Barrier disruption in the major cerebral arteries following experimental subarachnoid hemorrhage


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The effects of experimental subarachnoid hemorrhage (SAH) on the blood-arterial wall barrier in the major cerebral arteries were studied in 20 normotensive dogs. Horseradish peroxidase (HRP) was given intravenously before the animals were sacrificed to assess the integrity of the barrier. Transient elevation of intracranial pressure (ICP) produced by cisternal injection of saline solution resulted in HRP leakage at the branching points of the major cerebral arteries. Extensive disturbance of the blood-arterial wall barrier was consistently observed in the major cerebral arteries after SAH, with or without elevation of ICP. These results suggest that both subarachnoid clot and a sudden rise in the ICP are important factors causing the breakdown of the blood-arterial wall barrier, but that the effect of the clot is the most profound. Electron microscopy revealed that opening of the interendothelial junctions is one of the important mechanisms responsible for the HRP leakage in the major cerebral arteries following SAH. Disturbance of arterial permeability in the major cerebral arteries following SAH probably accounts for the abnormal post-contrast enhancement that occurs in patients who are prone to develop vasospasm following aneurysm rupture, and is probably involved in the pathogenesis of vasospasm.

Key Words • subarachnoid hemorrhage • vasospasm • horseradish peroxidase • blood-arterial wall barrier • dog

PERMEABILITY changes in the major cerebral arteries following subarachnoid hemorrhage (SAH) may be important in the pathogenesis of cerebral vasospasm. However, the effect of SAH on the blood-arterial wall barrier in the major cerebral arteries has not been precisely studied. Information about the permeability change in major cerebral arteries following SAH may also be useful for identifying the mechanisms responsible for the abnormal post-contrast enhancement in the region of the basal subarachnoid cisterns seen on computerized tomography (CT) scans in SAH patients.

The present study was undertaken to elucidate the mechanisms responsible for the permeability changes of the major cerebral arteries following SAH.

Materials and Methods

Twenty normotensive dogs of either sex, each weighing 4.0 to 6.8 kg, were anesthetized with sodium pentobarbital (30 mg/kg). The animals were intubated and ventilated with a Harvard respirator.* Light anesthesia was maintained with 60% nitrous oxide and 40% oxygen. End-tidal CO2 was monitored continuously by a Hewlett-Packard 47210A capnometer.† Arterial blood gases were checked frequently, and arterial pH, PaO2, and PaCO2 were maintained within the physiological range. If necessary, PaCO2 was adjusted by addition of CO2 to the inspired gas mixture.

Catheters were introduced into the right femoral artery and vein. The arterial line served for blood pressure monitoring and gas sampling, and the venous line for the injection of drugs and horseradish peroxidase (HRP). Intracranial pressure (ICP) was measured through a No. 21 butterfly needle inserted into the cisterna magna. Arterial blood pressure and ICP were

† Hewlett-Packard capnometer, Model 47210A, manufactured by Hewlett-Packard, Palo Alto, California.
arterial pressure to the baseline level.

diphenhydramine hydrochloride (5 mg/kg body weight, Sigma Type II; RZ: 1.52) was injected intravenously prior to injecting the blood slowly.

The animals were divided into seven groups as shown in Table 1. The SAH was produced either by a single 7-ml injection of fresh autologous nonheparinized arterial blood into the cisterna magna or by two successive 7-ml injections given 72 hours apart. In the isovolemic group, 7 ml of cerebrospinal fluid was withdrawn just prior to injection of the blood. The animals were tilted with the head down for 30 minutes to facilitate settling of the blood in the basal cisterns by gravity.

Cisternal injection of 7 ml saline solution in a non-iso- volemic manner transiently elevated the ICP and mean arterial pressure by about 130 mm Hg and 20 mm Hg, respectively. The non-iso- volemic SAH induced a sudden rise of both the ICP and mean arterial pressure by about 150 mm Hg and 80 mm Hg, respectively, which lasted for several minutes. In the isovolemic SAH dogs, both the ICP and mean arterial pressure were controlled to increases of less than 20 mm Hg by injecting the blood slowly.

