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Multimodal MRI characterization of experimental subarachnoid hemorrhage

Yuhao Sun, M.D., Ph.D.^{1,2}, Qiang Shen, Ph.D.², Lora Talley Watts, Ph.D.^{2,3,4}, Eric R. Muir, Ph.D.², Shiliang Huang, Ph.D.², Guo-Yuan Yang, M.D., Ph.D.^{1,5}, Jose I Suarez, M.D.⁶, and Timothy Q. Duong, Ph.D.²

¹Departments of Neurosurgery, Stereotactic and Functional Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

²Research Imaging Institute, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA

³Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA

⁴Department of Neurology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA

⁵Neuroscience and Neuroengineering Research Center, Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, China

⁶Division of Vascular Neurology and Neurocritical Care, Department of Neurology, Baylor College of Medicine, Baylor St Luke's Medical Center, Houston, TX 77027, USA

Abstract

Subarachnoid hemorrhage (SAH) is associated with significant morbidity and mortality. We implemented an in-scanner rat model of mild SAH in which blood or vehicle was injected into the cistern magna, and applied multimodal MRI to study the brain prior to, immediately after (5mins to 4hrs), and up to 7 days after SAH. Vehicle injection did not change arterial lumen diameter, apparent diffusion coefficient (ADC), T₂, venous signal, vascular reactivity to hypercapnia, or foot fault scores, but mildly reduce cerebral blood flow (CBF) up to 4hrs, and open-field activity up to 7 days post injection. By contrast, blood injection caused: i) vasospasm 30mins after SAH but not thereafter, ii) venous abnormalities at 3hrs and 2 days, delayed relative to vasospasm, iii) reduced basal CBF and CBF response to hypercapnia 1–4hrs but not thereafter, iv) reduced ADC immediately after SAH but no ADC and T₂ changes on day 2 and 7, and v) reduced open-field activities in both SAH and vehicle animals, but no significant differences in open-field activities

[§]Corresponding author: Timothy Q. Duong, Ph.D., Research Imaging Institute, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, Texas 78229, USA, Tel: 210-567-8115 duongt@uthscsa.edu.

Disclosure Statement

No conflict of interest.

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and foot fault tests between groups. Mild SAH exhibited transient and mild hemodynamic disturbances and diffusion changes, but did not show apparent ischemic brain injury nor functional deficits.

Keywords

magnetic resonance imaging; subarachnoid hemorrhage; cerebral ischemia

Introduction

Subarachnoid hemorrhage (SAH) is a neurologic emergency associated with significant morbidity and mortality representing the deadliest type of acute stroke.¹ The hallmarks of SAH include increased intracranial pressure (ICP), hypoperfusion, and delayed cerebral ischemia (with or without vasospasm).^{2–7} The pathophysiology of acute SAH, particularly in the milder forms, is poorly understood because it is challenging to study acute SAH in a systematic and controlled manner in humans. Animal models of SAH are important to unravel the underlying pathophysiological mechanisms that could inform clinical SAH conditions. Two common animal models of SAH are arterial perforation typically via the internal carotid artery, and blood injection typically into the cistern magna. The values of the blood injection model are that it models the effects of bleeding in the subarachnoid space, the degree of injury can be controlled, and it has relatively high survival rates.^{8–10} The disadvantage is that it does not mimic the processes surrounding aneurysmal rupture.¹¹

Magnetic resonance imaging (MRI) provides non-invasive structural, physiological and functional imaging data of the whole brain in a longitudinal fashion. MRI studies of SAH have reported vasospasm using magnetic resonance angiography (MRA),¹² reduced cerebral blood flow (CBF),^{13,14} reduced cerebrovascular reserve by hypercapnic challenge,^{15,16} ischemic brain injury using diffusion-weighted MRI^{17,18}, and cerebral edema or infarction using T₂ MRI.^{12,19,20} However, these changes in the blood-injection model to the cistern magna have not been widely explored, especially in its hyperacute phase. Moreover, the effects of SAH on the veins have not been reported to our knowledge. We suspect that SAH could affect venules and veins resulting in changes that could contribute to pathophysiology of SAH. This is supported by the fact that vascular oxidative stress and tissue hypoxia after SAH increase thrombogenicity, tissue inflammation, and neurodegenerative changes.²¹ In addition, the release of vasoactive agents after SAH are likely to interfere with both arterioles and venules tone. MR venography could offer a unique non-invasive means to visualize changes in the veins associated with SAH.

The goal of our study was to implement an in-scanner rat model of mild SAH in which blood is injected into the cistern magna, and to apply multimodal MRI to investigate SAH pathophysiology in the hyperacute to subacute phase. The in-scanner SAH model enabled MRI measurements before and immediately after SAH in the same animals. We chose to investigate a relatively mild SAH model to achieve good survival rate for longitudinal studies. MRA, MR venography (MRV), cerebral blood flow, and cerebrovascular reserve (by measuring CBF response to hypercapnia), diffusion and T₂ were evaluated. Functional

measures using the open-field and foot-fault tests were also performed. Comparisons were made with the vehicle group injected with artificial cerebrospinal fluid.

Methods

Animal preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio. The study was written following ARRIVE guidelines. Two groups of male Sprague-Dawley rats (350–400 g) were studied: *i*) a SAH group injected with blood into the cisterna magna (N=10) and *ii*) a vehicle group injected with artificial cerebral-spinal fluid (ACSF) (N=10). Power analysis (G Power, version 3.1, Heinrich Heine University, Germany) yielded 9 animals per group to be the minimal sample size needed. The study was randomized and vehicle-controlled, but the experimenter was not blinded to the conditions of the animal group assignment.

