Topical Therapy with Mesenchymal Stem Cells Following an Acute Experimental Head Injury Has Benefits in Motor-Behavioral Tests for Rodents

P.K. Lam, Kevin K.W. Wang, Anthony W.I. Ip, Don W.C. Ching, Cindy S.W. Tong, Henry C.H. Lau, Themis H.C.S. Kong, Paul B.S. Lai, George K.C. Wong, and W.S. Poon

Abstract *Background*: The neuroprotective effects of mesenchymal stem cells (MSCs) have been reported in rodent and in preliminary clinical studies. MSCs are usually transplanted to patients by systemic infusion. However, only a few of the infused MSCs are delivered to the brain because of pulmonary trapping and the blood–brain barrier. In this study, MSCs were topically applied to the site of traumatic brain injury (TBI) and the neuroprotective effects were assessed. *Materials and Methods*: TBI was induced in Sprague–Dawley (SD) rats with an electromagnetically controlled cortical impact device after craniotomy was performed between the bregma and lambda, 1 mm lateral to the midline. We applied 1.5 million MSCs, derived from the adipose tissue of transgenic green fluorescent protein (GFP)-SD rats, to the exposed cerebral cortex at the injured site. The MSCs were held in position by a thin layer of fibrin. Neurological function in the test ($n = 10$) and control ($n = 10$) animals was evaluated using the rotarod test, the water maze test, and gait analysis at different time points. *Results*: Within 5 days following topical application, GFP-positive cells were found in the brain parenchyma. These cells co-expressed with markers of Glial fibrillary acidic protein (GFAP), nestin, and NeuN. There was less neuronal death in CA1 and CA3 of the hippocampus in the test animals. Neurological functional recovery was significantly improved. *Conclusion*: Topically applied MSCs can migrate to the injured brain parenchyma and offer neuroprotective effects.

Keywords Mesenchymal stem cells • Topical application • Traumatic brain injury

P.K. Lam
Division of Neurosurgery, Department of Surgery,
The Chinese University of Hong Kong, Prince of Wales Hospital,
Shatin, Hong Kong, China

Department of Anatomical and Cellular Pathology,
The Chinese University of Hong Kong, Prince of Wales Hospital,
Shatin, Hong Kong, China

K.K.W. Wang
Department of Psychiatry and Neuroscience,
Center of Neuroproteomics and Biomarkers Research, University of Florida, Gainesville, FL, USA

A.W.I. Ip
Chow Tai Fook-Cheng Yu Tung Surgical Stem cell Research Center, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China

D.W.C. Ching • C.S.W. Tong • H.C.H. Lau • T.H.C.S. Kong
Division of Neurosurgery, Department of Surgery,
The Chinese University of Hong Kong, Prince of Wales Hospital,
Shatin, Hong Kong, China

Chow Tai Fook-Cheng Yu Tung Surgical Stem cell Research Center, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China

P.B.S. Lai • G.K.C. Wong • W.S. Poon (✉)
Division of Neurosurgery, Department of Surgery,
The Chinese University of Hong Kong, Prince of Wales Hospital,
Shatin, Hong Kong, China
e-mail: wpoon@surgery.cuhk.edu.hk

Introduction

Traumatic brain injury (TBI) is a serious neurological disorder involving contusion, diffuse axon injury, subdural hematoma, cerebral ischemia, and inflammatory reactions with the resultant death of neurons and oligodendrocytes, a type of glial cell [1]. Worldwide, TBI causes significant morbidity and mortality. Survivors suffer from debilitating long-term motor, cognitive, and behavioral deficits, and functional neurological impairment [2]. To date there have been no effective pharmacological agents that target only a pathophysiological pathway of a given disease, to reverse the sequelae of TBI at either a cellular or a subcellular level. On the other hand, stem cell therapy restores organ function via multiple mechanisms and represents one of the potential avenues for treating TBI.

