Arterial Changes in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) in Relation to Pathogenesis of Diffuse Myelin Loss of Cerebral White Matter

Examination of Cerebral Medullary Arteries by Reconstruction of Serial Sections of an Autopsy Case

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Background and Purpose—There is little information regarding the pathogenesis underlying diffuse myelin loss in the cerebral white matter and sparing of the U fibers in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), in which the medial smooth muscle cells of systemic arteries are characteristically involved. We sought to examine the precise extent and severity of changes in the cerebral arteries in an autopsy case of CADASIL in relation to pathogenesis of the diffuse myelin loss.

Methods—We reconstructed 1000 serial sections of the frontal cerebral medullary arteries of an autopsy subject, which was the first identified Japanese case of CADASIL, as confirmed by the presence of ultrastructural deposits of granular osmiophilic material in the media of some visceral arteries and by genetic analysis.

Results—We reconstructed 11 medullary arteries of the frontal lobe showing diffuse myelin loss and atrophy of the white matter with sparing of the U fibers. All of these showed complete loss of medial smooth muscle cells over their entire length and severe adventitial fibrosis. Although intimal fibrosis or hyalinosis was present, luminal occlusion was scarce. These changes were also observed in the small and large arachnoidal arteries but were relatively mild in the latter and in the cortical and subcortical medullary arteries.

Conclusions—These arterial changes resulted in transformation of the cerebral arteries, in particular almost all the medullary arteries, to a so-called earthen pipe state. This supports the reported findings of a reduction in vascular reactivity to fluctuations in CO2 levels and systemic blood pressure in CADASIL. (Stroke. 2002;33:2565-2569.)

Key Words: Binswanger’s disease • CADASIL • cerebral medullary artery

Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a systemic disease characterized by deposition of granular osmiophilic material (GOM) and loss of smooth muscle cells (SMCs) of the arterial media, with preferential involvement of the central nervous system. Genetic mutation, such as point mutation of the Notch3 gene of 19q13.1, has been nominated as the etiology, 1,2 and >28 missense mutations have been reported.2,3 However, there are some phenocopies in which such a mutation is not recognized but in which deposition of GOM has been noted pathologically.4 Therefore, the origin of CADASIL seems to be genetically heterogeneous. Neuro-pathologically, diffuse myelin loss, atrophy of the cerebral white matter with sparing of the subcortical U fibers, and small infarcts in the subcortical gray matter are all characteristic of CADASIL but not specific to it; these pathological signs are also seen in association with acute carbon monoxide poisoning, a potential hazard of general anesthesia such as in accidents of artificial ventilation, amyloid angiopathy, and Binswanger’s disease. We have reported that in the case of Binswanger’s disease, such changes in the cerebral white matter are caused by mural stiffening due to intimal fibrosis and loss of medial SMCs of the proximal portion of the medullary arteries, and neither luminal stenosis nor occlusion plays an important role.5 In CADASIL, the medial SMCs of the systemic arteries, especially those in the central nervous system, are also involved, but the severity and extent of damage caused have not been studied precisely, with information available only from several randomly dissected specimens.

The aim of this study, therefore, was to examine the degree and extent of the pathological changes to the cerebral med-
ullary arteries in CADASIL in relation to pathogenesis of the diffuse myelin loss of the cerebral white matter in this disease. The entire length of the medullary arteries was examined by reconstruction of serial sections of the frontal lobe from an autopsy case of CADASIL. This case is the first autopsy case of CADASIL in Japan in which GOM deposition has been demonstrated by electron microscopy in the media of the cerebral and other visceral arteries.

