Ultrastructural Characteristics of Occluded Perforating Arteries in Stroke-Prone Spontaneously Hypertensive Rats

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We studied ultrastructurally cerebral perforating arteries in 60 stroke-prone spontaneously hypertensive rats (SHRSP), which were sequentially killed at 4–52 weeks of age before showing symptoms of stroke. Another 24 SHRSP were killed soon after they showed symptoms of cerebral infarction. The initial vascular lesions observed in the asymptomatic group included focal cytoplasmic necrosis in the outer layers of the media. This change progressed to widespread medial necrosis with time. In the infarction group, numerous monocytes were seen adhering to the endothelium of the arteries having advanced medial damage. Following the adherence of monocytes to the endothelium, large amounts of plasma components were visible in the arterial wall. The accumulation of the plasma components (especially fibrin) thickened the wall, narrowed the lumen, and resulted in occlusion. These results suggest that monocytes may affect the endothelium, perhaps disturbing the so-called blood–brain barrier to proteins. The monocytes may therefore be closely related to the occurrence of arterial occlusion with resultant cerebral infarction. (Stroke 1987;18:733–740)

There are many studies on hypertensive vascular lesions in humans and experimental animals. Only a few investigators, however, have focused on the long-term effects of hypertension on the perforating arteries in the brain. In the present study we sequentially analyzed the ultrastructure of the perforating arteries in stroke-prone spontaneously hypertensive rats (SHRSP) as developed by Okamoto et al in 1974. We discovered numerous monocytes that adhered to the endothelium. We have therefore tried to clarify the characteristic changes that develop into occlusions and have attempted to explain the role of monocytes in arterial lesions, especially those resulting in occlusion.

Materials and Methods

Okamoto et al and Yamori et al developed SHRSP in which stroke (cerebral hemorrhage and/or infarction) develops spontaneously in > 80% of the rats. The cerebral lesions resemble those found in humans. Since the development of this rat strain, we have continued to inbreed them selectively. At present we have > 1,500 SHRSP in our laboratory. A group of 24 SHRSP 28–50 weeks of age with symptoms of stroke composed a cerebral infarction group, and 60 asymptomatic SHRSP 4–52 weeks of age served as controls. Almost half the rats in the infarction group were in an irritable state denoted as “Stage 2,” while the other half were lethargic, labelled “Stage 3.” Rats in the infarction group were anesthetized with pentobarbital, and the cerebral arteries were perfused with a 1% formaldehyde-1.25% glutaraldehyde fixative in 0.1 M cacodylate buffer at pH 7.6 at room temperature via the descending aorta at 180 mm Hg pressure for 4–5 minutes. After perfusion, the brains were carefully removed and fixed with a 2% formaldehyde-2.5% glutaraldehyde mixture in 0.1 M cacodylate buffer for 1 hour at 4°C. The infarcted cortices were then taken out and placed in the same fixative for 3 hours at 4°C and then washed overnight at 4°C in 0.1 M cacodylate buffer. Afterwards, the infarcted cortices were sectioned into small pieces under a microscope as previously reported. Several sections from each group were cut on a tissue chopper without freezing and were collected in chilled fixative. Chopped sections were then washed for 60 minutes in 3 changes of cacodylate buffer and incubated for 120 minutes at room temperature in a modification of the medium originally described by Karnovsky for the demonstration of horseradish peroxidase, consisting of 5 mg 3,3′-diaminobenzidine tetrachloride (DAB; Sigma Chemical Co., St. Louis, Mo.) in 9.9 ml Tris-HCl buffer (pH 7.6) to which 0.1 ml of a freshly prepared 1% solution of H2O2 had been added just prior to its use. After 60 minutes of incubation, the stale medium was replaced with fresh. Control chopped sections were incubated in a medium...
lacking DAB but containing H₂O₂. After incubation, chopped sections were rinsed with cacodylate buffer for 15 minutes.

All of the sections were post-fixed with 2% OsO₄ buffered with 0.1 M cacodylate buffer for 2 hours. They were then stained with 2% uranyl acetate in 50% ethanol for 1 hour at 4°C, dehydrated in graded ethanol, and embedded in Epon 812. The epoxy blocks were cut on a Sorvall Porter-Blum MT-2B Ultratome. Ultrathin sections were examined, either unstained except for the original block staining or after double staining with uranyl acetate and lead citrate, under an Hitachi HS-9 electron microscope.

