

The investigation of vasospastic effect of hemostatic matrix used in intracranial operations on cerebral arteries

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Abstract

Aim: We aimed to investigate the vasospastic effect of thrombin-based hemostatic matrix on the basilar arteries in rats.

Material and Methods: A total of 28 female albino Wistar rats were used in the study. The rats were randomly assigned to four groups. The rats in group I (control group) were sacrificed without surgical manipulation. We injected 0.1 mL of nonheparinized autologous arterial blood into the cisterna magna of the rats in group II, 0.1 mL of hemostatic matrix into the cisterna magna of the rats in group III, and 0.1 mL of a mixture of nonheparinized autologous arterial blood and the hemostatic matrix into the cisterna magna of the rats in group IV. The experimental rats were sacrificed 48 hours after injections. Three sections were obtained from each rat's basilar arteries and photographed under a light microscope. Basilar artery cross-section areas were measured using computerized image analysis systems.

Results: Mean basilar artery cross-sections of all groups revealed statistically a significant difference between only group I and group II. The mean basilar artery cross-section areas of groups II, III, and IV decreased by 36%, 19%, and 22%, respectively, in comparison with those of group I.

Conclusion: Although our results pertaining to thrombin-based hemostatic matrix were not statistically significant, it is hypothesized that this matrix has a vasospastic effect on arteries. Therefore, it is important to remember that when such a matrix is used in cranial surgery, the thrombin contained therein could have a vasospastic effect on cerebral arteries.

Keywords: Cerebral vasospasm; hemostatic matrix; thrombin; rat; subarachnoid hemorrhage.

INTRODUCTION

Cerebral vasospasm (CVS) is an extended and reversible arterial constriction that develops after subarachnoid hemorrhage (SAH), traumatic brain damage, intraventricular hemorrhages, meningeal infections, and craniotomy procedures (1-4). Mortality and morbidity attributed to CVS are often caused by decreased perfusion of affected arteries (5). Although the pathogenesis of CVS is still controversial, it is thought to be linked to extravasated platelet and erythrocyte lysis products, along with inflammatory mediators, in the subarachnoid space (6).

Thrombin (activated coagulation factor II) is a protein

that has many effects in the coagulation cascade. A large amount of thrombin is produced during SAH. In addition, the well-known potent spasmogenic effect of thrombin causes CVS (7). In current surgical practice, thrombin-based hemostatic matrix agents are widely used for the purpose of hemostasis. In particular, they are used not only in neurosurgery (8) but also in urologic (9), cardiothoracic (10), endoscopic (11), and gynecological procedures (12). When used in neurosurgery, hemostatic matrix agents may cause cerebral arterial CVS because of their high thrombin content. There is a gap in literature regarding the effects of hemostatic matrix agents on CVS after intracranial surgery. In this study, we aimed to investigate the vasospastic effect of thrombin-containing hemostatic matrix on the basilar arteries in rats.

Received: 24.05.2019 Accepted: 07.08.2019 Available online: 09.08.2019

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MATERIAL and METHODS

The experiment was performed in accordance with institutional guidelines for the care and use of laboratory animals, and our University Hospital Animal Research Ethics Committee approved all protocols.

In total, 28 adult male albino Wistar rats, weighting 250-300 g, were used the study. To determine groups and numbers, we followed the guidelines of the "Experimental Design" page at the website of the National Center for the Replacement, Refinement & Reduction of Animals in Research (<http://www.nc3rs.org.uk/experimental-design>).

Animal groups

Rats were randomly assigned to four groups. Those in group I (control; n=7) underwent no procedure; those in group II (SAH group; n=7) received only SAH-inducing material; those in group III (hemostatic matrix group; n=7) received only thrombin-based hemostatic matrix; and those in group IV (SAH + hemostatic matrix group; n=7) received an equal mixture of SAH-inducing material and hemostatic matrix. All animals were kept at the same environmental temperature (22 °C) and humidity. Throughout the experiment, the rats were allowed free access to food and water.

