Thrombin-soaked gelatin sponge and brain edema in rats

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Previous work from this laboratory has shown that injection of thrombin into rat basal ganglia causes brain edema. This study investigates the effect on rat brain of thrombin-soaked gelatin sponge (used for intraoperative hemostasis in clinical situations) at a concentration similar to that used in humans. Three models were developed to evaluate this effect. In the first model, a gelatin sponge soaked with vehicle or thrombin (100 U/cm³) was placed on the intact pia of the right frontal lobe in rats without cortical lesions. In the second model, frontal cortex was excised (3 mm³) and the exposed brain was cauterized with electrocoagulation. Gelatin sponge was soaked with vehicle or thrombin (1000, 100, 10, or 1 U/cm³) and placed in the lesion site. In the third model, hirudin, a specific thrombin antagonist, was added to the thrombin-soaked gelatin sponge and placed in a similar cortical lesion to determine if the observed effects were specific to thrombin. The dose-response range for thrombin was determined qualitatively by magnetic resonance (MR) imaging and quantitatively by brain edema formation 24 hours after exposure. We found no edema in the cortically intact rats. The rats given cortical lesions developed significant edema when subjected to 1000, 100, and 10 U/cm³ thrombin as seen on MR imaging and at 100 and 10 U/cm³ thrombin as revealed by wet/dry weight and ion studies of brain tissue. Topical hirudin prevented thrombin-induced edema. It is concluded that thrombin-soaked gelatin sponges cause or enhance significant brain edema in rats at concentrations typically used for human neurosurgery.

KEY WORDS • thrombin • gelatin sponge • magnetic resonance imaging • brain edema • rat

Materials and Methods

Preparation and Anesthesia

Adult male Sprague–Dawley rats weighing 250 to 350 g were each anesthetized in a closed chamber with 2.5% isoflurane. A 16-gauge catheter was inserted perorally into the trachea. The rats were ventilated with a rodent ventilator using a mixture of room air (21% O₂) and isoflurane (2%–3%). A catheter was inserted into the femoral artery. The arterial blood pressure was monitored continuously and titrated with isoflurane to maintain a mean pressure of 100 mm Hg. Arterial blood was obtained from the femoral artery catheter for blood gas analysis. The ventilator rate, tidal volume, and inspired gas concentrations were adjusted to maintain normal blood oxygen (O₂ saturation > 95%) and carbon dioxide tensions (PCO₂ = 35–45 mm Hg). Body temperature was maintained at 37.5°C using a rectal thermometer and a feedback-controlled heating pad. Each rat was placed prone in three-point cranial fixation, and a 2.0-cm sagittal skin incision was made aseptically. Microscissors were then used to incise the dura with the aid of an operating microscope.

Experimental Groups

The animals were divided into two groups to observe the effects of topical thrombin on the brain in cases with an intact pial surface and in cases with deeper cortical lesions. In the group of rats with
cortical lesions, hirudin, a thrombin antagonist, was added to see if the effects were specific to thrombin.

**Group 1.** The first group consisted of rats with no cortical lesion and intact pia. After the dura was opened, a 2-mm³ piece of gelatin sponge soaked with 10 µl of vehicle (0.15 M NaCl, 0.05 M Na citrate in a 1:10 dilution of normal saline (five animals)) or 10 µl of 100 U/cm³ thrombin (five animals) was placed on the intact pia. The dura was left open. The bone flap was replaced and secured with interrupted No. 2.0 vicryl sutures attached to the temporalis fascia. The skin was then closed with a No. 2.0 silk suture. The animals were extubated and returned to their cages with free access to food and water.

**Group 2.** The second group consisted of rats with a standardized cortical resection. After the dura was opened, 3 mm³ of brain cortex from the right frontoparietal region was excised using a No. 11 scalpel blade with the aid of the operating microscope. The exposed parenchymal surface was then cauterized using bipolar cautery with the coagulation current at 35 mA. Pieces of gelatin sponge (2 mm³) soaked with 10 µl of thrombin at concentrations of 1000, 100, 10, or 1 U/cm³ (two rats at each concentration) or 10 µl of vehicle (two rats) were placed in the surgical bed. The wound was closed as in Group 1 and the anesthetic was discontinued.

The rats underwent coronal T₂-weighted magnetic resonance (MR) imaging at 24 hours after the operation. Right frontal cortical and basal ganglia sections from animals subjected to these same thrombin concentrations: 1000, 100, 10, and 1 U/cm³ (six rats each at 1000 and 100 U/cm³, five each at 10 and 1 U/cm³) or 10 µl of vehicle were then evaluated with wet/dry weight and ion studies. A final group of five rats received pledgets of gelatin sponge soaked with 100 U/cm³ of thrombin mixed with 900 U/cm³ of hirudin (10 µl). Hirudin is a specific thrombin antagonist. The stock solution vehicle consisted of 0.15 M NaCl and 0.05 M Na citrate at pH 6.5 and osmolarity 330 mOsm. A stock solution vehicle was used for comparison with the 1000 U/cm³ thrombin group, whereas serial 10-fold dilutions in normal saline were used to duplicate thrombin concentrations of 100, 10, and 1 U/cm³. There were five rats in each vehicle dilution group for a total of 20 vehicle-treated rats. Closure and extubation procedures were identical to those used in Group 1.

