MULTIMODAL MRI CHARACTERIZATION OF EXPERIMENTAL SUBARACHNOID HEMORRHAGE

Y. SUN, a,b,c Q. SHEN, d L. T. WATTS, c,d,e E. R. MUIR, f S. HUANG, g G.-Y. YANG, a,b,f J. I. SUAREZ g AND T. Q. DUONG a,b,c

a Department of Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
b Department of Stereotactic and Functional Neurosurgery, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
c Research Imaging Institute, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
d Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
e Department of Neurology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
f Neuroscience and Neuroengineering Research Center, Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, China
g Division 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, 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 4 h, and open-field activity up to 7 days post injection. By contrast, blood injection caused: (i) vasospasm 30 min after SAH but not thereafter, (ii) venous abnormalities at 3 h and 2 days, delayed relative to vasospasm, (iii) reduced basal CBF and to hypercapnia 1–4 h but not thereafter, (iv) reduced ADC immediately after SAH but no ADC and T2 changes on days 2 and 7, and (v) reduced open-field activities in both SAH and vehicle animals, but no significant differences in open-field activities 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. © 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: 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 (Suarez et al., 2006). The hallmarks of SAH include increased intracranial pressure (ICP), hypoperfusion, and delayed cerebral ischemia (with or without vasospasm) (Eide and Sorteberg, 2006; Ansar and Edvinsson, 2009; Westermaier et al., 2011; Kelly et al., 2013; Danura et al., 2015). 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 (Prunell et al., 2002; Reilly et al., 2004; Vatter et al., 2006). The disadvantage is that it does not mimic the processes surrounding aneurysmal rupture (Sehba and Piuta, 2013).

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) (van den Bergh et al., 2005), reduced cerebral blood flow (CBF) (Guresir et al., 2010, 2013), reduced cerebrovascular reserve by hypercapnic challenge (Reinprecht et al., 2005; da Costa et al., 2014), ischemic brain injury using diffusion-weighted MRI (Busch et al., 1998; Piepgras et al., 2001), and cerebral edema or infarction using T2 MRI (van den Bergh et al., 2005; Okubo et al., 2013; Tiebosch et al., 2013). 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 the 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 (Ostergaard et al., 2013). 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), CBF, and cerebrovascular reserve (by measuring CBF response to hypercapnia), diffusion and T_2 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.

**EXPERIMENTAL PROCEDURES**

**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 nine animals per group to be the minimal sample size needed. The study was randomized and vehicle-controlled, but the experimenters 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 midline burr hole was made just rostral to the atlas and the atlanto-occipital membrane. A small air. The nuchal muscle layers were divided at the

**MRI experiments**

Imaging was performed on a Bruker Biospec 11.7 Tesla/16 cm scanner with a 76G/cm BGA9S gradient insert (Billerica, MA, USA) using a custom-made surface coil for brain imaging and a neck coil for perfusion labeling (Tanaka et al., 2011; Shen et al., 2011a,b, 2014).

**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 min) were acquired during baseline and 40 pairs (4 min) during hypercapnic challenge (5% CO_2 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 three 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).
Apparent diffusion coefficient (ADC): Diffusion weighted images were obtained with five $b = 0$ and 30 directions with a 1200 s/mm. Single-shot, spin-echo EPI scans with partial Fourier (3/4) were acquired using the following settings: seven 1.5-mm coronal images, FOV = 25.6 $\times$ 25.6 mm, matrix 96 $\times$ 96 and reconstructed to 128 $\times$ 128, TR = 3 s, TE = 28 ms, separate between diffusion gradient $\Delta = 9$ ms, diffusion gradient duration $\delta = 3$ ms, and two transients for signal averaging.

$T_2$: $T_2$-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° FA), matrix = 128 $\times$ 128, FOV = 25.6 $\times$ 25.6 mm, echo train length = 4, and four signal averages.

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

Horizontal angiograms (reconstructed to 50 $\times$ 50 $\mu$m) along the rostrocaudal axis were generated by maximum intensity projections from the MRA data (Shen et al., 2011a,b, 2014). 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 $T_2$ and hypercapnia-induced percent change maps were calculated as previously described (Shen et al., 2003, 2004, 2005, 2011a; Tanaka et al., 2011). Regions of interests (ROIs) were drawn in the cortex, striatum and hippocampus across multiple image slices.

