Cytotherapy, 2014; 0: 1–13

International Society for Cellular Therapy
ISCT

Transplantation of olfactory ensheathing cells promotes the recovery of neurological functions in rats with traumatic brain injury associated with downregulation of Bad

YOU-CUI WANG[1,*], QING-JIE XIA[2,*], YING-CHUN BA[3], TING-YONG WANG[3], NA LIN[3], YU ZOU[2], FEI-FEI SHANG[2], XIN-FU ZHOU[5], TING-HUA WANG[1,2,3], XUE-MEI FU[4,*] & JIAN-GUO QI[1,*]

[1]*Department of Histology, Embryology and Neurobiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China,* [2]*Institute of Neurobiological Disease, Translational Neuroscience Center and the State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu, China,* [3]*Institute of Neuroscience, Kunming Medical University, Kunming, China,* [4]*Shenzhen Children's Hospital, Shenzhen, Guangdong, China, and* [5]*School of Pharmacy and Medical Sciences, Division of Health Sciences, University of South Australia, Adelaide, SA, Australia*

Abstract

Background aims. The neuroprotective effects of olfactory ensheathing cells (OECs) after transplantation have largely been known in the injured nervous system. However, the underlying mechanisms still must be further elucidated. We explored the effects of OEC transplantation on the recovery of neurophysiologic function and the related anti-apoptosis mechanism in acute traumatic brain injury. *Methods.* The OECs from neonatal Sprague-Dawley rats were isolated, identified and labeled and then were immediately transplanted into the regions surrounding the injured brain site that is resulted from free-weight drop injury. *Results.* Nerve growth factor and it's recepor, p75 was expressed in cultured OECs. Transplanted OECs survived, migrated around the injury site and significantly improved the neurological severe scores compared with the control group ($P < 0.05$). OEC transplantation significantly increased the number of GAP-43–immunopositive fibers and synaptophysin-positive vesicles ($P < 0.05$) but significantly decreased the number of apoptotic cells ($P < 0.05$). On the molecular level, the expression of Bad in the OEC transplantation group was significantly downregulated ($P < 0.05$). *Conclusions.* OEC transplantation could effectively improve neurological deficits in TBI rats; the underlying mechanism may be related with their effects on neuroprotection and regeneration induction, which is associated with the downregulation of the apoptotic molecule Bad.

Key Words: *behavior, olfactory ensheathing cells, rats, transplantation, traumatic brain injury*

Introduction

Traumatic brain injury (TBI) is caused by a blow impact or penetrating injury to the head that disrupts normal brain structure and function; it is also called mechanical cerebral injury. It is commonly encountered in the clinic of neurosurgery. With the development of society, the incidence of TBI has increased to become a leading cause of morbidity and mortality among all traumas (1,2). About 1.7 million people sustain a TBI annually in the United States (2). The pathophysiological process of TBI includes a primary response and a secondary response (3). The primary brain damage that occurs at the time of injury is characterized by diffuse degeneration and necrosis of the affected neurons and the interruption of the normal neurophysiological processes. The secondary brain damage occurs subsequently, leading to additional pathological features such as cellular apoptosis and necrosis. In the meantime, the activated astrocytes form glial scars, which last for several months or even several years. These damages result in serious and irreversible neurological dysfunctions, such as sensory-motor disorder and cognition-memory disorder (4). The pathological cascade is associated with changes in a certain number of gene expressions

*These authors contributed equally to this work.
Correspondence: **Jian-Guo Qi**, PhD, Department of Histology, Embryology and Neurobiology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, 610041, China. E-mail: jgqi@scu.edu.cn; **Xue-Mei Fu**, PhD, Shezen Children's Hospital, China. E-mail: fxm2j2004@163.com

(Received 20 December 2012; accepted 26 December 2013)

http://dx.doi.org/10.1016/j.jcyt.2013.12.009

such as neurotrophic factor—encoding genes (5). Currently, therapeutic strategies for TBI mainly include controlling the secondary damage with the administration of neurotrophic drugs and promoting rehabilitation training of neurological function (5). However, these therapeutic effects were less than optimal, and novel strategies must be found.

