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Capillary beds are decreased in Alzheimer's disease, but not in Binswanger's disease

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Abstract

Morphological abnormalities of the cortical microvessels have been reported in Alzheimer's disease (AD), but not in Binswanger's disease (BD), a form of vascular dementia. Therefore, we compared the capillary beds in AD and BD brains, using a modified Gallyas silver impregnation method and immunohistochemistry for β amyloid. Eight autopsied brains with AD and seven with BD were compared with six control brains. The cortical microvessels in AD were frequently narrowed, and torn off, especially in close proximity to the senile plaques. The capillary densities in AD were significantly decreased as compared with the control brains. In contrast, there were no significant changes in the capillary densities and their morphologies in BD brains. Immunohistochemistry for β amyloid revealed numerous deposits in the vascular wall and perivascular neuropil exclusively in AD brains. Cortical microvascular changes in AD and their absence in BD may indicate a role of β amyloid for the microvessel pathology in AD.

Keywords: Microvessel; Alzheimer's disease; Vascular dementia; Binswanger's disease; Silver impregnation; Amyloid protein

Alzheimer's disease (AD) and vascular dementia are major causes of dementia and disabilities in the elderly. These two conditions have been believed to have an independent pathoetiology. However, in recent studies, co-morbid factors have been revealed in AD and vascular dementia [10,12]. These factors include hypertension, diabetes mellitus, hyperlipidemia, apo E4 ε genotype, cholinergic deficits, and white matter lesions. In addition, patients with vascular lesions reportedly develop dementia more frequently than those without vascular lesions among those subjects with senile changes [18]. Taken together, this evidence has shed light on the interrelationship between AD and vascular dementia, and raised the hypothesis that vascular factors may have a role in the pathogenesis of AD. In concordance with this hypothesis, previous electron microscopic studies have reported thickening of the basement membrane, denervation of the perivascular nerves, and bulging or narrowing of the cortical microvessels in AD brains [14,17].

Binswanger's disease (BD) is a form of vascular dementia, featured by diffuse white matter lesions, lacunar infarcts and fibrohyaline thickening of the microvessel [13]. Fibrohyaline thickening of the microvessels is marked in BD, and significant but less severe in AD in the cerebral white matter [19]. However, with respect to the cortical microvessels, there are no studies in BD. Therefore, we aimed to compare the alterations of the cortical microvessels in AD and BD using a modified Gallyas silver impregnation method and immunohistochemistry for β amyloid, which enable us to examine the network of the brain capillaries, and senile plaques.

We examined 21 brains, including 8 from patients with AD (3 males), 7 from patients with BD (4 males), and 6 from patients who did not have any neuropsychiatric symptoms or brain lesions (3 males). The age was 79 ± 12 years (mean ± S.D.) in the AD, 74 ± 13 years in the BD and 73 ± 4 in the control groups, respectively, among which no significant differences were observed ($p < 0.05$). The brain weight was 1020 ± 111 g in the AD, 1093 ± 112 g in the BD, and 1244 ± 57 g in the control groups, respectively. The brain weight in the AD group was significantly lower than in the control and BD groups ($p < 0.05$). The patients with AD and BD, but not the control patients, met

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the diagnostic criteria for dementia (diagnostic and statistical manual of mental disorders; DSM-IV) [1] in the occasion of diagnosis and, most of these patients suffered in a bed-ridden condition in their terminal stages.

The diagnosis of AD was made based on the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) diagnostic neuropathologic criteria [15] and excluded the brains with large cerebral infarctions. The diagnosis of BD was made clinico-pathologically, and retrospectively met the pathological inclusion criteria including (1) presence of diffuse white matter lesions, (2) lacunar infarctions in the perforator territory, (3) arteriolosclerosis such as fibrohyalinosis and fibrinoid necrosis and (4) absence of cortical infarctions, as well as the clinical criteria by Bennett et al. [3], and excluded the brains with significant pathologic hallmarks of AD. The control group included two patients with pneumonia, one patient each with renal cancer, lung cancer, chronic renal failure and pulmonary emphysema.

