Blood–Brain Barrier Disturbance Following Subarachnoid Hemorrhage in Rabbits

Hiroo Johshita, MD, DMSc, Neal F. Kassell, MD, and Tomio Sasaki, MD, DMSc

We studied disruption of the blood–brain barrier after experimental subarachnoid hemorrhage induced by an injection of 4 ml autologous arterial blood into the cisterna magna of rabbits. The animals were killed at 40 minutes, 6 hours, 1 day, 2 days, 4 days, or 6 days after subarachnoid hemorrhage. We assessed the integrity of the barrier function of intraparenchymal vessels in the ventral brain stem and cerebral hemispheres morphologically with transmission electron microscopy, using horseradish peroxidase as a tracer. In the ventral brain stem, which was in direct contact with the cisternal clots, markedly increased peroxidase staining toward the core of the brain stem was observed on the first day after subarachnoid hemorrhage. In an area of the cerebral hemispheres distant from the clots, barrier disturbance was prominent in the 6-hour specimens, and permeation of the tracer was spotty. From the distribution and morphological findings of these lesions, permeability changes in the ventral brain stem may have been caused by a direct effect of the cisternal clots; in the cerebral hemispheres, hemodynamic factors and changes in intracranial pressure associated with the early stages of subarachnoid hemorrhage seemed to be responsible. These results suggest that barrier disturbance associated with subarachnoid hemorrhage may be multifactorial in time course and location. (Stroke 1990;21:1051–1058)

A neurysmal subarachnoid hemorrhage (SAH) causes various life-threatening complications such as cerebral vasospasm, brain swelling and increased intracranial pressure, edema, and ischemia, all of which may be interrelated. Disruption of the blood–brain barrier (BBB) may play a significant role in the pathophysiology of SAH, because the BBB is important for regulating the internal environment of the central nervous system. However, studies concerning the BBB disturbance associated with SAH are relatively few compared with a variety of studies on vasospasm, and the pathophysiologic significance of the changes in the permeability of intraparenchymal small vessels following SAH has still not been fully elucidated.

To assess the function of the BBB following SAH, we examined changes in the permeability of intraparenchymal vessels in rabbits at varying intervals up to 6 days after experimental SAH, using horseradish peroxidase as a tracer.

Materials and Methods

Twenty-nine male New Zealand White rabbits, each weighing 2.5–3.5 kg, were anesthetized with an intramuscular injection of 6 mg/kg xylazine and 30 mg/kg ketamine hydrochloride. Each animal was intubated, immobilized with 0.1 mg/kg pancuronium bromide, and mechanically ventilated. General anesthesia was maintained with the same mixture of anesthetics. One of the auricular arteries was cannulated with a 24-gauge Teflon catheter for the continuous monitoring of blood pressure and for blood sampling. A marginal ear vein was cannulated for injection of drugs and tracer. Arterial blood gases were checked before the SAH was induced, and arterial pH, Pco₂, and Po₂ were maintained within the physiologic range. Subsequently, the animal was placed in a 30° head-down position. Under aseptic conditions, a 23-gauge butterfly needle was inserted percutaneously into the cisterna magna. Approximately 4 ml fresh autologous arterial blood was introduced over a period of 30 seconds. The rabbit was maintained in that position to allow distribution of the blood in the subarachnoid cistern.

After awakening from anesthesia, the rabbits were examined daily to evaluate their general condition and to check for neurologic abnormalities. For morphological observations, the rabbits were given horseradish peroxidase as described below and divided into experimental groups that were killed under general anesthesia at 40 minutes, 6 hours, 1 day, 2 days, 4 days, and 6 days after SAH. Control rabbits received no intrathecal injection and were killed 20...
mean arterial blood pressure by 30-60 mm Hg. The cisternal injection of blood transiently elevated the rabbits recovered uneventfully from the insult, and electron microscope.

U.S.A. Inc., Peabody, Mass.) or Hitachi HU-12A (Hitachi Scientific Instruments, Mountain View, Cal-

stained with uranyl acetate in a JEOL-100CX (JEOL Ultrathin sections were examined unstained or osmium tetroxide and embedded in Epon 812. ples for electron microscopy were postfixed in 1% peroxide (Graham and Karnovsky procedure9). Sam-

ronds that were incubated 1 hour at 4° C in a medium consisting of 5 mg of 3,3’-diaminobenzidine-tetrahydrochloride, 10 ml of 0.05 M Tris-hydrochloride buffer (pH 7.4) at 37° C. The brain was removed, immersed in a solution containing 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). The brain was cut into 50-μm-thick sections that were incubated 1 hour at 4° C in a medium consisting of 5 mg of 3,3’-diaminobenzidine-tetrahydrochloride, 10 ml of 0.05 M Tris-hydrochloride buffer (pH 7.4), and 0.1 ml of 1% hydrogen peroxide (Graham and Karnovsky procedure9). Samples for electron microscopy were postfixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were examined unstained or stained with uranyl acetate in a JEOL-100CX (JEOL U.S.A., Inc., Peabody, Mass.) or Hitachi HU-12A (Hitachi Scientific Instruments, Mountain View, Calif.) electron microscope.

