazosentan

[49, 50]. Three studies found improved neurologic outcome despite an insignificant effect on delayed cerebral ischemia/vasospasm/infarction including studies of cilostazol [41], methylprednisolone [44], and fasudil [58].

Among the cardiac arrest trials, 13% (6/46) demonstrated neurologic or mortality benefit. Improved mortality rates were demonstrated with mild therapeutic hypothermia [89], coenzyme Q10 [68], vasopressin plus epinephrine plus methylprednisolone [71], active compression-decompression CPR [93], and hemofiltration [108]. Improved neurological outcome was demonstrated with early mild therapeutic

hypothermia for ventricular fibrillation and pulseless ventricular tachycardia arrests [88, 89], and one study of active compression-decompression CPR [93], though a larger study of active compression-decompression was negative [94].

3.5. Trial Overlap. Though nimodipine has demonstrated mortality and functional outcome benefit in SAH [4–6, 8, 110], it has shown no benefit in cardiac arrest trials [64, 65, 67]. Similarly, intracisternal thrombolysis showed some benefit in reducing delayed cerebral ischemia and infarction after SAH [36, 38], but intravenous tenecteplase showed no long-term benefit and, in fact, increased intracranial hemorrhage after cardiac arrest [69, 70]. Neither magnesium [53, 54, 81–85] nor intensive insulin [59, 87] has proven beneficial after SAH or cardiac arrest. Though hypothermia [88, 89] has been the single most effective treatment for cardiac arrest (the number needed to treat to prevent one death is 7 and the number needed to treat to produce favorable neurological outcome is 6), it has not proven useful in the context of aneurysm surgery after SAH [60]. There is little mechanistic overlap in ongoing randomized, controlled trials of SAH and cardiac arrest patients.

4. Discussion

In this paper, a direct comparison is made between randomized, controlled clinical trials that evaluate mortality or neurologic outcome after SAH and cardiac arrest. Though 28% of SAH studies showed some neurologic outcome benefit in the intervention group, only nimodipine [4–6, 8, 110], fasudil [57, 58], and endovascular coiling [29–31] have been found to consistently improve outcome in multiple, multicenter randomized controlled trials. Smaller studies [8, 41, 58], single center [21, 44], or phase II safety and feasibility studies [13, 25, 45] have shown outcome benefit, but still require larger efficacy trials before integration into standard practice. Among cardiac arrest trials, only mild therapeutic hypothermia has been shown to improve both mortality and neurologic outcome [88, 89]. Little overlap in trial results or mechanisms of study was identified in these different patient populations.

Methodological differences in the timing, duration, neurological severity, and outcomes studied may explain some of the differences in trial results between SAH and cardiac arrest populations. First, the timing of intervention for SAH and cardiac arrest trials is quite different. With the exception of aneurysm repair and aneurysm rebleeding trials (some of which were carried out in the era of delayed surgical treatment), the vast majority of SAH trials focus on the delayed cerebral ischemia period. Conversely, all cardiac arrest trials are directed at intervening against early brain injury. The difference in time frames studied may explain, in part, the variable results for mild therapeutic hypothermia in each population. Unlike the cardiac arrest trials, which applied hypothermia either prior to ED arrival [88] or within a median of 105 minutes from return of spontaneous circulation (ROSC) [89] for a duration of 12–24 hours, hypothermia was applied in the IHAST trial at a median of two days from

SAH onset and only for a brief time (median 5-6 hours) [60]. Second, patient selection may result in variable trial results for hypothermia. For example, hypothermia for cardiac arrest was used for comatose survivors, while relatively neurologically intact patients (WFNS I–III) were studied in the IHAST trial. Finally, outcome measures differ in the cardiac arrest and SAH literature. Many cardiac trials measure 30-day or discharge mortality or neurologic outcome, while SAH trials measure outcomes from 3 months to 1 year. Though the majority of cardiac arrest trials measure neurologic outcome using the Pittsburgh cerebral performance scale, while SAH trials utilize the Glasgow outcome scale or modified Rankin scale, all of these scales are very similar and provide gross estimates of disability. Despite the aforementioned methodological differences, certain interventions, such as magnesium and intensive insulin, have not proven effective in either population.

Another reason for variable outcome in clinical trials may be due to pathophysiological differences in SAH and cardiac arrest. Though early brain injury in SAH may mechanistically mirror the cascade of injury occurring after cardiac arrest, SAH differs from cardiac arrest in that it is not a monophasic disease. Break down of blood products initiates a distinctive series of delayed clinical events that characteristically can lead to ischemia or infarction between SAH days 3–14. The fact that nimodipine has been so successful in SAH trials, but shown no effect at similar doses in cardiac arrest trials suggests it is acting on a distinct pathway. Indeed, the absolute risk reduction for poor outcome after SAH in a meta-analysis of 16 trials of nimodipine is 5.3% with a number needed to treat for benefit of 19 [111]. No such signal for benefit was seen in cardiac arrest trials [64, 65, 67]. The mechanism of beneficial effect of nimodipine in SAH has been widely debated and may be related to its effect on fibrinolysis [112], spreading cortical depression [113], or excitotoxicity. Though nimodipine improves ischemic neurological deficits by clinical criteria and CT-documented infarction (with a pooled relative risks of 0.66 (95% CI 0.59–0.75) and 0.78 (95% CI 0.70–0.87), resp.) [111], it has little effect on angiographic vasospasm or cerebral blood flow [4, 5]. The corollary to this observation is that interventions that improve angiographic vasospasm, such as clazosentan, do not necessarily improve cerebral infarction or outcome. [49, 50, 114, 115]. While angiographic vasospasm seems to be related to infarction [116], other mechanisms may play a role in neurological deficits, cerebral infarction, and outcome. Such pathophysiological differences may make extrapolation of results from cardiac arrest trials to an SAH population problematic. Indeed, delayed cerebral ischemia (DCI) may blunt the positive effect of hypothermia on early brain injury. Further animal research may better identify mechanistic differences of early brain injury in cardiac arrest and SAH.

Despite a second wave of neurological injury in SAH, poor-grade (Hunt Hess 4-5) SAH patients, who are at higher risk for secondary neurological injury, still have comparable, if not better, outcomes compared to cardiac arrest patients who are not cooled. Among Hunt-Hess grade 4-5 patients, the 12-month mortality rate with aggressive treatment is 43%, while 40% had no or slight-moderate disability (mRS 0–3)

[117]. By comparison, the 6-month death rate in the control (nonhypothermia) group of the HACA trial was 55%, while good neurologic outcome (defined as Pittsburgh cerebral performance scale 1-2; good outcome or moderate disability) occurred in 26–39% [88, 89]. We have additionally shown that DCI does not predict mortality after SAH with aggressive vasospasm treatment, while early brain injury (measured by Hunt-Hess grade) does [1]. Thus, despite secondary neurologic insults and delayed cerebral ischemia risk, poor-grade SAH patients do at least as well as normothermic cardiac arrest patients, who may face risks to survival and functional outcome related to the underlying cause of the cardiac arrest. Also, the median age of cardiac arrest patients tends to be older than SAH patients, which may also explain why even the sickest SAH patients have relatively good outcomes by comparison. If nihilism can be overcome in the management of poor-grade SAH patients, the early application of mild therapeutic hypothermia may improve outcomes further.

There are some limitations to this review that should be mentioned. A medical librarian was not used and only MEDLINE/PubMed and clinicaltrials.gov were used to identify literature for review. An Embase search was not performed. Additionally, an exhaustive search for all neurologic outcome based RCTs was not performed, rather only English studies in humans were included.

In conclusion, while the mechanisms of early brain injury after SAH and cardiac arrest may be similar, the preponderance of SAH clinical trials do not focus on interventions addressing early brain injury. Clinical trials in SAH assessing interventions that have proven successful in the cardiac arrest literature, such as early mild therapeutic hypothermia, are warranted.

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Review Article

Early Brain Injury: A Common Mechanism in Subarachnoid Hemorrhage and Global Cerebral Ischemia

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Early brain injury (EBI) has become an area of extreme interest in the recent years and seems to be a common denominator in the pathophysiology of global transient ischemia and subarachnoid hemorrhage (SAH). In this paper, we highlight the importance of cerebral hypoperfusion and other mechanisms that occur in tandem in both pathologies and underline their possible roles in triggering brain injury after hemorrhagic or ischemic strokes.

1. Introduction

Aneurysmal subarachnoid hemorrhage (SAH) is associated with significant morbidity and mortality, accounting for up to ~5% of all stroke cases [1, 2]. The mortality from SAH is estimated at 40–45% by 30 days after hemorrhagic onset and up to 15% mortality before hospital admission [3]. After years of research and extensive pathophysiological investigations of SAH, much is known in animal models about pathways that are activated after SAH and that may contribute to brain injury. However, few have proven to be effective therapeutic targets in humans [4, 5].

SAH has been suggested in multiple reports to be complex, multisystem, and multifaceted pathogenesis that likely has multiple ongoing processes activated contributing to its final pathogenesis and highly morbid manifestations [4–8]. There are some common effects, however, such as vasoconstriction of both large and small cerebral arteries. As a result, it is difficult to research one pathway, one protein, and one target for potential therapeutic benefits. There has been a shift in research to understand how all the manifestations connect, interact, and further contribute to this pathology.

Many strides have been made to understand the common secondary complications that occur after SAH, especially focusing on complications that occur early on, often known as early brain injury (EBI) [9, 10]. Some of the complications that EBI encompasses are delayed neuronal injury/death (DND), oxidative stress and inflammatory destruction of the parenchyma, and ischemic deficits leading to cortical spreading depression (CSD). These complications have been theorized to play a major role in the pathogenesis and may contribute significantly to poor morbidity and outcome after SAH.

Individual studies on several secondary complications have shed light on shared mechanisms and pathways that may be activated after or during or even before the hemorrhage, which may explain a number of these secondary manifestations. Research has also shifted from considering primary angiographic vasospasm as a major contributor to poor outcome to other secondary mechanisms that may also occur early on during the hemorrhage and interact with angiographic vasospasm and predispose the brain to significant delayed injury and poor outcome [10–13].

