Vascular Cell Components of the Medullary Arteries in Binswanger’s Disease Brains: A Morphometric and Immunoelectron Microscopic Study

Jin-Xi Lin, MD; Hidekazu Tomimoto, MD; Ichiro Akiguchi, MD; Akinori Matsuo, MD; Hideaki Wakita, MD; Hiroshi Shibaasaki, MD; Herbert Budka, MD

Background and Purpose—It has been hypothesized that fibrohyalinosis of the medullary arteries may cause white matter lesions in Binswanger’s disease (BD). However, previous reports have been inconsistent on the pathological alterations of the cellular components, which may vary in terms of vessel sizes. We therefore quantitatively examined vasculopathy in the medullary arteries of a defined caliber in BD brains with a quantitative technique.

Methods—A total of 20 brains were examined: 10 from patients with BD and 10 from age-matched nonneurological control patients. The alterations in the vascular cell components were examined with quantitative immunohistochemistry and immunoelectron microscopy for collagen and smooth muscle actin.

Results—The nonneurological control patients showed no white matter lesions. In contrast, the patients with BD invariably had marked white matter lesions, as well as fibrohyalinosis of the medullary arteries. The ratio of the area immunolabeled for collagen type I and type IV to the cross-sectional area was 2-fold higher in the BD patients than in the control patients, regardless of the vessel caliber (P<0.005). Although the ratio for smooth muscle actin in the BD brains was increased in arteries of <100 μm (P<0.0001), there was no corresponding increase in the arteries of >100 μm. However, in the ultrastructure of these vessels, the cell bodies immunolabeled for smooth muscle actin were hypertrophic and segregated from each other by proliferated fibrils. The basal lamina appeared multilayered, and the endothelial cells were swollen. Collagen type I and type IV immunoreactive fibrils also proliferated in the pericapillary space of the BD brains.

Conclusions—The proliferation of collagen fibrils in the media and adventitia of the blood vessels in BD brains was not specific to small arteries and arterioles but also occurred in the pericapillary spaces. Pericapillary sclerosis, smooth muscle cell proliferation in the terminal arterioles, and their morphological transformation in the proximal arteries may alter the shear rates and thus cause profound microcirculatory disturbances in BD brains. (Stroke. 2000;31:1838-1842.)

Key Words: Binswanger’s disease ▪ dementia ▪ leukoencephalopathy ▪ white matter

Subcortical arteriosclerotic encephalopathy, or Binswanger’s disease (BD), is characterized pathologically by widespread white matter lesions, multiple lacunae, and fibrohyalinosis of the medullary arteries.1-4 There is a longstanding assumption that white matter lesions are pathogenically attributable to changes in the small vessel of the white matter.5 Because small arteries and arterioles are the main vessels that resist the intraluminal perfusion pressure, especially in hypertension,6 it is conceivable that fibrohyalinosis in these vessels has an influence on vascular resistance and flow.

Although numerous studies have characterized the white matter lesions in BD, which consist of myelin degeneration, gliosis, edema, and necrotic foci,4,7-11 the vasculopathy associated with this syndrome has not been entirely clear in previous publications.1,12-16 Little information is available as to the extent of the adventitial fibrosis in BD with respect to the vessel sizes.17 In addition, there have been inconsistent reports on the medial changes of the medullary arteries, such as hypertrophy,18 degeneration, and lipohyalinosis,1,19,20 which might have resulted from caliber differences or from difficulties in defining the media-adventitia border on routine histological staining.

In the present study, we performed a quantitative estimation of the vascular cell components of medullary arteries of a defined caliber by using collagen and smooth muscle actin as immunohistochemical markers. The ultrastructural features were further characterized with immunoelectron microscopy. The results indicated that the vasculopathy observed in BD brains was accompanied by changes in the cellular constituents, which are dependent on the external diameter, and that these changes may profoundly impair the cerebral microcirculation.
Materials and Methods

Human Tissues

We examined 20 autopsied brains obtained from the Wien University and Kyoto University Hospitals. Ten brains were obtained from nonneuropsychiatric patients aged 65 to 85 years (mean±SD 73±6 years), and 10 were from BD patients aged 52 to 85 years (mean±SD 67±12 years). The diagnosis of BD was made clinicopathologically and retrospectively fulfilled all of the clinical criteria proposed by Bennett et al. Briefly, all of the patients had dementia, bilateral diffuse subcortical hyperintense lesions on T2-weighted MRI, and at least 2 of the following 3 clinical findings: (1) a vascular risk factor or evidence of systemic vascular disease, (2) evidence of focal cerebrovascular disease, and (3) evidence of subcortical cerebral dysfunction such as gait disorders, parkinsonism, or incontinence. The neuropathological criteria used in the present study included diffuse white matter lesions, brain atrophy, and lacunae in the white matter and basal ganglia with evidence of arterioarteriosclerosis of the cerebral vessels.

