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The Role of the Coagulation Cascade in Brain Edema Formation after Intracerebral Hemorrhage

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Summary

The coagulation cascade has a potential role in brain edema formation due to intracerebral hemorrhage. In this study blood and other solutions were injected stereotactically into the right basal ganglia in rats. Twenty-four hours following injection, brain water and ion contents were measured to determine the amount of brain edema. Intracerebral blood resulted in an increase in brain water content. The amount of brain edema surrounding the intracerebral hematoma was reduced by a thrombin inhibitor Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperidide, (α-NAPAP) infused into the hematoma after the clot had been allowed to solidify. The inhibitor did not alter the actual size of the clot mass. An artificial clot composed of fibrinogen, thrombin, and styrene microspheres also produced brain edema. A fibrin clot led to edema formation even in the absence of mass effect provided by the microspheres. The single component responsible for production of brain edema in all these models was thrombin. The edema was formed in response to a fibrinogen-independent pathway. These results indicate that the coagulation cascade is involved in brain edema that develops adjacent to an intracerebral hematoma.

Keywords: Brain edema; brain water; coagulation cascade; fibrinogen; Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperidide; α-NAPAP; rat; thrombin.

Introduction

Many patients deteriorate after an intracerebral hemorrhage because of the secondary formation of brain edema. A hematoma in the brain may produce edema through either chemical toxicity or mass effect or both. The hematoma and its associated edema combine to cause focal ischemia and intracranial hypertension.

In order to investigate the injury produced by a hematoma, various animal models have been developed [6–8, 16, 17, 23, 24]. Hematomas have been produced using several techniques, including direct injection of blood into the brain, disruption of an intracerebral artery, and diversion of blood flow from a peripheral artery into the brain through flexible catheters. In addition to blood, other substances have been placed in the brain to mimic the mass effect of a hematoma, including microballoons, silicone, saline, paraffin, and plasma clots.

These studies have implicated several different blood components in the pathogenesis of brain edema from intracerebral hemorrhage. Recent investigations indicate that the clotting factor, thrombin, produces brain edema through chemical toxicity [9]. Conversely, hirudin, a naturally occurring thrombin inhibitor, blocks brain edema formation due to intracerebral hemorrhage [10, 11].

In this study, the effect of a synthetic thrombin inhibitor, Nα-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalaninepiperidide (α-NAPAP), on brain edema from intracerebral hemorrhage was investigated. In addition, an artificial clot produced from thrombin, fibrinogen and synthetic microspheres was developed to serve as a model of brain injury due to intracerebral hemorrhage. This model was used to determine whether the edema response due to thrombin is dependent on the presence of fibrinogen.

Materials and Methods

General Preparation

Seventy-four adult male Sprague Dawley rats (Charles River, Portage, MI) were each anesthetized in a closed chamber with 5% isoflurane. A 16 gauge catheter was inserted orally into the trachea, and the rats were ventilated with a Harvard rodent ventilator using a mixture of room air (oxygen 21%, nitrogen 76–77%) 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 and intracerebral injection. The ventilator rate, tidal volume, and inspired gas concentrations were adjusted to maintain normal blood oxygen (O_2 saturation > 95%) and carbon dioxide tensions (pCO_2 = 35–45 mm Hg). Body temperature was maintained at 37.5 °C using a rectal thermometer and a feedback-controlled heating pad.

Intracerebral Injection

In a stereotactic frame, a 1 mm burr hole was drilled in the skull near the right coronal suture 3 mm lateral to the bregma. Solutions were infused into the right basal ganglia using a Harvard pump (coordinates; 0.2 mm anterior, 6.0 mm ventral, and 3.0 mm lateral to the bregma). The rate of infusion was 10 µl per minute. During the infusion, the brain was observed through the burr hole and no significant back flow of fluid was noted among the animals in this study.

The incisions were closed with suture. The inhalational anesthetic was discontinued and the animals were extubated. The rats were allowed to recover and given food and water.

