Experimental Vasospasm Produced Without Blood Cell Components

—Hypothesis for the Development of Cerebral Vasospasm—

Mitsuru HONDA and Hideo TERAO

First Department of Neurosurgery, Toho University School of Medicine, Tokyo

Abstract

A canine model of cerebral vasospasm using noncellular blood material (fibrin glue) was designed to investigate the effect of cerebrospinal fluid obstruction. The arachnoid membrane covering the cerebral arteries in the basal cistern was dissected and fibrin glue was applied to the adventitial surface of the arteries in three groups of animals. In Group 1, the arachnoid membrane was extensively dissected and fibrin glue was widely applied to the cerebral arteries. In Group 2, the dissection and coating was less extensive. Group 3 was a control group in which the arachnoid membrane was dissected but fibrin glue was not applied. Cerebral angiography 1 week later clearly demonstrated vasospasm in all six dogs in Group 1 and in four of six dogs in Group 2. Vasospasm did not occur in Group 3. The dogs were sacrificed and the arteries in the basal cistern were removed. Histological investigation showed typical findings of vasospasm and inertness of fibrin glue to the tissue. Cerebral vasospasm can be induced by a noncellular material from the blood densely applied to the arterial surface suggesting that obstruction of cerebrospinal fluid circulation around the artery may be important in the development of cerebral vasospasm.

Key words: vasospasm, canine cerebral artery, fibrin glue, cerebrospinal fluid circulation

Introduction

Cerebral vasospasm is one of the major complications following subarachnoid hemorrhage, but its pathogenesis is not clearly understood. Cerebral vasospasm is a peculiar phenomenon that only occurs in the cerebral arteries which lack vasa vasorum. Postangiographic bleeding from the femoral artery or carotid artery is a frequent occurrence in neurosurgical practice, but vasospasm of these arteries rarely develops. Therefore, the microstructural peculiarity of the cerebral arteries and/or a function of cerebrospinal fluid (CSF) is most probably crucial in the development of vasospasm.

Electron microscopy of the feline basilar artery revealed that the adventitia of the basilar artery, unlike systemic arteries, has a distinct adventitial cell layer on the external surface, with many small stomata on the surface and a wide labyrinthine space beneath the cell lining. Rapid diffusion of CSF and high molecular weight substances was found to occur through the adventitial structures, suggesting that the CSF is important in the nutrition of these arteries, and disturbance of local CSF circulation may be involved in some pathological processes such as cerebral vasospasm. Experimental subarachnoid hemorrhage caused the adventitial surface of the feline basilar artery with stomata to be densely covered by the debris of blood corpuscles, resulting in severely inhibited CSF diffusion into the vascular wall associated with vasospasm.

Various studies have attempted to identify a chemical substance responsible for vasospasm without convincing results. However, noncellular components of whole blood can also induce cerebral vasospasm. Experimental vasospasm in the canine basilar artery was caused by bead-stabilized plasma injectate, due to the inflammatory process resulting from the beads of the foreign body. The plasma clot in the basal cistern was easily pushed out from the cistern by the CSF circulation. A study involving a control group of dogs with fibrin in the basal cisterns showed no vasospasm, although the fibrin clot was usually dissolved within 1 to 2 days.

This study produced an experimental model of cerebral vasospasm in the dog by disturbing the CSF...
circulation around the segments of the anterior and middle cerebral arteries in the basal cisterns using fibrin glue which has no blood cell components.

Materials and Methods

Twenty mongrel dogs weighing 7 to 10 kg were anesthetized with intravenous sodium pentobarbital (25 mg/kg). Oral intubation and artificial ventilation with room air was instituted. The arterial blood gas level was monitored to prevent variations in PCO₂. The brachial artery was exposed aseptically, and a cerebral angiography catheter was inserted through the brachial artery. The catheter was connected to an injector set to deliver 20 ml of contrast medium over 1.3 seconds. Preoperative angiography was performed for comparison with subsequent postoperative angiograms. Serial angiograms (Angioskop A33, Polydoros 100; Siemens, Erlangen, Germany) in the anteroposterior projection were taken at one exposure per second for 5 successive seconds at 70 kV and 3.06 mA.

An aseptic craniectomy was performed in the right frontotemporal area. The frontal lobe was elevated and the basal cistern was opened under the surgical microscope. The arachnoid membrane was meticulously dissected along the proximal segment of the anterior cerebral artery and middle cerebral artery. Fibrin glue (fibrin tissue adhesive Beriplast® P; Behringwerke, Marburg, Germany) (2 ml) was applied on and around the arteries. Eighteen dogs were divided into three groups of six animals each. In Group 1, the arachnoid membranes on the internal carotid artery, A1 segment of the anterior cerebral artery, and M1 segment of the middle cerebral artery were dissected extensively, and fibrin glue was applied to the stripped arteries. In Group 2, the arachnoid membrane dissection was confined to the internal carotid artery and A1 portion, and fibrin glue was applied to the stripped portion of the arteries. In Group 3 (the control group), the arachnoid membranes on the internal carotid artery and A1 and M1 portions were dissected extensively, but fibrin glue was not applied. The remaining two dogs were used to investigate the reaction of a systemic artery to applied fibrin glue. The right common carotid artery was exposed and 4 ml of fibrin glue was applied around the artery. The dogs were allowed to recover in a sanitary environment for 7 days.