Recent research has proposed additional mechanisms behind brain predisposition to injury and poor outcome, some of which include global ischemia, delayed cerebral ischemia (DCI), and cortical spreading depression (CSD) [14–16]. Recent work has also focused on trying to delineate the fundamental differences between ischemic deficits and hemorrhagic insult and how early brain injury (EBI) after SAH may be linked to transient global ischemia or may be actually a result of an ischemic deficit introduced early on by the hemorrhage. Does transient global ischemia occur before or during the hemorrhage, and thus predisposing the brain to the secondary complications mentioned? Or is transient global ischemia a separate entity that has its own manifestations, mechanisms, and complications, separate from those pertaining to SAH?

In this paper we discuss the secondary complications that arise after SAH, its relationship to the pathogenesis, and recent work that has been done to decipher their triggers and roles in poor outcome. Additionally, we will discuss the similarities in pathogenesis between global ischemia and SAH.

2. Global Cerebral Ischemia and Stroke

Ischemia is generally defined as a diminution of cerebral blood flow (CBF) below critical thresholds, resulting in a damage to the entire brain (global ischemia which is necessarily transient if the patient is to survive, and thus it is this type of global ischemia that is often investigated in animal models) or a focal region to which perfusion is relatively low [17, 18]. Global cerebral ischemia occurs when the blood supply to the entire or large part of the brain is impeded [19]. Global cerebral ischemia may also arise from a number of clinical conditions such as cardiac arrest that lasts more than about 10 minutes [19]. This transient insult may result in permanent brain damage and other parenchymal changes that are not completely understood. Since the majority of global cerebral ischemic insults occur due to cardiac arrest, a substantial effort has been allotted to establish protocols for proper management and efficient resuscitation protocols for cardiac arrest patients [19]. Despite optimal resuscitation and adequate ongoing supportive measures, the postarrest period is often accompanied by ongoing cerebral ischemia or no reflow to multiple regions in the brain. This phase of cerebral ischemia is followed by a short phase of cerebral hyperaemia and a prolonged phase of hypoperfusion that lasts from several hours to days and which correlates with significant neurocognitive, behavioural, sensory, and motor deficits [19].

Other types of stroke including SAH are associated with a similar pattern of ischemic insult to the brain and may share similarities in cellular pathophysiology. SAH in rats was associated with an upregulation of vasoconstriction-mediating receptors, endothelin B (ET_B), and serotonin receptors ($5-HT_{1B}$) and with reductions in vasodilators like nitric oxide (NO) [20, 21]. Similarly, in a model of transient global ischemia in rats, Johansson and colleagues demonstrated that animals had prolonged neurological deficits as well as functional upregulation of the same ET_B and $5-HT_{1B}$

receptors in forebrain cerebral arteries. These findings suggest the contribution of cerebral artery vasoconstriction, cerebral hypoperfusion, and neuronal damage to transient global ischemia, which mimics similar findings in SAH [22].

3. Secondary Complications after SAH

3.1. Early Brain Injury: Delayed Neuronal Injury. Cells die after stroke primarily by apoptosis or necrosis [23]. Both are thought to occur after global cerebral ischemia and SAH. The exact pathways activated in these types of stroke are not entirely worked out and there may be different contributions of apoptotic and necrotic cell death. It is documented that transient global cerebral ischemia can trigger multiple cellular events and activate pathways which lead to both apoptotic and necrotic cell death in endothelial, glial, and neuronal cells [24].

During aneurismal rupture causing SAH, the intracranial pressure can increase enough to cause global cerebral ischemia. In some cases, if the bleeding continues and the intracranial pressure does not decrease, then the patient dies immediately, probably secondary to acute cardiac changes secondary to the increased intracranial pressure and near-instantaneous brain death. In survivors, however, the contribution of transient global ischemia to brain injury is variable. Some patients have very small hemorrhages, do not loose consciousness, and thus do not have transient global ischemia. They are still at risk for DCI [25]. Interestingly, patients who become transiently unconscious at the time of their SAH and then awaken have likely had a transient global cerebral ischemic event and may have been at a higher risk of developing DCI [26]. Patients also only develop acute focal cerebral ischemia immediately after SAH in about 3% of cases [27].

Cellular apoptosis is reported to be a mechanism of EBI after the SAH and has been investigated in several studies. These studies focused on large cerebral arteries and found endothelial cell apoptosis after SAH [28, 29]. Neuronal apoptosis in the cortex and hippocampus has been detected after SAH in humans [30]. In animal studies, neurons, astrocytes, and oligodendroglia also exhibited apoptosis after SAH [31]. In some studies, there were fewer neurons in the hippocampus and inner cortical layers 5 days after SAH in rats [32].

The pathways involved in apoptosis after SAH have not been widely investigated. The apoptotic pathways include intrinsic (caspase-independent and mitochondrial) and extrinsic (cell-death receptor) pathways [33–35].

Ischemia caused by increased intracranial pressure (ICP) is probably the first process that activates apoptosis. Apoptosis was observed within minutes of SAH in a rat endovascular perforation model of SAH and persisted for at least 24 hours [12, 35]. Ischemia following a SAH causes apoptotic cell death within the brain through several pathways such as induction of heat shock protein 70 (HSP70) [36]. HSP70 is a sensitive biomarker, which is activated diffusely throughout the brain one day after SAH is induced by endovascular perforation in rats. It continues to be activated 5 days after the SAH [36]. Ischemia also is associated with excitotoxic

mechanisms that are mediated through the efflux of the amino acid glutamate. Glutamate activates the n-methyl-d-aspartate (NMDA) receptor following ischemia, resulting in an influx of sodium and calcium into neurons and subsequent neuronal death [37]. This mechanism has been suggested to cause neuronal apoptosis *in vitro* and *in vivo* [38].

The death receptor pathway has been implicated in apoptosis after cerebral ischemia and SAH. This pathway is activated by multiple cell membrane receptors, including the tumor necrosis factor receptor (TNFR), Fas, and DR3-5 [6, 29]. The ligands for these receptors include TNF-α, TNF-related apoptosis-inducing ligand, and Fas ligand. This pathway is activated by cerebral ischemia [23]. It has been shown that TNF-α is upregulated in the endothelium of dog basilar artery after SAH, and the inhibition of this with broad spectrum apoptosis inhibitors prevented vasospasm [39]. The dogs also had improved neurological outcomes [39]. TNF-α binding to TNFR activates caspase 8 and in some cases caspase 10. Downstream caspases are then activated, including caspases 3 and 9. Caspase 3 is a common essential component in the apoptotic pathway [39]. Cleaved caspase 3, a component of the intrinsic, caspase-dependent pathway, was detected in hippocampus and cortex after experimental SAH [12, 40]. The mitochondrial apoptotic pathway is likely involved in cerebral ischemia. Akt (protein kinase B) and mitogen-activated protein kinase (MAPK) are protein kinases that, when activated, inhibit apoptosis by interacting with Bax, Bad, glycogen synthase kinase-3, apoptosis signal-regulating kinase 1, and caspase 9. Akt activity is reduced after cerebral ischemia and its prevention reduced ischemic neuronal death [35]. Inhibiting Akt phosphorylation, which activates it, was associated with EBI after experimental SAH, and overexpression of Akt reduced brain injury [34, 41]. The MAPK may also be involved in EBI [35].

Other mechanisms include caspase-independent intrinsic cell death pathway involved mitochondrial apoptosis-inducing factor (AIF), endonuclease G, and Bcl2/adenovirus E1B 19 kDa-interacting protein (BNIP3) [35]. Nuclear translocation of AIF was found after cerebral ischemia, suggesting the activation of this pathway; however, its role in SAH is less well studied [42].

Autophagy is a process where cells form a multimembrane bound structure called the autophagosome, which sequesters cytoplasm and cell organelles in order to degrade them and recycle cytoplasm [43]. It occurs at basal levels in many tissues and is important in development, differentiation, and remodeling of organs and tissues. Autophagy is linked to apoptosis, but it is unclear if it causes cell death or is activated by some apoptotic pathways [44]. Autophagy has been suggested to provide a neuroprotective role in maintaining cellular homeostasis [43]. On the other hand, under certain conditions, it can have deleterious neurodegenerative effects [45]. After experimental SAH, autophagy has been observed in neurons and astrocytes of the basal frontal cortex on electron microscopy and in brain homogenates by an increase in the amount of membrane-bound microtubule-associated protein I light chain 3 [46]. Cathepsin D, an enzyme associated with degradation of damaged proteins and beclin-1, is also associated with autophagy

and also is significantly higher after SAH [46]. Beclin-1 is a protein that interacts with Bcl-2 which is integral in the autophagic process [47]. Activation of autophagy with rapamycin reduced brain injury markers after SAH, whereas inhibition of autophagy with 3-methyladenine aggravated brain injury. This suggests that autophagy plays a neuroprotective role following SAH [44, 47].

As discussed above, all of the apoptosis pathways are also likely important in global ischemic deficits after ischemic stroke [19] and may indeed account for some of the EBI after SAH. While neurons and other brain cells die by apoptosis after cerebral ischemia, the predominant mechanism of cell death is caused by necrosis, especially in the core of the ischemic brain [24]. Furthermore, activation of the death receptor pathway in apoptotic-deficient situations causes a sort of a combined form of cell death called necroptosis. Reports have demonstrated that neurons in the core tend to demonstrate liquefaction necrosis, while neurons in the penumbra tend to undergo apoptosis [23, 48, 49]. Apoptosis after cerebral ischemia occurs through intrinsic and extrinsic pathways [48, 50]. In the intrinsic pathway, ischemia results in the generation of permeability pores in the inner mitochondrial membrane, which results in the release of a number of proapoptotic factors and ultimately results in deoxyribonucleic acid (DNA) fragmentation and necrosis [51, 52]. The mitochondrial independent pathways after global ischemia tend to activate death receptors such as TNFR and Fas. Caspases also tend to play a major role in apoptotic activation in both cerebral ischemia and SAH [52, 53].