Results

The mean arterial blood pressure before induction of the SAH was 80.9±2.5 mm Hg (mean±SEM). Cisternal injection of blood transiently elevated the mean arterial blood pressure by 30–60 mm Hg. The rabbits recovered uneventfully from the insult, and no overt neurologic deficits were observed in the experimental groups at the time of fixation. The rabbits killed 40 minutes to 2 days after SAH had thick subarachnoid clots on the ventral surface of the brain stem that were large enough to cover the entire length of the basilar artery. In rabbits killed at 4 and 6 days after SAH, the subarachnoid clots were partly absorbed and remained predominantly on the upper half of the brain stem and the surface of the piriform cortex.

In the control rabbits, no horseradish peroxidase reaction product was observed at the light microscopic level. Electron microscopy showed only limited tracer-labeled vesicles in the endothelium of brain stem arterioles and capillaries. There was no permeation of horseradish peroxidase reaction product into the basal lamina of vessels (Figure 1). In the specimens from rabbits killed 40 minutes after SAH, horseradish peroxidase reaction product was inconspicuous in the ventral brain stem (Figure 2A) at the light microscopic level. Electron microscopy revealed a limited number of plasmalemmal vesicles labeled with tracer in the endothelium of arterioles and capillaries. The perivascular lamina of these vessels was also devoid of horseradish peroxidase reaction product. In the cerebral hemispheres, horseradish peroxidase reaction product was occasionally observed in the thalamus and parasagittal cortex. In specimens taken from rabbits killed 6 hours to 2 days after SAH, a belt-like localization of reaction product directed toward the core of the brain stem was noted along the basilar artery. This horseradish peroxidase extravasation, which was evident by the concentration of reaction product around the perforating arterioles seen under light microscopy (Figure 2B), was most noticeable in specimens taken from rabbits killed 1 day after SAH. The sites of peroxidase leakage observed in brain stem specimens at the light microscopic level were also documented as having massive extravasation of the tracer under electron microscopy (Figure 3). The interendothelial spaces were only partly filled with reaction product. No evidence of endothelial cell damage was observed.

Light microscopic examination of the cerebral hemispheres revealed that spotty staining with reaction product was most remarkable in specimens taken from rabbits killed 6 hours after SAH (Figure 4A). Electron microscopic observations showed that the change in the permeability of the hemispheres was similar to that of the ventral brain stem (Figure 4B). In rabbits killed 1 day after SAH, spotty diffuse staining, which was relatively reduced compared with specimens from rabbits killed 6 hours after SAH, was also observed in the cerebral hemispheres.

In rabbits killed 2 days after SAH, staining with horseradish peroxidase reaction product was observed to be considerably reduced in the ventral brain stem, although electron microscopy showed well-demarcated permeation into the basal lamina of arterioles and capillaries. Further light microscopic examination of sections from rabbits killed on the

### Table 1. Summary of Experimental Rabbit Groups and Results

<table>
<thead>
<tr>
<th>Time after SAH</th>
<th>No. of rabbits</th>
<th>Ventral brain stem</th>
<th>Cerebral cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>4</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>40 minutes</td>
<td>4</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>6 hours</td>
<td>4</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>1 day</td>
<td>4</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>1 day†</td>
<td>2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2 days</td>
<td>4</td>
<td>+</td>
<td>±</td>
</tr>
<tr>
<td>4 days</td>
<td>3</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>6 days</td>
<td>4</td>
<td>±</td>
<td>+</td>
</tr>
</tbody>
</table>

