Involvement of Internal Elastic Lamina in Development of Induced Cerebral Aneurysms in Rats

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To elucidate the role of the internal elastic lamina in the development of cerebral aneurysm, the bifurcation of the anterior cerebral artery and olfactory artery was histologically studied in control and experimental rats treated with unilateral carotid ligation and renal hypertension. Various stages of aneurysm formation were compared, and it was found that early aneurysmal changes were always present just distal to the apical intimal pad on the anterior cerebral artery side. The internal elastic lamina was thinned and fragmented just distal to the pad even in the very early stage of aneurysm formation when the medial layer was still present. In control rats, the internal elastic lamina had a tendency to thin and fragment at the site where aneurysms would develop in experimental rats. Our study shows that changes of the internal elastic lamina were present just distal to the pad even in control rats, which never develop cerebral aneurysms. Under hemodynamic stress augmented by experimental treatments, further degenerative changes of the internal elastic lamina and involvement of the medial layer are considered to occur and result in aneurysm formation there. (Stroke 1988;19:507-511)

Materials and Methods

Cerebral aneurysms were produced in 13 male Sprague-Dawley rats ranging from 7 to 9 weeks of age.

ACA-OA Junctions in Control Rats

Most components of the arterial wall were weakly stained gray, while elastic fibers were strongly stained a dark blue.

In each rat, the left common carotid artery and the posterior branches of both renal arteries were ligated under 40 mg/kg i.p. sodium pentobarbital anesthesia. One week after the operation, 1% saline was substituted for drinking water. The controls were 21 age-matched rats. All rats were killed approximately 3 months after the operation.

At the end of the experiment, the rats were perfused with heparinized saline from the descending aorta, followed by a mixed solution of 2% glutaraldehyde and 1.5% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After perfusion fixation, the major arteries at the base of the brain were carefully freed under a dissecting microscope. The anterior cerebral artery-olfactory artery (ACA-OA) junctions on both sides were removed for histologic examination. The specimens were immersed in a mixture of 2% tannic acid and 2% glutaraldehyde for 24 hours, washed in 0.1 M phosphate buffer (pH 7.4), and postfixed in 0.1% osmium tetroxide for 1 hour. After dehydration in graded concentrations of alcohol, they were embedded in epoxy resin. Serial semithin (1-μm) sections were stained with 1% toluidine blue for light microscopy.

The nomenclature of the various branching vessels is shown in Figure 1.

Results

ACA-OA Junctions in Control Rats

Most components of the arterial wall were weakly stained gray, while elastic fibers were strongly stained a dark blue.

The ACA-OA junctions in control rats had an acute angle ranging from 20 to 50 degrees. The lateral angle was much wider on the OA than on the ACA side. At the bifurcation, the ACA was about two times larger
in diameter than the OA. The walls of these arterial bifurcations were composed of endothelial cells, the internal elastic lamina, the medial muscle layer, and the adventitial layer. The internal elastic lamina was thick, smooth, and strongly stained, and the medial layer consisted of three to five strata of smooth muscle cells.

In the apical region, there was an intimal protrusion, or pad, consistently located near the apex on the distal side of the ACA. This pad was composed of spindle-shaped cells similar to the medial smooth muscle cells, rich in interstitial tissue. The internal elastic lamina was continuous along the curvature of the apex, while at the proximal margin of the intimal pad it was split into several layers and considerably fragmented under the intimal pad. Just distal to the intimal pad on the side of the ACA, the internal elastic lamina was thinned and fragmented for a short distance; further distally, it was again thick, smooth, and strongly stained. Thus, there always existed a portion without intact internal elastic lamina under and just distal to the intimal pad on the side of the ACA. Around the apex, a defect of the medial layer was not found in any bifurcation in the control group (Figure 2).

**ACA-OA Junction in Experimental Rats—Nonligated Side**

Twelve ACA-OA junctions of the nonligated side were studied in 13 experimental rats, and various changes related to aneurysmal formation were observed.

In two bifurcations, there were apparent aneurysmal bulges near the apex. The walls of the bulges were composed of connective tissue, and normal arterial components such as smooth muscle cells and the internal elastic lamina were not recognized. The internal elastic lamina and the medial muscle layer either ended abruptly at the entrance to the aneurysmal bulges or tapered off. In both bifurcations, the intima was thickened at the entrance to the aneurysm (Figure 3).

In three bifurcations, a small evagination of the arterial wall was found just distal to the apex on the side of the ACA. The intimal pad was either just proximal to this evagination or partly involved in the wall of the evagination, which was composed mainly of connective tissue. Under the intimal pad or just proximal to the entrance of the evagination, the internal elastic lamina was split into layers and tapered off. At and distal to the entrance of the evagination on the ACA side, a thin, fragmented, and weakly stained internal elastic lamina was observed. Further distal, a thick and strongly stained internal elastic lamina was identified. At the entrance, the medial muscle layer tapered into the wall of the lesion. The defect of the internal elastic lamina was always wider than that of the medial layer at the evaginations (Figure 4).

In five bifurcations, a shallow but apparent depression of the luminal aspect of the arterial wall, which we previously described as a juxta-apical depression, was found just distal to the intimal pad on the side of the ACA. At the proximal margin of the intimal pad, the internal elastic lamina split into several layers, then fragmented and disappeared under the pad. In the shallow juxta-apical depression, fragmented and weakly stained elastic tissue was visualized with

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**FIGURE 1.** Nomenclature of various parts of anterior cerebral artery-olfactory artery junction. OA, olfactory artery; ACA, anterior cerebral artery; Ap, apex; IP, intimal pad.