Experimental model of subarachnoid hemorrhage

The rats were initially anesthetized with intraperitoneal injections of ketamine hydrochloride (60 mg/kg) and xylazine (10 mg/kg); spontaneous respiration continued throughout the procedures. In addition, all rats were injected with 50 mg/kg cefazolin sodium for preoperative antibiotic prophylaxis.

Experimental SAH was induced according to a modified model of single injection in rats (13). Under sterile conditions, a linear incision was made, the posterior cervical muscles were dissected, and the atlantooccipital membrane was exposed. Under microscopic view, the atlantooccipital membrane was opened and the dura mater was located (Figure 1). A needle was inserted into the cisterna magna, and 0.1 mL of cerebrospinal fluid was withdrawn. For rats in group II, the same amount of fresh nonheparinized autologous blood obtained from the tail artery was injected into subarachnoid space. For rats in group III, the same amount of thrombin-based hemostatic matrix (FLOSEAL; Baxter Healthcare Corporation, Deerfield, Ill.) was injected into the subarachnoid space. For rats in group IV, a mixture of equal amounts (0.1 mL) of the fresh nonheparinized autologous blood obtained from the tail artery and thrombin-based hemostatic matrix was injected into subarachnoid space to simulate a real operative site. The rats were euthanized 48 hours after the injections and were transcardially perfused first with 50 mL of phosphate-buffered saline and then with 100 mL of

4% paraformaldehyde to fix the basilar arteries at their in situ diameters.

Histopathologic examination

All tissue specimens were kept for 10 days in containers that contained 10% formaldehyde solution. The tissue specimens were embedded in paraffin blocks and cut into 0.5- μ m sections. Then the specimens were stained with hematoxylin and eosin. Three sections were taken from the proximal, middle, and distal portions of all basilar arteries. The basilar artery sections were evaluated and digitally photographed by an examiner (unaware of experimental condition) under a light microscope (Eclipse Cli; Nikon, Minato, Tokyo). A cross-sectional area (CSA) of each basilar artery was measured with NIS Elements 04.00 (Nikon) software. Means of all three sections' measurements were calculated for all specimens.

Statistical evaluation

One-way analysis of variance was performed to compare the mean basilar artery CSAs of the groups. For dual comparison of mean basilar artery CSAs the groups, Tukey's honest significant difference test was used. A p level of less than 0.05 was accepted as level of significance. Statistical Analysis and Service Solutions (SPSS) version 22.0 was used for statistical analysis.

RESULTS

Macroscopic inspection of the harvested brains from groups II and IV revealed diffuse SAH in the basal cisterns, confirming the adequate subarachnoid distribution of blood from the cisterna magna injection site (Figure 1).

Figure 2 is the microscopic view of each group's basilar artery cross-sections stained with hematoxylin and eosin. One-way analysis of variance for comparing the mean basilar artery CSAs of the groups yielded statistically significant results ($p = 0.014$). Table 1 shows mean values and rates of decrease in basilar artery CSAs. The mean basilar artery CSAs for group I and group II were 10.980.86 μ m² and 7014.43 μ m², respectively. The difference between these two groups was statistically significant ($p=0.008$). The mean basilar artery CSA in group II was 36% smaller that of group I.

The mean basilar artery CSA for group III was 8868.29 μ m², which was 19% smaller than that of group I; however, the difference between these CSAs was not statistically significant ($p=0.252$). Table 2 shows the results of Tukey's honest significant difference test. The mean basilar artery CSA for group IV) was 8568.86 μ m², which was 22% smaller than that of group I; however, the difference between these two groups was statistically insignificant ($p=0.158$). The difference between the mean basilar artery CSAs for groups II (7014.43 μ m²) and IV (8568.86 μ m²) was statistically insignificant ($p=0.509$). The difference between the mean basilar artery CSAs for groups III (8868.29 μ m²) and IV (8568.86 μ m²) was statistically insignificant ($p=0.993$). The difference between the mean basilar artery CSAs for groups III (8868.29 μ m²) and II (7014.43 μ m²) was statistically insignificant ($p=0.359$).