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Mesenchymal stem cells (MSCs) exhibit two major characteristics that define stem cells: multipotentiality and self-renewal [3]. The neuroprotective effects of MSCs on TBI have been demonstrated in a number of animal and preliminary clinical studies [4–6]. MSCs are usually given to patients by systemic infusion, which is minimally invasive. MSCs have chemotactic properties of homing to the sites of injury/inflammation. However, only a small amount of the infused MSCs can reach the brain because of pulmonary trapping and the blood–brain barrier [7]. Our previous study showed that topically applied MSCs in the contralateral hemisphere migrated along the corpus callosum to the site of the TBI [8]. In this study, the neuroprotective effects of topical MSCs on TBI were investigated.

Materials and Methods

Adipose-Derived MSCs

The MSCs were derived from the subcutaneous adipose tissue of male transgenic Sprague–Dawley (SD) rats expressing green fluorescent protein (GFP; SD-Tg(CAG-EGFP)CZ-0040sb; SLC, Hamamatsu, Japan). Briefly, the adipose tissue was washed extensively with sterile phosphate-buffered saline (PBS) and treated with 0.1 % collagenase (type I; Sigma-Aldrich) in PBS for 30 min at 37 °C with gentle agitation. After filtration through a 100-μm mesh filter to remove debris, the filtrate was washed and suspended in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. The MSC cultures were maintained in an incubator with a humidified atmosphere of 5 % CO_2. The cell phenotype was tested with CD45 and CD90 (Abcam, Cambridge, UK) and CD 29 (Biolegend, San Diego, CA, USA). The adipogenic, chondrogenic, and osteogenic differentiation potentials of MSCs were evaluated.

Experimental Traumatic Brain Injury

Male, wild-type SD rats (weighing 250–300 g) were anesthetized with intraperitoneal administration of ketamine (125 mg/kg) and placed on a stereotactic frame. A midline cranial incision was made to expose the skull. A 5-mm × 5-mm craniotomy was made between the bregma and the lambda. TBI was induced over the right parietal cortex of the anesthetized animals ($n = 10$) by impacting a 3-mm tip of an electromagnetic controlled cortical impact device at a rate of 4 m/s and 2.5 mm of compression [9]. Within 1 h, 1.5 million GFP-MSCs were applied to the surface of the exposed cortex. A thin layer of fibrin (Tisseel®; Baxter, Volketswil, Switzerland) was applied to fix the cells in position. In the control group ($n = 10$), no treatment was given to the animals.

Behavioral Testing

Rotarod Test

Two days before TBI, all rats were trained with the walking and balancing abilities on the rotarod machine (Stoelting, Wood Dale, IL, USA). The rotating rod was started off at a speed of 10 rpm. The speed was accelerated up to 30 rpm within 100 s. The machine stopped at 150 s. The latency to fall (in seconds) was measured at days 3, 7, and 10.

Morris Water Maze Test

The acquisition of spatial learning was studied in accordance with Morris [10] with minor modifications. The ANY-Maze (Stoelting) consisted of a circular water tank divided into four quadrants. A circular platform was hidden under the water at a fixed location in the center of one quadrant. On the day after the TBI, all test and control rats were placed on the platform and then released into the water so that they could find the hidden platform. The rats were given ten consecutive daily trials to build up this memory before a probe test was conducted (with removal of the platform). Distance traveled (in meters) to find the hidden platform was measured in each trial.

Microscopic Examination

Trafficking of the topical MSCs was studied with immunohistochemistry staining using anti-GFP. The trans-differentiation potential of the engrafted MSCs was investigated with immunofluorescence staining. The injury at the hippocampus was examined using Cresyl violet staining.

Statistical Analysis

All quantitative data of the behavioral tests were expressed as mean ± 1 standard deviation (SD) using IBM SPSS Statistics software (version 20, SPSS, Chicago, IL, USA) and compared using the paired samples t test. Statistical significance was set at $p < 0.05$.

