Short Communications

Does Vasospasm Occur in Small Pial Arteries and Arterioles of Rabbits?

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Background: Vasospasm is a serious complication associated with subarachnoid hemorrhage. Successful management of vasospasm will ultimately depend on a clear understanding of the scope of this phenomenon, including whether arterial elements of different calibers are equally affected. We therefore examined the responses to subarachnoid hemorrhage in rabbit basilar arteries, small pial arteries, and arterioles.

Summary of Report: We compared the brain stem pial arteries of 10 perfusion-fixed male New Zealand White rabbits after experimental subarachnoid hemorrhage to those of five control rabbits using morphological analysis of cross-sections of plastic-embedded vessels. After subarachnoid hemorrhage, the internal elastic lamina was highly corrugated in all basilar arteries (mean diameter 319±51 μm). These arteries were severely constricted in comparison with the control group, in which the mean diameter was 691±17 μm, and corrugation of the internal elastic lamina was not present. In contrast, small pial arteries and arterioles very rarely demonstrated a vasoconstrictive configuration after subarachnoid hemorrhage. The contractility of the smaller vessels was confirmed by injecting 2 mg/kg BaCl₂ intracisternally. Following BaCl₂ injection, corrugation of the internal elastic lamina was detected in the small arteries and arterioles as well as the basilar arteries.

Conclusions: We conclude that experimental chronic vasospasm after subarachnoid hemorrhage in rabbits tends to occur in large conducting arteries rather than in smaller pial arteries and arterioles. (Stroke 1991;22:1419–1425)

Vasospasm after subarachnoid hemorrhage (SAH) was first reported almost 40 years ago, but it still remains a serious problem to be resolved in the management of SAH patients. Vasospasm is generally observed by angiogram, which has unsatisfactory resolution for arteries having an inner diameter of <200 μm. It remains unclear, therefore, how extensively the cerebral arterial tree is longitudinally affected by vasospasm.

Small pial arteries are frequently the subject of study in the investigation of vasospasm using a cranial window technique. The importance of small arteries is emphasized by the speculation that Ca²⁺ antagonists may reduce the ischemic complication of vasospasm by dilating small arteries rather than conducting arteries. One important question remains unanswered, however. Considering recent reports indicating that the pharmacological and physiological characteristics of the small pial arteries and arterioles differ from those of the large conducting arteries, is the reaction of the small arteries and arterioles after subarachnoid hemorrhage similar to that of large conducting arteries? Namely, does vasospasm occur in small arteries and arterioles as in the large conducting arteries?

According to the report of Van Citters et al., vasocostriction is defined morphologically by the deformity of internal elastic lamina, namely, the existence of corrugation. From previous morphometric studies of vasospasm, our attention was drawn to the apparent discrepancy in morphological vasoconstriction between major conducting arteries and small pial arteries. For this brief report, the longitudinal variability of vasospasm in a rabbit SAH model was analyzed.

Materials and Methods

Fifteen male New Zealand White rabbits (3–4 kg body weight) were divided into three groups of five animals each. Experimental subarachnoid hemorrhage was induced in animals as previously described. Briefly, animals were anesthetized with an intramuscular injection of a mixture of 5 mg/kg xylazine and 30 mg/kg ketamine, intubated orotracheally, immobilized with 0.1 mg/kg pancuronium...
FIGURE 1. Electron micrographs of rabbit basilar artery (panels A and B) and surrounding arteries (panels C and D). Panel A: Basilar artery after double subarachnoid hemorrhage (SAH). Panel B: Basilar artery after intracisternal injection of BaCl₂. Panel C: Enlargement of small artery inset in panel A. Panel D: Enlargement of small artery inset in panel B. Note that both basilar arteries are similarly constricted. However, surrounding small artery from double SAH rabbit does not show the constricted configuration, whereas small artery from BaCl₂ rabbit has a highly corrugated internal elastic lamina. Other small arteries in BaCl₂ rabbit appear to be occluded completely (arrows). Bars=100 μm.
bromide, and mechanically ventilated (Rodent Ventilator, model 683, Harvard Instrument Co., South Natick, Mass.). A central ear artery was catheterized with a 22G intravenous catheter to monitor blood pressure and obtain fresh arterial blood. After shaving the nuchal area, the cisterna magna was punctured with a 23G butterfly needle and autologous nonheparinized blood was injected. In the first group, two injections, one of 5 ml and one of 3 ml, were performed at an interval of 2 days. This double-hemorrhage model of SAH was chosen to induce severe vasospasm. The second group was prepared to ascertain whether small arteries have enough contractility to show corrugation of the internal elastic lamina. Rabbits were injected intracerebrally with 2 mg/kg BaCl₂ diluted with 1 ml cerebrospinal fluid. Animals were killed 10 minutes after the injection. The third group was perfusion-fixed without treatment and served as the control group.