* Rabbits that received no intrathecal injection and were killed 20 minutes after tracer administration.
† Rabbits that did not receive horseradish peroxidase.
Figure 1. Electron micrographs showing an arteriole (panel A) and capillary (panel B) from the ventral brain stem of a control rabbit. No horseradish peroxidase reaction product is present in the basal lamina (bl) of the arteriole or capillary. A tracer-laden vesicle (V) is seen in an endothelial cell of the arteriole. L, lumen; E, endothelium; sm, smooth muscle; sbl, subendothelial basal lamina. Bar, 1 μm.
FIGURE 2. Light micrographs showing sagittal sections (50 µm thick) of the ventral brain stem taken from animals killed 40 minutes (panel A) and 1 day (panel B) after subarachnoid hemorrhage. In panel A, no extravasation of horseradish peroxidase reaction product is present around the arterioles (arrows). However, in panel B, marked extravasation of horseradish peroxidase reaction product is noted, and tracer is present within the walls of penetrating arterioles (arrows). C, cisternal clots. Magnification, ×135.
Figure 3. Electron micrographs showing an arteriole (panel A) and capillary (panel B) from the ventral brain stem of rabbits killed 1 day after subarachnoid hemorrhage. Extensive permeation of horseradish peroxidase reaction product into the basal lamina (bl) and extracellular spaces of the neuropil are evident. L, lumen; E, endothelium; sbl, subendothelial basal lamina; sm, smooth muscle. Bar, 1 μm.
FIGURE 4. Light micrograph (panel A) and electron micrograph (panel B) showing the cerebral cortex from a rabbit killed 6 hours after subarachnoid hemorrhage. Extravasation of horseradish peroxidase reaction product is shown in panel A. In panel B, horseradish peroxidase reaction product is present within endothelial vesicles, the basal lamina (bl), and intercellular spaces of the neuropil. L, lumen; V, a representative vesicle. Magnification (panel A), ×135. Bar (panel B), 1 μm.
fourth and sixth day after SAH showed no staining in the ventral brain stem. In the cerebral hemispheres, occasional staining of the parasagittal cortex was observed.

**Discussion**

The function of the BBB is important in maintaining the integrity of the central nervous system; however, studies concerning barrier disruption associated with SAH are relatively sparse.\(^3\)\(^-\)\(^7\) There has even been controversy with regard to the influence of SAH on the BBB. Peterson and Cardoso\(^2\)\(^,\)\(^4\) reported an inhibitory effect of SAH on preexisting BBB disturbance; Dócz et al\(^5\) and Trojanowski\(^6\) have demonstrated that BBB disturbance evolves in the acute stage of SAH. The results observed in our study are similar to those in the latter reports.\(^5\)\(^,\)\(^6\) With regard to the time course of barrier disruption, SAH appears to be different due to its initial delay. Other insults, such as osmotic opening of BBB,\(^10\) hypertension,\(^10\) trauma,\(^11\) and ischemia,\(^12\) are reported to show permeability changes immediately. Consequently, the dissociation of the experimental findings may have partly originated from the difference in experimental time windows. Peterson and Cardoso\(^2\)\(^,\)\(^4\) examined the effects of SAH in a relatively early phase.

It is interesting to note the intense peroxidase-staining of the perforating arterioles in the ventral brain stem, which was in direct contact with the cisternal clot. This finding is in agreement with that of Dócz et al\(^5\) in both time peak and localization. Several possibilities can be speculated. First, mechanical compression\(^13\) by the clot may have caused this barrier disturbance. However, in that case, mechanical injury may have resulted in changes in the very early stage after insult.\(^13\) The second possibility is a focal susceptibility of the penetrating arterioles,\(^11\)\(^,\)\(^14\) since the ventral brain stem is especially rich in perforating arterioles running vertically to the neural axis.\(^15\) The presence of SAH may have interfered with the circulation of cerebrospinal fluid in the subarachnoid cisterns and perivascular spaces known as the Virchow-Robin space.\(^16\)\(^,\)\(^17\) around these arterioles. Although this possibility is highly speculative, penetration and stagnation of spasmogenic substances\(^18\) or disturbance of extracellular fluid exchange\(^19\) may have caused dysfunction of endothelium (compare with “Results”). Further, these may be responsible for the delay of the barrier disturbance. From the results obtained, the time course of barrier disturbance in intraparenchymal vessels after SAH is considerably different from that of the major cerebral arteries.\(^20\)\(^,\)\(^21\) Even pathophysiologic responses of intraparenchymal vessels to SAH\(^22\)\(^,\)\(^23\) are far less understood than those of the major cerebral arteries.\(^1\) Therefore, it is important for further studies to elucidate the pathophysiologic effects of subarachnoid blood at the arteriolar level.

In the cerebral hemispheres, on the other hand, the most prominent barrier disturbance was found 6 hours following SAH, that is, slightly earlier than in the ventral brain stem. It should also be noted that the distribution of lesions was spotty in its location. The features of the lesion appear to be similar to those of the permeability changes examined by Trojanowski\(^6\) and Shigeno et al\(^7\) who used cat SAH models. We did not assess changes in intracranial pressure and cerebral blood flow in the present study; however, the location and time course of hemispheric lesions not in direct contact with the cisternal clot are likely related to these factors.\(^7\)

In conclusion, the present study suggests a difference in barrier disruption of the intraparenchymal vessels located proximal or distal to cisternal clots following SAH. These phenomena may have possible pathophysiologic implications in relation to brain swelling and microcirculatory disturbances occurring during the early clinical course of patients suffering with SAH.

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**References**


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