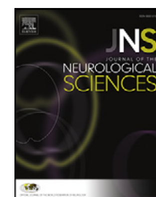
Journal of the Neurological Sciences 348 (2015) 166–173

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns



Effect of the treatment of focal brain ablation in rat with bone marrow mesenchymal stromal cells on sensorimotor recovery and cytokine production

Helder Teixeira de Freitas, Mariana Gomes Rebel, Bárbara Paula Coelho, Viviane Gomes da Silva, Gisela Garcia Cabral Galaxe-Almeida, Arthur Giraldi-Guimarães *

Laboratório de Biologia Celular e Tecidual (Laboratory of Cell and Tissue Biology) - Centro de Biociências e Biotecnologia (Center of Biosciences and Biotechnology) - Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) (State University of North Fluminense) - Campos dos Goytacazes - RJ, Brazil

ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 15 November 2014
Accepted 24 November 2014
Available online 3 December 2014

Keywords:
Brain injury
Cerebral cortex
Cell therapy
Mesenchymal stem cells
Collagen scaffold
Functional recovery

ABSTRACT

Increased efficacy of the multipotent mesenchymal stromal cells (MSCs) for the treatment of CNS injuries has been shown when they are administrated within a collagen scaffold, an environment in three dimensions (3D), when compared to the cultivation over a plastic surface (2D). We evaluated the MSC therapeutic effect in the 2D and 3D conditions using the model of focal cortical ablation. Male Wistar rats were submitted to the ablation by aspiration. Intravenous injection (IV) of MSC cultured in 2D, and the intralesional administration (IL) of MSC cultured in 2D or 3D were tested. Administrations were made 24 h after ablation. Unskilled and skilled forelimb movements were evaluated by sensorimotor tests. The level of cytokines was measured two days after ablation in the 2D IV groups. Only the MSC 3D IL promoted recovery of the skilled movements. MSC 2D IV promoted recovery of the unskilled movements in all tests, and the MSC 3D IL promoted it only in the adhesive test. MSC 2D IL was unable to promote any recovery. DAPI-stained MSC was found in the perilesional parenchyma at the third post-ablation day after 2D and 3D IL. A significant reduction in the levels of cytokines by the MSC 2D IV was observed in the plasma. Our study strengthens the evidences of the MSC as a prospective therapeutic approach for the CNS injuries.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The use of the multipotent mesenchymal stromal cells (MSCs), also widely known as mesenchymal stem cells [12], is a promising therapeutic approach to treat injuries of the Central Nervous System (CNS) [27], e.g., stroke [16], spinal cord injury [7], and traumatic brain injury (TBI) [51]. The MSC has been described as multipotent cells, with the possible capability to regenerate lost tissues [20]. However, their main mechanisms of action have been shown to be the chemoattraction to lesioned tissues and release of cytokines and trophic factors [27].

Intraarterial, intravenous and intralesional administrations of the MSC have yielded the same efficacy to promoted tissue rescue and functional recovery after CNS injuries [7,16,27,33,46]. For intraarterial and intravenous administrations, cells should be solubilized in physiological solution (PBS, saline or culture medium). Regarding the intralesional administration, cells can also be implanted into the lesion enclosed in a scaffold. The administration of cells into a three-dimensional scaffold is a strategy for cell therapy that has been widely studied for tissue engineering and repair [21]. Collagen, the most abundant protein in the animal kingdom and the principal structural element of the animal extracellular matrix [22], has been shown to be a suitable three-dimensional support for cell delivery in several studies [17]. For example, positive results have been shown in animal models of limb ischemia [48], stroke [14], and TBI [11,33].

