Edema from intracerebral hemorrhage: the role of thrombin

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The mechanism by which intracerebral hemorrhage leads to the formation of brain edema is unknown. This study assesses the components of blood to determine if any are toxic to surrounding brain. Various solutions were infused stereotactically into the right basal ganglia of rats. The animals were sacrificed 24 hours later; brain edema and ion contents were measured. Whole blood caused an increase in brain water content and ion changes consistent with brain edema. Concentrated blood cells, serum from clotted blood, and plasma from unclotted blood all failed to provoke edema formation when infused directly into the brain. On the other hand, activation of the coagulation cascade by adding prothrombinase to plasma did produce brain edema. The edema response to whole blood could be prevented by adding a specific thrombin inhibitor, hirudin, to the injected blood. This study indicates that thrombin plays an important role in edema formation from an intracerebral blood clot.

KEY WORDS • intracerebral hemorrhage • thrombin • prothrombinase • blood • brain edema • hirudin • rat

In the United States, 15% of deaths due to cerebrovascular disease are the result of spontaneous intracerebral hemorrhages. Currently, spontaneous intracerebral hemorrhage is more common than subarachnoid hemorrhage (SAH) and has a similar mortality rate. Intracerebral hemorrhage is also commonly associated with head trauma.

Many patients with an intracerebral hematoma deteriorate progressively after the hemorrhage because of the accumulation of edema fluid around the mass. The reason an intracerebral hematoma causes brain edema is unknown. The hematoma and its associated edema may combine to cause intracranial hypertension from progressive mass effect, producing brain shifts and secondary ischemia.

Better understanding of the pathophysiology of edema formation due to intracerebral hemorrhage might lead to improved clinical management of these patients. Our study sought to determine whether or not individual blood components cause edema formation or contribute to it through the coagulation cascade. If a substance in blood or a mechanism induced by blood clotting could be identified, blockade of the process might be possible.

Materials and Methods

Animal Preparation

In all, 92 adult male Sprague-Dawley rats, each weighing between 250 and 350 g, were anesthetized in a closed chamber with 5% isoflurane. A 16-gauge catheter was inserted into the trachea by the oral route. The rats were ventilated with a rodent ventilator using a mixture of oxygen (21%), nitrogen (76%–77%), and isoflurane (2%–3%). A catheter was inserted into the femoral artery and arterial blood pressure was monitored continuously. The blood pressure was 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 and as a source of blood for infusion. Arterial blood gas analysis and as a source of blood for infusion. Arterial blood gas analysis and as a source of blood for infusion. Body temperature was maintained at 37.5°C using a rectal thermometer and a feedback-controlled heating pad.

Experimental Model

The rats were positioned in a stereotactic frame, and the scalp was incised along the sagittal midline. An operating microscope was used for the remainder of the procedure, and hemostasis was maintained with bipolar electrocautery. A 1-mm burr hole was placed in the skull near the right coronal suture 3.0 mm lateral to the midline. Needles were inserted into the right basal ganglia using stereotactic guidance (coordinates: 0.2 mm anterior, 6.0 mm ventral, and 3.0 mm lateral to the bregma). In part 1 of the experiment, a single 26-gauge needle was used for each animal. In parts 2 and 3, the first needle was removed and then 5 minutes later a second 26-gauge needle was inserted using the same coordinates. Solutions were infused into the brain using a commercially available pump. The stereotactic needle and arterial catheter were removed after infusion. The incisions were closed with sutures. The anesthetic was discontinued and the animals were extubated. The rats were allowed to recover and given free access to food and water.

Experimental Groups

This study was performed in three parts. The first part evaluated the effect of the major blood components on edema formation. In the second part the role of the coagulation cascade in brain injury...
from intracerebral hemorrhage was investigated. The third part was concerned specifically with the involvement of thrombin in brain edema formation after intracerebral hemorrhage.