With regard to the demographic information, the control patients died of malignancy (n=3), cardiac failure (n=2), and pulmonary embolism, suppurative spondylitis, and renal failure (n=1). The BD patients died of pneumonia (n=7), malignancy (n=1), and cardiac failure (n=1). The direct cause of death was not specified in 2 control patients and 1 BD patient. All of the BD patients had either hypertension or a history of hypertension. The duration of illness ranged from 19 months to 5 years 10 months for the BD patients. The postmortem delay did not differ significantly between the control and BD patients: from 2 to 6 hours and from 3 to 10 hours, respectively.

The brains were fixed in formalin and then embedded in paraffin. Each paraffin section was cut coronally at the level of the anterior horn of the lateral ventricles and included the frontal cortex and the underlying white matter. Standard histological examinations were then performed with Klüver-Barrera and Masson trichrome stains.

Light and Electron Microscopic Immunohistochemistry

The sections were incubated overnight with the following primary antibodies: a mouse monoclonal antibody directed against human smooth muscle actin (diluted 1:100; DAKO) and rabbit polyclonal antiserum raised against human collagen type I (diluted 1:100; Chemicon) and collagen type IV (diluted 1:100; Chemicon). These sections were subsequently incubated with the appropriate secondary antibody (diluted 1:200) for 1 hour and then an avidin-biotin peroxidase complex solution (diluted 1:200) for 1 hour. After each incubation, the sections were rinsed for 15 minutes in 0.1 mol/L PBS (pH 7.4). Finally, the immunoreaction products were visualized in a mixed solution of 0.02% 3,3’-diaminobenzidine tetrahydrochloride and 0.005% H2O2 in 0.05 mol/L Tris buffer (pH 7.6).

For immunoelectron microscopy, the brains were initially perfused with 0.01 mol/L PBS (pH 7.4) and then with 4% paraformaldehyde, 0.2% picric acid, and 0.1% glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.4). These brains were postfixed overnight at 4°C in 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) and then sectioned on a vibratome into 50-μm-thick slices. The sections were immunostained as described for the light microscopic immunohistochemistry. They were osmicated, stained en block with 1% uranyl acetate, and then flat embedded in Spurr’s embedding medium. Ultrathin sections were finally examined under a transmission electron microscope.

Morphometry

The immunoreactive areas from monochromatic photo images were digitized with an Apple personal computer (PC7500) with a LS-1000 film scanner (Nikon) at a resolution of 1350 dots per inch and were saved as 8-bit gray scale TIFF files (256 shades of gray). The image files were converted to PICT files and analyzed with NIH Image analyzer software. The medullary arteries were classified into 3 groups according to their external diameters (<50 μm, 50 to 100 μm, and >100 μm). In all patients, 10 arteries from each diameter group were examined in the deep white matter regions ≥2 mm distant from the gray and white matter border at the level of the anterior horn.

Results

The vessel walls immunoreactive for collagen type I were thicker in the BD patients than in the control subjects (Figure 1). Although the capillaries in the control brains were immunonegative for collagen type I, those in the BD brains were intensely immunoreactive (Figure 1C, inset). Similarly, the immunostaining for collagen type IV was more intense in the intima-media border of the BD brains than the control brains (Figure 2). The adventitia was also immunostained for collagen type IV exclusively in the BD brains. In the medullary arteries with a diameter of 100 μm, the vessel walls immunolabeled for smooth muscle actin were thicker in the BD brains than in the control brains (Figure 3). In those >100 μm, no differences were noted between the BD and control brains in terms of the wall thickness immunolabeled for smooth muscle actin. However, the immunoreactivity was distributed in a heterogeneous stippled pattern in the BD brains.

Immunoelectron microscopy of the BD brains confirmed that the fibrils immunoreactive for collagen type I proliferated in the pericapillary space (Figure 4A) and in the media and adventitia of the medullary arteries of the BD brains (Figures 4B and 4C). The fibrils immunoreactive for collagen type IV were localized adjacent to the endothelial cells in the control brains (not shown), but in the BD brains they extended into the adventitia (Figure 4D). Electron-dense immunoreaction products for smooth muscle actin were observed in a few layers of the hypertrophic cytoplasm in the BD brains (Figure 4F) compared with the spindle-shaped ones in the control brains (Figure 4E). In the BD brains, the
basal lamina was thickened beneath the endothelial cells and in the surroundings of the smooth muscle cells, resulting in a multilayered appearance. The endothelial cells appeared swollen occasionally.