Rats were orally intubated, mechanically ventilated and anesthetized with 2% isoflurane mixed with room air. The nuchal muscle layers were divided at the midline, retracted laterally to expose the lamina of the atlas and the atlanto-occipital membrane. A small midline burr hole was made just rostral to the interparietal-occipital suture using a high-speed drill. A PE-10 catheter attached to a 1 ml syringe was introduced along the inner table of the occipital bone into the cisterna magna. Under manual manipulation, 100µl of cerebral-spinal fluid was gently aspirated. To collect autologous blood, the rat's tail was immersed in warm water for 2 mins, cleaned with 70% ethanol, and 1.5 cm of the tail tip was then removed with a pair of sterile surgical scissors. We did not attempt to increase blood flow by rubbing the tail, as this will result in leukocytosis and increase the risk of tissue fluid contamination. Then, blood or ACSF^{22,23} was loaded in PE-10 tubing, which had been pretreated with heparin-doped saline (100 USP/ml) and then rinsed with saline. The tubing was fixed with tissue glue without any leakage and the wound was closed. Anesthesia was reduced to 1.2–1.5% isoflurane and maintained at this level during all MRI studies.

Rats were placed in a stereotaxic frame equipped with ear and tooth bars and secured in the supine position using an MRI-compatible rat stereotaxic headset. While the animal was in the magnet, the 300µL of blood or ACSF was injected using a micro-pump over a period of 7 mins. Imaging was performed before, repeatedly from 15 mins to 4hrs after the blood or ACSF was injected. During MRI, end-tidal CO₂, rectal temperature, heart rate and arterial oxygen saturation were recorded and maintained within normal physiological ranges. At the end of 4 hours, the animals were extubated and allowed to recover in home cages.

MRI studies were repeated on again on day 2 and 7. For studies on subsequent days, animals were re-anesthetized also under 1.5% isoflurane. The ACSF group, which also experienced repeated anesthetics, was the control group.

MRI experiments

Imaging was performed on a Bruker Biospec 11.7 Tesla/16 cm scanner with a 76G/cm BGA9S gradient insert (Billerica, MA) using a custom-made surface coil for brain imaging and a neck coil for perfusion labeling.^{24–27}

CBF—Basal CBF and CBF response to hypercapnic challenge were measured using the continuous arterial spin labeling (ASL) technique with gradient echo-planar imaging (EPI). ASL used a 2.7 s square radiofrequency pulse to the labeling coil with a post-labeling delay of 250 ms. Paired images were acquired alternately – one with spin labeling and the other without. Other MRI parameters were: single shot, matrix = 96×96 (reconstructed to 128×128), field of view (FOV) = 25.6×25.6 mm, seven 1.5 mm thick slices, 90° flip angle (FA), bandwidth = 300 kHz, repetition time (TR) = 3 s, and echo time (TE) = 10.2 ms. For basal CBF, 100 pairs of images were averaged. For measuring CBF responses, 60 pairs of images (6 mins) were acquired during baseline and 40 pairs (4 mins) during hypercapnic challenge (5% CO₂ in air).

MRA—3D fast low angle shot (FLASH) sequence was used with TE/TR = 2.125/15 ms, FA = 25°, bandwidth = 100 kHz, FOV = 25.6×20.8×12.8 mm, matrix = 256×256×256 (100×80×50 μm) and 3 signal averages.

MRV—For MRV imaging, a 3D FLASH sequence was used with TE/TR = 12/40 ms, FA = 20°, bandwidth = 20 kHz, FOV = 25.6×25.6×12.8 mm, matrix = 256×256×128 (100×100×100 μm).

ADC—Diffusion weighted images were obtained with five b=0 and 30 directions with a 1200 s/mm² b_{max}-value. Single-shot, spin-echo EPI scans with partial Fourier (3/4) were acquired using the following settings: seven 1.5-mm coronal images, field of view (FOV) = 25.6 × 25.6 mm, matrix 96×96 and reconstructed to 128 x 128, TR = 3 s, TE = 28 ms, separate between diffusion gradient = 9 ms, diffusion gradient duration δ = 3 ms, and 2 transients for signal averaging.

T₂—T₂-weighted images were acquired using a fast spin-echo pulse sequence with four effective TEs (25, 32, 75 and 96 ms), TR = 3 s (90° flip angle), matrix = 128 x 128, FOV = 25.6× 25.6 mm, echo train length = 4, and 4 signal averages.

Data analysis

MRI measurements were analyzed using Matlab (version R2013a, MathWorks, Natick, MA) and STIMULATE (version 6.0, University of Minnesota, Minneapolis, MN). Images from each rat at different time points were co-registered.

Horizontal angiograms (reconstructed to 50×50 μm) along the rostrocaudal axis were generated by maximum intensity projections from the MRA data.^{25–27} A customized Matlab code was used to measure artery diameter. Profiles perpendicular to the vessel were obtained over a segment of the artery, and the diameter was measured as the full-width at half maximum from the profiles. Diameters were measured for the bilateral anterior cerebral artery, bilateral middle cerebral artery and basilar artery were utilized to calculate the relative diameter change.

Axial venograms were generated by minimum intensity projections of every 15 slices from the MRV data. To quantify the MRV changes, a large ROI encompassing the neocortex was used and volume with intensity below the fixed threshold was obtained. ROI intensity in the

cortex was normalized to unity. The relative volume of pixels with hypointensity was obtained using an objective but arbitrary threshold < 0.7 within the cortex based on the intensity histogram profile which reasonably separated the vein and tissue signals.

Quantitative CBF, ADC and T2 and hypercapnia-induced percent change maps were calculated as previously described.^{24,26,28–30} Regions of interest (ROIs) were drawn in the cortex, striatum and hippocampus across multiple image slices.

Functional assessment

Sensorimotor function was assessed using the open field³¹ and foot-fault tests.^{32–34} Testing was conducted prior to SAH and again 1, 2, 4 and 7 days post SAH.

