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Cerebral ischemia in experimental subarachnoid hemorrhage

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ABBREVIATIONS

AP	Anterior-posterior
BBB	Blood brain barrier
CBF	Cerebral blood flow
CBF-V	Cerebral blood flow velocity
CO_2	Carbon dioxide
CSF	Cerebrospinal fluid
CT	Computed tomography
DIND	Delayed ischemic neurological deficit
$etpCO_2$	End-tidal partial pressure of carbon dioxide
GFAP	Glial fibrillary acid protein
ICA	Internal carotid artery
MCA	Middle cerebral artery
MCAO	Middle cerebral artery occlusion
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
NO	Nitric oxide
rtPA	Recombinant tissue plasminogen activator
SAH	Subarachnoid hemorrhage
TCD	Transcranial Doppler

SUMMARY

Compared to other cerebrovascular events, subarachnoid hemorrhage (SAH) accounts for a quarter of productive life years lost to stroke due to the relatively young age of affected patients. Delayed ischemic neurological deficit (DIND) is an important cause of morbidity and mortality after SAH. DINDs occur several days after hospitalization while patients are under medical surveillance. Nonetheless, no definitive treatment exists to prevent this condition. Animal models of this disease have so far only provided information on a particular aspect of DIND, e.g. the occurrence of delayed cerebral vasospasm. The present work focuses on the characterization of cerebral blood flow (CBF), cerebrospinal fluid (CSF) dynamics and neuropathological findings in a widely-used preclinical animal model of SAH.

INTRODUCTION

Aneurismal subarachnoid hemorrhage

SAH occurs due to rupture of an intracranial aneurysm in 85% of cases. In 10%, hemorrhage is confined to the pretruncal area with no identifiable vascular malformation. This subtype of SAH is referred to "perimesencephalic", or more precisely, "pretruncal" SAH and has a benign course. The remaining 5% are attributable to a number of cerebrovascular conditions as reviewed elsewhere (van Gijn, Kerr et al. 2007).
SAH has an incidence of 6-7 per 100,000 person-years and represents 5% of all strokes. In contrast to the more prevalent ischemic stroke, half of the affected patients are younger than 55 years. SAH thus accounts for a quarter of all productive life years lost to stroke (Johnston, Selvin et al. 1998).

Mortality and morbidity from aneurismal subarachnoid hemorrhage

Overall mortality from SAH is about 50%. An estimated 15% of patients die before reaching a hospital. If a ruptured aneurysm is left untreated, the risk of a potentially fatal rebleed is about 40% in the first two weeks. Swift treatment of an aneurysm is mandatory to minimize this risk (van Gijn, Kerr et al. 2007).
Temporary neurological fluctuations in the first two weeks after the bleed (defined as a drop in 5 points of the National Institutes of Health (NIH) Stroke Scale) following aneurysm treatment occur in up to 80% (Schatlo et al, unpublished data) of SAH patients. The patient's status can change due to the presence of hydrocephalus, brain edema, raised intracranial pressure, embolic events or delayed cerebral ischemia. SAH can also be associated with severe systemic complications such as neurogenic stress cardiomyopathy, hyponatremia or neurogenic pulmonary edema (Solenski, Haley et al. 1995).

Delayed ischemic neurological deficits

DIND occur in 33 to 38% of patients after SAH and accounts for disability in 6.3% and death in 7.2% (Haley, Kassell et al. 1997; Lanzino, Kassell et al. 1999). Clinical deficits develop gradually over several hours in contrast to thromboembolic stroke where symptoms appear more abruptly. Hemispheric focal deficits occur in a quarter of patients, the other quarter shows a reduced level of consciousness. The remaining half of patients show both symptoms (Hijdra, Van Gijn et al. 1986). Prior to establishing the diagnosis of DIND, one needs to rule out re-bleeding, hydrocephalus, hyponatremia and surgical or interventional complications. The term "delayed" reflects the fact that DINDs occur mostly between days 5 to 14 after aneurysm rupture. Predisposing factors for DIND include loss of consciousness at the time of hemorrhage, hypovolemia and hypotension and a history of smoking. The influence of age on DIND has been discussed controversially (van Gijn, Kerr et al. 2007). The most important known independent parameter associated with DIND is the amount – not the localization - of blood on admission computed tomography (CT) scans (Fisher, Kistler et al. 1980). Hemolysis of the blood clot in the subarachnoid space leads to a release of deoxyhemoglobin and potassium which reach their maximum concentrations on day 7 (Pluta, Afshar et al. 1998). Deoxyhemoglobin is a potent scavenger of the endogenous vasodilating molecule nitric oxide (NO), and may thus decrease the vasodilatory reserve. In turn, potassium favors depolarization of cells surrounding the blood clot, affecting both vascular smooth muscle cells as well as the neuronal and glial compartment. The release of erythrocyte products has been found to cause decreased vasoreactivity and, ultimately, the development of delayed cerebral vasospasm (Macdonald and Weir 1991). This characteristic narrowing of the basal cerebral arteries after SAH occurs in up to 70% of patients and peaks at days 5 to 10. The gold standard for the detection of delayed cerebral vasospasm is arteriography. Other methods such as contrast CT and bedside transcranial Doppler (TCD) are less reliable, but more available and non-invasive methods. Vasospasm can lead to DIND. However, low positive predictive values ranging from 33% to 50% (Vora, Suarez-Almazor et al. 1999; Unterberg, Sakowitz et al. 2001) suggest that the presence of vasospasm alone does not necessarily translate into a neurological deficit. Besides their effect on smooth muscle cells, components of the subarachnoid blood clot may trigger a wave of depolarization on the cortical surface (Dreier, Korner et al. 1998). This wave of depolarization is termed "cortical spreading depolarization" (Leao 1951) and predisposes to metabolic failure and ischemia. Repeated spreading depolarizations are associated with DIND in patients with SAH (Dreier, Woitzik et al. 2006; Dreier, Major et al. 2009) and represent another mechanism for the multifactorial etiology of DIND.

Treatment of DIND

It is currently recommended to perform a number of preventive measures in patients with SAH to decrease the risk of DIND (Raabe, Beck et al. 2005). In a Cochrane meta-analysis, prophylactic administration of calcium-channel antagonists (nimodipine) appears to reduce the risk of poor outcome (relative risk 0.81 (95% confidence interval 0.72 to 0.92)). Oral calcium antagonists prevent DIND in one out of 19 treated patients (Dorhout Mees, Rinkel et al. 2007). Hypovolemia and hypotension should be avoided.

