HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2015.05.001

Effect of mesenchymal stem cells transplantation combining with hyperbaric oxygen therapy on rehabilitation of rat spinal cord injury

Cheng-Kui Geng[1,2], Hong-Hua Cao[3], Xiong Ying[1], Hua-Lin Yu[2*]

[1]*Department of Orthopedics, Yan'an Hospital of Kunming City, the Affiliated Hospital of Kunming Medical University, Kunming 650051, China*

[2]*Department of Minimally Invasive Neurosurgery, the First Affiliated Hospital of Kunming Medical University, Kunming 650032, China*

[3]*Department of Hematology, Tumor Hospital of Yunnan Province & The Third Affiliated Hospital of Kunming Medical University, Kunming 650118, China*

ARTICLE INFO

Article history:
Received 15 Mar 2015
Received in revised form 20 Apr 2015
Accepted 15 May 2015
Available online 25 June 2015

Keywords:
Spinal cord injury
Mesenchymal stem cells
Transplantation
Hyperbaric oxygen
Locomotive function

ABSTRACT

Objective: To investigate the effect of BMSCs transplantation plus hyperbaric oxygen (HBO) on repair of rat SCI.

Methods: Seventy five male rats were divided randomly into five groups: sham, vehicle, BMSCs transplantation group, combination group, 15 rats in each group. Every week after the SCI onset, all animals were evaluated for behavior outcome by Basso–Beattle–Bresnahan (BBB) score and inclined plane test. Axon recovery was examined with focal spinal cord tissue by electron microscope at 6 weeks after the SCI onset. HE staining and BrdU staining were performed to examine the BMSCs and lesion post injury. Somatosensory evoked potential (SEP) testing was performed to detect the recovery of neural conduction.

Results: Results from the behavior tests from combination group were significant higher than rats which received only transplantation or HBO treatment. Results from histopathology showed favorable recovery from combination group than other treatment groups. The number of $BrdU^+$ in combination group were measureable more than transplantation group ($P < 0.05$). The greatest decrease in TNF-α, IL-1β, IL-6, IFN-α determined by Elisa assay in combination group were evident too.

Conclusions: BMSCs transplantation can promote the functional recovery of rat hind limbs after SCI, and its combination with HBO has a synergistic effect.

1. Introduction

Spinal cord injury (SCI) is the most serious complication in spinal injury which caused hemiparalysis or general paralysis and jeopardize the living quality of patients. SCI usually causes neuron death and axonal damage resulting in dyskinesia or somatosensory loss. The adverse microenvironment in focal area such as ischemia, neuroinflammation and glial scarring which made the neural system regeneration even more difficult [1,2]. Neuroinflammation is a pathological process principally involving activation of microglia and astrocytes by inflammatory mediators in various CNS disorders, including brain trauma, ischemia, and SCI [3,4]. To date, there is still lack of effective therapy to treat traumatic SCI.

Stem cells transplant is a promising therapy for the repair of damaged nervous system [5–7]. Bone marrow derived mesenchymal stem cells (BMSCs) are one of the most commonly used cell to treat injured spinal cord. The transplants of BMSCs in neural systems are considered to repair the injury because of the various immunoregulatory macromolecules secreted by BMSCs that contributing to structure regenerative microenvironments in fields of injury [8–10]. However, these effects and axonal regeneration are limited due to the damage from cytokines and immunological rejection. Hyperbaric oxygen (HBO) is occasionally used as a prognosis therapy for the treatment of SCI. The beneficial effects of HBO in SCI

*Corresponding author: Hua-Lin Yu Ph.D, MD, Department of Minimally Invasive Neurosurgery, the First Affiliated Hospital of Kunming Medical University, Kunming 650032, China.
Tel: +86 0871 65399138
E-mail: 15877990976@163.com

Peer review under responsibility of Hainan Medical University.
Foundation project: This study was supported by a grant from Yun Nan Province United Foundation (2008CD006); Yun Nan Province Education Department Foundation (2011y184).

stem from lower endothelial adhesion molecule expression and decreased neutrophil activation [11]. Moreover, HBO stabilizes metabolism, improves angiogenesis and collateral circulation which enhance the focal area recovery [12,13]. Base on the above reasons, the purpose of this experimental study was to investigate the effectiveness of HBO either alone or in combine with BMSCs transplant on the recovery of locomotive in a SCI model in rat.

2. Materials and methods

2.1. Experimental animals

The experimental animals were purchased from Institute of Laboratory Animal Science, license NO: SCXK20060023. The experimental procedures were in according with the "Opinion on the treatment of experimental animal" published by Chinese National Technical Department, 2006.

