Aneurysmal subarachnoid hemorrhage (SAH). Hydrocephalus can result in increased intracranial pressure that may lead to decreased cerebral blood flow and clinical deterioration.¹,² Hydrocephalus portends a worse prognosis in patients with aneurysmal SAH.¹ Few studies have investigated acute hydrocephalus after SAH in animal models. Here we report a rat model of acute hydrocephalus after SAH induced by endovascular perforation that may prove useful for developing potential therapies.

**Materials and Methods**

Animal use protocols were approved by the University Committee on the Use and Care of Animals. A total of 44 adult male Sprague-Dawley rats (n=36) were used in this study. SAH induction (n=36) was performed using an endovascular perforation technique.³⁵ Sham rats (n=8) underwent the same procedure without perforation. MRI was performed 24 hours after SAH and the volume of the ventricular system and extent of T²⁺ hypointensity lesions were measured. We define hydrocephalus as ventricular volume > +3 SDs above the mean in sham animals. SAH grade was determined and brains were used for histology, immunohistochemistry, Perls staining, and Western blot analysis. Ventricular wall damage was defined as percentage of ependymal surface disruption.

**Results**

All surviving rats (n=27) after SAH had ventricular enlargement (33.6±4.7 versus 13.5±1.4 mm³ in sham animals, P<0.01). Ventricular volume correlated with SAH severity (r=0.48; P<0.05). Out of 27 SAH rats, 12 demonstrated hydrocephalus and all had intraventricular blood accumulation. Rats with hydrocephalus had more severe ventricular wall damage (7.4±1.2%) than the sham animals (0.6±0.2%; P<0.01) and rats without hydrocephalus (1.1±0.2%; P<0.01). Periventricular iron deposition was observed and heme oxygenase-1 and Iba-1 expression were markedly increased in hydrocephalus rats.

**Conclusions**

SAH causes ventricular enlargement in a rat endovascular perforation model, with hydrocephalus occurring in 44% of animals at 24 hours. Rats with hydrocephalus had more severe SAH, intraventricular hemorrhage, and greater ventricular wall damage. (Stroke. 2013;44:547-550.)

**Key Words:** acute hydrocephalus • iron • rats • subarachnoid hemorrhage
Figure 1. Coronal T2 and T2* images, photomicrographs, and hematoxylin and eosin sections 24 hours after endovascular perforation (A) or sham procedure (B). HE indicates hematoxylin and eosin staining.

(sham animals =3, SAH with hydrocephalus =4, SAH without hydrocephalus =4). We used polyclonal anti-rat HO-1 IgG (StressGen), and polyclonal anti-rat Iba-1 IgG (Abcam) at 1:2000 dilution.

Values are presented as the mean±SEM. Data were analyzed with Student t test, ANOVA, and Spearman rank correlation test. Statistical significance was set at P<0.05.

Figure 2. Ventricular volume 24 hours after endovascular perforation or sham procedure (A). Correlation of ventricular volume and subarachnoid hemorrhage (SAH) grade at 24 hours (B). Coronal T2* images of sham and SAH animals with or without hydrocephalus at 24 hours. Rats with hydrocephalus have a larger hypointensity volume than the sham animals or rats without hydrocephalus (C). Hematoxylin and eosin staining of sham and SAH animals with or without hydrocephalus. Note the presence of intraventricular hemorrhage in the hydrocephalic rat. Boxes show intact ependyma (sham, SAH without hydrocephalus) and disrupted ependyma with intraventricular hemorrhage (SAH with hydrocephalus). SAH animals with hydrocephalus have more ventricular wall damage compared with sham or SAH animals without hydrocephalus (D). *P<0.05 and #P<0.01. Values are mean±SEM. Scale bar=200 µm. HC indicates hydrocephalus.

Results

The mortality in this study was 25% (9 of 36 rats) at 24 hours after endovascular perforation. No sham animals died (n=8). Endovascular perforation induced SAH in all cases (n=27) and results in ventricular enlargement at 24 hours.
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(33.6±4.7 mm³) compared with sham controls (13.5±1.4 mm³; n=8; *P*<0.01; Figures 1 and 2A). The ventricular volume in SAH animals correlated with SAH grade (*r*=0.48; *P*<0.05; Figure 2B). Hydrocephalus was defined as ventricular volume >+3 SD above the mean in sham animals and was present in 44% (12/27) of SAH animals. All animals with hydrocephalus (n=12) had intraventricular hemorrhage confirmed by MRI and histology. Rats with hydrocephalus had larger T2* hypointensity volume (4.9±1.8 mm³; n=12) than the sham animals (0.6±0.1 mm³; n=8; *P*<0.05; Figure 2C). The percentage of ventricular wall damage was greater in SAH animals with hydrocephalus (7.4±1.2%; n=8) compared with those without hydrocephalus (1.1±0.2%; n=11; *P*<0.01) and sham controls (n=5; 0.6±0.2%; n=5, *P*<0.01; Figure 2D). Using Perls staining, iron-positive cells were found in ependyma and subependyma of hydrocephalic SAH rats (Figure 3A). Periventricular HO-1 immunoreactivity was also observed in hydrocephalic SAH animals. By Western blotting, HO-1 levels were increased in rats with hydrocephalus (4119±384 pixels) compared with sham controls (1911±348 pixels; *P*<0.05) and those without hydrocephalus (1889±307 pixels; n=3–4; *P*<0.05; Figure 3C).

**Discussion**

We have demonstrated a rat model of SAH induced by endovascular perforation that results in hydrocephalus in 44% of animals at 24 hours. Hydrocephalus is associated with SAH grade, intraventricular hemorrhage, and ventricular wall damage. Periventricular iron deposition was observed. HO-1 (a stress marker, also called heat shock protein 32) and Iba-1 (a marker of microglia and macrophages) expressions were increased in animals with hydrocephalus, suggesting that hydrocephalus may exacerbate SAH-induced brain injury. Clinical studies have shown that amount of blood in the subarachnoid space and the presence of intraventricular hemorrhage increase the risk of acute hydrocephalus.2,3 Similar to these reports, ventricular volume was correlated with the severity of SAH and all animals with hydrocephalus had intraventricular blood in our study. Our results suggest that the amount of subarachnoid blood or presence of intraventricular blood may cause obstruction of cerebrospinal fluid flow. However, ependymal deficits and periventricular iron deposition were also observed in the
animals with hydrocephalus. Ependymal damage and iron deposition may lead to increased periventricular brain injury, which may play a role in hydrocephalus. High periventricular accumulation of iron in the hydrocephalic SAH rats may indicate a role of iron in producing hydrocephalus because our previous study has shown that iron causes hydrocephalus in a rat intraventricular hemorrhage model.6

Conclusions
Induction of SAH by endovascular perforation in rats results in a 44% rate of hydrocephalus at 24 hours. Intraventricular hemorrhage, damage to the ventricular wall, periventricular iron deposition, and tissue stress were observed in this rat model of acute hydrocephalus after SAH. This model should prove useful in examining potential therapeutics, including iron chelators and anti-inflammatory agents, for SAH-induced hydrocephalus.

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Disclosures
None.

References
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