For the therapy of DIND, hypertension up to a mean arterial pressure of 130 mmHg improves cerebral oxygenation and has a clinical benefit. Standalone hypervolemia without hypertension only increases the risk of systemic complications. If angiographic vasospasm is diagnosed as a cause of DIND, angiographic intervention with either balloon angioplasty or intra-arterial injection of vasodilators is a viable therapeutic option to attempt reversal of neurological symptoms. In many centers angioplasty is performed for DIND although we lack data from large randomized controlled trials. The complication rate of 5% has to be weighed against an impending neurological deficit (Bederson, Connolly et al. 2009). Future efforts will focus on the prevention of cortical spreading ischemia which was recently identified as a player in the development of DIND (Dreier, Major et al. 2009). Resistance vessels respond to spreading depolarization with tone alterations, causing either (i) transient hyperperfusion (physiological hemodynamic response = spreading hyperemia) in healthy tissue or (ii) severe hypoperfusion (inverse hemodynamic response = spreading ischemia) in tissue at risk for progressive damage. The latter may contribute to lesion progression in patients with SAH. Thus, spreading ischemia could be an interesting target for treatment. Experimentally, spreading ischemia is induced by decreased NO availability in combination with increase of basal potassium. Thus, e.g., NO donors could cause spreading ischemia to revert to spreading hyperemia in patients with SAH (Dreier, Petzold et al. 2001).

DIND as a "model disease" for cerebral ischemia

Despite improvements in neurointensive care, DIND continues to affect patients who are hospitalized and monitored. Anticipating such an event significantly facilitates our ability to study the conditions of ischemia. It provides an opportunity to (1) identify precursors of ischemia and to (2) apply neuroprotective agents prior to or during ischemia onset. One of the major hurdles for advances in the treatment of ischemic stroke is the significant delay from ictus to treatment. The overly optimistic use of short ischemia times in basic research was recently criticized. Positive results from such studies may be part of the reason for the high rate of disappointing results of neuroprotective drugs against ischemia (Dirnagl 2006). In this respect, SAH and DIND are unique and may well prove to become a pioneering avenue to the treatment of cerebral ischemia in a broader sense.

Goal of this thesis

No animal model exists which reproduces the timecourse and pattern of pathological lesions observed in patients with DIND (Megyesi and Findlay 2001). The aim of my thesis is to evaluate a widely used preclinical primate model of SAH. We herein (1) characterized the effect of vasospasm on CBF in this model. (2) We evaluated to what degree a subarachnoid blood clot limits free circulation of cerebrospinal fluid. We then (3) performed a neuropathological workup to assess whether this animal model shows infarcts resembling those of patients with DIND. In addition, in a separate experiment, (4) we assessed the efficacy of a novel therapeutic strategy against ischemia in a rodent model of stroke with serial magnetic resonance imaging.

Part 1: Hemodynamics of ischemia and vasospasm

From: Bawarjan Schatlo, Sven Gläsker, Alois Zauner, Byron Gregory Thompson, Edward H. Oldfield, Ryszard M. Pluta: Continuous neuromonitoring using transcranial Doppler reflects blood flow during carbon dioxide challenge in primates with global cerebral ischemia. Neurosurgery, 2009. 64 (6): 1148-1154.

Methods

We employed a primate model of human SAH which has been in wide use to study consequences of SAH with particular focus on delayed cerebral vasospasm (Espinosa, Weir et al. 1982; Pluta, Dejam et al. 2005). A right pterional craniotomy is performed in cynomolgus monkeys. After careful neurosurgical dissection of the Sylvian fissure, we place a clot of

autologous arterial blood around the MCA. Arteriography was performed before SAH and on day 7 after surgery (Fig. 1). To assess the degree of vasospasm, the areas of the proximal 14 mm of the R MCA on preoperative and postoperative anterior-posterior (AP) arteriography were measured using a computerized image analysis system (NIH Image 1.25) and compared (Pluta, Zauner et al. 1992). All procedures described herein were validated and approved by the Animal Care and Use Committee of the National Institute for Neurological Disorders and Stroke at the NIH.

CBF and cerebral blood flow velocity (CBF-V) were measured simultaneously in three groups with a total of n=16 Cynomolgus monkeys. The first two groups served as controls to confirm the validity of the model showing that in healthy primates (1) autoregulation and (2) chemoregulation are intact. Then, we tested the working hypothesis of this study whether chemoregulation combined with continuous TCD recordings could reflect CBF during (3) vasospasm or (4) ischemia. A thermal probe developed by Brawley (Brawley 1969) and modified by Carter (Carter, Erspamer et al. 1981) was used to assess CBF continuously. The CBF probe (Saber thermomonitoring ®, Flowtronics, Phoenix, AZ) was slipped between the dura and brain to lie over a region perfused by the MCA. TCD measurements of flow velocities in the MCA were made through an anterior temporal window at a depth of 25-35 mm. Blood pressure, end-tidal partial pressure of CO_2 ($etpCO_2$), CBF and CBF-V were recorded simultaneously.

Results

The alteration of $etpCO_2$ in healthy primates was performed for validation purposes and produced the well-known changes in CBF. CBF decreased during hyperventilation. Hypoventilation caused in increase in CBF. At very high values of $etpCO_2$, CBF decreased again (Fig. 3). In our global ischemia protocol, responses of the vasculature in response to $etCO_2$ changes (chemoregulation) were absent. CBF and CBF-V were strongly correlated ($r^2>0.8$; $p<0.001$). During vasospasm after SAH, we observed active chemoregulation when $etpCO_2$ reached lower values, but vasoparalysis in normocapnia and high $etpCO_2$. The overall correlation between CBF and CBF-V during vasospasm after SAH was low.

Discussion

Continuous TCD recordings during changes in $etpCO_2$ cannot provide an estimate of CBF when cerebrovascular regulatory mechanisms are present, which was confirmed in healthy primates. Chemoregulation appeared to be partly preserved in our primate model of vasospasm, comparable to the dissociative vasoparalysis described in humans. In consequence, correlations between continuous TCD measurements and CBF were poor, even during modifications in $etpCO_2$. Only in cerebral ischemia, during which vessel reactivity is known to be abolished, modification of $etpCO_2$ during chemoregulation produced a strong correlation of TCD with CBF.

Part 2: Cerebrospinal fluid dynamics

From: Ryszard M. Pluta, John A. Butman, Bawarjan Schatlo, Dennis L. Johnson, Edward H. Oldfield: Subarachnoid hemorrhage and the distribution of drugs delivered into the cerebrospinal fluid. Laboratory investigation. Journal of Neurosurgery, 2009. 111(5): 1001-1007.