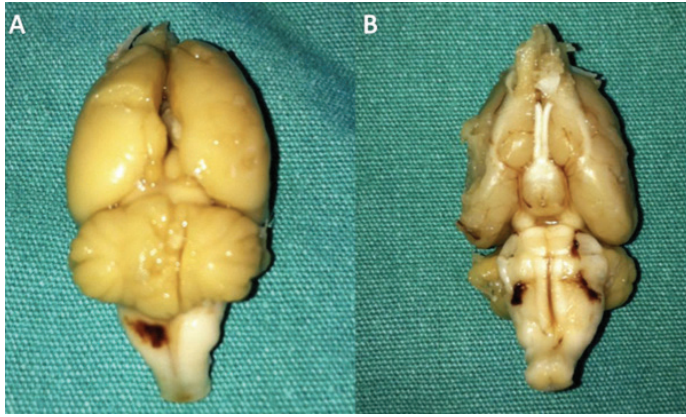


Figure 1. Blood clots in the subarachnoid space (A) Ventral surface of the rat brain. (B) Dorsal surface of the rat brain

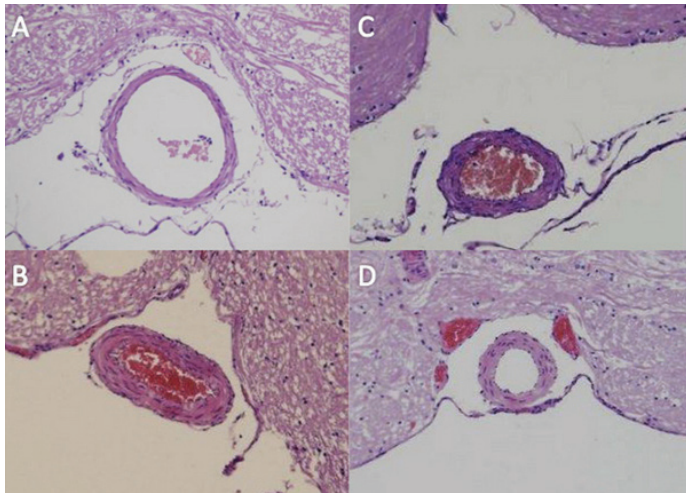


Figure 2. Microscopic views of each group basilar artery cross-sections stained with Hematoxylin & Eosin. (A) Group 1: Control group. (B) Group 2: having only SAH. (C) Group 3: Only thrombin-based hemostatic matrix administered group. (D) Group 4: Equal mixture of the SAH + Hemostatic matrix administered group

Groups	Mean \pm SD (μm^2)	Decrease (frequency; %)	Minimum	Maximum
Control	10980.86 \pm 1764	-	8631	13563
SAH	7014.43 \pm 1404	36	5252	9238
FloSeal®	8868.29 \pm 1057	19	6581	11325
FloSeal® + SAH	8568.86 \pm 2999	22	2685	11416

SAH; subarachnoid hemorrhage SD; standard deviation

Comparison of the Groups		Significance
Group 1	Group 2	.008
	Group 3	.252
	Group 4	.158
Group 2	Group 1	.008
	Group 3	.359
	Group 4	.509
Group 3	Group 1	.252
	Group 2	.359
	Group 4	.993
Group 4	Group 1	.158
	Group 2	.509
	Group 3	.993

DISCUSSION

The thrombin-based hemostatic matrix is commonly used for various surgical interventions. The risk of CVS must be taken into account, especially in cranial surgery. This study demonstrated that thrombin-based hemostatic matrix causes a statistically insignificant degree of CVS.

