学 位 論 文 の 要 旨

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学 位 論 文 名 : A Mesenchymal Stem Cell Line Transplantation Improves Neurological Function and Angiogenesis in Intraventricular Amyloid β-Infused Rats

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論 文 内 容 の 要 旨

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory disturbance and cognitive impairment. The main pathological feature of AD is the presence of extracellular amyloid-β (Aβ) peptide, and intra-neuronal fibrillary tangles composed mainly of phosphorylated tau protein in the brain. Several studies have suggested Aβ peptide deposition as a causative factor of AD. Aβ is produced from amyloid precursor protein (APP) by β- and γ-secretase enzyme activities, and then clear up from the brain through perivascular pathway. Increased production of Aβ is a cause of its deposition in brains and found in familial AD. Also, impaired clearance due to vascular dysfunction and other causes could result its deposition and found in sporadic AD. Several animal models have been developed to explore the pathology of AD, such as APP transgenic and APP-processing enzymes transgenic mice with various AD-related mutations. In these mouse models, Aβ production is increased, which results the deposition of aggregated peptide in the hippocampus and cortex along with neurodegeneration. Additionally, direct infusion of Aβ into the ventricle of rat brains resulted deposition of the peptide along with neurodegeneration in the hippocampal and cortical areas and impairment of memory similar to AD models. Generation of Aβ-infused AD model is cost effective and less time consuming than that of transgenic model. Moreover, Aβ deposition is time sensitive and found only in relatively older APP transgenic animals. Since, vascular function is altered during aging process; it is difficult to distinguish the role of increased production from decreased clearance of Aβ as a cause of deposition in transgenic AD models. But in infusion model, the peptide can be infused in animals of any age, thereby age-related vascular dysfunction

as a possible cause of deposition can be eliminated. Hence, in this study we prepared AD animal model by continuous infusion of Aβ peptide into the lateral ventricle of 8-weeks old rats.

Recently stem cell-based therapies are gaining interest as a treatment option for neurodegenerative diseases, because of their ability to replace damaged neurons, neuroprotection, immunomodulation and angiogenesis. In this study we investigated the efficacy of a mesenchymal stem cell (B10) line for the therapy of AD using an Aβ-infusion AD rat model. Our results showed that B10 transplantation increased angiogenesis and clear up deposited Aβ from the vessel wall of the rat brains.

MATERIALS AND METHODS

All experimental procedures and protocols were approved by the Ethical Committee of Shimane University of School of Medicine (Reference number: IZ30-4). Eight weeks old Wister rats were used to prepare AD model. $A\beta_{1-42}$ peptide was infused continuously to the left lateral ventricle using osmotic pump for 2 weeks (n= 20). Then, 2 x 10^5 B10 cells in 20 μl PBS were transplanted through right lateral ventricle (n= 10). As controls, $A\beta_{1-42}$-infused PBS-treated (n= 10), and sham operated (n= 10) rats were used. Memory-related behaviour was evaluated weekly using shuttle avoidance test apparatus for 4 weeks. Training for behaviour study was started 2 weeks before AD model preparation. After 4 weeks, rats were sacrificed and brain tissues were collected for staining and mRNA analysis. The mRNA expression of angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), hypoxia inducible factor-1-alpha (HIF-1α), vascular endothelial growth factor (VEGF), placental growth factor (PlGF), platelet-derived growth factor (PDGF), fms-like tyrosine kinase 1 (FLT1), tyrosine protein kinase receptor 2 (Tie2), interlukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor α (TNFα), brain derived neurotrophic factor (BDNF), glial cell line derived neurotrophic factor (GDNF), nerve growth factor (NGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin like growth factor-1 (IGF1) in hippocampus were checked by real time polymerase chain reaction (RT-PCR). Astrocytes and microglia were evaluated by glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule-1 (iba1) immunostaining, respectively. Levels of Aβ, VEGF and IL-1β proteins, and endoglin positive new endothelial cells were checked by immunostaining using specific antibodies. Hippocampal neurons were counted by Nissl staining. Vessel counting was done after solanum tuberosum lectin (STL) staining.

RESULTS AND DISCUSSION

Evaluation of memory by shuttle avoidance showed that the memory performance was decreased after 4 weeks in PBS treated Aβ-infusion AD model rats compared to sham. Moreover, Aβ was deposited around the vessel along with the reduction of neuron number in the CA1 area. These results are suggesting that in this model, infused Aβ might be absorbed from ventricle into the brain parenchyma, and then cleared through perivascular pathways; where the clearance

pathway is affected leading to neurodegeneration and decreased memory related performance. In B10 transplanted Aβ-infusion AD model rats, memory performance and neuronal cell number was not statistically different than sham rats. Also, Aβ deposition around the vessel was decreased. Hence, B10 might improve perivascular clearance of Aβ, which leads to decreased Aβ burden and neurodegeneration, and improved neurological performance.

Since Aβ deposition around the vessels was decreased by B10 transplantation in this AD model rats, we investigated the vessel density. STL staining results showed that in PBS treated AD model rats, vessel number was decreased compared to sham rats. Vessel density was increased by B10 transplantation, especially small-sized vessels in the cortex and hippocampus. Further experiments showed that endoglin positive cell number was increased in B10 transplanted AD model rat brains, suggesting an active angiogenesis process in this model. Such process could be vital, since disturbance perivascular clearance is a putative mechanism of Aβ deposition and neurodegeneration in this AD model, which is recovered by newly formed vessels. Additionally, angiogenesis is intimately related to neural proliferation and neuroprotection, which could also be important for increased neuronal number in B10 transplanted AD model rats. To investigate the underlying mechanism of increased neural cell number and angiogenesis, we checked the mRNA expression of various cytokines, growth factors and angiogenesis regulators. mRNA of IL-1β, IL-6, Ang2, Tie2, PlGF and IGF-1 were increased by B10 transplantation. Tie2 is an angiopoietin receptor, also can be activated by VEGF. Further, PlGF is a VEGF family protein, and IL-1β can induce VEGF. Hence, VEGF family protein signaling at least has some role in B10 induced angiogenesis.

Our immunostaining results showed that the number of astrocytes and microglia in the CA1 area was decreased in PBS treated AD model rats, which could be due to acute toxicity of Aβ. Conversely, in B10 transplanted AD model rats, their numbers were similar like sham. Decreased Aβ level by B10 transplantation could explain such protective effects on astrocyte and microglial cell numbers. Both microglia and astrocytes are known to produce growth factors that have neuroprotective effects. Indeed, we have found that IGF-1 mRNA was specifically increased by B10 transplantation, which is reported to protect neurons from amyloidogenic derivatives. Moreover, microglia has the ability to clear up deposited Aβ. Therefore, angiogenesis may induce the supply of macrophage/microglia to the Aβ deposited areas in the brain of AD model rats and facilitates microglia-mediated Aβ clearance.

CONCLUSION

In conclusion, we have shown in this study that transplantation of B10 in AD model rats provide neuroprotection, prevent Aβ deposition and improve neurological function probably through angiogenesis. Therefore, angiogenesis related therapy could be a novel strategy for the management of neurodegenerative diseases like AD.