Methods

To assess the effects of a subarachnoid blood clot on the free circulation of CSF, we performed intrathecal injections of CT contrast agent (1ml of Isovue-M 300) and Evan's blue dye (0.5ml of a 3% solution). Animals were randomly assigned to SAH (n=5) or sham (n=5) groups. Animals in the SAH group underwent clot placement as described previously. Surgery in the sham group was terminated after arachnoid dissection around the Sylvian fissure and internal carotid artery (ICA) bifurcation. Animals were sacrificed at different timepoints after surgery (SAH group: Day 1 (n=2), day 3 (n=1), day 7 (n=28); sham group: day 1 (n=1), day 7 (n=3) and day 28 (n=1)). In all animals, CT cisternography with intrathecal contrast injection was performed for two hours with serial CT to assess CSF dynamics. In the animals surviving for 28 days, the procedure was repeated on days 7, 14, 21 (only in the SAH group) and 28.

Results

Within the first week, CT cisternography consistently showed contrast distribution in the Sylvian fissure contralateral to the blood clot in both SAH and the sham group (Fig. 4). In 4/5 animals with SAH, contrast agent did not penetrate sufficiently into the Sylvian fissure ipsilateral to the clot to achieve preset threshold values for contrast enhancement.

Contrast distribution was significantly lower on sulci of the convexity ipsilateral to the clot ($p<0.05$). Color intensity comparison of Evan's blue staining on gross macroscopy reflected CT cisternography findings with little staining on the brain surface and the arteries ipsilateral to the blood clot.

Discussion

The pattern of changes and differences in contrast distribution between animals in the SAH and sham groups as well as between the hemispheres (with and without clot) confirmed our hypothesis that intrathecal CSF circulation is substantially limited by SAH. Thus, with intrathecal drug delivery after SAH, vasoactive drugs are unlikely to reach sufficient concentrations around the arteries encased by the blood clot or in the cortex covered with blood.

Part 3: Neuropathology

From: Bawarjan Schatlo*, Jens P. Dreier*, Sven Gläsker, Ali-Reza Fathi, Travis Moncrief, Edward H. Oldfield, Ryszard M. Pluta: Report of selective cortical necrosis in the primate clot model of vasospasm after subarachnoid hemorrhage. Neurosurgery, 2010. 67 (3): 721-8.

Methods

We performed a systematic pathological assessment of brains of n=16 cynomolgus species which have previously undergone craniotomy for blood clot placement (n=13) or sham surgery (n=3) (Pluta, Butman et al. 2009). The brains were cut in coronal sections of approximately 0.5 to 1cm starting from the frontal pole to the beginning of the occipital lobe at the level of the cerebral peduncles. Multiple samples were obtained from the right (exposed to the clot) and left cortex and underlying white matter and paraffinized for histological workup (Fig. 2). All slides were stained using hematoxylin and eosin. Sections with pathological appearance were stained selectively using glial fibrillary acid protein (GFAP) and KP-1 (CD68). Pathological evaluation was performed in a blinded manner by a neuropathologist.

Results

We identified two types of cortical infarcts which occurred in a large portion of animals with SAH, but not in control animals undergoing surgery without placement of a clot. "Horizontal infarcts" corresponded to laminar cortical necrosis which affected one or multiple layers of cortex, but not white underlying matter. These infarcts were contiguous to subarachnoid blood clots. Small sized, longitudinal cortical infarcts were observed which we termed "vertical infarcts" (Fig. 5). The presence of infarcts was not correlated with angiographic vasospasm.

Discussion

We provided evidence for the frequent occurrence of horizontal and vertical cortical infarcts after experimental SAH in primates. The lesions share a characteristic morphology with human autopsy findings. The pathogenesis of these lesions cannot be exclusively ascribed to delayed cerebral vasospasm. Thus, we hypothesize that infarcts in this model develop as a result of other known causes of infarcts after SAH, including microembolism, distal vasospasm, and cortical spreading ischemia. Our findings show that this modified nonhuman primate model of SAH may be a tool to examine the role of these mechanisms in the development of delayed ischemia after SAH.

Part 4: Rodent model of ischemia with delayed reperfusion

From: Bawarjan Schatlo, Erica C. Henning, Ryszard M. Pluta, Lawrence L. Latour, Nahal Golpayegani, Marsha J. Merril, Naomi Lewin, Yong Chen, Edward H. Oldfield: Nitrite does not provide additional protection to thrombolysis in a rat model of stroke with delayed reperfusion. Journal of Cerebral Blood Flow and Metabolism, 2008. 28(3): 482-489.

Methods

This experiment was based on an existing model of stroke and reperfusion (Longa, Weinstein et al. 1989) in rats. We modified this widely-used model by increasing the time of ischemia to a maximum of six hours followed by reperfusion. We investigated adjuvant intravenous sodium nitrite with recombinant tissue plasminogen activator (rtPA) in middle cerebral artery occlusion (MCAO) with 6 and 2 hours of ischemia followed by reperfusion in Sprague-Dawley rats (n=59). Rats were randomized to receive treatment or vehicle solutions, and the investigators were blinded until analysis

was completed. Quantitative diffusion, T_1-, T_2-weighted and semi-quantitative perfusion MRI were performed before and after reperfusion and at 48 hours after ischemia to determine the spatiotemporal evolution of stroke. After 48 hours animals were sacrificed and examined to evaluate infarct size and evidence of hemorrhagic transformation. Factor VIII immunostaining was performed to assess vessel morphology.

Results

Nitrite treatment (6 hour group: 37.5µmol over 90 minutes; 2 hour group: 26.25 and 1.75µmol over 60 minutes) did not reduce infarct volume 48 hours after MCAO compared to saline-treated placebo groups after 6 hours or 2 hours of MCAO. Stroke progression from baseline to 48 hours, based on the apparent diffusion coefficient and relative CBF deficits before and after reperfusion and T_2-weighted hyperintensity at 48 hours, did not differ between treated and control animals.

Discussion

To address patients with delayed admission (later than 3h after stroke symptoms), our study examined delayed co-administration of nitrite and rtPA. The rationale for delayed treatment was that an experimental therapy that is effective 6 hours after stroke onset while co-administered with rtPA, could be promising for clinical trials (DeGraba and Pettigrew 2000). Adding nitrite to thrombolysis should reduce reperfusion injury by increasing blood flow and quenching free oxygen radicals, protecting endothelial cells and preventing disruption of the blood brain barrier (BBB) (Pluta, Rak et al. 2001). However, we did not detect a beneficial effect. Possibly, this was due to interactions between the two drugs. For this purpose, the timing of nitrite administration should be adjusted in future studies. Although nitrite alone has been reported to be neuroprotective up to 90 mins after ischemia (Jung, Chu et al. 2006), the absence of an effect in the 6 hour and 2 hour groups suggests that there is a time limit of this potentially beneficial effect. Moreover, BBB integrity deteriorates with ischemia duration. Because both rtPA (Yepes, Sandkvist et al. 2003) and NO affect endothelial integrity (Weyerbrock, Walbridge et al. 2003), a detrimental effect on the BBB of rtPA combined with NO released from nitrite cannot be excluded. Further research is warranted to examine possible interactions of nitrite with rtPA in this setting.

