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Neuroprotective effects of placenta-derived mesenchymal stromal cells in a rat model of experimental autoimmune encephalomyelitis

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Abstract

Background. Current therapies for multiple sclerosis (MS) are largely palliative, not curative. Mesenchymal stromal cells (MSCs) harbor regenerative and immunosuppressive functions, indicating a potential therapy for MS. A preparation of MSCs derived from full-term human placenta (PDMSCs) is a new approach in the treatment of patients with MS. *Objective.* This study aimed to rule out the possible therapy by PDMSCs in experimental autoimmune encephalomyelitis (EAE), a rat model of MS. *Methods and results.* Thirty-five female Wistar rats were classified into the following groups: I, control; II, EAE untreated; III and IV, EAE treated with phosphate-buffered saline (PBS) at 9 and 16 days post-immunization (dpi), respectively; V and VI, EAE treated with PDMSCs at 9 and 16 dpi, respectively. Intravenous administration of PDMSCs at 9 or 16 dpi significantly ameliorated the disease course, decreasing brain inflammation and degenerating neurons. A reduction of axonal damage as well as an increase of oligodendrocyte precursors were recorded. Moreover, there was an engraftment of the PDMSCs into the brain tissue. Human brain–derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin 3 (NTF3) were significantly expressed in brains of rats treated by PDMSCs. *Conclusions.* Human PDMSCs have demonstrated striking therapeutic effects when delivered at the onset or at the peak of the disease. PDMSCs have direct neurotrophic support after their engraftment within the lesion through expression of the neurotrophins.

Key Words: *autoimmune encephalomyelitis, neurotrophic factors, placenta-derived mesenchymal stromal cells, rat*

Introduction

Multiple sclerosis (MS) is an autoimmune disease, characterized by inflammatory demyelinating lesions of the central nervous system (CNS) and loss of motor function [1]. The immune system has a crucial role in pathogenesis of MS. T-helper-1, T-helper-17 and cytotoxic cells play an important part in promoting neuroinflammation and degeneration. Furthermore, complex interactions with other peripheral immune cells and CNS resident cells are responsible for repeated episodes of autoimmune-mediated damage to myelin and oligodendrocytes [2].

Experimental autoimmune encephalomyelitis (EAE) correlates with chronic and progressive MS and is considered as an appropriate model for MS [3]. EAE is usually induced by immunization with myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG) and adjuvants [4,5]. The finding of inflammatory cells and their secreted molecules in the brain of patients with MS and of animals with EAE has supported the widely accepted notion that MS is mediated by pathogenic T cells, which react with myelin antigens. These autoreactive T cells then migrate and cross the blood-brain barrier (BBB) to destroy the neurons and their myelin sheaths as well as axons [6].

Until now, there is no cure for MS. Current therapies consist of anti-inflammatory drugs that are mainly effective in early disease but do not decrease neurodegeneration or promote CNS repair [7]. Stem cell therapy has emerged as a promising treatment approach for MS. Mesenchymal stromal cells (MSCs) have been investigated with regard to their potential for tissue regeneration as well as their trophic support and immunomodulatory functions. Many sources of MSCs have been estimated and the most significant source is bone marrow (BM). However, cells from the BM may only be obtained through an invasive procedure and their numbers decrease significantly with age [8]. For this reason, alternative sources from where

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MSCs may be isolated have been shown including the placenta. Several studies indicated that placenta-derived MSCs (PDMSCs) are similar to BM-MSCs with respect to their cell characteristics and multi-lineage differentiation potential. It fulfills many advantages: it obtains as high as possible number of cells, it has non-invasive methods for their harvesting and also it is without ethical issues [9]. As placenta-derived multipotent cells are fetal in origin, they may generate less of an immune response [10]. These characteristics make PDMSCs a potential candidate for clinical application in cell-based therapies. Although the therapeutic application of adult MSCs has been extensively reported in the EAE model [11–14], limited data are currently available on the effect of extra-embryonic fetal stem cell types such as placenta and umbilical cord in this condition.