Results
Morphology of Perforating Arteries in SHRSP Without Symptoms of Stroke

We obtained at least 10 cerebral sections from the anteromedial and occipital cortices of 60 asymptomatic SHRSP. We studied >50 perforating arteries 20–100 μm in diameter in every rat. During the examination of more than 3,000 total perforating arteries, we discovered that there were no abnormal lesions in SHRSP of <12 weeks of age. Initial arterial lesions in SHRSP at 16 weeks of age were focal cytoplasmic necrosis in the outer layers of the media (Figure 1), characterized by circumscribed, electron-dense, granular or filamentous materials with or without necrotic cell organelles. Focal cytoplasmic necrosis became widespread necrosis of the outer layer of the media followed by atrophy. As a result, the medial muscle cells were replaced by increased amounts of basement membrane-like materials, collagen fibers, and cell debris. These medial changes, which were frequently observed in SHRSP of >28 weeks of age, worsened with time and finally led to the complete destruction of the medial muscle cells. As a result, the highly damaged arterial wall was composed of endothelial cells, intercellular matrices, and fibroblasts. Even in these highly damaged arteries, the endothelial cells were morphologically well-maintained (Figure 2). In SHRSP of >28 weeks of age, we also observed that monocytes adhered to well-maintained endothelial cells and that fibrin depositions occurred in the subendothelial spaces. The monocytes adhering to the endothelium contained many peroxidase-positive granules.

Morphology of Perforating Arteries in SHRSP With Symptoms of Stroke

Every brain of the 24 SHRSP with symptoms of stroke was macroscopically swollen; 35 cerebral infarctions were found in these brains. We obtained at least 20 cerebral sections from the infarcted areas and observed >50 perforating arteries 20–100 μm in diameter in every rat with symptoms. This study, using serial ultrathin section techniques, revealed that both multiple occluded arteries and highly damaged ones abounded in the infarcted areas. Several monocytes were observed adhering to the well-maintained endothelial cells in the arteries in which medial smooth muscle cells had completely disappeared (Figure 3). Following adherence of the monocytes, plasma components including fibrinogen infiltrated the subendothelial space and then the media. Monocytes were then projected toward the endothelial junctions and entered the subendothelial space.

FIGURE 1. Perforating artery of stroke-prone spontaneously hypertensive rats without symptoms of stroke at the age of 16 weeks. Initial arterial lesions (★) in the outer layers of the media consist of increased deposition of collagen. L, lumen; EC, endothelial cell; SM, smooth muscle cell. Bar = 10 μm.
FIGURE 2. Perforating artery of stroke-prone spontaneously hypertensive rats without symptoms of stroke at the age of 30 weeks. Medial smooth muscle cells (SM) have almost completely disappeared. The media is replaced by basement membrane-like materials, collagen fibers, and cell debris. In contrast, endothelial cells (EC) are well-maintained. Bar = 10 μm.

(Figures 4 and 5); therefore, numerous monocytes accumulated in the subendothelial space and sometimes in the media. The emigrating blood monocytes surrounded by plasma components, especially fibrin, extended many processes and enveloped plasma components. At the same time, they lost their peroxidase-positive granules (Figure 6). Subendothelial and medial fibrin deposition thickened the arterial wall and narrowed the arterial lumen (Figure 7). The narrowed lumen was occupied by monocytes and platelets adhering to the endothelial cells and was then occluded by thrombi composed of monocytes, platelets, red blood cells, and fibrin (Figure 8). Complete occlusion with massive fibrin deposition was frequently observed at the bifurcation of the perforating arteries. In the cerebral cortex, marked expansion of the extracellular space was constantly observed around the highly damaged arteries. We did not find any pseudoaneurysms in the walls of affected arteries.

Discussion
Numerous studies have been performed on hypertensive lesions in humans and experimental animals. Most investigators have examined the lesions in the visceral arteries and the intracranial extracerebral arteries.

