Acetazolamide Attenuates Thrombin-Induced Hydrocephalus

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Introduction

Studies have found intraventricular hemorrhage (IVH) is a predictor of poor outcome after intracerebral hemorrhage (ICH) [2, 8]. For example, the presence of IVH in patients with ICH lowered the rate of favorable outcome from 31 to 15 % and was an independent predictor of worse outcome in the International Surgical Trial in ICH [2]. That trial also showed that hydrocephalus develops in more than 50 % of patients with IVH [2]. IVH is also an independent prognostic factor for poor outcome in SAH patients and acute hydrocephalus occurs in 20–30 % of such patients [15]. However, the mechanisms of brain hemorrhage-induced hydrocephalus are not well understood.

Thrombin is a serine protease and an essential component in the coagulation cascade. Thrombin forms immediately after brain hemorrhage. Our recent study found that thrombin has a role in hydrocephalus development after IVH [6]. Acetazolamide is a carbonic anhydrase inhibitor and can reduce CSF production [3]. This study examined the effect of acetazolamide on thrombin-induced hydrocephalus.

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Materials and Methods

Animal Preparation and Intraventricular Injection

Animal use protocols were approved by the University of Michigan Committee on the Use and Care of Animals. A total of 24 male Sprague-Dawley rats (Charles River Laboratories, Portage, MI, USA), at the weight of 270–300 g, were used in this study. Animals were anesthetized with pentobarbital (50 mg/kg intraperitoneally (IP)) and the right femoral artery was catheterized to monitor arterial blood pressure, blood pH, PaO_2, $PaCO_2$, hematocrit, and glucose levels. Core body temperature was maintained at 37.5 °C with a feedback-controlled heating pad. Rats were then positioned in a stereotaxic frame. A cranial burr hole (1 mm) was drilled and a 26-gauge needle was inserted stereotaxically into the right lateral ventricle (coordinates: 0.6 mm posterior, 4.5 mm ventral, and 1.6 mm lateral to the bregma). Saline or thrombin was infused using a microinfusion pump. The needle was removed after injection, the burr hole was filled with bone wax, and the skin incision was sutured closed.

Experimental Groups

There were two parts in this study. First, rats had an injection of either 50 µl saline ($n=6$) or 3 U of rat thrombin in 50 µl saline ($n=6$) into the right lateral ventricle. Second, rats had an injection of 3 U thrombin into the right lateral ventricle and were treated with either vehicle ($n=6$) or acetazolamide (30 mg/kg, IP, $n=6$) at 1 h after thrombin infusion. All rats underwent magnetic resonance imaging (MRI) 24 h after the intraventricular injection and were then euthanized. Lateral ventricle volumes were measured in T2-weighted MRI images and the brains were used for histology.

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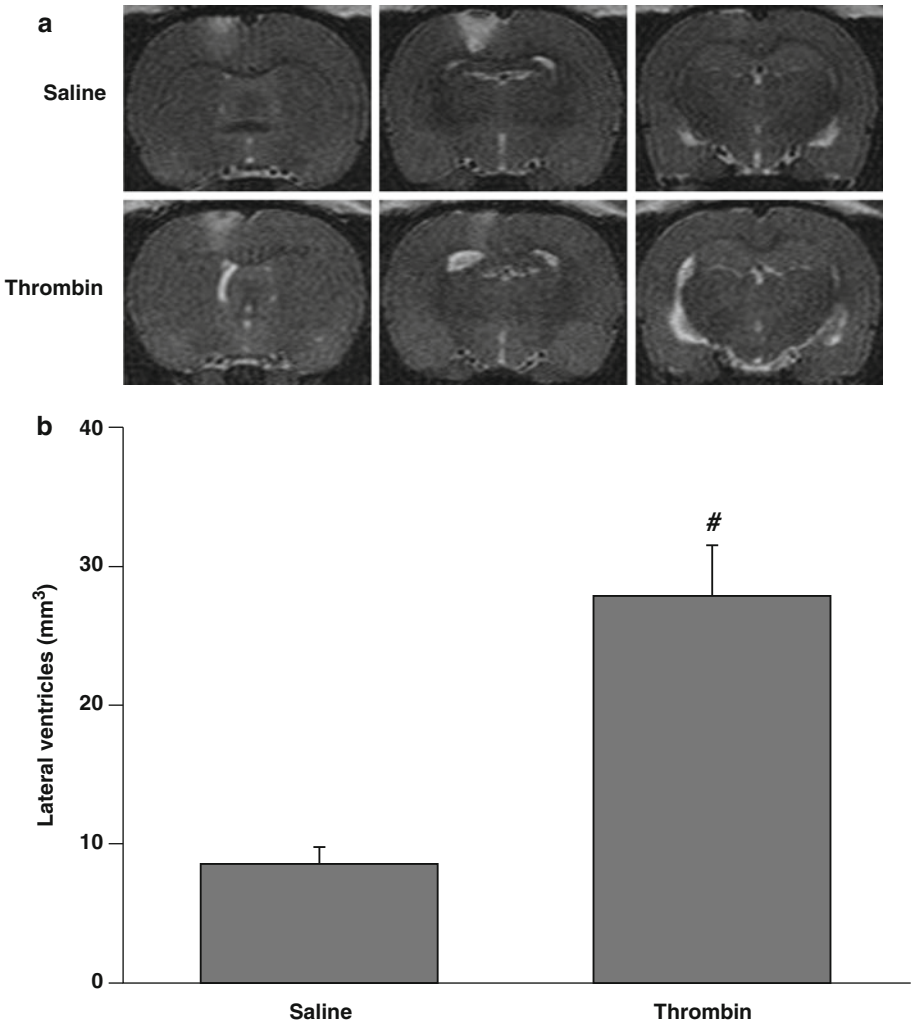

