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Research Report

The distribution of transplanted human mesenchymal stem cells in the CNS of young *Macaca fascicularis*

Jiamei Li[a,b], *Hua Zhu*[a], *Yunxin Chen*[a], *Wei Deng*[a], *Qin Li*[c], *Shan Lu*[d], *Yanfeng Xu*[a], *Lan Huang*[a], *Chunmei Ma*[a], *Chunhua Zhao*[d], *Renzhi Wang*[e], *Chuan Qin*[a,*]

[a]*Comparative Medical Center, Institute of Laboratory Animal Science, Peking Union Medical College (PUMC) and Chinese Academy of Medical Science (CAMS), Beijing 100005, China*
[b]*Department of Pathology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, China*
[c]*Motac Collaborative Laboratory, Institute of Laboratory Animal Science, Peking Union Medical College (PUMC) and Chinese Academy of Medical Science (CAMS), Beijing 100005, China*
[d]*Institute of Basic Medical Sciences and School of Basic Medicine, Center of Excellence in Tissue Engineering, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100005, China*
[e]*Peking Union Medical College Hospital, Beijing 100730, China*

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ABSTRACT

Mesenchymal stem cell (MSC)-based therapies have generated much hope and promise as a potential source of cells for cell-based therapeutic strategies in pediatric degenerative diseases. However, the distribution and migratory routes of MSCs are unknown. Here, real-time PCR and microscopy were used to observe the migration and distribution of labeled human MSCs (hMSCs) transplanted into the striatum of young *Macaca fascicularis*. Moreover, the differentiation of hMSCs was also detected using immunofluorescence. We found that hMSCs were mainly located near the injection site in the brain and in the anterior brain after 2 weeks. After 4 weeks, the hMSCs had dispersed and could be detected in each brain slice and were more uniformly distributed than after 2 weeks. The hMSCs showed a preference for migration towards blood vessels, which may be one of the migratory routes used by hMSCs. Additionally, hMSCs could be observed to give rise to NeuN- and GFAP-positive cells. Transplanted hMSCs also increased the expression levels of N-cadherin in the host brain tissue, which may be one factor that drives the migration and differentiation of hMSCs after transplantation. These results provide preclinical evidence that MSC-based therapies may represent an efficacious alternative to more conventional treatment regimens for a variety of pediatric neurologic disorders.

Abbreviations: hMSCs, human bone-marrow-derived mesenchymal stem cells; HLA-DR, human leukocyte antigen-DR; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei

*Corresponding author. Fax: +86 10 67710812.
E-mail address: qinchuan@pumc.edu.cn (C. Qin).

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1. Introduction

Pediatric degenerative diseases and cerebral trauma of the central nervous system (CNS) are common clinical neurologic disorders. These disorders include cerebral palsy, movement disorders, hypoxic–ischemic encephalopathy and cerebral trauma. Hence, there is considerable interest in protecting neurologic function and promoting neuronal regeneration. Experimental clinical and animal studies have begun to elucidate the utility of stem cell-based therapies to prevent or repair brain injuries (Castillo-Melendez et al., 2013; Dailey et al., 2013; Hsu et al., 2013). Mesenchymal stem cells (MSCs) are multipotent precursors that are present in adult bone marrow, and they have generated hope and promise as a potential source of cells for cell-based therapeutic strategies (van Velthoven et al., 2013, 2012). MSCs have the potential to migrate toward injured tissues in animal models of CNS disorders after either intravenous or transcerebral transplantation (Okuma et al., 2013; Liu et al., 2013; Forostyak et al., 2013). MSC-based therapies represent a safe alternative for clinical intervention in patients with CNS disorders (Li et al., 2010; Forostyak et al., 2013; Wang et al., 2013). Successful MSC transplantation has been applied successfully in animal models of adult neurological disorders, including cerebral ischemia, Alzheimer's disease and Parkinson's disease (Isakova et al., 2006; Yun et al., 2013; Kim et al., 2013; Glavaski-Joksimovic and Bohn, 2013). Stem cell transplantation seems to be feasible, effective and safe with encouraging functional outcome improvements in cerebral palsy patients (Purandare et al., 2012). Our previous studies have also demonstrated that MSC transplantation can be beneficial for treating cerebral ischemic stroke in adult *Macaca fascicularis* (Li et al., 2010).

