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Platelet-rich plasma-derived scaffolds increase the benefit of delayed mesenchymal stromal cell therapy after severe traumatic brain injury

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Abstract

Background. Cell therapy using mesenchymal stromal cells (MSCs) offers new perspectives in the treatment of traumatic brain injury (TBI). The aim of the present study was to assess the impact of platelet-rich plasma scaffolds (PRPS) as support of MSCs in a delayed phase after severe TBI in rats. *Methods.* TBI was produced by weight-drop impact to the right cerebral hemisphere. Two months after TBI, four experimental groups were established; saline, PRPS, MSCs in saline, or MSCs in PRPS was transplanted into the area of brain lesion through a small hole. All groups were evaluated in the course of the following 12 months after therapy and the animals were then humanely killed. *Results.* Our results showed that a greater functional improvement was obtained after the administration of MSCs in PRPS compared with the other experimental groups. *Discussion.* PRPS enhanced the benefit of cell therapy with MSCs to treat chronic brain damage in rats that suffered a severe TBI. The present findings suggest that the use of intralesional MSCs supported in PRPS may be a strategy of tissue engineering for patients with established neurological severe dysfunction after a TBI.

Key Words: *brain injury, mesenchymal stromal cells, platelet-rich plasma scaffolds, transplantation*

Introduction

Traumatic brain injury (TBI) is a devastating disease that produces an important public health concern, being one of the leading causes of death and disability around the world [1,2]. Currently, aside from supportive care measures, there are no available pharmacological treatments for TBI [3]. Finding a treatment to the devastating consequences of TBI is currently one of the main challenges in neurobiology.

In our experience, the effectiveness of mesenchymal stromal cell (MSC) transplants to influence neurological behaviour after nervous system damage may be due to number of cells, route of transplantation or transplantation technique, both in animal models of spinal cord injury (SCI) [4–7] and TBI [8–10].

Previously, we suggested for the first time a relationship between scores of brain damage and effectiveness of cell therapy with MSCs for the treatment of chronic TBI [11]. Once the treatment was administered, we obtained different results depending on whether the animals belonged to the group of severe or moderate lesions. In the moderate lesion group, administration of MSCs showed evident and progressive recovery of neurological functions with respect to controls, but, in the severe lesion group, saline and MSCs showed similar neurological recovery with no improved functional outcome.

This study was designed to try to find an effective therapy for severe brain injury. We addressed the problem from two different approaches. First, we tried to optimize cell transplantation with platelet-rich plasma scaffolds (PRPS) [12]. Second, we studied the late effect of cell transplantation, through the establishment of a long-term period of follow-up of 12 months.

Materials and methods

Animal model

All animal studies were approved by the experimentation committee of Puerta de Hierro-Majadahonda Hospital. Experiments were conducted using 3-month-old female Wistar rats weighing 225–250 g (Charles River Laboratories International, Inc.).

The experimental TBI was performed according to a technique previously described [11]. Once the animals were anesthetized, a 1 cm incision was made along the middle line of the scalp. The animals were subjected to a 10-mm diameter craniotomy on the right hemisphere, adjacent to the central suture, midway

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between lambda and bregma. The dura was cut with microsurgery scissors to expose the subjacent brain. The brain injury was achieved by a 12 mm^2 thick cylindrical steel bar weighing 25 g, which was allowed to fall vertically onto the brain surface from a height of 20 cm, guided by a hollow cylinder, pointed at the craniotomy area. After surgery, the rats were placed overnight in a temperature- and humidity-controlled chamber.

Before surgery, rats underwent different tests to establish their basal state and an additional test to measure their balance and coordination capacity. After experimental TBI (see below), the rats recovered for 1 week and then underwent the same weekly series of behavioral tests, during the following 2 months, to determine their neurological level post-TBI. Once the different neurological severities were established, 50 rats with severe brain injury were distributed into four experimental groups (PRPS-MSCs group, PRPS group, MSCs group and saline group). After the treatments, all animals were re-assessed using the same weekly series of behavioral tests, following their neurological evaluation for 12 months.

Neurological evaluation

A double-blind study was performed to assess the degree of lesion of the animals and the evolution of the different treatments.

To determine the degree of brain damage, all animals were evaluated during the next 2 months after the traumatic injury using two different tests (modified neurological severity scores [mNSS] and video tracking box [VTB] test); an additional test (Rotarod test) was used to measure their balance and coordination capacity.

mNSS test was performed as we have previously described [8]. The test is suitable for evaluating the long-term neurological function after unilateral TBI. It is a composition of motor (muscle status and abnormal movement), sensory (visual, tactile and propioceptive) and reflex tests. Neurological function was graded on a scale of 0 to 19 (normal score 0; maximal deficit score 19). To rate the severity of the injury, one point is awarded for the exhibition of a specific abnormal behavior or for the lack of reflexes. The higher the score, the higher the brain injury.

For a more objective assessment of possible improvements and neurological deficits in animals, we used a computer program (Smart) connected to a VTB test. This method adequately quantifies parameters related to locomotors activity and orientation in brain-injured rats, and has been used in our laboratory to measure behavioral outcome after TBI in adult rats [9,11]. VTB test analyzes the images captured by a video camera in a 35×45 cm closed box where animals are deposited. Different parameters were defined: 90 seconds time and top speed of 10 cm/s. The closed box was divided into different areas to study differences in the movement and orientation of animals: inner zone and outer zone. Rats without brain injury prefer the outer zone, whereas animals with brain injury use any of the zones. We studied the time the animals remain in the inner zone (inner zone Permanence Time, izPT) as a measure of brain injury.

In the Rotarod test, the time the animals remained on a rotating roller was measured. The speed was slowly increasing from 4 to 40 rpm for 1 min. The test ended when the animals fell from the roller.

High score in the mNSS, little time on the Rotarod cylinder or more time in the inner zone are associated with a severe brain injury in rats. Because in each group the data present a great variability, to be able to compare them the tests' data are presented, in each case, as a percentage of recovery: 100% represents baseline of a healthy animal and 0% represents the maximum damage acquired by the animals after TBI.

Isolation and characterization of MSCs

The MSCs were harvested from adult male Wistar rats weighing 200–250 g. The procedures have been reported previously [5,8,11,13]. The cells were cultured *in vitro* for approximately 4 weeks.

Using a 1-mL syringe and a 21-gauge needle, whole bone marrow was harvested aseptically from tibias and femurs. Both ends of the bones were burst and the marrow was extruded with 5 mL of alpha-MEM (Minimum Essential Medium) (Lonza Group Ltd.). Bone marrow was mechanically dissociated to obtain a homogeneous marrow. The cell suspension was filtered through a 70 μm mesh nylon strainer and placed in a 75 cm^2 flask for tissue culture with 12 mL alpha-MEM medium containing 20% fetal bovine serum (FBS; Lonza Group Ltd.), 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin and 25 ng/mL amphotericin B. The cells were incubated at 37°C in 5% CO_2 for 3 days. At this time, nonadherent cells were removed by replacing the medium. The culture medium was replaced three times a week.

After the primary cultures reached confluence, they were rinsed three times with phosphate-buffered saline (PBS) and dissociated with 0.25% trypsin solution and 1 mmol/L ethylenediaminetetraacetic acid (EDTA) for 5 min, at 37°C. The cells were washed two times with alpha-MEM medium/2.5% FBS at 1000 rpm during 5 min and cultivated at 37°C in 5% CO_2.

To obtain MSCs for transplants, cells corresponding to passage (P) 1 were rinsed three times with PBS and dissociated with 0.25% trypsin solution and