Cellular or tissue transplantation has been considered as one of the most promising strategies for the therapy of TBI (6—8). Transplantation of olfactory ensheathing cells (OECs) has attracted the widest attention among medical researchers. This could be attributed to the distinct biological features of OECs and their apparent effects on promoting axonal regeneration (8,9). OECs, as a special kind of neuroglial cells, are not only present in the olfactory epithelium of the nose and olfactory nerve but also in the olfactory bulb in the central nervous system. OECs share the features of both Schwann cells and astrocytes (8—13) and could secrete many kinds of neurotrophic factors to support the survival and development of neurons. These neurotrophic factors include nerve growth factor (NGF), brain-derived neurotrophic factor and glial cell line—derived neurotrophic factor (14,15). In addition, certain molecules are also expressed on the cell membrane of OECs. These molecules, such as N-CAM, are involved in cellular adherence and axonal growth and may mediate the elongation of olfactory axons (16). After nerve injury, OECs could inhibit the secretion of inflammatory factors and induce neural stem cells to differentiate into neurons to replace dead ones. They could also promote the growth and remyelination of injured or degenerative neurons (17,18). An *in vivo* experiment has shown that the transplanted OECs had stronger migratory features and were better integrated with host tissues (19). OECs transplantation significantly decreased the local reactive hyperplasia of astrocytes, contributed to neural remyelination and accelerated the conductive velocity of neural tissue. However, the molecular mechanisms of OEC transplantation in central nervous system repair remains unclear.

In this study, we propose that OECs could be a good source of cells for transplantation in TBI. We investigated the effects of OEC transplantation in the acute stage of TBI rats and sought to explore the underlying molecular mechanisms to provide critical evidence for the therapy of TBI by OEC transplantation.

Methods

Culturing, purification and identification of OECs

As described previously (20), the olfactory nerves were obtained from 2- to 3-d-old Sprague-Dawley rats (provided by the Center of Experimental Animal, Sichuan University, Chengdu, China) and were digested with 0.1% trypsin. The tissues were added to Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F-12; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco) and were mechanically dissociated into a single-cell suspension. The cells were then inoculated into glass flasks not covered with polylysine. After the addition of DMEM/F-12 containing fetal bovine serum, the flasks were cultured for 12 h in air containing 5% CO_2 at 37°C. The culture supernatants along with non-adhered cells were then transferred into another glass flask not covered with polylysine, followed by another 12 h of culturing. After that, the cell density was adjusted to 1×10^6/mL and the cells were implanted into six-well plates pretreated with polylysine. After 5 d of culturing, the cells were treated with Arabinofuranosylcytosine (Ara-C; final concentration, 10^{-5} mol/L; Invitrogen, Carlsbad, CA, USA) for 36 h (21). When the adherent cells grew to 80% confluence, they were passaged (1:3) into different culture flasks. OECs of passages 2 were used for the study. After approximately 10 d of culturing, the cells were transplanted and identified by immunohistochemistry staining with anti—nerve growth factor receptor (NGFRp75) antibody (diluted 1:100; Chemicon, Billerica, MA, USA) to determine cell purity.

Immunofluorescent staining

For immunofluorescent staining, the cultured OECs were washed with phosphate-buffered saline (PBS) three times. This was followed an incubation with 0.3% Triton X-100 for 30 min at room temperature (RT), then blocking with 5% normal goat serum for 30 min. Afterward, OECs were incubated with rabbit anti-NGF (1:500, Santa Cruz Biotechnology, Dallas, Texas, USA) overnight at 4°C. After washing three times, OECs were incubated with fluorescent secondary antibody immunoglobulin G (1:200, goat anti-rabbit CY3: red) for 2 h at 37°C; red fluorescence was observed under fluorescence microscope. The images were collected by use of the Leica imagine analysis system.

Animals and experimental groups

Forty-eight healthy adult female Sprague-Dawley rats (the Center of Experimental Animals, Sichuan University, Chengdu, China) weighing 220—250 g were randomly divided into three groups: group A was sham-operated (n = 16); group B, rats that underwent injury at the cortical motor area (n = 16), is also called the TBI group or control group; group C,