Functional assessment
Sensorimotor function was assessed using the open field (Boyko et al., 2013) and foot-fault tests (Long et al., 2014; Watts et al., 2014a,b). 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 $\times$ 35 $\times$ 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 min by a digital camera with post-recording analysis performed using Anymaze software (Stoelting Co., Wood Dale, IL, USA) 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 $\times$ 11 inches with grid openings of 1.56 $\times$ 1.00 inches) for 5 min. 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 a 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-tailed $p < 0.05$ 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 h and 6/9 survived to 7 days. In the ACSF group, 9/9 survived 24 h and 8/9 survived up to 7 days. The heart rate, arterial oxygen saturation, and rectal temperature were 360 $\pm$ 16 bpm, 96.5 $\pm$ 4.4%, and 36.8 $\pm$ 0.5 °C, respectively, in the SAH group, and 355 $\pm$ 18 bpm, 96.4 $\pm$ 4.4%, and 36.8 $\pm$ 0.5 °C in the ACSF group. These physiological parameters were not significantly different between the ACSF and SAH groups across all time points studied ($p > 0.05$) and are consistent with our previous studies under the same isoflurane preparation in normal animals and various injury models (Shen et al., 2003, 2004, 2005, 2011a; Tanaka et al., 2011; Talley Watts et al., 2014; Long et al., 2015). Although end-tidal CO$_2$ was monitored, the long sample line resulted in reduced recorded CO$_2$ 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 (Fig. 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 min ($p < 0.05$), but not thereafter at 150 min, 2 and 7 days ($p > 0.05$).
Arterial lumen diameters between the blood and ACSF groups were not significantly different pre-injection, 150 min, 2 and 7 days after injection ($p > 0.05$), but were significantly different at 30 min after injection ($p < 0.05$).

Veins

MRV and the volume fraction with hypointense pixels in the neocortices are shown in Fig. 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 h 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 h, and 7 days after injection, but were significantly different at 3 h and 2 days after injection ($p < 0.05$).

CBF

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

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Fig. 1. MRA of pre- and 30 min 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, $^*P < 0.05$.

Fig. 2. (A) Representative temporal evolution of venogram before and 3 h 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.
2 and 7 days after injection ($p > 0.05$), but were significantly different at 15 min, 2 and 4 h after injection ($p < 0.05$).

**Vascular reactivity (VR)**

VR data, as measured by CBF responses to hypercapnia, are shown in Fig. 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 h, but not at 2 and 7 days.

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

**Diffusion and $T_2$ MRI**

Group ADC and $T_2$ data are shown in Fig. 5 for the cortex, hippocampus and striatum. Pre-injection ADC and $T_2$ values were not significantly different between the ACSF and SAH groups ($p > 0.05$). ACSF did not affect ADC and $T_2$ at all time points studied. ADC values were significantly different between the blood and ACSF groups only at 5 min after injection ($p < 0.05$) but not at subsequent time points ($p > 0.05$) in all brain structures analyzed. $T_2$ 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 Fig. 6. After ACSF injection, travel distances were mildly reduced compared to pre-injection. After blood injection, travel distances were

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**Fig. 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 15 min, 2 h, 4 h, 2 d and 7 d in the (B) cortex, (C) hippocampus and (D) striatum (D). Error bars represent SEM, n = 9 each group, *$P < 0.05$.

**Fig. 4.** Group-averaged CBF changes in the region of cortex, hippocampus and striatum. Error bars represent SEM, n = 9 each group, *$P < 0.05$. 

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**Fig. 5.** Group ADC and $T_2$ data are shown for the cortex, hippocampus and striatum. Pre-injection ADC and $T_2$ values were not significantly different between the ACSF and SAH groups ($p > 0.05$). ACSF did not affect ADC and $T_2$ at all time points studied. ADC values were significantly different between the blood and ACSF groups only at 5 min after injection ($p < 0.05$) but not at subsequent time points ($p > 0.05$) in all brain structures analyzed. $T_2$ values were not significantly different between the blood and ACSF groups at all post-injection time points in all brain structures analyzed ($p > 0.05$).

