Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Research Report

Restoration of tissue damage, and never activity after hypoxia–ischemia by implantation of peripheral blood mononuclear cells

Yu-xiao Liu[a,1], Xiao-ming Guo[a,1], Jun-feng Li[a], Yu Meng[a], Hai-tao Zhang[a], Ai-jun Liu[a], Shou-chun Li[a], Yuan-lin Liu[b], Heng Zhu[b], Jing-hui Xue[a,*], Yi Zhang[b,**], Zhi-wen Zhang[a,*]

[a]Department of Neurosurgery, First Affiliated Hospital of Chinese PLA General Hospital, Beijing 100048, China
[b]Department of Cell Biology, Institute of Basic Medical Sciences, Beijing 100850, China

ARTICLE INFO

Article history:
Accepted 26 November 2013
Available online 27 December 2013

Keywords:
Hypoxia–ischemia encephalopathy
Peripheral blood mononuclear cells
Cell transplantation
Migration
Stromal cell-derived factor-1

ABSTRACT

Hypoxia–ischemia (HI) encephalopathy is a frequent cause of disability and mortality with limited therapeutic options. Here, we collected peripheral blood mononuclear cells (PB-MNCs) from healthy donors and labeled them with CM-DiI before implanting these cells by tail-vein injection into rats at day 3 after hypoxia–ischemia (HI). For immune-suppression the animals received daily injections of cyclosporine throughout the experiment, commencing 24 h before cell transplantation. Then we observed the PB-MNCs by fluorescent microscopy, examined motor function of rats by rotarod and cylinder tests, measured the lesion volume using image-pro plus software, and analyzed the apoptosis of neural cells in HI rats by tunnel assay. The results showed PB-MNCs could survive in the brain of hosts, migrate to the damage area and express neural marker. In addition, The HI rats that received PB-MNCs showed a reduction in motor function impairment, lesion volume and neural cell apoptosis. To better understand the mechanism of cell migration, PB-MNCs were also injected into normal rats via tail-vein. The expression of stromal cell-derived factor-1 (SDF-1) in the brain of normal and HI rats was measured by RT- PCR and western-blot, while the response of PB-MNCs in vitro to HI or normal brain extracts were measured by cell migration assay. Collectively these data suggest that the migration of PB-MNCs is directed to the damaged brain through an SDF-1-dependent pathway. Our results suggest that intravenous transplantation of PB-MNCs may be a feasible candidate for HI therapy.

Abbreviations: ANOVA, analysis of variance; BM-MNCs, bone marrow mononuclear; CB-MNCs, cord blood mononuclear; CsA, cyclosporine A; G-CSF, granulocyte colony-stimulating factor; GFAP, glial fibrillary acidic protein; HE, hematoxylin and eosin; HI, hypoxia–ischemia; PB-MNCs, peripheral blood mononuclear cells; SD, Sprague-Dawley; SDF-1, stromal cell-derived factor-1; TTC, triphenyltetrazolium chloride

*Correspondence to: The First Affiliated Hospital of PLA General Hospital, 51 Fucheng Road, Beijing, P.R. China, 100048. Fax: +86 1066848315.
**Correspondence to: Department of Cell Biology, Institute of Basic Medical Sciences, 27 Taiping Road, Beijing 100850, P.R. China.
E-mail addresses: xuejh2009@yahoo.com.cn (J.-h. Xue), zhangyi_612@126.com (Y. Zhang), zhangzhiwen_301@yahoo.com.cn (Z.-w. Zhang).
[1]These authors contributed equally to this work.

0006-8993/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.brainres.2013.11.026

1. Introduction

To date, no mechanism for restoring the damage of brain after hypoxia–ischemia (HI) has been identified, and as a result there are no successful therapies for brain injury. Thus, it is crucial to explore new methods for the clinical treatment of brain injury. Cell replacement is a novel therapeutic strategy for neural diseases (Daadi et al., 2010; Meier et al., 2006; Pimentel-Coelho et al., 2010; Van Velthoven et al., 2010). However, cell transplantation strategies require the safe and ethical acquisition of human donor cells in large numbers.

Mononuclear cells (MNCs) comprise hematopoietic progenitor cells, mesenchymal stem cells, and monocytes. Most clinical studies to date have been performed with MNCs, because MNCs are easily obtained from a range of tissues and do not induce the kinds of ethical debates that are prompted by some other cell types (Battistella et al., 2011; Brenneman et al., 2010; Savitz et al., 2011). Adult peripheral blood is the most safe and abundant source of MNCs. However, the quantity of stem cells in PB-MNCs is limited. Thus, many studies have used MNCs from bone marrow and cord blood but few studies have used MNCs from adult peripheral blood in brain injury (Elżbieta et al., 2011; Park et al., 2009; Vendrame et al., 2004).

