Etiology and Treatment of Vasospasm following Subarachnoid Hemorrhage

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Summary

In our recent study, biochemical analysis of spasmogenic substances released from blood or a blood-CSF mixture has been performed. These vasoactive substances were applied in both in vitro and in vivo experiments and it was found that oxyhemoglobin or its allied polypeptide, produced in the process of clot lysis or during the breakdown of blood corpuscles, was the main causative substance for prolonged vasospasm.

In this study, morphological examination using the fluorescent antibody and the ferritin antibody methods were undertaken to gain a better understanding of the mechanism of spasmogenic reaction of hemoglobin on cerebral vessels. Hemoglobin was found to be distributed in the adventitia and in the smooth muscle layer of the media. Haptoglobin, which is a normal constituent of serum, is known to bind with hemoglobin to form a chemically stable hemoglobin-haptaglobin compound. Vasospasm was released by the application of haptoglobin both in vitro and in vivo. Based on these experimental results, clinical use of haptoglobin was made in 27 patients with vasospasm. In 17 cases, angiographical spasm was progressively increased or decreased on the day prior to surgery. Fourteen of the 17 cases showed angiographical improvement of vasospasm after use of haptoglobin. We also discuss the development of postoperative vasospasm due to the operation itself.

Key words: Vasospasm, hemoglobin, fluorescent antibody, ferritin antibody, haptoglobin, postoperative vasospasm

Introduction

At present, cerebral vasospasm following subarachnoid hemorrhage (SAH) is one of the most important problems in the treatment of ruptured intracranial aneurysm. Its etiology, however, is not fully clear and so there is no effective treatment for this condition.

For the past few years, we have been engaged in research to isolate and identify spasmogenic substances contained in blood and in blood CSF mixtures using biochemical analysis techniques. We have reported both in vitro and in vivo results which support the concept that oxyhemoglobin is one of the most essential causative substances producing cerebral vasospasm,12,13 and also results concerning the spasmolytic effects of haptoglobin (Hp) on vasospasm.12,17,21

The present report describes the results of a morphological study using the fluorescent antibody and the ferritin antibody methods to better understand the mechanism of spasmogenic reaction of hemoglobin (Hb) on the cerebral vessels. The results of clinical use of Hp to treat vasospasm in 27 patients with intracranial aneurysm, and furthermore, discussion of postoperative vasospasm found through postoperative angiography is presented.
Materials and Methods

Experimental study

Adult dogs were anesthetized by intravenous administration of nembutal (25mg/kg). An appropriate amount of human Hb (50mg) was atraumatically injected into the cisterna magna. Having confirmed the presence of diffuse vasospasm of the basilar artery and the circle of Willis by vertebroangiography on the 3rd day after injection of Hb, the animals were sacrificed by perfusion with 1% glutaraldehyde in 0.15M phosphate buffer (pH 7.4). The basilar arteries were removed.

a) Conjugation of serum globulin with fluorescein isothiocyanate (FITC).

Human Hb was prepared according to Drabkins method.4) The anti-human Hb serum was produced by Green Cross Corporation. The globulin was prepared by the Sober and Peterson method.18) FITC was produced by ICN • NBC Laboratories, Inc.

The globulin and FITC mixture was first gelfiltrated using Sephadex G-50 column chromatography (2.0 x 30cm) eluted with 0.01M phosphate buffered saline (pH 7.2). The first peak of the chromatographically eluted fractions was collected and then this sample was passed through a column of DEAE cellulose (2.0 x 30cm) by stepwise elution with 0.005M phosphate buffer (pH 8.4), 0.05M phosphate buffer (pH 6.4) and 0.1M phosphate buffer (pH 6.4). The first peak was collected and concentrated by the carbon wax method. Then the conjugate was passed through a bacterial filter and stored at 4°C. The specificity of this conjugate was examined by the blocking test.

