学 位 論 文 の 要 旨

氏名　SHIBLY ABU ZAFFAR

学　位　論　文　名　Analysis of Cerebral Small Vessel Changes in AD Model Mice
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著　　者　　名　Abu Zaffar Shibly, Abdullah Md. Sheikh, Makoto Michikawa, Shatera Tabassum, Abul Kalam Azad, Xiaojing Zhou, Yuchi Zhang, Shozo Yano and Atsushi Nagai

論 文 内 容 の 要 旨

INTRODUCTION

Alzheimer's disease (AD) causes a gradual decline in memory and other cognitive functions. Deposition of amyloid (Aβ) peptides in the brain is the major pathology. Aβ deposition begins in high-metabolic-activity neocortical neurons. Deposition spreads from neocortex to allocortex. β- and γ-secretase activities convert amyloid precursor protein (APP) into Aβ. APP or processing enzyme mutations that boost Aβ production and deposition cause familial AD. Aβ aggregates are neurotoxic and neuroinflammatory. Aggregation may contribute to AD pathogenesis due to neuroinflammation and neurodegeneration. In familial AD, excessive Aβ production may overwhelm clearance, causing aggregation and deposition.

Enzymatic breakdown, cell-mediated clearance, and perivascular routes remove Aβ. Neither APP nor its processing enzymes are mutated in sporadic AD, and Aβ production is equivalent to healthy people. Gene association studies show a strong relationship between an ApoE (*ApoE ε4*) variant and the disease. Subsequently, it was found that the variant impedes Aβ clearance. Aβ deposits in AD's parenchyma and cerebral arteries. Such findings suggest that disturbances in the clearance associated with the vessels could be important. Moreover, it was shown that ApoE is associated with vessel-mediated clearance of Aβ, and the efficiency of *ApoE ε4* is less than that of *ApoE ε3*. Hence, sporadic AD is suggested to be caused by the disturbance in the vessel-dependent perivascular clearance of Aβ.

The Brain lacks lymphatics. The perivascular glymphatic system removes waste from the brain. Leptomeningeal cells around perforating arterioles create cerebrospinal fluid's perivascular space (CSF). Virchow-Robin space is replaced by a porous basal lamina where CSF can easily exchange constituents, including waste products, with interstitial fluid. The brain's perivascular fluid drains to cervical lymph nodes. All brain arterioles, venules, and capillaries are surrounded by astrocytic end-feet outside the basal lamina, which creates the perivascular space's outer wall. Aquaporin 4 (AQP4) helps transport fluid in the perivascular region. Perivascular space clears

Aβ. Increased load or ApoE variation disrupts perivascular clearance, creating vessel wall deposition that interacts with vascular cells. These cytotoxic and inflammatory interactions can change vascular anatomy, function, and disease pathology. All these features can be seen in AD brains. However, there is a lack of understanding about which events appear early in AD pathology. Therefore, we have done a detailed time-dependent study using an AD model mouse to investigate the sequence of events in the pathology. We have found that at a very early age, BBB changes appear due decreased expression of tight junction protein, followed by changes in the vessel leakage and astrocytes' expression of AQP4.

MATERIALS AND METHODS

We used the J20 strain as an AD model and non-transgenic WT littermates (WT) as a control. We used four age groups (2, 3, 6, and 9 months) of J20 and WT for the light microscopy studies. Each age group had five animals. Immunostaining and Western blotting were used to evaluate pathological changes in the brain and blood vessels in J20 mice brain from 2 to 9 months old. To prepare tissue, isoflurane-anesthetized mice were perfused transcardially with normal saline, followed by 4% PFA in 0.1 M PBS (pH = 7.4). The mice's brains were removed and post-fixed for 6-24 hours. The brain samples were cryoprotected for 48 hours with 30% sucrose in 0.1 M PBS. Brains were serially sectioned coronally into 2 mm tissue blocks and 10 μm tissue slices using a cryostat (Leica, Wentzler, Germany). We checked pathological changes and expression of vessel-related molecules claudin-5, collagen 4, AQP4, endothelial cells, and vascular endothelial growth factor (VEGF) through immunohistochemistry, immunofluorescence and western blotting. Albumin extravasation was used to check vessel leakage. Lectin staining was used to check vessel density. Digital microscope images were captured at (x40) magnification in the same plane of all experimental mouse brains from both hemispheres, the cortex, and the hippocampus. NIH ImageJ software analyzed, counted, and quantified the immunoreactive area. All numerical data are expressed as the mean ± standard deviation (SD). Statistical analysis was performed to compare mean values using the student's *t*-test between different age-matched groups. Statistical significance was denoted as $p < 0.05$. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University (Approval number: IZ29-28).