Limas et al7 examined the aorta and intrarenal arteries of spontaneously hypertensive rats (SHR) and stated that medial change, especially thickening, was first noted at 10 weeks of age and became more pronounced with time in both the aorta and the renal arteries. Takebayashi21 examined 4 different experimental groups of hypertensive rats and showed that focal cytoplasmic necrosis was present from the early stage of hypertension and that it induced fibrin deposition which resulted in fibrinoid necrosis. He suggested that focal cytoplasmic necrosis was caused by toxic chemical factors. Similar medial changes in arterial walls have also been observed in the gastric arteries of humans and animals exposed to repeated electric stimulation as well as to restrained stress. Some investigators have proposed that medial necrosis was attributable to inappropriate responses of the arterial walls to vasospasm and its sequential hemodynamic changes.22-24

In the present study, we examined the cerebral perforating arteries in SHRSP. The initial lesions were focal cytoplasmic necrosis in the outer layers of the media, but with time focal cytoplasmic necrosis developed into widespread necrosis and atrophy of the medial smooth muscle cells. To clarify the relation between medial necrosis and hypertension, the anatomic characteristics of the perforating arteries require consideration. These arteries are lined by endothelial cells that restrict the penetration of plasma components, that is, the so-called blood–brain barrier.17,25 Furthermore, the arteries do not have vasa vasora, which are essential for nutrition and oxygen supply in the outer layers of arteries. This suggests that perforating arteries are not sufficiently supplied with nutritional elements or oxygen, which can only be obtained from the arterial lu-
FIGURE 3. Perforating artery of stroke-prone spontaneously hypertensive rats with symptoms of stroke at the age of 38 weeks. Numerous monocytes (arrows) adhere to well-maintained endothelial cells (EC) and project toward endothelial junctions (★). Bar = 5 μm.

FIGURE 4. Perforating artery of stroke-prone spontaneously hypertensive rats with symptoms of stroke at the age of 38 weeks. A monocyte is beginning to penetrate the endothelial cell (EC). The upper half of the monocyte (arrows) is in the arterial lumen, and the lower half (★) is in the subendothelial space (SES). Bar = 1 μm.
men by diffusion. Our previous study demonstrated that cerebral blood flow decreased in SHRSP with development of hypertension. This study revealed that arterial lesions begin in the outer layers of the media, where both nutrition and oxygen concentration are probably minimal because of the anatomic features described. This suggests that deficiency of nutrients and oxygen may be the cause of the medial necrosis.

To elucidate a mechanism for the role of monocytes, we performed a cytochemical assessment of peroxidase because, unlike lymphocytes, blood monocytes possess peroxidase-positive granules in their cytoplasm. We discovered that monocytes with peroxidase-positive granules adhered to well-preserved endothelial cells of the perforating arteries in which medial muscle cells had completely disappeared. Thereafter, the monocytes were directed toward the endothelial junctions and then migrated into the subendothelial spaces.

Other researchers have examined perforating arteries. Ooneda et al., working in autopsied cases, showed that intracerebral hemorrhage was caused by intracerebral microaneurysms resulting from "plasmatic arterionecrosis," defined as loss of medial smooth muscle cells, blood plasma insudation, lysis of the internal elastic lamina and intimal collagenous fibers, fibrin deposition, and luminal dilatation. Takeshita et al. studied 11 freshly removed brains and 20 lenticulostriate arteries collected from surgical biopsies in cases of intracerebral hemorrhage and concluded that primary rupture was caused by "arteriosclerosis" accompanied by degeneration of the medial smooth muscle cells; they also showed that rupture from microaneurysms was infrequent. These investigators were not in agreement on the pathogenesis of the ruptures, though both commented carefully on the medial smooth muscle changes. Neither group remarked on the endothelial changes.

In animal experiments, two investigators studied the perforating arteries. Amano attempted to clarify the pathogenesis of so-called hyaline and fibrinoid degeneration in the brain of SHR. He classified the arterial changes designated as hyalinosis into 3 types: hyaline, fibrinoid, and atypical fibrinoid degeneration. Ogata et al., studying the brains of SHRSP, reported that the intracerebral arterioles had fibrinoid necrosis and occluded lumens. The predominant tissue alterations were rarefaction and cyst formation in the white matter and rarefaction of the neuropil and preserved neurons in the neocortex at the paramedian regions of the cerebral hemispheres. Edema fluid was present in and around the lesions. Amano and Ogata et al. also examined the medial muscle cells but ignored the endothelium as well as the reports of Ooneda et al. and Takeshita et al. In summary, these 4 reports suggest that pronounced medial changes are the cause of both rupture and occlusion of the perforating arteries.