Fig. 1 T2-weighted MRI images (**a**) and lateral ventricular volume measurements (**b**) from rats 24 h after injection of 50 µl of saline or thrombin (3 U) into the right lateral ventricle. Values are means ± SD, $n = 6$, # $p < 0.01$ vs saline group

MRI Scanning and Ventricle Volume Measurement

Rats were anesthetized with 2 % isoflurane throughout the MRI examination. MRI scanning was performed in a 7.0-T Varian MR scanner (Varian Inc., Palo Alto, CA) with a T2 fast spin-echo sequence, using a view field of 35 × 35 mm and 25 coronal slices [17]. Ventricular volumes were measured and calculated as described previously [4, 12, 13]. Bilateral ventricles were outlined and the areas were measured. Ventricular volume was calculated by summing the ventricle areas over all slices and multiplying by the section thickness. All image analysis was performed using the ImageJ program by a blinded observer.

Ventricular Wall Damage Analysis

Ventricular wall damage was analyzed by calculating the percentage of the ependyma that was damaged, as previously described [13]. Briefly, brain sections underwent hematoxylin and eosin staining. The length of the ependyma that was disrupted or detached from the periventricular parenchyma was determined and divided by the total ventricular surface perimeter. All the analysis was performed using ImageJ software by a blinded observer.

Statistical Analysis

Values are given as means ± standard deviation (SD). Student's *t*-tests or Mann-Whitney *U* tests were used to analyze the data. Differences were considered significant at $p < 0.05$.

Results

MRI showed that intraventricular injection of thrombin (3 U) resulted in hydrocephalus. Thus, lateral ventricular volume in thrombin-injected rats was significantly larger than in saline-injected rats at 24 h (27.8 ± 3.7 vs 8.5 ± 1.3 mm^3 in saline, $p < 0.01$, Fig. 1). In addition, intraventricular thrombin

