Elastic Skeleton of Intracranial Cerebral Aneurysms in Rats

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In an attempt to clarify the developmental mechanism of cerebral aneurysms, we studied the elastic skeleton of experimentally induced cerebral aneurysms in rats under scanning electron microscopy after hot formic acid extraction followed by freeze-drying. We produced cerebral aneurysms in 19 rats by unilaterally ligating the common carotid artery, inducing renal hypertension, and feeding β-aminopropionitrile fumarate. The first noted change was the loss of folds protruding from the internal elastic lamina. Morphologic changes of the internal elastic lamina, considered to be primarily responsible for aneurysmal formation, occurred after the loss or disintegration of the elastic skeleton of first the intima, then the media. In large aneurysms with thick domes, we found proliferation of elastic lamellae that may reduce the risk of rupture. It seems probable that the complex elastic skeleton of the arterial wall may account for the mechanical properties of the artery and that growth of an aneurysm occurs due to disintegration of the elastic skeleton and not simply to rupture of the internal elastic lamina. We believe that such changes in the elastic skeleton are a property of the functional state of the cells that produce elastin. (Stroke 1990;21:1722–1726)

Although the pathogenesis of cerebral aneurysms is still a matter of debate, many investigators have considered degenerative changes of the internal elastic lamina, including the enlargement of fenestrations, to be responsible for the development of aneurysms.1-4 The results of our previous study of normal cerebral arteries under scanning electron microscopy after hot formic acid extraction5 showed that the internal elastic lamina was not the sole element, but a part of the complicated architecture of the elastic skeleton. Based on these observations, we considered that the process of various vascular pathologies might be re-evaluated by analyzing morphologic changes of the elastic skeleton in cerebral arteries. Our current study was designed to clarify changes of the elastic skeleton at various stages of aneurysmal formation using experimentally induced cerebral aneurysms.6

Materials and Methods

To produce cerebral aneurysms, in 19 male Sprague-Dawley rats aged 8 weeks and weighing 250–300 g we ligated the left common carotid artery and posterior branches of both renal arteries under anesthesia with 40 mg/kg i.p. sodium pentobarbital. One week after surgery 1% saline was substituted for the drinking water, and 2 weeks after surgery, the rats were fed a diet containing 0.12% β-aminopropionitrile fumarate (Tokyo Kasei Co., Tokyo, Japan). We used 12 age-matched rats as controls. Four to 16 weeks after surgery, the rats were perfused with heparinized 0.1 M phosphate buffer (pH 7.4) from the descending aorta, followed by fixation with 3% glutaraldehyde in the same buffer.

After perfusion and fixation, the major arteries at the base of the brain were carefully freed under a microscope and immersed in the same fixative for 24 hours at 4°C. The internal carotid artery bifurcation, the anterior cerebral artery–olfactory artery bifurcation, and the anterior cerebral artery–anterior communicating artery bifurcation on both sides were dissected out and the luminal surfaces were exposed. According to the method of Hass,7 the specimens were incubated in 90% formic acid at 45°C for 60 hours, washed several times in 0.002N HCl to avoid swelling, rapidly frozen in liquid nitrogen, and freeze-dried in a vacuum evaporator. They were then coated with gold-palladium alloy 10 nm thick in an Eiko IB-3 ion coater (Ibaraki, Japan) and examined under an Hitachi S-570 scanning electron microscope (Tokyo, Japan).
Results

Figure 1 shows the nomenclature of the different regions of the cerebral arterial bifurcations.

The major cerebral arteries of the 12 control rats and the ligated side of the 19 experimental rats had one distinct internal elastic lamina, connected with sponge-like medial elastic tissue. The internal elastic lamina had a few fenestrations and folds into the lumen. At the apical intimal pad, the internal elastic lamina was continuous, showing an overall honeycomb appearance.

When observed from the luminal side, eight bifurcations from experimental rats showed shallow depressions that were always located just distal to the intimal pad near the apex (Figure 1). Folds in and around the depressions were decreased in both number and height compared with normal structures in control rats (Figure 2). Residual folds delineated the depressions. The internal elastic lamina was continuous, and there were no apparent changes in the

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

**Figure 1.** Schematic drawing showing nomenclature of different regions of cerebral arterial bifurcations in rats. AP, apex; IP, intimal pad; AC, aneurysmal change.

