Computer-Assisted Three-Dimensional Image Analysis of Cerebral Amyloid Angiopathy

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Background and Purpose: Microaneurysms and fibrinoid necrosis of cerebral cortical arteries have been reported to be related to the pathogenesis of intracerebral hemorrhage associated with cerebral amyloid angiopathy. To elucidate the pathogenesis of such vascular lesions, we conducted the present study.

Methods: Five hundred serial sections from brain tissue of a patient with severe amyloid angiopathy and intracerebral hemorrhage were analyzed histologically and immunohistochemically. Three-dimensional reconstructions of the vascular lesions were performed using a computer-assisted image analysis system.

Results: The microaneurysms were found to develop in small cortical arteries with diameters of about 40 to 50 μm. They were spindle-shaped dilatations, with a maximum diameter of about 200 μm, and appeared within vascular segments bearing severe amyloid deposition. In the walls of the aneurysms, the intima was thickened, and the media and adventitia showed thinning and disruption. Fibrinoid necrosis was found in the vascular walls of the most dilated, middle portions of the aneurysm. The vascular walls undergoing fibrinoid necrosis did not show any β/A4 or cystatin C but presented with fibrinogen-like immunoreactivities, indicating invasion of plasma components.

Conclusions: These results suggested the following sequential events for the pathogenesis of the cerebral amyloid angiopathy-associated vascular lesions leading to hemorrhage: (1) damage of the media and adventitia due to severe amyloid deposition results in dilatation of the cortical arteries, (2) the vascular dilatation progresses and is accompanied by thickening of the intima and disruption of the media and adventitia (microaneurysm formation), (3) plasma components invade to the vascular wall (fibrinoid necrosis), and (4) finally, hemorrhage develops. (Stroke. 1993;24:1857-1864.)

Key Words • amyloid • aneurysm • computer-assisted image processing • intracerebral hemorrhage

Cerebral amyloid angiopathy (CAA), the cerebrovascular amyloid deposits frequently found in elderly individuals and patients with the senile dementia of the Alzheimer type, has been found to be associated with intracerebral hemorrhage. CAA-related intracerebral hemorrhage is characterized by its location in cerebral lobes and cerebellum and by its association with secondary rupture of the hemorrhage to the subarachnoid space. In brains from patients with CAA and intracerebral hemorrhage, microaneurysms and fibrinoid necrosis of cerebral blood vessels are frequently observed in addition to a severe degree of CAA, and such vascular changes have been reported to be important as causative lesions of CAA-related intracerebral hemorrhage. In our series of 11 patients with CAA-related intracerebral hemorrhage, microaneurysms with fibrinoid necrosis of the cortical vessels were observed in 9 patients (82%) (M. Yamada and Y. Itoh, unpublished data, 1993). Vonsattel et al reported that fibrinoid necrosis was seen in the brains of patients with CAA and hemorrhage (12 of the 17 brains) but not in those without hemorrhage. These data indicate that the development of such vascular lesions in the brains of patients with CAA would at least in part contribute to the pathogenesis of the intracerebral hemorrhage.

The development of microaneurysms and fibrinoid necrosis (angioneurosis) has been well documented in brains from hypertensive patients and has been found to be a pathological basis of hypertensive cerebral hemorrhage. In the brains of patients with CAA, however, the mechanism for formation of the microaneurysms and fibrinoid necrosis leading to hemorrhage remains unclear.

To elucidate the pathogenesis of the microaneurysms and fibrinoid necrosis in CAA, we performed histological and immunohistochemical studies of such vascular lesions using serial sections of a brain showing evidence of CAA and computer-assisted three-dimensional image analysis. Here we described for the first time the three-dimensional structure of the microaneurysmal vascular dilatation with fibrinoid necrosis that is associated with CAA. Such vascular lesions were also analyzed immunohistochemically with antibodies to amyloid, plasma, and glial proteins.

Subjects and Methods

The brain from a patient with a severe degree of CAA and CAA-related intracerebral hemorrhage was

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**Figure 1.** Microaneurysmal vascular dilatation with fibrinoid necrosis of a cerebral cortical artery stained with Congo red (a through d and f) and elastica-Masson (e) stains. Dilatation is serially shown from a to f; d and e are adjacent sections. Congo red-positive materials in b through d and f show green birefringence under polarized light (not shown) and are identified as amyloid. Artery (arrow) is 45 μm in diameter at point of perforation of cerebral surface and is free of amyloid deposition (a). Before dilatation, artery (arrow) is heavily deposited with amyloid (b). The vessel is dilated up to 200 μm (c). Amyloid deposits (arrowheads) in media and adventitia of dilated vascular segment are found to be fragmented (c and d). In the dilated portion, thickened intima shows fibrinoid necrosis (asterisk) (e). In the vascular segment distal to the maximum dilatation, amyloid deposits appear again in entire media and adventitia (arrow) (f). (Panels a through d and f, Congo red, ×120; e, elastica-Masson, ×120; bar=100 μm.)
studied for microaneurysmal formations and fibrinoid necrosis of the cerebral blood vessels.

