Increased Vulnerability of the Blood-Brain Barrier to Experimental Subarachnoid Hemorrhage in Spontaneously Hypertensive Rats

Tamás Dóczi, M.D.,*, Ferenc Joó, M.D.,‡, Sándor Sonkodi, M.D.,† and Géza Ádám, M.D.‡

SUMMARY The influence of chronic arterial hypertension upon the permeability to albumin of the cerebral capillaries, i.e., the blood-brain barrier (BBB), was studied in normotensive Wistar and spontaneously hypertensive rats with experimental subarachnoid hemorrhage. The blood-brain barrier permeability to albumin was assessed quantitatively by spectrophotometric determination of Evans blue extravasation. Subarachnoid hemorrhage was produced by injecting autologous blood into the cortical subarachnoid space. A significant increase of Evans blue albumin extravasation was found in the spontaneously hypertensive rats with subarachnoid hemorrhage as compared with normotensive animals suffering from subarachnoid hemorrhage. Subarachnoid hemorrhage in this model alone caused a significant Evans blue extravasation, whereas sham-operation did not. These findings emphasize the necessity for effective attempts to reduce the leakage of the capillary system in the early stage of subarachnoid hemorrhage.

PREVIOUS CLINICAL STUDIES OBSERVED that the outcome of subarachnoid hemorrhage (SAH) in chronic hypertensive patients was less favourable than in those with normal arterial pressure.1,2 More recently, SAH was found to bring about disruption of the blood-brain barrier (BBB) in about two-fifths of selected SAH patients in the acute stage and the majority of these patients developed vasospasm and ischemic complications in the later phase of SAH, and had a poor prognosis.3-5 The incidence of early BBB disruption was significantly higher in chronic hypertensive patients.6 Under experimental conditions, SAH caused marked BBB damage in cats and rats, although contradictory data have also been published.6-9 The controversy in the literature concerning the state of the BBB after SAH should be ascribed to the fact that induction of SAH in these studies was produced in different ways.10

During acute increases of arterial pressure, when the autoregulatory capacity of cerebral vessels is exceeded, there is an increase in cerebral blood flow and disruption of the BBB.11 Cerebral vessels of spontaneously hypertensive rats (SHRs) were less susceptible to disruption of the BBB during acute hypertension than normotensive Wistar rats, i.e., their capillary system displayed a protective adaptation during the course of acute hypertension.11

The high incidence of BBB disruption in hypertensive SAH patients might be one of the causes of the poor outcome, as the increased capillary permeability not only leads to vasogenic brain edema, but also allows the passage into the brain of substances normally restricted.

The aim of this experimental study was to establish the degree and possible difference of BBB damage to albumin in normotensive and spontaneously hypertensive Wistar rats with SAH.

Materials and Methods

The experiments were performed on adult Wistar and spontaneously hypertensive Wistar rats of either sexes, ranging in weight from 250 to 300 g. The animals were subjected to light ether anesthesia during operations, as well as later, before perfusion and decapitation. The rectal temperature was monitored, and cooling was prevented with an electric heating pad. Arterial blood gases were also monitored. SAH was produced by administration of autologous blood onto the surface of the cerebral cortex. Details of the procedure were published earlier.6,10 In brief, a burr hole was drilled over the right cerebral convexity 3 mm caudal from the coronal and 2 mm lateral from the sagittal suture. Care was taken to keep the dura intact. The burr hole was sealed with bone wax, and the dura was pierced. 200 μl of autologous blood drawn from the tail was injected within 60-80 seconds. The bone wax prevented leakage back through the burr hole. The rats were killed by decapitation. The proper position and the extension of the subarachnoid blood clot were confirmed by various methods: macroscopic evaluation, light microscopic and electronmicroscopic studies. At sham-operation 200 μl of 0.45% saline, or 200 μl of cell free fraction of rat blood (serum) was injected instead of autologous blood. Intracranial (ICP) and systemic mean arterial blood pressure (MABP) were measured in a separate group. A chronic cannula was inserted into the left lateral ventricle. One week later the animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg) and the right femoral artery was cannulated. Both cannulas were connected to Statham P23D pressure transducers, and the ICP and MABP were recorded continuously with a Hellige recorder. The pressures were generally monitored for 6 hours after induction of SAH. The status of the cerebral microcirculation in the rat SAH model was studied previously with carbon black perfusion.6,10 Characterization of the model by means of morphological methods (light microscopic and elec-
tronmicroscopic studies, albumin immunohistochemistry) have been published elsewhere.12

Quantitative assessment of the BBB permeability to albumin was performed 6 hours after production of SAH by spectrophotometric detection of Evans blue albumin: 1 hour after operation 50 mg/kg Evans blue was administered intravenously under light ether anesthesia. Before decapitation of the animals, the Evans blue was washed out through the aorta by perfusion with 0.9% saline in a volume of 100 ml/100 g body weight. The amount of extravasated Evans blue remaining in the brain was determined with the spectrophotometric method of Rössner and Tempel.13

The blood pressure of SHRs was monitored daily by tail plethysmography with a W and W 8005 recorder. The SHRs were classified according to their blood pressure as follows: Group 1: 220-260 mm Hg; Group 2: 150-180 mm Hg; Group 3: below 150 mm Hg. The "control" Wistar groups and the SHR groups were compared by means of the two-tailed unpaired Student's t-test.