After washing in 0.15M phosphate buffer (pH 7.4), the arterial strips were fixed in 95% ethanol with 1% glacial acetic acid for 2 hours at 4°C. They were dehydrated with absolute alcohol and embedded in paraffin at 52°C to 54°C for 30 minutes. Sectioning was carried out (4 μ thick). The paraffin was removed from the sections by xylene and then the sections were overlaid with FITC labelled antibody for 24 hours at 4°C. Thin sections were examined with a Nikon-FL microscope.

b) Preparation of the ferritin-antibody conjugates

Ferritin, Cd Free was produced by ICN • NBC Laboratories, Inc. m-Xylylene diisocyanate (XC) was produced by Iwai Chemical Industries, Ltd.

The ferritin-XC conjugates and the conjugation of ferritin to globulin were carried out by the Rifkind procedure.16) Characterization of these conjugates was carried out through immunoelectrophoresis in Special Noble agar buffered with barbital buffer (pH 8.6). Finally, the specificity of this conjugate was examined by the blocking test. The arterial strips were applied in ferritin-antibody conjugates for 30 min at 4°C. After a brief washing in the phosphate buffer, they were fixed in 2% glutaraldehyde for 1 hour at 4°C. After a second brief washing in the phosphate buffer, they were postfixed in 1% osmium tetroxide in 0.15M phosphate buffer (pH 7.4) for 2 hours at 4°C. They were dehydrated with ethanol and embedded in Epon 812. Sections were cut with a Porter-Blum ultramicrotome. Thin sections were stained with lead citrate for 5 min and then with uranyl acetate for 10 min. They were examined with a JEM-100B electron microscope.

Clinical study

a) Clinical application of Hp

Clinical use of Hp was attempted in 27 patients with intracranial aneurysm. In all patients, the preoperative angiograms were taken more than twice; the last one was taken within 24 hours prior to surgery.

Hp was applied topically to the arteries around the aneurysm after the neck of the aneurysm had been clipped. In early surgery cases, infusion of Hp was repeated for 2 days following surgery through a catheter left in the basal cistern. Postoperative angiogram was taken within 24 hours and again on the third postoperative day.

The effects of Hp were determined by measuring and comparing the size of the arteries on the preoperative and postoperative angiograms. When the diameter of a given artery on the postoperative film had increased over 50% compared to that of the preoperative angiogram, this was classified as “excellent.” In like manner, dilatation of the artery between 25% and 50% was classified as “good,” between 0 and 25% as “fair” and no change or reduction in diameter of the artery as “no effect.” The 27 patients were
divided into 4 groups. Group 1 consisted of 5 patients who were operated on within 48 hours following aneurysm rupture. Group 2 consisted of 7 patients in whom it was found by repeated angiography that the degree of vasospasm was progressively increasing at the time of surgery. Group 3 consisted of 10 cases, all of whom had obvious vasospasm at the time of surgery but in whom it was demonstrated by repeated preoperative angiography that the degree of vasospasm was progressively decreasing up until the date of operation. Group 4 consisted of 5 patients, in whom the degree of vasospasm remained virtually unchanged angiographically for a period of 7 to 21 days prior to the operation.

Results

1) Experimental study
   a) Fluorescent antibody method
   Sections of arteries showed specific fluorescent staining of the adventitia and no staining of the smooth muscle layer of the media (Fig. 1).

b) Ferritin antibody method
   Each thin section showed numerous ferritin granules surrounding the collagen fibers in the adventitia and the basement membrane of smooth muscle cells at the outermost layer of the media (Fig. 2). There were numerous ferritin granules surrounding the nerve fibers at the border between the adventitia and the layer of smooth muscle (Fig. 2). In several thin sections, there were numerous ferritin granules surrounding the basement membrane of smooth muscle cells at the inner layer of the media (Fig. 3).

2) Clinical study
   a) Clinical application of Hp
      Group 1. Though vasospasm was not found at the time of surgery in these cases (except one case), Hp was applied with the hope of preventing subsequent development of vasospasm. In one case of this group, a single administration of Hp failed to prevent the occurrence of vasospasm: this patient developed motor weakness and his consciousness deteriorated again in parallel to the degree of vasospasm as seen angiographically from the fifth postoperative day. In other cases of this group, infusion of Hp was repeated for 2 days following surgery through a catheter left in the basal cistern. Postoperatively, repeated angiography showed the occurrence of vasospasm (Fig. 4). These patients, however, were clinically well and were discharged with no neurological deficits.

