Experimental Cerebral Vasospasm After Subarachnoid Hemorrhage

Development and Degree of Vasospasm

SHUNRO ENDO, M.D. AND JIRO SUZUKI, M.D.

SUMMARY

Vasospasm of the basilar artery in 57 cats was induced by application of fresh blood, or a blood and cerebrospinal fluid (CSF) mixture incubated at 37°C for 2 to 16 days. In animals treated with fresh blood or mixtures incubated for over 15 days, the severity of induced vasoconstriction is slight and duration short. Mixtures incubated 5 to 10 days induced severe and prolonged vasoconstriction. This incubation period for blood and CSF mixtures induces severe and prolonged vasoconstriction. This experimental study suggests the existence of a vasospasmogenic substance, which possesses the potential to produce severe and prolonged vasoconstriction.

VASOSPASM associated with subarachnoid hemorrhage (SAH) from a ruptured intracranial aneurysm plays an important role in determining prognosis. However, not only the prevention, but also the true nature and cause of vasospasm remains unclear.

We based this experiment upon the hypothesis that a vasospasmogenic substance might be produced from the blood components in the subarachnoid space by sequential chemical changes and be temporally related to clinical data which suggest that vasospasm develops about 7 days after SAH. Experimental vasospasm was induced in the basilar arteries of cats by applying fresh blood or blood and cerebrospinal fluid (CSF) mixtures incubated for various durations at 37°C. The frequency and severity of vasospasm and its correlation with the incubation time of the mixtures was studied. Results are discussed mainly with regard to the developmental time of vasospasm after SAH and to the possibility of the existence of a vasospasmogenic substance.

Methods

Fifty-seven adult cats weighing from 2.5 to 5.0 kg, were anesthetized with intramuscular pentobarbital, and placed on an electric temperature-controlled surgical table. Tracheostomies were performed. Spontaneous respiration was permitted. Body temperature, femoral arterial pressure, respiratory rate and acid-base balance were measured. In experiments lasting more than 3 hours, a polyethylene catheter was placed in the femoral vein for infusions.

By the transcervical, transclival approach, a bone window

From the Division of Neurosurgery, Institute of Brain Diseases, Tohoku University School of Medicine, 5-13-1, Nagamachi, Sendai, Japan.
of about 2 × 2 cm was made in the clivus with an electric drill. Then the dura was opened in the midline and traction sutures were placed to retract the dura laterally. About two-thirds of the proximal portion of basilar artery and the distal portion of vertebral artery were exposed. Care was taken to leave the arachnoid as untouched as possible. An incubated blood-CSF mixture or fresh blood was poured around the basilar-vertebral artery 15 minutes after opening the dura to induce experimental vasospasm.

Equal amounts of autogenous blood and CSF were extracted respectively from femoral artery and cisterna magna. A mixture of these was incubated for varying periods at 37°C. In 6 cases the mixtures were incubated for 2 days, in 5 cases for 3 days, in 10 for 5 days, in 12 for 7 days, in 4 for 10 days, in 4 for 15 days and in 3 cases for 16 days. Since these mixtures had both clotted and fluid components, they were used after mixing. Then 0.3 ml of the fluid was introduced into the subarachnoid space around the basilar-vertebral artery. To avoid the mechanical stimulation of the artery, the mixture was applied to the area as distant as possible, from the artery with a 27G needle. The fluid was allowed to be diluted by CSF gradually. In 5 cases the fluid plus clot mixture incubated for 7 days, was injected into the subarachnoid space diffusely from several points through torn arachnoid.

Photographs of the exposed artery were made using Fuji R-100 color film in a 35 mm camera attached to an operative microscope. Photographs were taken before and at

![Figure 1](http://stroke.ahajournals.org/)

Figure 1. "Diffuse type" vasospasm (upper) and "local type" vasospasm (lower) of basilar artery in the cat induced by application of blood and CSF mixture incubated for 7 days. (left: control; right: treated)
5 minute intervals after the application, and the caliber of the basilar artery was measured. The results were expressed as percent change (+ for dilation and — for constriction) in the caliber of the artery relative to the control caliber immediately preceding the application. The severity of induced vasospasm was classified according to the degree of constriction. In Grade I the constriction is less than 10%, Grade II, greater than 10 to 30%, Grade III, more than 30 to 50% and Grade IV, more than 50%. When segmental constriction occurred, the area of maximum constriction was selected for measurement.