Case Report

A 79-year-old woman suddenly developed disturbance of consciousness. The patient had been suffering from slowly progressive dementia since the age of 78 and was diagnosed as having senile dementia of the Alzheimer type. She had no history of hypertension or heart disease. Her blood pressure was 152/60 mm Hg. She was in a semicoma. Right hemiparesis and right-sided hype reflexia with a positive Babinski’s sign were noted. A computed tomographic (CT) scan revealed a large intracerebral hemorrhage with rupture to the subarachnoid space in the left temporoparietal region. The patient’s condition was complicated by gastrointestinal bleeding, and she died 53 days after the onset of cerebral hemorrhage.

Pathological Examination at Autopsy

A complete postmortem examination was carried out 2 hours after death. The formalin-fixed tissues were investigated with routine pathological techniques. Neuropathological specimens were obtained from all the cerebral lobes, basal ganglia, thalamus, brain stem, cerebellum, and spinal cord. The brain sections were stained with hematoxylin-eosin, Klüver-Barrera, Bodian, elastica-Masson, and Congo red stains. The Congo red-positive materials that showed green birefringence with polarized light were identified as amyloid.

Serial Sections for Analyses of CAA-Associated Vascular Lesions

A 26×20 mm-sized tissue block was obtained from the right occipital lobe. The formalin-fixed, paraffin-embedded tissue was sectioned serially at a thickness of 8 μm. A total of 500 serial sections were obtained. Every fifth section was stained with Congo red and elastica-Masson and was examined, with particular attention to the microaneurysmal vascular dilatation and fibrinoid necrosis of the vessel walls as well as cerebrovascular amyloid deposition.

Three-dimensional Reconstruction of the Vascular Lesions

Three-dimensional images of the vascular lesions showing microaneurysmal dilatation and fibrinoid necrosis were reconstructed from the serial sections. We used the computer-assisted three-dimensional image analysis system OZ (Olympus, Tokyo, Japan).

Immunohistochemical Studies

Immunohistochemical studies were performed on the sections containing aneurysmally dilated vessels with fibrinoid necrosis, using the following primary antibodies: rabbit polyclonal antibody to amyloid β/A4 protein\(^{30}\) (dilution, 1:1000), rabbit polyclonal antibody to human cystatin C (a gift from Dr A.O. Grubb, Lund, Sweden)\(^{31}\) (dilution, 1:1000), rabbit polyclonal antibody to human fibrinogen (Dako, Carpinteria, Calif) (dilution, 1:400), and rabbit polyclonal antibody to glial fibrillary acid protein (GFAP) (Dako) (dilution, 1:400). The antibody to fibrinogen recognizes the epitope shared by both the fibrinogen and fibrin, according to the vendor’s information. The avidin-biotin-peroxidase complex (ABC) method\(^{32}\) was applied using a Vectastain ABC Kit (Vector Laboratories, Burlingame, Calif). Sections were treated sequentially with the following: 5% normal goat serum in phosphate-buffered saline for 30 minutes at room temperature, the primary antibodies or normal rabbit serum (a negative control) for 16 hours at 4°C, 1:40 biotinylated goat antibody to rabbit immunoglobulin G for 30 minutes at room temperature, ABC for 30 minutes at room temperature, and diaminobenzidine (Sigma, St Louis, Mo) –H\(_2\)O\(_2\). The sections were counterstained with hematoxylin. Samples of brain tissue from a normal brain and from patients with Alzheimer’s disease without severe CAA and hereditary cerebral hemorrhage with amyloidosis, Icelandic type (HCHWA-I, a gift from Dr O. Jensson, Reykjavik, Iceland), were included as positive and negative controls for the immunohistochemistry.