For morphological examination, the animals were sacrificed by perfusion-fixation according to the time schedule shown in Table 1. Ten minutes before fixation, 2% HRP solution in saline (HRP: 200 mg/kg body weight, Sigma Type II; RZ: 1.52) was injected intravenously. Preliminary experiments revealed that the intravenous HRP injection decreased the arterial pressure. Accordingly, in order to prevent the HRP-induced hypotension, diphenhydramine hydrochloride (5 mg/kg body weight) was injected intravenously prior to HRP administration. When the arterial pressure still decreased after HRP injection, small amounts of norepinephrine solution (2 mg norepinephrine dissolved in 500 ml saline solution) were administered to return arterial pressure to the baseline level.

After a brief period of perfusion with heparinized saline solution, the perfusion-fixation was started from the left ventricle of the heart with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), under a pressure of 150 cm H2O. The brain was removed and immersed in a cacodylate-buffered fixative (pH 7.4) for 5 hours at 4°C, and was then kept overnight at 4°C in 0.1 M sodium cacodylate buffer (pH 7.3). The next day, the major cerebral arteries were dissected from the brain under magnification. The HRP was localized by Graham and Karnovsky's procedure using a medium consisting of 4 mg of 3,3-diaminobenzidine-tetra-HCl, 10 ml of 0.05 M Tris-HCl buffer (pH 7.6), and 0.1 ml of 1% hydrogen peroxide.

Samples were postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in acetone, and embedded in Spurr's epoxy resin. Ultrathin sections were cut on a DuPont-Sorvall MF-5000 ultramicrotome equipped with a DuPont diamond knife mounted on uncoated 200-mesh copper grids. Sections were examined unstained or stained with uranyl acetate and lead citrate in a Hitachi H-600 electron microscope. The regions with HRP-reactive products in the major cerebral arteries were easily identified by inspecting the brown-stained area under magnification, and the exact location of these HRP-reactive products was identified using electron microscopy.

In preliminary experiments, it was shown that no positive reaction to Graham and Karnovsky's procedure was observed in the major cerebral arteries of dogs with isovolemic SAH which did not receive HRP. This result suggests that the canine cerebral artery does not possess "endogenous" peroxidase activity.

Results

Macroscopic Changes

In control animals, proximal segments 1 to 3 mm beyond the origin of the intradural sections of both the internal carotid and vertebral arteries were stained with HRP-reactive products (Fig. 1 left). Occasionally, the branching points of the major cerebral arteries were weakly stained. Non-iso- volomic cisternal injection of saline solution slightly increased the permeability to

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<tr>
<th>Animal Group</th>
<th>Time of Sacrifice</th>
<th>Barrier Breakdown</th>
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<tr>
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<tr>
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</tr>
<tr>
<td>single SAH (non-iso- volomic)</td>
<td>72 hrs after SAH</td>
<td>diffuse, moderate</td>
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<tr>
<td>double SAH</td>
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* SAH = subarachnoid hemorrhage; ICA = internal carotid artery; BA = basilar artery; — = none.

† Bell and Howell transducers, Model 4-327, manufactured by Bell and Howell Company, Pasadena, California.

**TABLE 1**

Summary of experimental groups and results

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HRP of the major cerebral arteries at the branching points (Fig. 1 center). The leakage of HRP into the vessel wall was observed at either 40 minutes or 72 hours after the saline injection. In dogs with non-isovolemic single SAH, HRP leakage into the vessel wall was observed mainly at the branching points of the major cerebral arteries in the basal subarachnoid cisterns at 72 hours after the SAH. The HRP leakage induced by the non-isovolemic single SAH appeared to be a little more extensive than in the animals that received non-isovolemic cisternal injection of saline solution.

In dogs with non-isovolemic double SAH, extensive and marked disturbance of arterial permeability to HRP was consistently observed in the major cerebral arteries at both 40 minutes and 72 hours after the second SAH. Such marked leakage of HRP into the vessel wall was also observed at 72 hours after the second SAH in the major cerebral arteries of the dogs with isovolemic double SAH (Fig. 1 right).

**Microscopic Changes**

Electron microscopy studies in control animals demonstrated no HRP-reactive products in the major cerebral arteries except at the proximal segments of the internal carotid and vertebral arteries and at the branching points of the major cerebral arteries (Fig. 2). At the proximal segments, plasmalemmal vesicles containing HRP-reactive products were occasionally observed at the abluminal front of the endothelium. In some areas, the interendothelial spaces were partly filled with HRP-reactive products.