Results

In the five control cases, the sequential changes of caliber between 3 to 24 hours were from +3% to —4%, and the reactivity of vessel to mechanical stimulation remained unchanged. Abnormal changes of arterial blood pressure, body temperature, respiratory rate and acid-base balance were not observed in these experiments. Following the application of fresh blood or incubated mixture, the change of vessel size began immediately and reached its peak 5 or 10 minutes later. The shapes of the vasospasm with constriction of over 10% in 36 cats (Grade II—IV) were classified into 3 types. Sixteen cats (diffuse type) exhibited diffuse constriction of the basilar artery in the photographed area (fig. 1), 5 cats (local type) showed segmental constriction, even though the blood-CSF mixture had been equally applied along the artery (fig. 1). Fifteen cats had diffuse and focal spasm (combined type). The severity and pattern of the vasospasm in any single cat was always almost the same after repeated applications.

Caliber changes within 20 minutes following the application of fresh blood or fluid components of the incubated mixture in 49 cats are shown in figure 2. They are classified into 4 groups, those receiving fresh blood, incubated blood-CSF mixture, for 2 or 3 days, for 5 to 10 days, and for 15 or 16 days. The relation between the grade of vasospasm and the incubation period is shown in table I. In the group treated with fresh blood, no severe vasospasm of grade III and IV was observed. The mean maximum change in caliber was 8.29 ± 4.53%. This value showed significant constriction when compared with the control cats (P < 0.05).

In the group treated with a blood and CSF mixture incubated for 5 to 10 days, the frequency of severe vasospasm, Grade III and IV, was extremely high — 80%. The mean reduction in caliber was 41.37 ± 14.20%, P < 0.01. In the group receiving the mixture incubated for 2 or 3 days, severe vasospasm was seen in 2 of the 11 cats, and the mean caliber reduction was 21.44 ± 18.06%. In the group of cats treated with the mixture incubated for 15 or 16 days, only one had constriction of Grade III and the others were all Grade I.

In the cats treated with fresh blood, or the blood-CSF mixture incubated for 15 or 16 days, vasospasm almost completely disappeared in all cases 20 minutes after the application. At the end of 20 minutes the cats treated with the mixture incubated for 5 to 10 days still exhibited an average reduction in vessel caliber of 34.5%. In the group treated with this mixture incubated for 2 or 3 days the results show an intermediate tendency between the two described groups in duration as well as in severity.

A more detailed and longer observation of caliber changes in the group treated with the blood-CSF mixture incubated for 7 days is shown in figure 3. Vasospasm induced by the application of the mixture without clotted components

![Figure 2. Percent change in the caliber of the basilar artery in relation to the time following application of fresh blood, or incubated mixture of blood and CSF.](http://stroke.ahajournals.org/)

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gradually reduced over 1 hour after the application, and thereafter a constriction of about 20% was observed continuously. The vasospasm induced by the blood-CSF mixture with clotted components, showed very little recovery. It continued almost unchanged during the observation period which ranged from 5 hours to a maximum of 24 hours in 2 cases.

In cats with prolonged vasospasm of Grade III and IV, swelling of brain through the bone window of the clivus was observed beginning about 5 hours after the application and increasing gradually. These changes in brain volume were not seen in the control group or in the cats without severe vasospasm.