**Sample Collection and Measurements**

Twenty-four hours postoperatively, the rats were anesthetized with 5% isoflurane in room air and killed by decapitation in accordance with the University Committee on Use and Care of Animals guidelines.

**Wet/Dry Weights.** The brains were removed immediately. The gelatin sponge pledgets were then carefully removed and the most anterior 5 mm of cortex was excised from both hemispheres. Each brain was sectioned into six regions: right and left cortex, and right and left basal ganglia, and right and left cerebellum. These tissue samples were weighed on an electronic analytical balance with 0.0001 mg precision to obtain wet weight (ww). The tissue was then dried in a gravity oven at 95°C for 24 hours and reweighed to obtain dry weight (dw). The water content (Table 1) was calculated as follows: Water % = (ww - dw)/ww × 100.

**Ion Content.** The dehydrated section was digested in 1 ml of 1 N nitric acid for a minimum of 5 days. Then a 0.2-ml aliquot was removed and diluted to 2 ml with deionized water and 3 mM CsCl solution. The sodium ion contents were measured in this solution using an automatic flame photometer. Another 0.2-ml aliquot was removed and diluted with chloridometer acid reagent solution.
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TABLE 1

Percentage of water content in brain samples in rats treated with gelatin sponges soaked with various concentrations of thrombin and vehicle*

<table>
<thead>
<tr>
<th>Sample Site†</th>
<th>1000 U/cm³</th>
<th>100 U/cm³</th>
<th>10 U/cm³</th>
<th>1 U/cm³</th>
<th>100 U/cm³</th>
<th>Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rt cortex</td>
<td>ND</td>
<td>80.2 (0.37)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>80.1 (0.15)</td>
</tr>
<tr>
<td>rt basal ganglia</td>
<td>ND</td>
<td>78.9 (0.18)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>78.5 (0.23)</td>
</tr>
<tr>
<td>rt cerebellum</td>
<td>ND</td>
<td>78.0 (0.14)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>78.1 (0.13)</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rt cortex</td>
<td>81.4 (0.58)</td>
<td>81.5 (0.24)</td>
<td>81.0 (0.35)</td>
<td>81.0 (0.26)</td>
<td>82.5 (0.36)</td>
<td>81.1 (0.14)</td>
</tr>
<tr>
<td>rt basal ganglia</td>
<td>80.6 (0.56)</td>
<td>82.3 (0.39)</td>
<td>82.4 (0.55)</td>
<td>79.4 (0.30)</td>
<td>81.0 (0.30)</td>
<td>79.9 (0.13)</td>
</tr>
<tr>
<td>rt cerebellum</td>
<td>78.1 (0.17)</td>
<td>77.7 (0.14)</td>
<td>78.3 (0.10)</td>
<td>77.8 (0.16)</td>
<td>77.7 (0.34)</td>
<td>78.0 (0.14)</td>
</tr>
</tbody>
</table>

* Values in parentheses are expressed as means ± standard error of the means. Abbreviation: ND = not done.
† Group 1 = no cortical lesion, right craniotomy; Group 2 = right cortical lesion.
‡ p < 0.05.

Chloride content was measured in this solution using a digital chloride meter. The milliequivalents of each ion were then expressed as milliequivalents per kilogram of dry weight.

Magnetic Resonance Imaging. We obtained MR images in rats from Group 2 24 hours after operation, just prior to their decapitation. These rats received pentobarbital intraperitoneally (0.02 mg/100 g body weight) for sedation prior to placement in the 2-tesla laboratory MR imaging system. Thirty-two T₁-weighted images, each 1 mm thick, were obtained in the coronal plane.

Analysis of Data

The brain wet/dry weights and ion content comparisons were made using analysis of variance with a Scheffé F-test. The differences were considered significant at probability values of less than 0.05. All MR imaging data were evaluated for edema in a qualitative fashion independently by two investigators (G.P.C. and T.L.C.).

Sources of Supplies and Equipment

The rats were obtained from Charles River Laboratories, Portage, MI. The rodent ventilator was supplied by Harvard Apparatus, Inc., S. Natick, MA, and the electric drill by Pfingst & Co., S. Plainfield, MI. The rodent ventilator was supplied by Harvard Apparatus, Inc., S. Plainfield, NJ. The Malis CMC-II-PC bipolar cauterizing device was purchased from Codman Co., Randolph, MA. The electronic balance, model AF100, was obtained from Mettler Instrument Co., Heights-town, NJ. The gravity oven from Blue M Co., Blue Island, IL; the flame photometer, model IL943TM, from Instrumentation Lab, Lexington, MA; and the chloridometer, model 442-5000, from Haake-Buchler Inc., Paramus, NJ. Thrombin and hirudin were purchased from Sigma Chemical Co., St. Louis, MO.