**Part 1.** Four groups of animals were studied in the first part of the study. Each rat received a 50-μl infusion of either normal saline (six animals), plasma (five animals), concentrated blood cells (six animals), or whole blood (seven animals). The whole blood or blood component injected in each case was autologous. To obtain plasma or concentrated blood cells (hematocrit = 87 ± 2), whole blood was immediately centrifuged at 14,000 G for 10 seconds before coagulation occurred. The plasma appeared in the upper layer and concentrated blood cells in the lower layer of the centrifuged blood. The rate of thrombin formation in plasma is decreased markedly by removal of the cellular elements, and, therefore, plasma is considered to be thrombin poor. The decrease in thrombin production is most likely due to the loss of phospholipids, which are an important cofactor in the conversion of prothrombin to thrombin.24

**Part 2.** In the second part of the experiment five groups of animals were studied. Each rat received a 50-μl infusion followed 5 minutes later by a 20-μl infusion. The first group of six animals received only normal saline. The second group of six animals received 50 μl of serum followed by 20 μl of saline. Serum was produced by allowing whole blood to clot and then centrifuging at 14,000 G for 10 seconds. The third group of five animals received 50 μl of plasma obtained by centrifuging unclotted blood followed by an injection of 20 μl of saline. The fourth group of nine animals received 50 μl of plasma followed by 20 μl of prothrombinase complex. The prothrombinase complex injection was composed of 10 μl of 0.04 U/ml activated factor X added to 10 μl of phospholipids (rabbit brain cephalin) with 2.5 mM CaCl. One unit of factor X is the amount contained in 1 ml of normal human plasma. The prothrombinase complex consists of activated factor X and several cofactors. Phospholipids and calcium are two cofactors that increase the rate of thrombin production in the presence of activated factor X by 50-fold.25 To determine if these cofactors have an independent effect on brain edema, a second group of five sham-treated animals received 50 μl of saline followed by 20 μl of a solution containing 2.5 mM CaCl, and phospholipids prepared as above, but without activated factor X.

**Part 3.** Four groups of animals were studied. Fifty microliters of blood or saline were infused and the needle was removed. A second needle was inserted and the next injection started 5 minutes later. This period of time allowed for clotting of the injected blood. In the second infusion, rats received either 20 μl of saline or 20 μl of 0.5 U/μl hirudin, a thrombin inhibitor. Therefore, the animals receiving hirudin were injected with a total of 10 U. One unit of hirudin is the amount that will bind and inhibit 1 U of thrombin. The four groups of animals included: no hematoma plus saline (six animals), no hematoma plus hirudin (six animals), hematoma plus saline (seven animals), and hematoma plus hirudin (seven animals). The quantity of hirudin infused was theoretically sufficient to inhibit the amount of thrombin that could be produced by the hematoma. Investigators have reported 260 to 360 μl of prothrombin in 1 ml of plasma.124 One unit of prothrombin will produce 1 U of thrombin. Because a 50-μl blood clot contains at least 30 μl of plasma, the potential amount of thrombin produced in this size clot is calculated to range from 8 to 10 U. Therefore, 10 U of hirudin should inhibit all of the thrombin formed in a 50-μl clot.

**Brain Water, Sodium, Potassium, and Chloride Contents**

The rats were sacrificed by decapitation 24 hours after intracerebral infusion. The brains were removed and a coronal slice 3 mm from the frontal pole was cut approximately 4 mm thick. This section of brain was divided along the midline, and the cortex was separated from the basal ganglia bilaterally. The brain tissue surrounding the hematoma in the basal ganglia was isolated.

The tissue samples were immediately weighed on an electronic analytical balance to the nearest 0.1 mg to obtain the wet weight (WW). The tissue was then dried in a gravity oven at 95°C for 24 hours and weighed again to obtain the dry weight (DW). The formula (WW − DW)/WW · 100 was used to calculate the water content expressed as percentage of WW.

The dehydrated samples were digested in 1 ml of 1 M nitric acid for a period of 1 week to release the ions into the solution. The sodium and potassium contents of this solution were measured by flame photometry, and chloride content was measured using a digital chloride meter. Ion content was expressed in microequivalents per gram of dehydrated brain (μEq/g DW).