**Fig. 6.** Behavioral assessments using open field and foot-fault tests are presented. 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 the 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 min–4 h), 2 and 7 days. All measured pre-injection parameters were not significantly different between the ACSF and SAH group. ACSF injection did not affect arterial lumen diameter, ADC, \( T_2 \), venous signal, VR, and foot-fault scores, but mildly reduce CBF only at 1–3 h compared to pre-injection. Mild reduction in CBF is not surprising because ACSF likely transiently increased ICP, thereby reducing perfusion pressure (Auer et al., 1987; Steiner and Andrews, 2006; Ansar and Edvinsson, 2009). Animals with ACSF injection tended to freeze and

**EFFECTS OF ACSF INJECTION**

ACSF injection did not affect arterial lumen diameter, \( T_2 \), ADC, MRV signal, VR, but mildly reduce CBF only at 1–3 h compared to pre-injection. Mild reduction in CBF is not surprising because ACSF likely transiently increased ICP, thereby reducing perfusion pressure (Auer et al., 1987; Steiner and Andrews, 2006; Ansar and Edvinsson, 2009). Animals with ACSF injection tended to freeze and

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**Fig. 5.** (A) ADC and (B) \( T_2 \) of ACSF and blood injection groups pre-injection, 5 min, 2 h, 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 groups.

**Fig. 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, \( * \ P < 0.05 \).
stay close to the walls (i.e., astigmatotaxis) 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 (Morrow et al., 1990, 1992).

**EFFECTS OF BLOOD INJECTION**

**Vasospasm:** Vasospasm is often thought to be the prime mechanism of delayed cerebral ischemia after aneurysmal SAH (Suarez et al., 2006). Vasospasm was detected immediately after SAH (30 min) but not thereafter, indicating that vasospasm is transient in this mild SAH model. Previous studies using ex vivo arteries (Bederson et al., 1998; Alkan et al., 2001; Yang et al., 2010) and in vivo angiography (Koktekir et al., 2010; Zhao and Wu, 2012) 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 (van den Bergh et al., 2005). 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 (Sehba and Friedrich, 2011). 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 (Woitzik et al., 2012). Another possibility would be microthrombi formation that reduces overall CBF by affecting the microcirculation (Ostergaard et al., 2013). 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 spasmodgens from lysed blood clot and inflammatory response of the vessel wall have been suggested to play a role in vasospasm in SAH (Kozniewska et al., 2006).

**CBF:** Basal CBF was reduced 1–4 h after SAH induction, but returned toward normal thereafter. Although CBF was reduced, it did not reach the 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 (Naveri et al., 1994; Bederson et al., 1995; Sehba et al., 1999; Prunell et al., 2004; van den Bergh et al., 2005).

**Cerebrovascular reactivity:** Cerebrovascular reactivity offers the means to evaluate cerebrovascular reserve. CBF changes in response to hypercapnia were reduced 1–4 h 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 MRI (da Costa et al., 2014) and SPECT (Reinprecht et al., 2005). In animal studies, the CBF reactivity to CO₂ inhalation was intact after SAH, whereas autoregulation on blood pressure changes was deranged after SAH (Diringer et al., 1993). Hassler and Chioffi found that there are downstream of segmental vasospasm and small peripheral vessels were near-maximum dilation in aneurysmal SAH (Hassler and Chioffi, 1989). 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 (Carrera et al., 2010). Together, these findings indicate that there could be significant compromise of cerebrovascular reserve after SAH.

**MRV:** Venous changes were detected but they were delayed compared to vasospasm, occurring at 3 h and 2 days. The relative hypointense MRV signals could arise from increased venous volume or decreased venous oxygen saturation (Eide and Sorteberg, 2006; Westermaier et al., 2014). Given the venous blood T₂ is likely much shorter than 14 ms echo time used (Lin et al., 2012), 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. (1998) 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 1–3 min. From 30 min 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 Bergh et al. (2005) 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 h 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 was not significantly different between SAH and ACSF groups, 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 (Prunell et al., 2003; Ansar and Edvinsson, 2009; Lee et al., 2009). 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 MRV signals in the ACSF group varied over small ranges, providing confidence to the analysis of the MRV data. Although SAH did not cause neurological disorders up to 60 Y. Sun et al. / Neuroscience 316 (2016) 53–62

**DISCLOSURE STATEMENT**

No conflict of interest.

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