Mouse Model of Cerebral Aneurysm
Experimental Induction by Renal Hypertension and Local Hemodynamic Changes

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Background and Purpose—Rupture of cerebral aneurysm (CA) is the major cause of subarachnoid hemorrhage. Molecular mechanisms of this disease, however, remain unknown. To make possible genetic analysis of CA formation with genetically altered mice, we have successfully established a mouse model of saccular CA that recapitulates the essential features of human saccular CA.

Methods—In C57black/6 male mice, various stages of CAs were experimentally induced at the right anterior cerebral artery–olfactory artery bifurcations by ligations of left common carotid arteries and posterior branches of bilateral renal arteries with high salt diet. Both light and electron microscopic studies were performed with the longitudinal sections of anterior cerebral artery–olfactory artery bifurcations.

Results—In the treated group, various aneurysmal changes were detected in 14 of 18 mice. On the other hand, in the control group, no aneurysmal changes were found in 15 mice. In microscopic studies, aneurysmal changes were shown to include mainly fragmentation of internal elastic lamina, thinning of the smooth muscle cell layer, and degeneration of adventitial tissue, which were very similar to critical changes in human saccular CA.

Conclusions—This mouse model of CA will be useful for studying the effects of complex determinants on CA formation and makes it possible to understand the pathogenesis of CA at the molecular level. (Stroke. 2002;33:1911-1915.)

Key Words: cerebral aneurysm ■ genetics ■ hemodynamics ■ hypertension, renal ■ mice

Saccular cerebral aneurysm (CA) consists mainly of thinning degenerated vascular walls in contrast to aortic aneurysm, which consists of thick walls containing advanced atherosclerotic changes.1–3 Although recent reports have shown genetic analysis on aortic aneurysms formation,4,5 the molecular mechanisms involved in the pathogenesis of saccular CA remain unclear. The major reasons might be that human specimens are often too deformed to examine and that there have not been any suitable animal models for genetic analysis. We have previously established experimentally induced CAs in rats and monkeys,6–8 which were characterized by specific initial changes such as fragmentation of internal elastic lamina and thinning of the medial smooth muscle cell layer.9,10 Their pathological features appeared very similar to those of ruptured human CA11; in fact, these are the only models that have been accepted for studying the pathogenesis of human CA. However, compared with rats and monkeys, a mouse model has great advantages in genetic analysis because of a variety of established gene-modulated mice. In this report, we demonstrate the first mouse model of saccular CA that uses genetically altered mice to explore the molecular mechanisms of CA formation and provide insights into new CA prevention and treatment strategies.

Methods

Experimentally Treated Animals
As described in our previous reports,6–8 20 male C57black/6CrSlc mice (SLC), which were 7 to 9 weeks of age, were subjected to ligations of the left common carotid arteries and posterior branches of right renal arteries under general anesthesia with 1% to 2% halothane. The C57black/6 family mice are commonly used for manipulation of genes in the mouse genome to produce transgenic and gene-targeted animals. We took special care to maintain normal body temperature and not to induce excessively deep anesthesia on operation. One week after the first operation, the posterior branches of the left renal arteries were ligated. One week after these procedures, 1% saline was substituted for drinking water. At the same time, 15 age-matched untreated male mice served as control. All surviving mice were killed 4 months after the surgical procedure. In both groups, systolic blood pressure was measured by the tail-cuff plethysmographic method before operation and before sacrifice. Animal care and experiments complied with Japanese community standards on the care and use of laboratory animals.
Preparation for Light Microscopic Study
After deep anesthesia with ether, the mice in the 2 groups were perfused with phosphate-buffered saline containing 10 μmol/L p-amidinophenyl-methane sulfonyl fluoride and 10 μg/mL leupeptin for 3 minutes and subsequently with freshly prepared 2% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.3, for 15 minutes. The circles of Willis were isolated from the brains under surgical microscope and immersed in the same buffer for fixation. For light microscopic study, the right anterior cerebral artery–olfactory artery (ACA-OA) bifurcations were dissected from the circles, embedded in OCT, and frozen. Serial sections (3 μm) were stained with elastica–van Gieson.

Preparation for Electron Microscopic Study
Serial frozen sections were mounted on slides. The samples were washed and fixed with 0.5% osmium oxide in a buffer containing 100 mmol/L cacodylate buffer, pH 7.3. The sections were dehydrated by passing them through a graded series of ethanol (50%, 70%, 90%, and 100%) and propylene oxide and embedded in epoxy resin. From this sample, ultrathin sections were cut, stained with uranyl acetate and lead citrate, and then observed with an electron microscope (JEM-1200EX, JEOL).