Animal Groups

Part one. In the first part of the study four groups of animals were examined. Fifty µl of blood or saline were injected through a 26 gauge needle, the needle was removed, and 5 minutes allowed for clotting. A second 26 gauge needle was inserted along the same coordinates and 20 µl of saline or thrombin inhibitor were injected. A sham + saline group (n = 6) received 70 µl of saline. A sham + α-NAPAP group (n = 6) received 50 µl of saline and 20 µl of 0.2 mM Na-(2-Naphthalenesulfonylglycyl)-4-amidino-DL-phenylalanine-piperidide (α-NAPAP), a synthetic thrombin inhibitor (Sigma Chemical Co., St. Louis, MO). A hematoma + saline group (n = 7) received 50 µl of blood and 20 µl of saline. A hematoma + α-NAPAP group (n = 8) received 50 µl of blood and 20 µl of 0.2 mM α-NAPAP.

Part two. In the second part of the study, six groups of animals were examined. Solutions were infused simultaneously through two side by side needles (26 and 30 gauge). A blood clot group (n = 5) received 50 µl of autologous blood and 10 µl of saline simultaneously through the double-needle infusion system. An artificial clot group (n = 6) received a 50 µl injection of 40% (volume/volume) styrene microspheres (Bangs Laboratories, Inc, Carmel, IN) with a mean diameter of 14.6 microns mixed with fibrinogen (Sigma Chemical Co., St. Louis, MO) through one needle in the double-needle system. The fibrinogen concentration was equivalent to the amount found in normal blood (200–250 mg/dL). Ten µl (20 units) of bovine thrombin (Johnson and Johnson Medical Inc, Arlington, TX) was infused simultaneously through the second needle. The quantity of thrombin used was equal to the amount produced from the total conversion of prothrombin to thrombin in 50 µl of whole blood (300–360 units/ml of plasma) [1, 22]. The styrene microspheres were used to simulate the mass effect of red blood cells. The components of the artificial clot were each then studied. A vehicle group (n = 6) received 50 µl of saline and 10 µl of a solution containing 194 mM NaCl, 14 mM $CaCl_2$, 1.2% glycine (weight/volume), and 5% bovine serum albumin (weight/volume) through the double-needle system. The composition of this solution was identical to the additives present in the commercial thrombin formulation used in these experiments. The fibrinogen group (n = 5) received 50 µl of fibrinogen (200–250 mg/dL) in normal saline [19]. The thrombin group (n = 6) received 10 µl of bovine thrombin (20 units). The final group (n = 8) received a double-needle infusion of 50 µl of fibrinogen (250 mg/dL) and 10 µl of bovine thrombin (20 units).

Determination of Brain Water and Ion Contents

The rats were sacrificed by decapitation 24 hours following the intracerebral infusion. The brains were removed. A coronal slice 3 mm from the frontal pole was cut approximately 4 mm thick. This section of brain was divided along the midline. Tissue samples were weighed wet and dry (24 hours at 105 °C) to determine water content. The dehydrated samples were digested in 1 ml of 1 M nitric acid. Sodium and potassium contents were determined by flame photometry (IL943 Automatic Flame Photometer, Instrumentation Laboratory, Inc, Lexington, MA), and chloride content by a chloridometer (Model 442–5000, Haake Buchler Inc, Saddlebrook, NJ). The water and ion contents of the ipsilateral hemispheres were compared.

Hemoglobin Assay

The hemoglobin content of whole blood and intracerebral hematomas was measured spectrophotometrically [5] in animals receiving a 50 µl intracerebral injection of blood + 20 µl of saline (n = 5) or a 50 µl injection of blood + 20 µl of 0.2 mM α-NAPAP (n = 6). 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 an 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 (Sigma Chemical Co, St. Louis, MO) in a cuvette to form cyanmethemoglobin from hemoglobin. The 20 µl samples of whole blood were also mixed with this reagent. The absorbance of cyanmethemoglobin at 540 nm was measured with a spectophotometer. A standard curve was produced using a hemoglobin standard (Sigma Chemical Co, St. Louis, MO).

