学 位 論 文 の 要 旨

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学 位 論 文 名 Microglia Transplantation Attenuates White Matter Injury in Rat Chronic Ischemia Model via Matrix Metalloproteinase-2 Inhibition

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論 文 内 容 の 要 旨

INTRODUCTION

Chronic cerebral hypoperfusion and focal ischemia are vascular risk factors for white matter lesions (WMLs), which are clinically manifested as cognitive impairment. To investigate the pathogenesis of WMLs, a chronic cerebral hypoperfusion animal model, induced by permanent bilateral common carotid artery occlusion (BCAO), is widely used. In this model, profound white matter changes, including fiber disarrangement and vacuolation, are seen, with minimal changes in the cerebral cortex. In the pathology of BCAO-induced WMLs, proteases such as matrix metalloproteinases (MMPs) play a crucial role. For example, MMP-2 in the corpus callosum (CC) and caudoputamen (CP) participates in disruption of the white matter matrix and blood brain barrier, and in glial cell activation.

Several reports have shown that participation of activated microglial cells is crucial in WML pathology. As is the case in several other neurodegenerative and neuroinflammatory diseases, these immunocompetent cells of the central nervous system have been found to be activated in WMLs, releasing cytokines and proteases, including MMP-2, and causing progression of the disease process. However, under ischemic conditions, glial cells can modulate the temporal and spatial expression of neurotrophic factors, including brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF), resulting in attenuation of injury and neurological improvement. These reports indicate that the microglial expressional phenotype may become neuroinflammatory and neurodegenerative, or neurotrophic, depending on the type of injury and on environmental cues. Therefore, a detailed investigation of the role of microglia in WML pathology seems worthwhile. To investigate the microglial biology associated with neurodegeneration and neuroinflammation, we have generated a human microglia cell line, HMO6, by transfecting a retroviral vector encoding v-myc into human primary microglia isolated from human fetal telencephalon tissue. The morphological appearance, expressional phenotype

and migration ability of HMO6 were confirmed to be similar to those of human primary microglia.

Transplantation of multipotent stem cells, such as mesenchyamal stem cells (MSCs) and neural stem cells has recently been introduced as a therapeutic option for ischemic stroke. MSCs transplantation in ischemic animal brain, especially focal ischemia, showed functional and pathophysiological improvement by cell-replacement, inducing angiogenesis and neuroprotection by providing neurotrophic factors and cytokines. However, information about the function of MSCs in chronic hypoperfusion model is lacking.

In this report, we compare the effects of transplantation of human microglia (HMO6) and MSC cell lines.

MATERIALS AND METHODS

Human microglia cell line, HMO6, and human mesenchymal stem cell line, HM3.B10 (B10), which were established previously, show similar morphological and expressional characteristics to human primary microglia and mesenchymal stem cells, respectively. HMO6 was cultured in DMEM supplemented with 5% fetal calf serum, antibiotics and L-glutamine. B10 was cultured in MFmedium.

A microglial cell line (HMO6), mesenchymal stem cell line (B10) or vehicle (PBS) was intravenously injected in chronic cerebral hypoperfusion rat model, and the appearance and severity of WMLs were evaluated and compared among the groups. Under deep anesthesia, rats of the three groups at 5, 14, and 28 days after BCAO were perfused with 0.09% NaCl followed by a fixative solution. The brains were removed, postfixed in the same fixative solution for 24 hours, and frozen on dry ice. The distribution of transplanted HMO6 and B10 cells in the brain regions of the BCAO model were investigated by human nuclei staining. We investigated effects of the cell transplantation on accumulation of astrocytes and microglia in the CC and CP with anti-GFAP and Iba1 antibody. BCAO-induced WMLs were evaluated by means of a Klüver-Barrera (KB) staining-based scoring system.

Next we investigated gene expression of inflammatory cytokines and proteases in activated microglia in CC after BCAO by real-time PCR. Microglia were detected in ethanol-fixed coronal sections using anti-Iba1 antibody, and Iba1+ cells were isolated by LCM using a PixCell IIe LCM system (Arcturus, Mountain View, CA) equipped with an inverted base microscope under a 10x objective, and collected on an LCM Cap (Arcturus). Total RNA was isolated using a PicoPure RNA extraction kit (Arcturus) according to the manufacturer's instructions. MMP-2, MMP-9, cathepsin B immunoreactive were detected by Western blotting. Calpain-1 and cathepsin B activities in tissue lysates of CC and CP containing 100μg total protein was measured with a calpain and cathepsin B activity assay kit. Gelatin zymography using CC and CP homogenates was performed.

RESULTS AND DISCUSSION

Both transplanted cell lines were found to accumulate mainly in the CC region, though considerable accumulation also found in the CP region at 5 days after BCAO. The number of migrated HMO6 and B10 cells tended to decrease at Day 14, and no migrated cells

were found at Day 28. The number of HMO6 cells was less than that of B10 cells in both the CC and CP regions at all time points examined. These results suggested that the improvement of WMLs induced by HMO6 and B10 cell transplantation might not be due to neuronal differentiation of transplanted cells and integration into the CNS, but rather, other mechanisms could be involved.

HMO6 transplantation significantly decreased WMLs in both the CC and CP at Day 14 and Day 28. B10 transplantation also showed a tendency to reduce WMLs in both areas, but the effect reached significance only at Day 28. Moreover, HMO6-transplantation-induced WML improvement was more pronounced than B10-induced improvement in all regions at all time points, except for the CP at Day 28, where the difference did not reach significance. HMO6 transplantation induced an early, robust, and prolonged improvement of WMLs, while B10 transplantation showed a relatively delayed effect. Moreover, the inhibitory effect of human microglial cell transplantation on BCAO-induced WMLs appears to be greater than that of MSC transplantation.

HMO6 transplantation significantly decreased the accumulation of microglia and astrocytes mainly in the CC at Day 14 after BCAO, and the effect persisted up to Day 28. In the CP, HMO6 transplantation also decreased microglia and astrocyte accumulation at Day 14 and Day 28, but the changes did not reach significance.

Transplantation of HMO6 or B10 cells did not affect expression of inflammatory cytokines, including IL-6 and TNFα. Transplantation of both cell types reduced the level of matrix metalloproteinase MMP-2 mRNA in microglia of the CC. MMP-2 protein level and activity were also both greatly reduced in the same region. The results of gelatin zymography showed that MMP-2 activity was much higher than MMP-9 activity at Day 14 after BCAO. Transplantation of HMO6 and B10 decreased MMP-2 and MMP-9 activities in the CC, though MMP-9 activity was less markedly decreased than MMP-2 activity. In BCAO, activated microglia are believed to express proteases, including MMP-2, that influence WML pathology. Here, we found that in addition to MMP-2, MMP-9 and cathepsin B were inhibited by both HMO6 and B10 transplantation. Because gelatinolytic activity in the BCAO setting is predominantly due to MMP-2, it is tempting to speculate that HMO6 and B10 cell-transplantation-induced WML improvement is mainly mediated through the inhibition of MMP-2.

CONCLUSION

Our results indicate that transplantation of microglial cells and mesenchymal stem cells reduces the severity of chronic hypoperfusion-induced WMLs, and inhibits the accumulation and activation of microglia and astrocytes in the CC and CP regions in a rat model, through inhibition of glial activation and protease activities in the lesion areas. Microglia/macrophage lineage cells should be considered as a potential cell-therapy tool in the central nervous system due to their capacity to migrate to the sites of lesion.