4. Nitric Oxide and Nitric Oxide Synthases (NOS)

Nitric oxide has a physiological role as a vasodilator and inhibitor of platelet activation and inflammation [54]. Reduction in NO is thought to contribute to angiographic vasospasm after SAH [55, 56] as well as to EBI [57, 58]. Within 10 minutes of SAH in rats, there is acute vasoconstriction probably due to scavenging of NO [58]. NO concentrations subsequently increase above basal levels at 24 hours after SAH [59]. Another mechanism by which NO and NO synthases (NOS) can cause angiographic vasospasm and brain injury is by endothelial NOS uncoupling. This was demonstrated in the brain tissue of mice with SAH, in which there also was increased superoxide and nitrotyrosine production, and significant reduction in NO formation due to the dysfunction of eNOS [60]. Thus, while NO from eNOS might cause vasodilatation and reduce angiographic vasospasm and brain injury, under some conditions it could also be detrimental [61].

While most of the studies in SAH rely on pharmacological manipulations, the contribution of NO to ischemic stroke and transient global ischemia has been assessed in genetically manipulated mice [62, 63]. Mice with reduced neuronal or inducible NOS have reduced infarct sizes, whereas those with eNOS reduction have more [63].

5. Oxidative Stress

Reactive oxygen and nitrogen (such as peroxynitrite) species are hypothesized to be important in brain injury after cerebral ischemia and SAH. Multiple studies show that there is release of reactive oxygen species (ROS) after experimental and human SAH [64–69]. Reactive oxygen species can exacerbate inflammation and generalized oxidative stress after SAH by increasing lipid peroxidation, causing direct DNA damage and protein oxidation. These processes in turn activate apoptotic signals and inflammatory cascades that further damage the brain [66]. A major source of ROS after SAH is thought to be oxidation reactions catalyzed by the heme groups of hemoglobin that are obviously abundant in the subarachnoid space after SAH [67].

Reactive oxygen species can be generated by NOS isoforms (endothelial, neuronal, and inducible NOS). Multiple reports have demonstrated that under oxidative environments, NOS, particularly eNOS, can contribute to overproduction of peroxynitrite due to the reaction of NO and superoxide anion radicals [70]. Peroxynitrite oxidizes tetrahydrobiopterin, a cofactor for eNOS, and the zinc-thiolate complex in eNOS, which can uncouple eNOS and lead to generation of superoxide anion radicals [70]. Another source of superoxide anion radicals in the cerebral vasculature is the membrane-bound enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [71]. NADPH oxidase transfer electrons from NADH or NADPH to molecular oxygen through flavins that are present in the protein structure of the enzyme. This also generates superoxide anion radical which seems to be produced continuously at a low level in cerebral arteries. Structurally, NADPH oxidase has both membrane bound and cytosolic subunits. Functionally, it is a constitutively active enzyme that can mediate vasodilatation, for example, in rabbit cerebral arterioles in $vivo$ [72]. The role of NADPH oxidase in the pathophysiology of SAH has not been widely investigated. In one study, inhibition of NADPH oxidase with diphenyleneiodonium reduced middle cerebral artery vasospasm after SAH in rats [73]. Vascular production of superoxide anion radical and NADPH oxidase activity were increased 24 hours after SAH in this model, and this was associated with membrane translocation of p47phox, one of the NADPH oxidase subunits.

Another source of oxidative stress after SAH may be xanthine dehydrogenase. This enzyme is found in endothelial cells where it produces uric acid from purines [74]. Ischemia can convert it to xanthine oxidase, which produces uric acid, superoxide anion radical, and hydrogen peroxide. This is involved in the pathophysiology of brain injury after cerebral ischemia. After SAH, Marklund and colleagues found that delayed cerebral ischemia was associated with increased concentrations of hypoxanthine, allantoin, and uric acid in cerebral microdialysis samples, possibly due to xanthine oxidase activity [75]. However, after experimental SAH in dogs, uric acid was increased in the cerebrospinal fluid, and this was inhibited with allopurinol [76]. This did not reduce angiographic vasospasm.

Reactive oxygen species are also postulated to be important in cerebral ischemia and infarction. Nitric oxide also can be beneficial after ischemia by mediating vasodilation. However, it can also have toxic effects by, for example, inhibiting complexes I and II in the mitochondrial transport chain [19]. Also, as mentioned above, it can react with superoxide anion radical to produce peroxynitrite. Peroxynitrite generation promotes formation of other ROS such as hydroxyl-free radical and nitrogen dioxide. These nitrosylate tyrosine residues in proteins can result in further structural parenchymal damage [77]. NO has also been shown to upregulate the activity of poly (ADP-ribose) polymerase which leads to neuronal death through ATP consumption [77]. Additionally, in cerebral ischemia, constitutive NOS activity from the endothelial and neuronal NOS isoforms can be increased due to activation of various glutamate receptors, resulting in increased intracellular calcium and cytotoxicity [19]. In general, infarct volume and outcomes are worse in mice lacking eNOS, supporting the beneficial role of vascular endothelial NO [62]. On the other hand, genetic deletion of nNOS or iNOS tends to improve outcomes.

6. Inflammation

Inflammation is hypothesized to mediate brain injury and angiographic vasospasm after SAH [78, 79]. There is an increase in proinflammatory cytokines, including TNF-α, interleukin 1-β (IL1-β), and IL6 acutely after experimental SAH [80–82]. Additionally, it has been demonstrated that pharmacologic inhibition of TNF-α or IL1-β attenuated EBI and improved blood-brain barrier (BBB) function after SAH [80, 81]. Another protein involved in proinflammatory cascade activation is NF-κB, a transcription factor in endothelial cells, that becomes phosphorylated resulting in the subsequent inactivation of IκB-α [83]. When NF-κB was activated in the arterial wall, there was an increase in TNF-α, IL1-β, and adhesion molecules. Pyrrolidine dithiocarbamate, an inhibitor of NF-κB, reduced vasospasm and the increase in the inflammatory cytokines and adhesion molecules. Leukocytes play a role in the immune response following SAH through their role in activating cytokines such as endothelin-1, a power vasoconstrictor that becomes elevated in experimental and clinical SAH [84]. In a study of 224 patients with SAH, a leukocyte count of greater than 15×10^9/L was associated with a 3.3-fold increase in the probability of developing angiographic vasospasm [85].

Selectins are from a family of cellular adhesion molecules that play a role in the inflammatory response [79]. They are categorized into leukocyte (L) selectin, platelet (P) selectin, and endothelial (E) selectin, which together mediate the capture, rolling, and adhesion of leukocytes in blood vessels [79]. Functionally, E selectin acts through binding to a carbohydrate site on the leukocytes that helps leukocytes target the site of inflammation. An increase in selectins in cerebrospinal fluid of patients with SAH and in animal models of SAH supports their role in the recruiting of leukocytes to cerebral vessels and brain after SAH [86]. Immunohistochemistry of ruptured cerebral aneurysms found increased E selectin in the aneurysm wall, which could also be a contributor [87].

Brain damage after transient global ischemia involves similar pathways to those activated after SAH [19]. Cerebral ischemia leads to migration of peripheral neutrophils and monocytes into the brain. Multiple proinflammatory cytokines are released by neurons and glia, leading to increased selectin and adhesion molecules on cerebral blood vessels, similar to what is observed after SAH. Cytokines are also similarly involved in the pathogenesis of brain injury due to ischemia. Interleukin-1 beta (IL-1β) has been reported to play a detrimental role in brain injury, while proinflammatory IL-6 and anti-inflammatory cytokine IL-10 have uncertain roles [19]. Additionally, TNF-α has been found to either aggravate ischemic brain injury or to promote development of ischemic tolerance [19, 88].

7. Blood-Brain Barrier Disruption and Brain Edema

Brain edema is a well-documented phenomenon that occurs days after experimental SAH in multiple animal models [6, 89]. Claassen et al. also concluded, based on interpretation of CT scans, that about 10% of patients had global cerebral edema within 24 hours of SAH [90]. Global cerebral edema was an independent risk factor for poor outcome and mortality. Brain edema may develop due to BBB dysfunction, which is also documented after acute experimental SAH [7, 8, 29]. Multiple processes may contribute to BBB breakdown after SAH, including endothelial cell apoptosis [29]. Blood breakdown products such as oxyhemoglobin and oxidative stress caused by hemoglobin can contribute to BBB disruption [91]. Additionally, proinflammatory cytokines like TNF-α and thromboxane A$_2$ cause endothelial cell apoptosis and contribute to BBB dysfunction [92]. Inflammatory cytokines increase matrix metalloproteinases (MMP) that also disrupt the BBB. Yan et al. reported that inhibition of p53 ameliorated endothelial cell apoptosis and attenuated BBB disruption and brain edema after SAH in rats [93].

Accumulating evidence suggests a role for MMP-9 in the early disruption of the BBB after SAH [94]. MMP-9 degrades the extracellular matrix of the cerebral microvessel basal lamina, which includes collagen IV, laminin, fibronectin, and interendothelial tight junction proteins such as zona occludens-1 [95–97]. Basal lamina degradation starts as early as 6 hours and peaks 48 hours after experimental SAH created by endovascular perforation in rats [98]. Similar to after SAH, in after cerebral ischemia there is a release of proinflammatory cytokines like TNF-α and IL-1β from glia, leading to generation of adhesion molecules in the vasculature which can result in the weakening of the BBB [99, 100]. Thus, BBB disruption occurs after both a SAH and cerebral ischemia and predisposes to fluid/protein extravasation into the interstitial space resulting in cerebral edema.