Statistical Analysis

Differences between groups of rats in water and ion contents were analzyed using the Student's t-test and Dunnett t-test as indicated. A probability value of less than 0.05 was used to indicate a significant difference.

Results

The groups of rats did not differ significantly in any of the measured physiologic parameters. Normal blood gas and blood pressure values were recorded in all groups.

Part One

The importance of the coagulation cascade in brain edema formation was studied directly. Rats with intracerebral hematomas were treated with an injection of α-NAPAP. This thrombin inhibitor was injected into the clot. The injection was made 5 minutes

Table 1. *Clot and Blood Hemoglobin*

	Clot hemoglobin (mg)	Blood hemoglobin (mg/dL)
Hematoma + saline	4.5 ± 0.5	13.5 ± 1.4
Hematoma + α-NAPAP	4.5 ± 0.6	13.5 ± 0.5

Values are mean ± SEM. Comparisons performed using Student's t-test (two-tailed).

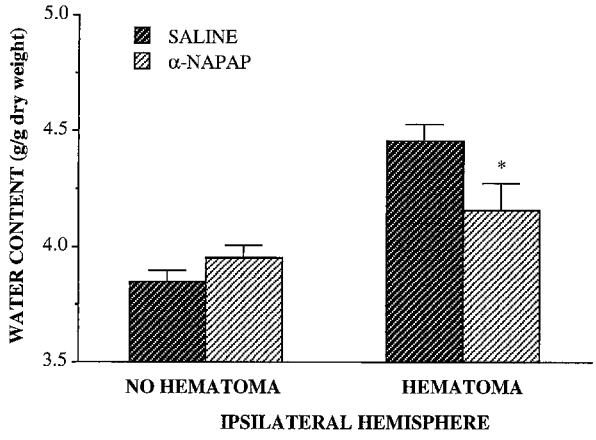

Fig. 1. Water content in the hemisphere ipsilateral to the site of intracerebral injection of saline or α-NAPAP in groups with and without intracerebral hematomas. The values shown are means ± standard errors of the means. *P < 0.005. The Student's t-test (two-tailed) was used for comparison

following placement of the hematoma in order to allow the clot to first solidify.

The total hemoglobin in the intracerebral hematomas in both the group receiving α-NAPAP and the animals receiving saline was compared in order to determine the relative volume of the clots (Table 1). No difference in the hemoglobin content of the blood clots between groups was noted. Since the hemoglobin concentration in the blood was the same in both groups, the similar total hemoglobin content of the

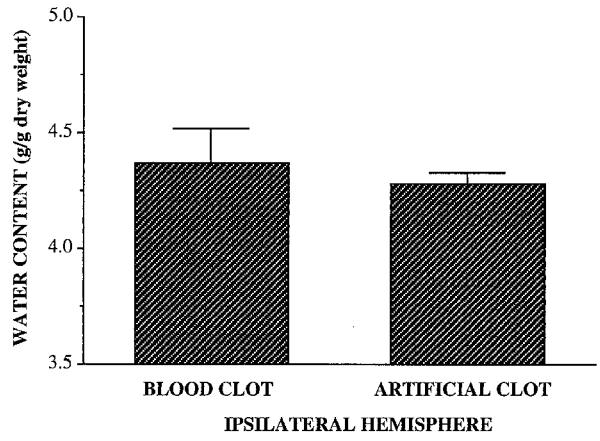

Fig. 2. Water content in the hemisphere ipsilateral to the placement of an intracerebral blood clot or an artificial clot. The artificial clots were composed of styrene microspheres, fibrinogen, and thrombin. The values shown are means ± standard errors of the means. The Student's t-test (two-tailed) was used for comparison

clots indicated that the clot mass was not significantly different in the two groups. This confirmed that the infused volumes were equivalent and that the α-NAPAP did not affect clot size.

Alpha-NAPAP prevented the increase in brain water (Fig. 1) as well as the ion changes (Table 2) in the brains of rats with hematomas. Increases in brain water were accompanied by an increase in sodium and chloride, and a decrease in potassium. In the absence of a hematoma, α-NAPAP had no effect on water or ion content. The coagulation cascade, therefore, appeared to be involved in edema formation with intracerebral blood. Since the clot sizes were equivalent the reduction in brain edema was due to a chemical mechanism, not mass effect.