DISCUSSION

Vasospasm, CBF and the pathological correlate of DIND

In 1951 Ecker and Riemenschneider provided the first evidence that neurological worsening of a patient with SAH is associated with angiographic narrowing of the basal cerebral arteries (Ecker and Riemenschneider 1951). We take care to distinguish angiographic vasospasm which may remain clinically silent from symptomatic (or “clinical”) vasospasm. In neurosurgical jargon, vasospasm may be used as a surrogate label for neurological worsening which is not due to hydrocephalus, electrolyte imbalance or other readily identified causes. However, this terminology is misleading. The neurological deterioration is better described by the term delayed cerebral ischemia. More than angiographic vasospasm or increased flow velocities as measured by TCD, cerebral ischemia is associated with poor outcome and death (Frontera, Fernandez et al. 2009). Even in the absence of angiographically proven vasospasm, ischemia may develop due to cortical spreading ischemia or microthrombosis. Thus, an invariable correlate of DIND is the cerebral infarct as described in a seminal pathological series (Neil-Dwyer, Lang et al. 1994). About 13% of patients with SAH develop delayed CT-proven infarcts (Rabinstein, Weigand et al. 2005). The significance of cortical lesions was probably underestimated in these studies due to limited detection of the cortical lesions on CT compared with MRI. Moreover, CBF studies after SAH show perfusion deficits in small, dispersed cortical areas (Ohkuma, Suzuki et al. 2003). Consistently, autopsy studies demonstrated that widespread cortical lesions adjacent to subarachnoid clot represent the predominant infarct pattern associated with DIND (Neil-Dwyer, Lang et al. 1994). The vicinity of the cortical lesions to subarachnoid clot suggested that direct toxic effects of the subarachnoid blood may cause these cortical lesions (Stoltenburg-Didinger and Schwarz 1987; Dreier, Windmuller et al. 2002; Weidauer, Vatter et al. 2008). Some authors suggest that large artery vasospasm is not a necessary condition for these infarcts to occur (Weidauer, Vatter et al. 2008).

Translational research on DIND

In animal models, large artery vasospasm or SAH either produces territorial infarcts or appears to have no effect. Most rodent models of vasospasm rely on (1) endovascular perforation of the MCA or (2) cisternal blood injection to produce SAH. Vascular penetration produces ischemia with a territory corresponding to the territory of the perforated artery (Busch, Beaulieu et al. 1998) which is readily explainable by traumatic vessel occlusion, dissection or immediate vasospasm. Although this model mimics immediate ischemia observed after rupture of an aneurysm, it does not occur in a delayed manner compatible with DIND strictu sensu. In contrast, cisternal injection models produce vasospasm, but have little effect on brain pathology (Sabri, Jeon et al. 2009). Even in larger animals, vasospasm rarely produces a sufficient reduction in CBF to produce infarcts (Sabri, Kawashima et al. 2008). The absence of infarcts in animal models may be due to the smaller size of the brain which allows for a better collateral circulation compared to humans. In the clot model of SAH used in this dissertation, we found that cortical CBF was not decreased during vasospasm. This can be explained by a reactive distal vasodilation which accelerates CBF velocity in the MCA and thus compensates for the decreased diameter of the proximal vasculature. It is also possible that some animal models do not take into account the multitude of factors occurring in patients including brain edema from the initial ictus, hydrocephalus or dysregulation of CBF.

We found two types of cortical infarcts which closely resembled those described in autopsy studies (Robertson 1949). Within the limitations of a small study, we were unable to find any correlation between those infarcts and vasospasm. The finding that infarcts are confined to cortical areas underlying the blood clot rather suggested a direct toxic effect of blood than an effect of proximal vasospasm. In comparison to the classic primate model of vasospasm, a wider dissection of the arachnoid membrane was performed which allowed us to expose more cortical surface to the blood clot. This likely promoted the occurrence of infarcts. The histomorphology of the infarcts suggests either spasm of perforating arterioles or local venous infarcts. Cortical microvascular spasm occurs *in vivo* during perforation of the MCA (Plesnila, personal communication) and superfusion with products of hemolysis (Dreier, Windmuller et al. 2002). The latter is triggered by spreading depolarization due to changed microvascular reactivity as explained above, and leads to spreading ischemia (Dreier, Korner et al. 1998; Petzold, Haack et al. 2008). Electrophysiological recordings obtained from patients with SAH suggest that spreading ischemia causes and accompanies DIND (Dreier, Major et al. 2009).

We demonstrated that CSF circulation is severely impaired in this model of SAH. In turn, a slowed decomposition of the blood clot may prolong the period of vasospasm and metabolic disturbance. Clinical efforts to dissolve the blood clot (Findlay and Jacka 2004) have so far produced inconclusive results. However, the limited penetration of contrast dye into the hemisphere affected by SAH in our primate model shows that intrathecal administration of therapy against vasospasm or DIND will most likely fail to reach the target area underlying the blood clot. More importantly, contrast dye accumulates in areas unaffected by the blood clot. Application of intrathecal vasodilating agents may thus more likely have an effect on brain areas not covered by blood and could lead to an undesired steal effect.

Limitations and outlook

Further study is necessary to assess a number of pending questions regarding our primate model. The pathogenesis of the infarcts may be elucidated by implanting subdural strip electrodes and CBF probes for continuous recordings. This would allow us to identify the role of spreading depolarization and alterations in microcirculation in the development of the observed infarcts. Moreover, due to restrictions of the model, we only measured vessel caliber of the M1 segment, while vasospasm of a distal MCA branch may well have favored the development of a more locoregional infarct. It appears very unlikely but cannot be excluded that arteriography on day 7 caused some of the observed lesions. Finally, our neuropathological conclusions cannot determine the precise timecourse of appearance of the lesions. An additional study with serial MRI would be ideally suited for this purpose.

CONCLUSION

Our hope is that the model of SAH described herein may serve as a preclinical drug evaluation tool not only for large artery vasospasm, but also for cerebral infarcts which ultimately constitute the pathological correlate of DIND. Further study is needed to assess the cause of these lesions and develop treatment strategies to target the mechanism of DIND. Although frustrating at first glance, the strategy of using more realistic animal models may prevent overly optimistic

bench to bedside translation, and ultimately improve the likelihood of successful clinical trials in the domain of cerebral ischemia.

