Early Changes of Experimentally Induced Cerebral Aneurysms in Rats: Scanning Electron Microscopic Study

Masayuki Kojima, M.D.,* Hajime Handa, M.D.,* Nobuo Hashimoto, M.D.,* Choegon Kim, M.D.,* Fumitada Hazama, M.D.†

SUMMARY To obtain information about the early changes of experimentally induced cerebral aneurysms in rats, the luminal surface of branching areas of their cerebral arteries was examined with a scanning electron microscope. At the branching sites of major cerebral arteries in the control animals, the intima just distal to the apex markedly protruded into the lumen forming a linear bank-like intimal pad. Along and distal to this pad, there was a shallow long groove (juxta-apical groove). Such grooves were much deeper and wider in experimental animals than those in the control rats. By studying various stages of early aneurysmal changes, cerebral aneurysms were proven to develop from such grooves. In deep juxta-apical grooves and small aneurysms, round regenerated endothelial cells with a large number of microvilli were diffusely present. Degenerated cells with balloons and craters were observed intermingled with such regenerated cells. Interendothelial gaps were also seen.

The present study showed the complex structure of the apex of arterial bifurcation in rats, including bank-like intimal pads. Such complex structures of the branching sites were considered to be responsible for the initiation of cerebral aneurysms due to endothelial injury possibly caused by turbulent flow there.

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THE ETIOLOGY and pathogenesis of saccular cerebral aneurysms are still debated. Many investigators have proposed various hypotheses such as medial defect, elastic lamellar, degenerative hypotheses, etc. Without studying early aneurysmal changes, the mechanisms of aneurysm formation and development cannot be clarified. However, it is very difficult to know whether minute pathological changes at the apex of human cerebral vessels obtained at operation or autopsy would ultimately develop into saccular aneurysms.

We have successfully induced cerebral aneurysms in rats by ligating one common carotid artery, inducing hypertension and/or administering β-aminopropionitrile (BAPN). We showed that hemodynamic stress, hypertension and abnormal metabolism of connective tissue play important etiological roles in the development of cerebral aneurysms. Using this animal model, it is now possible to study the developmental sequence of cerebral aneurysms.

Endothelial injury is known to play an important role in the development of atherosclerosis. We have shown that endothelial injuries induced by hypertension cause further vascular degenerative changes. It is also likely that endothelial injury plays a significant role in the development of cerebral aneurysms.

The purpose of the present study was to examine the intimal changes involved in the development of cerebral aneurysms. Using a scanning electron microscope (SEM), the luminal surfaces of branching areas of cerebral arteries were studied in rats in various stages of early aneurysmal formation. In some cases, plastic
A schematic drawing of the preparation of branching sites of the ACA-OA junction. The branching sites of the major cerebral arteries were cut along thin solid lines to expose the luminal surfaces. The arrow indicates the direction of observation of the lumen with SEM. ACA: the anterior cerebral artery, OA: the olfactory artery.

casts of such arteries were also studied with SEM to obtain further information about the three-dimensional structure of the apex of cerebral arterial bifurcations.

Materials and Methods

Aneurysms were produced in rats by the method of Hashimoto et al. In summary, 7-week-old male Sprague-Dawley rats were treated by ligation of the left common carotid artery and the posterior branch of both renal arteries under sodium pentobarbital anesthesia (50 mg/kg i.p.) in order to alter cerebral hemodynamics and induce hypertension, respectively. One week after the operation, 1% saline was substituted for drinking water to enhance the degree of hypertension.

For direct SEM observation of the luminal surface of branching sites, 20 rats, 2 to 12 weeks after operation and 10 age-matched control animals were perfused through the abdominal aorta with heparinized saline, followed by a solution of 2% glutaraldehyde and 1.5% paraformaldehyde in 0.1M phosphate buffer (PH 7.4). After perfusion fixation, the bifurcation of the internal carotid artery (ICA) and the anterior cerebral (ACA)-olfactory artery (OA) junction of both sides were dissected free from the brain. The specimens were immersed in the same fixatives for 24 hours. The luminal surfaces of these branching areas were exposed as shown in figure 1. They were then washed in 0.1 M phosphate buffer (PH 7.4) 3 times and postfixed in 1% OsO₄ for one hour. After dehydration in a graded concentration of alcohol, they were passed through isopropyl acetate, dried by the critical point dryer, coated with gold palladium and examined under a SEM (Hitachi Model S-500 AS).

