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A magnetic resonance imaging grading system for subarachnoid hemorrhage severity in a rat model

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Abstract

Background—The endovascular perforation model of subarachnoid hemorrhage (SAH) has a large variation in outcomes. This may reflect differences in the SAH size.

New method—Magnetic resonance imaging (MRI) was performed 24 hours after endovascular perforation in adult male (n = 58) and female (n = 58) rats. Rats were divided into five grades according to MRI characteristics: grade 0: no SAH or intraventricular hemorrhage (IVH); grade 1: minimal or thin SAH without IVH; grade 2: minimal or thin SAH with IVH; grade 3: thick SAH without IVH; grade 4: thick SAH with IVH. We investigated whether MRI grading scale reflected severity of SAH (determined post mortem) and neurological score.

Results—There was a strong correlation between MRI grading scale and current SAH grading scale ($P < 0.01$) and neurological score ($P < 0.01$) in male rats. In female rats, there was also a strong correlation between MRI grading scale and SAH grading scale ($P < 0.01$) but not with neurological score ($P = 0.24$).

Comparison with existing methods—The current grading system is based on the amount of SAH and needs animal euthanasia to evaluate SAH severity. There is no useful grading system to classify severity of SAH without decapitating animals.

Conclusions—We demonstrated a correlation between the MRI grading scale and the current SAH grading scale in an endovascular perforation rat model. The MRI grading scale allows evaluation of SAH severity without euthanizing animals.

Keywords

Subarachnoid hemorrhage; endovascular perforation; magnetic resonance imaging; rat

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1. Introduction

Spontaneous subarachnoid hemorrhage (SAH) usually occurs due to the rupture of a cerebral aneurysm, causing high mortality and morbidity. Although advanced surgical, endovascular and critical care techniques have improved outcome, early brain injury after SAH is still a common cause of death (Broderick et al., 1994). There is a consensus that evaluation of SAH severity in the acute phase is essential in the treatment of patients after SAH. In the clinical setting, evaluation of SAH severity using imaging modalities is well established. Computed tomography (CT) has come into routine use in the acute evaluation of SAH patients. The thickness and distribution of SAH clots and the presence of intraventricular hemorrhage (IVH) detected by CT are associated with clinical outcome and SAH-related complications (Claassen et al., 2001).

The endovascular perforation model is widely employed to study brain injury and cerebral blood flow following SAH in animals. Although this model mimics the rupture of a cerebral aneurysm by puncturing the internal carotid artery, it has major drawbacks including large variations in bleeding severity, high mortality rate and the possible failure to induce SAH. Especially, inter-animal variations in bleeding make it difficult to compare outcomes between different groups (Prunell et al., 2003; Titova et al., 2009). To overcome this drawback, a SAH grading scale developed by Sugawara et al. (2008) is commonly used to evaluate SAH severity in the endovascular perforation model (Sugawara et al., 2008). This grading scale has an advantage in evaluating correlations between subarachnoid blood clots and functional and morphological outcomes. In addition, this SAH grading system is easy to use in experimental models. However, as it assesses the distribution of blood and clots on the base of the brain, animals must be sacrificed for grading and this must be relatively soon after SAH because of clot resolution with time. Consequently, this grading system is unavailable to experimental studies that do not involve early euthanization.

In this study, we have outlined a new grading scale using magnetic resonance image (MRI) to assess SAH severity after endovascular perforation in rats. Our goal was to develop a MRI grading scale with predictive value for SAH severity which did not require euthanizing the animals. The MRI grading scale was evaluated functionally by correlating results with neurological scores.

2. Materials and methods

2.1. Animals and subarachnoid hemorrhage induction

Animal use protocols were approved by the University of Michigan Committee on the Use and Care of Animals. Rats were housed under standard 12:12 light-dark conditions and allowed free water and food. A total of 58 adult male and 58 adult female Sprague-Dawley rats were used in this study (body weight: 250-490g; Charles River Laboratories, Portage, MI).

