学 位 論 文 の 要 旨

氏名　若林　規良

学　位　論　文　名	Transplantation of Human Mesenchymal Stem Cells Promotes Functional Improvement and Increased Expression of Neurotrophic Factors in Rat Focal Cerebral Ischemia Model
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著　　者　　名	Kiryo Wakabayashi, Atsushi Nagai, Abdullah Md. Sheikh, Yuri Shiota, Dashdemberel Narantuya, Tatsuzo Watanabe, Junichi Masuda, Shotai Kobayashi, Seung U. Kim, Shuhei Yamaguchi

論 文 内 容 の 要 旨

INTRODUCTION

Mesenchymal stem cells (MSCs) have recently been investigated for their efficacy as a therapeutic tool in stem cell-based cell therapy in various disease settings. They exist primarily in the bone marrow and can differentiate into various cell types including osteoblasts, chondrocytes, adipocytes, hepatocytes, and neurons. MSCs are shown to pass through the blood-brain barrier and migrate throughout forebrain and cerebellum without disrupting the host brain architecture.

Previous studies have demonstrated that MSCs transplanted via intracerebral or intravenous route in animals with experimental stroke, migrate selectively to the ischemic boundary sites, differentiate into neurons and glial cells and promote functional improvement. MSCs are known to secrete neurotrophins and angiogenic growth factors, thereby transplanted MSCs could provide molecular reservoirs of neurotrophic factors within the ischemic brain and may indicate the important roles played by the neurotrophic factors in neuroprotection. Furthermore, transplantation of MSCs transduced with neurotrophic factor genes is more efficient than primary MSCs in inducing functional improvement, highlighting the neuroprotective role of neurotrophic factors. Then, the aim of the present study is to investigate the differential expressional levels of neurotrophic factors in endogenous host tissues and exogenous grafted MSCs in the ischemic brain tissues.

In the present study, we investigated neurological improvement in ischemic stroke model rats with transient middle cerebral artery occlusion (MCAO) following intravenous injection of human mesenchymal stem cell line and analyzed the gene expressions of neurotorphic factors and cytokines by quantitative real-time RT-PCR and Western blot in these animals.

MATERIALS AND METHODS

The expression of endogenous (rat-origin) and exogenous (human-origin) neurotorphic factors and cytokines was evaluated by quantitative real-time RT-PCR and Western blot analysis.

Adult male Wistar rats were used to prepare transient middle cerebral artery occlusion (MCAO) model. One day after MCAO, 3×10^6 B10 cells or PBS were injected into jugular vein. B10 human mesenchymal stem cell line used in the transplantation study was generated by v-myc gene transfer into primary human bone marrow cell cultures, obtained from human fetal spinal vertebrae of 15-weeks gestation.

Behavioral tests, infarction volume, and B10 cell migration were investigated at 1, 3, 7 and 14 days after MCAO. The modified neurological severity score system (mNSS) was used to grade the various aspects of neurological functions. To measurement of infarct volume, rat brains were sectioned and stained with 2% 2-3-5-triphenylterazolium (TTC) and then infarct volume was calculated indirectly, by subtracting intact area of the ipsilateral hemisphere from the area of contralateral hemisphere. To immunohistochemical analysis of transplanted MSCs, B10 MSCs were grown in medium containing 10-3M bromodeoxyuridine (BrdU) for two days, and transplanted via jugular vein. Transplanted B10 cells were detected by immunostaining with a monoclonal antibody against BrdU.

To investigate the differentiation of B10 cells to other cell types, double immunostaining was performed using cell type specific markers: neurofilament (NF) for neuron, glial fibrillary acidic protein (GFAP) for astrocyte, ionized calcium-binding adaptor molecule 1 (Iba1) for microglia or galactocerebroside (GalC) for oligodendrocytes.