Statistical Analysis
Values are expressed as mean±SD. Systolic blood pressure measured before operation and before sacrifice were compared and statistically analyzed by Student’s t test for paired data. Differences were considered statistically significant at P<0.05.

Results
Effects of Experimental Procedures
In the treated group (n=20), no mice died after the first operation. After the second operation, 2 of 20 mice (10%) died within 3 days; however, the other mice survived until sacrifice and could be analyzed. In the control group, all of the mice survived until sacrifice (n=15).

In the treated group (n=18), systolic blood pressure at 4 months after the operation was significantly higher than before surgery (before surgery, 84.4±4.8 mm Hg; before sacrifice, 117.0±12.1 mm Hg; P<0.001). On the other hand, in the control group (n=15), systolic blood pressure had not significantly changed after 4 months (before surgery, 82.6±2.9 mm Hg; before sacrifice, 83.7±1.9 mm Hg).

Light Microscopic Findings of CA
The circles of Willis in C57black/6 mice (Figure 1a and 1b) were anatomically similar to those of Sprague-Dawley strain rats, in which experimental CA was previously established.6,7 In the treated group, at right ACA-OA bifurcations, various stages of aneurysmal changes were detected in 14 of 18 mice (Figure 2a). These changes appeared very similar to pathological changes in experimentally induced CAs in rats and monkeys.6,8 In some cases, such changes were also found at the bifurcations of right ACA branches distal to ACA-OA bifurcation. No aneurysmal changes were found in 4 of the 18 mice in the treated group. In contrast, no aneurysmal changes were found at right ACA-OA bifurcations in the control group (n=15) (Figure 2b).

The ACA-OA bifurcations were dissected from the circles, and their serial sections (3 μm) were stained with elastica–van Gieson. The arterial walls protruded at various degrees at the distal area of ACA branches adjacent to the apex. These aneurysmal changes (n=14) were classified into 2 stages according to the pathological findings in light microscopic study: early stages (8 of 14 mice; Figure 3a) and advanced stages (6 of 14 mice; Figure 3b). In the early stages of CA (Figure 3a), the medial smooth muscle cell layer was thinning with discontinuity of internal elastic lamina, and the walls were not remarkably protruded. Initial changes were localized almost exclusively at the intimal pad and its neighboring distal portion. In the advanced stages of CA (Figure 3b), the wall protrusion was remarkable and consisted mainly of degenerated connective tissue (arrowheads). AP indicates apex. Scale bar=10 μm.
degenerated connective tissue. On the other hand, in the control group, the continuity of internal elastic lamina and the density of medial smooth muscle cells in all bifurcations were preserved (data not shown).

**Electron Microscopic Findings of CA**

Electron microscopic observations also have been carried out to examine in detail the development process of CA formation (Figure 4a). The normal wall of cerebral artery consisted of endothelial cell layer, internal elastic lamina, regularly arranged smooth muscle cells, and adventitial tissue in each layer (Figure 4b). The aneurysmal wall was composed mainly of degenerated smooth muscle cells and adventitia connective tissue (Figure 4a). At the proximal portion of the aneurysmal wall (Figure 4c), the internal elastic lamina disappeared, and smooth muscle cells could not be distinguished from adventitial tissue. At the distal portion (Figure 4d), severely degenerated smooth muscle cells and adventitial tissue mainly make up the aneurysmal wall; in

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**Figure 4.** Longitudinal section of CA in electron microscopic study. a, Aneurysmal change was localized at the neighboring distal portion to the intimal pad (arrowhead), which is at the distal side of ACA adjacent to the apex (AP). Its wall, which included smooth muscle cells and adventitia connective tissue, was severely degenerated and protruded outward. b, Wall of normal cerebral artery consisted of endothelial cell layer (ECL), internal elastic lamina (IEL), regularly arranged smooth muscle cells (SMC), and adventitial tissue from the luminal side. c, Proximal portion. Thinning wall consisted mainly of severely damaged smooth muscle cells (arrowhead) and degenerated adventitial tissue. They could not be distinguished from each other. d, Distal portion. Internal elastic lamina (asterisk) and a part of smooth muscle cells were preserved. At the luminal surface, irregular endothelial cell layer, including nucleus (arrowhead), was seen. e, Distal neck portion. Damaged smooth muscle cells (arrowhead) and adventitial tissue were the most remarkably mixed and degenerated in the aneurysmal wall. Scale bar=5 μm.
contrast, internal elastic lamina was still preserved. At the distal neck portion (Figure 4e), the medial smooth muscle cells and adventitia connective tissue were the most remarkably mixed and degenerated.