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FIGURES

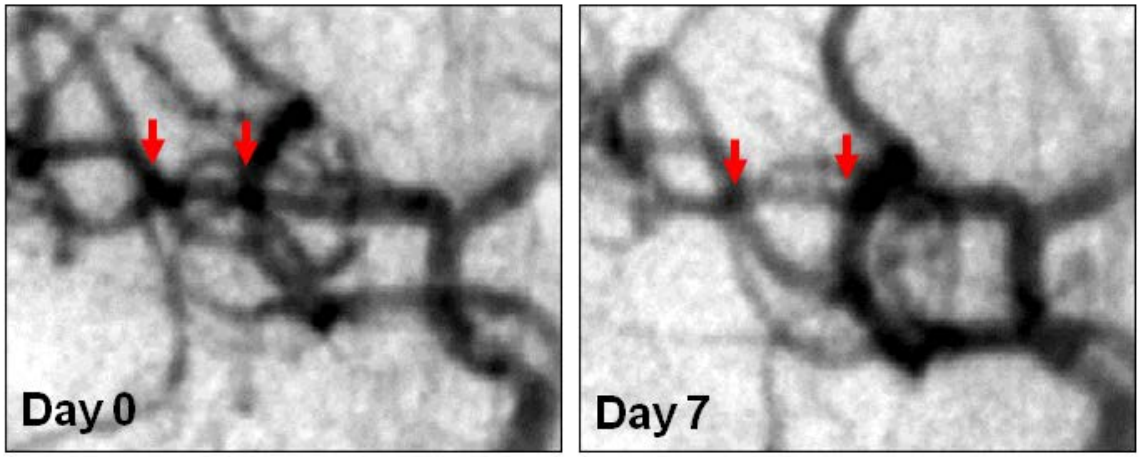

Figure 1
AP arteriography after right carotid artery injection in the primate model of SAH described herein. Left: Day 0 following surgery. The middle cerebral artery (MCA) segment just before the bifurcation is highlighted with red arrows. Right: Day 7 after surgery in a control animal. Scarce filling of the pre-bifurcation MCA segment indicates delayed cerebral vasospasm (Pluta RM, personal communication).

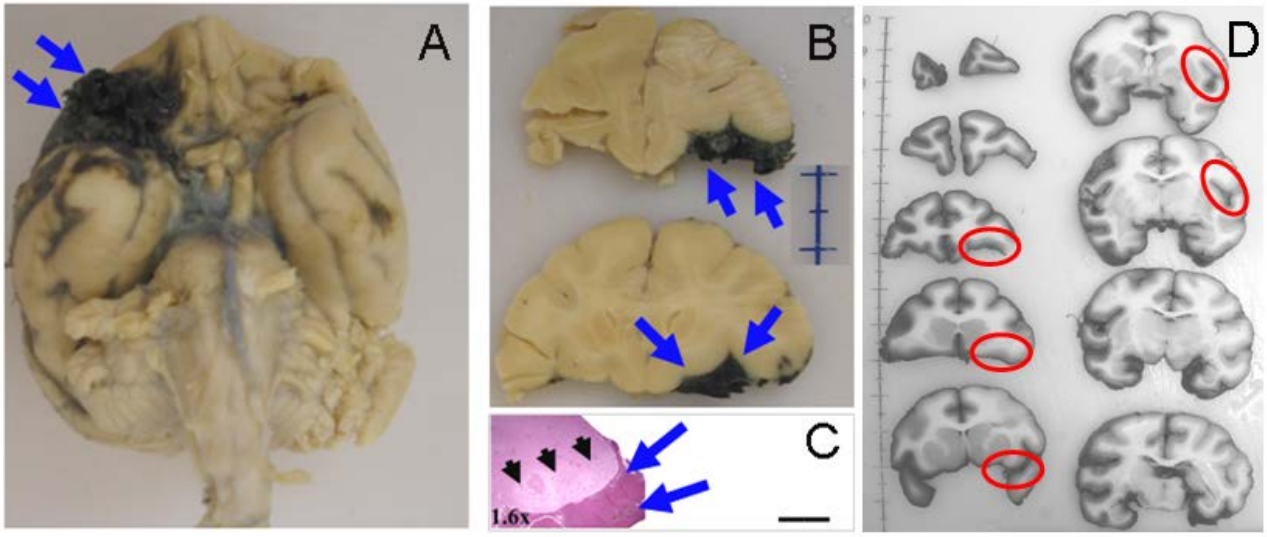

Figure 2
In this clot model of vasospasm, blood is contiguous to the fronto-temporal surface of the brain. A: The surface of the brain is covered with autologous blood (arrow) which still covers the brain 14 days after surgery. B: Gross aspect of coronal slices (arrow: blood clot; scale bar: 1 large interval=1cm). C: This figure represents a histological slide stained with H&E. On this slide, the brain is still covered with blood. The pale area of the otherwise pink-stained brain surface corresponds to the presence of “horizontal” cortical laminar necrosis (black arrows) underlying the blood clot (blue arrows; scale bar=2mm). D: The brains were cut into sections of 0.5 to 1cm ranging from the frontal pole to the level of the cerebral peduncles. The sections were embedded in paraffin for further processing and histopathological workup (scale bar=1cm). Areas of predilection for infarcts are marked with red circles.