The expression of endogenous (rat-origin) and exogenous (human-origin) neurotorphic factors and cytokines was evaluated by quantitative real-time RT-PCR and Western blot analysis. Total RNA was isolated from infarct core, ischemic boundary zone (IBZ: lateral region of TTC(+) area, just medial to infracted area and contralateral cortical brain tissues using Trizol reagent. To analyze mRNA level, real time PCR was performed using an ABI Prism 7000 Sequence Detector system. For quantification, a standard curve was prepared based on the serial dilution of one sample having highest GAPDH level. The target gene value of each sample was normalized by GAPDH value of same sample that was run simultaneously with target gene. Sixty μg of total protein was separated by 12.5% SDS polyacrylamide gel electrophoresis, transferred to PVDF membrane, and processed for immunoreaction using anti-human IGFor anti-rat EGF. Immunoreactive proteins were detected using an enhanced chemiluminescence system according to the manufacturer's protocol.

RESULTS AND DISCUSSION

Immunohistochemical analysis revealed that at 3 days after MCAO (2 days after intravenous transplantation), a large number of BrdU+ B10 cells were found in ipsilateral hemisphere, especially in infarct core and IBZ, with very few cells in ipsilateral caudoputamen and contralateral hemisphere. From 7 days onwards, BrdU+ B10 cells were not detected in the rat brains. Double immunostaining of B10-transplanted rat brain at 3 days after MCAO revealed that none of BrdU+ B10 cells expressed NF, GFAP, GalC or ED1 indicating that transplanted B10

cells failed to differentiate into neurons, astorcytes, oligodendrocytes or microglia in vivo.

However, as compared to PBS controls, rats receiving MSC transplantation showed improved functional recovery and reduced brain infarction volume at 7 and 14 days after MCAO.

In MSC transplanted brain, among many neurotrofic factors, only human insulin-like growth factor 1 (IGF-1) was detected in the core and ischemic border zone at 3 days after MCAO, while host cells expressed markedly higher neurotrophic factors (rat-origin) than control rats, especially vascular endothelial growth factor (VEGF) at 3 days, and epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) at 7 days after MCAO.

These findings imply that MSC-influenced induction of endogenous neurotrophic factors as well as MSC-secreted neurotrophic factors provides improvement in neurological outcome in MCAO ischemic rats.

High level expression of growth factors such as bFGF, BDNF, HGF and G-CSF was found in B10 cells under basal culture conditions, while IGF-1 expression was low. In contrast, only IGF-1 (human) was detected in vivo, indicating that IGF-1 was specifically induced in the transplanted B10 cells by the ischemic or inflammatory environment. IGF-1 is a growth factor that has been shown to have neurotrophic, anti-apoptotic and angiogenic properties and also to decrease the stroke volume by preventing apoptosis and increases the neuronal progenitor cell proliferation, resulting improvement of the neurological outcome. In the present study, we observed that the infarct volume tends to increase time-dependently in PBS group, while in B10 transplantation group, at 7 and 14 days after MCAO, infarct volume was markedly decreased, suggesting an anti-apoptotic and neuroprotective environment is provided by the grafted B10 MSCs.

Several rat origin growth factors such as VEGF, EGF, bFGF, BDNF and GDNF were found to increase in B10 transplanted rats. In particular, at 7 days after MCAO, EGF level in B10-transplanted group was markedly higher in IBZ and contralateral cortex. EGF has been shown to have the neurotrophic properties in in vitro ischemic condition. An in vivo study showed that exogenous EGF improves cerebral ischemic condition by inhibition of free radical generation and/or lipid peroxidation, leading to prevention of brain edema and neuronal damage. In our study, the infarct volume of B10 group after 7 and 14 days after MCAO were significantly reduced, might be due to prevention of delayed neuronal death by increased EGF or other neurotrophic factors.

CONCLUSION

The intravenous injection of human MSCs resulted in improved function and reduced ischemic damage in a rat model of MCAO. It appears that the functional recovery and reduction in infarct volume could have resulted from MSC graft-induced modulation of neurotorphic factors and cytokines in the host cells.