Results

Autopsy Findings

The brain weighed 1160 g. A massive hematoma was found in the left temporoparietal region with secondary

![Image of a brain with annotations](http://stroke.ahajournals.org/Download.jpg)
subarachnoid hemorrhage. There was no intraventricular hemorrhage. Atherosclerosis of the cerebral arteries was mild. There was moderate cortical atrophy and ventricular enlargement. Microscopically, many neurofibrillary tangles and senile plaques with neuronal loss were observed in the hippocampal and parahippocampal regions and in other cerebral cortices, which led to the neuropathological diagnosis of Alzheimer’s disease by the criteria.33,34 Furthermore, a severe degree of CAA was found in the cerebral and cerebellar cortexes. CAA was observed in most of the leptomeningeal and cortical arteries, arterioles, capillaries, and occasionally veins. Perivascular plaques (drusige Entartung of Scholz35) were frequently observed. Microaneurysmally dilated vascular lesions with fibrinoid necrosis were found scattered throughout the cerebral cortex. Such lesions sometimes accompanied microhemorrhages in the vicinity. No amyloid angiopathy was demonstrated in the basal ganglia, thalamus, brain stem, or spinal cord. General pathological examination revealed a perforating duodenal ulcer, bilateral pleural effusion, and slight atherosclerosis of the aorta and other systemic arteries. The heart showed mild enlargement of both atria but no left ventricular hypertrophy. The ratio of the heart weight (300 g) to body weight (44 kg) was 6.8, which was in the normal range in Japanese autopsy cases.36

**Observations of the Serial Sections of the Brain**

Cortical vascular changes were investigated in the 500 serial sections. Microaneurysmal dilatation was observed in 8 cortical arteries. Fibrinoid necrosis of the vessel walls as evidenced by elastica-Masson stain was found in 3 of the 8 arteries.

The microaneurysmal vascular dilatations with fibrinoid necrosis were found in the small cortical arteries. At the point where the arteries perforated the cerebral cortex, the diameters were 40 to 50 μm (Fig 1a). In the superficial portions of the cerebral cortex, the vessels were heavily coated with amyloid deposits (Fig 1b). The vessel walls were almost totally replaced by amyloid, with loss of elastic lamina and smooth muscle cells of the media, leaving endothelium. Aneurysmal dilatations were located in superficial-to-middle or middle portions of the cerebral cortexes. The vessels showed gradual dilatation with fibrous or hyalinus thickening of the intima. The vessels were dilated up to about 200 μm (Fig 1c through 1e). Around the portions of maximum dilatation, the thic-

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**Fig 3.** Immunostaining of an aneurysmally dilated vascular lesion with antibodies to β/A4 protein (a) and cystatin C (b). Amyloid deposits of a part of media and adventitia of the aneurysmal lesion (arrow) and of the other vessels (arrowheads) react with the antibody to β/A4 protein (a). Some of the vessels showing positive β/A4 protein–like immunoreactivities in panel a are less intensely stained with the antibody to cystatin C (arrow and arrowhead, b). The thickened intima with fibrinoid necrosis (asterisks) is negative for β/A4 protein and cystatin C (a and b) (a, anti–β/A4 protein, ×140; b, anti–cystatin C, ×140; bar=100 μm).
ened intima appeared with fibrinoid necrosis (Fig 1c). Such intimal lesions were occasionally associated with cellular infiltration, some of which had foamy cytoplasm and appeared to be macrophages. In contrast with the thickening of the intima, the media and adventitia were extremely thinned and frequently disrupted (Fig 1c and 1d). In such vascular segments showing marked dilatation, amyloid deposits were found scattered in rather small amounts or almost absent (Fig 1c and 1d). In the vascular segments distal to the maximum dilatation, the diameters of the vessels gradually decreased (Fig 1e). At the same time, amyloid deposits appeared again in the entire media and adventitia (Fig 1e). After that, when the vessels entered the white matter, the vessels were free of amyloid deposition.

Three-dimensional Reconstruction of Dilated Vessels

To elucidate the structure of the vessels exhibiting aneurysmal dilatation with fibrinoid necrosis, three-dimensional images were reconstructed using computer-assisted analysis. A representative image of such vascular lesions is shown in Fig 2. The spindle-shaped microaneurysms were formed within vascular segments involved with amyloid deposits. In the most dilated portions, however, amyloid deposits were sparse or almost absent. The fibrinoid necrosis of the vessel walls was located in the most dilated, middle portions of the aneurysmal lesions.

Immunohistochemical Studies

The aneurysmally dilated vascular lesions were analyzed immunohistochemically (Figs 3 through 5). Amyloid deposits in the vascular walls strongly reacted with the antibody to β/A4 protein (Fig 3a). The β/A4 protein immunostaining also disclosed intense immunoreactivities in perivascular amyloid deposits and senile plaques. In addition, a part of the vascular amyloid appeared labeled with the antibody to cystatin C (Fig 3b). However, the thickened intima with fibrinoid necrosis was negative for β/A4 protein and cystatin C (Fig 3). In the walls showing fibrinoid necrosis, fibrinogen-like immunoreactivities were present (Fig 4). Immunostaining with the antibody to GFAP demonstrated that many GFAP-positive reactive astrocytes surrounded the aneurysmally dilated vascular lesions but not the amyloid-laden vessels without dilatation (Fig 5).