Cardiac perfusion-fixation was carried out using 37°C solutions at a perfusion pressure of 100 cm H₂O. The flush solution consisted of 300 ml Hanks’ balanced salt solution and was followed by 500 ml 2% paraformaldehyde and 1.5% glutaraldehyde in Hanks’ balanced salt solution. Immediately before perfusion-fixation, arterial blood Pco₂ levels were confirmed to be within physiological range. Brain stems with surrounding vessels, including the basilar artery, were removed immediately after the perfusion-fixation and were kept in the same fixative for 48 hours. The middle third of the brain stem was blocked horizontally to analyze the configuration of the basilar and surrounding arteries oriented parallel to the basilar artery. The upper third of the brain stem was blocked sagittally to analyze cross-sectional views of the first branches of basilar artery that run tangentially to the basilar artery. Blocked sections of tissue were then embedded in Historesin (LKB-Produkter AB, Bromma, Sweden) for sectioning. Three-micron-thick sections were obtained with a microtome (Supercut, Reichert-Jung, Vienna, Austria) and stained with 0.5% toluidine blue. Representative low-magnification electron micrographs were taken of the lower third of the basilar arteries embedded in Epon using a JEOL-100S electron microscope (JEOL, Tokyo, Japan) to illustrate the more precise morphology of small pial arteries. The inner diameter and the length of internal elastic lamina in the basilar artery and some other arteries were analyzed morphometrically with a computer-assisted image analyzing system (IBAS, Carl Zeiss, Oberkochen, FRG).

Statistical analysis was performed with one-way analysis of variance and Student's unpaired t test.

**Results**

There were no significant differences between the three groups in physiological parameters, including arterial blood Pco₂ (Table 1).

From each animal, we observed more than 20 arteries: in total, we analyzed 135 vessels in the control group, 145 in the BaCl₂-injected group, and 179 in the SAH group. Neither the basilar nor surrounding small arteries of the control group rabbits were observed to be constricted. In SAH rabbits, the ventral side of the brain stem was completely covered with the subarachnoid clot, in which were buried the basilar artery and surrounding pial arteries. All basilar arteries after SAH showed significant vasoconstriction in contrast to control animals (p<0.01). The average inner diameter of the basilar artery (319 µm) decreased to 46% of the control animals (691 µm), and the internal elastic lamina was found to be highly corrugated (Figure 1, panel A). In three of five SAH rabbits, the basilar arteries were constricted to less than 30% of control, which was similar to the magnitude of the BaCl₂-treated group. Nevertheless, smaller pial arteries (diameter <120 µm) rarely showed a constricted configuration after SAH. The smallest artery that showed exceptional vasoconstriction was approximately 80 µm in diameter when it was constricted, whereas arteries less than 80 µm showed no constriction. Based on the morphometrically determined length of the internal elastic lamina, the original diameter of the 80-µm artery was estimated to be about 120 µm. The basilar arteries treated with BaCl₂ were found to be constricted more severely than those of the SAH group, the average diameter being 191 µm. Corrugation of internal elastic lamina was consistently observed, even in small pial arteries of <50 µm (Figure 1, panel B). Furthermore, it was not uncommon that arteries with diameters of 25 µm showed complete obstruction of the lumen when constricted.

Electron microscopic analysis yielded more precise images of the small arteries, showing that corrugation of the internal elastic lamina exists in the small pial arteries after administration of BaCl₂ but not after SAH (Figure 2). This corresponded with the light microscopic findings.

**Table 1. Physiological and Morphometric Parameters of Three Experimental Groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=5)</th>
<th>SAH (n=5)</th>
<th>BaCl₂ (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>3.34±0.05</td>
<td>3.26±0.07</td>
<td>3.32±0.07</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>87±4</td>
<td>78±4</td>
<td>83±5</td>
</tr>
<tr>
<td>Pco₂ (mm Hg)</td>
<td>41.2±2.1</td>
<td>40.4±1.2</td>
<td>40.1±1.2</td>
</tr>
<tr>
<td>Diameter of basilar artery (µm)</td>
<td>691±17</td>
<td>319±51*</td>
<td>191±8*</td>
</tr>
</tbody>
</table>

Data are mean±SE. SAH, subarachnoid hemorrhage. *p<0.01 different from control by Student's unpaired t test.

