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Research report

Neuroprotective effects of intravenous transplantation of bone marrow mononuclear cells from 5-fluorouracil pre-treated rats on ischemic stroke

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HIGHLIGHTS

- Bone marrow mononuclear cells (BMMNCs) from 5-fluorouracil (5-FU) pre-treated rats (named BMRMNCs) had a better therapeutic efficacy in ischemia/reperfusion rats as compared to BMMNCs from untreated rats.
- BMRMNCs have elevated secretion of growth factors as compared to BMMNCs.
- More BMRMNCs migrate to the injured brain early after transplantation as compared to BMMNCs.

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ABSTRACT

Our previous findings showed bone marrow mononuclear cells (BMMNCs) from 5- fluorouracil (5-FU) pre-treated rats (named BMRMNCs) had a better therapeutic efficacy in ischemia/reperfusion rats as compared to BMMNCs from untreated rats. This study was undertaken to explore the potential mechanisms underlying the neuroprotective effects of BMRMNCs in middle cerebral artery occlusion (MCAO) rat model. Rats were intravenously pre-treated with 5-FU and BMRMNCs were collected at different time points. The contents of growth factors in the supernatant and CXCR4 expression were detected by ELISA and flow cytometry, respectively. MCAO was introduced to rats, and BMMNCs and BMRMNCs collected at 7 days after 5-FU pre-treatment were independently transplanted via the tail vein 24 h later. The neurological function was evaluated before cell transplantation and at 24 h, 7 d and 14 d after cell transplantation. Rats were sacrificed at 14 d after cell transplantation, the brains were collected for TTC staining, infarct volume detection, NISSL staining, counting of viable cells in the CA1 region, and observation of transplanted cells. BMRMNCs had elevated expressions of growth factors as well as CXCR4 expression. Our results confirmed the better therapeutic effects of BMRMNCs in MCAO rats, demonstrated by reduction in infarct volume, improvement of neurological function and more viable cells in the hippocampus. In addition, more transplanted cells were found after BMRMNCs transplantation at 7 days and 14 days although there was no marked difference at 14 days. These findings indicate that BMRMNCs transplantation may protect ischemic stroke, at least partially, via increasing the secretion of growth factors and migration to the injured site.

1. Introduction

Stroke accounts for 9% of all deaths worldwide and has been the second most common cause of death [1]. A majority of survived patients following stroke will develop sequelae such as hemiplegia, aphasia, visual field defect, depression, etc, significantly

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increasing the economic burden to the family and society. It is estimated that stroke consumes about 2–4% of total health-care costs, and accounts for more than 4% of direct health-care costs in industrialized countries [2]. Because of the ageing population, the burden will increase greatly during the next 20 years, especially in developing countries.

Strokes can be divided into ischemic and hemorrhagic stroke. About 80% of all strokes are ischemic [3]. Although advances have been achieved in the management and treatment of stroke during the past decade, stroke continues to pose major therapeutic challenges, and no effective strategy has been developed for stroke. Early thrombolysis is an effective treatment for acute ischemic stroke, but the number of patients who might receive treatment and therefore potentially benefit is small because of the short therapeutic time window [4]. Thus, it is imperative to develop novel and effective strategies for the therapy of stroke.

In recent years, stem cell transplantation has been shown to be promising for the therapy of stroke [5]. Stem cells are a group of cells having the ability to differentiate into multiple functional cell types (multipotency) and to make identical copies of themselves via cell division (self-renewal). On one hand, the transplated stem cells may differentiate into different types of neural cells to replace dead or injured cells. On the other hand, these stem cells may secrete a variety of neurotrophic factors to promote tissue repair and improve neurological function [6,7]. To date, some types of stem cells have been used in the therapy of stroke in animal studies and clinical trials, including neural stem cells (NSCs), embryonic stem cells (ESCs), mesenchymal stem cells (MSCs) and bone marrow mononuclear cells (BMMNCs).

Our previous studies showed the BMMNCs collected from rats undergoing a single intravenous injection of 5-fluorouracil (5-FU) 7 days ago had a better therapeutic efficacy as compared to BMMNCs from untreated rats [8]. In this study, BMMNCs collected from 5-FU treated rats were named bone marrow regenerative mononuclear cells (BMRMNCs). On the basis of our previous findings, this study was undertaken to confirm the therapeutic efficacy of BMRMNCs in MCAO rats and to explore the potential mechanism.