Seven days later, postoperative cerebral and/or cervical carotid angiography was carried out with the same technique and method as described above. To quantify the degree of vasospasm, the angiograms were magnified and the images were recorded and digitized using a video transfer system coupled with a personal computer and an image analyzer. Vasospasm was defined as a decrease of 20% or more of the mean arterial cross section compared to the preoperative angiograms. Immediately after angiography, 5 ml of 10% diluted India ink was injected into the cisterna magna and the dogs were kept in the head down position for 30 minutes to investigate the patency of CSF circulation. Transcarotid perfusion of 10% formaldehyde at the physiological mean arterial pressure was used for sacrifice and fixation. The brain was removed carefully.

The presence of fibrin glue remaining in a gelatiniform at the original site was confirmed. The glue was then removed from the arteries and brain surface. India ink staining of the cerebral surface and cisterns was examined macroscopically. The large cerebrobasal arteries were dissected free of the brain and processed for light microscopic examination under the surgical microscope. The specimens were embedded in paraffin. Sections approximately 6 μm thick were cut on a microtome and stained with HE and Weigert’s method. The presence and degree of cerebral vasospasm is difficult to verify in a postmortem study, so morphometric analysis was employed to evaluate the degree of vasospasm. The photomicrographs were magnified (×50-260) and the images were recorded and digitized using a video transfer system coupled with a personal computer and an image analyzer. The cross-sectional area of the lumen and vessel wall were calculated by computer. The contralateral A1 portion of the same size on preoperative angiography was used as a control.

Results

Cerebral angiography showed that all six dogs in Group 1 had obvious narrowing of the A1 portion of the anterior cerebral artery (Fig. 1). Four of six dogs in Group 2 showed narrowing of the A1 portion. All dogs in Groups 1 and 2 showed no narrowing of the M1 and internal carotid artery. In contrast, no arterial narrowing was observed in six Group 3 and the two cervical common carotid artery group animals. Carotid angiography in the two dogs in which fibrin glue was applied on the cervical common carotid artery showed no vasospastic change or narrowing of the carotid artery (Fig. 2).

Macroscopic inspection of the brain in Groups 1 and 2 found the India ink staining was obviously less on the side exposed to the fibrin glue than on the contralateral intact side (Fig. 3). Histological examination of the arterial wall with angiographically confirmed vasospasm revealed the characteristic histo-
logical findings of vasospasm: intimal corrugation, thickening of medial smooth muscle layer, and increased collagen fibers in the adventitia (Fig. 4). The histological findings of vasospasm in our experimental model was same as that of post-subarachnoid hemorrhage. India ink was hardly visible in the periarterial space covered with fibrin glue in Groups 1 and 2 (Fig. 5 left). In contrast, the perivascular

Fig. 1 Cerebral artery angiograms, before (left) and 1 week after (right) applying fibrin glue around the right anterior cerebral artery in a Group 1 animal, showing resultant luminal narrowing of the right anterior cerebral artery (arrowheads).

Fig. 2 Cervical common carotid artery angiograms, before (left) and 1 week after (right) applying fibrin glue around a portion of the carotid artery, showing no resultant luminal narrowing of the carotid artery (arrows).

Fig. 3 Photograph of the ventral aspect of a typical canine brain from Groups 1 and 2, 1 week after fibrin glue application around the right anterior cerebral artery, showing India ink staining at the cerebral base on the right side was obviously less dense than on the left side. The fibrin glue was carefully removed before photography.

Fig. 4 Photomicrographs of the anterior cerebral artery, on the left without fibrin glue (left) and on the right with fibrin glue (right), showing intimal corrugation, thickening of the medial smooth muscle layer, and the increase of collagen fibers in the adventitia on the right. Weigert's stain, original magnification ×50.
space of the A1 portion of the contralateral hemisphere was darkly stained with India ink (Fig. 5 center). In the control group, the perivascular space of the A1 portion was also darkly stained with India ink (Fig. 5 right). Infiltration of inflammatory cells around the cerebral artery was meager in all groups. In addition, no histological changes suggesting vasospasm were found in the cervical carotid artery from the two dogs with fibrin glue applied to the common carotid artery showing the biological inertness of fibrin glue to the tissue (Fig. 6). In the cerebral parenchyma no histological changes were found (Fig. 7).