In dogs with either isovolemic or non-isovolemic double SAH, degenerative changes of the endothelium and the corrugation of elastic lamina were frequently observed (Figs. 3, 4, and 5). In places, endothelial cells were squeezed between tight folds of the internal lamina (Fig. 5). The endothelial bridges across the valley formed by the corrugated elastic lamina were also found in some specimens (Fig. 3A). Occasionally, the endothelial cells were separated from each other at the interendothelial junctions. These changes were not seen following saline injection.

Regarding the localization of HRP-reactive products, the microscopic studies in dogs with either isovolemic or non-isovolemic double SAH revealed HRP-reactive products in the interendothelial space, plasmalemmal vesicles, and subendothelial space of the major cerebral arteries in the basal subarachnoid cisterns (Figs. 3, 4, and 5). The HRP-reactive products in the subendothelial space were prominent beneath the HRP-labeled interendothelial space. In the area where elastic lamina

![Fig. 1. Photographs of the major cerebral arteries illustrating the areas stained dark by horseradish peroxidase (HRP)-reactive products. *Left*: Vessels from a control dog. *Arrows* indicate the HRP leakage at the proximal parts of the intradural internal carotid arteries. The cavernous portions of the internal carotid arteries were markedly stained. Bar = 1 cm. *Center*: Vessels from a dog sacrificed 40 minutes after the cisternal injection of 7 ml saline solution. Bar = 1 cm. *Right*: Vessels from a dog with isovolemic double subarachnoid hemorrhage sacrificed at 72 hours after the second injection of blood. The major cerebral arteries were extensively stained with HRP-reactive products. Bar = 1 cm.]
FIG. 2. Electron microscopic view of the proximal part of the intradural internal carotid artery (left) and the basilar artery (right) from a control dog. The arrow indicates a plasmalemmal vesicle containing horseradish peroxidase (HRP)-reactive products at the abluminal front of the endothelium. Arrowheads indicate the interendothelial space partly filled with HRP-reactive products. E = endothelium; SE = subendothelial space; EL = elastic lamina. Bar = 1 μm.

was exposed to the blood stream, however, HRP-reactive products were not evident (Fig. 3A). Plasmalemmal vesicles labeled with HRP were mainly observed in the cytoplasm and at the abluminal front of the endothelial cells (Figs. 3B and C and 4D). The vesicles or pits opening to the vessel lumen were devoid of HRP-reactive products. The HRP-labeled vesicles were also observed connected to the interendothelial space (Figs. 3C and 5). In some specimens, large cistern-like structures filled with HRP-reactive products were noted (Fig. 3D). These may represent channels cut en face. Similar observations of the localization of HRP-reactive products were obtained at the branching points of the major cerebral arteries of dogs with non-isovolemic single SAH or of dogs that received non-isovolemic single SAH or of dogs that received non-isovolemic cisternal injection of saline solution, although the severity of the lesions appeared less than that in dogs with double SAH.

Discussion

The effect of SAH on the blood-arterial wall barrier of the major cerebral arteries in the basal subarachnoid cisterns has not been studied. The present experiments reveal that: 1) the blood-arterial wall barrier in control dogs starts about 1 to 3 mm beyond the point at which the internal carotid and vertebral arteries penetrate the dura; 2) experimental SAH produces extensive disturbance in arterial wall permeability of the major cerebral arteries in the basal subarachnoid cisterns, which was more remarkable in dogs with non-isovolemic single SAH or of dogs that received non-isovolemic cisternal injection of saline solution, although the severity of the lesions appeared less than that in dogs with double SAH.

