The Microvasculature of the Cerebral White Matter: Arteries of the Subcortical White Matter

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Abstract. The microvascular architecture of the human cerebral subcortical white matter was studied. Most of the subcortical arteries ran straight through the cortex, but upon entering the white matter, they began to coil, loop, and spiral. Vascular stains showed wide spaces between the adventitial sheaths and blood vessels. The blood vessels coiled, looped, and spiraled within these wide adventitial spaces. This phenomenon was observed in the brains from persons ranging from the first to ninth decades of life and there were no statistically significant age-related correlations. Furthermore, there was no evidence of a reduction in the volume of white matter after fixation. Therefore, the observed tortuosity does not appear to be the result of shrinkage of brain tissue following fixation. While the mechanisms responsible for the subcortical arteries circuitry remain undetermined, this coiling architecture may serve as a trap for tumor cells and microorganisms passing through the blood stream, suggesting that these coiling arterial blood vessels may play a significant role in the pathogenesis of tumor metastasis and the brain abscesses that frequently occur in the gray-white matter junction.

Key Words: Cerebrum; Corrosion cast; Human brain; Microvasculature; Scanning electron microscopy; Subcortical white matter.

INTRODUCTION

We have previously published studies on the microvasculature of the human cerebral (1) and cerebellar (2) cortices and classified the arteries distributed in the cerebral cortex and white matter as cortical, subcortical, and deep white matter arteries. The cortical arteries were further classified into superficial, middle, and deep cortical branches according to the site of their termination. Many fountain-like rami were observed in the middle and deep cortical branches, as well as in the intracortical branches of the subcortical arteries. These fountain-like rami were composed of several to many fine branches evolved from repeated bifurcation within a short segment of a vessel’s course. Intertwinement of these branches, forming rope-like structures, increased in frequency with age, suggesting that this phenomenon correlates with age and/or is associated with brain atrophy. As part of a continuing project, our investigation was extended into the cerebral white matter.

Using transparent tissue preparation, Gallyas vascular stain (3), and scanning electron microscopic observation of corrosion casts of blood vessels, our goal was to demonstrate the architecture of the arterial supply in the cerebral white matter. Capillaries are readily identifiable by their size and extensive network formation, but small arteries and arterioles cannot be satisfactorily distinguished from each other because the size of a blood vessel tapers gradually. Thus, in this paper, we will group arteries of variable sizes together, including arterioles, and call all of them arteries.

MATERIALS AND METHODS

A total of 109 human brains obtained at autopsy were used for this study. All brains showed no gross abnormalities and the patients had no history of neurological diseases. Their age at death ranged from 9 mo to 89 yr.

Transparent Specimens

Fourteen brains were prepared for transparent specimens. The age of the patients ranged from 28 to 83 yr. At autopsy, immediately after removal of the brain from the skull, approximately 300 ml of physiological saline heated to 36°C was perfused through the right internal carotid artery, followed by injection of approximately 100 ml of barium sulfate suspension containing 1.5% gelatin. Two brains were injected with India ink. The brains were fixed in 10% formalin for 2 wk. Thereafter, 50-μm-thick frozen sections of the frontal lobe were made. These were dehydrated with alcohol and were cleared with xylol for light microscopic observation.

Fig. 1. a: Capillary networks are more dense in the cortex than in the white matter. The gyral white matter is rich in subcortical and deep white matter arteries, with concentrations of arteries penetrating from the summits and the lateral walls of the gyri. Diaphanized specimen. b: The subcortical arteries penetrate the cortex in a straight line. When they reach the subcortical white matter, they begin to coil, loop, and spiral. Diaphanized specimen. c: Coiling of a subcortical artery. Diaphanized specimen. Magnifications: a: ×35; b: ×40; c: ×330.
Vascular Staining with the Gallyas Method

In addition to the above 14 brains from which transparent specimens were made, 81 formalin-fixed brains were studied. The age of these patients also ranged from 9 mo to 89 yr at death (Table 1). One hundred and fifty-μm-thick frozen sections of the frontal lobe were stained with silver for cerebral blood vessels using physical development according to the method of Gallyas (3). Statistical analysis using χ² test and the Kruskal-Wallis test was performed on the results of the findings.

Paraffin Section

Blocks of formalin-fixed brains were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E), elastica-van-Gieson, and Masson’s trichrome stain.