Results

With the operation described above, a uniform, reliable, non-invasive simulated SAH with constant blood volume was produced in the rats. The blood clot was confined to the subarachnoid space over the right ipsilateral cortex, and did not extend into the subdural space or into the ventricles. The clinical course was uneventful: the animals were awake and able to drink during the study. The MABP decreased slightly for 5-10 minutes after SAH, but then returned to the control value. The ICP increased to a mean value of 4.7 mm Hg for 10-12 minutes, and then slowly returned nearly to the control levels. Blood gases remained at the control values. Extravasation of Evans blue albumin was performed 6 hours after production of SAH by spectrophotometric detection of Evans blue in untreated or sham-operated SHRs did not exceed that of control or sham-operated animals with blood pressure within normal limits. Evans blue accumulation secondary to SAH was of similar degree in rats with normal blood pressure and in animals with an arterial blood pressure of 150-180 mm Hg. On the other hand Evans blue albumin extravasation was statistically significantly higher in the Group 6 (SAH animals with a blood pressure of 220-260 mm Hg) than in Groups 3, and 8 (SAH animals with normal blood pressure) (p < 0.001) or in Group 7 (SAH animals with a blood pressure of 150-180 mm Hg: p < 0.05). In the most hypertensive rats (blood pressure: 220-260 mm Hg: Group 6), a statistically significant Evans blue albumin extravasation developed 6 hours postbleeding even in the contralateral hemispheres as compared with sham-operated hypertensive animals (Group 4, and 5) (p < 0.05).

Discussion

The influence on the major cerebral arteries of va-soactive substances released from the blood breakdown products in the cerebrospinal fluid after SAH has been evidenced in studies on experimental animals and the ensuing spasm has been claimed to be a pathoge-netic factor in the development of clinical ischemic complications.1, 14, 15 Nevertheless, the effects of these substances on the capillary system, i.e. on the BBB, are not yet fully understood.16 Although some authors have hinted at the possible clinical significance of the involvement of BBB in the pathomechanism of SAH, there is some controversy in the literature as concerns the state of the capillary system in the acute stage of SAH.3 4, 17 The influence of chronic arterial hypertension upon brain capillaries exposed to SAH has not been studied so far.16 The existing experimental SAH models employ larger and more expensive laboratory animals, such as the dog, cat, monkey, etc., in which often, though not always, rupture of a cerebral vessel is employed to produce SAH.18-20 In these cases SAH is often accompanied by a raised intracranial pressure,

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Arterial blood pressure (mm Hg)</th>
<th>Number of animals</th>
<th>Amount of Evans blue (μg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ipsilateral hemisphere</td>
</tr>
<tr>
<td>1. Untreated Wistar rats (control)</td>
<td>below 130</td>
<td>10</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>2. Sham-operated Wistar rats (control)</td>
<td>below 130</td>
<td>10</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>3. SAH in Wistar rats</td>
<td>below 130</td>
<td>10</td>
<td>9.5 ± 1.1†</td>
</tr>
<tr>
<td>4. Untreated SHRs (control)</td>
<td>220-260</td>
<td>5</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>5. Sham-operated SHRs (control)</td>
<td>220-260</td>
<td>5</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>6. SAH in SHRs</td>
<td>220-260</td>
<td>6</td>
<td>18.3 ± 3.0†</td>
</tr>
<tr>
<td>7. SAH in SHRs</td>
<td>150-180</td>
<td>6</td>
<td>12.1 ± 2.0†</td>
</tr>
<tr>
<td>8. SAH in SHRs strain with normotension</td>
<td>below 150</td>
<td>5</td>
<td>10.1 ± 0.7†</td>
</tr>
</tbody>
</table>

Mean ± SD.
* = statistically significant vs control at a level of p < 0.05.
† = statistically significant vs control at a level of p < 0.001.
arterial hypertension, autoregulatory vasodilatation, perfusion defects, etc., any of them alone capable of causing BBB disruption. \(^1\)\(^2\)\(^3\)

