Ultrastructural Studies of Cerebral Arteries and Collateral Vessels in Moyamoya Disease

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SUMMARY Moyamoya disease was originally defined as a characteristic syndrome of recurrent headaches, occlusion of the distal internal carotid arteries and the foggy (moyamoya) clusters of collateral vessels at the base of the brain as demonstrated by cerebral angiography. The etiology is unknown and pathobiology is poorly understood. We examined the intracranial arteries in 3 patients to demonstrate characteristic changes and to obtain a better understanding of the basic mechanisms of the disease. Controls were obtained from 3 normotensive patients who died as a result of cancer. Occluded internal carotid arteries were characterized by severe thickening of the intima with a dense luminal array of smooth muscle cells, a deeper less cellular zone, pronounced tortuosity of the internal elastica and thinning of the media. Collateral vessels were arterial in structure and were affected by similar proliferative changes in the intima, thinning of the media, and contorted internal elastica. Stainable lipids were not part of the typical components. Severe contortion of the internal elastica, medial damage and intimal proliferation may result from recurrent and sustained spasticity of the cerebral arteries. The distal lenticulostriate arteries showed severe medial damage similar to what is termed as a moth-eaten change in hypertensive patients dying of massive cerebral hemorrhage.

ARterial Changes in MOYAMOYA Disease, as shown by angiography of the distal internal carotid artery, reveal lipid-free fibromuscular thickening and the development of presumably newly formed collateral vessels at the base of the brain. These collaterals are so characteristic that the term moyamoya vessels has been applied to them. Occlusion of the internal carotid artery caused by atherosclerosis, is not accompanied by the appearance of collateral vessels and is characterized by the presence of stainable lipids.

The term moyamoya disease was coined for the "foggy" collateral channels demonstrable by cerebral angiography. The disease is relatively uncommon and shows no relationship to systemic hypertension. This disease was first reported in Japan. The condition is frequently familial, and may occur in infants and children as well as in adults. The pathogenesis is unknown and the possibilities include genetic factors. Intracerebral and subarachnoid hemorrhages are frequent complications. Moyamoya disease of the juvenile type often manifests itself by transient ischemic attacks, and the adult type usually presents as a massive intracerebral hemorrhage. Thus moyamoya disease provides an interesting model for studies of arterial disease and its complications. The histologic features and morphology of arterial changes in 22 patients with moyamoya disease have been reported by others.

The present study was undertaken to characterize ultrastructural changes of the circle of Willis and collateral moyamoya vessels, and to obtain a better understanding of the basic mechanisms of moyamoya disease.

Materials and Methods

Three patients who died with complications of moyamoya disease provided the material of this study. Additionally, the brain and vessels were obtained from 3 normotensive patients who died with malignancies and were used as controls. These patients were 22, 46, and 50 years old.

The patients with moyamoya disease were two women, 22 and 50 years old, and a man, 46 years old. Both of the women had had headaches since adolescence. None had known history of hypertension. All of the patients died after the sudden onset of severe headache followed by coma. Angiographic findings were those of typical moyamoya disease with occlusion of the intracranial internal carotid artery, clusters of moyamoya vessels at the base of the brain, and intracerebral, ventricular and subarachnoid hemorrhages. The 50-year-old woman had been blind since the age of 20 years because of an embolic infarct with hemorrhages in the left occipital lobe.

The brain and attached vessels were dissected within one hour postmortem. The primary fixative, 1.4% glutaraldehyde in phosphate buffer, pH 7.3, 300 mOs, was perfused at 100 mmHg for 20 minutes form the basilar and both of the internal carotid arteries. We then dissected the circle of Willis including the collateral vessels, lenticulostriate arteries and small cortical branches of the middle cerebral arteries. The lenticulostriate arteries were divided into the proximal, middle and distal segments. The proximal segments arose from the middle cerebral artery and were embedded in the anterior perforated substance. The distal segments were the arterioles within the basal ganglia.

Two to 3 mm segments of the aldehyde-fixed arteries were then rinsed in buffer and were further fixed in
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FIG. 1. The base of the brain of a 22-year-old woman showing the anterior cerebral arteries (AC) and mainly median collateral vessels (arrow). M, middle cerebral artery; CA, anterior communicating artery; IC, left internal carotid artery.

1% OsO₄ in phosphate buffer for one hour. Tissue blocks were dehydrated in graded series of ethanol and embedded in epoxy resin (Epon 812). One-μm sections were stained with alkaline methylene blue and basic fuchsin. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