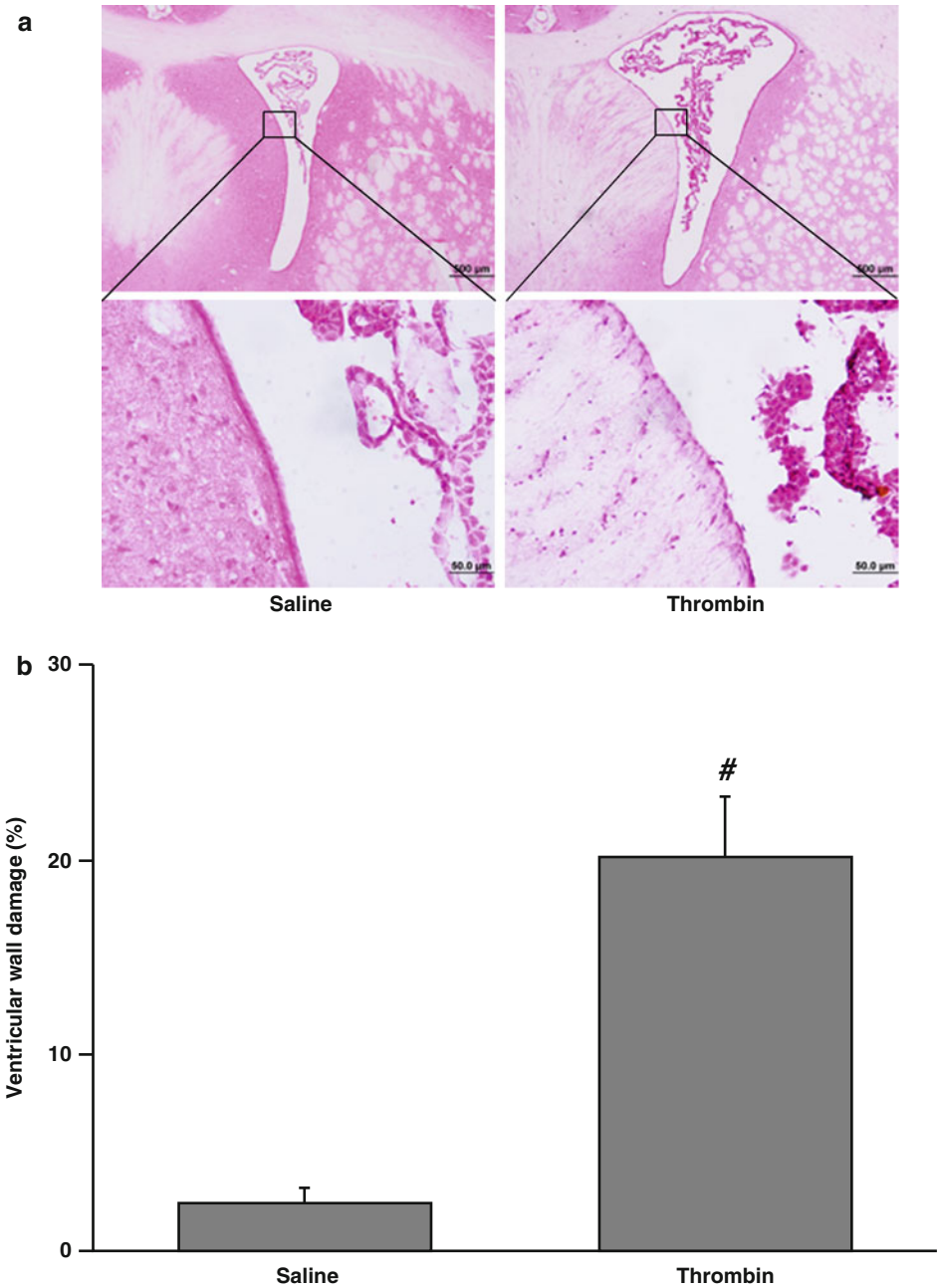

Fig. 2 The damaged ventricular wall in H&E-stained coronal sections (**a**) and the percentage of damage ventricular out of total ventricular wall (**b**) at 24 h after injection of 50 µl of saline or thrombin (3 U) into the right lateral ventricle. Values are means ± SD, $n=6$, # $p<0.01$ vs saline group

injection, but not saline injection, caused severe ventricular wall damage (20.2 ± 1.7 % vs 2.8 ± 0.4 % in saline, $p<0.01$) at 24 h (Fig. 2).

Systemic treatment with acetazolamide, a carbonic anhydrase inhibitor, 1 h after intraventricular injection of 3 U of thrombin resulted in less ventricular enlargement compared with vehicle treatment (16.1 ± 4.2 vs 29.5 ± 5.3 mm^3 in the vehicle-treated group, $p<0.01$) (Fig. 3).

Discussion

In this study, we found intraventricular injection of thrombin caused hydrocephalus and ventricular ependymal wall damage. Acetazolamide, given at 30 mg/kg IP 1 h after thrombin injection, attenuated thrombin-induced hydrocephalus.

Thrombin is a serine protease and an essential component in the coagulation cascade. Experimental investigations have indicated that thrombin formation plays a major role in ICH-induced injury [9, 11, 16]. Thrombin is responsible for early brain edema formation after ICH, and that edema results partly from a direct opening of the blood-brain barrier (BBB). We have demonstrated that intraventricular injection of thrombin can cause significant ventricular dilatation and periventricular parenchyma injury [6]. At present, the mechanisms associated with thrombin-induced ventricular dilatation are unknown. Ependymal injury such as reduced cilia and abnormal of organelle structure were observed in