Methods
The details of this case have been reported previously. Briefly, the proband was a 75-year-old man. His paternal grandfather had suffered from migraine and died at the age of 42 years, and his father had shown character changes at the age of 59 years, followed by gait disturbance, disorientation, dysphasia, dysarthria, and incontinence, and died at the age of 63 years. His father did not suffer from hypertension. The proband suffered from attacks of migraine between the ages of 10 and 55 years, followed by character changes (from moderate to aggressive temper) and development of a dragging walk. At the age of 69 years, multiple brain infarction was diagnosed, and his gait disturbance progressed. After the age of 70 years, dementia progressed, and pseudobulbar palsy with dysphasia and hoarseness occurred. He died of aspiration pneumonia. He had neither hypertension nor diabetes mellitus throughout his entire clinical course. He had no habits of alcohol drinking or smoking. MRI revealed atrophy of the cerebrum, corpus callosum, and brain stem, a diffuse low signal in enhanced T1 images, and a high signal in T2 images of the cerebral white matter.

Neuropathologically, the brain weighed 1530 g and exhibited tiny cystic infarcts scattered within the centrum semiovale, corpus callosum, basal nuclei, left brachium pontis and right tegmentum of the pons, and cerebellar cortex and white matter. The cerebral arteries showed slight to moderate atherosclerosis in these main trunks, which was compatible with that of the aged without risk factors. The histological findings were compatible with those of previously reported cases of CADASIL.

Other viscera were also examined ultrastructurally, and deposition of GOM was observed in the arteries of the heart, liver, and small intestine.

Genetic examination of Notch3 in this case was performed by Kotorii et al, and a missense mutation (nucleotide substitution 716G→A; Arg213Lys) was recognized in the EGF5 domain of exon 4.

Serial sections of the left superior frontal gyrus, including the cingulate gyrus and corpus callosum, were examined; the specimen containing these brain areas was embedded in paraffin, and 1000 serial sections were cut. Every fifth slice was stained with Elastica-Masson staining. All of the medullary arteries identified in these slices were individually traced from the penetrating site at the cortical surface to the distal portion, where they reached a diameter of 20 to 30 μm. The diameter, length, and pathological changes of the artery wall were depicted on graph paper. Loss of SMCs was ascertained by immunohistochemical staining with an antibody against SMC actin (monoclonal mouse antibody raised against human α-smooth muscle actin; Dako).

The severity (complete or incomplete) and distribution of SMC loss in the arachnoidal arteries were examined on 5 randomly

Figure 1. Reconstructed view of the frontal medullary arteries. The red zone and red dotted zone represent continuous and discontinuous loss, respectively, of medial SMCs. The green and blue zones show intimal fibrous thickening and adventitial fibrosis, respectively. Asterisks mark arteries showing an L-shaped bend in the subcortical white matter.
selected slides, which were stained immunohistochemically with an anti-SMC actin antibody. All cross-sectioned arachnoidal arteries in these slides were divided into 4 groups according to their diameter (>1000 μm, >500 to ≤1000 μm, 100 to ≤500 μm, and <100 μm), and the frequency of complete and incomplete loss of medial SMCs in each group was counted.

The deposition of granular material in the arterial wall, characterized of CADASIL (so-called small arterial granular degeneration), was ascertained by red floccular staining with Masson stain (Figure 2G) and purple-red staining with periodic acid–Schiff stain (Figure 2H) in the media, with loss of SMC contour and nucleus.

The distribution of cortical infarcts was also mapped by examination of these serial sections.