      Groups 2 and 3. It is noteworthy that beneficial effects of Hp application against vasospasm were noted in 14 out of 17 patients in these groups (Fig. 4).

      Group 4. Only one patient out of 5 responded favorably by Hp administration at the time of surgery in this group (Fig. 4).

   b) Postoperative vasospasm
      Six out of 19 patients (31.6%) showed vasospasm angiographically after surgery (Table 1). Vasospasm seemed more prone to develop in cases of anterior cerebral artery aneurysms than in those at other sites. In patients who were operated on after more than 3 weeks from the last SAH and who showed no vasospasm angiographically then, postoperative angiograms showed the development of several degrees of vasospasm (3/15) at the first or second day after surgery (Table 2). Such vasospasm, the so-called “postoperative vasospasm,” usually lasted for more
Fig. 2  Electron micrograph showing numerous ferritin granules surrounding the collagen fibers in the adventitia (C), the basement membrane of smooth muscle cells at the outer most layer of the media (M), and the nerve fibers at the border between the adventitia and the layer of the smooth muscle (N). (× 12000)

Fig. 3  Electron micrograph showing numerous ferritin granules surrounding the basement membrane of smooth muscle cells at the inner layer of the media. (× 5670)
than a week.

We present one case, a 54-year-old male who had an episode of SAH 24 days before admission. Angiography performed on the day of admission showed an aneurysm at the left middle cerebral artery with no vasospasm (Fig. 5a). He was operated on 7 days after admission. On the first day after surgery, angiograms showed marked diffused vasospasm at the left middle cerebral artery (Fig. 5b). On angiograms taken 3 days later, vasospasm was still noted, although the degree had become somewhat reduced (Fig. 5c).

**Discussion**

Brawley et al. from their experiments suggested that cerebral vasospasm was a biphasic phenomenon and this has been widely accepted. They reported that its chronic phase commenced at 3 hours after and reached the maximum stage 72 hours after SAH. In many clinical cases, however, cerebral vasospasm develops gradually after 3 or 4 days after SAH and persists for 7 to 10 days. This vasospasm, the so-called "late spasm," is a significant problem in the treatment of ruptured aneurysms. It has been noted by many investigators that the presence and degree of vasospasm corresponds well with CSF pigmentation. Consequently, the location where blood comes into contact with the vessels of the circle of Willis has been considered important in the causation of spasm. It has also been suggested that vasoactive substances are produced in the process of clot lysis or during the breakdown of blood corpuscles. We have performed biochemical analysis of vasoactive substances released from blood or blood-CSF mixtures and these vasoactive substances were applied in both in vitro and in vivo experiments. The absorption curves of these substances were analyzed with a self-recording spectrophotometer; prominent absorption was found at 415, 540 and 575 millimicrons. These were typical for oxyhemoglobin. Another biochemical analysis using polyacrylamide gel electrophoresis also showed chemical characteristics similar to oxyhemoglobin. From these studies, it may be safe to assume that vasoactive substances isolated from blood-CSF mixture are either polypeptides closely allied to oxyhemoglobin or are oxyhemoglobin itself. In this study,

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<th>GROUP-1</th>
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**Fig. 4** Angiographical response after clinical use of haptoglobin in four groups.

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<th>Table 1 Frequency of postoperative vasospasm and location of the aneurysm</th>
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<td>Location</td>
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<td>Internal Carotid</td>
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<td>Anterior Cerebral</td>
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<td>Multiple</td>
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<th>Table 2 Relationship between the frequency of postoperative vasospasm and the time from SAH to operation</th>
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<td>Time (Days) from SAH to Operation</td>
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it was found that Hb is distributed in the adventitia and in the smooth muscle layer of the media by morphological examination with light and electron microscope. It cannot be concluded at present, however, whether contraction of vascular smooth muscle is initiated by the direct action of Hb on smooth muscle cells or by the irritation of nerve endings by Hb.