SAH induction was performed using an endovascular perforation technique as previously described (Lee et al., 2010; Okubo et al., 2013). Rats were anesthetized with 5% isoflurane (VetOne Fluriso; MWI, Boise, ID). After intubation and initiation of mechanical ventilation,

isoflurane was titrated between 2.5 and 3%. Core body temperature was kept at 36.0 ± 1.0 °C with a controlled heating pad. In the supine position, the left external carotid artery was identified under a surgical microscope, transected distally, and reflected caudally in line with the internal carotid artery. A 3-0-nylon monofilament suture was inserted into the stump of external carotid artery through the common carotid artery bifurcation and into the internal carotid artery. The filament was advanced distally into the intracranial internal carotid artery until resistance was felt and the bifurcation of intracranial internal carotid artery was carefully perforated to create a SAH. The suture was then withdrawn into the external carotid artery and the internal carotid artery was reperfused. After surgery, rats were housed individually to recover.

2.2. Proposed MRI grading

MRI was performed 24 hours after SAH in a 7.0-T Varian MR scanner (Varian Inc, Palo Alto, CA) with acquisition of T2 fast spin-echo and T2* gradient-echo sequences using a field of view of 35×35 mm, matrix of 256×256 mm, and 25 coronal slices (0.5 mm thick). We defined MRI grading scales for SAH by modifying the Claassen CT rating scale (Claassen et al., 2001). MRI grades were classified as follows: grade 0: no SAH or intraventricular hemorrhage (IVH); grade 1: minimal or thin SAH without IVH; grade 2: minimal or thin SAH with IVH; grade 3: thick SAH without IVH; grade 4: thick SAH with IVH. Thick SAH was defined as there being at least 2 slices with clots thicker than 0.5 mm on T2* weighted MRI. IVH was defined as there being at least 1 hypointensity clot recognized in any ventricle on T2* weighted MRI.

2.3. Evaluation of SAH severity

The SAH severity score was assessed using a conventional grading scale established by Sugawara et al. (2008). In brief, rats were sacrificed after MRI and the brains rapidly removed. The basal brain including brainstem was divided into 6 segments. Each segment was assigned a grade from 0 to 3 depending on the amount of blood as follows: grade 0: no SAH; grade 1: minimal subarachnoid blood; grade 2: moderate blood clot with recognizable arteries; grade 3: massive hemorrhage covering the cerebral arteries. The minimum score is 0 and the maximum is 18.

2.4. Neurological score

Neurological scores were evaluated at 24 hours after SAH using a modified Garcia's scoring system as previously described (Garcia et al., 1995; Jeon et al., 2009; Park et al., 2004; Sugawara et al., 2008). We evaluated neurological scores using the following six tests: 1) spontaneous activity; 2) symmetry in the movement of four limbs; 3) forepaw outstretching; 4) climbing; 5) body proprioception; and 6) response to vibrissae touch. Each test was assigned from 0 to 3 (spontaneous activity, symmetry in the movement of four limbs, and forepaw outstretching) or 1 to 3 (climbing, body proprioception, and response to vibrissae touch). Animals were given a score of 3 to 18 in 1-number steps (higher scores indicate greater function).

2.5. Statistical analysis

The data in the text are presented as means $\pm$ SD. The Chi-square test was used to identify the categorical variable associated with mortality for both genders. Comparisons of gender differences in SAH severity and neurological score were tested using Student's t-test for the continuous variables. A Mann-Whitney U test was used to test the null hypothesis that the distribution of SAH clots based on proposed MRI grading does not differ with gender. The Spearman's rank correlation test was used to examine the correlation between MRI grading scale, SAH grading scale or neurological score. Statistical significance was set at $P < 0.05$. A value of r_s indicates Spearman's rank correlation coefficient. $r_s > 0.7$ was considered a strong correlation (Sugawara et al., 2008).