8. Excitotoxic Amino Acids

Excitatory amino acids may play a role in the pathogenesis of SAH. Germanò et al. reported that the NMDA receptor antagonist, felbamate, attenuated BBB disruption 48 hours after SAH [101]. In view of the known action of felbamate, this
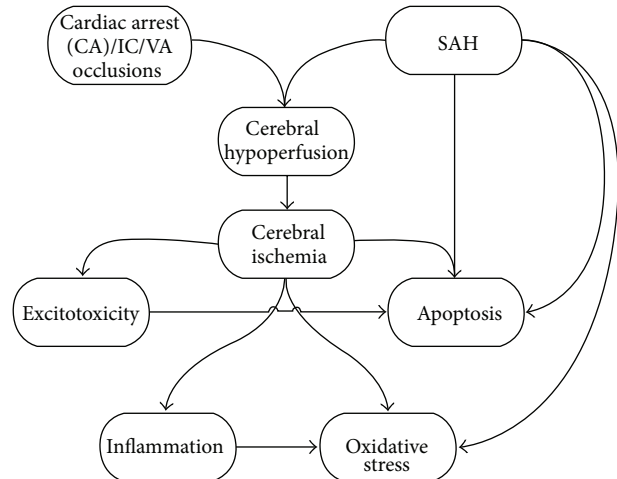

Figure 1: Some hypothetical relationships between cerebral ischemia due to cardiac arrest (CA) and subarachnoid hemorrhage.

suggests a role for NMDA receptor activation in BBB disruption after SAH. Additionally, Unterberg et al. found elevated brain glutamate by intracerebral microdialysis in patients with delayed ischemic deficits after SAH in humans [102]. Similar findings are observed in cerebral ischemia, where glutamate and other excitatory amino acids are increased in brain tissue [19]. The glutamate excitotoxicity hypothesis of brain injury after cerebral ischemia may not be proven, but the process likely occurs after SAH, especially in patients who develop focal ischemia due to delayed angiographic vasospasm or other complications or those with reduced cerebral perfusion pressure from brain swelling and edema. In the excitotoxicity hypothesis, there is brain energy depletion, like in the case with hypoxia-ischemia. Glutamate, one of the most abundant excitatory amino acids, is rapidly effluxed into the extracellular compartment due to neuronal depolarization. It activates NMDA receptors which causes increased intracellular calcium and sodium [19]. Increased calcium activates catabolic enzymes and cell death signaling pathways [38]. Blockade or retardation in the reuptake of excitotoxic amino acids like cysteine results in the depletion of antioxidant intracellular glutathione stores, purportedly causing neuronal injury and death [38]. Furthermore, the use of antiexcitotoxic agents such as NMDA and AMPA-R antagonists conferred neuroprotection through the amelioration of glutamate-induced excitotoxicity caused by hypoxic ischemic injury [38]. This success has not been translated into human ischemic stroke, however. These drugs also are not widely tested in clinical SAH, in part because they failed in human ischemic stroke.

9. Summary

The pathophysiology of SAH and cerebral ischemia share some common mechanisms. Cerebral ischemia is often seen as a complication of SAH as well. Early brain injury is also emerging as a key complication and a cause of morbidity and mortality after SAH. Again, common mechanisms may

be involved in EBI and cerebral ischemia. Indeed part of EBI may be transient global cerebral ischemia, or at least a common hypoperfusion mechanism that acts between both cerebral insults (Figure 1). Further research is required to help elucidate the differences between EBI in a SAH and ischemic brain injury.

Acknowledgments

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Research Article

CSF and Serum Biomarkers Focusing on Cerebral Vasospasm and Ischemia after Subarachnoid Hemorrhage

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Delayed cerebral vasospasm (CVS) and delayed cerebral ischemia (DCI) remain severe complications after subarachnoid hemorrhage (SAH). Although focal changes in cerebral metabolism indicating ischemia are detectable by microdialysis, routinely used biomarkers are missing. We therefore sought to evaluate a panel of possible global markers in serum and cerebrospinal fluid (CSF) of patients after SAH. CSF and serum of SAH patients were analyzed retrospectively. In CSF, levels of inhibitory, excitatory, and structural amino acids were detected by high-performance liquid chromatography (HPLC). In serum, neuron-specific enolase (NSE) and S100B level were measured and examined in conjunction with CVS and DCI. CVS was detected by arteriography, and ischemic lesions were assessed by computed tomography (CT) scans. All CSF amino acids were altered after SAH. CSF glutamate, glutamine, glycine, and histidine were significantly correlated with arteriographic CVS. CSF glutamate and serum S100B were significantly correlated with ischemic events after SAH; however, NSE did not correlate neither with ischemia nor with vasospasm. Glutamate, glutamine, glycine, and histidine might be used in CSF as markers for CVS. Glutamate also indicates ischemia. Serum S100B, but not NSE, is a suitable marker for ischemia. These results need to be validated in larger prospective cohorts.

1. Introduction

Besides acute brain injury [1], one-third of patients suffering from subarachnoid hemorrhage (SAH) develop secondary brain injury [2]. This secondary brain injury leading to the majority of morbidity and mortality after SAH seems to be due to delayed cerebral vasospasm (CVS), which results in delayed cerebral ischemia (DCI) [3]. There are a number of other causes of cerebral ischemia other than CVS after SAH [4], which may manifest clinically as delayed ischemic neurological deficits (DINDs).

CVS has been associated with DIND and DCI and was described for a long time as the underlying pathophysiology [5–8]. However, recent studies showed that ameliorating CVS is only partially effective in preventing DCI [9]. This might be explained by multifactorial mechanisms underlying DCI and the development of secondary brain injury. It further implies SAH and biomarker research aiming at a more comprehensive detection of secondary events after SAH; that one should not focus solely on CVS, but rather on evaluating CVS and DCI.

Although extensive research has been conducted over the last decades on monitoring tissue biochemistry in the injured brain and some studies have identified predictors of CVS following SAH (for review, see Lad et al., 2012 and Table 1) [10], no biomarkers predictive of CVS, DCI, or outcome have been incorporated into routine clinical work. Cerebral microdialysis has been demonstrated to be a useful method detecting biochemical changes associated with brain ischemia after acute brain injury [11]. Especially, the excitatory amino acid glutamate (Glu) has been predictive of ischemia [12]. However, microdialysis remains a focal indicator for intracerebral events, and its distribution and use among ICUs worldwide are limited [11]. Therefore, we sought to evaluate a possible panel of biomarkers in CSF and serum, including excitatory, inhibitory, and structural amino acids as well as neuron-specific enolase and S100B, which might facilitate to detect CVS and/or DCI after SAH and might help

TABLE 1: Summary of the literature focusing on S100B and NSE as biomarkers after SAH predictive of CVS and/or DCI.

Author	Year	Serum biomarker	Sample collection	CVS assessment	CVS	DCI assessment	DCI	Bad outcome
Herrmann et al. [20]	2000	S100B	First 4 days after ischemic stroke	NE	NE	CT	++	++
		No NSE detection			NE	for ischemia detection	(volume of lesion)	
Oertel et al. [21]	2006	S100B	First 3 days after SAH	TCD	↑	NE	NE	+ ↑
		NSE				NE	NE	↓
Weiss et al. [22]	2006	S100B	First 8 days after SAH	TCD + arteriography	−	NE	NE	++
		No NSE detection		(only CVS + S100B < 0.4 μg/L: no death)				
Sanchez-Peña et al. [23]	2008	S100B	First 15 days after SAH	TCD + arteriography	↑ in "ischemic vasospasm" patients			++ (↑)
		No NSE detection						(only mean 15 day S100B value)
Moritz et al. [24]	2010	S100B	Daily during ICU stay	TCD	−	CT	++	++
		NSE			−	CT	+	+ (only NSE peak value)

NE: not evaluated; ↑: increase; ↓: decrease; "−": no correlation; "+": positive correlation; "++": prognostic factor; TCD: transcranial doppler sonography.

to explain pathophysiological changes related to secondary brain injury after SAH.

2. Material and Methods

Stored serum and cerebrospinal fluid (CSF) samples of patients suffering from aneurysmal SAH ($n = 18$) and of controls ($n = 5$) with hydrocephalus after intracerebral hemorrhage, but without aneurysmal SAH, tumor, or trauma, were retrospectively analyzed. All SAH patients ($n = 18$) were Fisher grade III or IV [13] and had suffered from acute hydrocephalus after SAH which was treated by early placement of an external ventricular drainage (EVD) before or during aneurysm treatment. In control patients, a single CSF sample was collected during placement of EVD. Samples were immediately centrifuged, and supernatants were stored at $-70°$C until further assessment. Samples were collected between days 0 and 12 after SAH, depending on how long the EVD remained in situ. For 3 patients no CSF was accessible past day 9 after SAH. As stored CSF samples were also not accessible for every day, short time periods of 3 days each were defined: days 0–3, 4–6, 7–9, and 10–12 after SAH. Of every patient one sample was taken within each of these time periods for analysis. Additional selection criteria for samples drawn within each of the 3-day time periods were, that CSF collected at the day of arteriography and at the day CT scans were performed had to be available and were included for analysis. Serum samples were collected daily during intensive care stay and analysed from the first sample taken after admission until day 12 after SAH.

Sample collection and retrospective analysis were approved by the ethics committee of the University of Frankfurt/Main.

2.1. Biomarker Detection. In serum neuron-specific enolase (NSE) and S100B protein were determined using LIAISON Sangtec 100 assay and LIAISON NSE assay (Byk-Sangtec Diagnostica, Germany). In CSF high performance liquid chromatography (HPLC) was performed to detect the levels of free amino acids including the excitatory amino acids: aspartate (Asp) and glutamate (Glu), as well as the inhibitory amino acids: glycine (Gly) and γ-aminobutyric acid (GABA). Furthermore, the structural, nontransmitter AA glutamine (Gln), histidine (His), and serine (Ser) were detected. Chromatography conditions and quantification were previously described [14].

2.2. Clinical Assessment and Detection of Delayed Cerebral Vasospasm (CVS) and Cerebral Ischemia (DCI). Patients suffering from aneurysmal SAH were examined at admission using the Hunt and Hess classification [15] and the World Federation of Neurological Surgeons SAH scale (WFNS scale) [16] as well as at discharge using the Glasgow Outcome Scale (GOS) [17].

