Histological Features of Middle Cerebral Arteries From Patients Treated for Moyamoya Disease

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Abstract

Moyamoya disease (MMD) is a cerebrovascular occlusive disease characterized by progressive stenosis or occlusion at the distal ends of the bilateral internal carotid arteries. Vascular structural changes were previously detected using postmortem specimens. This study investigated 35 specimens of the middle cerebral artery (MCA) from 25 patients undergoing surgical treatment for MMD. Six MCA samples were also obtained from six control subjects. Histological examination showed that MCA specimens from patients with MMD had significantly thinner media and thicker intima than control specimens. In addition, abnormal findings of the internal elastic lamina and eosin-positive deposits in the intima were detected. Medial thinness and intimal hyperplasia occurred in the MCA of patients with MMD.

Key words: moyamoya disease, middle cerebral artery, media, intima

Introduction

Moyamoya disease (MMD) is a cerebrovascular occlusive disease characterized by progressive stenosis or occlusion at the distal ends of the bilateral internal carotid arteries (ICAs), resulting in the development of an unusual vascular network at the base of the brain (moyamoya vessels), which is called “moyamoya” because of its hazy, puff of smoke-like angiographic appearance due to the many collaterals of the basal perforating arteries and arterioles. The etiology of the disease is undefined. The incidence of the disease is highest in, but not confined to, the Japanese population and the condition is frequently familial, suggesting the involvement of a genetic factor in its pathogenesis.

Histological investigations of autopsy specimens have demonstrated that the main vascular lesion in MMD is stenosis or occlusion caused by fibrocellular intimal thickening. Patients with MMD are usually treated by superficial temporal artery-middle cerebral artery (STA-MCA) bypass surgery or indirect revascularization. During this surgery, the MCA is often observed to have a thin wall and to appear transparent. Several studies on extracranial vessels have been reported, but few on the intracranial arteries from patients with MMD except those based on autopsy specimens.

The present study histologically analyzed a series of samples of MCA walls obtained from patients treated for MMD to explore the characteristics of the intracranial arterial walls in MMD.

Methods

Twenty-five patients underwent surgical procedures for the standard indications of MMD at Kyoto University Hospital. All patients were symptomatic with reduction of cerebrovascular reserve assessed by single photon emission computed tomography with diamox challenge. Clinical data of the patients are summarized in Table 1. This study was performed under the guidelines of the ethics committee of Kyoto University School of Medicine. All patients gave informed consent.

During STA-MCA bypass surgery, an 11-0 nylon monofilament was passed around the wall of the recipient MCA (M portion, 0.5–1.0 mm in diameter). The vessel was pulled up by lifting the monofilament with forceps, and the operator (Y.T. or K.K.) performed arteriotomy with microscissors. Tiny samples of the MCA were obtained without disruption of the recipient MCA after the surgery. The 35 specimens were fixed in 10% formalin overnight and then embedded in paraffin the next day. The specimens were stored at room temperature. Six control MCA samples were also obtained in
Table 1  Summary of cases

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moyamoya disease</strong></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>35</td>
</tr>
<tr>
<td>Cases</td>
<td>25</td>
</tr>
<tr>
<td>Onset</td>
<td></td>
</tr>
<tr>
<td>ischemia</td>
<td>23</td>
</tr>
<tr>
<td>hemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>Age (mean ± SD, yrs)</td>
<td>38.4±15.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>7</td>
</tr>
<tr>
<td>female</td>
<td>18</td>
</tr>
<tr>
<td>Surgery</td>
<td></td>
</tr>
<tr>
<td>STA-MCA bypass</td>
<td>35</td>
</tr>
<tr>
<td>others</td>
<td>0</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
</tr>
<tr>
<td>Samples</td>
<td>6</td>
</tr>
<tr>
<td>Cases</td>
<td>6</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
</tr>
<tr>
<td>meningioma</td>
<td>1</td>
</tr>
<tr>
<td>astrocytoma</td>
<td>2</td>
</tr>
<tr>
<td>aneurysm</td>
<td>3</td>
</tr>
<tr>
<td>Age (mean ± SD, yrs)</td>
<td>59.1±9.3</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>1</td>
</tr>
<tr>
<td>female</td>
<td>5</td>
</tr>
</tbody>
</table>


the same way from the six control subjects described in Table 1.

Multiple, sequential, 6-μm thick tissue sections were cut from the paraffin blocks and deparaaffinized in xylene, rehydrated, and stained with hematoxylin and eosin. The sections were examined under a BX51 fluorescent microscope (Olympus Optical Co., Tokyo) or a Fluoview FV300 laser confocal microscope (Olympus Optical Co.) and the histological images were captured with a computer. Intimal and medial thickness was analyzed with an Image-Pro image-analyzing system (Media Cybernetics, Silver Spring, Md., U.S.A.).

Mann-Whitney and Fisher’s exact tests were used for statistical analysis (StatView; SAS Institute, Cary, N.C., U.S.A.). P < 0.05 was considered statistically significant.