Morphometric analysis of the luminal and mural area of the vasospastic vessels of the A1 portion in Groups 1 and 2 showed that the luminal space of the vasospastic arteries was significantly smaller than that of the control vessels \( (p < 0.01) \), and the wall

Fig. 5 Photomicrographs showing absence of India ink staining around an artery with fibrin glue (left), dark India ink staining (arrows) around an artery without fibrin glue (center), and dark India ink staining around an artery without fibrin glue (right). \textit{left}: Weigert's stain, \textit{center}: HE stain, \textit{right}: Weigert's stain, original magnification \( \times 260 \).

Fig. 6 Photomicrographs of cervical common carotid arteries, without fibrin glue (left) and with fibrin glue (right), showing no histological changes suggesting vasospasm. \textbf{Arrows}: vasa vasorum in adventitia, \textbf{arrowheads}: fibrin glue. Weigert's stain, original magnification \( \times 50 \).

Fig. 7 Photomicrograph of the cerebral parenchyma, on the vasospastic side of right hemisphere, showing no histological changes and India ink staining (arrows) on the brain surface. HE stain, original magnification \( \times 125 \).
area of the vasospastic arteries was significantly greater than that of the control arteries (p < 0.005) (Fig. 8).

**Discussion**

Fibrin glue has many components: fibrinogen, factor XIII, aprotinin, thrombin, calcium, albumin, L-arginine hydrochloride, L-isoleucine, and L-sodium glutamate. Fibrin glue causes no foreign body reaction or vasospasm when applied around the systemic arteries such as the mesenteric artery, as confirmed using the cervical common carotid artery in our study. Fibrin glue in our experimental model rapidly became a tight clot adhered to the adventitia of the artery, and remained as a gelatiniform in the perivascular space after 7 days. This dense application of fibrin glue on the arterial adventitia was expected to prohibit contact between the arterial wall and the CSF. The India ink staining study showed that the adventitia of the arteries on which fibrin glue was applied and vasospasm induced were less stained compared with the control arteries. Vasospasm occurred only in the A1 portion in our experimental dogs. The A1 portion runs in the basal cistern, whereas the M1 portion is firmly attached to the brain surface by perforators of the M1 portion, or is partly embedded in the cerebral parenchyma (Fig. 9). This anatomical peculiarity could explain why blockade of the CSF circulation was more complete in the A1 portion.

Electron microscopy shows wide networks or pathways from the subarachnoid space to the subendothelial layer in the wall of cerebral arteries that lack vasa vasorum and transendothelial retrograde transport function has been verified in normal cerebral arterial walls. The intima and inner third of the media layer imbibe plasma constituents constantly, whereas the outer layers, depending on the thickness of the vessel, are nourished by a CSF transport system. Therefore, it is reasonable to assume that subarachnoid hemorrhage and consequent obstruction of the CSF pathway will cause metabolic derangement in the cerebral artery due to disturbance of nutrient influx and waste efflux in the arterial wall. Increased permeability of the endothelium and entrance of plasma into the subendothelial layer of the constricted basilar artery have been observed. This increased permeability may also contribute to the metabolic derangement of the cerebral arteries. The energy sources of the large pial artery, glycogen, adenosine triphosphate, etc., are significantly decreased during cerebral vasospasm, suggesting an intramural metabolic derangement. A change of O2 content in the vasospastic wall has not yet been reported, but disturbances of CSF circulation must deprive the arterial wall of oxygen and facilitate metabolic derangement. Cerebral arteries are also more sensitive to hypoxia than systemic arteries.

The present study demonstrated that vasospasm can be produced without blood cell components by disturbing the contact of the arterial wall with the CSF circulation. Our tentative conclusion is that disturbance of CSF circulation surrounding the basal cerebral artery is the crucial link in the development...
of cerebral vasospasm. Many neurosurgeons have the clinical impression that cerebral vasospasm is likely to occur in patients with a large amount of blood clot in the basal cistern causing severe disturbance to the CSF circulation. There is a close association reported between vasospasm and the amount of blood in the subarachnoid space. Therefore, a promising way to prevent and treat cerebral blood in the subarachnoid space. Therefore, a promising way to prevent and treat cerebral vasospasm is restoration of the CSF circulation in the basal cisterns and around the cerebral artery by vasospasm.

References

16) Zervas NT, Liszczak TM, Mayberg MR, Black PM: Cerebrospinal fluid may nourish cerebral vessels through pathways in the adventitia that may be analogous to systemic vasa vasorum. J Neurosurg 56: 475-481, 1982

Commentary

This fascinating paper raises important new questions about the pathogenesis of vasospasm. An enormous amount of research in this field has concentrated largely on determining the nature of the elusive "breakdown product" or products of blood which initiate the cascade of events that finally lead to arterial narrowing several days after a subarachnoid hemorrhage. As pointed out, it has long been recognized, following the work of Fisher and others, that the risk of delayed vasospasm is related to the volume of subarachnoid blood visible on an early CT scan. This has always been assumed to mean that greater amounts of a chemical mediator are produced from a larger volume of blood; it could equally be the case that a larger volume of blood means more pronounced encasement of the adventitia of the arteries. In a similar way, more blood means more obstruction to the pathways of CSF absorption and thus a greater risk of communicating hydrocephalus.