Discussion

Development and rupture of CA include a complex biological response reflecting the interplay of various inherited and acquired factors. Some previous reports have shown that genetic factors might be implicated in the pathogenesis of CAs. Other factors such as hypertension, sex, age, and smoking have also been reported to affect this disease. In particular, hemodynamic stress has been shown in many investigations to be the major cause of various degenerative changes in CA formation. As for induced vascular remodeling of CA, multiple mechanisms such as endothelial injury, ischemia of arterial walls, and disturbed extracellular matrix synthesis have been found. Recently, some molecular mechanisms such as deficiency of collagens (types III and IV) and active expressions of matrix metalloproteinases have been shown to be associated with CA. However, most of the molecular mechanisms have not yet been conclusively identified. This mouse model of CA would be useful for solving this problem by use of gene-altered mice. Indeed, many reports have demonstrated various experimental models of CA by a vein pouch or a vein patch, direct injury, or toxic agents, but none of them were induced in basal cerebral arteries without direct manipulation and concomitant increases in wall shear stress.

We previously have succeeded in producing animal models of saccular CAs that were very similar to those of human beings and have provided a series of reports on such models. In these studies, we have revealed that the characteristic findings of CA formation in rats and monkeys were thinning of medial smooth muscle cell layer and disappearance of the internal elastic lamina in early stages. These changes might be caused by the increase in local wall shear stress, as well as wall tension. In addition, we have recently demonstrated some molecular mechanisms such as apoptosis of medial smooth muscle cells and production of nitric oxide by inducible nitric oxide synthase.

Here, we first demonstrate that aneurysmal change in intracranial vessels of mice could be experimentally induced by ligations of a common carotid artery and partial branches of bilateral renal arteries. The major causes of the induction might be increased hemodynamic stress at the specific region and renal hypertension, which is similar in rat and monkey models. In particular, the endocrine changes in the renin-angiotensin groups might play an important role in vascular remodeling at the sites of increased hemodynamic stress. We would like to carry out further studies to clarify the roles of such factors in CA formation.

To analyze the molecular mechanisms of CA formation with genetically altered mice, an ideal model must have the aneurysm induced in intracranial vessels without direct injury to recapitulate the essential features of human CA. The aneurysms in our models were experimentally induced at ACA-OA bifurcations without direct manipulation and contain the same pathological characteristics as our rat and monkey models, which have already been proven to grow into saccular aneurysm like human CAs. These facts indicate that this mouse model appears to be useful for studying the molecular mechanisms of CA formation with genetically altered mice.

In this study, we also could examine various stages of the CAs in mice by electron microscopy, which may help us to analyze CAs of gene-targeted mice in detail. We clearly found that the disappearance of internal elastic lamina and the damage of smooth muscle cells were more progressive at the proximal portion of the aneurysmal wall than at the distal portion in the early stage of CA development (Figure 4c and 4d). In addition, the induced wall remodeling was extreme at the distal neck portion, even though the endothelial cell layer and internal elastic lamina were preserved (Figure 4e). Our previous reports showed that wall shear stress was highest at the distal end of the aneurysmal wall in early stages of CA. Other reports have also demonstrated that hemodynamic stress might be an important factor underlying CA formation, and wall shear stress is highest at the ostium of CA in advanced stages. These data provide evidence to support the hypothesis that CAs in mice models, like human CA, might also be caused initially by an increase in hemodynamic stress, which might induce a complex, multifactorial remodeling through a variety of mediators and pathways.

CA formation in this mouse model would share common molecular mechanisms with human saccular CA, even though systemic hypertension is not always associated with CA formation or its risk in humans. As a matter of course, genes implicated in such remodelings need to be examined in human populations to look for alleles that may account for susceptibility. Analysis of the human genome is also rapidly advancing, and human polymorphisms related to CA should be tested with mice. The ability to compare the features of CA between humans and mice will play an integral role in the study of CA. Knowledge of the genetic determinants of CA from mice studies will allow us to identify disease-prone persons and envision gene-blocked agents to CA while avoiding clipping or other invasive surgeries.

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References


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