2.2. Experimental reagents & instruments

Dulbecco's modified Eagle's medium was purchased from Life Technology, USA. BrdU immunohistochemistry kit was purchased from Biyuntian biotechnology Co.

2.3. Preparation of acute SCI model

Rats were anesthetized by ip injection of pentobarbital sodium (20 mg/kg). Total laminectomy was performed at T9 spinous. The interscapular distance incision was acquired through the skin and subcutaneous tissues, via a 2-cm incision at the level of T8–T9 and dura mater was exposed by Rats vertebral wrench. Separated the dura mater and the spinal cord were half-cutted. After washing the wound by Penicillin, skin tissues were primarily sutured according to anatomic layers. Artificial urination was proceeded twice a day until micturition reflex recovery. The right hindlimb paralysis was considered as the model succeed.

2.4. BMSCs culture and transplantation

BMSCs were isolated from the bone marrow of tibias and femurs of 8-week old Sprague Dawley rats. Cell were harvested by trypsinisation and plated in six-well plates in 2 mL DMEM with high glucose, 10% FBS. The medium was changed after 24 h and cells were passaged for another 48 h. Fresh medium was changed every 48 h. BMSCs of passage 4 were used for transplantation.

Cell transplantation was proceeded 6 h post SCI. 10 μM BrdU were added into the BMSCs culture to mark the cell. The focal area was exposed before the cell transplantation. A microsyringe contained 10 μL cells (1×10^{10}/L) was lowered into the upper region central of the injured site. Needle retention should be operated for at least 5 min after the injection which was finished within 3 min.

2.5. Hyperbaric oxygen treatment procedure

Animals received HBO therapy in an animal monoplace chamber. Before pressurization, 100% medical oxygen was flushed through the chamber for 10 min to displace ambient air. The oxygen pressure was then increased slowly and reached 2.5 atm. in 5 min. The chamber was ventilated during HBO therapy to keep the oxygen volume fraction around 70%. The chamber was decompressed to normal atmospheric pressure in 10 min. The rats in HBO group received 4 times of treatment a day, 7 d in total.

2.6. Study groups

The experimental adult male Sprague–Dawley (SD) rats weighing 220–250 g were randomly allocated into five study groups (28 rats in each group). The HBO group received a single session of HBO treatment, the BMSC group received cell transplantation therapy; the HBO + BMSC group received both HBO and cell transplantation therapy; the vehicle group received only 0.9% saline and the fifth group was the sham group received only laminectomy.

2.7. Behavioral assessment

The Basso, Beattie, and Bresnahan (BBB) locomotor rating scale was used to assess the overground walking ability of the rats (n = 10/group) [14,15]. This scale measures hindlimb movements starting with a score of 0, indicating no observable movement. Increasing scores are given for movement of individual joints, limb coordination, weight-supported plantar stepping, *etc*., up to a maximum score of 21, which indicates normal movement. Rats were tested by double-blind assessment. Rats were familiarized with the open field and baseline values were determined before surgery. Locomotor functions were scored weekly from the 1st to the 6th week after SCI. The score was obtained by averaging the value of all limbs.

In the inclined plane test, the rats were put on a rubber tray parallel (8 mm thickness) to a flat surface. The unstable tray edge was raised so that the inclined level is increased. The highest angle during which the rats could stay stable for 5 s was the inclined plane angle. Angle was recorded for three times and the mean value was the final stay-time of one rat. Inclined plane test were scored weekly from the 1st to the 6th week after SCI.

2.8. Electron microscopy

6 weeks after the SCI surgery, randomly sacrificed 3 rats by heart perfusion with 25 g/L glutaraldehyde from each experimental group. Then the tissue were fixed in glutaraldehyde overnight and the spinal cord segment from 1 cm rostral to 1 cm caudal of the injury epicenter (2 cm total length) was harvested and post-fixed in the same fixative solution for 2 h. Then the samples were subjected to gradient dehydration by acetone and dyed by uranyl acetate for 4 h in 4 °C. The samples were embedded in epoxy resin 618 and observed using transmission electron microscopy.

2.9. HE staining and immunohistochemistry

6 weeks after the SCI surgery, randomly sacrificed 3 rats to determine the focal injury by immunohistochemistry. HE staining and BrdU antibody were used on tissue slice. Randomly chose 10 fields of vision under 200 × lens. Calculate $BrduU^+$ cell from every fields. The mean value was the final $BrduU^+$ cell number of one rat.