Two reports have described leukocyte migration in the arterial walls of experimental animals. Limas et al. examined the aorta of SHR and reported disruption of the endothelial junctions caused by mononuclear cells. Shiraishi et al., in sequential studies of gastric submucosal arteries resulting from stressful stimuli, noted 3 distinct acute changes of the media after the withdrawal of stress: 1) focal cytoplasmic necrosis, 2) leukocyte migration, and 3) fibrin insudation.

Following this description of leukocyte migration in the visceral arteries, we must examine the reasons why other researchers have not detected leukocyte migration in the endothelium of the perforating arteries. The first reason seems to be the lack of appropriate animal models for examining the hypertensive cerebrovascular lesions, especially ruptures and occlusions. Fortunately, we have maintained more than 1,500 SHRSP in our laboratory since the development of this strain. Therefore, we are able to regularly examine the brains of SHRSP that show symptoms of stroke. The second reason is that enormous efforts are essential for the examination of the perforating arteries by electron microscopy. We believe that more investigators will note leukocyte migration if more attention is paid to the endothelium in hypertensive humans and animals.

Several investigators have discovered chemoattractants in the arterial wall and in the medium in which
FIGURE 6. Perforating artery of stroke-prone spontaneously hypertensive rats with symptoms of stroke at the age of 40 weeks. Numerous monocytes, without peroxidase-positive granules (arrows), and large amounts of plasma components, especially fibrin (F), accumulate in the arterial wall, which thickens and the arterial lumen (L) narrows. Bar = 5 μm.

FIGURE 7. Perforating artery of stroke-prone spontaneously hypertensive rats with symptoms of stroke at the age of 40 weeks. A large amount of fibrin (F) accumulates in the arterial wall. Numerous monocytes (arrows) are in the arterial wall as well as in the arterial lumen (L), which is greatly narrowed. Bar = 5 μm.
smooth muscle cells or endothelial cells were maintained. They therefore suggest that chemotactic factors originating in the intima or media enhance monocyte adhesion to the endothelium. Other researchers have paid close attention to the endothelial degeneration caused by hyperlipidemia and suggest that endothelial degeneration, especially cell membrane injury, accelerates monocyte adhesion. However, it is not known how the monocytes adhere to the endothelium in the perforating artery.

We found that large pools of plasma components enter the arterial wall following the adhesion of the monocytes. One of the important roles of monocytes is their elaboration and release of a spectrum of hydrolytic enzymes including elastase and collagenase as well as the lipoprotein lipase. From our observations we suggest that monocytic enzymes may disturb the blood–brain barrier to proteins.

We discovered that penetration of the endothelium by the first monocyte with peroxidase-positive granules was followed by others. As a result, numerous monocytes accumulated in the subendothelial space, displayed enhanced phagocytic activity, and quickly lost their peroxidase-positive granules. These findings are similar to those observed in encephalitic lesions.

We also found that plasma components, especially fibrin, as well as numerous monocytes accumulated abundantly in the subendothelial space. Accumulation of both the monocytes and plasma components thickened the arterial wall, narrowed the lumen, and then led to occlusion. Furthermore, there was extensive edema around the affected perforating arteries. Brain samples from patients with hypertensive encephalopathy show fibrinoid necrosis of the wall of the cerebral arterioles with occlusion of the lumen, microinfarcts around these arterioles, and petechial hemorrhages. Many investigators have demonstrated cerebral edema in hypertensive encephalopathy. In this respect, our findings closely resemble those of human hypertensive encephalopathy.

From these observations we advance the following hypotheses: Deficiency of both nutrients and oxygen causes necrosis of the tunica media. Following the widespread medial necrosis, monocytes adhere to the endothelium. Enzymes produced by the monocytes alter the endothelial barrier function and enhance the penetration of plasma components as well as the monocytes themselves. Accumulation of both monocytes and plasma components results in the occlusion of the perforating arteries.

References

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