The morphometric analysis indicated that in vessels with a diameter of $<50 \, \mu m$, the ratio of the area immunoreactive for collagen type I to the cross-sectional area was $67.1 \pm 14.5\%$ in the BD patients, which was significantly higher than the ratio of $36.9 \pm 13.4\%$ (mean $\pm$ SD) observed in the control subjects (Figure 5a). There also was a significant increase in the ratio for collagen type I in the BD brains: $48.8 \pm 15.8\%$ and $25.0 \pm 10.6\%$ in the BD and control patients, respectively, for vessels with a diameter of $50$ to $100 \, \mu m$ and $31.8 \pm 13.6\%$ and $23.5 \pm 11.8\%$ in the BD and control patients, respectively, for vessels with a diameter of $>100 \, \mu m$. A similar increase was observed in the ratio for collagen type IV in the BD brains: $51.8 \pm 14.8\%$ versus $26.3 \pm 10.4\%$ for vessels with a diameter of $<50 \, \mu m$, $36.8 \pm 13.0\%$ versus $19.4 \pm 6.7\%$ for vessels with a diameter of $50$ to $100 \, \mu m$, and $23.9 \pm 11.0\%$ versus $11.7 \pm 5.2\%$ for vessels with a diameter of $>100 \, \mu m$ (Figure 5b), respectively.

The ratio for immunoreactive smooth muscle actin was higher in the BD patients than in the control subjects: $56.1 \pm 16.2\%$ versus $39.9 \pm 14.4\%$ (BD and controls, respectively) for vessels with a diameter of $<50 \, \mu m$ and $32.1 \pm 13.1\%$ and $24.6 \pm 12.2\%$ for vessels with a diameter of $50$ to $100 \, \mu m$. However, there were no statistically significant differences between the ratio of BD and control patients in vessels with a diameter of $>100 \, \mu m$ (16.7$\pm$11.3\% versus 15.4$\pm$9.0\%) (Figure 5c).

**Discussion**

In the present study, the ratios for both collagen type I and type VI were increased in the BD brains, regardless of the sizes of the vessels, including capillary. In contrast, the increase in the ratio for smooth muscle actin was seen only in vessels of $<100 \, \mu m$. These results are in agreement with the study by Zhang and Olsson, which reported that pronounced...
immunostaining for smooth muscle actin was frequently observed in the terminal arterioles of BD brains. Zhang and Olsson also reported reduced immunostaining for smooth muscle actin in the larger medullary arteries of BD patients. In hypertension and aging, the number of smooth muscle cell nuclei decreases in the larger medullary arteries. However, in the present investigation, there were no quantitative differences in the smooth muscle actin immunoreactive areas in the larger vessels. This might have been due to the hypertrophic changes in cell size compensating for the decrease in the number of cell bodies. The stippled pattern of immunostaining may be attributable to a proliferation of interstitial tissues, which segregate the cell bodies from each other. However, it still remains unclear whether these morphological changes reflect a degenerative process or a morphological transformation of the smooth muscle cells in response to chronic hypertension.

Fibrohyalinosis is a diffuse change in the medullary arteries that is likely to differ from fibrinoid necrosis, a focal or segmental microangiopathy that mainly involves the penetrating arteries in the deep gray matter and cerebral cortex. In experimental animals, fibrohyaline thickening occurs in association with a breakdown of the blood-brain barrier, which has been demonstrated in the white matter in BD. Although previous studies failed to detect any morphological abnormalities in the intima of the vessels from BD brains, the endothelial cell swelling and basal lamina thickening observed in the present investigation suggest the presence of blood-brain barrier dysfunction in BD. The proliferation of collagen fibrils may have been triggered by chronic hypertension and high flow rates, because endothelial cells are known to be one of the major sources of collagen production in response to shear stress, as well as the fibroblasts and smooth muscle cells. There are at least 14 distinct subtypes of collagen that are different gene products and have specific localizations and functions. Although there is little information available for BD, the composition of collagen has been shown to change in hypertensives; for example, collagen type IV, a constituent of the basal lamina, is ubiquitously increased. As demonstrated here, adventitial fibrosis was accompanied by an alteration of the matrix components; an increase in collagen type I, and an emergence of collagen type IV in the adventitia.

White matter lesions due to chronic cerebral hypoperfusion can be induced experimentally in the rat brain by bilateral clipping of the carotid arteries. A progressive reduction of the arterial pressure has been shown to produce a cessation of blood flow in the centrum semiovale while the cortical gray matter is perfused. Therefore, adventitial fibrosis that occurs in all 3 size categories of medullary arteries may impair the regulation of microvascular blood flow. These arteries may in turn be susceptible to a reduction in perfusion pressure and thus cause white matter lesions. It is also likely that these microcirculation disturbances may further reduce the blood flow in the larger vessels. Degeneration of the smooth muscle cells, which has been demonstrated in the larger medullary arteries of BD brains, may be compensated for by the thickened media of the terminal arterioles. These alterations in the vascular cytoarchitecture may cause a profound shear rate change and thus result in damage to the microvascular endothelium due to a higher shear stress and permeability changes in the blood-brain barrier.

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