The open field was made of a lusterless Perspex box (20×35×35 inches), which was divided into a 50% central zone and the surrounding border zone. Rats were placed in the corner of the open field. Experiments were recorded using a video camera suspended approximately 80 inches above the open-field arena. The apparatus was cleaned prior to the introduction of each animal. Their behavior (i.e. locomotor activity) was videotaped for 10 mins by a digital camera with post-recording analysis performed using Anymaze software (Stoelting Co., Wood Dale, IL) to measure total distance traveled.

The forelimb foot fault test was performed with videotaping to assess forelimb misplacement during locomotion. The rat was placed on an elevated grid floor (size 18×11 inches with grid openings of 1.56×1.00 inches) for 5 mins. The rat was allowed to move freely on the grid and the total number of steps and the number of times a forelimb fell below the grid opening were counted. The percentage of foot faults was calculated as the number of foot faults divided by the total number of steps taken.

Statistical analysis

Data are reported as mean $\pm$ SEM. For comparisons across the different time points, data were first analyzed by one-way ANOVA. If the data were normally distributed this was followed by a Tukey's test; if the data were not normally distributed we utilized a Kruskal Wallis test. Mann-Whitney U tests were used to compare differences at each time point between blood and ACSF injected animals. Two tail $p < 0.05$ was taken to be statistically significant.

Results

One animal from each group was excluded due to failed surgery before MRI. In the SAH group, 7/9 survived 24 hrs and 6/9 survived to 7 days. In the ACSF group, 9/9 survived 24 hrs and 8/9 survived up to 7 days. The heart rate, arterial oxygen saturation, and rectal temperature were 360 ± 16 bpm, 96.5 ± 4.4 %, and $36.8 \pm 0.5^\circ\text{C}$, respectively, in SAH group, and 355 ± 18 bpm, 96.4 ± 4.8 %, and $37.0 \pm 0.6^\circ\text{C}$ in the ACSF group. These physiological parameters were not significantly different between the ACSF and SAH group across all time points studied ($p > 0.05$) and are consistent with our studies under the same isoflurane preparation in normal animals and various injury models.^{24,26,28–30,35,36}

Although end-tidal CO₂ was monitored, the long sample line resulted in reduced recorded CO₂ values and thus it was not reported.

Arteries

Mean arterial lumen diameters were analyzed for the bilateral anterior cerebral arteries, bilateral middle cerebral arteries and the basilar artery and the percent changes in diameters between pre and post injection were tabulated (Figure 1). After ACSF injection, the arterial lumen diameters were not significantly different across all time points studied ($p>0.05$). After blood injection, the arterial lumen diameters significantly narrowed at 30 mins ($p<0.05$), but not thereafter at 150 mins, 2 and 7 days ($p>0.05$).

Arterial lumen diameters between the blood and ACSF groups were not significantly different pre-injection, 150 mins, 2 and 7 days after injection ($p>0.05$), but were significantly different at 30 mins after injection ($p<0.05$).

Veins

MRV and the volume fraction with hypointense pixels in the neocortices are shown in Figure 2. After ACSF injection, MRV showed no significant changes in the volume fraction of hypointense pixels compared to pre-injection at all time points studied. After blood injection, MRV showed significantly enlarged hypointensity bands compared to pre-injection ($p<0.05$), with the volume fraction of hypointense pixels on MRV peaking at 3 hrs and returned toward pre-injection value on days 2 and 7.

The volume fractions of hypointense pixels on MRV were not significantly different between the blood and ACSF groups at pre-injection ($p>0.05$), 1 hr, and 7 days after injection, but were significantly different at 3 hrs and 2 days after injection ($p<0.05$).

Cerebral blood flow

Representative CBF maps and group CBF data from the neocortex, hippocampus, and striatum at 15 mins, 2 hrs, 4hrs, 2 days and 7 days from blood and ACSF groups are shown in Figure 3. After ACSF injection, CBF from the cortex, hippocampus, and striatum were reduced slightly but not significantly (except in the striatum at 15 mins after injection) up to 7 days compared to pre-injection. After blood injection, CBF was reduced at 15 mins, 2 hrs and 4hrs ($p<0.05$) but returned toward normal and was not significantly different from pre-injection at 2 and 7 days ($p>0.05$).

CBF values were not significantly different between the blood and ACSF groups at pre-injection ($p<0.05$), 2 and 7 days after injection ($p>0.05$), but were significantly different at 15 mins, 2 and 4 hrs after injection ($p<0.05$).

Vascular reactivity (VR)

Vascular reactivity data, as measured by CBF responses to hypercapnia, are shown in Figure 4 for the cortex, hippocampus and striatum. After ACSF injection, CBF responses were not significantly different from pre-injection responses across different time points in the three

regions analyzed ($p>0.05$). After blood injection, CBF responses were significantly different from pre-injection at 1 and 3 hrs, but not at 2 and 7 days.

CBF responses were not significantly different between the blood and ACSF groups at pre-injection, 150 mins, 2 and 7 days after injection ($p>0.05$), but were significantly different between the blood and ACSF groups at 1 hr for all three structures analyzed and at 3 hrs for the striatum after injection ($p<0.05$).

Diffusion and T2 MRI

Group ADC and T₂ data are shown in Figure 5 for the cortex, hippocampus and striatum. Pre-injection ADC and T₂ values were not significantly different between the ACSF and SAH group ($p>0.05$). ACSF did not affect ADC and T₂ at all time points studied. The ADC values were significantly different between the blood and ACSF groups only at 5 mins after injection ($p<0.05$) but not at subsequent time points ($p>0.05$) in all brain structures analyzed. T₂ values were not significantly different between the blood and ACSF groups at all post-injection time points in all brain structures analyzed ($p>0.05$).