**Results**

**Arterial Changes**

**Cerebral Medullary Artery**

Eleven arteries could be traced, 8 of which included the penetrating site at the cortical surface, and 5 of which included a distal end with a diameter of 20 to 30 μm (Figure 1). The predominant pathological changes were loss of medial SMCs and adventitial fibrosis, which are shown in Figure 1 as red and blue zones, respectively. The former was generally circumferentially complete and continuous (Figure 2A to 2F) or incomplete and discontinuous, as shown in Figure 1 by the red zones and red dots, respectively. Most arteries showed continuous complete loss of SMCs from the penetrating site at the cortical surface, and 5 of which included a distal end interposed by a few short segments of discontinuous SMC loss. Segments of discontinuous SMC loss or intact segments were located preferentially at the cortical (proximal) level of the medullary arteries. Severe adventitial fibrosis (shown as a blue zone in Figure 1) occurred in all arteries (with a thickness exceeding that of the media) but was restricted exclusively to the white matter (Figure 2G). In addition, luminal dilatation occurred in 5 arteries along their length, not only at the cortical level but also at the level of the white matter, varying in its extent. The intima showed segmental fibrous or hyalinous thickening (Figure 2C), the distribution being shown in Figure 1 as a green zone, but it was mild, and stenosis or occlusion was absent. Intimal deposition of foamy macrophages was rare. Moreover, subendothelial myointimal cells were well preserved, even in severely involved arteries (Figure 2D). Microaneurysms were also absent. Both the straight and L-shaped medullary arteries were equally involved (Figure 1).

**Arachnoidal Artery**

There appeared to be a general increase in the number of arachnoidal arteries (Figure 2K) because of luminal dilatation, mural thickening due to intimal fibrosis or hyalinosis to various degrees, severe adventitial fibrosis, and intramedial deposition of a red substance on Masson staining. Immunostaining of SMC actin revealed incomplete or complete loss of medial SMCs in all arachnoidal arteries (Table), but myointimal cells in the intimal fibrosis expressed actin strongly in a manner different from that of medial SMCs. All large arteries with a luminal diameter >500 μm presented moderate SMC loss that was often associated with slight segmental intimal fibrosis (Figures 2I and 2J); complete loss of SMCs was not observed. The small arteries were classified into 2 groups according to diameter—100 to ≤500 μm and <100 μm—in which the frequency of complete SMC loss was 42% and 61%, respectively (Table). The difference was statistically significant. Therefore, small arteries <100 μm in diameter showed complete loss of SMCs significantly more frequently than the larger arteries did. This was often accompanied by mild to moderate segmental intimal fibrosis or hyalinosis in these small arteries, but severe stenosis (>75% of the section area surrounded by the internal elastic mem-

<table>
<thead>
<tr>
<th>Diameter, μm</th>
<th>Complete Loss</th>
<th>Incomplete Loss</th>
<th>Preserved</th>
<th>n</th>
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<tbody>
<tr>
<td>&gt;1000</td>
<td>0 (0%)</td>
<td>7 (100%)</td>
<td>0 (0%)</td>
<td>7</td>
</tr>
<tr>
<td>&gt;500 to ≤1000</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td>5</td>
</tr>
<tr>
<td>100 to ≤500</td>
<td>28 (42%)</td>
<td>39 (58%)</td>
<td>0 (0%)</td>
<td>67</td>
</tr>
<tr>
<td>&lt;100</td>
<td>72 (61%)*</td>
<td>46 (39%)</td>
<td>0 (0%)</td>
<td>118</td>
</tr>
</tbody>
</table>

*n* indicates total number of counted arteries.

*Significantly (*P*<0.05) more frequent than that of complete SMC loss of arteries with larger diameter.*
Although the genetic mutation of Notch3 where granular atrophy was observed. No focal infarcts were present. Myelin loss and the corpus callosum was atrophic, but the U fibers were spared. The density of the axons decreased diffusely, but the severity was slight compared with that of myelin loss. Diffuse but slight astrocytic gliosis was present in the white matter. Macrophages were scarce, and lipid deposition suggestive of leukodystrophy was absent. Microscopic foci of cystic infarcts or elective neuronal loss with gliosis were scattered in the cortex, but these were distributed preferentially in the lateral side of the superior frontal gyrus, where granular atrophy was observed. No focal infarcts were present in the cerebral white matter of this specimen.