**Hemoglobin Determination**

Hemoglobin content of whole blood and intracerebral hematomas was measured spectrophotometrically in animals receiving a 50-μl intracerebral injection of blood plus 20 μl of 0.5 U/μl hirudin (six animals) or a 50-μl injection of blood plus 20 μl of saline (five animals). The animals were prepared as described above. Blood was removed from the femoral artery catheter and divided into three 20-μl samples. The animals were sacrificed within 1 hour of the intracerebral injections. The brains were removed and the clot separated from the brain. The hematomas were each mixed with 5 ml of Drabkin’s reagent in a cuvette to form cyanmethemoglobin from hemoglobin. The 20-μl samples of whole blood were also each mixed with this reagent. The absorbance of cyanmethemoglobin at 540 nm was measured with a spectrophotometer.

**Statistical Analysis**

Differences in brain water and ion contents between groups of rats were evaluated using analysis of variance (ANOVA) and the Scheffé F-test of significance. The hemoglobin data were compared using the Student’s t-test. A two-tailed probability value of less than 0.05 was used to indicate a significant difference.

**Sources of Supplies and Equipment**

Sprague-Dawley rats were obtained from Charles River Laboratories, Portage, MI. The rodent ventilator and the pump were manufactured by Harvard Apparatus, Inc., S. Natick, MA. Biorex 70 columns, 125 mm × 0.8 mm, were obtained from Bio-Rad Laboratories, Richmond, CA. The digital chloride meter, model EL420, was manufactured by Mettler Instrument Co., Hightstown, NJ. The gravity oven was manufactured by Blue M Electric Co., Blue Island, IL, and the automatic flame photometer, model IL943, by Instrumentation Laboratory Inc., Lexington, MA. The digital chloridometer, model 4420-5000 was manufactured by Haake Buchler Inc., Saddlebrook, NJ.

**Results**

**Physiological Parameters**

The physiological parameters for the groups of rats were all within normal range. Table 1 shows the mean values for the rats used in each of the three parts of the study. Normal blood gas and blood pressure values were recorded in all groups during the anesthetic period.

**Experimental Groups**

**Part 1.** Figure 1 shows the effect of the specific blood components on brain water and ions. Plasma alone and concentrated blood cells (red blood cells, platelets, and white blood cells) alone did not produce brain edema. However, whole blood composed of both plasma and blood cells produced a significant amount of brain edema and total ion changes. In general, brain sodium and chloride changes corresponded directly to the degree of edema fluid produced.

**Part 2.** Figure 2 shows the effect of the coagulation cascade on brain edema formation. Two sham-treated control
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Figure 3 shows the effect of a thrombin inhibitor on brain edema and ions in the presence of an intracerebral hematoma. There was an increase in brain water content in the group treated with heparin, while the group treated with hirudin demonstrated significantly less local accumulation of brain water in the group treated with hirudin than controls.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (Saline)</th>
<th>Plasma + Saline</th>
<th>Blood Cells + Saline</th>
<th>Whole Blood (Saline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>97.5 ± 0.2</td>
<td>97.3 ± 0.2</td>
<td>97.3 ± 0.2</td>
<td>97.3 ± 0.2</td>
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<tr>
<td>pH (mm Hb)</td>
<td>7.38 ± 0.02</td>
<td>7.38 ± 0.02</td>
<td>7.38 ± 0.02</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td>CO₂ (mm Hb)</td>
<td>40.4 ± 0.9</td>
<td>40.4 ± 0.9</td>
<td>40.4 ± 0.9</td>
<td>40.4 ± 0.9</td>
</tr>
<tr>
<td>bicarbonate (mmol/L)</td>
<td>23.2 ± 1.2</td>
<td>23.2 ± 1.2</td>
<td>23.2 ± 1.2</td>
<td>23.2 ± 1.2</td>
</tr>
</tbody>
</table>

* Values are means ± standard errors of the means.