Behavioral scores

Behavioral assessments using open field and foot-fault tests are presented in Figure 6. After ACSF injection, travel distances were mildly reduced compared to pre-injection. After blood injection, travel distances were markedly reduced compared to pre-injection across all time points ($p < 0.05$). Travel distances were not significantly different between the blood and ACSF groups at pre-injection, 2 and 4 days after injection ($p>0.05$), but were significantly different at 1 and 7 days after injection ($p<0.05$).

Foot fault scores were not significantly different compared to pre-injection across all time points studied ($p>0.05$). The foot fault scores on day 1 increased in the blood group albeit non-significantly compared to ACSF group ($p>0.05$).

DISCUSSION

We implemented an in-scanner rat model of SAH and applied multimodal MRI to longitudinally study the brain prior to SAH, immediately after SAH (5 mins – 4 hrs), 2 and 7 days. All measured pre-injection parameters were not significantly different between ACSF and SAH group. ACSF injection did not affect arterial lumen diameter, ADC, T₂, venous signal, vascular reactivity, and foot fault scores, but mildly reduce CBF at 1–3 hrs and open-field activity up to 7 days. By contrast, blood injection caused: i) vasospasm at 30 mins after SAH but not thereafter, ii) venous signal abnormalities at 3 hrs and 2 days but delayed relative to vasospasm, iii) reduced basal CBF and CBF changes in response hypercapnia at 1–4 hrs, iv) decreased ADC 5 mins after blood injection but no apparent ischemic brain injury up to 7 days as measured by ADC and T₂, and v) reduced open-field activities in both SAH and ACSF animals, but no significant differences in open-field activities and foot fault scores between the ACSF and SAH group up to 7 days, suggesting that while there was likely surgery-related trauma, there were no apparent neurological deficits in this mild SAH model.

Effects of ACSF injection

ACSF injection did not affect arterial lumen diameter, T₂, ADC, MRV signal, vascular reactivity, but mildly reduce CBF only at 1–3 hrs compared to pre-injection. Mild reduction in CBF is not surprising because ACSF likely transiently increased ICP, thereby reducing perfusion pressure.^{3,37,38} Animals with ACSF injection tended to freeze and stay close to the walls (i.e., asthigmotaxis) rather than exploring the environment compared to pre-injection. As such they exhibited reduced open-field activity up to 7 days, indicative of post-surgical trauma. ACSF injection however did not affect foot fault scores, suggesting no apparent neurological deficits.^{39,40}

Effects of blood injection

Vasospasm—Vasospasm is often thought to be the prime mechanism of delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage.¹ Vasospasm was detected immediately after SAH (30 mins) but not thereafter, indicating that vasospasm is transient in this mild SAH model. Previous studies using ex vivo arteries^{41–43} and in vivo angiography^{44,45} in relatively more severe SAH models have detected vasospasm. Specifically, an MRI study of a SAH model by puncturing the vessel found narrowing of vessels but vasoconstriction only reached significance in the right proximal ICA in the acute phase and returned to normal on day 9.¹² In our SAH model, large vessel vasospasm per se is unlikely the main cause of cerebral hypoperfusion after SAH. It remains possible however that there was vasoconstriction in smaller blood vessels that could have contributed to the observed hypoperfusion.⁴⁶ Some patients have been reported to develop delayed cerebral ischemia in the absence of angiographic vasospasm, but show significant correlation with the number of spreading depolarizations.⁴⁷ Another possibility would be microthrombi formation that reduces overall CBF by affecting the microcirculation.²¹ Blood injection into the cisterna magna has been widely used to study SAH. Although the exact mechanisms of vasospasm in this model remain incompletely understood, in other animal models of SAH and in patients, dysfunction of endothelium resulting in enhanced production of vasoconstrictors, release of spasmogens from lysed blood clot and inflammatory response of the vessel wall have been suggested to play a role in vasospasm in SAH⁴⁸.

CBF—Basal CBF was reduced 1–4 hrs after SAH induction, but returned toward normal thereafter. Although CBF was reduced, it did not reach ischemic level at any time points studied. In addition to possible small vessel vasoconstriction, mild intracranial hypertension could contribute to the observed hypoperfusion. In more severe SAH models, hypoperfusion has been reported and has been linked to subsequent cerebral ischemia.^{12,49–52}

Cerebrovascular reactivity—Cerebrovascular reactivity offers the means to evaluate cerebrovascular reserve. CBF changes in response to hypercapnia were reduced 1–4 hrs after blood injection but returned toward normal thereafter. Cerebrovascular reserve after SAH has been examined in experimental models and SAH patients. Feasibility studies in human SAH patients showed reduced cerebrovascular reserve using MRI¹⁵ and SPECT.¹⁶ In animal studies, the CBF reactivity to CO₂ inhalation was intact after SAH, whereas autoregulation on blood pressure changes was deranged after SAH.⁵³ Hassler and Chioffi found that there are downstream of segmental vasospasm and small peripheral vessels were

near-maximum dilation in aneurysmal SAH.⁵⁴ They concluded that the dilatational capacity of small blood vessels during hypercapnia was low and hyperventilation was hazardous because a contraction of peripheral vessels might rapidly cause ischemia in the presence of a spastic proximal vessel segment. Carrera and coworkers found that the loss of normal CO₂ reactivity in SAH predicted a high risk for the eventual development of cerebral ischemia.⁵⁵ Together, these findings indicate that there are significant compromise of cerebrovascular reserve after SAH.

MRV—Venous changes were detected but they were delayed compared to vasospasm, occurring at 3 hrs and 2 days. The relative hypointense MRV signals could arise from damages of the venous volume or decreased venous oxygen saturation.^{5,56} The relative hypointense MRV signals could arise from increased venous volume or decreased venous oxygen saturation.^{5,56} Given the venous blood T2* is likely much shorter than 14ms echo time used⁵⁷, signals from the veins are likely invisible and any small changes in venous oxygen saturation is unlikely to significantly affect MRV signals. Thus, the observed MRV in SAH likely arises from changes in venous volume. To our knowledge, this is the first report that suggests venous changes in SAH. Further studies are needed to identify the underlying mechanisms for SAH's effects on the venous system.