All patients underwent either early clipping ($n = 13$) or coiling ($n = 5$) of the detected ruptured aneurysm, within 72 hours after the initial bleed, followed by hypertensive hypervolemic hemodilution therapy to prevent vasospasm-induced brain ischemia.

Delayed cerebral vasospasm (CVS) was detected arteriographically: an early baseline cerebral arteriography,

performed between days 0 to 2 after SAH, was compared with a subsequently performed arteriography 7 ± 1 days after SAH. The time point of the second arteriography depended on the individual clinical course and was influenced by clinical symptoms and transcranial Doppler sonography (TCD) signs for cerebral vasospasm (increase in flow velocity >30 cm/sec compared to previous days or an overall increase >200 cm/sec). Arteriographic CVS was quantified relative to each patient's baseline arteriogram and was measured by two blinded examiners as described previously. CVS was graded as none, mild, moderate, or severe arteriographic cerebral vasospasm [18].

Delayed cerebral ischemic events (DCIs) were assessed by follow-up computed tomography (CT) scans and determined as hypointensive changes reflecting partial or total involvement of the territory of a cerebral artery on CT scans [19]. To differentiate between treatment-induced ischemic events and SAH-induced delayed cerebral ischemia (DCI) a CT scan was performed within 24 hours after clipping or coiling. DCI included all ischemic lesions detected in subsequent follow-up CT scans, more than 24 hours after treatment. Cerebral ischemia was graded 0 if no hypointensive changes were detected. A small perforator infarction was graded as I and a territorial infarction as grade II.

2.3. Statistical Analysis. Data are presented as mean value ± standard deviation (SD). Statistical analysis of the data was performed using two-tailed Student's *t*-test and analysis of variance (ANOVA) followed by Tukey's test for *post hoc* comparisons of mean values. Pearson's correlation coefficient was used to assess correlations. Statistical significance was defined as $P < 0.05$.

3. Results

Of 18 retrospectively analyzed SAH patients, thirteen developed arteriographic CVS. 6 patients showed cerebral ischemic events which were related to treatment and visible in the early CT scans 24 hours after aneurysm treatment. All treatment-related infarctions were perforator infarctions and were classified as grade I. One patient with a treatment-related perforator infarction developed CVS. Follow-up CT scans of this patient revealed a territorial infarction in the distribution of the formerly detected CVS. Altogether, five patients developed DCI on computed tomography several days after clipping or coiling. These infarctions were all big territorial infarctions (grade II). None of the patients showed small, grade I delayed cerebral ischemia in follow-up CT scans. All patients who developed DCI suffered also from moderate or severe arteriographic vasospasm. Furthermore, patients without signs of arteriographic vasospasm showed no delayed ischemic events on follow-up CT scans (Table 2). Clinical examination at admission (WFNS grade as well as Hunt and Hess grade) was not correlated with outcome measures (GOS) at discharge.

3.1. Biomarkers. CSF glutamine (Gln), glycine (Gly), serine (Ser), and histidine (His) concentrations significantly

TABLE 2: Summary of patient characteristics.

Patient	Gender	CVS	DCI	Pop ischemia
1	M	+	+	−
2	M	+	+	+
3	M	+	+	−
4	M	+	−	−
5	F	+	+	−
6	F	+	−	−
7	F	+	+	−
8	M	+	−	−
9	M	+	−	−
10	M	+	−	−
11	F	+	−	−
12	F	+	−	+
13	F	+	−	+
14	M	−	−	+
15	F	−	−	+
16	F	−	−	−
17	F	−	−	−
18	F	−	−	−

M: male; F: female; CVS: cerebral vasospasm; DIND: delayed ischemic neurological deficit detected in alert patients; DCI: delayed cerebral ischemia; pop ischemia: postoperative/treatment-related ischemia; "+": with; "−": without the characteristic measure.
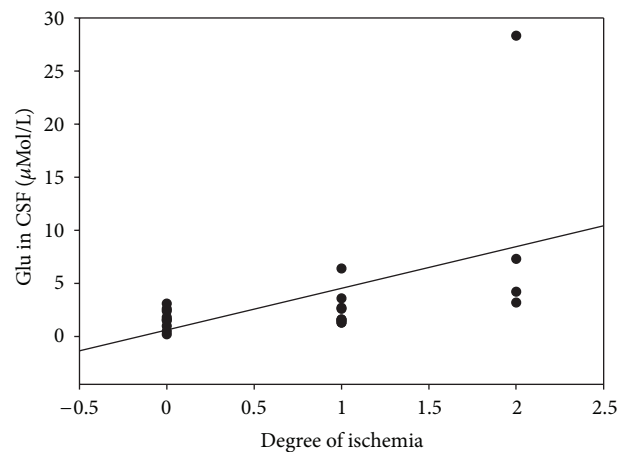
FIGURE 1: The graph depicts CSF Glu in association with the degree of ischemia.

increased after SAH. CSF γ-aminobutyric acid (GABA) significantly decreased compared to control values ($0.22 \pm 0.13\,\mu$mol/L) after an initial increase ($20.6 \pm 36.4\,\mu$mol/L, $n = 18$) on days 0–3 after SAH. Glutamate (Glu) showed in all SAH patients a trend to increase, which did not reach statistical significance. Furthermore, aspartate (Asp) remained unchanged after SAH. However, Glu (CC: 0.48; $P = 0.03$), Gln (CC: 0.47; $P = 0.04$), Gly (CC: 0.53; $P = 0.02$), and His (CC: 0.66; $P = 0.001$) were correlated with the occurrence of arteriographic CVS at the day arteriography was performed. In addition Glu was correlated with the size of ischemia (CC: 0.51; $P = 0.02$) (Figure 1) on the day CT scans are performed. However, no difference could be observed between treatment-related ischemia or SAH-related DCI.
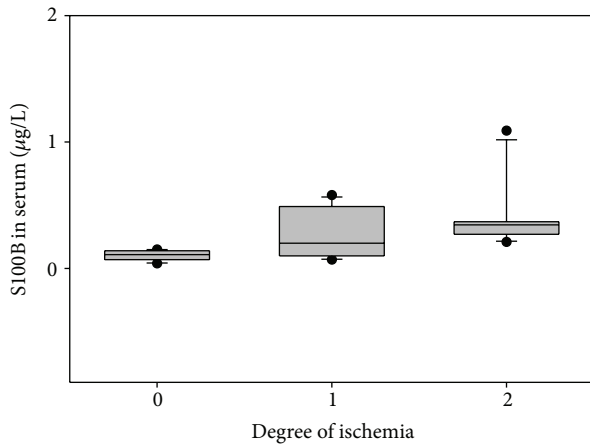
FIGURE 2: The graph depicts S100B in serum in association with the degree of ischemia.

Although Ser significantly increased after SAH, it showed in addition to Asp no correlation with CVS or ischemia.

In serum, no significant changes could be detected for S100B during the time course after SAH, and no correlation was detectable with the development of CVS on the day arteriography was performed (CC = 0.51; $P = 0.052$). However, S100B serum levels were associated with ischemic events detected in follow-up CT scans, irrespective of whether the ischemic lesion was treatment-related or supposedly SAH-related DCI. Serum S100B concentrations were further correlated with the size of ischemic lesions (CC = 0.54; $P = 0.03$) (Figure 2). However, NSE in serum was neither correlated with CVS ($P = 0.3$) nor with ischemia ($P = 0.7$).

No association could be detected between clinical parameter (WFNS as well as Hunt and Hess grades) and the different CSF amino acid (AA) levels at admission. Furthermore, no correlation was detectable between CSF AA levels and GOS outcome parameter at discharge.

4. Discussion

Secondary brain injury exacerbating morbidity and mortality after SAH seems to be due to CVS and DCI. Delayed ischemic neurological deficits (DINDs) seem to result from tissue ischemia. In addition, DIND and DCI have been associated with vascular territories in which CVS has been documented arteriographically, suggesting a causal relationship [5–8]. In accordance with these former studies, 5 patients who derived from the SAH group with arteriographic vasospasm developed DCI, detected as delayed ischemic lesions on follow-up CT scans. Recent reports, however, on CVS after SAH cast doubt on the assumption that DCI is caused only by CVS [25, 26]. Vergouwen et al. showed that cerebral infarction after SAH had a direct effect on outcome independent of arteriographic CVS and suggested that coexisting factors might be involved in the pathogenesis of DIND and DCI [27]. For example, delayed spreading ischemia was suggested as an additional possible source of DIND [28–30]. However, data of this study do not suffice to give any hint concerning this hypothesis. In particular, we did not use MRI to detect ischemic lesions. Furthermore, Jordan and Nyquist proposed that CVS could be an epiphenomena or a contributing factor to parenchymal destruction [31]. In accordance with these observations detected associations between CSF amino acids and CVS, as seen for Gln, Gly, and His, are often not accompanied by an association with DCI. Although we agree that an association between CVS and DCI does not prove a causal relationship, it seems conspicuous that only patients with arteriographic vasospasm developed DCI detected as delayed ischemic lesions on follow-up CT scan in this study. In this perspective, monitoring brain injury and clinical course after SAH demands biomarkers to detect delayed cerebral vasospasm and delayed cerebral ischemia/infarctions. To identify impending secondary injury and to explain neuropathological changes, markers reflecting global processes within the brain, as expected from CSF and serum markers, could be advantageous.