RESULTS AND DISCUSSION

Compared to wild-type (WT), vessel density was increased at 2 months but decreased at 9 months in J20 mice, claudin-5 levels were decreased, and vascular endothelial growth factor (VEGF) levels were increased in the cortex and hippocampus of J20 mice brain at all time points. Albumin extravasation was evident from 3 months in J20 brains. Collagen 4 was increased at 2 and 3 months. Aquaporin 4 was spread beyond the vessels starting from 3 months in J20, which was restricted around the vessel in WT mice. At 2 months, Aβ was only intraneuronal, whereas

vessels were positive from 3 months in J20 mice. At 2 months, VEGF levels and vessel number increased in J20 brain. Such findings show an active angiogenic process in J20 mice brains that reduces claudin-5 levels. Claudin-5 immunoreactivity was positive for endothelial cell, where its expression was lower in J20 mice brains at all the time points. Claudin-5 expression decreased without affecting the endothelial cell density, suggested by decreased claudin-5/STL expression data. Albumin immunostaining was used as a leakage marker, and its expression was perivascular at 2 months and extravascular at 3 months, indicating that the BBB was impaired. As a result, blood constituents leak out into the brain parenchyma and contribute to the Aβ aggregation and deposition process. From 3 months, Aβ was found to be deposited around vessel-like structures along with intracellular and extracellular deposition. Aβ deposits extracellularly as insoluble aggregates in brain parenchyma. It is reported soluble oligomeric aggregates are more toxic. Aβ is a highly aggregation-prone peptide that is cleared from the brain via the perivascular pathway. Due to its high concentration, the peptide may oligomerize during clearance, or by contact with blood constituents that leak from blood vessels. Oligomerized peptides can interact with endothelial cells and astrocytes, causing perivascular changes. Such changes could hinder clearance, causing Aβ deposition in brain parenchyma.

This study also found AQP4 redistribution from 3 months in J20 mice brain. In the WT mice brain, AQP4 immunoreactivity was polarized and concentrated around the vessel; it was dense, concentrated, and localized within the astrocytic end feet, unsheathing the BBB interface of cerebral microvessels at all time points. In the J20 mouse brain, AQP4 expression extended beyond the vessel into the parenchyma, and there was loss of localized expression of AQP4 from perivascular end-foot processes. Redistribution of AQP4 indicates the activation of astrocytes that participate in the neuroinflammatory process seen in AD. Taken together, such redistribution of AQP4 could start a vicious cycle by increasing the Aβ burden in brains, leading to activation of a neuroinflammatory process, which further increases the deposition. Collagen 4 is an important constituent of the vessel basement membrane that forms the perivascular space. Our immunostaining results increased collagen 4 levels in J20 mice brains during early time points. Due to the vascular degenerative process and prolonged VEGF expression can be pathogenic might affect the collagen levels, showing overall similar levels to their WT counterparts later. Collagen 4 interacts with Aβ and inhibits the fibril formation process. Such interaction might impede the clearance through perivascular space and cause the deposition of Aβ.

CONCLUSION

In conclusion, we found that the blood-brain barrier is disrupted early in an AD mouse model and persists over time. Such a disruption of the barrier function may contribute to AD's perivascular changes and astrocytic neuroinflammatory process, creating a vicious cycle of Aβ deposition. Restoring the blood-brain barrier and clearing Aβ could improve AD therapy.