Scanning Electron Microscopy

Nine other brains from individuals aging 53 to 83 yr were examined by scanning electron microscopy (SEM). At autopsy, immediately after removal of the brain from the skull, about 300 ml of physiological saline heated to 36°C was injected into the right internal carotid artery, followed by an injection of approximately 100 ml methacrylate resin (Mercox CL-2R, Dainippon Ink, Tokyo, Japan). After fixation in 10% formalin solution for 2 wk, approximately 7-mm-thick coronal sections containing both cortex and white matter were prepared from the middle and inferior frontal gyri. These sections were immersed in 20% KOH solution for 10 to 14 days to dissolve the brain tissue. After washing with running tap water, the blood vessel cast obtained was freeze-dried. The specimens were coated with gold-palladium in a vacuum evaporator before SEM evaluation.

Volume Change of White Matter in Fixative

A cubic mass of fresh white matter was obtained from the frontal lobe of 5 brains. The age of the individuals ranged from 42 to 65 yr. The cubes were immersed in 10% formalin and their volume was measured daily using a graduated cylinder filled with fixative.

RESULTS

Transparent Specimens

The capillary networks in the cortex were more dense than those in the white matter (Fig. 1a). Arterial blood vessels penetrated the cortex from the leptomeninges at almost right angles to the cortical surface. Many blood vessels terminated in the cortex and subcortical white matter. Some large blood vessels passed through the cortex and extended into the white matter toward the ventricular angle. Cortical, subcortical, and deep white matter arteries were thus distinguished. Most of the trunks of the subcortical and deep white matter arteries ran straight through the cortex.

The gyral white matter was rich in subcortical and deep white matter arteries and contained condensed arteries penetrating the crests of gyri and walls of sulci. The arteries penetrating the walls of sulci made an almost right angle turn when they reached the subcortical white matter, and at that point coursed the deeper white matter (Fig. 1a).

When the subcortical arteries entered the subcortical white matter, they began to coil, loop, and spiral (Fig. 1b, c). This was noted both in arteries penetrating the convexities of the gyri and those entering through the walls of sulci. After giving out a few intracortical branches in the deeper cortex, they tapered and branched out to capillaries in the subcortical white matter.

Gallyas Capillary Staining

Since these sections were thinner than the transparent specimens, it was possible to trace the course of blood capillaries in the subcortical white matter. The capillary networks in the cortex were more dense than those in the white matter (Fig. 1a). Arterial blood vessels penetrated the cortex from the leptomeninges at almost right angles to the cortical surface. Many blood vessels terminated in the cortex and subcortical white matter. Some large blood vessels passed through the cortex and extended into the white matter toward the ventricular angle. Cortical, subcortical, and deep white matter arteries were thus distinguished. Most of the trunks of the subcortical and deep white matter arteries ran straight through the cortex.

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vessels only for a short length within the same section. The cortex had more dense capillary networks than the white matter. The trunks of the subcortical and deep white matter arteries that traversed the cortex were characterized by tight adhesion between the thin adventitial sheaths and the vascular walls; most of which penetrated straight through the cortex (Fig. 2a). At the point where the subcortical arteries entered the subcortical white matter, the spaces between the adventitial sheaths and vascular walls widened. Blood vessels began to coil, loop, and spiral within these wide adventitial spaces (Fig. 2b, c). After giving off a few side branches to the cortex, the arteries branched out to capillaries in the subcortical white matter.

We counted the number of coiled arteries per 10 subcortical arteries in sections where the coiled arteries were most frequently observed. The numbers of coiled arteries (Table 1) and the frequency of coiled arteries per 10 arteries (Table 2) were tabulated by age. These data were statistically analyzed using the $\chi^2$ test for the numbers of coiled arteries and the Kruskal-Wallis test for the frequency of coiled arteries per 10 arteries. In brains from the first to the ninth decades there was no statistically significant relation between the number or frequency of this phenomenon and the age of the patients ($\chi^2 = 6.250; \text{DF} = 7; p = 0.511$ and $p = 0.7582$ for the Kruskal-Wallis test, respectively).

**Scanning Electron Microscopy (SEM)**

As the cortex was rich in capillary networks, the vascular frames of the corrosion casts were well preserved, but they tended to collapse easily in the white matter because the capillary networks of the white matter were not as dense as those in the cortex. Nevertheless, the subcortical and deep white matter arteries were easily identified, possessing the same features seen in the transparent specimens. In the cortex many cortical arteries were noted. After branching in the deep cortical layer, the subcortical arteries reached the subcortical white matter and bifurcated into capillaries. Within the white matter, the deep white matter arteries continued straight toward the angle of the ventricle. The subcortical arteries penetrated straight through the cortex. At the point where they entered the subcortical white matter, they began to coil, loop, and spiral (Fig. 3a, b). The blood vessels penetrating the walls of sulci made an almost right angle turn when they reached the subcortical white matter.