Results

The MSCs were positive in CD29 (70 %) and CD90 (75 %) and negative in CD45. Adipogenic, chrondrogenic, and osteogenic differential potentials were expressed in MSCs. Within 5 days of topical application, GFP-positive cells were found in the brain parenchyma at the injury site. These cells showed expression of GFAP, nestin and NeuN. Cresyl violet

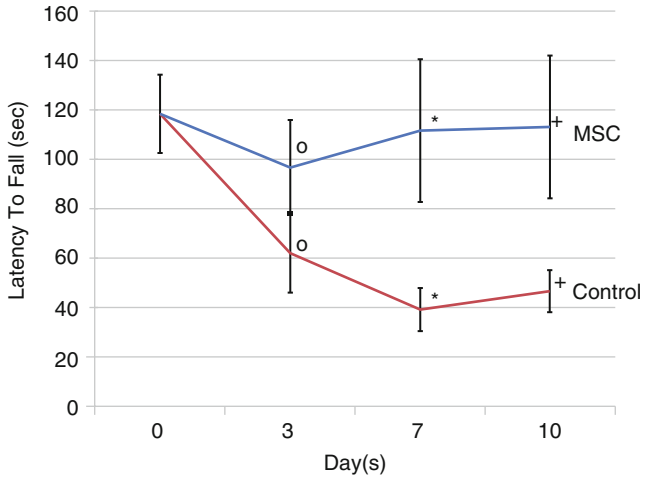

Fig. 1 Rotarod performance of animals with traumatic brain injury (TBI). Data are expressed as mean and standard deviation. Test animals had a significantly longer latency to fall ($p<0.05$)

staining showed less neuronal loss at the CA1 and CA3 areas of the hippocampus when MSCs were topically applied onto the brain (data not shown).

The power to grasp the rotating rod decreased in both test and control animals after TBI. In the former animals, the balance and motor coordination started to recover at day 3 and returned to 90 % normal at day 10. The latency to fall of the test animals was significantly longer ($p<0.05$) than that of control groups at all time points (Fig. 1).

The Morris water maze assessment consisted of the memory acquisition test, which measured the mean distance traveled (*m*) to reach the hidden platform, and the probe test, which assessed the ability to retrieve information learned in the previous hidden platform test. Between trial 4 (day 6) and trial 10 (day 14), the test animals traveled a significantly shorter distance to reach the hidden platform (Fig. 2a). When the hidden platform was removed in the probe test at day 15, the animals treated with MSCs were able to find the correct quadrant where the platform was previously placed. On the contrary, a nonspatial strategy was found in the control animals. They failed to find the correct quadrant and swam in concentric circles at a fixed distance from the tank wall (Fig. 2b, c).