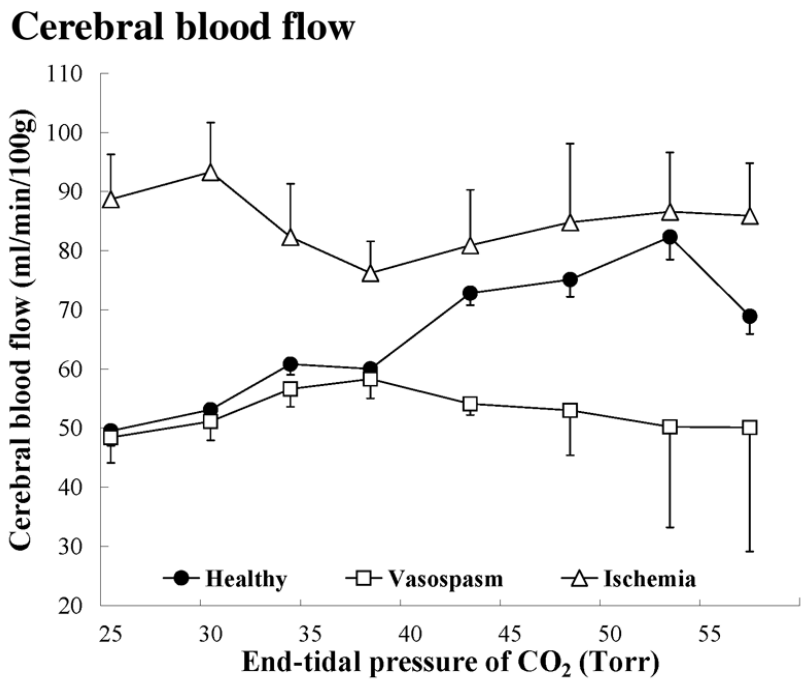

Figure 3
CBF during changes in etpCO_2 in control animals (n=9, filled circles), animals with vasospasm (n= 4, open squares), and after ischemia (n= 3, open triangles). CBF in normal animals shows the well-known CO_2 dependent response: Under hypocapnia, CBF decreases while it increases at higher CO_2 levels. At values above 50 Torr, however, CBF reaches a plateau and decreases again, indicating a limitation of perfusion due to raised intracranial pressure. In animals with delayed cerebral vasospasm, a decrease in CO_2 produces a vasoconstriction, while a rise in CO_2 produced no significant changes, suggesting an abolished vasodilatory reserve. Since vasoconstriction was at least partly preserved, this reactivity pattern corresponds to a “dissociated vasoparalysis”. Interestingly, vasospasm did not produce a significant overall drop in CBF. It appears likely that reactive distal vasodilation with concomitant rise in CBF-V compensate for the decreased diameter of the conductive arteries. In animals who suffered from global cerebral ischemia, CBF showed no significant alteration due to changes in etpCO_2, indicating complete vasoparalysis.

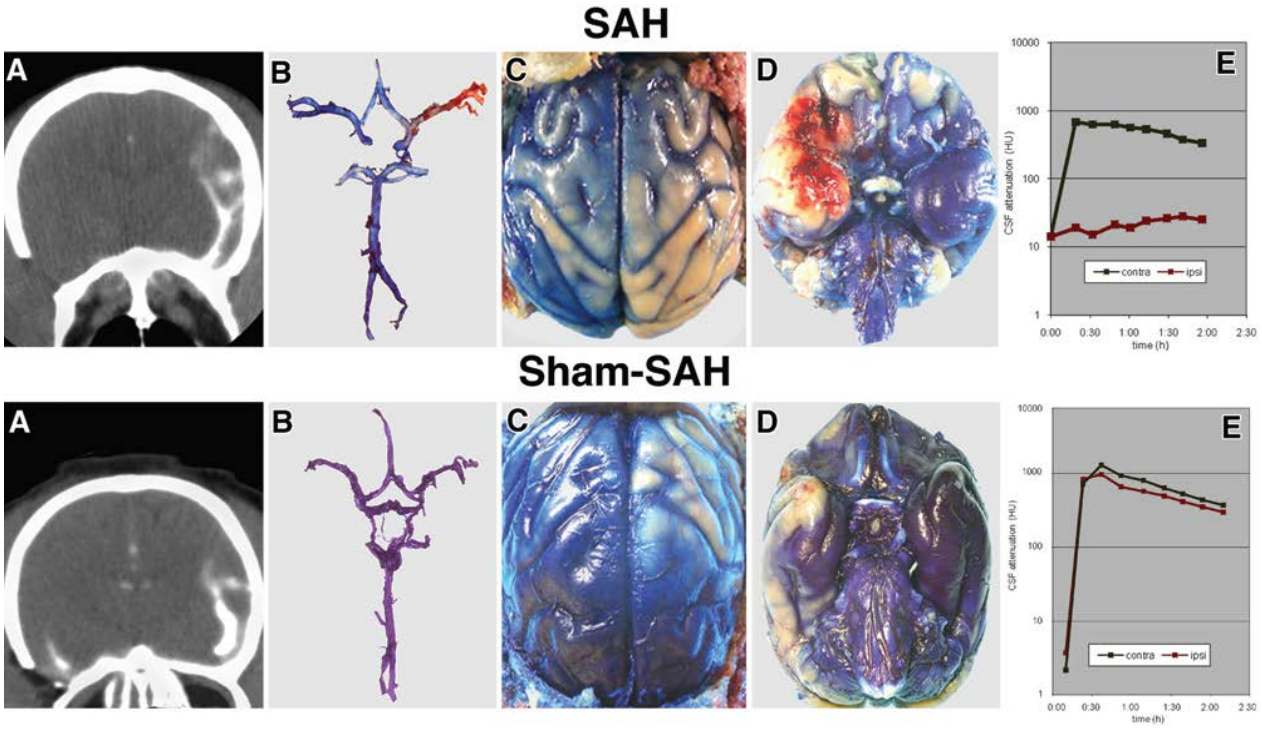

Figure 4
Images from the animals in the SAH *(top row)* and sham SAH *(bottom row)* groups. SAH Group: On day 7 after SAH, CT cisternography (A) revealed contrast material entering the Sylvian fissure and distributed over the surface of the cerebral hemisphere on the side opposite the SAH, but no contrast present in the vicinity of the right MCA. There is significantly less contrast on the surface of the right hemisphere. Evans blue dye injected 3 hours before death (B) stains blue the proximal (M1) portion of the right MCA, the segment close to the ICA, leaving the M2 branches unstained. The convexity of the right hemisphere (C) and the base of the frontal and temporal lobes near the right Sylvian fissure (D) are also less stained than the corresponding areas of the left cerebral hemisphere. The graph (E) depicts the CSF attenuation in the Sylvian fissure on the CT cisternographies performed every 30 minutes for 2 hours on postoperative day 7 after SAH in the same animal. There was a significant difference ($p < 0.05$) in the CSF attenuation between the right (ipsilateral) and left (contralateral) sides. Sham SAH Group: On day 7 after sham SAH, CT cisternography (A) revealed contrast material entering the Sylvian fissure along the left MCA and distribution of the material over the convexity of the cerebral hemisphere on the side opposite surgery. Contrast is also present in the vicinity of the right MCA in the right Sylvian fissure. There is less contrast on the surface of the right cerebral hemisphere. Evans blue dye injected 3 hours before death (B) evenly stained blue the right and left MCAs as well as the rest of the cerebral arteries, although there is an appreciable difference in dye distribution over the convexity of the right and left hemispheres (C) and at the base on the brain (D). The graph (E) depicts the CSF attenuation in the Sylvian fissure on the CT cisternographies performed every 30 minutes for 2 hours on postoperative day 7 after sham SAH surgery in the same animal. There was no difference ($p > 0.05$) in the CSF attenuation between the right (ipsilateral) and left (contralateral) sides but there was diminished contrast in the parietal region on the ipsilateral compared with contralateral side ($p < 0.02$).