It has previously been shown that granulocyte colony-stimulating factor (G-CSF) mobilization is an alternative approach to collect a large number of stem/progenitor cells from peripheral blood for cell transplantation in many diseases (Gordon et al., 2003; Huang et al., 2004). Against this backdrop, we have witnessed an explosion in a novel therapeutic approach for HI using the transplantation of G-CSF mobilized PB-MNCs. First, PB-MNCs were isolated from healthy, G-CSF treated donors using a CS-3000 blood cell separation machine. Second, the isolated cells were labeled with CM-DiI and transplanted into HI rats by tail-vein injection 3 days after surgery. Before transplantation, the rats were treated with immune-suppressive agent to promote donor cell survival. Third, we observed the migration and differentiation of human PB-MNCs in vivo and analyzed the effect of PB-MNC treatment on motor function, brain damage and cell apoptosis of HI rats (Supply 1). Finally, we analyzed the effect of normal and HI brain environments on the migration of human PB-MNCs. The results showed human PB-MNCs could survive and improve neural function after grafting into immuno-suppressed HI rats.

2. Result

2.1. G-CSF increase the quantity of stem cells in PB-MNCs

G-CSF is the “gold standard” for mobilizing stem cells from the bone marrow to the peripheral blood for transplantation. Here we used flow cytometry to assess the effect of G-CSF on the quantity of hematopoietic stem cells in PB-MNCs from healthy individuals. This analysis revealed that G-CSF administration induced a more than 4-fold increase of $CD34^+$cells (hematopoietic stem cells) as compared with the same donor without stimulation (Fig. 1A and B).

2.2. Transplanted PB-MNCs survive and migrate in the brain of HI rats

To observe the migration of PB-MNCs directly, the cells were labeled with CM-DiI, a fluorescence marker, before transplantation. After labeling, more than 95% percent of PB-MNCs were CM-DiI positive (Fig. 1C).

Rats then received an intravenous graft of human PB-MNCs 3 days after HI. We investigated the fate of PB-MNCs after transplantation by using CM-DiI labeling. CM-DiI-positive cells were clearly observed in the brain 7 days after HI, most labeled cells were located in the pallium (Fig. 2A and Supply 3A). Interestedly, CM-DiI -positive cells were found in the central and deep cortex on day 14 and in the damaged region of the brain on day 21 (Fig. 2B and C and Supply 3B and C). However, there was a significant decrease in the number of cells on day 28 (Fig. 2D and Supply 3D).

To further identify the transplanted cells, immunofluorescence examination was carried out using an antibody raised against human vimentin to recognize the cells from human but not rat, which revealed identifiable human grafts in all transplanted HI rats. The results were in accordance with the CM-DiI trace (Fig. 2).

2.3. PB-MNCs could express neural marker after transplantation

Before transplantation, we investigated whether human PB-MNCs expressed neural markers by flow cytometric analysis and did not detect GFAP in the human PB-MNCs (Fig. 3A). However, 4 weeks after HI, some CM-DiI^+/human $GFAP^+$ cells were found in hippocampus and cortex of HI treated rats by confocal microscope.

2.4. Transplanted PB-MNCs reduced infarct volume and cell apoptosis of HI brain

HI caused damage to either the entire brain or part of the brain in untreated and treated rats and led to the loss of brain tissue. Representative photographs of HE-stained brain sections from control, untreated and treated rats on day 28 are shown in Fig. 4A. Lesion volume was estimated on the images of HE stained sections using image-pro plus software. A significant decrease ($P<0.05$) in tissue loss was observed after 28 days in rats treated with PB-MNCs ($4.91\%\pm0.65\%$) compared to the untreated group of rats ($9.78\%\pm1.05\%$).

In addition, to investigate the effect of cell transplantation on cell apoptosis in the brain, we measured the apoptosis of neural cells in different groups by tunnel assay on day14 and 28. We found that treatment with PB-MNCs significantly decreased the number of Tunnel-positive cells in the treated group after HI (Supply 4A and Fig. 5A), with about 14% and 10% fewer apoptotic cells when compared with the untreated group on day14 and 28 respectively (Supply 4B and Fig. 5B). These results suggested that PB-MNCs may diminish brain damage and the apoptosis of nervous cells caused by HI.