3. Results

3.1. Mortality, SAH severity, Neurological score

Mortality rates after SAH were 28% (16/58) in male and 22% (13/58) in female rats ($P = 0.52$, Chi-square test). In rats that survived 24 hours, SAH severity scores were 10.5 ± 4.4 in male and 12.1 ± 4.4 in female experimental rats ($P = 0.09$, Student's t-test). Neurological scores were 13.8 ± 3.1 in male and 14.7 ± 3.0 in female experimental rats ($P = 0.18$, Student's t-test).

3.2. The correlation between MRI grading and the SAH severity and neurological scores

Figure 1 show representative T2 and T2* weighted MRI scans and corresponding brain sections and whole brain photos for each MRI grading scale. We classified all experimental rats in accordance with MRI grading (Table 1). There was no significant difference in the distribution of bleeding between male and female rats with this MRI grading criteria ($n = 42-45$, $P = 0.69$, Mann-Whitney U test, Table. 1) with most rats in both genders being MRI grades 1, 3 and 4.

In male rats, there was a strong correlation between MRI and SAH grading scales (r_s (Spearman's rank correlation coefficient) = 0.86, $P < 0.01$, Fig. 2A). Neurological scores were also significantly correlated to MRI grading scale ($r_s = 0.46$, $P < 0.01$) and the SAH grading scale ($r_s = 0.51$, $P < 0.01$) in males (Fig. 2B & C). In female rats, there was also a strong correlation between MRI grading scale and SAH grading scale ($r_s = 0.82$, $P < 0.01$, Fig. 2D). However, in females, there was no significant correlation between either MRI grade or SAH grade and neurological score (Fig. 2E & F).

4. Discussion

Two major methods are used to induce SAH in animals: (1) blood injection at a preselected blood pressure or volume into the cisternal system and (2) endovascular perforation (Prunell et al., 2003). The blood injection model is useful for inducing reproducible SAH in animals by controlling the blood volume or pressure of injection. However, with the blood injection model it is difficult to make the abrupt changes in brain that mimic clinical SAH and there is no underlying vascular rupture. In contrast, the endovascular perforation method more closely mimics the events after an aneurysmal rupture, but there is a large variation in the

extent of SAH and outcome severity (Prunell et al., 2003; Titova et al., 2009). To address this drawback, Sugawara et al. (2008) developed an SAH grading scale and they found a strong correlation between that scale and the extent of both neurological deficits and cerebral vasospasm in male rats (Sugawara et al., 2008). However, to determine that SAH grading scale, animals need to be euthanatized.

The present study developed a new grading scale based on MRI to allow assessment of the extent of SAH without sacrificing the animals. The scale was based around the Claassen human SAH CT rating scale. Notably, the proportion of each grade based on our MRI grading in experimental rats (Table. 1) was comparable to that in human after SAH (Claassen et al., 2001). In addition, there was a highly significant correlation between the MRI grading scale and the current post-mortem SAH grading scale for both genders (Fig. 2A & D). These results suggest that the endovascular perforation model can imitate the human SAH and our MRI grading system may be available to investigate severity of bleeding in experimental SAH studies without animal euthanasia. This is important in allowing determination of SAH severity in individual animals in medium- to long-term studies. There is a relatively rapid clearance of blood from the subarachnoid space limiting the utility of post-mortem evaluation of SAH extent in medium- and long-term studies. The MRI grading scale can be used to assess potential therapies in animals with pre-defined degrees of SAH in the perforation model.

In male rats, we found a strong correlation between MRI grade and neurological score and we also found a correlation between the post-mortem SAH scale and neurological score (Fig. 2B & C), confirming the results of Sugawara et al (Sugawara et al., 2008). The findings indicate that rats with thick SAH clots or IVH tend to have worse neurological score than those with thin SAH or without IVH. Our previous report demonstrated that the ventricular volume correlated with the post-mortem SAH scale and rats with hydrocephalus had IVH in that SAH study (Okubo et al., 2013). A previous clinical report showed that the amount of subarachnoid hemorrhage and the presence of IVH was a significant predictor of 30-day mortality (Broderick et al., 1994). Our current findings support those reports.