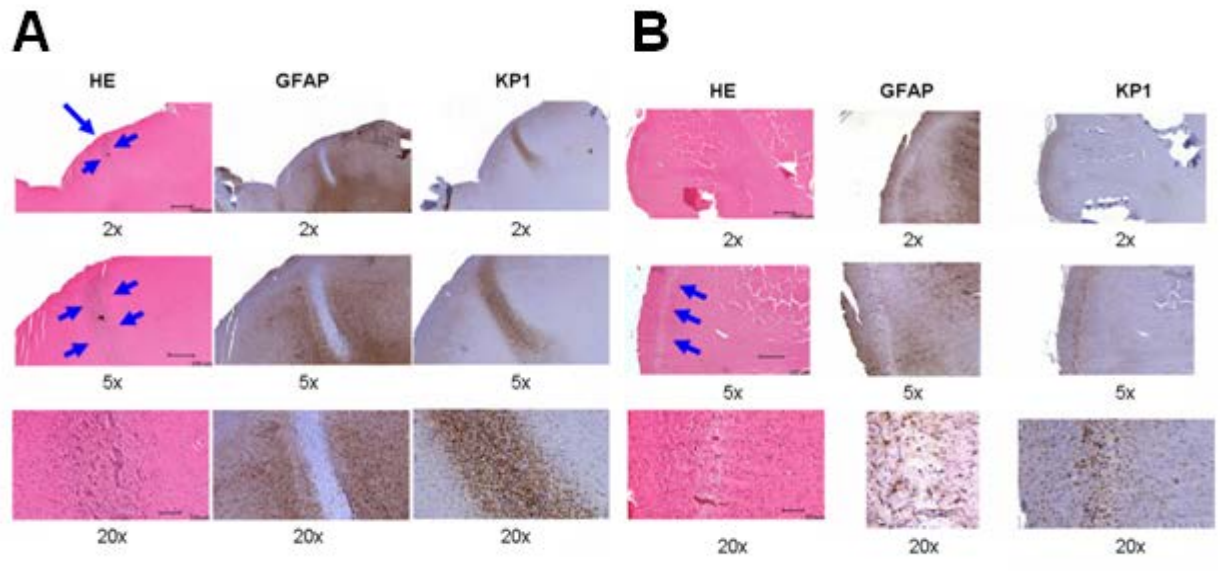

Figure 5
Types of necrosis occurring in the primate model of SAH. We observed mainly cortical changes which we categorized as (A) vertical and (B) horizontal infarcts. Vertical infarcts are wedge- or pillar-shaped and affect an area which corresponds to a single perforating arteriole. Horizontal infarcts stretch along one or multiple cellular layers of cortex while respecting the grey-white matter boundary. (*Scale bars: 2x magnification: 1mm; 5x magnification: 500µm; 10x magnification: 100µm*).

ANTEILSERKLÄRUNG

Bawarjan Schatlo hatte folgenden Anteil an den vorgelegten Publikationen:

Publikation 1:
Bawarjan Schatlo, Sven Gläsker, Alois Zauner, Byron Gregory Thompson, Edward H. Oldfield, Ryszard M. Pluta
Continuous neuromonitoring using transcranial Doppler reflects blood flow during carbon dioxide challenge in primates with global cerebral ischemia.
Neurosurgery, 2009. 64 (6): 1148-1154.

30 Prozent
Beitrag im Einzelnen: Datenerhebung und Analyse, Verfassen des Manuskripts

Publikation 2:
Ryszard M. Pluta, John A. Butman, Bawarjan Schatlo, Dennis L. Johnson, Edward H. Oldfield
Subarachnoid hemorrhage and the distribution of drugs delivered into the cerebrospinal fluid. Laboratory investigation.
Journal of Neurosurgery, 2009 111(5): 1001-1007.

15 Prozent
Beitrag im Einzelnen: Assistenz bei den Experimenten und Korrektur des Manuskripts

Publikation 3:
Bawarjan Schatlo*, Jens P. Dreier*, Sven Gläsker, Ali-Reza Fathi, Travis Moncrief, Edward H. Oldfield, Ryszard M. Pluta
Report of selective cortical necrosis in the primate clot model of vasospasm after subarachnoid hemorrhage.
Neurosurgery, 2010. 67 (3): 721-8.

35 Prozent
Beitrag im Einzelnen: Konzept, histopathologische Aufarbeitung und Färbung und Verfassen des Manuskripts

Publikation 4:
Bawarjan Schatlo, Erica C. Henning, Ryszard M. Pluta, Lawrence L. Latour, Nahal Golpayegani, Marsha J. Merril, Naomi Lewin, Yong Chen, Edward H. Oldfield
Nitrite does not provide additional protection to thrombolysis in a rat model of stroke with delayed reperfusion.
Journal of Cerebral Blood Flow and Metabolism, 2008. 28(3): 482-489.

50 Prozent
Beitrag im Einzelnen: Assistenz bei der Antragstellung, Tierexperimente (eigenständig), Durchführung der Kernspintomographie-Bildgebung, histologische Aufarbeitung, Datenanalyse und Verfassen des Manuskripts

Promovend	Betreuender Hochschullehrer
Bawarjan Schatlo	Prof. Dr. med. Jens Dreier

LITERATURVERWEIS

Folgende Arbeiten sind Teil dieser Publikationspromotion:

Publikation 1:
Bawarjan Schatlo, Sven Gläsker, Alois Zauner, Byron Gregory Thompson, Edward H. Oldfield, Ryszard M. Pluta
Continuous neuromonitoring using transcranial Doppler reflects blood flow during carbon dioxide challenge in primates with global cerebral ischemia.
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Journal of Cerebral Blood Flow and Metabolism, 2008. 28(3): 482-489.

CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

LIST OF PUBLICATIONS

Original contributions (peer-reviewed)

1. Ferrier MC, Sarin H, Fung SH, Schatlo B, Pluta RM, Gupta SN, Choyke PL, Oldfield EH, Thomasson D, Butman JA. Validation of dynamic contrast-enhanced magnetic resonance imaging-derived vascular permeability measurements using quantitative autoradiography in the RG2 rat brain tumor model. Neoplasia 2007;9(7):546-55.

2. Schatlo B, Glasker S, Zauner A, Thompson GB, Oldfield EH, Pluta RM. Correlation of end-tidal CO2 with transcranial Doppler flow velocity is decreased during chemoregulation in delayed cerebral vasospasm after subarachnoid haemorrhage--results of a pilot study. Acta Neurochir Suppl 2008;104:249-50.

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5. Schatlo B, Glasker S, Zauner A, Thompson BG, Oldfield EH, Pluta RM. Continuous neuromonitoring using transcranial Doppler reflects blood flow during carbon dioxide challenge in primates with global cerebral ischemia. Neurosurgery 2009;64(6):1148-54; discussion 1154.

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1. Panciani PP, Fontanella M, Crobeddu E, Schatlo B, Bergui M, Ducati A. Spontaneous occlusion of a spinal arteriovenous malformation: is treatment always necessary? J Neurosurg Spine 2010;12(4):397-401.