We have recently used the focal cerebral cortex lesion by ablation, a model that mimics the clinical condition of the instantaneous removal of brain tissue, e.g., surgical removal of a brain tumor [8]. The cortical ablation is a type of brain injury less frequent than stroke or TBI in humans. However, it should also be a useful model for studies about the efficacy of cell therapies. First, it leads to neurological impairments similar to those found after stroke or TBI [29]. Moreover, since the formation of a lesion cavity is direct, the intralesional delivery of cells within different

Abbreviations: 2D, 2-dimensional; 3D, 3-dimensional; ANOVA, analysis of variance; CNS, Central Nervous System; DAPI, 4′,6′-diamidino-2-phenylindole; FA, form of administration; FBS, fetal bovine serum; IL, intralesional placement; IV, intravenous injection; MSC, mesenchymal stromal cell; PAD, post-ischemic day; PBS, phosphate-buffered saline; PF, paraformaldehyde; RCPR, reaching chamber/pellet retrieval; SEM, standard error mean; TBI, traumatic brain injury

* Corresponding author at: Setor de Apoio da Unidade de Experimentação Animal (Biologia Celular) - sala 094 do Hospital Veterinário - UENF - Av. Alberto Lamego, 2000 - Parque Califórnia - Campos dos Goytacazes - RJ - CEP: 28013-602, Brazil. Tel.: +55 22 2739 7340; fax: +55 22 2739 7178.

E-mail addresses: agiraldi@uenf.br, agiraguima@gmail.com (A. Giraldi-Guimarães).

http://dx.doi.org/10.1016/j.jns.2014.11.032
0022-510X/© 2014 Elsevier B.V. All rights reserved.

types of scaffolds can be easily verified, chiefly in the acute phase of the disease. The intravenous injection of bone marrow-derived cells (MSC or mononuclear cells) 24 h after the ablation promoted sensorimotor recovery [8]. Here, we used the model of focal cortical ablation and tested only the bone marrow-derived MSC, given the large number of previous studies about the transplantation of the MSC with collagen scaffold in different models of tissue injury. Thus, we compared the MSC efficacy to promote sensorimotor recovery after intravenous or intralesional administration. Moreover, the intralesional delivery of the MSC into a collagen scaffold was also evaluated. For the analysis of the sensorimotor recovery, the effectiveness of the treatment on the skilled and unskilled forepaw movements was verified [36]. The effect of the MSC treatment in the cytokine production was checked out as well.

2. Experimental procedures

2.1. Animals

Male Wistar rats with two months of age at the beginning of the experiment were used. All animals were housed in a colony room with controlled temperature, and with food and water available ad libitum. Our experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of our institution.

2.2. Surgery

The ablation was performed by aspiration, as previously described [8,43]. Briefly, after anesthesia with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.), and placed in a stereotaxic apparatus (Insight Ltda., Ribeirão Preto, SP, Brazil). The skull was surgically exposed and a craniotomy was performed, exposing the frontoparietal cortex contralateral to the preferred forelimb in the RCPR test (see Section 2.7) (+2 to −6 mm A.P. from bregma; [31]). The cortical tissue was aspirated with a pipette tip (1 ml) attached to a vacuum pump. After the procedure, a piece of collagen hemostatic sponge was placed inside the lesion, and the skin was sutured. The animals were kept warm under a hot lamp and returned to the colony room after recovery from anesthesia.

2.3. MSC culture

Bone marrow was harvested aseptically from the tibias and femurs of naive male Wistar rats weighing 400–500 g to obtain cultures enriched of MSC, as previously described [45]. Briefly, bone marrow was extracted and collected with serum-free DMEM-F12 (GIBCO BRL, Grand Island, NY, USA). Cells were mechanically dissociated, centrifuged and resuspended in serum-free DMEM-F12. Separation of mononuclear fraction was made by centrifugation in Histopaque 1083 (Sigma-Aldrich, St. Louis, MO, USA). Mononuclear cells were collected, washed with phosphate-buffered saline (PBS), and plated in flasks with DMEM-F12 supplemented with 10% fetal bovine serum (FBS) (GIBCO BRL). After reaching confluence, the cells of each flask were harvested and re-plated in two new flasks for expansion. After reaching confluence in the third re-plating, the MSC was harvested, suspended in PBS and counted.