**Paraffin Sections**

These sections were too thin to trace and follow the course of arteries. When the coiled subcortical arteries were longitudinally sectioned, chains of blood vessels cross sections could be observed (Fig. 3d).

**Change in the White Matter Volume in Fixative**

The volume of all specimens increased slightly 1 day after fixation, but remained unchanged for 30 days thereafter (Fig. 4).

**DISCUSSION**

The density of the arteries and capillary networks was greater in the cortex than in the white matter. According to Ranson and Clark (4), the capillary networks in the cortex are 3 times more dense than those of the white matter.

The branching pattern of the subcortical arteries differed from that of the cortical arteries. The characteristic features of the arteries in the cerebral cortex were the fountain-like rami and the intertwining of the branches (1). The subcortical arteries branched and tapered into...
capillaries in the subcortical white matter after a few branchings in the deeper cortex.

The subcortical arteries ran straight within the cerebral cortex, but began to loop and coil immediately after penetrating the white matter. In the Gallyas stains, the trunks of subcortical arteries that traversed the cortex were characterized by tight adhesion between the adventitial sheaths and vascular walls. However, when these arteries entered the subcortical white matter, the spaces between the adventitial sheaths and vascular walls became widened, and the blood vessels coiled, looped, and spiraled within this wide adventitial space.

Hassler (5, 6) examined the brains from individuals with senile dementia, using a micro-angiographic method, and found similar vascular changes, which he described as glomerular loop formation in the brains of senile dementia cases and controls. He observed these changes in most of the senile dementia brains and in the control brains from individuals over 40 years old. Changes were noted in both the cortex and white matter. Duvernoy et al (7) also noted these changes in the subcortical arteries of normal brains. Challa et al (8) reported similar arteriolar coiling, looping, and spiraling and detected a 4-fold increase in arteriolar tortuosity in an older age group (over 60 years of age). They further suggested the possibility that these tortuous blood vessels had been misinterpreted as Charcot-Bouchard aneurysms, which were considered to be the cause of hypertensive cerebral hemorrhage. In the present study, however, these circuitous arterial changes were observed only in the subcortical arteries. Spangler et al (9) also reported arterial tortuosity, 70% of which was noted in the gray-white interface; incidence increased with age, but not significantly. There was no correlation between the vascular tortuosity and either hypertension, sex, or race. The presence and frequency of tortuosity per 10 arteries by age and the relevant statistical analyses are shown in Tables 1 and 2, respectively. In the brains from the first to the ninth decades, there was no statistically significant correlation between the presence and frequency of this phenomenon and the age.

The coiling changes were not seen in arteries distributed in the basal ganglia (10), which are the most common sites for Charcot-Bouchard aneurysms (11–13). Charcot-Bouchard aneurysms have been previously noted in the subcortical white matter (11–13), possibly the result of miscellaneous vascular changes, including specimen preparation artifacts.

The occurrence of these coil, loop, and spiral changes is easily attributed to shrinkage of brain substance due to fixation. The volume of all specimens increased slightly one day after fixation and remained unchanged for 30 days thereafter. However, the result does not support the idea of arterial tortuosity being a fixation artifact.

While the exact mechanisms responsible for arterial tortuosity remain unclear, it appears that it is not a pathological phenomenon because it was noted in the normal brains—even those from individuals in their first decade of life—and did not correlate with advancing age. Tortuosity may be due to the different consistency of the cortex and white matter and the size of the arteries. The cerebral cortex is compact, with densely packed neuronal processes connected by synapses; extracellular spaces are restricted to an interval of 10 to 20 nm. In contrast, the cerebral white matter consists of parallel bundles of myelinated fibers and extracellular spaces of 80 nm or more. The diameter of these coiling arteries is limited to 30 to 40 μm.

Although the arteries penetrating the walls of sulci make an almost right angle turn when they reach the subcortical white matter, the circuitry is also present in these arteries. Tumor cells and microorganisms in the bloodstream may be easily trapped in the vascular coils. The presence of these coiled arteries may be the reason why metastatic tumors and brain abscesses tend to occur at the gray-white matter junction (14, 15).

ACKNOWLEDGMENTS

We wish to express our thanks to all members of the Department of Pathology, Toho University School of Medicine, for their collaboration; to Prof. Chiaki Nishimura, Department of Medical Informatics, Toho University School of Medicine, for statistical analysis; and to Prof. Emeritus Cheng-Mei Shaw, Department of Neuropathology, University of Washington, for his useful comments on the manuscript. We would also like to thank Mrs. Toshie Shimozeki and Mr. Akira Maeda for their skillful technical assistance.

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Received December 20, 2001
Revision received May 8, 2002 and October 10, 2002
Accepted October 17, 2002