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Abstracts

1. Golpayegani N, Gläsker S, Li J, Schatlo B, Oldfield EH, Vortmeyer AO, Pluta RM. Neuroglobin, a neuron-specific hemoglobin, is present in glial brain tumors. Paper presented at: NIH Research Symposium, 2005; Bethesda, MD.

2. Schatlo B, Henning EC, Sarin H, Latour LL, Lewin N, Despres D, Golpayegani N, Vortmeyer AO, Angstadt M, Oldfield EH, Pluta RM. Sustained blood brain barrier damage in a rat model of delayed cerebral reperfusion injury - a magnetic resonance imaging study. NIH Research Symposium. Bethesda, MD; 2005.

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14. Kotowski M, Schulz S, Schatlo B, Jägersberg M, Bijlenga P, Rüfenacht D, Schaller C, Hofmann-Apitius M, Rumpf K, Fluck J. A data-model for structuring and mediation of knowledge on risk factors for intracranial

aneurysm formation and rupture as part of the integrated biomedical informatics for the management of cerebral aneurysms (Abstract ID: 88). Swiss Archives of Neurology and Psychiatry 2008;159(4):291.

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21. Gläsker S, Vortmeyer AO, Schatlo B, Lonser RR, Pluta RM. Growth mechanisms of CNS hemangioblastomas (Abstract ID: DocP08-02). 60th annual meeting of the German Society for Neurosurgery. Münster; 2009.

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1. Golpayegani N, Gläsker S, Li J, Schatlo B, Oldfield EH, Vortmeyer AO, Pluta RM. Neuroglobin, a neuron-specific hemoglobin, is present in glial brain tumors. Paper presented at: NIH Research Symposium, 2005; Bethesda, MD.
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10. Kotowski M, Schulz S, Schatlo B, Jägersberg M, Bijlenga P, Rüfenacht D, Schaller C, Hofmann-Apitius M, Rumpf K, Fluck J. A data-model for structuring and mediation of knowledge on risk factors for intracranial aneurysm formation and rupture as part of the integrated biomedical informatics for the management of cerebral aneurysms (Abstract ID: 88). 100th Anniversary Meeting of the neurosurgical and neurological societies of Switzerland. Montreux; 2008. p. 291.

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12. Schatlo B, Henning EC, Latour LL, Merrill MJ, Oldfield EH, Pluta RM. Microvascular factor VIII staining pattern correlates with the duration of infarct after ischemic stroke and thrombolysis in rats FENS. Geneva; 2008.

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15. Schatlo B, Vortmeyer AO, Gläsker S, Oldfield EH, Pluta RM, Dreier J. Cortical infarct pattern in a primate model of vasospasm resembles necrosis in patients with delayed deficits after subarachnoid hemorrhage (Abstract ID: 105). 100th Anniversary Meeting of the neurosurgical and neurological societies of Switzerland. Montreux; 2008.

16. Schatlo B, Kotowski M, Schaller K, Tessitore E. Safety and accuracy of robot-assisted spinal instrumentation. Annual Meeting of the Congress of Neurological Surgeons. San Francisco, CA; 2010.

17. Tenan M, Schatlo B, Kotowski M, Marino D, Schaller K, Radovanovic I, Clément V. Gliomas derived from human glioma-initiating cells show no specific expression of GFAP, YKL-40 and S100b. 61st Annual meeting of the German Society for Neurosurgery Mannheim; 2010.

Oral presentations/Lectures/Conferences

1. Schatlo B. Animal models of ischemic stroke - defining clinically useful endpoint. Paper presented at: National Institutes of Neurological Disorders and Stroke Research Rounds, 2006; Bethesda, MD.

2. Schatlo B. Continuous transcranial Doppler ultrasound assesses cerebral blood flow after cerebral ischemia but not in vasospasm or in healthy primates. Paper presented at: 9th International conference on cerebral vasospasm, 2006; Istanbul, Turkey.

3. Schatlo B, Gläsker S, Zauner A, Thompson BG, Oldfield EH, Pluta RM. Transcranial Doppler assesses cerebral blood flow in primates with exhausted vasomotor capacity. 56th annual meeting of the Congress of Neurological Surgeons. Chicago, IL; 2006.

4. Schatlo B. Mechanisms of delayed ischemic deficits after subarachnoid hemorrhage. Paper presented at: Neurosurgery Chairman Lecture, 2007; Mayo Clinic, Rochester, MN.

5. Schatlo B. Cortical infarct pattern in a primate model of vasospasm resembles necrosis in patients with delayed deficits after subarachnoid hemorrhage. Paper presented at: 100th Anniversary Meeting of the neurosurgical and neurological societies of Switzerland, 2008; Montreux.

6. Schatlo B. Cortical necrosis in a primate model of subarachnoid hemorrhage – is it a model of delayed ischemic neurological deficits? Paper presented at: Annual meeting of the American Association of Neurological Surgeons, 2008; Chicago, IL.

7. Schatlo B. Cortical necrosis occurs independently of vasospasm in a primate model of subarachnoid hemorrhage – the first animal model of delayed ischemic deficits? Paper presented at: 59th Annual meeting of the German Society of Neurosurgery, 2008; Würzburg.

8. Schatlo B. Markers of glial tumors in biological fluids – Opportunities for diagnosis and treatment. Paper presented at: Neuroscience rounds, 2008; Lausanne, Switzerland.

9. Schatlo B. A preclinical randomized, blinded, placebo-controlled study of sodium nitrite as an adjunct to delayed thrombolysis for ischemic stroke. Paper presented at: 59th Annual meeting of the German Society of Neurosurgery, 2008; Würzburg.

10. Schatlo B, Vortmeyer AO, Gläsker S, Oldfield EH, Pluta RM, Dreier J. Cortical infarct pattern in a primate model of vasospasm resembles necrosis in patients with delayed deficits after subarachnoid hemorrhage (Abstract ID: 105). 100th Anniversary Meeting of the neurosurgical and neurological societies of Switzerland. Montreux; 2008.

11. Schatlo B. Classifying brain damage in an animal model of vasospasm after subarachnoid hemorrhage. Paper presented at: 10th International Conference on Cerebral Vasospasm, 2009; Chongqing, China.

12. Schatlo B. The use of transcranial Doppler ultrasound to guide treatment after subarachnoid hemorrhage. Paper presented at: 10th International Conference on Cerebral Vasospasm, 2009; Chongqing, China.

13. Schatlo B. Hydrocephalus through the ages. Paper presented at: Neuroclub, 2010; Geneva, Switzerland.

STATUTORY DECLARATION

„Ich, Bawarjan Schatlo, erkläre, dass ich die vorgelegte Dissertation mit dem Thema:

Zerebrale Ischämie nach experimenteller Subarachnoidalblutung

(Cerebral ischemia in experimental subarachnoid hemorrhage)

selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

24.10.2011 _____

Unterschrift