**Discussion**

Due to insufficient resolution of angiography in the detection of vasospasm, it remains unclear how far the cerebral arterial tree is affected by vasospasm. Despite this fact, small pial arteries have frequently been selected for analysis in the research of vaso- spasm. According to a previous report concerning the contraction of intraparenchymal arterioles after

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FIGURE 2. Electron micrographs of small arteries. The size of both vessels at rest is similar according to length of internal elastic lamina. Panel A: After double subarachnoid hemorrhage. Internal elastic lamina is smooth, but there are vacuoles in endothelial cells (arrow). Panel B: After treatment with intracisternal injection of BaCl₂. Internal elastic lamina is corrugated. Bars=10 μm. Magnification, ×2,100.
topical application of 4–5-day-old whole blood, the control and test groups did not show statistically significant differences in mean arteriolar diameter. It was therefore concluded that the test arteries seem to be in a phase of vasoconstriction, based on the lumen-to-wall ratio. The increase of cerebral blood volume in the SAH patient with vasospasm is attributed to the dilation of small arteries and arterioles while large arteries are constricted.

The morphometric technique has been developed as a suitable method for the investigation of morphological changes of small vessels for which vasospasm is very difficult to visualize with angiography. In light of recent morphometric studies of experimental vasospasm, we have become interested in the configuration of the small arteries. As explained here, morphological observation of arteries after SAH does not show corrugation of the internal elastic lamina, even when the basilar artery has a >70% reduction in diameter. However, small arteries undoubtedly have enough contractility to develop corrugation of the internal elastic lamina because severe vasoconstriction and, occasionally, complete occlusion of small arteries have been observed after the intracisternal injection of BaCl₂, a nonspecific potent smooth muscle contractive agent. Therefore, it is suggested that larger arteries are affected by vasospasm after SAH more readily than smaller arteries.

Initially, before the start of this project, we reviewed material from our previous morphometric studies, including those of contracted monkey middle cerebral arteries and rat basilar arteries after SAH. We found no morphological constriction in the smaller arteries that surround the large conducting arteries. Thus, our observation that vasospasm occurs in large conducting arteries seems to be consistent beyond the interspecies differences.

Although small arteries are not observed to be constricted severely enough to show corrugation of internal elastic lamina, there might be some question of whether they are dilated, rested, or constricted to some extent. The morphometric analysis was not performed in this study because of disadvantages in the combination of Historesin with toluidine blue staining. The internal elastic lamina will not stain and appears as a translucent area, which caused some analytical difficulties in vessels with diameters of <50 μm. In addition, the speed of polymerization of Historesin is affected by both temperature and humidity, which might cause variability. An advanced project using Epon for morphometric analysis of vasospasm after SAH is now being processed and is expected to determine the configuration of the small arteries and arterioles after SAH.

It is well known that a cerebral artery has various characteristics depending on its size, anatomical location, and species. For example, the distribution of γ-aminobutyric acid receptor, α-adrenoeceptor, and the activity of choline acetyltransferase are reported to differ according to precise conditions. It has been shown that there is longitudinal variation of vascular responsiveness to vasoactive substances in the cerebrovascular tree. From the pharmacological viewpoint, serotonin or histamine induce greater contraction in larger arteries than in smaller ones, and, furthermore, smaller vessels tend to dilate more readily than their parent vessels. Therefore, it may be speculated that there exists longitudinal variability in the contractile response to vasospasm after SAH due to the easy-to-dilate, hard-to-constrict character of the smaller artery, which suggests that vasospasm after SAH occurs in a size-dependent manner.

In the clinical setting, pathological changes after vasospasm were considered to affect larger arteries more readily than smaller arteries.

The pathomechanism of vasospasm has been considered to be activated initially by certain spasmogenic substances derived from the clot, which packs the subarachnoid space around the vessels. Both large and small arteries are surrounded by this clot and are assumed to be similarly exposed to the spasmogen. The fact that only larger conducting arteries are constricted cannot be explained to be a result of the spasmogen, and it suggests that the character of artery itself may play an important role in the pathomechanism of chronic vasospasm. Future studies about the discrepancy between large conducting and small resistance arteries in vasospasm are expected to further elucidate the pathomechanism of vasospasm.

Acknowledgments
The authors gratefully acknowledge the technical assistance of Sarah B. Hudson, BS, Grace A. Lehman, BA, and Michi Machi, MD.

References

KEY WORDS • basilar artery • cerebral vasospasm • subarachnoid hemorrhage • rabbits
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Stroke. 1991;22:1419-1425
doi: 10.1161/01.STR.22.11.1419

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