Part Two

The experiments in part one demonstrate that thrombin in blood produces brain edema. The edema may be either a direct effect of thrombin or may be

Table 2. *Brain Ion Contents in the Ipsilateral Hemisphere*

	Sodium (μEq/g dry wt)	Potassium (μEq/g dry wt)	Chloride (μEq/g dry wt)
Sham + Saline	216 ± 11	457 ± 14	158 ± 9
Sham + α-NAPAP	248 ± 13	433 ± 11	187 ± 12
Hematoma + Saline	469 ± 20	369 ± 14	331 ± 11
Hematoma + α-NAPAP	284 ± 20*	407 ± 14*	213 ± 11*

Values are mean ± SEM. *P < 0.005. Comparisons performed using Student's t-test (two-tailed).

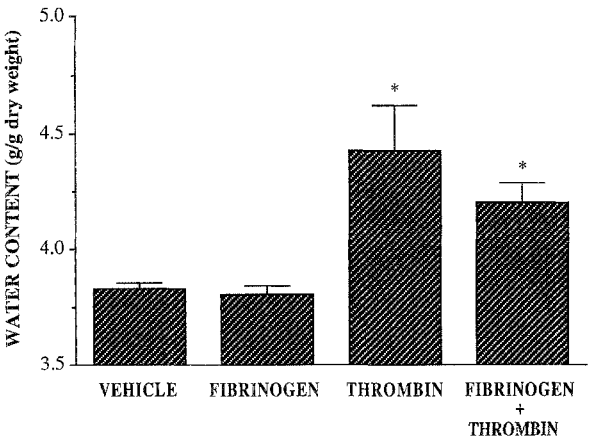

Fig. 3. Water content in the hemisphere ipsilateral to the placement of various factors of the coagulation cascade. The values shown are means ± standard errors of the means. *P < 0.05. The Dunnett t-test (two-tailed) was used for comparison of the treated groups to the vehicle group

due to its interaction with fibrinogen. Fibrinogen either in combination with thrombin or alone may be involved in edema formation.

In order to determine if the edema-effect of thrombin is fibrinogen-dependent or not, an artificial clot was used. The clot contained physiologic amounts of thrombin and fibrinogen and included styrene microspheres to produce a sustained mass effect. The same amount of brain edema was produced in the hemisphere ipsilateral to the site of injection in the group of animals receiving the artificial clot as the group of animals receiving blood clots (Fig. 2). This model, using an artificial clot, simulated the edema-response of blood.

The components of the artificial clot were examined to determine if the edema due to thrombin was dependent on the presence of fibrinogen or not (Fig. 3). Fibrinogen alone did not produce brain edema. On the other hand, thrombin produced a large amount of brain edema when administered alone. Thrombin and fibrinogen together also produced edema, but to no greater degree than thrombin alone. These findings indicate that the edema associated with thrombin is fibrinogen-independent.

An infusion of 10 µl of thrombin produced the same amount of brain edema as the artificial clot containing microspheres, fibrinogen and thrombin (thrombin, 4.42 ± 0.20 versus artificial clot, 4.28 ± 0.04). The edema therefore was totally accounted for by the chemical effect of thrombin. Mass effect did not appear to contribute significantly to the brain edema from the artificial clot.

Discussion

In this study the coagulation cascade was shown to be a source of brain edema. Alpha-NAPAP, a potent and specific inhibitor of thrombin [12, 14, 20], reduced the amount of brain water and ion changes when infused following placement of a bloot clot. Alpha-NAPAP did not influence clot size and therefore did not affect brain edema by a change in mass effect. These results, along with previous studies using hirudin [10, 11], indicate that thrombin is primarily involved in the brain edema that develops around an intracerebral hematoma.