**Part 1** Figure 1. Bar graphs showing water content in percent of wet weight (A) and brain ion contents (B) in the basal ganglia ipsilateral to the site of intracerebral injection of various blood components with five to seven rats per group. The values shown are means ± standard errors of the means. *, sham-treated versus whole blood (water = p < 0.0005, ions = p < 0.0001); †, plasma versus whole blood (water = p < 0.005, ions = p < 0.0001); §, blood cells versus whole blood (water = p < 0.0001, ions = p < 0.0001). The Scheffé F-test was used for comparison.
in the saline-treated controls (hirudin, 79.6% ± 0.3%; saline, 82.4% ± 0.3%). Similar inhibition of edema formation was observed in the ipsilateral cortex and contralateral basal ganglia and cortex with hirudin treatment.

Increases in brain sodium and chloride concentrations corresponded to increases in brain water content in saline-treated rats with intracerebral hematomas. The changes in brain ion content in the local brain tissue surrounding the intracerebral hemorrage were reduced in the hirudin-treated rats as compared to the group receiving saline.

Hematoma Size

The total hemoglobin in the intracerebral hematomas in groups receiving hirudin (six animals) and saline (five animals) was compared to determine the relative volume of the clots (Table 2). No difference in the hemoglobin content of the blood clots between groups was noted (saline, 4.52 ± 0.47 mg; hirudin, 4.13 ± 0.42 mg). Because the hemoglobin concentration in the blood was the same in both groups, the similar total hemoglobin content of the clots indicates that the clot mass was not significantly different in the two groups.

Discussion

Previous studies of intracerebral hemorrhage in the rat characterized the effects of blood on brain water and ion contents as well as cerebral blood flow and blood-brain barrier (BBB) permeability. Brain edema increases progressively in the first 24 hours. The edema remains elevated for 4 to 5 days, then begins to resolve. The BBB is disrupted between 4 and 12 hours, aggravating the rate of edema formation.

Part 1 of this study demonstrated that an intraparenchymal blood clot causes edema surrounding the clot when both plasma and cells are present. Plasma alone and concentrated blood cells alone did not produce significant edema. Thus, edema caused by a whole blood clot might
be due to a chemical response generated by the clot itself or to its mass effect.

Red blood cells are responsible for the majority of the mass effect created by the clot. If mass effect were the principal cause of edema formation, a concentrated blood cell mixture might be expected to cause even more edema. To the contrary, animals receiving concentrated red blood cells did not have more brain water than sham-treated animals, suggesting that at a volume of 50 μL, mass effect from cells did not generate much edema around the hematoma. At this clot volume chemical toxicity must have been primarily responsible for the brain edema. What biochemical reaction in blood might be activated by hemorrhage and, at the same time, require both plasma and blood cells for activation? One such reaction is the coagulation cascade. We know that coagulation does play some role in the edema process because our earlier studies showed that thrombin from an exogenous source causes brain edema to form and that thrombin inhibitors prevent that thrombin-induced edema.14,15

In the second part of this study, blood components with varying degrees of thrombin production were tested. Plasma that is unclotted is thrombin poor. Serum, on the other hand, contains bound and unbound thrombin. In this study serum and plasma did not produce brain edema. Therefore, the amount of active thrombin in autologous serum must have been insufficient to produce brain edema.

The thrombin potential (the sum of the prothrombin and thrombin contents) of blood is greater than the amount required for clotting.24 The total quantity of thrombin that can be produced by whole blood has been shown to be 260 to 360 U/mL of plasma.1,2,24 Only 1 U of thrombin is actually required for a milliliter of blood to clot over a period of 15 seconds.

Prothrombinase is a complex of cofactors that catalyzes the conversion of prothrombin to thrombin.21 The prothrombinase complex is formed from constituents of plasma and cells; activated factor X is from plasma, phospholipids from cell membranes, and calcium from plasma. When prothrombinase is added to plasma and the mixture is injected into brain parenchyma, significant edema forms around the injection site (Fig. 2). Intracerebral injections of calcium and phospholipids, in the absence of activated factor X, do not produce brain edema. Our data from part 2 support the theory that the thrombin released from autologous blood in the brain is largely responsible for edema formation around the clot.