2. Materials and methods

2.1. Separation of BMRMNCs

Healthy male Spraque–Dawley rats ($n = 25$) weighing 120–150 g were purchased from the Experimental Animal Center of Medical School of Shanghai Jiaotong University and routinely housed for 1 week before use. These animals were divided into 2 groups: control group ($n = 5$) and 5-FU group ($n = 20$). In control group, rats did not receive any treatment, and bone marrow was collected; in 5-FU group, rats were intravenously injected with 5-FU (Shanghai Xudong Haipu Pharmaceutical Co., Ltd) at 150 mg/kg, and bone marrow was collected at 3, 7, 11 and 14 days ($n = 5$ at each time point). BMRMNCs and BMMNCs were separated by density gradient centrifugation (Hao Yang Biological Products Co., Ltd). Then, these cells were maintained in L-DMEM containing 15% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere with 5% CO_2. The protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Shanghai Jiaotong University.

2.2. Enzyme-linked immunosorbent assay

Cells were maintained for 24 h, and then the supernatant was harvested for the detection of growth factors (VEGF, bFGF, BDNF and NGF) by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's instructions (R&D, USA).

2.3. Detection of cell viability

The cell density was adjusted to 1×10^7/ml, and then 5 μL of cell suspension was mixed with 5 μL of 2% trypan blue solution (Beyotime Biotech Co., Ltd.), followed by incubation for 2–3 min. These cells were observed under a light microscope. A total of 200 mononuclear cells were counted, and the cell viability was calculated as follow: cell viability = viable cells/total cells × 100%.

2.4. Detection of CXCR4 by flow cytometry

BMRMNCs were harvested at 7 days after 5-FU injection, and CXCR4 was measured in BMRMNCs and BMMNCs. In brief, cells were re-suspended in 0.1% FBS and incubated at room temperature for 1 h. Goat anti-rat CXCR4 antibody (Abcam, UK) was added (1:100), followed by incubation at 4 °C for 30 min. Following washing in PBS twice, cells were harvested by centrifugation at 1000 rpm for 5 min. Then, Cy3 conjugated rabbit anti-goat IgG (1:100) was added, followed by incubation at 4 °C for 30 min. Following washing in PBS twice, cells were harvested by centrifugation at 1000 rpm for 5 min. Finally, cells were re-suspended in PBS at a density of $2–10 \times 10^4$/mL, and then subjected to flow cytometry.

2.5. Middle cerebral artery occlusion (MCAO) and grouping

MCAO was surgically performed using a previously described method [9]. Rats were anesthetized via an intraperitoneal injection of 10% chloral hydrate (0.35 ml/100 g weight). First, the right common carotid artery was exposed and the external carotid artery cut. Then, a 4/0 nylon monofilament with a rounded tip was thrusted into the exposed right external carotid artery and advanced into the internal carotid artery. Body temperature was maintained at 37.0 ± 0.5 °C during the surgery with a homeothermic blanket. After 120 min of occlusion, the intraluminal monofilament was withdrawn to allow reperfusion. A sham surgery was performed similarly with the exception that the monofilament was immediately removed after it was introduced into the internal carotid artery.

Animals undergoing MCAO were divided into three groups randomly with random number table: control group ($n = 8$), BMMNCs group ($n = 20$) and BMRMNCs group ($n = 20$). In Control group, MCAO rats did not received therapy; in BMMNCs group, MCAO rats received intravenous transplantation of BMMNCs at 24 h after reperfusion; in BMRMNCs group, rats received intravenous transplantation of BMRMNCs at 24 h after reperfusion.

2.6. Labeling and transplantation of BMRMNCs and BMMNCs

BMRMNCs were collected at 7 days after 5-FU injection. Then, BMRMNCs and BMMNCs were independently incubated with 50 mg/L DAPI at 4 °C for 60 min, and then washed with Hank's solution to remove unbound DAPI. Cells were harvested by centrifugation and re-suspended in PBS at a density of 1×10^7 cells/ml. At 24 h after reperfusion, cells were injected via the tail vein. At 7 and 14 days after cell transplantation, rats were sacrificed and the brain was collected for the detection of transplanted cells.

2.7. TTC staining and detection of infarct volume

Rats were sacrificed at 14 days after cell transplantation and the brains were collected for TTC staining and detection of infarct volume. In brief, brains were sectioned into 2-mm coronal slices by a brain matrix, and the infarct volume was determined by 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) staining as previously described [10]. The stained slices were photographed by a digital