**Discussion**

In experimental vasospasm, the contraction appears immediately after the subarachnoid hemorrhage and disappears in a short time. Late vasospasm is distinct from the
early form. It appears about 3 days after SAH and is prolonged. Late vasospasm may be compared to clinical vasospasm observed after SAH in man. 6, 9, 10, 26, 27 In our experiments it is possible that the vasospasm induced by the fresh blood is similar to early vasospasm, and vasospasm induced by the mixture of blood and CSF incubated 5 to 10 days is similar to late vasospasm.

The method of observing the exposed basilar artery is very useful and has been used since the report of Echlin. 8 In reports of this method there are conflicting results as to the severity of vasospasm induced by the application of fresh blood. The appearance of severe vasospasm was reported by Echlin, 8 Osaka, 17 and Kapp et al.; but not by Symon, 14 Suzuki 19 and Kimura 24 et al. However, investigators are in agreement about the duration of vasospasm. According to the reports the early vasospasm had a short duration disappearing within 30 minutes to 2 hours after fresh blood application. The appearance of severe vasospasm after the application of fresh blood along with mechanical stimulation and the puncture or the tear of artery has been reported. 8, 9, 10 The stimulation by the mechanical component is believed to have a multiplicative effect inducing severe vasoconstriction. Because of these reports, we did our best to avoid mechanical stimulation to the artery during the experiments; as a consequence all vasospasm induced by fresh blood was less than 20% and disappeared almost completely within 20 minutes after application. The differences in the degree of vasospasm in some of the reports may be due to the degree of mechanical stimulation of vessels during experiments. Great importance lay in whether or not the arachnoid was removed and how the blood application was performed. The genesis of early vasospasm is suspected to be the result of a mechanical factor 8, 9, 10 acting on the vessel’s wall and a chemical 8, 9, 10, 11, 12, 13 factor in blood, i.e. serotonin. The results from this study show that the mechanical factor played a fairly important role.

The vasospasm induced by the application of the mixture of blood and CSF incubated for 5 to 10 days at 37°C was the most severe and prolonged, and the vasospasm induced by the blood-CSF mixture incubated for 2 or 3 days was moderate. These patterns of vasospasm were definitely different from those induced by fresh blood both in severity and duration. These findings indicate the possible existence of strong vasospasmogenic substances in these mixtures. After SAH, blood and CSF combined, in essence, are incubated at body temperature in the subarachnoid space. Consequently a vasospasmogenic substance develops and/or becomes active from about 2 or 3 days after the hemorrhage, the activity progressively intensifies to reach a peak 5 to 10 days after the hemorrhage, then declines and disappears after 15 days.

Most clinical data on the developmental time of vasospasm after the rupture of an aneurysm show that vasospasm occurs within three weeks, usually about 7 days after hemorrhage. 4, 5, 10, 11 The data reported by Suzuki 19 showed that vasospasm usually occurs between the 4th and 14th days after SAH. The data of Wilkins 26 showed that no vasospasm occurred within 24 hours after hemorrhage. These clinical reports coincide closely with the results of this experiment.

Echlin and Suzuki et al. pointed out a species difference in vascular reactivity in the cat, dog and monkey. The cat shows the strongest vasoreactivity. In our experiments with cats, the degree of constriction of the vasospasm induced by the mixture incubated for 5 to 10 days ranged from 20% to 70%. This wide range suggests the presence of individual differences in reactivity even in one kind of animal. With reference to the duration of vasospasm the contraction induced by the blood-CSF mixture without clotted components continued longer than that induced by a blood-CSF mixture without clotted components.

In Osaka’s report 17 vasospasm continued 6 hours following the continuous application of incubated lysed red cells. These findings suggest that the existence of a certain amount of vasospasmogenic substance is necessary for the prolongation of vasospasm. It is possible to say that the severity of vasospasm depends on the reactivity of a vessel and that prolongation of vasospasm depends on the quantity of vasospasmogenic substances. This assumption may explain clinical findings as follows: generally the frequency of vasospasm is high in patients with severe SAH with a large hematoma around a vessel. This relationship does not always hold, since individual differences in the frequency or severity of vasospasm exist, as demonstrated in our experimental model.