Responsible for the disturbance of the blood-arterial wall barrier in the major cerebral arteries following SAH. One is a sudden rise in ICP since both the non-isovolemic cisternal saline injection and the non-isovolemic SAH (which produced a sudden rise in ICP) increased the permeability of the major cerebral arteries. A second important factor is the direct effect of subarachnoid clots around the arteries. The marked disturbance of the arterial permeability was observed in dogs with both isovolemic and non-isovolemic SAH. In dogs with isovolemic SAH, the ICP and arterial blood pressure were not increased markedly by the induction of SAH. These observations suggest that the subarachnoid clot around the arteries contributes to the interruption in the blood-arterial wall barrier. Subarachnoid clot produces constriction of the major cerebral arteries resulting in corrugation of the elastic lamina. The corrugated lamina may squeeze the endothelial cells and inhibit the metabolism of the cells. If this occurs, vasoconstrictor substances released into cerebrospinal fluid following SAH may also affect the permeability of the major cerebral arteries. It has recently been revealed in our laboratory (unpublished data) that cisternal injection of 0.3 ng synthetic thromboxane A2 produced acute breakdown of the blood-arterial wall barrier of rabbit basilar arteries. This amount of synthetic thromboxane A2 is comparable to that found in cerebrospinal fluid following SAH. Histamine may also play a role in the barrier breakdown. Previous studies indicate that, in regions of increased hemodynamic shearing stress, there is increased histamine synthesis in the endothelial layer and an increase in vascular permeability. Hemodynamic shearing stress is presumably increased in the major intracranial arteries following SAH, since endothelial bridges and

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FIG. 3. Electron micrographs from dogs with non-isovolemic double subarachnoid hemorrhage (SAH). A and B: The internal artery from a dog sacrificed 72 hours after the second SAH. A: The subendothelial space exposed to the vessel lumen (arrowhead) is devoid of horseradish peroxidase (HRP)-reactive products. Note the endothelial bridge. EL = elastic lamina; SM = smooth muscle. Bar = 3 μm. B: The interendothelial space seen in A (asterisk) filled with HRP-reactive products at higher magnification. Bar = 0.5 μm. C and D: The basilar artery from a dog sacrificed 40 minutes after the second SAH. C: An interendothelial space is almost completely filled with HRP-reactive products. Plasmalemmal vesicles connected to the interendothelial space are also filled with HRP-reactive products. E = endothelium. Bar = 1 μm. D: Note the channel-like structure (arrow) filled with HRP-reactive products. Bar = 1 μm.

marked corrugation of the elastic lamina are frequently seen in these arteries following SAH. The present results, opening of endothelial tight junctions appears to be the most important mechanism responsible for the breakdown of the blood-arterial wall barrier in the major cerebral arteries following SAH. The present electron microscopy studies in SAH dogs demonstrated degenerative changes of the endothelial cells in the major cerebral arteries. Some HRP-reactive products were evident in the interendothelial and subendothelial spaces of the arteries. The HRP-reactive products in the subendothelial space were generally localized in the area beneath the labeled interendothelial space. Occasionally, HRP-reactive products were not evident in the subendothelial space beneath the opened interendothelial junctions.

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FIG. 5. Unstained micrographs of basilar arteries from dogs with isovolemic double subarachnoid hemorrhage (SAH) sacrificed 72 hours after the second SAH. Left: Endothelial cells appear to be squeezed between tight folds of elastic lamina (EL). An interendothelial space is almost completely filled with horseradish peroxidase (HRP)-reactive products from the vessel lumen to the subendothelial space. Some endothelial cells (arrowheads) appear to be degenerated. SM = smooth muscle; E = endothelium. Bar = 1 \( \mu \text{m} \). Right: All three electron micrographs show the staining of interendothelial spaces with HRP-reactive products. L = vessel lumen. Bar = 0.2 \( \mu \text{m} \).

may be that HRP, once having leaked into the subendothelial space through the opened interendothelial junctions, was washed out during the perfusion of the brain with heparinized saline solution and fixatives.

The present electron microscopy studies in SAH dogs also demonstrated the HRP-labeled plasmalemmal vesicles in the cytoplasm and at the abluminal front of the endothelial cells of the major cerebral arteries. Such HRP-labeled vesicles were not found in the major cerebral arteries of control dogs except at the proximal segments of the internal carotid and vertebral arteries and at the branching points of the cerebral arteries. These results suggest that SAH may activate endothelial cell pinocytosis which would contribute to the breakdown of the blood-arterial wall barrier of the major cerebral arteries. However, the present data seem to be inadequate to conclude that endothelial cell pinocytosis is actively participating in the barrier breakdown, particularly since the HRP-labeled vesicles opening at the luminal surface were not observed. The direction of the HRP transport cannot be determined from the present study. The HRP-labeled vesicles observed at the abluminal front and in the cytoplasm of the endothelial cells may represent reverse transport of the HRP; that is, from the abluminal front to the lumen. Further studies will be necessary in order to clarify the role of endothelial cell pinocytosis in the barrier breakdown.

