Morphological Analysis of the Pathogenesis of Hypertensive Cerebrovascular Lesions: Role of Monocytes and Platelets in Intracerebral Vessel Occlusions

MOTOKI TAGAMI, M.D. AND YUKIO YAMORI, M.D.

We performed ultrastructural studies of intracerebral vessels in stroke-prone spontaneously hypertensive rats (SHRSP). The initial vascular lesions observed in the asymptomatic SHRSP were focal cytoplasmic necrosis in the outer layers of the media. Focal cytoplasmic necrosis progressed into widespread medial necrosis with time. In the SHRSP with cerebral infarctions we discovered that numerous monocytes adhered to the endothelium of the arteries having advanced medial damage. Following the adherence of the monocytes to the endothelium enormous amounts of plasma components entered and accumulated in the arterial wall. The accumulation of the plasma components, especially fibrin, thickened the wall, narrowed the lumen and resulted in occlusion with resultant cerebral infarctions. A lot of activated platelets are seen adhering to the endothelium of the capillaries and the venules around the cerebral infarctions. These results suggest that the monocytes and the platelets may be closely related to the occurrence of cerebrovascular occlusions. The occlusions may result in further cerebral blood flow reduction and continuous deterioration.

There are various views concerning the direct cause of hypertensive vascular lesions in humans and experimental animals!–3 Only a few investigators, however, have focused on the long term effects of hypertension on the intracerebral vessels?–10 In the present study we performed sequential ultrastructural analysis of the intracerebral vessels in stroke-prone spontaneously hypertensive rats (SHRSP) as developed by Okamoto et al in 1974.11 We discovered numerous monocytes which adhered to the endothelium of the intracerebral arteries and arterioles. Furthermore we discovered many activated platelets which adhered to the endothelium of the intracerebral capillaries and venules. We have therefore tried to clarify the mechanisms of increased monocyte and platelet adhesion and have attempted to explain the roles of monocytes and platelets in vascular lesions, especially those resulting in occlusion.

MATERIALS AND METHODS

Okamoto et al11 and Yamori et al12 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 the rat strain, we have continued to inbreed them selectively. At present we have >1500 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. Rats in the infarction group were anesthetized with pentobarbital and the cerebral arteries perfused with a 1% formaldehyde = 1.25% glutaraldehyde fixative in 0.1M cacodylate

---

Key words:
Cerebral infarction
Intracerebral vessel
Monocyte
Platelet
Stroke-Prone SHR

Department of Medicine, Sanraku Hospital, Tokyo; Department of Pathology, Shimane Medical University, Shimane, Japan
Mailing address: Motoki Tagami, M.D., Department of Medicine, Sanraku Hospital, Chiyoda-ku, Tokyo 101, Japan

Japanese Circulation Journal Vol. 52, November 1988 1351
buffer at pH 7.6 at room temperature via the descending aorta at 180 mmHg pressure for 4–5 min. 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. In the control group we killed rats every 4 weeks from 4 to 52 weeks of age by the perfusion fixation method as previously reported.14

Several sections from each group were cut on a tissue chopper without freezing and collected in chilled fixative. Chopped sections were incubated for 120 min at room temperature in a modification of the medium originally described by Karnovsky for the demonstration of horseradish peroxidase.

All of the sections were post-fixed with 2% OsO4. 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. Ultrathin sections of epon-embedded tissue were mounted on gold grids. These were oxidized or bleached with 4.7% performic acid for 30 min at 4°C, thoroughly washed in distilled water and stained in 1% phosphotungstic acid for 30 min at room temperature (PFP reaction).16

RESULTS
I. 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. Then 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. 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.

II. 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 infarction 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 infarction areas. Several monocytes were observed adhering to the well-maintained endothelial cells in the arteries in which medial smooth muscle cells had completely disappeared (Fig. 1). 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. 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

*Japanese Circulation Journal Vol. 52, November 1988*
processes and enveloped plasma components. At the same time they lost their peroxidase-positive granules. The monocytes, which acted as scavengers, lost cell organella and became electron-dense. Some of the degenerated monocytes returned to the blood stream from the arterial wall (Fig. 2). Subendothelial and medial fibrin deposition thickened the arterial wall and narrowed the arterial lumen. The narrowed lumen was completely occluded by thrombi.

III. Morphology of Capillaries and Venules in SHRSP with Symptoms of Stroke

We discovered that petechial hemorrhages were frequently present around the infarctions. Many red blood cells were seen within the cerebral matrices. Activated platelets were observed without exception in the capillary next to the petechial hemorrhages. The activated platelets put out many pseudopods, closely associated with each other and adhered to the endothelial cells. As a result both the capillaries and the venules were packed with numerous platelets (Fig. 3). Furthermore we performed PFP reaction on ultrathin sections in order to examine the area of adhesion between platelets and endothelial cells. PFP positive substances, that is glyccocaryx, were observed at the sites of platelet adhesion to the endothelial cells as well as to each other.
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.

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 wide-spread 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. 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 lumen 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. The penetration of 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. Furthermore it became clear that the monocytes, which acted as scavengers, lost cell organella, became electron-dense and then returned to the blood stream from the arterial wall. These findings are similar to those observed in aortic lesions of hyperlipidemic animals.\textsuperscript{19,20}

A few researchers have examined perforating arteries. Ooneda et al, working in autopsied cases\textsuperscript{8} showed that intracerebral hemorrhage was caused by intracerebral microaneurysms resulting from "plasmatic arterionecrosis". "Plasmatic arterionecrosis" was 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. Takebayashi et al studied 11 freshly removed brains and 20 lenticulostriate arteries collected from surgical biopsies in cases of intracerebral hemorrhage\textsuperscript{10} 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.

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\textsuperscript{5} Shiraishi et al, in sequential studies of gastric submucosal arteries resulting from stressful stimuli\textsuperscript{21} 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 which 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\textsuperscript{22} and in the medium where smooth muscle cells or endothelial cells were maintained\textsuperscript{23–25} They therefore suggest that chemoattractants 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\textsuperscript{26,27} 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.\textsuperscript{28–30} From our observations we suggest that monocytic enzymes may disturb the blood-brain barrier to proteins. As a result 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.

Moreover we discovered that petechial hemorrhages were frequently present around the infarctions. A lot of platelets, possibly activated by ATP from red blood cells, were observed in the capillaries and the venules. The activated platelets adhered to the endothelial cells and then blocked the lumen of both the capillaries and the venules.

From these observations we advance the following hypotheses (Fig. 4):

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 with resultant cerebral infarctions. The cerebral infarctions are frequently accompanied by petechial hemorrhages. A lot of platelets, activated by ATP released from red blood cells in the hemorrhages, occlude the capillaries and the venules. The occlusions result in further cerebral blood flow reduction and continuous deterioration.

REFERENCES

22. GERRITY R, GOSS J, SOBY L: Control of monocyte recruitment by chemotactic factor in lesion-prone areas of swine aorta. Arteriosclerosis 5: 55, 1985
23. MAZZONE T, JENSEN M, CHAIT A: Human arterial wall cells secrete factor that are chemotactic for monocytes. Proc Natl Acad Sci USA 80: 5094, 1983

Japanese Circulation Journal Vol. 52, November 988