References

REGULATION OF cerebral blood flow is a complex mechanism adjusted to yield a flow rate which precisely matches the metabolic requirements of the brain. Following a period of cerebral ischemia, this mechanism is severely disturbed. Immediately after ischemia, blood flow is increased (postischemic hyperemia) although brain metabolism initially is considerably reduced. After longer recirculation times, when the electroencephalogram begins to recover, blood flow gradually decreases despite the rise in the metabolic requirements of the brain (postischemic hypoperfusion syndrome). This situation may provoke a misrelationship between oxygen consumption and oxygen availability to the brain, and bears the risk of delayed metabolic disturbances.

Postischemic reactive hyperemia has been studied extensively before and appears to be the consequence of both a loss of cerebrovascular autoregulation and of CO₂ responsiveness, resulting in total paralysis of the cerebral vasculature. The pathomechanism of the postischemic hypoperfusion syndrome is less clear. Factors which may be involved, are vasoconstriction, changes in blood viscosity, brain swelling, or functional disturbances such as the dissociation between an already recovered autoregulation and the still severed CO₂ reactivity.

Numerous attempts have been made to improve cerebral blood flow pharmacologically after ischemia (for review see Refs. 9, 10), but there is considerable disagreement about the efficiency of such treatment. Some of the controversy may be due to the route of application because many of the drugs used have a more pronounced effect on the extracerebral than on the cerebrovascular smooth muscle which is protected by the blood-brain barrier. Systemic application, therefore, may provoke steal effects of the blood from the brain to extracerebral tissues. Another point of uncertainty is the fact that the permeability properties of the blood-brain barrier, as well as the responsiveness of the cerebral

SUMMARY Normothermic adult cats were submitted to 30 min complete cerebral ischemia by arterial inflow occlusion, and the brains subsequently recirculated with blood for 4 hours. Cortical blood flow was recorded with a heated thermocouple, cortical oxygen pressure fell from 44 ± 5.8 to 0 torr (means ± S.E.), cortical heat conductance decreased from 15.1 ± 0.8 to 10.2 ± 0.5 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹, and the pial arteries constricted to less than 50% of their initial diameter. Recirculation of the ischemic brain initiated reactive hyperemia (heat conductance 23.6 ± 3.6 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹) which lasted for about 45 min. and which was accompanied by an increase in arterial Po₂ to 74 ± 14 torr, and a dilatation of the pial arteries by about 50%. Hyperemia was followed by postischemic hypoperfusion during which cortical Po₂ returned to, or slightly below, normal (34 ± 4 torr), cortical heat conductance decreased to 12.9 ± 0.4 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹, and pial arteries constricted by about 10%.

The pharmacological responsiveness of the pial vasculature was tested before ischemia and during postischemic hypoperfusion by intra-arterial administration of alpha- and beta-adrenergic stimulating and blocking agents, the antiserotonergic agent metergolin, and papaverine. The autonomic drugs and metergolin induced only minor changes in cortical blood flow or oxygenation but there was distinct vasodilatation and an increase in blood flow with papaverin both before and after ischemia. It is concluded that treatment of postischemic hypoperfusion is more efficient with drugs which cause direct relaxation of the vascular smooth muscle than with those which act on the autonomic receptor sites.

Blood Recirculation and Pharmacological Responsiveness of the Cerebral Vasculature Following Prolonged Ischemia of Cat Brain

S. Takagi, M.D., L. Cocito, M.D. and K.-A. Hossmann, M.D., Ph.D.

**From the Max-Planck-Institut für Hirnforschung Abt. Allgemeine Neurologie, 5000 Köln 91, West Germany.**

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*Dr. Takagi's present address is Juntendo University, Neurosurgical Dept., Tokyo, Japan. Dr. Cocito's present address is Università di Genova, Istituto di Clinica Malattie Nerve e Mentali, Genova, Italy. Reprint requests to Dr. K.-A. Hossmann, Osterheimerstrasse 200, 5000 Köln 91, West Germany.*

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S Endo and J Suzuki

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