Diffusion and T2 MRI—Despite significant hemodynamic perturbations, we found only transient ADC reduction at hyperacute phase and no apparent ischemic brain injuries as measured by ADC and T2 in this SAH model up to 7 days. Possible subtle diffusion and T2 changes could occur beyond day 7 and thus it would be important to monitor MRI changes beyond day 7 in future studies. In more severe SAH models, persistent ADC and T2 changes have been reported. Busch et al.¹⁷ for example studied perforation of the circle of Willis via an endovascular monofilament in nonheparinized and heparinized animals. The SAH group showed a sharp decline of ADC within 2 min of SAH was observed in the ipsilateral somatosensory cortex. These decreases in diffusion then spread within minutes over the ipsilateral hemisphere. Similar ADC decreases on the contralateral side started with a further time delay of 1 to 3 min. From 30 mins onward, the extent of the diffusion abnormality decreased progressively in the nonheparinized animals. No recovery was observed in heparinized rats. Persistent hemorrhage in heparinized animals was reflected by early decline of ADC values throughout the entire brain.

van den Bergh¹² studied the effects of advancing a sharpened prolene 3.0 suture to perforate the intracranial bifurcation of the internal carotid artery. They found that the ischemic lesion volume increased significantly between 1 and 48 hours after SAH from 0.039 to 0.26 ml. These findings indicated that cerebral ischemia after SAH are highly dependent on the severity of the injury.

Behavioral tests—Open-field activities were significantly different between pre- and post-injection in the ACSF group, suggesting that these changes may be associated with post-surgery trauma. Open-field activities were significantly different between SAH and ACSF group at 1 and 7 days. By contrast, foot fault score were not significantly different between SAH and ACSF group, suggesting that this mild SAH model has no apparent and

persistent neurological deficits. These functional data are consistent with quantitative MRI data where there was no apparent ischemic brain injury up to 7 days.

Limitations of the study

A limitation of this study is that ICP was not measured. However, most previous studies using similar SAH models reported no or only transient (few minutes to hours) changes in MABP and ICP.^{3,58,59} Another limitation is that quantitative analysis of MRV signal is inherently challenging and our analysis is susceptible to error for cross-day comparisons. That said, a fixed intensity threshold and a larger ROI were used, and the MRV signals in the ACSF group varied over small ranges, providing confidence to the analysis of the MRV data. Although SAH did not cause T2 and ADC changes on day 7, SAH could cause long-term damage and subtle neurodegeneration not detectable by MRI. Future study extending MRI and behavioral measurements to the chronic phase as well as histological validation would be helpful. Another limitation is that sensitivity to changes in the hyperacute time points where measurements were made before and after blood injection without moving the animals differed from those of subsequent time points. This could have contributed to larger uncertainty and non-significant differences between ACSF and blood group at later time points. The differences in sensitivities likely affect some parameters (i.e., MRV) with low signal-to-noise ratio more than others. Finally, we did not study other possible mechanisms such as distal vascular dysautoregulation, micro-thrombi, direct neurotoxic effects, inflammation, cortical spreading depolarizations, and disruption of blood-brain barrier.^{21,60}

Conclusions

To our knowledge, this is the first report of an MRI study of an in-scanner SAH model using blood injection via the cistern magna, enabling measurements at pre-SAH and hyperacute SAH phase in the same animals. The volume of blood injected in this study resulted in mild SAH injury, where transient and mild vascular and hemodynamic disturbances, and transient changes in diffusion were detected, but there was no apparent ischemic brain injury. Behavioral functional tests showed reduced open-field activities in both SAH and sham animals but no apparent neurological disorders up to 7 days. This in-scanner SAH model along with the MRI protocols may prove useful for longitudinal study of SAH pathophysiology and to evaluate novel treatment strategies. Future studies will investigate SAH with larger volume of blood injection, monitor changes in more chronic phase, employ histology to validate MRI findings, and evaluate novel treatment strategies.

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GLOSSARY

ACSF artificial cerebral-spinal fluid

ADC	apparent diffusion coefficient
ASL	arterial spin-labeling
CBF	cerebral blood flow
EPI	echo planar imaging
MRI	Magnetic resonance imaging
SAH	subarachnoid hemorrhage