CVS is defined as a reversible, self-limited narrowing of the intracranial arteries several days after a subarachnoid hemorrhage. In addition, CVS may occur after traumatic brain damage, intraventricular hemorrhages, meningeal infections, and craniotomy procedures (1-4) It is associated with clinical worsening as a result of delayed cerebral ischemia and can affect up to 40% of patients with subarachnoid hemorrhage (14-16). In addition, CVS may occur without angiographically confirmed narrowing (16).

CVS is hypothesized to be caused by spasmogenic agents that arise from subarachnoid blood degradation (17). This hypothesis is supported by findings in the literature of clot removal and cisternal irrigation (18). Active thrombin is normally present in immeasurable amounts in cerebrospinal fluid. It reaches very high levels after SAH, and the level is strongly correlated with the severity of vasospasm (19); thus thrombin is regarded as a potent spasmogenic enzyme (7). A recent study of the effect of thrombin on cerebral arteries in rats with experimental SAH revealed that thrombin is an independent factor in the development of CVS (20).

Thrombin-based FLOSEAL Hemostatic Matrix is a new-generation topical hemostatic agent that is obtained by combining a high dose (2500 IU) of human thrombin and gelatinized bovine collagen. It has proved effective in stopping bleeding and shortening operation duration, thereby lessening damage to surrounding neural tissue in cranial and spinal operations (21-23)

Large numbers of rats can be used in studies, are easy to handle, and are inexpensive (24). Considering these rationales, we used rats in this study. With regard to endovascular vessel perforation and experimental models of SAH involving single and double injection into the cisterna magna, the mortality rate is lower in the single-injection model (0%) than in the double-injection model (9%) or in endovascular perforation (56.7%) in studies previously performed on rats (25). In our study, in which the single-injection method was used, the mortality rate was 0%. In other studies, vasospasm was most severe 48 hours after development of experimental SAH in rats (24). Therefore, the rats were sacrificed 48 hours after the induction of SAH, at the time of maximal vasospasm. It was showed that the most appropriate clinically correlated method of quantifying vasospasm on rats is basilar artery CSA measurement. In view of the aforementioned literature, we evaluated the severity of CVS by measuring basilar artery CSA.

The statistically significant difference between the mean basilar artery CSAs in group I and group II (36%; p=0.008)

verifies the validity of our experimental SAH model. The 19% difference between the mean basilar artery CSAs in groups I and III was not statistically significant; the reason for the decrease might be explained by the vasospastic effect of thrombin. The amount of thrombin in CSF after SAH was reported to be closely related to the incidence and severity of vasospasm (26,27). The difference between the mean basilar artery CSAs of groups II and III was also statistically insignificant. However, the difference between the mean basilar artery CSAs of groups I and III was 19%, which indicates that vasospasm is a process that does not depend solely on thrombin; inflammatory mediators and blood lysis products also play a role. Likewise, the difference between the mean basilar artery CSAs of groups III and IV was statistically insignificant, but the difference between basilar artery CSAs of groups I and IV was 22%. This result demonstrates that the vasospastic effect is stronger under conditions that best resemble the operative site, in which thrombin-based hemostatic matrix is present with blood, in comparison with thrombin-based hemostatic matrix injection alone.

Limitations of this study include a small sample size and the use of animals rather than human subjects. Clinical trials and long-term animal studies with higher numbers of "subjects" would distinguish the indications and contraindications of hemostatic agents more precisely manner and demonstrate side effects more clearly.

CONCLUSION

Although the results pertaining to thrombin-based hemostatic matrix were not statistically significant, the matrix does have a vasospastic effect on rat basilar arteries. It is therefore important to remember that when this matrix is used in cranial surgery, the thrombin contained therein could have a vasospastic effect on cerebral arteries.

Acknowledgement

English language editing of this article was partly supported by Turkish Neurosurgical Society.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports

Ethical approval: Dokuz Eylul University Faculty of Medicine Experimental Animals Local Ethics Committee No: 70/2013

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