4.1. CSF Marker. After traumatic brain injury the release of the excitatory amino acid glutamate (Glu) and aspartate (Asp), measured in interstitial fluid of the brain and in CSF, was strongly correlated with increased ICP, secondary brain injury, and poor outcome [32–34]. Asp and Glu have been reported as markers of cellular degradation [35], and Glu has been discussed as a predictive biomarker for secondary brain injury and has been demonstrated to be a useful parameter in microdialysis for detection of brain ischemia after SAH [11, 12]. Consistent with this observation Glu CSF concentrations were correlated with CVS and DCI in this study. Excitotoxicity has been suggested as a mechanism of ischemic secondary brain injury, mediated by excessive calcium influx via glutamate-mediated ion channels [33]. Glu further participates in multiple biochemical pathways. It plays a role in neuron-glia communications: the released Glu is taken up into the glia and is converted to glutamine (Gln) which is transported back to the presynaptic neuron and then reconverted to Glu. Glu and Gln CSF concentrations detected were comparable to those described previously [36]. Furthermore, GABA derives from Glu and vice versa. Therefore, alterations in glutamate metabolism might take effect on GABA metabolism [36]. Hutchinson et al. described increased GABA levels, measured by microdialysis, in SAH patients who suffered from DCI, while GABA levels under basal conditions were low. In addition a correlation between GABA and Glu was observed [37]. In contrast CSF Glu increased and CSF GABA decreased after SAH in this patient collective, and no association could be observed. This difference, between microdialysis and CSF examination results, might be due to rapid clearance and limited diffusion of GABA from its neuronal and synaptic origin [38, 39].

Glycine (Gly) also belongs to the inhibitory amino acids and represents the major amino acid found in collagens and thus in cell membranes. Next to its function as precursor of a variety of metabolic products serine (Ser) is also found in high concentration in cell membranes [40]. Histidine is an essential amino acid and the precursor of histamine. Furthermore it is involved in synthesis of hemoglobin. Because of its free radical scavenging characteristics it was reported to attenuate CVS in a rabbit model of SAH [41]. The release

and increase of these structural amino acids might be an indicator for progressive cell membrane degradation. Under experimental conditions excitatory amino acids release has normalized rapidly after global ischemia with reperfusion [42]. Ischemia-induced release of neuroactive amino acids has been suggested to result from energy substrate depletion which is related to reduction in regional blood flow [43], leading to a Ca^{2+}-dependent efflux of neurotransmitters [44] and to inhibition of the neurotransmitter uptake system [45]. Therefore, the more blood flow is reduced, the more efflux of amino acids is expected. Thus, excitatory and inhibitory amino acid detection should increase.

4.2. Serum Marker. S100B and NSE in serum have been discussed as prognostic marker after SAH [21–24] (Table 1). Oertel et al. tried to predict CVS and outcome within the first 3 days after SAH by measuring S100B in serum. Although they did not succeed to differentiate between favorable and unfavorable outcomes, they found significantly higher S100B levels in serum in patients who did not develop CVS as well as in those who died [21]. Moritz et al. showed that serum S100B but not serum NSE allows for determination of good and bad outcomes after SAH [24]. Furthermore, serum S100B allowed the detection of cerebral infarction but not of CVS [24]. Although the low number of patients with grade II ischemia as well as the variance in samples led only to a weak correlation between S100B and degree of ischemia in this study, we could confirm S100B in serum as an indicator for cerebral ischemia. S100B concentrations above 0.15 μg/L were associated with the occurrence and size of ischemic lesions in follow-up CT scans. Similar to Moritz et al., S100B was not associated with arteriographic CVS, and serum NSE did not correlate neither with ischemia nor with vasospasm. Therefore, NSE seems not to be useful as a biomarker for monitoring SAH patients. Herrmann et al. measured S100B after acute stroke using the same assay. They reported that patients who suffered from stroke which was completely reversible within a few days had no increased serum S100B levels. These findings are comparable with those of patients of this study who develop CVS but showed no DCI in follow-up CT scans suggesting a possible pathophysiological point of no return in DCI development from CVS. The fact that S100B is correlated only with ischemic events and not with arteriographic assessed CVS points to possible different degrees of tissue degradation among the wide range of CVS going from reversible mild narrowing to severe constriction leading to ischemia needed to detect S100B in serum. In manifest stroke serum S100B levels described a decelerated increase compared with GFAP [20]. The different expression patterns have been explained by different release patterns under pathological conditions: necrotic cell death leading to leakage from cytosol, breakdown of membrane integrity in the penumbra of infarcts due to cytotoxic, and vasogenic edema as well as brain repair mechanisms [20].

4.3. Limitations of the Study. In this study, selection criteria as, for example, Fisher Grade III and IV and acute hydrocephalus, might lead to study bias. Furthermore, no MRI data could be used to assess ischemia because of the retrospective nature of this study. In addition, the cohort is relatively small and contains only a small amount of patients with DCI, matching the usual distribution of DCI after SAH. In addition, outcome of the patients was assessed at discharge. Therefore the results are limited to short-term and not to long-term outcome. Furthermore, WFNS grade as well as Hunt and Hess grades was not correlated with short-term outcome parameter GOS at discharge. This may be due to the small number of patients in each WFNS/Hunt and Hess subgroup or the limitation of this study to only "poor grade" patients.

5. Conclusions

After SAH glutamate, glutamine, glycine, and histidine might in addition to microdialysis be used in CSF as markers for arteriographic CVS. Glutamate also indicated ischemia. Serum S100B, but not NSE, was associated with delayed cerebral ischemia, but was not correlated with arteriographic CVS. These results need to be validated in a larger prospective cohort.

Conflict of Interests

The authors of this paper, do not have a direct financial relation with the commercial identities mentioned, that might lead to a conflict of interests. Therefore, no conflict of interests exists.

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Review Article

Calcium and Potassium Channels in Experimental Subarachnoid Hemorrhage and Transient Global Ischemia

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Healthy cerebrovascular myocytes express members of several different ion channel families which regulate resting membrane potential, vascular diameter, and vascular tone and are involved in cerebral autoregulation. In animal models, in response to subarachnoid blood, a dynamic transition of ion channel expression and function is initiated, with acute and long-term effects differing from each other. Initial hypoperfusion after exposure of cerebral vessels to oxyhemoglobin correlates with a suppression of voltage-gated potassium channel activity, whereas delayed cerebral vasospasm involves changes in other potassium channel and voltage-gated calcium channels expression and function. Furthermore, expression patterns and function of ion channels appear to differ between main and small peripheral vessels, which may be key in understanding mechanisms behind subarachnoid hemorrhage-induced vasospasm. Here, changes in calcium and potassium channel expression and function in animal models of subarachnoid hemorrhage and transient global ischemia are systematically reviewed and their clinical significance discussed.

1. Introduction

Despite current treatment options, delayed cerebral ischemia following aneurismal subarachnoid hemorrhage (SAH) is still associated with a high morbidity and mortality [1]. The narrowing of cerebral blood vessels by vasospasm represents the main cause of delayed cerebral ischemia [2]. Because vasospastic smooth muscle cells are known to be depolarized compared to controls [3, 4], the expression and function of ion channels in these cells after SAH are of great interest. Furthermore, the inhibitor of L-type calcium channels nimodipine remains gold standard in treatment and prophylaxis of vasospasm after SAH. However, recent studies have revealed that several ion channels of different subfamilies are impacted by SAH and may contribute to delayed vasospasm. The goal of the present analysis is to review ion channel expression and function in healthy cerebral blood vessels as well as after SAH.

2. Ion Channels Healthy Cerebral Vessels

2.1. Expression and Function of Potassium Channels in Healthy Cerebral Vessels. Membrane potential of cerebrovascular smooth muscle cells and thus dilation and constriction of cerebral arteries are directly dependent on potassium conductance [5, 6]. Members of four potassium superfamilies have been shown to be expressed in smooth muscle cells of healthy cerebral vessels: inwardly rectifying (K_{ir}), ATP-dependent- (K_{ATP}), voltage-gated (K_v), and large-conductance calcium-activated (BK) potassium channels. $K_{ir}2.1$ mRNA and protein could be identified in basilar arteries of rats and dogs [7, 8], whereas the presence of K_{ATP} in cerebrovascular smooth muscle has been determined electrophysiologically [9] and reviewed in detail by Ploug et al. 2008 [10]. Transcripts of K_v channel subunits $K_v1.1$ to $K_v1.6$, $K_v2.1$, $K_v2.2$, were detected in healthy rat cerebral vessels and $K_v3.1$, $K_v3.4$, and $K_v4.3$ in healthy dog cerebral vessels but only protein of $K_v1.2$, $K_v1.5$, $K_v2.1$, and $K_v2.2$

subunits could be identified [7, 11, 12]. In situ hybridization revealed the presence of different BK channel splice variants ($X1_{+24}$, $X2_{+92}$, $SS2_{+174}$ and $SS4_{+81}$) in combination with $\beta1$, $\beta2$, and $\beta4$ subunits in rat cerebral arteries [13]. Transcripts of both $K_{ir}2.1$ and $K_{ir}2.2$ have been identified in cerebrovascular smooth muscle [14, 15], where they are thought to play an essential role in neurovascular coupling by mediating local vasodilation as a response to increased neuronal activity [16–18].

Functional studies of the physiological role of K_v and BK channels in healthy cerebral vessels show that they contribute to vascular tone by regulating resting membrane potential of vascular myocytes, limiting depolarization by promoting K^+ efflux [19]. BK channels are particularly vital in cerebral resistance arteries, where raised intracellular calcium at depolarization elicits outward BK currents representing a negative feedback loop, which antagonizes vasoconstriction [20, 21]. Furthermore, BK channels also play an important role in the principle vasorelaxation pathway (nitric oxide synthase pathway), as they are activated by cyclic GMP-dependent protein kinase (PKG), which is stimulated by the NO-induced increase of cGMP [22]. Interestingly, a recent study of mouse cerebral parenchymal arterioles found small-conductance (SK) and intermediate-conductance (IK) calcium-activated potassium currents in isolated endothelial cells in addition to the BK currents in isolated myocytes [23]. Both appear to contribute to vasorelaxation, as superfusion of the cerebral cortex with SK and IK channel inhibitors apamin and TRAM-34, respectively, reduces resting cortical CBF [23].