The time course for thrombin production in the brain after intracerebral hemorrhage is not known. Thrombin may be produced continuously over time until the prothrombin supply is depleted. In vitro, prothrombin converts to thrombin over a period of several hours.24 The rate of that reaction might be controlled by the rate of release of phospholipids from blood cell membranes, that is, the rate of thrombin production may be directly related to the rate of cell breakdown. Eventually the amount of thrombin produced may exceed the fixed amount of thrombin inhibitors. Thus brain edema may increase over the first 24 hours after injury because the rate of cell breakdown increases, leading to a higher concentration of phospholipids and more thrombin production. The gradual production of thrombin in blood suggests that a time period for treatment may exist, during which thrombin activity may be inhibited.

The third part of this study shows that animals with intracerebral hematomas treated with hirudin develop less brain edema than animals with hematomas that received an equal amount of intracerebral saline. The difference in brain water content in the basal ganglia between these two groups was approximately 2.8%. This represents a 20% increase in the volume of the basal ganglia, a clinically significant change.

Hirudin, an anticoagulant agent found in leeches, is the most potent and specific thrombin inhibitor.5,16,18 In addition to inhibition of water accumulation, hirudin reduces the brain ion changes that accompany brain edema. The inhibitory effect of hirudin does not appear to be mediated by mass effect. Extracted clots from rats receiving hematomas with or without hirudin had similar hemoglobin contents and, therefore, were of similar volume. The effectiveness of hirudin as an inhibitor of brain edema from intracerebral hematomas provides further evidence that thrombin plays an important role in the pathogenesis of edema resulting from a parenchymal clot.

Thrombin is a serine protease derived from prothrombin. Fibrinogen is converted to fibrin by thrombin. Other clotting factors from the coagulation cascade, such as fibrin, may participate in the formation of brain edema. In addition to coagulation, thrombin has functions related to inflammation and wound healing, including induction of mitosis, chemotaxis of leukocytes, regulation of adhesion molecules, platelet aggregation, and cytokine release.26 Thrombin also has vasoactive properties.2,8,11,28 It causes vasoconstriction in some vessels through a direct effect on vascular smooth-muscle cells or through the release of endothelin. In other vessels thrombin causes vasodilation secondary to the release of endothelium-derived relaxing factor. An additional vascular effect of thrombin involves modulation of endothelial cell permeability.4,9,17 Brain edema due to thrombin may be from a direct opening of the BBB.

There is also evidence that thrombin is involved in vasospasm caused by SAH. The naturally occurring inhibitor, antithrombin III, reverses vasospasm due to SAH in rabbits.26 Heparin accelerates the reaction of thrombin with antithrombin III,23 which might explain reports that delayed ischemic events due to vasospasm are reduced by the addition of heparin.

Thrombin causes inflammation, scar formation, and reactive gliosis in the brain.20 The brain and spinal cord have a large number of thrombin-binding sites.19 Thrombin contributes to cell damage by causing retraction of

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Arterial Blood (g/dL)</th>
<th>Brain Hematoma (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hematoma + saline</td>
<td>13.50 ± 1.44</td>
<td>4.52 ± 0.47</td>
</tr>
<tr>
<td>hematoma + hirudin</td>
<td>13.45 ± 0.29</td>
<td>4.13 ± 0.42</td>
</tr>
</tbody>
</table>

*Values are means ± standard errors of the means for 11 rats.*

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Conclusions

These studies indicate that a parenchymal clot containing both plasma and blood cells is necessary to produce brain edema. Full activation of the coagulation cascade, which requires both plasma and cells, appears to lead to edema formation around the clot. The level of activation required to produce brain edema, however, is probably greater than the level necessary to produce simple clotting. Thus, serum derived from clotted blood does not produce edema. On the other hand, rapid and massive conversion of prothrombin to thrombin in plasma produces significant edema around the clot. The thrombin inhibitor hirudin appears to limit edema production in rats with intracerebral hematomas. Thus, thrombin is directly involved in the pathogenesis of cerebral edema after intracerebral hemorrhage. How thrombin actually causes the formation of brain edema in intracerebral hemorrhage requires more study.

References


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