Study of the Elastic Skeleton of Intracranial Arteries in Animal and Human Vessels by Scanning Electron Microscopy

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We studied the elastic skeleton of major cerebral arteries in rats, monkeys, and one human using scanning electron microscopy after hot formic acid extraction followed by freeze-drying. For comparison, we also examined the thoracic aorta and femoral artery of rats. The cerebral arteries of rats had one distinct internal elastic lamina connected to the thin adventitia with sponge-like medial elastic tissue. This internal elastic lamina had fenestrations, which we found to be less frequent in cerebral arteries than in extracranial arteries, and fold-like protrusions into the lumen. This finding has not been recognized before. Such protrusions were more prominent in cerebral arteries than in extracerebral arteries. At the apical intimal pad, the internal elastic lamina appeared to be continuous, making a honeycomb-like structure. The folds and fenestrations were numerous at the apex. There were no essential differences among species. Our study shows that the internal elastic lamina is not a simple sheet but part of the complicated architecture of the elastic tissue of the vessel wall. These differences in the elastic skeleton, including fenestrations and fold-like structures, in various sites of different arteries may explain the development of various localized vascular diseases. (Stroke 1990;21:765–770)

Classically, the elastic tissue of the cerebral vasculature has been recognized as only a major structural component contributing to elasticity and tensile strength.1–3 Recent studies indicate that the internal elastic lamina may also act as a barrier to the diffusion of large molecules such as fibrinogen and low density lipoprotein.4,5 The internal elastic lamina also has fenestrations between the intimal and medial layers6 that may be related to its mechanical properties and may permit the exchange of cellular and noncellular components through it.

Degenerative changes of the internal elastic lamina are considered to play a primary role in the development of cerebral aneurysms.7–9 During the development of atherosclerosis, elastic tissue is remodeled, which includes formation of new intimal plaque and degradation of the medial elastic lamellae.10–12 Other pathologic changes of the cerebral arteries, such as vasospasm after subarachnoid hemorrhage,13 fusiform aneurysm,7 arteriovenous malformation,14 fibromuscular dysplasia,15 or moyamoya disease,16 also accompany changes of elastic tissue. Quantitative and qualitative changes of the elastic tissue of arteries should be more clearly elucidated in these vascular diseases.

We demonstrate the three-dimensional architecture of the elastic tissue of normal rat cerebral arteries by scanning electron microscopy, using the hot formic acid extraction method.17,18 We compare the structure of rat cerebral arteries with that of rat extracranial arteries and with that of cerebral arteries from monkeys and a human.

Materials and Methods

We used 25 male Sprague-Dawley rats aged 2–6 months and weighing 250–520 g. All rats were sacrificed under 40 mg/kg i.p. sodium pentobarbital anesthesia and were perfused with heparinized 0.1 M phosphate buffer (pH 7.4) from the descending aorta at a constant pressure of 120 mm Hg, followed by fixation with 3% glutaraldehyde in the same buffer. After perfusion-fixation, the major arteries from the base of the brain, the aorta, and the femoral artery were carefully dissected free 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 also dissected free. The aorta and femoral artery were cut transversely into several semicircular pieces. According to the method of Hass,17 several specimens of the rat cerebral arteries and all specimens of the extracranial arteries were incubated in 90% formic acid at 45°C for approximately 60 and 100 hours, respectively. The incubation period was chosen from a pilot study. The extracellular matrix other than elastic tissue and all cellular components were completely dissolved during the incubation.

We obtained the major cerebral arteries from two female monkeys (Macaca fascicularis) estimated to be 5 years of age and weighing 3.0 and 3.5 kg and from one woman 52 years of age with no known cerebrovascular disease. At autopsy, the dissected brains were perfused and fixed through the basilar artery. We then dissected out the internal carotid artery–posterior communicating artery bifurcation, the internal carotid artery–anterior choroidal artery bifurcation, and the internal carotid artery bifurcation as well as the first and second major bifurcations of the middle cerebral artery and the anterior cerebral artery–anterior communicating artery bifurcation on both sides. The specimens were incubated for approximately 100 hours in hot formic acid.

For electron microscopy, specimens treated with hot formic acid were washed several times in 0.002N HCl,18 rapidly frozen in liquid nitrogen, and freeze-dried in a vacuum evaporator. The specimens were then coated with a gold-palladium alloy to a thickness of 10 nm in an Eiko IB-3 ion coater (Ibaraki, Japan) and examined under a scanning electron microscope (S-570, Hitachi, Tokyo, Japan). For light microscopy, the rat cerebral artery specimens not treated with hot formic acid were postfixed in 1% osmium tetroxide in the phosphate buffer for 1 hour, dehydrated in a graded series of ethanol, and embedded in epoxy.
FIGURE 2. Scanning electron micrographs showing elastic skeleton of internal carotid artery of rat, luminal views. Left: Distal to apex. Fold-like structures (white arrows) run basically parallel to longitudinal axis of artery and are sometimes divided or fused with each other. Fenestrations of internal elastic lamina are rarely found. X2,000. Right: Near apex. Fold-like structures are more developed than in distal portion. Fenestrations of internal elastic lamina (white arrows) are not frequent. X2,000.
FIGURE 3. Light micrograph of intimal pad in anterior cerebral artery–olfactory artery bifurcation of rat, cross-sectional view. At intimal pad, internal elastic lamina is fragmented into two or three layers. x400, toluidine blue stain.