In healthy cerebral blood vessels activation of K_{ATP} channels causes hyperpolarization of vascular myocytes and thus vasodilatation [5, 18, 19]. Several antihypertensive drugs like the vasodilators diazoxide, cromakalim, and pinacidil exert their therapeutic effect by activating K_{ATP} channels [24, 25]. K_{ATP} channels appear to play an important role in cerebral autoregulation, as in rats their inhibition impairs healthy autoregulatory vasomotor responses to hypotension and its reverse. Furthermore, K_{ATP} channel activation is also associated with several pathophysiological responses such as reactive hyperemia in cerebral circulation after hypoxia [26] (reviewed by Ko et al., 2008 [27]).

2.2. Expression and Function of Calcium Channels in Healthy Cerebral Vessels. L-type voltage-gated calcium channels (VGCCs) were traditionally believed to predominantly control Ca^{2+} influx in cerebrovascular smooth muscle cells; however recent studies have revealed expression of various Ca^{2+} channels and their isoforms. Protein and transcripts of the alpha1 subunit of the $Ca_v1.2$ (L-type) VGCC are expressed strongly in basilar arteries of the dog [28] and the rat [29]. Interestingly, however, in the rat basilar artery (and lateral branches), transcripts of the low-voltage activated channel $Ca_v3.1$ were the strongest expressed VGCCs, exceeding relative mRNA levels of the other four identified VGCCs in the following order: $Ca_v3.1$ (T-type) > $Ca_v1.2$ (L-type) > $Ca_v1.3$ (L-type) > $Ca_v3.2$ (T-type) > $Ca_v2.3$ (R-type). The same study found that, at the protein level, $Ca_v3.1$

and $Ca_v1.2$ were both clearly expressed basilar artery smooth muscle cells, while $Ca_v3.2$ protein expression was much lower, $Ca_v2.3$ protein was confined to the surface of the vessel, and $Ca_v1.3$ protein was not detectable at all. However, the authors did not find evidence for $Ca_v2.2$ (N-type) VGCC protein or mRNA expression as was found in basilar arteries of the dog [28]. In dogs inhibition of L- and T-type Ca^{2+} channels with nimodipine and mibefradil, respectively leads to a relaxation of healthy arteries under isometric tension, whereas blockade, of N-type Ca^{2+} channel has no effect [30].

It is notable that expression of VGCCs appears to be heterogeneous in cerebrovascular smooth muscle cells: in the dog basilar artery low-voltage activated (LVA) current made up more than 50% of the total current in 12% of myocytes, less than 10% in 26% of myocytes, and between 10% and 50% in 62% of myocytes [28]. Additionally VGCC expression may vary depending on vessel size: Kuo and coworkers [31] described a high-voltage-activated Ca^{2+} current showing T-type channel kinetics, which is insensitive to nifedipine and nimodipine and is blocked by the T-type blocker mibefradil. Interestingly, the fraction of this current is higher in smaller vessels and decreases with vessel size. These currents could represent low-voltage-activated T-type currents, but also "intermediate-voltage-" activated R-type currents, which are insensitive to dihydropyridines but are also antagonized by mibefradil [32]. This vessel-size-dependent difference of expression patterns of VGCCs in cerebral blood vessels implies that the contribution of L-type VGCCs to vasoconstriction is greatest in large basal cerebral vessels, while dihydropyridine-insensitive VGCCs play a more important role in smaller vessels. Other investigators suggest that L-type Ca^{2+} channels could be responsible for vasomotion, while non-L-type Ca^{2+} channels control vascular tone [29].

3. Early Ion Channel Dysfunction after SAH

In addition to delayed cerebral vasospasms, acute hypoperfusion immediately after rupture of an aneurysm causing subarachnoid blood represents another characteristic of SAH pathology [33–35]. Relative hypovolemia, impaired cerebral circulation due to elevated intracranial pressure, abnormal autoregulation, as well as early vasospasm have been discussed as possible etiologies behind acute hypoperfusion after SAH [33, 35–38]. Some insights into the underlying molecular mechanism could be gained from animal experiments. Data from a rat SAH model found evidence for acute vasoconstriction after even minor subarachnoid hemorrhage [35]. In cultured primate cerebrovascular smooth muscle cells a significant increase of free intracellular Ca^{2+} is observed as early as 2 minutes after exposure to oxyhemoglobin (oxyHb) and sustains for 7 days [39]. Similarly Takenaka and colleagues found that endothelin, oxyHb, 5-hydroxytryptamine, norepinephrine, prostaglandin F2 alpha, and leukotrienes C4 and D4 but not bilirubin produced acute dose-dependent increases in intracellular Ca^{2+} concentration [40] in cultured cerebrovascular smooth muscle cells. Furthermore, Takenaka and coworkers report that the L-type Ca^{2+} channel blocker verapamil

does not inhibit the oxyHb-induced rise in intracellular Ca^{2+}, implying non-L-type calcium channels in acute vasoconstriction after exposure of the vessel to blood [41]. This finding may be explained by data from Ishiguro and coworkers [42] that demonstrates that, in isolated cerebral arteries, acute oxyHb exposure induces vasoconstriction and suppression of K_v currents but does not influence VGCCs. Long-term (5 days) oxyHb exposure on the other hand enhanced expression of VGCCs, pointing toward important roles of K_v channels in acute vasoconstriction and VGCCs in delayed vasoconstriction after SAH.

4. Changes in Ion Channel Expression and Function in Delayed Cerebral Vasospasm

4.1. The Pathophysiological Role of Potassium Channels in the Genesis of Delayed Cerebral Vasospasm

4.1.1. Voltage-Gated Potassium (K_v) Channels in SAH. Reduced K^+ conductance causing depolarization of cerebrovascular myocytes was amongst the earliest hypotheses behind delayed cerebral vasospasm after SAH, and indeed many modern studies support this model [43–45]. However, it has become increasingly evident that members of different potassium channel families are affected in different ways after SAH, raising many new questions. Several authors emphasize a loss of functional voltage-gated K^+ channel (K_v) in response to SAH, as mainly responsible for the disturbance of K^+ conductance. Seven days after SAH, $K_v2.2$, and $K_v2.1$ transcripts and protein were found to be reduced in basilar arteries of dogs [7, 43]. Immunohistochemical staining of rabbit cerebral arteries revealed a reduction of surface-expressed $K_v1.5$ protein 5 days after oxyHb exposure [46]. Furthermore, Ishiguro describes redistribution of $K_v1.5$ protein after oxyHb exposure: in unexposed vessels $K_v1.5$ was observed within large defined regions of the cell membrane and was associated with phosphotyrosine-rich vesicular compartments adjacent to the plasma membrane, whereas OxyHb exposure caused a decrease in $K_v1.5$ surface staining and a redistribution of the remaining $K_v1.5$ into smaller foci that appeared fused with phosphotyrosine-enriched vesicles. This stands in support of the hypothesis that oxyHb-induced suppression of $K_v1.5$ channels is mediated by a mechanism involving increased tyrosine phosphorylation-dependent trafficking of the channel from the cell surface [46].

4.1.2. Inwardly Rectifying Potassium (K_{ir}) Channels in SAH. Next to K_v channels, expression of an inwardly rectifying potassium channel is found to be influenced by SAH. Seven days after SAH, dog basilar artery myocytes display enhanced expression of $K_{ir}2.1$ protein and transcripts [7, 45]. Accordingly, blockage of $K_{ir}2.1$ channels in arteries under isometric tension produced a greater contraction in SAH than in control arteries. It is thus possible that increased expression of $K_{ir}2.1$ channels after SAH may represent an adaptive response reducing disturbance of the cellular K^+ balance and consecutively cerebral vasospasm.

4.1.3. Large Conductance Calcium-Activated (BK) Potassium Channels in SAH. Whether BK channels are impacted by SAH and contribute to vasospasm is a matter of debate, as data on this subject has proven to be somewhat contradictory. It has been reported that in dog basilar artery myocytes, BK current density, kinetics, Ca^{2+} and voltage sensitivity, single-channel conductance, and apparent Ca^{2+} affinity are unaffected by SAH [44]. Aihara et al. report that although the expression of the BK channel alpha subunit is unchanged after SAH, expression of BK channel $\beta1$ subunit mRNA (but not protein) is reduced 7 days after SAH in dog basilar artery myocytes and correlates with the degree of vasospasm [7]. Koide et al. found that although SAH does not alter BK channel density or single channel properties in rabbits, SAH does cause a distinct reduction in Ca^{2+} spark-induced transient BK currents, corresponding to decreased expression of ryanodine receptor type-2 protein [47]. Ca^{2+} sparks are focal Ca^{2+} releases through ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR), which oppose the contractile actions of global cytosolic Ca^{2+} by activation of BK channels leading to hyperpolarization and decreased Ca^{2+} influx through VGCCs [48, 49]. Findings by Koide et al. suggest that impaired subcellular signaling from the SR to BK channels at the cell surface, due to reduced expression of RyRs causing less focal Ca^{2+} spark discharges, could be a key mechanism in vasospasm after SAH.

4.1.4. ATP-Dependent (K_{ATP}) Potassium Channels in SAH. An important role of K_{ATP} channels in animal models of SAH-induced vasospasm appears likely, as several experimental studies have shown that pharmacologic activation of K_{ATP} channels can significantly attenuate vasospasm. The K_{ATP} channel activator levcromakalim increased vasorelaxation in rabbit basilar arteries three days after SAH [4] and in dog basilar arteries seven days after SAH [50]. Furthermore, the endogenous K_{ATP} channel activator calcitonin gene-related peptide (CGRP) displayed therapeutic effects reversing vasospasm after SAH in rabbits and monkeys [51, 52] but failed to significantly attenuate vasospasm to a greater degree than standard of care (nimodipine) in a clinical trial comprising 117 patients [53].