In a previous study we investigated the suitability of the rat (a relatively small, easily available, not too expensive laboratory animal) in studies of the effects of a non-invasive simulated SAH, produced by the introduction of blood into the cortical subarachnoid space on the BBB permeability, blood pressure, intracranial pressure and brain perfusion. \(^9\)\(^12\) This approach was taken to ensure an easily standardizable model appropriate for quantitative studies. Cortical subarachnoid administration proved to be a reproducible method, ensuring a constant subarachnoid blood volume with minimal brain damage. The proper location of the blood clot in the subarachnoid space was confirmed by various morphological methods. Our results supported the findings of Krisch et al who used the same route of administration in their morphological studies of the meninges. While the tracer injected below the dura always entered the subarachnoid space, no opening and filling of the subdural space occurred. This was very reasonable, because the virtual subdural space opens only in the vicinity of penetrating vessels as happens in clinical situations. Over the convexity, the virtual subdural space cannot be widened easily, as the injected fluid flows in the direction of least pressure, i.e. towards the CSF space, where its draining away is also best ensured. \(^22\) Internal CSF pathways were not blocked, and the subarachnoid clot lying widely above the hemispherical grey matter (which contains four times more capillaries than the white matter) came into very close contact with the nervous tissue. \(^9\)\(^12\)\(^24\) The non-specific effects of a possible compression injury could be ruled out, as in all experiments (sham-operatives) the blood-brain barrier damage in the acute stage of SAH.

This SAH model was employed in normotensive Wistar and SHRs of the Wistar strain to assess the influence of chronic arterial hypertension and the vasodilatory breakdown products of a subarachnoid clot upon the capillary system. Quantitative determinations of Evans blue extravasation revealed a significant difference between animals with normotension and those with high blood pressure. Evans blue injected into the blood binds to albumin normally confined to the intravascular space. Albumin extravasation not only indicates the development of vasogenic brain edema, with its resulting effects on ICP, brain shift, cerebral perfusion, direct toxic influence on the neuropil, but also shows the pathological passage into the brain of substances normally restricted to the blood stream. \(^25\)\(^26\)

These findings indicate that the frequent development of BBB disruption in hypertensive patients suffering from a subarachnoid hemorrhage may be one of the causes of the worse prognosis of these patients. It emphasizes the necessity for more effective attempts to reduce the leakage of the capillary system in the early stage of SAH.

Acknowledgments
Dr. Dóczi is indebted to Professor Lindsay Symon for initiating and developing his interest in subarachnoid hemorrhage, and the authors are grateful to him for reviewing the manuscript prior to submission.

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Effect of Nimodipine on Cerebral Functional and Metabolic Recovery Following Ischemia in the Rat Brain

Hideo Mabe, M.D., Hajime Nagai, M.D., Terumasa Takagi, M.D., Satoshi Umemura, M.D., and Masahiro Ohno, M.D.

SUMMARY Whether the calcium entry blocker, nimodipine, prevents the increase in the concentration of free fatty acids and metabolic disturbances during ischemia and promotes functional and metabolic recovery after recirculation were examined.

Severe forebrain ischemia in rats was induced by four-vessel occlusion with mild hypotension. After 30 minutes of ischemia, recirculation was started by removal of the arterial clamps and by increasing blood pressure to the preschismic level.

Recovery of EEG activity following recirculation was better in the nimodipine-treated group than in the control group. During the ischemic period, there were no significant differences in accumulation of free fatty acids or in depletion of ATP between treated and control groups. At 120 minutes following recirculation, recovery of the ATP level was significantly better in the treated group than in the control group.

Therefore, the promotion of functional and metabolic recovery by nimodipine-treatment is suggested to be not due to the prevention of an accumulation of free fatty acids nor to the depletion of ATP during the ischemic period, but to either improvement of postschismic hyperperfusion or a direct action on metabolic processes during reperfusion period.

Stroke Vol 17, No 3, 1986

RECENTLY, considerable interest has been centered on the role of calcium in irreversible ischemic brain damage. Ischemic depolarization of cell membrane is associated with a precipitous influx of calcium from the extracellular to the intracellular compartment, and, as a consequence, intracellular calcium increase. The elevated cytosolic calcium induces impairment of the mitochondrial function that leads to failure of ATP production and induces activation of Ca\(^{2+}\) - ATPase resulting in a further reduction of ATP. Increase in Ca\(^{2+}\) leads to activation of phospholipase A\(_2\) with formation of free fatty acids, in particular, arachidonic acid which is metabolized to prostaglandins and leukotrienes. The free fatty acids are assumed to have detrimental effects on mitochondrial and plasma membrane functions and to induce brain edema. Moreover, it is suggested that the products of free fatty acids oxidative metabolism may further aggravate ischemic brain damage during the reperfusion period.

Calcium entry blockers appear to inhibit calcium entry into cells by so-called slow channels to the cell membrane and to prevent metabolic disturbance and accumulation of free fatty acids during ischemia. The present study was designed to test whether a calcium entry blocker, nimodipine, prevents increased free fatty acids and metabolic disturbances during the ischemic period and promotes functional and metabolic recovery after recirculation.

From the Department of Neurosurgery, Nagoya City University Medical School, 1 Kawasaki, Mizuho-cho, Mizuho-ku, Nagoya, Japan.

Address correspondence to: Hideo Mabe, M.D., Department of Neurosurgery, Nagoya City University Medical School, 1 Kawasaki, Mizuho-cho, Mizuho-ku, Nagoya, Japan.
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Stroke. 1986;17:498-501
doi: 10.1161/01.STR.17.3.498

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

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