Results

The major cerebral arteries of the rats had one distinct internal elastic lamina approximately 1 μm thick and no apparent external elastic lamina. The media had radially oriented fibrous or septum-like elastic tissue connecting the internal elastic lamina and the thin adventitia (Figure 1). On the luminal surface of the internal elastic lamina, fold-like structures approximately 3–4 μm high and 0.5 μm wide protruded from the lamina and ran basically parallel to the longitudinal axis of the artery. However, the fold-like structures were often divided or fused with each other (Figure 2), and fenestrations, varying in diameter from 2 to 10 μm, were seen sporadically on the surface of the internal elastic lamina.

At the apex of bifurcations of the rat cerebral arteries, the intima protruded markedly into the lumen. Such an intimal pad was located just distal to the apex on the side of the major branch (Figure 1). Under light microscopy the internal elastic lamina was fragmented into two or three layers (Figure 3), but under scanning electron microscopy the laminae were not fragmented but connected with thin septa, making many small chambers and showing an overall honeycomb appearance; small or large defects of the septa connected the chambers (Figure 1). Around the intimal pad, the fold-like structures were larger and more numerous than in other areas. The fenestrations of the internal elastic lamina were also larger and more numerous here than elsewhere along the artery (Figure 2, right). Findings were essentially the same in all rat intracranial artery bifurcations examined.

The aorta and femoral arteries of the rats were composed of multiple concentric elastic laminae, including the internal and external elastic laminae. Each lamina was 0.5–2.0 μm thick. The adventitia of the extracranial arteries was composed of randomly oriented elastic lamellae that were much more abundant than in the cerebral arteries. On the luminal surface, there were numerous fenestrations varying from 2 to 20 μm in diameter but only sparse fold-like structures.

In the cerebral arteries of the monkeys and the human, the internal elastic lamina was double at several points and the media and adventitia were thicker than in the rats. Although the intimal pad was not apparent as a bulge, it was recognized as a complicated elastic structure at the apex, as in the rats. Fenestrations and fold-like structures were also numerous at the apex (Figure 4).

Discussion

We demonstrate that the structure of elastic tissue in both the cerebral and the extracranial arteries is much more complicated than classically recognized. Although transmission electron microscopy has shown details of the structure of elastic tissue in arteries, such studies have not demonstrated the elastic tissue's complex three-dimensional appearance.
To visualize the elastic skeleton under scanning electron microscopy, it is necessary to eliminate other tissue components, including endothelial cells, smooth muscle cells, collagen fibers, and ground substances. Several methods have been developed for this purpose. We adopted Hass's hot formic acid extraction method of isolating elastic tissue from perfused and fixed materials to preserve their fine and fragile structure. The residua after hot formic acid extraction is considered to be elastic tissue, although this has not been confirmed by biochemical qualitative analysis.

Fold-like structures protruding from the internal elastic lamina were more prominent in the cerebral arteries than in the extracranial arteries. Several studies on the elastic skeleton of extracranial arteries after extraction failed to visualize such folds. This may be partly due to the rarity and small size of folds in the extracranial arteries or to differences in extraction conditions. The size, interval, and well-defined margin of the folds indicate that they were located between endothelial cells. The folds were more prominent at the apex, where degeneration and regeneration of endothelial cells are most apparent. Although the nature and significance of such folds should be elucidated further, it is possible that they reflect the condition of cells lining the internal elastic lamina.

Near the apex of cerebral arteries of the rats, an intimal pad composed of smooth muscle cells covered by endothelial cells was clearly identified as a marked protrusion into the lumen. Under light microscopy, the internal elastic lamina appeared to be fragmented into two or three layers. These results show that the internal elastic lamina with its underlying elastic tissue forms a complicated honeycomb network of elastic tissue not only in rats but also in monkeys and a human. In contrast to the findings of light microscopy, the elastic skeleton at the intimal pad appears to be able to resist hemodynamic or mechanical stresses.
Large fenestrations have been considered to weaken the internal elastic lamina of cerebral arteries. New research shows that the elastic skeleton of cerebral arteries is composed, not of a simple sheet of elastic lamina, but of a more complex structure. This finding indicates that simple enlargement of the fenestrations does not affect the mechanical strength of the vascular wall but may be responsible for the exchange of cells and substances between the intimal and medial layers.

Although our study shows only morphologic aspects of the elastic skeleton of cerebral vessels, further studies in various vascular pathologies, especially of their early stages, may reveal the functional roles of each structure more clearly.

References

Key Words • microscopy, electron, scanning • cerebral arteries • elastic tissue
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