Discussion

In this report, we focused on the pathomechanism by which the CAA-associated vascular changes (ie, microaneurysm and fibrinoid necrosis) develop in brains with CAA. For this purpose, the brain from an autopsied normotensive patient with severe CAA and cerebral lobar hemorrhage was investigated with three-dimensional image analysis and immunohistochemical studies.

Our observations using the image analysis system clearly demonstrated that the microaneurysmal dilatations of the cortical small arteries were spindle-shaped and were formed in the middle of amyloid-laden vascular segments (Fig 2). Amyloid deposition in the vessels was severe, and the media and adventitia were almost totally replaced by amyloid, with loss of elastic lamina and smooth muscle cells of the media. We suggest that such damage to the media resulted in the dilatation of the vessels. The vascular dilatation was always accom-
panied by the fibrous and hyaline thickening of intima, which was probably a reactive change after the medial damage caused by amyloid deposition.

Interestingly, in the most dilated portions of the vessels, amyloid deposits in the vascular walls were sparse (Fig 1c and 1d). Similar findings have been previously described. Our observations of the serial sections suggested that this sparsity of amyloid would be associated with thinning and disruption of the media and adventitia caused by marked vascular dilatation. Amyloid deposits of the media and adventitia would be secondarily fragmented in the process of the vascular dilatation.

Our results revealed that fibrinoid necrosis occurred in the thickened intima of the most dilated, middle portion of the spindle-shaped aneurysmal lesions (Fig 2). Deposition of fibrinogen and/or fibrin was demonstrated immunohistochemically in the vascular walls with fibrinoid necrosis (Fig 4). This indicated the infiltration of plasma proteins into the vascular lesions. Similar cerebrovascular changes were reported in the brain with hypertensive cerebral hemorrhage. There is no doubt that such necrotic vascular lesions would be a pathological basis of hemorrhage.

How can plasma proteins infiltrate the vessel walls in the brains with CAA? In cerebral vessels deposited with amyloid, endothelial abnormalities suggesting dysfunction of blood-brain barrier have been reported. The altered vascular permeability would induce invasion of various plasma elements, including proteases, and result in necrosis of vessel walls. Furthermore, additional factors, including hemodynamic disturbance such as hypertension, might play a role in the development of change in vascular permeability.

An amyloid protein of CAA found in the elderly individuals and patients with dementia of the Alzheimer type is β/A4 protein. A cerebrovascular amyloid protein in HCHWA-I is a variant of cystatin C, a cysteine protease inhibitor. Recently, colocalization of cystatin C with β/A4 protein in sporadic CAA has been reported. In our studies, strong β/A4 protein immunoreactivities were observed in CAA and plaques. Furthermore, immunohistochemistry using the antibody to CC suggested some immunoreactivities of cystatin C in some of the blood vessels, although the significance remains unclear. Some authors have postulated that deposits of cystatin C might be related to pathogenesis of CAA-related cerebral hemorrhage. However, the vessel walls undergoing fibrinoid necrosis, which would be a pathologic basis of CAA-related hemorrhage, did not show any cystatin C–like immunoreactivity (Fig 3b).

Many GFAP-positive astrocytes were found surrounding the dilated vascular segments (Fig 5). In contrast, around amyloid-laden vessels without dilatation, there was no significant proliferation of astrocytes, which is consistent with a report by Mandybur. Therefore, we suggest that the astrocytic proliferation is reactive to the abnormal vascular dilatation.

Finally, the results have led us to a proposal for the pathomechanism of CAA-related intracerebral hemorrhage (Fig 6). Amyloid deposits in the media and adventitia of cerebral cortical small arteries damage the vascular smooth muscle cells, resulting in the vascular dilatation; the spindle-shaped vascular dilatation (microaneurysm) is associated with thinning and disruption
Amyloid deposition in the media and adventitia of cortical arteries

Loss of smooth muscle cells of the media

Vascular dilatation (spindle-shaped microaneurysm)

Intimal thickening with thinning and disruption of the media and adventitia

Change of vascular permeability

Additional factor(s) (e.g., hypertension)

Invasion of plasma components (e.g., proteases) to the vascular wall (fibrinoid necrosis)

Hemorrhage

**Fig 6. Hypothesis for the mechanism of cerebral amyloid angiopathy–related intracerebral hemorrhage.**

of the media and adventitia and with reactive thickening of the intima; thereafter, vascular integrity is disrupted with or without the contribution of additional factors such as hypertension, followed by invasion of plasma proteins, including proteases (fibrinoid necrosis); and the fibrinoid necrosis of the thickened intima and the disrupted media and adventitia easily induce the development of hemorrhage.

**References**

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