References

1. Suarez JJ, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. *The New England journal of medicine*. 2006; 354(4):387–96. [PubMed: 16436770]
2. Cetas JS, Lee DR, Alkayed NJ, Wang R, Iliff JJ, Heinricher MM. Brainstem control of cerebral blood flow and application to acute vasospasm following experimental subarachnoid hemorrhage. *Neuroscience*. 2009; 163(2):719–29. [PubMed: 19539726]
3. Ansar S, Edvinsson L. Equal contribution of increased intracranial pressure and subarachnoid blood to cerebral blood flow reduction and receptor upregulation after subarachnoid hemorrhage. *Laboratory investigation Journal of neurosurgery*. 2009; 111(5):978–87. [PubMed: 19408972]
4. Westermaier T, Jauss A, Eriskat J, Kunze E, Roosen K. The temporal profile of cerebral blood flow and tissue metabolites indicates sustained metabolic depression after experimental subarachnoid hemorrhage in rats. *Neurosurgery*. 2011; 68(1):223–9. discussion 229–30. [PubMed: 21099709]
5. Eide PK, Sorteberg W. Intracranial pressure levels and single wave amplitudes, Glasgow Coma Score and Glasgow Outcome Score after subarachnoid haemorrhage. *Acta neurochirurgica*. 2006; 148(12):1267–75. discussion 1275–6. [PubMed: 17123038]
6. Kelly ME, Rowland MJ, Okell TW, Chappell MA, Corkill R, Kerr RS, et al. Pseudo-continuous arterial spin labelling MRI for non-invasive, whole-brain, serial quantification of cerebral blood flow following aneurysmal subarachnoid haemorrhage. *Translational stroke research*. 2013; 4(6): 710–8. [PubMed: 24323425]
7. Danura H, Schatlo B, Marbacher S, Kerkeni H, Diepers M, Remonda L, et al. Acute angiographic vasospasm and the incidence of delayed cerebral vasospasm: preliminary results. *Acta neurochirurgica Supplement*. 2015; 120:187–90. [PubMed: 25366622]
8. Reilly C, Amidei C, Tolentino J, Jahromi BS, Macdonald RL. Clot volume and clearance rate as independent predictors of vasospasm after aneurysmal subarachnoid hemorrhage. *Journal of neurosurgery*. 2004; 101(2):255–61. [PubMed: 15309916]
9. Prunell GF, Mathiesen T, Svendgaard NA. A new experimental model in rats for study of the pathophysiology of subarachnoid hemorrhage. *Neuroreport*. 2002; 13(18):2553–6. [PubMed: 12499866]
10. Vatter H, Weidauer S, Konczalla J, Dettmann E, Zimmermann M, Raabe A, et al. Time course in the development of cerebral vasospasm after experimental subarachnoid hemorrhage: clinical and neuroradiological assessment of the rat double hemorrhage model. *Neurosurgery*. 2006; 58(6): 1190–7. discussion 1190–7. [PubMed: 16723899]
11. Sehba FA, Pluta RM. Aneurysmal subarachnoid hemorrhage models: do they need a fix? *Stroke research and treatment*. 2013; 2013:615154. [PubMed: 23878760]
12. van den Bergh WM, Schepers J, Veldhuis WB, Nicolay K, Tulleken CA, Rinkel GJ. Magnetic resonance imaging in experimental subarachnoid haemorrhage. *Acta neurochirurgica*. 2005; 147(9):977–83. discussion 983. [PubMed: 15900401]
13. Guresir E, Raabe A, Jaiimsin A, Dias S, Raab P, Seifert V, et al. Histological evidence of delayed ischemic brain tissue damage in the rat double-hemorrhage model. *Journal of the neurological sciences*. 2010; 293(1–2):18–22. [PubMed: 20421120]

14. Guresir E, Vasiliadis N, Konczalla J, Raab P, Hattingen E, Seifert V, et al. Erythropoietin prevents delayed hemodynamic dysfunction after subarachnoid hemorrhage in a randomized controlled experimental setting. *Journal of the neurological sciences*. 2013; 332(1–2):128–35. [PubMed: 23907045]
15. da Costa L, Fierstra J, Fisher JA, Mikulis DJ, Han JS, Tymianski M. BOLD MRI and early impairment of cerebrovascular reserve after aneurysmal subarachnoid hemorrhage. *Journal of magnetic resonance imaging : JMRI*. 2014; 40(4):972–9. [PubMed: 24243534]
16. Reinprecht A, Czech T, Asenbaum S, Podreka I, Schmidbauer M. Low cerebrovascular reserve capacity in long-term follow-up after subarachnoid hemorrhage. *Surgical neurology*. 2005; 64(2): 116–20. discussion 121. [PubMed: 16051000]
17. Busch E, Beaulieu C, de Crespigny A, Moseley ME. Diffusion MR imaging during acute subarachnoid hemorrhage in rats. *Stroke*. 1998; 29(10):2155–61. [PubMed: 9756598]
18. Piepgras A, Elste V, Frietsch T, Schmiedek P, Reith W, Schilling L. Effect of moderate hypothermia on experimental severe subarachnoid hemorrhage, as evaluated by apparent diffusion coefficient changes. *Neurosurgery*. 2001; 48(5):1128–34. discussion 1134–5. [PubMed: 11334280]
19. Tiebosch IA, van den Bergh WM, Bouts MJ, Zwartbol R, van der Toorn A, Dijkhuizen RM. Progression of brain lesions in relation to hyperperfusion from subacute to chronic stages after experimental subarachnoid hemorrhage: a multiparametric MRI study. *Cerebrovascular diseases*. 2013; 36(3):167–72. [PubMed: 24135525]
20. Okubo S, Strahle J, Keep RF, Hua Y, Xi G. Subarachnoid hemorrhage-induced hydrocephalus in rats. *Stroke; a journal of cerebral circulation*. 2013; 44(2):547–50.
21. Ostergaard L, Aamand R, Karabegovic S, Tietze A, Blicher JU, Mikkelsen IK, et al. The role of the microcirculation in delayed cerebral ischemia and chronic degenerative changes after subarachnoid hemorrhage. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2013; 33(12):1825–37.
22. Davson, H. *Physiology of the cerebrospinal fluid*. Churchill; London: 1967.
23. Altman, PL.; Katz, DD. *Federation of American Societies for Experimental Biology. Biology data book. 2. Federation of American Societies for Experimental Biology; Bethesda, Md: 1974.*
24. Tanaka Y, Nagaoka T, Nair G, Ohno K, Duong TQ. Arterial spin labeling and dynamic susceptibility contrast CBF MRI in postischemic hyperperfusion, hypercapnia, and after mannitol injection. *J Cereb Blood Flow Metab*. 2011; 31(6):1403–11. [PubMed: 21179070]
25. Shen Q, Du F, Huang S, Duong TQ. Effects of cerebral ischemic and reperfusion on T2*-weighted MRI responses to brief oxygen challenge. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2014; 34(1):169–75.
26. Shen Q, Du F, Huang S, Duong TQ. Spatiotemporal characteristics of postischemic hyperperfusion with respect to changes in T1, T2, diffusion, angiography, and blood-brain barrier permeability. *J Cereb Blood Flow Metab*. 2011; 31(10):2076–85. [PubMed: 21540871]
27. Shen Q, Huang S, Du F, Duong TQ. Probing ischemic tissue fate with BOLD fMRI of brief oxygen challenge. *Brain Res*. 2011; 1425:132–41. [PubMed: 22032876]
28. Shen Q, Fisher M, Sotak CH, Duong TQ. Effects of reperfusion on ADC and CBF pixel-by-pixel dynamics in stroke: characterizing tissue fates using quantitative diffusion and perfusion imaging. *J Cereb Blood Flow Metab*. 2004; 24(3):280–90. [PubMed: 15091108]
29. Shen Q, Meng X, Fisher M, Sotak CH, Duong TQ. Pixel-by-pixel spatiotemporal progression of focal ischemia derived using quantitative perfusion and diffusion imaging. *J Cereb Blood Flow Metab*. 2003; 23(12):1479–88. [PubMed: 14663344]
30. Shen Q, Ren H, Cheng H, Fisher M, Duong TQ. Functional, perfusion and diffusion MRI of acute focal ischemic brain injury. *J Cereb Blood Flow Metab*. 2005; 25(10):1265–79. [PubMed: 15858531]
31. Boyko M, Azab AN, Kuts R, Gruenbaum BF, Gruenbaum SE, Melamed I, et al. The neuro-behavioral profile in rats after subarachnoid hemorrhage. *Brain research*. 2013; 1491:109–16. [PubMed: 23123210]