Discussion

Although the genetic mutation of Notch3 reported in this case is novel compared with cases reported previously, the family history and clinicopathological findings, including the ultrastructural deposition of GOM, support the description of this as a typical case of CADASIL. However, it should be determined in other cases of CADASIL whether this missense mutation is a rare polymorphism or whether it is related to the pathogenesis. Moreover, we have ascertained histologically that the specimen sampled in this study (the frontal lobe) showed diffuse myelin loss and atrophy of the corpus callosum with sparing of the subcortical U fibers.

The arachnoidal and medullary arteries nourishing the cerebral deep white matter presented widespread and severe medial pathology that is characteristic of CADASIL and is associated with dilatation and intimal and adventitial fibrosis. The luminal dilatation and intimal and adventitial fibrosis are also recognized in cases ofBinswanger’s disease and hypertensive cerebral bleeding; in both of these disorders loss of the medial SMCs is commonly present, but the severity is significantly greater in Binswanger’s disease than in hypertensive cerebral bleeding. Therefore, these changes may be secondary to loss of medial SMCs. In this study we found that all of the examined medullary arteries showed diffuse loss of medial SMCs with severe adventitial fibrosis along almost their entire length, from the penetrating site at the cortical surface to the distal end. Therefore, these arteries are considered to have been transformed almost totally to the so-called earthen pipe state, in which neither dilatation nor constriction is possible, and the blood flow of the cerebral white matter is thus devoid of autoregulation and is blood pressure dependent. In Binswanger’s disease, the proximal portion of the cerebral medullary arteries is selectively and severely affected by hypertensive damage, such as loss of the medial SMCs and intimal fibrosis with or without atheroma. Although these medial changes are more severe in CADASIL, in both CADASIL and Binswanger’s disease they may induce hemodynamic derangement of the distal vascular bed whenever there is a fluctuation in systemic blood pressure or CO2 tension. Since the deep cerebral white matter belongs to the farthest territory from the heart in the central nervous system, this would result in disruption of the blood-brain barrier and edema-induced destruction of the myelin.10 The pathological findings of the white matter of this case, such as the diffuse myelin loss and atrophy with mild gliosis and sparing of the subcortical U fibers and no evidence of leukodystrophy, are also compatible with this deduction. The significance of arterial SMC loss with respect to hemodynamic derangement in CADASIL is supported by the reduced cerebrovascular CO2 reactivity associated with this disease;11 it has been shown that CO2 reactivity is significantly lower in disabled than in nondisabled CADASIL individuals. This suggests that the severity and extent of SMC loss in the cerebral arteries progress at the shift from the nonsymptomatic to the symptomatic state. In addition, it has been reported that there is a reduction in blood flow to the cerebrum and in the middle cerebral artery in CADASIL, but these findings may merely reflect the final devastated state of the brain. Cerebrovascular reactivity to fluctuations not only in CO2 but also in systemic blood pressure during the clinical course of CADASIL should be examined further.

Although small infarcts were found in the cortex of this case, these occurred preferentially in the lateral side of the superior frontal cortex, which belongs to the watershed region of the anterior and middle cerebral arteries, where the cortical surface was granular (granular atrophy). The cortical arteries supplying these infarcted areas showed no severe stenosis or occlusion in this examination. Therefore, such a preferential distribution of cortical infarcts in the watershed region is consistent with the idea that not only the cerebral medullary arteries but also the arachnoidal arteries are transformed to so-called earthen pipes and become blood pressure dependent.

It is interesting to note that the subendothelial myointimal cells were well preserved, even in the severely involved medullary arteries. These cells, similarly to the SMCs of the intestinal muscle layer, may be spared from the influence of the genetic mutation associated with CADASIL and may be biologically different from the medial SMCs of arteries. Moreover, the relative immunity of medial SMCs of the cortical and subcortical medullary arteries is also interesting, and an issue for future research would be whether these medial SMCs are biologically different from those of other cerebral arteries.

References

cortical infarcts and leukoencephalopathy maps to chromosome 19q12.


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*Stroke*. 2002;33:2565-2569
doi: 10.1161/01.STR.0000032620.91848.1C

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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