Transendothelial channels are another possible route for HRP leakage into the vessel wall. The channel structures in brain capillaries have been described in acute hypertension and in ischemic conditions. It has also been demonstrated that biogenic amines trigger the mechanism that opens endothelial channels in brain arterioles and capillaries. It is well known that SAH induces acute hypertension and cerebral ischemia and that the level of catecholamines in plasma or cerebrospinal fluid increases after SAH. Furthermore, transport of protein through endothelial channels is a passive process which does not require energy. Based on these facts, it seems reasonable to assume that transendothelial channels participate in permeability changes of the major cerebral arteries following SAH. In the present studies, however, the channel-like structures filled with HRP-reactive products were only occasionally observed. Therefore, transendothelial channels seem to play a limited role, if any,
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in the permeability changes of the major cerebral arteries following SAH.

Breakdown of the blood-arterial wall barrier in the major cerebral arteries following SAH is of special interest with regard to pathogenesis of cerebral vasospasm. Previous studies in SAH patients have demonstrated subendothelial thickening of the major cerebral arteries resulting in a concentric narrowing of the arterial lumen.4,12 Our present results suggest that SAH produces the breakdown of the blood-arterial wall barrier in the major cerebral arteries, which would allow penetration of plasma factors into the vessel wall. Some of these plasma factors may stimulate the proliferation of undifferentiated mesenchymal cells presumably derived from medial smooth muscle, since it is well known that low-molecular-weight plasma lipoproteins23 or a platelet-derived factor24 stimulate smooth muscle proliferation. Furthermore, intravascular vasoactive substances, such as prostaglandins3 or catecholamines,1,16 may also penetrate into the smooth muscle layer and stimulate vasoconstriction of the cerebral arteries, in association with the decreased production of a vasodilator, prostacyclin.19,25

In addition, our results suggest a mechanism to account for the abnormal post-contrast enhancement in the region of the basal subarachnoid cisterns on contrast-enhanced CT, which has been documented in SAH patients.5-7,11,20,27,29,34 In 1978, Fox and Ko7 first reported such abnormal post-contrast enhancement on contrast-enhanced CT in three SAH patients, and suggested a relationship between the abnormal post-contrast enhancement and the development of cerebral vasospasm. Their observation has been explored by several investigators:5,6,10,13,20,27,29,34 It has been reported that the abnormal post-contrast enhancement was clearly observed for several days after SAH1,27,29 and was associated with the development of angiographic spasm, poor clinical condition, and a poor outcome.6,7,29 Thus, contrast-enhanced CT in SAH patients appears to be useful in predicting the subsequent development of clinically symptomatic vasospasm.

However, the pathogenesis of the abnormal post-contrast enhancement is not clearly understood. Two possible mechanisms have been postulated: 1) leakage of contrast medium into the cisterns from the vessels and the aneurysmal wall;7 and 2) meningeal hyperemia,5,20,34 although no evidence has been shown for either hypothesis. The present electron microscopy study demonstrated that HRP injected intravenously passed into the vessel wall of the major cerebral arteries after SAH. Between the subendothelial space of cerebral arteries and the cerebrospinal fluid cisterns, there is no barrier to protein transport.35 These findings support the possibility that contrast medium leaks into the cisterns after SAH, through the vessel wall of the major cerebral arteries, resulting in the abnormal post-contrast enhancement in the region of the basal subarachnoid cisterns on contrast-enhanced CT.

In conclusion, these results revealed that SAH produced the breakdown of the blood-arterial wall barrier of the major cerebral arteries in the region of the basal subarachnoid cisterns. Both subarachnoid clots and a sudden rise in ICP leading to acute arterial hypertension seem to be important factors responsible for the breakdown of the blood-arterial wall barrier following SAH. One of the main routes for the HRP leakage was shown by electron microscopy to be through opened interendothelial junctions.

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References


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