On the other hand, we failed to detect the significant association between MRI grading scale or the post-mortem SAH scale with the neurological score in female rats (Fig. 2E & F). The reason for this result is considered that some female experimental rats had a better neurological score in spite of a worse SAH severity in this study. Little is known about the relationships between gender and mechanisms of brain injury after SAH in clinical and experimental studies. In a clinical study, the overall outcomes between women and men were similar (Hamdan et al., 2014). In an experimental study, Friedrich and colleagues found that gender influences the impact of SAH with male rats having greater bleeding and worse brain injury in comparison to female rats (Friedrich et al., 2013). In addition, a recent report showed that progesterone, a sexual hormone, attenuated early brain injury and improved neurological deficits at 24 hours after SAH in rats (Yan et al., 2013). Whether gender may play a role in the outcome of patients with SAH is controversial. However, it is possible that gender differences may influence early brain injury after SAH and affect neurological outcome. Further experiments are needed to determine whether gender influences early brain injuries after SAH in basic and clinical research.

5. Conclusion

We developed a grading scale based on MRI in the rat endovascular perforation model of SAH. This MRI grading scale strongly correlated with a post-mortem SAH grading scale. This method may be useful for evaluating initial SAH severity in long-term studies using the endovascular perforation model in rats.

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Abbreviations

SAH	subarachnoid hemorrhage
MRI	magnetic resonance image
IVH	intraventricular hemorrhage

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Highlights

- We developed a MRI grading scale to allow assessment of the extent of SAH in rats.
- There was a strong correlation between MRI grading and post-mortem SAH grading.
- The MRI grading system can evaluate severity of SAH without euthanizing animals.

Figure 1.

Representative T2 and T2* weighted magnetic resonance images (MRI) and corresponding brain sections and whole brain photos for each MRI grade. MRI grade was categorized as follows: grade 0: no SAH or intraventricular hemorrhage (IVH); grade 1: minimal or thin SAH without IVH; grade 2: minimal or thin SAH with IVH; grade 3: thick SAH without IVH; grade 4: thick SAH with IVH. Thick SAH was defined as there being at least 2 slices with clots thicker than 0.5 mm on T2* weighted MRI. Arrows indicate the subarachnoid blood clots and arrowheads indicate intraventricular hemorrhage in T2* weighted MRI.

Figure 2.

Graphs showing the correlations between MRI grading score and subarachnoid hemorrhage (SAH) grading scale (A, D), MRI grading score and neurological score (B, E) and SAH grading scale and neurological score (C, F). Measurements were made in male (A, B, C) and female (D, E, F) rats 24 hours after SAH. There was a significant correlation between MRI grade and SAH scale in both male ($P < 0.01$) and female ($P < 0.01$) rats. In male rats, there were also significant correlations between MRI grade and SAH scale with neurological score ($P < 0.01$). However, in female rats, neither MRI grade nor SAH scale correlated with neurological score. r_s indicates Spearman's rank Correlation Coefficient.

Table 1

Proposed MRI grading scale for SAH

MRI		Male	Female	P value [#]
Grade	Criteria	% (n)	% (n)	
0	No SAH or IVH	7.1 (3)	8.9 (4)	0.69
1	Minimal/thin SAH, no IVH	28.6 (12)	20.0 (9)	
2	Minimal/thin SAH with IVH	7.1 (3)	6.7 (3)	
3	*Thick SAH, no IVH	31.0 (13)	37.8 (17)	
4	*Thick SAH, with IVH	26.2 (11)	26.6 (12)	
Total		100 (42)	100 (45)	

* Thick subarachnoid hemorrhage (SAH): at least 2 slices are recognized as more than 0.5 mm thickness clots on T2* weighted MRI.

Intraventricular hemorrhage (IVH): at least 1 clot is recognized in any ventricle on T2* weighted MRI.

[#] Statistical analysis was performed using a Mann-Whitney U test.