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MULTIMODAL MRI CHARACTERIZATION OF EXPERIMENTAL SUBARACHNOID HEMORRHAGE

Y. SUN, [a,b,c] **Q. SHEN,** [c] **L. T. WATTS,** [c,d,e] **E. R. MUIR,** [c] **S. HUANG,** [c] **G.-Y. YANG,** [a,b,f] **J. I. SUAREZ** [g] **AND T. Q. DUONG** [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 (5 min to 4 h), and upto 7 days after SAH. Vehicle injection did not change arterial lumen diameter, apparent diffusion coefficient (ADC), T_2, 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 T_2 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.

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 Pluta, 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 T_2 MRI (van den Bergh et al., 2005; Okubo et al., 2013; Tiebosch et al., 2013). However, these changes in the

*Corresponding author. Address: Research Imaging Institute, The University of Texas Health Science Center at San Antonio, 8403 Floyd Curl Drive, San Antonio, TX 78229, USA. Tel: +1-210-567-8115.
E-mail address: duongt@uthscsa.edu (T. Q. Duong).

Abbreviations: ACSF, artificial cerebral–spinal fluid; ADC, apparent diffusion coefficient; ASL, arterial spin-labeling; CBF, cerebral blood flow; EPI, echo planar imaging; FA, flip angle; FLASH, fast low-angle shot; FOV, field of view; ICP, intracranial pressure; MRA, magnetic resonance angiography; MRI, magnetic resonance imaging; MRV, MR venography; ROIs, regions of interests; SAH, subarachnoid hemorrhage; TE, echo time; TR, repetition time; VR, vascular reactivity.

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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 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, 100 μl of cerebral–spinal fluid was gently aspirated. To collect autologous blood, the rat's tail was immersed in warm water for 2 min, 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 (Davson, 1967; Altman and Katz, 1974) 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, 300 μL of blood or ACSF was injected using a micro-pump over a period of 7 min. Imaging was performed before, repeatedly from 15 min to 4 h after the blood or ACSF was injected. During MRI, end-tidal CO_2, rectal temperature, heart rate and arterial oxygen saturation were recorded and maintained within normal physiological ranges. At the end of 4 h, the animals were extubated and allowed to recover in home cages.

MRI studies were repeated again on days 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, 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 = $25.6 \times 20.8 \times 12.8$ mm, matrix = $256 \times 256 \times 256$ ($100 \times 80 \times 50$ 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 = $25.6 \times 25.6 \times 12.8$ mm, matrix = $256 \times 256 \times 128$ ($100 \times 100 \times 100$ μm).