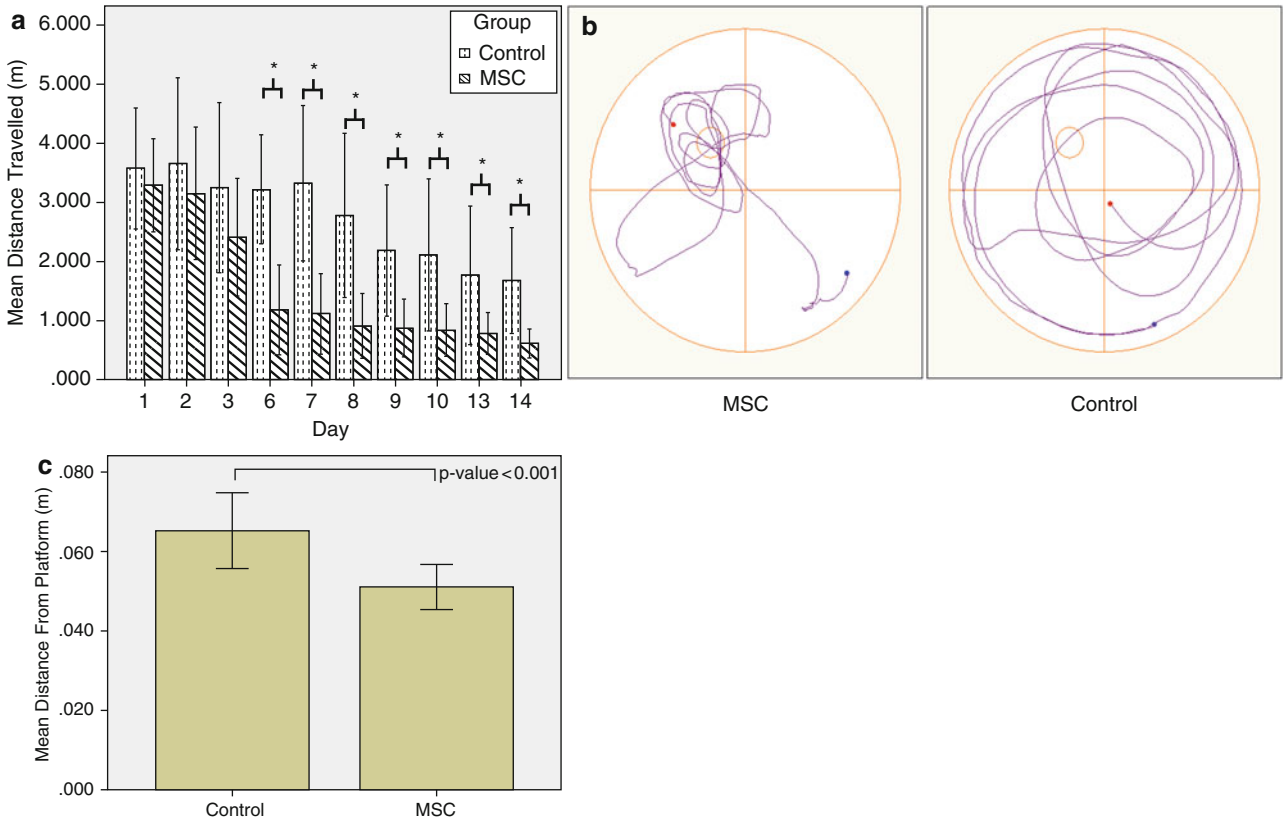

Fig. 2 Water maze test. In the learning period, test animals traveled a shorter distance to find the hidden platform ($p<0.05$; **a**). Representative swimming patterns of the test and control groups in the probe test (**b**). The test animals found the platform more precisely than the control animals ($p<0.05$; **c**)

Discussion

Mesenchymal stem cells, available from various adult tissues, are attracting increasing interest with regard to regenerative medicine [11, 12]. However, the clinical efficacy of MSCs is inconsistent and modest because of poor homing and the heterogeneity of MSCs [13, 14]. In our previous studies, we conceptualized the topical application of MSCs onto the surfaces of various somatic organs [15]. The MSCs had migrated to the injured parenchymal tissues a few days after topical application.

The degree of neurological motor deficit of TBI is an indicator of the severity of brain injury [16]. In this study, the posttraumatic motor functions were compared in animals with and without topical MSCs. Accelerating rotarod is a measurement of the coordination and integration of movements. A significant improvement in neurological motion was found in the animals treated with topical MSCs. In the automated water maze test, brief preliminary training was given to the animals before TBI to ensure that they could swim and climb onto the platform. The distance they traveled to find the platform was an indicator of visual–spatial learning, which was jeopardized after TBI. The ability to retrieve the information learned in the hidden platform test was studied using the probe test. The animals with topical MSCs benefited from the neuroprotective effects of MSCs. These animals could recall their memory regarding the location of the platform. The control animals failed to find the location and traveled randomly in the probe test. Topical application offers definite advantages over systemic infusion. As millions of MSCs can be directly delivered to the brain in a single procedure, the therapeutic potential of MSCs in the treatment of TBI should be greatly enhanced.

Conflict of Interest No conflict of interest exists for any of the authors.

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