An artificial clot simulating coagulation and mass effect from a blood clot was shown to produce the same amount of brain edema as a hematoma of similar size. The edema formed from this moderate-sized artificial clot in the rat brain was due primarily to chemical toxicity.

Whether the thrombin effect was fibrinogen-dependent or independent was then tested. Thrombin is a serine protease produced from prothrombin [19]. The final common pathway of the coagulation cascade involves the conversion of fibrinogen to fibrin through the enzymatic activity of thrombin. We found that thrombin alone and in combination with fibrinogen produced brain edema. Fibrinogen alone, however, did not cause edema formation. We conclude therefore that thrombin causes brain edema through a fibrinogen-independent pathway.

The precise mechanism by which thrombin produces brain edema is unknown. Studies have shown that the brain and spinal cord have a large number of thrombin binding sites [15]. Thrombin modulates endothelial cell permeability through receptor-mediated pathways and may, as a consequence, affect the blood-brain barrier directly [4, 13]. Brain edema formation then follows. In cerebral arteries, thrombin causes vasoconstriction which may produce ischemia and secondary brain edema formation. Thrombin receptors are also present on neurons and glia which may produce direct cell injury when activated. Thrombin contributes to cell damage by causing retraction of cell processes of neurons and glia [21]. Finally, an inflammatory response due to thrombin has been demonstrated in the brain, which includes induction of mitosis, chemotaxis of leukocytes, production of adhesion molecules, platelet aggregation, and cytokine release [2, 3, 18].

Inhibition of thrombin activity in a cerebral hematoma limits brain edema formation around the clot.

The artificial blood clot produced from thrombin, fibrinogen, and synthetic microspheres simulates the edema formation due to a hematoma, and serves as a reliable model for the mass effect and chemical toxicity of blood. Study of the components of the clot shows that the edema effect of thrombin is fibrinogen-independent. The current study supports the concept that the coagulation cascade in general and thrombin specifically play a key role in edema formation resulting from intracerebral hemorrhage. Identification of the precise mechanisms by which thrombin causes the formation of brain edema will require further study.