32. Watts LT, Long JA, Chemello J, Van Koughnet S, Fernandez A, Huang S, et al. Methylene Blue Is Neuroprotective against Mild Traumatic Brain Injury. *J Neurotrauma*. 2014; 31(11):1063–71. [PubMed: 24479842]
33. Watts LT, Shen Q, Deng S, Chemello J, Duong TQ. Manganese-enhanced MRI of traumatic brain injury. *J Neurotrauma*. 2014
34. Long JA, Watts LT, Chemello J, Huang S, Shen Q, Duong TQ. Multiparametric and Longitudinal MRI Characterization of Mild Traumatic Brain Injury in Rats. *J Neurotrauma*. 2014
35. Talley Watts L, Long JA, Chemello J, Van Koughnet S, Fernandez A, Huang S, et al. Methylene blue is neuroprotective against mild traumatic brain injury. *Journal of neurotrauma*. 2014; 31(11): 1063–71. [PubMed: 24479842]
36. Long JA, Watts LT, Chemello J, Huang S, Shen Q, Duong TQ. Multiparametric and longitudinal MRI characterization of mild traumatic brain injury in rats. *J Neurotrauma*. 2015; 32(8):598–607. [PubMed: 25203249]
37. Steiner LA, Andrews PJ. Monitoring the injured brain: ICP and CBF. *British journal of anaesthesia*. 2006; 97(1):26–38. [PubMed: 16698860]
38. Auer LM, Ishiyama N, Hodde KC, Kleinert R, Pucher R. Effect of intracranial pressure on bridging veins in rats. *Journal of neurosurgery*. 1987; 67(2):263–8. [PubMed: 3598685]
39. Morrow BA, Holt MR, Starcevic VP, Keil LC, Severs WB. Mechanism of delayed intracranial hypertension after cerebroventricular infusions in conscious rats. *Brain research*. 1992; 570(1–2): 218–24. [PubMed: 1617414]
40. Morrow BA, Starcevic VP, Keil LC, Seve WB. Intracranial hypertension after cerebroventricular infusions in conscious rats. *The American journal of physiology*. 1990; 258(5 Pt 2):R1170–6. [PubMed: 2337198]
41. Yang X, Chen C, Hu Q, Yan J, Zhou C. Gamma-secretase inhibitor (GSI1) attenuates morphological cerebral vasospasm in 24h after experimental subarachnoid hemorrhage in rats. *Neuroscience letters*. 2010; 469(3):385–90. [PubMed: 20026381]
42. Alkan T, Tureyen K, Ulutas M, Kahveci N, Goren B, Korfali E, et al. Acute and delayed vasoconstriction after subarachnoid hemorrhage: local cerebral blood flow, histopathology, and morphology in the rat basilar artery. *Archives of physiology and biochemistry*. 2001; 109(2):145–53. [PubMed: 11780775]
43. Bederson JB, Levy AL, Ding WH, Kahn R, DiPerna CA, Jenkins AL 3rd, et al. Acute vasoconstriction after subarachnoid hemorrhage. *Neurosurgery*. 1998; 42(2):352–60. discussion 360–2. [PubMed: 9482187]
44. Zhao WJ, Wu C. Nimodipine attenuation of early brain dysfunctions is partially related to its inverting acute vasospasm in a cisterna magna subarachnoid hemorrhage (SAH) model in rats. *The International journal of neuroscience*. 2012; 122(10):611–7. [PubMed: 22694164]
45. Koktekir E, Erdem Y, Akif Bayar M, Gokcek C, Karatay M, Kilic C. A new approach to the treatment of cerebral vasospasm: the angiographic effects of tadalafil on experimental vasospasm. *Acta neurochirurgica*. 2010; 152(3):463–9. [PubMed: 19841856]
46. Sehba FA, Friedrich V. Early micro vascular changes after subarachnoid hemorrhage. *Acta neurochirurgica Supplement*. 2011; 110(Pt 1):49–55. [PubMed: 21116914]
47. Woitzik J, Dreier JP, Hecht N, Fiss I, Sandow N, Major S, et al. Delayed cerebral ischemia and spreading depolarization in absence of angiographic vasospasm after subarachnoid hemorrhage. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2012; 32(2):203–12.
48. Kozniewska E, Michalik R, Rafalowska J, Gadamski R, Walski M, Frontczak-Baniewicz M, et al. Mechanisms of vascular dysfunction after subarachnoid hemorrhage. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*. 2006; 57(Suppl 11):145–60. [PubMed: 17244946]
49. Prunell GF, Mathiesen T, Svendgaard NA. Experimental subarachnoid hemorrhage: cerebral blood flow and brain metabolism during the acute phase in three different models in the rat. *Neurosurgery*. 2004; 54(2):426–36. discussion 436–7. [PubMed: 14744290]
50. Naveri L, Stromberg C, Saavedra JM. Angiotensin IV reverses the acute cerebral blood flow reduction after experimental subarachnoid hemorrhage in the rat. *Journal of cerebral blood flow*

and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 1994; 14(6):1096–9.