The tissue blocks were sectioned in a cryostat (30 or 100 μm-thick) and kept in 0.1 M phosphate buffer (pH 7.4). The tissue sections were processed according to the silver impregnation method described by Gallyas [8]. Briefly, the sections were incubated for 30 min in 5% periodic acid (HIO4) solution. The sections were then immersed in 4% sodium hydroxide solution for 30 min, and washed in 0.5% acetic acid solution in double-distilled water for 5 min. They were further incubated in a mixture (pH 13.0) of 90 ml of an ammoniated silver nitrate solution (AgNO3, 0.5 g and ammonium nitrate, 2.5 g in 900 ml double-distilled water), and 10 ml of 4% sodium dihydroxide in double-distilled water for 30 min at 20 °C. These sections were left in a physical developer solution, which was composed of a mixture of 10 ml of solution A, 5 ml of solution B and 5 ml of solution C at 25 °C. The composition of each solution was as follows; sodium carbonate, 50 g in 1000 ml of distilled water (solution A); ammonium nitrate 1.9 g, silver nitrate, 2.0 g; tungsto-silicic acid ($SiO_2 \cdot 2WO_3$), 10 g in 1000 ml of distilled water (solution B); and ammonium nitrate, 1.9 g; silver nitrate, 2.0 g; tungsto-silicic acid ($SiO_2 \cdot 2WO_3$), 10.0 g; and 6.1 ml of 40% formalin solution in 1000 ml of distilled water (solution C). The reaction was terminated in 0.5% acetic acid solution, and the extent of silver impregnation was monitored intermittently under light microscopy.

For immunohistochemistry, autoclaved paraffin sections were incubated with a mouse anti-amyloid β protein antibody (Dakopatts, diluted 1:200), biotinylated anti mouse IgG (Vector laboratories, diluted 1:200) and an avidin biotinylated peroxidase complex (Vector Laboratories, diluted 1:200). They were finally visualized with 0.01% diaminobenzidine tetrahydrochloride and 0.005% H_2O_2 in 0.05 M Tris–HCl (pH 7.6). To test for the specificity of the immunohistochemical reaction, control sections were incubated with normal mouse IgG instead of the primary antibody.

The density of the capillary beds was determined by the test grid method [7], in which the number of vascular intersections were counted against 6 × 6 square test grids each with a 50 μm width. The average counts from five representative fields in the layers II–IV of the frontal and parietal cortices, respectively, were used as the capillary densities in each patient. The data were expressed as means ± S.D. and the Mann–Whitney *U*-test was used to compare between the groups.

Using the modified Gallyas stains, the microvessels in the cerebral cortices appeared smooth and regular in diameter in the non-neurological control and BD brains (Fig. 1A and C, respectively). There were no senile plaques nor neurofibrillary tangles. In contrast, the brains with AD had numerous senile plaques and neurofibrillary tangles, which were intermingled by irregularly-shaped microvessels in both frontal and parietal cortices (Fig. 1B). The microvessels were frequently narrowed and irregular in diameter for a variable length of the vessel (Fig. 1D–F). These vessels often showed bulging of their walls. In close proximity to the senile plaques, the microvessels were blunted and torn off in the sections with a thickness of 100 μm (Fig. 1E). These microscopic changes were not observed in the non-neurological control and BD groups.

With immunohistochemistry, β amyloid-immunoreactivity was localized in senile plaques which accumulated numerously in the superficial layer, as well as perivascular deposits in the vascular wall itself and perivascular neuropil in the AD group (Fig. 1G–I). Beta amyloid-immunopositive fine texture fibrils were distributed in the neuropil with or without contact to the microvessels. In contrast, there was almost no deposit of β amyloid in the cerebral cortices of the non-neurological control and BD groups. In the semi-quantitative measures of the microvessels, the microvascular densities were significantly lower in the AD group as compared to the other two groups in both frontal and parietal cortices (Fig. 2).

The capillaries in AD have been shown to exhibit thickening of their basement membrane, atrophy, perivascular fibrosis and degeneration of the pericytes [5,6,16], which may correspond to the bulging of the microvessels observed here. In semi-quantitative measures, some authors have not observed any decrease in the capillary densities [2], while others showed a decrease in selected or non-selected regions of AD brains [4,7]. The present study underscored the morphological abnormalities of the capillaries, and further revealed their numerical decrease in AD and absence of capillary damages in BD. The actual reduction rate in the capillary density of AD brains may be more severe, because significant atrophy in this group should have ameliorated the reduction ratio. The fact that there were no β amyloid-deposits nor damages in the cortical microvessels in BD brains was not contradictory to the major site of the pathologic process, which involve subcortical white matter and perforator territory in BD. However, in previous studies, slight but significant neuronal dysfunction has been noted in the cerebral cortex, such as a decrease in the synaptic densities and neuronal viabilities [11,22].

The reduction in the vascular densities and the spatial proximity of β amyloid deposits to the microvascular changes may suggest some vascular toxicity due to β amyloid. Indeed, preamyloid deposits were found in the extracellular space and extended directly into the capillaries [16]. Vinters and Farag [20] raised a neurovascular hypothesis, in which β amyolid accumulates on the outer side of the basement membrane and