Results

All animals survived for the 24-hour period following the operation. The rats receiving the higher concentrations of thrombin (1000, 100, and 10 U/cm³) were qualitatively more lethargic at 12 to 24 hours after operation but were still able to take food and water.

Group 1

Group 1 (rats with no cortical lesion and intact pia) showed no difference in water content of the brain as measured by wet/dry weight and MR imaging when thrombin- and vehicle-soaked gelatin sponge treatments were compared (Fig. 1).

Group 2

The rats with cortical lesions evaluated by means of MR imaging 24 hours postinjury showed that thrombin in concentrations of 1000, 100, and 10 U/cm³ caused brain edema, which was especially evident tracking through the white matter (Fig. 2). The animals subjected to either 1 U/cm³ of thrombin or vehicle alone showed less brain water accumulation on the side of resection and did not have edema in the white matter tracts. The animals treated with hirudin/thrombin had some tracking of edema in the white matter but less than the group treated with 100 U/cm³ thrombin. All animals had similar perilesional edema surrounding the cortical operative bed. There was no difference in brain water between the groups receiving vehicle at various dilutions. Hence, the vehicle groups were combined for statistical purposes.

Rats with cortical injury showed significant edema around the lesion on the operated compared with the unoperated side. No difference in water content was found by wet/dry weight studies between groups receiving various thrombin concentrations or vehicle (Fig. 3). Water content and ion studies showed that edema developed in the right basal ganglia in the 100 and 10 U/cm³ groups (p < 0.01) but not in the 1000, 1 U/cm³, or vehicle groups (Fig. 4). The rats subjected to a cortical lesion and the gelatin sponge pledget soaked in hirudin/thrombin showed less edema when compared with the 100 U/cm³ thrombin group on both brain water (p < 0.05) and ion studies (p < 0.01) (Fig. 5). There was no difference in edema in the hirudin/thrombin group when compared with the vehicle group (Fig. 5).

Discussion

In the course of investigations of the edemogenic effect of intracerebral hemorrhage, our laboratory has shown that thrombin injected into the basal ganglia of rats causes brain edema at concentrations of the drug commonly used in neurosurgical operations. This is interesting for two reasons, both clinically relevant. First, thrombin release from a developing blood clot in the brain parenchyma may account for edema formation around the...
hematoma and the progressive neurological deterioration often seen after parenchymal hemorrhage. Second, topical thrombin-soaked gelatin sponge is commonly used for hemostasis in clinical neurosurgery, but little is known about its effects on the brain. The typical intraoperative thrombin concentrations used for topical hemostasis range from 100 to 1000 U/cm³ applied on a gelatin sponge carrier. Our data indicate that this dose range causes edema over a 24-hour period based on T1-weighted MR imaging analysis, even at a dilute concentration (100 U/cm³). The hypothesis that this effect is specifically due to thrombin is supported by the ability of a specific thrombin inhibitor, hirudin, to suppress or prevent edema formation. Hirudin alone has no edemogenic effect on the brain.8

Topical thrombin caused edema to form, especially in the white matter tracts, at concentrations of 1000, 100, and 10 U/cm³ of thrombin as seen on MR imaging, and at 100 and 10 U/cm³ as detected by water content and ion studies. Surprisingly, edema did not develop at the 1000 U/cm³ concentration according to wet/dry weight studies, although there was a trend for the thrombin group to show more edema than its control. Because previous work with stereotactic injection of thrombin showed edema only at very high concentrations (10,000–1000 U/cm³, but not at 100 U/cm³), the method of thrombin delivery might account for the failure of a high dose (1000 U/cm³) to cause significant edema. Furthermore, stereotactic injection involves high local concentrations that diffuse quickly, whereas a thrombin-soaked pledget concentrates the drug locally for longer periods of time. Because thrombin is a vasoconstrictor,3,10 it might decrease local blood flow when delivered slowly from a soaked pledget containing high concentrations of drug. The expected changes in regional ion and water content that follow lower thrombin concentrations might have been impeded for the same reasons.

Our data have clinical implications. First, thrombin did not cause significant edema when placed on intact pia-arachnoid. This is probably because of a barrier effect from the leptomeninges. Second, edema caused or enhanced by topical thrombin was seen only with brain injury as shown in our animals receiving cortical lesions. Third, the gelatin sponge itself had no deleterious effects in our study even though it may have in different clinical situations.5,6 Fourth, thrombin is known to have deleterious effects on central nervous system tissue at a cellular level.9,11

We believe that topical thrombin should not be in contact with brain parenchyma because it may contribute to significant peri- and postoperative edema. Cauterization or mechanical hemostasis, or both, should be used to control bleeding, and thrombin should be applied only if all other options have been attempted and bleeding persists. Thrombin-soaked gelatin sponges should probably
be reserved as an adjunct for hemostasis in procedures involving intact pia, such as subarachnoid dissection of the sylvian fissure or sinus oozing.

References


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