The experimental design appears valid, and with the results presented it would be difficult to postulate a mechanism for the production of vasospasm in this model apart from the mechanical obstruction to CSF bathing the arteries. The differences between cerebral and systemic arteries, and the possible dependence of cerebral vessels on CSF for nutrition and waste removal via adventitial pores, are well discussed.

One may wonder whether some other component of a commercial fibrin glue, such as a preservative, was a commercial fibrin glue, such as a preservative, was a commercial fibrin glue, such as a preservative, was a commercial fibrin glue, such as a preservative, was a commercial fibrin glue, such as a preservative.
could cause an inflammatory reaction and contribute to vasospasm in that way. However, the absence of any such reaction in systemic arteries, as shown by the authors and others, makes this unlikely. It would still be interesting to see the experiments done again with fibrin glue made up from the experimental animal's own blood.

This study also sounds a warning for when it is planned to use fibrin glue clinically. If a significant artery is nearby, care will be needed to avoid encasing it accidentally in the glue.

Nicholas W. C. Dorsch, M.D., F.R.C.S., F.R.A.C.S.
Department of Neurosurgery
Westmead Hospital
Sydney, Australia

In this article, Honda and Terao demonstrated in dogs that local application of fibrin glue to the adventitial surface of cerebral arteries (internal carotid artery, A1 segment, and M1 segment) following dissection of arachnoid membranes induced chronic vasospasm of A1 segment. The vasospasm was confirmed by both cerebral angiography and morphometric analysis 1 week after fibrin glue application. Based on the results that the adventitial layer in dogs with application of fibrin glue was less stained with cisternally injected India ink than in control dogs, they concluded that disturbance of CSF circulation surrounding the basal cerebral artery is the crucial link in the development of cerebral vasospasm. This is a unique and interesting study. However, there are several questions. They explained why vasospasm occurred in the A1 segment but not in the M1 segment as follows: tighter adhesion of fibrin glue around the A1 segment, which is probably due to the anatomical peculiarity that the space between the A1 segment and brain parenchyma is bigger than that between the M1 segment and brain parenchyma, could induce more complete blockade of the CSF circulation. If that is true, vasospasm could also occur in the internal carotid artery. However, they did not mention it. Why was the presence or absence of vasospasm not described in the internal carotid artery? As application of fibrin glue to the cervical common carotid artery did not induce vasospasm, they excluded the possible vasoconstrictor effect of fibrin glue. However, there are differences in pharmacological responses between the common carotid artery and cerebral arteries. Therefore, if they had evaluated the direct vasoconstrictor effect of fibrin glue in vitro, this study would have been better.

Although they clearly demonstrated that local application of fibrin glue to cerebral arteries induced chronic vasospasm, Higuchi et al.1) have previously reported contrary results in SAH patients, in that the local application of fibrin glue prevented the occurrence of vasospasm following SAH. Is this due to species differences? Further studies will be necessary to clarify the true effect of local application of fibrin glue to cerebral arteries.

Reference

Tomio Sasaki, M.D.
Department of Neurosurgery
Faculty of Medicine, The University of Tokyo
Tokyo, Japan

Genetic factors of cerebral vasospasm are still unknown but it is thought to be a multifactorial event. Honda and Terao demonstrated that vasospasm can be produced without blood cell components by disturbing the contact of arterial wall with the CSF circulation using topical application of fibrin glue. This experiment can explain one factor of the cerebral vasospasm which is likely to occur in patients with a large amount of blood clot in the basal cistern causing severe disturbance to the CSF circulation. This paper also shows that effect of cisternal irrigation with clotlysis can be achieved not only by removal of vasospasmogenic substances but also by restoration of the CSF circulation in the basal cistern and around the cerebral arteries. I wonder how important the role of CSF circulation on cerebral vasospasm is in humans. Higuchi et al.1) reported the efficiency of fibrin glue coating therapy, which was carried out to prevent cerebral vasospasm by coating the main arteries with the glue to isolate the arteries from blood clots. Based on this experimental study, fibrin glue coating therapy could not have any effect on vasospasm. I have no idea how to resolve this discrepancy. I expect a further study to explain this controversial fact.

Reference

Nobuyuki Yasui, M.D.
Department of Surgical Neurology
Research Institute for Brain and Blood Vessels-Akita
Akita, Japan

Neurol Med Chir (Tokyo) 37, May, 1997