MSCs can migrate towards an injured tissue by following chemotactic guidance cues provided by cytokines. Wang et al. (2008) reported that intravenously injected MSCs could migrate to an ischemic lesion in the brain along the olfactory-thalamus and hippocampus-cortex routes. CNS lesions can specifically attract MSCs and mediate their migratory behaviors. MSC engraftment caused no adverse effects on rhesus macaque health, behavior, postural and locomotor patterns, or upper limb motor performance, as evaluated over a 6-month period post-transplantation. Furthermore, the injected MSCs could persistently engraft and disseminate throughout the CNS (Isakova et al., 2006). MSCs are distributed across a wide range of tissues following systemic infusion into adult baboons (Devine et al., 2003). Our previous study confirmed that MSCs can mainly migrate along the axes of the corpus callosum external capsule and the subependyma layer in the healthy rat brain (Li et al., 2011). A lingering problem in the field of cell-based therapies is the development of methods to deliver cells to the site of injury. Injured tissues can specifically attract MSCs and mediate their migratory behavior. However, MSCs can also migrate throughout the brain in the absence of injury. There has been no report on the distribution and migratory routes of MSCs in young healthy *M. fascicularis*.

In summary, this study aimed to observe the distribution, migratory routes and differentiation of transplanted hMSCs in the CNS of young healthy *M. fascicularis*, and to analyze N-cadherin expression after hMSC transplantation. This study provides preclinical evidence that MSC-based therapies can be an efficacious alternative to conventional treatment regimens for a variety of pediatric neurologic disorders.

2. Results

2.1. *Characterization of hMSC cultures*

After the fifth passage, we trypsinized and collected hMSCs. The hMSCs used in this experiment were positive for the following cell surface antigens: Flk-1, CD44, CD29 and CD105. The cells were negative for hematopoietic cell surface antigen, CD31, CD34 and human leukocyte antigen (HLA-DR; data not shown here). We could label 95% of hMSCs by CM-DiI.

2.2. *Effects of MSC engraftment on animal health and neurologic function*

No notable changes in routine urinalysis were detected before or after surgery. No significant differences in the contents of hemoglobin, packed cell volume, mean corpuscular volume, mean hemoglobin content, eosinophil counts, granulocyte counts, basophil counts, or thrombocytes counts before or after hMSC transplantation were observed.

Neurologic functions of the animals were evaluated using a modified Kito et al. (2001) scoring scale. There were no significant differences in neurologic deficit scoring between the hMSC-transplanted group and control group 4 weeks after surgery.

To define the response of hosts to donor cells, serial sections from injection sites were stained with hematoxylin and eosin for histological examination. No inflammatory cells were found near the injection sites. To further examine whether the host rejected the donor cells, immunohistochemistry to detect CD57, CD45, CD4 and CD8 was performed. However, no positive cells were detected in the brain (data not shown).

2.3. *The migration and distribution of hMSCs*

To observe the distribution of transplanted hMSCs in the *M. fascicularis* brain, we initially injected 1×10^6 CM-DiI-labeled cells unilaterally into the right caudate nucleus. Brain tissues were sliced into 12–14 coronal sections of alternating thickness (2, 3, 4 or 6 mm) according to the method of Isakova et al. (2006); Fig. 1A). Brain slices were cut into smaller sections for further study (Fig. 1B). An examination of the brain sections revealed that the hMSCs engrafted into the brain and then dispersed over time. Plotting the fluorescent signals detected in each brain slice onto a physical map of the brain more clearly illustrated appreciable signals near the injection site, but also within the forebrain after 2 weeks (Fig. 1C). After 4 weeks, hMSCs migrated throughout the brain and could be detected in each brain slice, including the cerebellum. The distribution of engrafted hMSCs was more uniform compared to 2 weeks post-transplantation (Fig. 1D). For example, we detected CM-DiI fluorescent signals in the