**FIGURE 2.** Anterior cerebral artery-olfactory artery (ACA-OA) junctions in control rats. Intimal pad (large arrow) is just distal to apex on distal side of ACA. Internal elastic lamina is thinned and fragmented for short distance just distal to intimal pad (small arrows). Defect of medial layer is not found (×280).
difficulty. The medial layer was thinner than that in other portions and was intermingled with connective tissue (Figure 5).

Two bifurcations showed neither aneurysmal bulges nor shallow depressions distal to the intimal pad on the side of the ACA. At these sites, however, the internal elastic lamina was thinned and partly fragmented to a more significant degree than in the controls. The underlying medial muscle layer was as thick as that in the controls, and focal fibrosis was observed (Figure 6).

**ACA-OA Junction in Experimental Rats — Ligated Side**

Twelve ACA-OA junctions of the ligated side were studied in 13 experimental rats. Neither apparent aneurysmal bulges nor shallow depressions were observed.

Just distal to the intimal pad on the side of the ACA in these bifurcations, the internal elastic lamina was thinned and fragmented in the same fashion as in the bifurcations without bulges or depressions of the contralateral side. Here, the medial muscle layer was as thick as that in the controls (Figure 7).

**Discussion**

Degenerative changes of the internal elastic lamina have been thought to play an essential role in the development of cerebral aneurysms. These changes have been considered in relation to the so-called medial defect or gap and have generated three hypotheses concerning the mechanism of degeneration of the internal elastic lamina and aneurysm formation. One hypothesis is that cerebral aneurysms are "acquired" lesions produced by the disintegration of the internal elastic lamina, resulting from its overdistension at the medial defect. Another hypothesis is that these changes are usually due to atheroma and saccular aneurysms develop at the site of elastic degeneration only when a congenital medial defect coexists there by chance. The third hypothesis is that focal degeneration of the internal elastic lamina is caused by atheroscler-
To clarify the developmental mechanism of cerebral aneurysms, early aneurysmal changes should be studied. Using our rat model of the disease, it is possible to study many bifurcations at the same anatomic location in animals under the same biologic conditions. In our study, histopathologic changes of the ACA-OA junction, one of the most frequent sites of aneurysm development, were compared among experimental rats and between experimental and control rats.

Various changes related to aneurysmal development at the ACA-OA junctions in rats were classified into four groups according to the degree of changes in the wall: 1) an apparent aneurysmal bulge, 2) a small evagination, 3) a shallow depression, and 4) no depression. These early aneurysmal changes were always found adjacent to the apex on the distal side of the ACA. In cases of apparent aneurysmal bulge and small evagination, both the internal elastic lamina and the medial muscle layer ended or tapered off at the entrance to the aneurysm. The aneurysmal wall was composed of connective tissue. In the walls of shallow depressions, the internal elastic lamina was thinned, fragmented, and partly absent. The underlying medial layer was continuous throughout the lesion, although it was thinner than that at other portions.

As shown in this article, aneurysmal development was clearly demonstrated in various stages from a fully developed aneurysm, to a small evagination, to a shallow depression. The site of aneurysmal formation was always restricted to the site just distal to the apical intimal pad. It was apparent even in arteries without any depression that the internal elastic lamina was thinned, fragmented, and partly absent at this site. Under this degenerated internal elastic lamina, the medial muscle layer looked intact. Thus, it can be said that the portion with degenerated internal elastic lamina is the site of a very early stage of aneurysmal development.

It is interesting that even in the ACA-OA junction of control rats, the internal elastic lamina at the site where aneurysms developed in experimental rats also showed some degenerative changes such as thinning and fragmentation. Our previous study with scanning electron microscopy showed that a shallow juxta-apical groove was present even in control rats. In our present study, the site of the juxta-apical groove was shown to correlate with the portion of thinning and fragmentation of the internal elastic lamina just distal to the apical intimal pad. Although the mechanism of degeneration of the internal elastic lamina at the juxta-apical groove is not clear, both previous and present studies show that the site of degeneration of the endothelial cells correlates with the site of degenerative changes of the
internal elastic lamina and that aneurysms develop from this portion distal to the intimal pad.

We have reported that in experimental aneurysms many leukocytes were present adhering to the interendothelial gaps, which may represent the participation of leukocytes in degradation of the elastica. Cajander and Hassler also found extracellular lysosome-like granules closely connected to the disintegrated elastic lamella in the mouths of aneurysms and hypothesized that discharged leukocyte granules containing elastase help to destroy the elastic lamella. Enhanced activity of elastase in the arterial wall may also participate in the degenerative changes of the internal elastic lamina, as in the case of hypertension.

In conclusion, the thinning and fragmentation of the internal elastic lamina were proved to be ubiquitously present in rats at the site just distal to the apical intimal pad. Under hemodynamic stress augmented by experimental treatments, further degenerative changes of the internal elastic lamina and involvement of the medial muscle layer are considered to result in aneurysmal development there.

References

KEY WORDS • cerebral aneurysms • rats • internal elastic lamina
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