Plastic casts of the arteries were made by the injection of polyester resin (Mercox CL-2R-5, Dainippon Ink & Chemical Inc.). For this purpose, 11 operated and 9 control rats were used. Immediately after perfusion fixation, 20 ml of polyester resin with hardening agent was injected through the abdominal aorta. Two hours later, the brain was taken out and kept in 20% NaOH solution for 2 days to dissolve the brain tissue and obtain arterial casts. In an ultrasonic cleaner, debris on the arterial casts was completely removed. Casts of the ICA bifurcation and ACA-OA junction were taken, coated with gold palladium, and examined under the same microscope.

Immediately before sacrifice, blood pressure was measured by the tail-cuff, auto-pickup method in an unanesthetized state.

Results

a) Blood Pressure

In the operated animals, blood pressures (mean ± SD) were 160 ± 26 mmHg, 176 ± 17 mmHg, 220 ± 12 mmHg, and 216 ± 15 mmHg, at 2, 4, 8, and 12 weeks after the operation, respectively. The blood
pressure of the control rats was 96 ± 10 mmHg. Irrespective of timing of sacrifice, blood pressures in the operated rats were significantly higher than those in the control ones (p < 0.005).

b) Direct Observation of Luminal Surface

1) Control Animals

In the control animals, the intima of the ACA just distal to the apex of the ACA-OA junction markedly protruded into the lumen and this protrusion extended obliquely to the lateral angle forming a linear bank-like intimal pad (fig. 2). At the center of the apex, the protrusion was less prominent than the remaining parts. Most endothelial cells in this region were oriented longitudinally, but some were not. Endothelial cells on the protrusion were round, thick, and, in parts, polypoid in appearance. Some of them showed mild degenerative changes such as crater-like depressions and balloon-like bulges.

Just distal to this apical protrusion, a shallow long groove was always found (juxta-apical groove) (fig. 2). Endothelial cells within this groove were flat with numerous finger-like or granular microvilli, particularly in the center of these cells. Endothelial cells on the surface distal to the groove were also flat, but with sparse microvilli.

At the ICA bifurcation of the control animals, the bank-like intimal pad and juxta-apical groove were not so marked as those at the ACA-OA junction, but endothelial cells in the apical area were also round and thick. Those of the middle cerebral artery (MCA) and the ACA were flat and often had a small number of microvilli and marginal folds.

2) Treated Animals

On the ACA branch at the ACA-OA junction of the non-ligated side, intimal pads in the treated animals were more prominent than those in the control. In half of the treated rats, a shallow and narrow transverse groove was located between the apex and intimal pad (fig. 3A&B).

In three of 20 treated animals, full berry aneurysms were observed at the apex of bifurcations under the dissecting microscope. On SEM, they were located just distal to the intimal pads. Another 17 cases had no outward bulge macroscopically, but in 12 of these, the juxta-apical grooves were much deeper and wider than those in the control animals (fig. 3A&B). In the other 5 rats, the grooves were not different from those in the controls. Occasionally the grooves showed a deep, focal indentation laterally (fig. 4).

In only two of 20 ACA-OA junctions on the ligated side, the juxta-apical grooves were deep and wide, and only one of 20 ICA bifurcations on the non-ligated side had a definite groove.

On the bank-like intimal pads in the treated rats, endothelial cells were round and polypoid in shape (fig. 5A). They were not well oriented along the longitudinal axis of the vessel. Some had many small, shallow craters and were devoid of microvilli. Clusters of
platelets with long processes often adhered to the surface of the pad (fig. 5B).

The surface of deep juxta-apical grooves at the ACA-OA junction on the non-ligated side were rugged and consisted of irregularly arranged, round endothelial cells (fig. 6). Microvilli were most numerous in the center of these cells over the nucleus. At a higher magnification, these microvilli were mushroom-shaped. Some endothelial cells were atrophic and devoid of microvilli. They were intermingled with cells with a large number of microvilli. Some cells were completely collapsed and connected each other by interendothelial bridge-like structures due to enlargement of the interendothelial gaps. On these cells, many small and shallow craters were also visible.

On the distal edge of the grooves, endothelial cells were also round and irregularly arranged. Some of them had balloon-like protrusions or large and deep craters (fig. 6).

Endothelial cells distal to the branching area were flat and regularly arranged along the longitudinal axis of the vessel. These cells had a large number of microvilli and linear marginal folds. Degenerated cells with balloons or craters were less frequent than those on the pads and grooves.