In the present study, we studied the beneficial effect of PDMSCs as a potential therapy in MS-like disease, EAE in rats. These effects were performed by histological and biochemical studies of cerebral cortex. We also assessed the neurotrophic factors expressed by PDMSCs on neuro-protection effects of stem cells.

Materials and methods

This study was performed in the Histology & Cell Biology and Medical Biochemistry departments, Faculty of Medicine, Zagazig University.

Induction of EAE

Female Wistar rats (6–8 weeks old, weighing 75–110 g) were used in this study. Rats were maintained according to the standard guidelines of Institutional Animal Care and Use Committee and after institutional review board approval. Rats were immunized with 300 µL of an emulsion composed of 150 µL incomplete Freund's adjuvant (Sigma) supplemented with 0.8 mg/mL Mycobacterium tuberculosis (strain H37RA; Difco) and 200 µg of MOG35-55 (Sigma) diluted with an equal volume of phosphate-buffered saline (PBS) and injected subcutaneously into the flanks at two sites. They received 50 ng of pertussis toxin (Sigma) intravenously at the immunization time (day 0) and 48 h later [11].

Isolation of PDMSCs

After receiving informed consent, placentas were obtained from women following uncomplicated full-term pregnancies. A total of 15 placentas were screened for isolation of MSCs. Briefly, it was dissected following the drainage of umbilical cord blood. Its chorionic plate was exposed by stripping off the amnion. This plate was then washed with PBS, pH 7.2, to remove traces of blood. Placental tissue was cut into smaller pieces; these pieces were digested with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA; Lonza Bioproducts) for 30 min at 37°C on a magnetic stirrer [15]. The digest was then centrifuged at 1000 rpm to separate the cells. These cells were washed three times with PBS, and finally collected by centrifugation (1000 rpm). The cell pellet was cultured in low-glucose Dulbecco's Modified Eagle's Medium (DMEM; Lonza Bioproducts) supplemented with 10% fetal bovine serum (FBS), 50 U/mL penicillin and 50 µg/mL streptomycin, and incubated at 37°C in a 5% CO_2 atmosphere. After 48 days, the non-adherent hematopoietic cells were discarded and the adherent cells were preserved for further expansion [16].

Labeling of stem cells with PKH26 dye

PDMSCs at passage 2 were labeled with the red fluorescent dye PKH26 (Sigma Aldrich) according to the manufacturer's protocol. Briefly, the detached stem cells were washed with PBS and resuspended in 1 mL of dilution buffer from the manufacturer's labeling kit. The cell suspension was mixed with an equal volume of the labeling solution containing 4×10^{-6} mmol/L PKH26 in the dilution buffer and incubated for 5 min at room temperature. Then cells were washed 3 times with the DMEM solution.

Experimental protocol

Thirty-five healthy animals were divided into the following groups: Group I, the normal un–induced (n = 10; subdivided into two subgroups: negative with no treatment and positive received 1 mL of PBS); Group II, the EAE (n = 5); Group III, the EAE rats injected intravenously with 1 mL PBS at 9 days post-immunization (dpi; n = 5); Group IV, the EAE rats injected with 1 mL PBS at 16 dpi (n = 5); Group V, the EAE rats injected intravenously with PDMSCs at 9 dpi (n = 5); and Group VI, the EAE rats injected with PDSMCs at 16 dpi (n = 5) [17]. For groups V and VI, 1 x 10^6 PDMSCs were re-suspended in 1 mL PBS and injected through the tail vein. All rats were euthanized unless moribund at the end of the study (30 dpi). Brains were removed immediately, after authorizing, and cleaned with physiological salt buffers (0.9% NaCl). Each brain was divided into two cerebral hemispheres; one was fixed in 10% neutral-formalin immediately for 2 days, then the specimens were dehydrated, cleared and embedded in paraffin for histopathologic analysis. The second half was used for DNA and RNA extraction. Approximately 1 g of brain was homogenized in 750 µL of lysis buffer containing 0.5% Triton X-100, 150 mmol/L NaCl, 15 mmol/L Tris, 1 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$, pH 7.4, with a tissue homogenizer.

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