At the base of the brain there were characteristic clusters of collateral vessels resembling the fibrous roots of plants as previously described. These moyamoya vessels were concentrated on the median side of the cerebral arteries as shown in figure 1. The component arteries of the circle of Willis in all 3 cases showed severe mound-like to concentric circumferential intimal lesions. These lesions were characterized, in large part, by the compact layer of smooth muscle cells near the lumen (fig. 2). The deep zone of the lesion near the internal elastica showed nearly acellular areas with a loose matrix (fig. 3 a). The compact cellular zone near the lumen consisted of 15 to 30 layers of circumferentially aligned, contractile form of smooth muscle cells. They were separated by a minimal amount of collagen and basement membranes. Scattered cellular debris and pyknotic dead cells were also noted (fig. 4a). Deep intimal cells were widely separated by the matrix which consisted of fine granular electron-dense material, small amounts of collagen and particulate or lamellar elastica (fig. 4 b). These deeper intimal cells had only part of the usual features of smooth muscle cells. Most of them had partial or circumferential basement membrane, but the shape was either bizarre and elongated with 10 nm cytoplasmic filaments, or roughly round with minimal amounts of organelles. Although the pressure perfusion fixation at 100 mmHg, the internal elastica of the arteries of the circle of Willis of moyamoya patients showed marked tortuosity, extended loops of the elastica projecting far towards the lumen in places (fig. 2 and 3 a). This marked tortuosity of the internal elastica was not seen in nor-

Fig. 2. Middle cerebral artery, 500μm in diameter, of a 22-year-old woman is occluded by concentric intimal fibromuscular thickening. The grey band (O) appears to be the partially persistent, old internal elastica. The internal elastica is black and is extremely tortuous. The media (M) is thin and atrophic. The adventitia is normal. Methylen blue — basic fuchsin stain.

Fig. 3 A. The anterior cerebral artery as shown in Fig. 1 is stenosed by intimal fibromuscular thickening. The internal elastica is markedly tortuous. The media is atrophic. L, lumen; M, media; Ad, adventitia. Epoxy section, methylene blue — basic fuchsin stain. 500 x.

Fig. 3 B. Portion of the same anterior cerebral artery as shown in Fig. 3 A. Medial smooth muscle cells (SM) are distorted and sparse. A large portion of the media is occupied by an amorphous material (*). IE, internal elastica. Uranyl acetate and lead citrate. 6000 x.
and numerous in the 22-year-old woman and least prominent in the 50-year-old. These collateral vessels were indisputable arteries having an internal elastic lamina and 3 to 5 layers of medial smooth muscle cells. They ranged from 0.2 to 0.6 mm in diameter. Most of these moyamoya vessels in the 46- and 50-year-old showed segmental intimal thickenings, especially at the points of junction to the circle of Willis and penetration into the brain substance. These intimal thickenings were characterized by marked tortuosity of the internal elastica and compactness of the superficial layer of intimal smooth muscle cells, as in occluded intracranial internal carotid arteries. Some of the moyamoya vessels adjacent to the occluded internal carotid artery in the 46-year-old man were occluded by hyalinized fibrosis (fig. 5). The media of such occluded collateral vessels showed sparse smooth muscle cells and many pyknotic dead cells. The internal elastica persisted in large part, but was barely visible having only slight electron-density with loss of fibrillar components. The adventitia appeared hyalinized.

The proximal segments of the lenticulostriate arteries with moyamoya disease were similar to those in normotensive controls who died with malignancies. These arteries showed an average diameter of 0.8 mm, a thin intima with attenuated endothelial cells and scattered collections of 1 to 2 layers of smooth muscle cells, and internal elastica with occasional fenestrae. The internal elastica measured from 7 to 10 μm in thickness. The media consisted of 5 to 7 layers of circumferential smooth muscle cells and minimal amounts of collagen and basement membranes. The adventitia was normal (fig. 6 B).

The distal segments of the lenticulostriate arteries of moyamoya patients showed diffuse changes similar to those observed in hypertensive patients who died with massive intracerebral hemorrhage (fig. 6 A). 11 The distal segments showed diffuse intimal thickening with 1 to 3 layers of smooth muscle cells. The internal elastica was thin, measuring 4 to 5 μm. There were more fenestrae than in the proximal segments. The

Collateral moyamoya vessels were most prominent motensive arteries of the controls which showed only slight scalloping at most and were normal otherwise. What appeared to be the old internal elastica in moyamoya disease patients was partially preserved near the lumen. It was wavy and less electron-dense and less clearly defined that the contorted internal elastica. The underlying media was thin, especially in areas where intimal thickening was severe (fig. 3). The media in normotensive controls who died with malignancies averaged 80 μm in thickness having many smooth muscle cell layers. The media of corresponding segments in moyamoya disease patients averaged 70 μm in thickness having 2 to 6 smooth muscle cell layers. Medial smooth muscle cells appeared atrophic and were widely separated by fine granular electron-dense materials, small bundles of collagen, particulate elastica, and cellular debris. Many of the cells showed cytoplasmic blebs and there were scattered pyknotic cells exhibiting coagulation necrosis. 7 The adventitia and endothelium were normal.