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Scanning electron micrograph showing earliest change in development of cerebral aneurysm in rats. Shallow depression (*) was located just distal to intimal pad. Fold-like structures in and around depressions decreased in both number and height relative to normal structures in control rats. Internal elastic lamina (open black arrows) and medial elastic skeleton preserved their normal structures. Fenestrations of internal elastic lamina (white arrowheads) did not increase in size or number. ×1,500 magnification.
elastic skeleton of the medial layer. The fenestrations were not enlarged or more common. The intimal pad had no apparent change in its architecture.

In 11 bifurcations from experimental rats, the depressions were apparently deeper than those described above and were slightly invaginated into the vascular wall. The internal elastic lamina was thinned, but still continuous (Figure 3). The intimal pad was involved in most of these invaginations. In the thinned medial layer, septum-like elastic tissue was destroyed. On the luminal surfaces, the folds were markedly reduced in both number and height and were irregularly arranged, although they were larger and more numerous in areas adjacent to the invagination. There was no enlargement of the fenestrations.

In 11 bifurcations from experimental rats, the depressions were much deeper than small invaginations and were recognized as apparent aneurysms bulging from the outside of the artery wall. Not only the apex, but also the distal branches were involved in these aneurysmal bulgings. The internal elastic lamina was disrupted considerably. In some large aneurysms, the aneurysmal wall was thickened and composed of multiple concentric elastic lamellae, which were completely different from the normal media (Figure 4). On the luminal surface, normal folds were completely absent. Fine elastic lamellae were numerous and covered the inner surface of the wall.

Discussion

In our present study, the earliest change in the development of cerebral aneurysms was found to be a loss of folds protruding from the internal elastic lamina. In a more advanced stage, while the internal elastic lamina was still continuous, the medial layer lost its septal structures. Although the nature and significance of the folds are still unclear, their site and size indicate that they are located between
endothelial cells. The area of loss of the folds corresponds to the area of degeneration of endothelial cells in the early stage of aneurysmal formation and appears to correspond to the degenerative change of endothelial cells. Medial sponge-like structures are also located between smooth muscle cells and are destroyed during the course of degeneration of these cells. It is speculated that the degeneration of endothelial and smooth muscle cells is responsible for the alterations of such folds and sponge-like structures.

In previous studies under light microscopy, the internal elastic lamina was disrupted or absent even in a very early stage of aneurysm formation and was considered to play the most important role in maintaining mechanical strength. Our results show that morphologic changes of the internal elastic lamina occur after changes of the elastic skeleton in the intima and media. The fragmentation and disappearance of the internal elastic lamina in the very early stage of aneurysmal development found under light microscopy may be partly due to a loss of stainability and do not mean a simple disruption under scanning electron microscopy.

In rats, there were a few fenestrations of the internal elastic lamina near the apex. Hassler reported that the fenestrations were enlarged at the mouth of aneurysms in humans. Campbell and Roach also proposed that the presence of enlarged fenestrations contributes to the initiation of microaneurysms. Although there are differences in species and fixation methods, our study in rats failed to show enlarged fenestrations not only in the very early stage, but also in relatively well-developed aneurysms. It is therefore difficult to explain the pathogenesis of cerebral aneurysms only from the point of mechanical weakness caused by enlarged fenestrations.
The complex elastic skeleton of the vasculature indicates that the mechanical strength of the vascular wall is supported not only by the internal elastic lamina but also by the elastic skeleton of the media. In large aneurysms, which no longer have an internal elastic lamina, we observed significant proliferation of elastic lamellae. The elastic lamellae appear to serve as a source of mechanical strength in the aneurysm wall beyond that supplied by thickness alone.

**References**


**KEY WORDS** • microscopy, electron scanning • elastic tissue • cerebral aneurysm • rats
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*Stroke.* 1990;21:1722-1726
doi: 10.1161/01.STR.21.12.1722

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/21/12/1722

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