51. Sehba FA, Ding WH, Cheresnev I, Bederson JB. Effects of S-nitrosoglutathione on acute vasoconstriction and glutamate release after subarachnoid hemorrhage. *Stroke; a journal of cerebral circulation*. 1999; 30(9):1955–61.
52. Bederson JB, Germano IM, Guarino L. Cortical blood flow and cerebral perfusion pressure in a new noncraniotomy model of subarachnoid hemorrhage in the rat. *Stroke; a journal of cerebral circulation*. 1995; 26(6):1086–91. discussion 1091–2.
53. Diringer MN, Kirsch JR, Hanley DF, Traystman RJ. Altered cerebrovascular CO₂ reactivity following subarachnoid hemorrhage in cats. *Journal of neurosurgery*. 1993; 78(6):915–21. [PubMed: 8487074]
54. Hassler W, Chioffi F. CO₂ reactivity of cerebral vasospasm after aneurysmal subarachnoid haemorrhage. *Acta neurochirurgica*. 1989; 98(3–4):167–75. [PubMed: 2500837]
55. Carrera E, Kurtz P, Badjatia N, Fernandez L, Claassen J, Lee K, et al. Cerebrovascular carbon dioxide reactivity and delayed cerebral ischemia after subarachnoid hemorrhage. *Archives of neurology*. 2010; 67(4):434–9. [PubMed: 20385909]
56. Westermaier T, Stetter C, Kunze E, Willner N, Holzmeier J, Kilgenstein C, et al. Controlled transient hypercapnia: a novel approach for the treatment of delayed cerebral ischemia after subarachnoid hemorrhage? *Journal of neurosurgery*. 2014; 121(5):1056–62. [PubMed: 25148012]
57. Lin AL, Qin Q, Zhao X, Duong TQ. Blood longitudinal (T₁) and transverse (T₂) relaxation time constants at 11.7 Tesla. *MAGMA*. 2012; 25(3):245–9. [PubMed: 22071580]
58. Lee JY, Sagher O, Keep R, Hua Y, Xi G. Comparison of experimental rat models of early brain injury after subarachnoid hemorrhage. *Neurosurgery*. 2009; 65(2):331–43. discussion 343. [PubMed: 19625913]
59. Prunell GF, Mathiesen T, Diemer NH, Svendgaard NA. Experimental subarachnoid hemorrhage: subarachnoid blood volume, mortality rate, neuronal death, cerebral blood flow, and perfusion pressure in three different rat models. *Neurosurgery*. 2003; 52(1):165–75. discussion 175–6. [PubMed: 12493115]
60. Macdonald RL. Delayed neurological deterioration after subarachnoid haemorrhage. *Nature reviews Neurology*. 2014; 10(1):44–58. [PubMed: 24323051]

- Subarachnoid hemorrhage (SAH) results in transient vasospasm and venous abnormality.
- SAH transiently reduces basal cerebral blood flow and its response to hypercapnia.
- SAH causes transient diffusion change but no ischemic brain injury.
- Both SAH and control rats show reduced open-field activities.
- There are however no differences in functional deficits between groups.

Figure 1.

MRA of pre- and 30mins after blood (A) or ACSF (B) injection. ROIs show the bilateral anterior cerebral artery (arrow head), basilar artery (hollow arrow) and middle cerebral artery (arrow). The decrease in diameter in the artery following the blood injection represents a vasospasm as evidenced by the decreased conspicuity in main cerebral vessels. (C) Group-averaged arterial diameter differences from ACSF and blood injection (the mean value measured on bilateral ACAs, bilateral MCAs and basilar artery). Error bars represent SEM, $n=9$ each group, $*P<0.05$.

Figure 2.

(A) Representative temporal evolution of venogram before and 3 hrs after injection of ACSF or blood (SAH). The increased conspicuity of hypointense cortical veins following the blood injection represents a venous dilation. (B) Group-averaged relative venous volume over time. Error bars represent SEM, n=9 each group, *P<0.05. The dotted ROIs show the tabulated data. Rectangular ROIs show the expanded regions.

Figure 3.

(A) Representative temporal evolution of cerebral blood flow (CBF) maps of a rat subjected to cistern magna blood injection. Group-averaged CBF before blood or ACSF injection and after 15mins, 2hrs, 4hrs, 2d and 7d in the (B) cortex, (C) hippocampus and (D) striatum (D). Error bars represent SEM, n=9 each group, *P<0.05.

Figure 4.

Group-averaged CBF changes in the region of cortex, hippocampus and striatum. Error bars represent SEM, n=9 each group, *P<0.05.

Figure 5.

(A) ADC and (B) T2 of ACSF and blood injection groups pre-injection, 5 mins, 2 hrs, 2 and 7 days after injection for the cortex, hippocampus and striatum. Error bars represent SEM, $n=9$ each group. * $P < 0.05$ compared between ACSF and SAH group.

Figure 6.

Behavior parameters of ACSF and blood injection groups evaluated before and 1, 2, 4 and 7 days after blood or ACSF injection showing (A) distance traveled in the open field test, (B) percentage of faults from the foot-fault test. Error bars represent SEM, n=9 each group, *P<0.05.