Acknowledgement

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References

1. Arand AG, Sawaya R (1986) Intraoperative chemical hemostasis in neurosurgery. Neurosurgery 18: 223–233
2. Bar-Shavit R, Benezra M, Sabbah V, Bode W, Vlodavsky I (1992) Thrombin as a multifunctional protein: induction of cell adhesion and proliferation. Am J Respir Cell Mol Biol 6: 123–130
3. Carney DH, Redin W, McCroskey L (1992) Role of high-affinity thrombin receptors in postclotting cellular effects of thrombin. Semin Thromb Hemost 18: 91–103
4. DeMichele MA, Minnear FL (1992) Modulation of vascular endothelial permeability by thrombin. Semin Thromb Hemost 18: 287–295
5. Drabkin DL, Austin JH (1935) Spectrophotometric studies. II. Preparations from washed blood cells; nitric oxide hemoglobin and sulfhemoglobin. J Biol Chem 112: 51
6. Kaufman HH, Pruessner JL, Bernstein DP, Borit A, Ostrow PT, Cahall DL (1985) A rabbit model of intracerebral hematoma. Acta Neuropathol 65: 318–321
7. Kaufman HH, Schochet S, Koss W, Herschberger JE, Bernstein D (1987) Efficacy and safety of tissue plasminogen activator. Neurosurgery 20: 403–407
8. Kingman TA, Mendelow AD, Graham DI, Teasdale GM (1988) Experimental intracerebral mass: description of model, intracranial pressure changes and neuropathology. J Neuropathol Exp Neurol 48: 128–137
9. Lee KR, Betz AL, Keep RF, Chenevert TL, Kim S, Hoff JT (1995) Intracerebral infusion of thrombin as a cause of brain edema. J Neurosurg 83: 1045–1050
10. Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT (1996) Edema from intracerebral hemorrhage: the role of thrombin. J Neurosurg: 84: 91–96
11. Lee KR, Keep RF, Betz AL, Hoff JT (1994) A thrombin antagonist, hirudin, inhibits brain edema formation in a model of intracerebral hemorrhage. In: Nagai H, Kamiya K, Ishii S (eds) Intracranial pressure IX. Springer, Berlin Heidelberg New York Tokyo, pp 70–75
12. Maffrand JP (1992) Direct thrombin inhibitors. Nouv Rev Fr Hematol 34: 405–419
13. Malik AB, Fenton JW (1992) Thrombin-mediated increase in vascular endothelial permeability. Semin Thromb Hemost 18: 193–199
14. Markwardt F (1988) Pharmacology of selective thrombin inhibitors. Nouv Rev Fr Hematol 30: 161–165
15. Mckiney M, Snider RM, Richelson E (1983) Thrombin binding to human brain and spinal cord. Mayo Clin Proc 58: 829–831
16. Mendelow AD, Bullock R, Nath FP, Jenkins A, Kingman T, Teasdale GM (1986) Experimental intracerebral hemorrhage: intracranial pressure changes and cerebral blood flow. In: Miller JD, Teasdale GM, Rowan JO (eds) Intracranial pressure VI. Springer, Berlin Heidelberg New York Tokyo, pp 515–520
17. Nehls DG, Mendelow AD, Graham DI, Sinar EJ, Teasdale GM (1988) Experimental intracerebral hemorrhage: progression of hemodynamic changes after production of a spontaneous mass lesion. Neurosurgery 23: 439–444
18. Nishino A, Suzuki M, Ohtani H, Motohashi O, Umezawa K, Nagura H, Yoshimoto T (1993) Thrombin may contribute to the pathophysiology of central nervous system injury. J Neurotrauma 10: 167–179
19. Seegers WH (1962) Prothrombin. Harvard University Press, Cambridge
20. Svendsen L, Brogli M, Lindeberg G, Stocker K (1984) Differentiation of thrombin- and factor Xa-related amidolytic activity in plasma by means of a synthetic thrombin inhibitor. Thromb Res 34: 457–462
21. Vaughan PJ, Cunningham DD (1993) Regulation of protease nexin-1 synthesis and secretion in cultured brain cells by injury-related factors. J Biol Chem 268: 3720–3727
22. Wagner RH, Graham JB, Penick GD, Brinkhous KM (1955) Estimation of prothrombin by the two-stage method. In: Tocantins LM, Kazal LA (eds) Blood coagulation, hemorrhage, and thrombosis methods of study. Grune and Stratton, New York, pp 159–164
23. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT (1993) Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. J Neurosurg 81: 93–102
24. Yang GY, Betz AL, Hoff JT (1994) The effects of blood or plasma clot on brain edema in the rat with intracerebral hemorrhage. Acta Neurochir (Wien) [Suppl 60]: 555–557

Comments

This is another experimental work from a group of investigators that made relevant contributions to our understanding of the factors leading to brain oedema development following spontaneous intracerebral haemorrhage.

They assume that brain oedema is not only related to the local mass effect (dynamic forces) caused by the clot, but also to the release of clot-derived toxic factors which spread into the adjacent brain tissue. In the present work they analysed the possible role played by the coagulation cascade in the formation of brain oedema. From a series of well-planned experiments they conclude that thrombin is the single component responsible for oedema formation. This action is through a fibrinogen-independent pathway. They have also shown that a specific thrombin inhibitor (alpha-NAPAP) significantly reduces the amount of water and ion changes in the surrounding brain tissue. Though the intimate mechanism by

which thrombin causes brain oedema remains unknown it is clear that the present findings are of clinical significance.

R. D. Lobato

The information that thrombin is responsible for brain oedema around an intracerebral haemorrhage is important. It brings the reader to several comments as:

– If thrombin affects the blood-brain barrier, this effect could be evaluated using the familiar blood-brain barrier indicators.

– Neurosurgeons currently use thrombin with fibrinogen as a gel in a wound cavity in order to prevent a postoperative bleeding. Is this an appropriate procedure? What is the smallest quantity of thrombin, sufficient to start the coagulation cascade, but not enough to produce brain oedema?

This would require dose-effect studies for thrombin.

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