Vascular corrosion casts mirroring early morphological changes that lead to the formation of saccular cerebral aneurysm: an experimental study in rats

MOHAMMAD A. JAMOUS, M.D., SHINJI NAGAHIRO, M.D., PH.D., KEIKO T. KITAZATO, B.S., KOICHI SATOH, M.D., PH.D., AND JUNICHIRO SATOMI, M.D., PH.D.

Department of Neurosurgery, School of Medicine, The Institute of Health Bioscience, The University of Tokushima, Tokushima, Japan

Materials and Methods

We used 20 male 7-week-old Sprague–Dawley rats for this experiment. After induction of anesthesia (inhalation of 2–4% isoflurane mixture), each animal underwent ligation of the right CCA and the bilateral posterior renal arteries. One week later, a 1% NaCl solution was provided for their drinking water. Ten additional male 7-week-old Sprague–Dawley rats served as a control group.

Blood pressure was measured once a month in unanesthetized animals by using the tail-cuff auto-pickup method. Two months after the surgical procedure, the animals were killed and vascular corrosion casts of the cerebral arteries were prepared. Animal care and the experimental protocol complied with Japanese standards on the care and use of laboratory animals.

Vascular Corrosion Casts

A previously described method was adopted to create the vascular corrosion casts.11,15 After anesthesia had been induced (inhalation of a 2–4% isoflurane mixture), the rats underwent laparotomy and thoracotomy. The tip of a plastic cannula (19-gauge caliber with a length of 1.25 in) was inserted into the left ventricle and secured in the ascending aorta. After the right atrium had been cut for drainage, the rats were perfused with 100 ml heparin/phosphate-buffered saline (20 U/ml) by using a perfusion pump at a rate of 10 ml/minute. This procedure was followed by manual injection of 10 ml Batson No. 17 plastic (Polysciences, Inc., Warrington, PA). After a 24-hour period of polymerization at room temperature, the entire brain was removed and digested in 20% KOH for 24 to 72 hours, with intermittent water rinses. The vascular cast that remained was mounted on the stub of a scanning electron microscope (model S-100; Hitachi, Tokyo, Japan) by using colloidal silver paste, sputter-coated with...
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Morphological Changes at Artery Bifurcations

Based on our morphological findings we classified changes at artery bifurcations in the following manner.

Endothelial Changes (Stage I). Compared with normal bifurcations (Fig. 2A) the endothelial surface in experimental animals was rough and irregular endothelial cell imprints were evident (Fig. 2B). These changes were located just distal to the apical intimal pad. No arterial wall dilation was identified.

Intimal Pad Elevation (Stage II). The next change was a slight elevation of the apical intimal pad. This was manifested as a wedge- or fusiform-shaped shallow dilation of the apical intimal pad (Fig. 2C). The dome of the swelling was covered by abnormal endothelial cell imprints.

Saccular Dilation (Stage III). As the swelling of the dilated intimal pad progressed, a saccular area of swelling formed and irregular endothelial cell imprints continued to cover its dome (Fig. 2D).

All surgically treated rats manifested systolic hypertension; at 182 ± 15.9 mm Hg (mean ± standard deviation) their mean systolic blood pressure was significantly higher than that measured in control animals (117 ± 14.3 mm Hg; p < 0.005).

Using scanning electron microscopy, we were able to obtain 3D images of cerebral vascular casts. Imprints of the lining endothelial cells were clearly visible (Figs. 2 and 3). Table 1 lists the frequency and location of cerebral aneurysmal changes. Of the 20 surgically treated rats, 11 (55%) displayed aneurysmal changes. In five of these animals only changes in endothelial cell imprints could be identified. In the other six rats morphological changes in the endothelial cells were associated with different stages of aneurysm dilation: in two rats saccular dilation of the artery bifurcation (Stage III) developed and in four intimal pad elevation (Stage II) was observed.

The ACA–OA bifurcation was the main site of these aneurysmal changes. Of the 11 observed aneurysmal changes, seven (63.6%) were localized at that site and the other four were found at ACA fenestrations. None of the control rats manifested any aneurysmal dilation.

All aneurysms were located on the left side, that is, contralateral to the side of CCA occlusion. An ACA fenestration was found in approximately 50% of surgically treated and control rats, and there was no correlation between ACA fenestrations and the development of cerebral aneurysmal
changes. Junctional dilation, found in three rats, was differentiated from aneurysmal changes by the normal endothelial markings that cover the area of dilation (Fig. 3). The site of these dilations was the right ACA–OA bifurcation in two rats and the left internal carotid artery–posterior cerebral artery bifurcation in one rat.

Discussion

Increased shear stress is regarded as a major factor in the development of intracranial saccular aneurysms. We used the aneurysm induction model described by Hashimoto, et al.,4–7 in which renal hypertension and unilateral ligation of the CCA are performed to exacerbate hemodynamic forces on one side of the circle of Willis. The pathological characteristics and the distribution of cerebral aneurysms in this model were found to be similar to those found in humans.