Many clinical methods to release vasospasm have been developed, but as yet none are fully satisfactory. Recent studies by Allen¹¹ have shown that simultaneous use of alpha blocker sodium nitroprusside (3.0–4.5 μg/kg/min) and alpha stimulator phenylephrine (0.38 μg/kg/min) can be effective in the treatment of delayed vasospasm. He reported that angiographically there was a dramatic increase in the caliber of the cerebral vessels after treatment. Flamm et al.⁶) recently reported clinical success in the treatment of vasospasm with isoproterenol and amino phyline, which have increased the levels of cyclic adenosine monophosphate (cAMP) in vascular smooth muscle by stimulating adenylate cyclase (isoproterenol) and inhibiting cAMP phosphodiesterase (aminophyline) in 9 out of 12 patients. Fleischer et al.⁷) reported that 10 positive responses were obtained in 14 patients using three agents when treatment was begun within 14 hours after the development of spasm symptoms. Increased levels of available cAMP within vascular smooth muscle are known to cause relaxation of the muscle by promoting binding of the vascular myoplasmic calcium.¹⁴) Sundt et al.¹⁹-²⁰) suggested that simultaneous use of lidocaine hydrochloride (2 g/500 ml) and isoproterenol (0.6–1.2 mg/150 ml) would protect cardiac arrhythmias and cause clinical improvement in patients with vasospasm. The search for spasmylytic substances which can release vasospasm is being made in hopes of finding a method appropriate for the treatment of vasospasm. Haptoglobin, α₂-globulin, is a normal constituent of serum. It is known that Hp has the property of binding with Hb stoichiometrically to form a chemically stable Hp-Hb compound quite easily. We have reported on the spasmylytic effects of Hp on vasospasm in both in vitro and in vivo studies.¹²,²¹) Based on these results, clinical use of Hp on vasospasm was attempted in 27 cases. In early surgery cases (Group 1), repeated application of Hp seemed effective, but it failed to prevent the occurrence of vasospasm as seen angiographically. In Groups 2 and 3, beneficial effects were noted. In these cases, the degree of vasospasm demonstrated by repeat angiography pro-

Fig. 5  Postoperative Vasospasm.

a)  Left:  Angiogram on 24th day after SAH showing an aneurysm at the left middle cerebral artery and no vasospasm.

b)  Middle:  Angiogram on the 1st day after surgery showing marked diffuse vasospasm at the left middle cerebral artery.

c)  Right:  Angiogram on the 4th day after surgery showing mild vasospasm at the left middle cerebral artery.
gressively increased or decreased up to the date of operation. It was thus apparent that such vasospasm belonged in the category of functional rather than organic spastic change. On the other hand, in Group 4, no effect of Hp (except for one case) was noted. In these cases, the degree of vasospasm remained unchanged for a period of 7 to 20 days. Such vasospasm, thus, is assumed to have already undergone considerable organic changes. The number of patients we experienced, however, is evidently too small to draw any definite conclusions. Nonetheless, we believe that Hp is an effective measure in treating vasospasm provided that careful selection of patients is made. As a rule, we treat ruptured aneurysm patients in the acute phase, in accordance with the so-called "intentional delayed surgery" formula advocated by Ransohoff et al. and others. This significantly reduces the incidences of focal rebleeding. Still a considerable number of patients may survive or die as the result of long-lasting vasospasm. Drake suggested that cases operated on within 3 days after SAH lead to unsuccessful results. However, if the condition of the patient after SAH is fair, some advocate early operation. Especially when consciousness seems clear, early surgery should be recommended to prevent occurrence of vasospasm due to the removal of subarachnoid clots in the basal cistern and Sylvian fissures, and topical application of Hp to the arteries around the aneurysm should be made after the neck of the aneurysm has been clipped. Following surgery, infusion of Hp should be repeated for 2 days through a catheter left in the basal cistern.

It is possible that causes of pre- and postoperative vasospasm differ, especially in patients experiencing the last SAH more than 3 weeks prior to surgery. The two conditions, therefore, must be considered separately. Significant effects of the operation itself on postoperative angiographic findings must also be considered. Vasospasm caused by the operation itself should also be considered postoperative vasospasm.

References