4.1.5. VGCCs in SAH. The role of VGCCs in vasospasm may seem obvious in clinical practice where L-type Ca^{2+} channel blockers, such as nimodipine, are the gold standard of prophylaxis and treatments of cerebral vasospasm. This is indeed reflected in experimental investigations offering evidence in support of a large contribution of L-type VGCCs to vasoconstriction in certain cerebral blood vessels. However, recent findings have revealed the importance of R-type and T-type channels in vasospasm. Although typically classed with the high-voltage-activated Ca^{2+} channels, R-type calcium channels are activated at potentials between those of low and high VGCCs, representing an intermediate VGCC. This channel is of interest in vasospasm, as its expression is directly linked to SAH and it may be available for opening at the depolarized resting potential of vasospastic cerebrovascular myocytes.

Intravenous administration of nimodipine five minutes after SAH improves circulation and attenuates vasospasm in rats [54]. Nicardipine (dihydropyridine) pellets positioned next to the basal arteries have been shown to reduce the occurrence of angiographic vasospasm in a dose-dependent manner in patients suffering from SAH [55, 56]. However, L-type antagonists alone cannot reverse SAH-induced vasospasm completely. In this regard, the finding is that although L-type VGCC antagonists abolish cerebral artery constriction and block VGCC currents in cerebral artery myocytes from healthy rabbits, the lack of their efficacy in rabbits after SAH corresponds to an increase in R-type currents and alpha$_{1E}$ (Ca$_v$2.3 pore forming subunit) protein and mRNA [57]. This is in line with recent findings by Nikitina et al. who observed that high-voltage-activated (HVA) Ca^{2+} channel currents were significantly decreased and low-voltage-activated (LVA) currents increased during vasospasm 4, 7, and 21 days after SAH in dogs [28]. This study revealed an increase in protein expression of T-type (Ca$_v$3.1 and Ca$_v$3.3 alpha subunits) and R-type VGCCs and a decrease in L-type (Ca$_v$1.2 and Ca$_v$1.3 alpha subunits) VGCCs in dog basilar arteries after SAH. Interestingly however, differently to Nikitina et al., Ishiguro et al. could not observe an increase in R-type protein and mRNA in the basilar artery or other larger diameter vessels after SAH, but only in smaller vessels. Several authors suggest that the functional significance of R-type channels may lie within small diameter blood vessels and that blood vessels of different sizes are impacted differently by SAH [58]. Furthermore, exposure of organ cultured rabbit cerebral arteries to oxyHb induces the expression of R-type VGCC mRNA in small vessels rendering the vessels sensitive to SNX-482 (R-type antagonist) and less sensitive to diltiazem [59]. SNX-482 was also found to attenuate CBF reduction after SAH in rats [60].

In addition to R-type VGCCs, the low-voltage-activated (T-type) channels Ca$_v$3.1 and Ca$_v$3.3 have been shown to be upregulated in the dog basilar artery after SAH [28]; however the functional significance of this finding is a matter of debate: the increased expression of T-type VGCC channels was proposed to be functionally irrelevant because these channels should be inactivated in depolarized cells. In fact, T-type channels were reported to inactivate at resting membrane potentials of most smooth muscle cells at about -75 to -65 mV [17]. Cisternal application of nicardipine but not of the T-type antagonist mibefradil reduced CT angiography measured vasospasm in cynomolgus macaques [61], which is in agreement with the functional insignificance of T-type VGCCs in depolarized cells.

5. Changes of Ion Channel Expression and Function following Transient Global Ischemia after SAH

Increased ICP and decreased CPP immediately following SAB cause a transient global ischemia (TGI) [62–64].

Distinguishing which molecular changes can be attributed to subarachnoid blood and which to TGI is difficult; however studies of TGI (without SAH) can be of assistance. Very little is known about changes in ion channel expression and function in cerebral vessels following transient global ischemia. The only study describing direct impact of TGI on ion channels in cerebral arteries found that in piglets arteriolar response (i.e., dilation) of K$_{ATP}$ channels to their activators aprikalim and iloprost is impaired 1 hour after TGI but normalizes over 2–4 hours [65]. Interestingly, this reduction of cerebral arteriolar dilation to activation of K$_{ATP}$ channels could be prevented with the nonsteroidal anti-inflammatory drug indomethacin. In addition to this acute provasoconstrictive effect, TGI has recently been described to have a delayed provasoconstrictive effect. In the two-vessel carotid artery occlusion model, transient forebrain ischemia caused a functional upregulation of ET$_B$ and 5-HT$_{1B}$ receptors in the ACA and MCA of the rat 48 hours after the insult [66]. In the case of SAH-induced TGI, an upregulation of vasoconstrictor receptors could contribute to vasospasm and thus to delayed cerebral ischemia.

More is known about the effects of TGI on neuronal ion channels. Transient forebrain ischemia in rats leads to a downregulation of L-type VGCCs in vulnerable hippocampal CA1 neurons by oxidation modulation, whereas L-type Ca^{2+} channels in the CA3 are not affected [67]. Interestingly, blockade of L-type but not of N- or P-/Q-type VGCCs worsened neuronal survival, while, more importantly, L-type calcium-channel agonists applied after reperfusion significantly decreased neuronal injury in rats subjected to forebrain ischemia [67]. These results stand in strong contrast to the widely accepted view of excitotoxic mechanisms after brain ischemia, which make glutamate-induced intracellular calcium overloading responsible for induction of apoptotic proteins and toxic molecules [68, 69], but shed light on possible region-specific involvement of calcium signaling in cell survival. Indeed other studies give weight to this hypothesis of L-type downregulation after ischemia and may ultimately lead to a modification of the view of calcium-mediated neurotoxicity [70–72]. R-type VGCCs may also mediate neuroprotection in focal ischemia, as mice lacking the R-type VGCC display larger infarct volume size than wild-type mice after occlusion of the MCA [73]. Although N-type VGCCs (but also L-type, P/Q-type) have been reported to be upregulated in the hippocampus and cortex after global ischemia [74, 75], neuroprotective effectiveness of their inhibitors is a matter of debate, as evidence is contradictory [76]. Furthermore, pharmacologic inhibition of T-type VGCCs has been shown to have a neuroprotective effect in hippocampal neurons after global ischemia in rats and also an in vitro model of ischemia-induced delayed cell death in rat organotypic hippocampal slice cultures [77, 78]. However, a 2012 meta-analysis of effectivity of calcium channel antagonists on ischemic stroke including 7731 patients in 34 trials concluded that calcium channel antagonists have no effect on primary outcome or survival after stroke but that nimodipine at high doses is associated with poorer outcome [79].

6. Conclusions

It is apparent that the decrease of K^+ conductance and the shift from HVA Ca^{2+} currents to LVA Ca^{2+} in cerebrovascular myocytes represent key phenomena in SAH-induced vasospasm; however we have yet to put together the pieces to establish a model of the complex mechanisms behind SAH pathology. This paper focuses on ion channels and thus on processes at the cell surface, but one must not overlook the downstream effects of ion channel signaling via interacting proteins like protein kinase C (PKC), an important regulator of VGCCs. Several VGCCs and nearly all K^+ channels are highly regulated by PKC. After SAH, hemoglobin alters expression levels of different PKC isoforms and induces their translocation from the cytosol to the plasma membrane (PKC-δ on day 4 and PKC-α on day 7) [80]. It has been suggested that PKC-δ is involved in initiation of SAH-induced vasospasm whereas PKC-α plays a role in its endurance [81, 82]. PKC phosphorylates the $Ca_v1.2$ subunit of L-type calcium channels and leads to dual modulation with inhibitory and stimulatory effects in vascular smooth muscle cells. R-type VGCCs also underlie PKC-mediated Ca^{2+}-dependent stimulation [83, 84]. But also calmodulin, another regulatory protein of voltage-gated Ca^{2+} channels, is significantly impacted by SAH, displaying a decrease within the first 48 hours after SAH [85]. One may speculate that imbalance of calmodulin-mediated inactivation and PKC-mediated Ca^{2+}-dependent stimulation of R-type Ca^{2+} channels might lead to self-perpetuating Ca^{2+} influx during vasospasm. The calmodulin antagonist trifluoperazine was demonstrated to reduce severity of cerebral vasospasm following SAH but at doses far in excess of the normal accepted therapeutic range in humans [86].

Transient global ischemia after SAH may contribute to neurologic injury by downregulation of L-type VGCCs in the CA1 region of the hippocampus [67] but also may contribute to the occurrence of vasospasm by the increase of vasoconstrictor receptors and the functional impairment of K_{ATP} channels in cerebrovascular myocytes [65, 66].

In the effort of developing better pharmacologic therapies and prophylaxes of vasospasm, it is very likely that patients will ultimately benefit from *in vitro* studies investigating ion-channel signaling and protein interaction partners in great detail. As in every disease, identifying exact targets in order to develop specific modulators is key, and the lack thereof may be the root of difficulties in treating vasospasms with L-type antagonists, such as nicardipine or nimodipine, which also have substantial modulatory effects on several other ion channels [87, 88]. Furthermore, the extent to which vasospasm contributes to poor outcome after SAH remains a matter of debate. Although several authors falsely cite CONSCIOUS-1 as evidence that vasospasm does not contribute to poor outcome (the study was not powered to detect changes in morbidity, mortality, or clinical outcome), recent evidence showing that a reduction of cerebral infarction but not of vasospasm correlated with better neurological outcome [89] fuels the debate on causality of the pathological phenomena following SAH. In this regard, it may be necessary to consider further mechanisms by which nimodipine enhances clinical outcome. Several experimental studies of different animal models of cerebral ischemia have found neuroprotective effects of nimodipine [90–92]; however clinical studies remain inconclusive. Although nimodipine was found to have no effect on primary outcome or survival after stroke in a recent meta-analysis of 34 clinical trials, one study has found nimodipine to reduce relative risk of the frequency of CT-scan-documented cerebral infarction and of ischemic neurologic deficit after aneurysmal SAH but not of angiographically detected cerebral vasospasm [93]. Taken together, these results underline the need for both experimental and clinical investigations of the molecular mechanisms behind the therapeutic effect of nimodipine and thus calcium channel blockade.

Authors' Contribution

M. A. Kamp and M. Dibué contributed equally to this work.

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