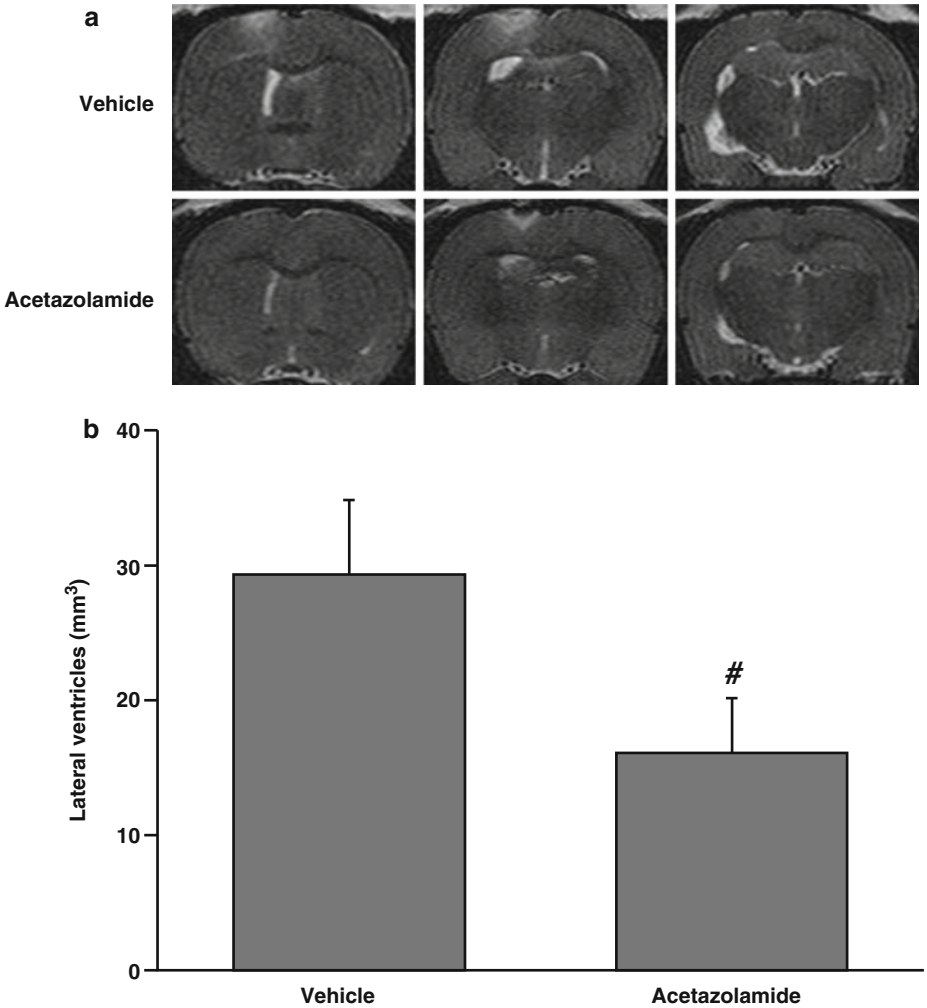

Fig. 3 T2-weighted MRI images (**a**) and lateral ventricular volumes (**b**) 24 h after injection of thrombin (3 U). Rats were treated systemically (IP) with vehicle or acetazolamide 1 h after the thrombin was injected. Values are means ± SD, $n=6$, # $p<0.01$ vs vehicle-treated group

thrombin-injected rats [6]. Ependymal damage may lead to increased periventricular brain injury and to hydrocephalus [4, 14]. Activities of normal ependymal cilia are thought to direct cerebrospinal fluid (CSF) current toward the ventricular outlets. Previous study showed absent or functionally defective ependymal cell motile cilia may be a cause of hydrocephalus in a mouse model [1]. Therefore, ependymal damage and abnormal ependymal cilia may play a role in hydrocephalus induced by thrombin.

Acetazolamide, a carbonic anhydrase inhibitor, decreases CSF production in animal models [5, 10, 17]. Acetazolamide has a rapid onset of action. Treatment with 10 mg/kg of acetazolamide resulted in a significant decrease in CSF production and absorption within 3 h of ingestion in dogs [17]. Acetazolamide is also used in both children and adults to treat hydrocephalus and pseudotumor cerebri [6, 7]. We have shown that co-injection of acetazolamide reduced ICH-induced brain injury [7]. In the present study, acetazolamide treatment significantly reduced thrombin-induced hydrocephalus, most likely by reducing CSF production.

Conclusions

Intraventricular injection of thrombin caused ventricular wall damage and hydrocephalus in rats. Inhibition of carbonic anhydrase by acetazolamide effectively reduced thrombin-induced hydrocephalus. The results suggest that thrombin-induced hydrocephalus may result from reduced absorption and/or increased production of CSF.

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