Collateral moyamoya vessels were most prominent

![Fig. 4 A. The intima of anterior cerebral artery in a moyamoya disease patient is thickened by a compact array of smooth muscle cells. D is a pyknotic and dead smooth muscle cell. The intercellular matrix is small in amount. Uranyl acetate and lead citrate. 4000 X.](http://stroke.ahajournals.org/)

![Fig. 4 B. The deep zone of the thickened intima shows sparse cells with bizarre shape and 10 nm intermediate filaments. The basement membrane (BM) is present around most of the cells. Some of the cells are roughly round and show minimal amounts of organelles. Uranyl acetate and lead citrate. 4000 X.](http://stroke.ahajournals.org/)

![Fig. 5. Moyamoya vessels in a 46-year-old man. The vessel on the left shows moderate intimal thickening with lamellar elastica, and hypocellularity of the media (M). The vessel on the right is occluded by hyalinized collagenous tissue. IE, internal elastica with severe tortuosity. Epoxy section, methylene blue and basic fuchsin.](http://stroke.ahajournals.org/)
Fig. 6. Longitudinal sections of proximal (A) and distal (B) segments of lenticulostriate artery of a 22-year-old woman with moyamoya disease.

The proximal segment is normal. The media consists of compact layers of smooth muscle cells. The internal elastica (IE) is only slightly wavy. The distal segment shows atrophy of the medial smooth muscle cells and cellular debris. IE, diffuse internal elastica.

Fig. 7. Medial damage of distal lenticulostriate artery, 46-year-old man with moyamoya disease. Smooth muscle cells are atrophic, bizarre in shape and are surrounded by redundant basement membrane densities (*). Granular and vesicular debris (†) are seen in wide areas of electron-lucent matrix. 6000 ×.

Some of the larger aggregates of cellular debris were surrounded by the basement membranes that were continuous with those around smooth muscle cells and were sufficiently large as to represent remains of single cells. The adventitia appeared unchanged. The actual site of necrosis and rupture of the lenticulostriate arteries was not demonstrated in the 3 cases studied.

Discussion

The basic cellular events in moyamoya disease and atherosclerosis seem similar in that both types of lesions are the composite of tissue injury and repair with proliferation. Atherosclerotic lesions are mainly concentric intimal lesions consisting of various combinations of smooth muscle cells, macrophages, intra- and extracellular stainable lipids, and connective tissue matrix materials. The internal elastica may be clearly demonstrable with scalloping as in normal arteries, or partially or totally obscured. Arterial lesions in moyamoya disease resemble those in atherosclerosis with intimal fibromuscular thickening. They differ, however, in several respects. Both slight and severe stenotic thickenings of the intima were overwhelmingly fibromuscular with a paucity, if any of stainable lipids. Such stenotic lesions have been reported not only in adult, but also in infants and children. The internal elastica provided a point of distinction by being markedly tortuous with looplike extensions toward the lumen beyond the level of aggravation of scalloping. Medial changes in moyamoya disease were profound and much more severe than those usually seen in atherosclerotic arteries. A histological survey and morphometry of cerebral arteries in 22 patients with moyamoya disease indicated a prevalence of severe stenotic lesions in older patients and dilatation and attenuation of the media in younger patients. The current study revealed the ultrastructural basis for this medial attenuation and dilatation. Moreover, these medial changes in moyamoya disease were similar to those in hypertensive patients with massive cerebral hemorrhages. Such ultrastructural medial changes have been termed descriptively moth-eaten change to indicate a combination of irregular atrophy and damage of the medial smooth muscle cells accompanied by an increase in the amount of matrix which included cellular debris, sparse collagen and electron-lucent background substance.

The pathogenesis of the moth-eaten change is unknown. Possibilities include sustained focal cytoplasmic necrosis of the media smooth muscle cells causing arterial spasm and/or hypoxia. Similar degenerative changes of the media and marked tortuosity of the internal elastica can be induced by repeated electrical stimulation and spasm of the gastric artery in rabbits. Hypoxia compounded by the intimal thickening would further enhance proliferation of intimal cells and stroma.

No occlusion, but stenosis was demonstrable in the 22-year-old woman's moyamoya vessels. In the older patients complete occlusions were observed. The basic mechanisms of occlusion appear to be the same in both
the moyamoya vessels and intracranial internal carotid arteries. Occlusion of the internal carotid artery is probably responsible for the formation of collateral moyamoya vessels. Development and later obliteration of moyamoya vessels have been ascertained by sequential angiography.5

Pathogenesis of massive cerebral hemorrhages in moyamoya disease remains poorly understood.15 In the series of 22 patients with moyamoya disease 13 vessels in 6 patients were found to be ruptured.7 Microaneurysm and fibrin deposits were observed in only 1 and 2 vessels of these 13. On the other hand, the majority of these 6 and the rest of the patients showed fibrosis and attenuation of the media. These findings together are in accord with our working hypothesis that the characteristic moth-eaten change and attenuation of the media predispose to cerebral arteriolar rupture leading to massive cerebral hemorrhage. Studies in hypertensive patients suggest that microaneurysm and fibrinoid necrosis are secondary to rupture and circumscribed hemorrhage.11

None of our patients with moyamoya disease had a history of hypertension. None of them had left ventricular myocardial hypertrophy or renal contraction, such as due to arteriolar nephrosclerosis. Further investigations are warranted to determine whether these two conditions, medial damage and attenuation in hypertensive cerebral hemorrhage and nonhypertensive cerebral hemorrhage in moyamoya disease, share a common cause.

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