**Results**

The mean thickness of the MMD intima was 19.4 ± 9.7 μm and significantly greater than that of the control intima of 8.0 ± 4.7 μm (p = 0.0041). The mean thickness of the MMD media was 23.0 ± 7.7 μm and significantly less than the control intima of 61.8 ± 30.4 μm (p = 0.0009). Histological examination of the MMD samples showed the internal elastic lamina was normal in seven samples, disrupted in 10 samples, and thin in 14 samples compared to the control samples, with eosin-stained intimal deposits in five samples (Fig. 1).

**Discussion**

The present study of surgical samples of MCAs from patients with MMD found thinner media and thicker intima compared to the control specimens. In
addition, changes in the internal elastic lamina were more frequent in MMD specimens than in control specimens. These results indicate that remodeling of vascular wall occurred in the intima and media of the MCA in patients with MMD as previously reported using autopsy samples.

Examination of autopsy samples of the MCA from patients with MMD found fewer than normal smooth muscle cells in the media and thick intima. Fragmented elastic lamina and attenuated media were also reported. The present study also found intimal hyperplasia and medial thinness in the M4 portion of the MCA. These changes were predominantly observed in the terminal portion of ICA in the previous autopsy studies. Therefore, the characteristics of arterial changes are similar between the M4 and ICA. We analyzed the thicknesses of intima and media in patients under 30 years old, from 30 years old to 40 years old, and over 40 years old, and in males and females, but could not detect any significant differences between the groups (results not shown). The incidence of abnormal internal elastic lamina was also assessed. Twenty percent of the patients under 30 years old, 40% of the patients from 30 years old to 40 years old, and 11% of the patients over 40 years old showed abnormal internal elastic lamina. These results may indicate intimal hyperplasia and medial thinness even in young patients with MMD. However, this abnormality of internal elastic lamina may occur as a secondary effect.

The molecular mechanism underlying MMD has been investigated, but not fully clarified. We reported that basic fibroblast growth factor (bFGF) may be involved, as bFGF expression is elevated in the cerebrospinal fluid (CSF) and STA of patients with MMD. In addition, FGF receptors are overexpressed in MMD. FGF is a strong mitogen and is thought to promote intimal thickening and angiogenesis. Transforming growth factor-beta (TGF-β) is also involved in MMD, because elevated levels of TGF-β in the CSF and TGF-β expression in the STA are found in patients with MMD. TGF-β mediates MMD through elastin synthesis, as elastin accumulates via the TGF-β pathway and results in the intimal thickening in MMD. Moreover, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and elastin levels increase in the CSF of patients with MMD. Other findings indicate that an inflammatory response or endothelial activation occurs, as the production of prostaglandin E2 and interleukin-1β is greater in the smooth muscle cells of patients with MMD.

Patients with MMD often present systemic arterial lesions including the STA or renal artery.

However, the main characteristic of MMD is intracranial stenosis, so intracranial arterial samples are the most important. Autopsy specimens tend to be degraded during the fixation process, so proteins and messenger ribonucleic acid in good condition are very difficult to collect. Our specimens were fresh and messenger ribonucleic acid was easy to collect. In this study, we only showed the evidence of reorganization of the arterial walls. We are planning to investigate the molecular mechanisms of intimal thickening, medial thinning, and angiogenesis in the future analysis using our samples.

References

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Commentary

The authors histologically analyzed 35 specimens of the middle cerebral arteries from patients with moyamoya disease, which were obtained during bypass surgery and were freshly fixed with formalin. They found hyperplasia of the intima and narrowing of the media of the middle cerebral arteries. These findings are consistent with previous histological findings in autopsy specimens. In autopsy cases, these histological changes are typically observed in the terminal portion of the internal carotid arteries, which is the essential characteristic of moyamoya disease. The similarity of histological findings may justify the importance of investigating such specimens in search of the causes of moyamoya disease.

The unique feature that the specimens were fresh would have great advantages in investigating proteins, DNAs, and RNAs in the vessels, because they are less likely to be denatured. As they have stated in the last part of the discussion, further investigation using these specimens is awaited.

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Moyamoya disease with its characteristic feature of bilateral progressive stenosis and occlusion of the supraclinoidal internal carotid arteries has been recognized for many decades. Yet, the etiology of this disease is not well understood.

In this article, instead of using specimens from autopsy, Takagi and colleagues from Kyoto studied specimens of intracranial arterial wall obtained from arteriotomy during extracranial-intracranial vascular bypass surgery. The results of the present study revealed the peripheral branch of the middle cerebral artery (M4 segment) in patients with moyamoya disease has a thinner media and thicker intima than the normal controls. Changes such as disruption and thinning of the internal elastic lamina and eosin-positive deposits in intima were also found. These findings are similar to the previous autopsy studies on distal internal carotid arteries.

However, whether these results represent the etiology or the consequences of moyamoya disease cannot be elucidated from this morphological study. It also cannot be explained why similar morphological changes are observed in proximal and distal arteries, but not in the clinical setting where stenosis and occlusion always start from the supraclinoidal internal carotid artery. As the authors mentioned, further molecular study would probably provide solutions for these questions.

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