At the ICA bifurcation on the non-ligated side in the treated animals, shallow juxta-apical grooves were occasionally observed on the MCA just distal to banklike intimal pads. The grooves were not so marked as those at the ACA-OA junction, while the endothelial
cells over the grooves also showed degenerative changes. Even in the branches without such grooves, endothelial cells also showed degenerative changes at the place where shallow grooves were expected.

c) Indirect Observation of Plastic Casts

In the control animals, casts of the ACA-OA junction showed deep linear imprints in the area corresponding to the bank-like intimal pad found on the luminal surface of the arteries (fig. 7A). Such imprints were found in every case and were almost uniform in size and shape. They started from the apical area and extended through the face and dorsum of the ACA to the lateral angle. At the ICA bifurcation, such structures reached the face and dorsum of the MCA, but not the lateral angle (fig. 7B). Their size and shape varied from one animal to another, but they were not so marked as those at the ACA-OA junction.

Findings of the casts of the ACA-OA junction in the operated rats correlated well with the findings of the luminal surface. Small outward bulges at the ACA-OA junction on the non-ligated side were found in 7 of 11 operated animals. They were located distal to the linear imprints of the bank-like intimal pads and were crescent-like or semioval in shape (fig. 8A&B). Endothelial cells over the surface of such outward bulges were irregularly oriented and had rugged surfaces. In cases with advanced bulges, the imprint reflected the bulges. Such changes were not found at the ICA bifurcation on the non-ligated side or the ACA-OA junction on the ligated side.

Discussion

Intimal thickenings are known to be present at the branching sites of the major cerebral arteries, not only in man but also in various animals. Various names have been given to these structures, such as intimal pads, intimal cushions, intimal proliferations, etc. The pathophysiological significance of such structures is unclear. They have been regarded as physiological structures which play an important role in the regulation of blood flow. On the other hand, it has been pointed out that intimal pads are formed in early life, possibly as a result of focal hemodynamic conditions, and that they may be precursors of atherosclerotic lesions.

In the present study, the branching sites of the major cerebral arteries in rats were observed three-dimensionally with SEM. The intimal thickening in the apical area was much more prominent than that expected by conventional light microscopic studies. Scanning electron microscopically, the intimal pad was shown to be a linear bank-like structure and the name of “bank-like protrusion” may be proper to represent the structure. Just distal to such protrusions, there were long shallow depressions which we called “juxta-apical grooves.” They were much deeper and wider in the treated rats than in the controls. They were much more prominent at the ACA-OA junction, where saccular aneurysms are frequently induced and less prominent at the ICA bifurcation, where aneurysms infrequently develop.

In our previous experiments, saccular cerebral aneurysms were frequently found at the branching sites on the non-ligated side of the circle of Willis and showed a tendency to occur just distal to intimal pads on the larger branches. The location of the deep juxta-apical grooves found in the present study was the same as the site of origin of the experimental aneurysms. Transitional forms between grooves and aneurysms were observed. Therefore, such juxta-apical grooves may be considered to represent the early changes of cerebral aneurysm formation. The complex structures at the apex of arterial bifurcations may be responsible for the initiation of cerebral aneurysm development.

Endothelial cells were round and had a large number of microvilli in the juxta-apical grooves or small aneurysms of the ACA branch of the ACA-OA junction. The presence of microvilli on the endothelial cells may be an expression of the immaturity of these cells, because such profuse microvilli have been reported in the fetal aorta, ductus arteriosus and pulmonary arteries. Many collapsed and degenerated endothelial cells with balloons and craters were also seen intermingled with such cells. The round cells with profuse microvilli may be newly formed cells replacing injured cells. Balloons, craters and interendothelial gaps have been regarded as non-specific reactions of endothelial...
FIGURE 7. Casts of branching sites in the control rat. A–The ACA-OA junction. (×150). There was a linear deep imprint (arrows) in the area corresponding to the bank. It extended from the apical area to the lateral angle. B–The ICA bifurcation. (×150). A similar structure (arrows) reached the face and dorsum of the MCA, but not the lateral angle. ICA: the internal cerebral artery, ACA: the anterior cerebral artery, MCA: the middle cerebral artery, OA: the olfactory artery.

cells to various injuries. Thus, endothelial injuries may play a significant role in the development of cerebral aneurysms, as in the case of atherosclerosis and hypertensive vascular changes.

In summary, the present study showed that the apex of arterial bifurcation in rats had a complex structure, including bank-like intimal pads. Juxta-apical grooves were always situated just distal to intimal pads, and endothelial cells on this groove showed degenerative changes. Some hemodynamic stress, possibly turbulent flow or secondary flow, due to such complex branching structures may injure the endothelial cells located distal to the pad, and such injured endothelial cells in turn develop saccular cerebral aneurysms.

References
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M Kojima, H Handa, N Hashimoto, C Kim and F Hazama

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