The tendency to develop cerebral aneurysms differs from one rat to another; whereas in some rats aneurysmal changes developed early after the aneurysm induction procedure, in others additional time is required to show such changes. Because of these variations, we proposed that these aneurysmal changes, although observed at one single time post-mortem, represent sequential changes.

Exposure of endothelial cells to shear stress results in both morphological and functional changes. Although physiological fluid–induced shear stress transforms polygonal, cobblestone-shaped endothelial cells of random orientation into fusiform endothelial cells aligned in the direction of blood flow, decreases endothelial cell proliferation, and increases the production of vasodilators, the exposure of endothelial cells to supraphysiological high shear stress levels results in endothelial cell dysfunction and a reduction in the production of vasodilators, with the possible progression to endothelial cell degeneration.2,3,14–16,19,20

Changes in endothelial cell markings were the earliest structural alterations we observed at artery bifurcations opposite the occluded CCA. Roughening of the arterial vascular surface and irregularities in the shape of endothelial cell imprints preceded the outward bulging of the artery wall (Fig. 2B). These observed changes may reflect a disturbance in blood flow, exposure to high shear stress, and possibly endothelial cell dysfunction. Because these morphological changes in endothelial cells occurred just distal to the apical intimal pad, rather than at the apex per se, we hypothesize that this area is the primary site of blood flow disturbances and maximum shear stress. Abrogation of functional and regenerative abilities of endothelial cells, which may be affected by genetic, environmental, and hormonal factors, plays a major role in the appearance of these early changes. Even in the absence of swelling or dilation of the artery bifurcation, these endothelial changes are reflective of a weakness in the artery wall and render it vulnerable to outward bulging.

The observed endothelial cell changes were followed by a shallow elevation of the intimal pad (Fig. 2C), which represents the first step in artery wall dilation and may indicate the failure of endothelial cells to neutralize hemodynamic shear stress. Consequently, the shape of the artery wall at the intimal pad area starts to be directly affected by intramural pressure and blood flow patterns. The shape of the elevation may reflect the blood flow pattern at this early stage of aneurysmal dilation. We propose that aneurysmal blood flow patterns at this stage consist mainly of inflow and outflow, with minimal intra-aneurysm circulating flow. Abnormal endothelial cell markings of the apical intimal pad continue to cover this elevation.

Progressive expansion of the fusiform dilation resulted in a gradual increase in intraaneurysm circulating flow. This in turn increases intraaneurysm pressure and leads to the formation of well-developed saccular aneurysms (Fig. 2D), which appear to affect the apex of the bifurcation rather than the apical intimal pad, the site of earlier changes (Stages I and II). We posit that intraaneurysm pressure and blood flow patterns control the migration of the aneurysmal dilation toward the bifurcation apex. The aneurysm dome continued to exhibit irregularly shaped endothelial imprints and there were areas devoid of endothelial cell markings. We posit that the absence of endothelial cell imprints is more pronounced in larger aneurysms, and may be related to the increased incidence of these aneurysms to rupture.

We were able to differentiate early aneurysmal dilation (Stage II) from junctional dilation, considered by some investigators to be a preaneurysmal lesion.10,11 Junctional dilation was present in three rats and differed from aneurysmal dilation because in the former, the area of dilation was covered by normal endothelium (Fig. 3). Of the three junction-
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al dilations, two were on the right side, that is, the low shear stress side. Based on these observations we suggest that true junctional dilations do not progress to Stage III without undergoing the changes described as Stages I and II.

Methods previously used to investigate the pathological characteristics of cerebral aneurysms in clinical and experimental studies relied on the appearance of artery wall dilation, identified by either angiography or dissecting microscopy, for the diagnosis of aneurysmal changes. These approaches do not allow the detection of early changes that occur before the manifestation of visible swelling of the artery wall, however. Using scanning electron microscopy to screen vascular corrosion casts, we were able to observe early ultrastructural changes at artery bifurcations that precede the appearance of well-developed saccular aneurysms. The advantage of our technique is that it does not require fixing, dehydrating, or drying of the vessels; consequently, their in vivo geometry is preserved and artifacts attributable to these procedures and to mechanical distortion of the endothelial cells can be avoided. Our technique yields material to study the ultrastructural and 3D morphological characteristics of large areas of the vascular tree.

In this article we present the first demonstration of the sequence of ultrastructural morphological changes that leads to the formation of saccular cerebral aneurysms in vivo and in 3D geometry. These morphological changes reflect functional changes related to endothelial cells and blood flow patterns. Detailed knowledge regarding these changes is crucial for an understanding of the mechanisms underlying the formation and progression of cerebral aneurysms, and for the development of new preventive and therapeutic strategies.

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References


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