2.4. Cultivation of MSC into the collagen gel

Collagen Type I was obtained from rat tail tendon, similar to the protocol previously described by Rajan et al. [34]. Briefly, the tails were washed with 70% ethanol and dissected into a laminar flow cabinet for the removal of the tendons, which contained the collagen fibrils. The fibrils were solubilized in 200 ml of 1% acetic acid for 4–7 days at 4 °C. Then, the solution containing the fibrils was ultracentrifuged twice at 30,000 $\times g$ for 45 min, and the supernatant was submitted to dialyzed with 600 ml of 1% chloroform for 1 h. The chloroform solution was discarded and replaced by 600 ml of sterile 0.02 N acetic acid and kept in a laminar flow cabinet for 3 days. The acetic acid solution was changed every 24 h. After dialysis the material was lyophilized for 48 h, the dry weight of collagen was determined and stock solutions were made with sterile 0.02 N acetic acid.

MSC harvested after the third re-plating was suspended in DMEM-F12 and supplemented with collagen solution. The cell solution was neutralized with 0.1 N NaOH, and the final concentrations of the collagen and MSC were 1.5 μg/μl and 1.5×10^4 cells/μl, respectively. In 24-well plates, 200 μl of the solution was placed in a well, which contained 3×10^6 MSC. The same solution without the MSC was prepared and also placed in the 24-well plates (200 μl/well) for control. The plates were set at 37 °C for approximately 60 min in 5% CO_2 for jellification of the collagen. After the formation of the collagen gel, DMEM plus 10% FBS was added to each well, and the plates were returned for incubation at 37 °C in 5% CO_2 for approximately 24 h.

2.5. Experimental groups

Two different routes of administration of MSC were compared: intravenous injection (IV), and intralesional placement (IL). Moreover, two forms of MSC cultivation were used: conventional cultivation in plastic flasks (see Section 2.3), considered as the 2-dimensional (2D) condition and the cultivation in the 2D condition followed by the cultivation into the collagen gel for 24 h (see Section 2.4), considered as the 3-dimensional (3D) condition. The route of administration and form of cultivation were combined, forming the following experimental groups: MSC 2D IV, animals that received IV of 2D cultivated MSC; control 2D IV, animals that received IV of the vehicle (PBS); MSC 2D IL, animals that received IL of 2D cultivated MSC; control 2D IL, animals that received IL of vehicle (PBS); MSC 3D IL, animals that received IL of 3D cultivated MSC; and control 3D IL, animals that received IL of the vehicle (collagen gel).

2.6. Administration of MSC

All administrations were made approximately 24 h after ablation. The ablated animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.). For the 2D IV, 3×10^6 2D cultivated MSC in 500 μl of PBS, or 500 μl of PBS, was injected through the jugular vein ipsilateral to the ablation. For the IL, the skin incision was reopened, and the hemostatic sponge was removed. For the 2D IL, 3×10^6 2D cultivated MSC in 30 μl of PBS, or 30 μl of PBS, was placed inside the lesion cavity. We carefully observed with a stereoscopic microscope the accommodation of the cell solution inside the lesion cavity for several minutes. The cell solution was very concentrated, and it was possible to check the precipitation of the cells above the bottom of the lesion. For the 3D IL, a collagen gel containing 3×10^6 3D cultivated MSC, or a cell-free collagen gel, was placed inside the lesion cavity. The skin was sutured, and the animals were returned to the colony room after recovery from anesthesia.

2.7. Behavioral tests

The ablated animals from all experimental groups ($n = 6$ for each group) were submitted to the "reaching chamber/pellet retrieval" (RCPR) task to assess the skilled forelimb motor function, as previously described [36,37]. Briefly, the animal was placed in a Plexiglas box with a front window, and platform attached outside the box in the front window (see details in [36]). There were five holes on this platform, where food pellets (45 mg; Dustless Precision Pellets®/Rodent, Grain-Based; Bio-Serve, Frenchtown, NJ, USA) were placed. A daily task was standardized with 20 trials or 20 min of task. A trial consisted to grasp and lift a food pellet placed on the external platform and take it to the mouth, inside the box. The whole experiment was divided into three phases.

Download English Version:

https://daneshyari.com/en/article/1913420

Download Persian Version:

https://daneshyari.com/article/1913420

Daneshyari.com