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IMPROVED DIFFERENTIATION OF OLIGODENDROCYTE PRECURSOR CELLS AND NEUROLOGICAL FUNCTION AFTER SPINAL CORD INJURY IN RATS BY OSCILLATING FIELD STIMULATION

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Abstract—Oscillating field stimulation (OFS) has been used in attempts to treat spinal cord injury (SCI) and has been shown to improve remyelination after SCI in rats. However, some controversies regarding the effects of OFS have been presented in previous papers. Oligodendrocytes (OLs) are the main cell for remyelination and are derived from the differentiation of oligodendrocyte precursor cells (OPCs). To date, it has been unclear whether the differentiation of OPCs can be regulated by OFS. The goal of this study was to determine if OFS can improve the differentiation of OPCs and promote the recovery of neurological function after SCI in rats. Immature and mature OLs were observed in spinal cord slices through immunofluorescence staining. Levels of adenosine triphosphate (ATP) and the cytokine leukemia inhibitory factor (LIF) were detected by enzyme-linked immunosorbent assay (ELISA). Basso–Beattie–Bresnahan (BBB) scores and transcranial magnetic motor-evoked potentials (tcMMEPs) were used to evaluate the locomotor outcomes of rats after SCI. Our results showed a significant improvement in the differentiation of OPCs and the content of ATP and LIF in the injured spinal cord in the OFS group. Furthermore, BBB scores and tcMMEPs were significantly improved in the rats stimulated by OFS. These findings suggest that OFS can improve the differentiation of OPCs and promote the recovery of neurological function following SCI in rats. **© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: oscillating field stimulation, oligodendrocyte progenitor cell, ATP, LIF, differentiation, spinal cord injury.

INTRODUCTION

In the central nervous system (CNS), traumatic spinal cord injury (SCI) is a catastrophic injury. Treatments to improve neurological function after SCI still present some obstacles. The treatment strategies include both non-invasive therapies and invasive methods, such as methylprednisolone, nerve growth factor, stem cells, decompression of the spinal cord and electrical stimulation (Oliver et al., 2012; Fitzharris et al., 2014).

One of the major purposes of these treatments is focused on changing the microenvironment of the injured spinal cord for axonal regeneration and remyelination after SCI (Falnikar et al., 2014). Remyelination plays a very important role in the reconnection of neural pathways following SCI (Falnikar et al., 2014). Remyelination could be regulated by endogenous and exogenous factors (Whittaker et al., 2012). In previous studies, electrical stimulation has been shown to enhance remyelination and to lead to the recovery of motor function in CNS injury (Becker et al., 2010; Li et al., 2010). As an exogenous factor, electrical stimulation was considered a promising method for the treatment of SCI (Becker et al., 2010; Beaumont et al., 2014). However, some limitations in the effects of electrical stimulation on nerve regeneration have been proposed by different authors (McCaig, 1987; Fehlings et al., 1988). These limitations could be eliminated if Oscillating field stimulation (OFS) was applied instead of electrical stimulation for nerve regeneration (Borgens et al., 1999). OFS has been used to treat SCI in clinical trials, and positive results have been reported (Shapiro et al., 2005). However, some researchers questioned the results because methylprednisolone was used in the trial (Hamid and Hayek, 2008).

Oligodendrocytes (OLs) are the main cell for remyelination and are derived from the differentiation of oligodendrocyte precursor cells (OPCs) (Dusart et al., 1992). OPCs can differentiate into OLs in a suitable microenvironment in the spinal cord (Islam et al., 2009). Our previous study and other studies have shown that remyelination could be improved by OFS after SCI in rats (Zhang et al., 2014). However, to date, it has been unclear whether the differentiation of OPCs in the process of remyelination can be regulated by OFS.

In this study, the goal was to investigate if OFS can improve the differentiation of OPCs into OLs and promote the recovery of neurological function after SCI in rats.

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Abbreviations: ATP, adenosine triphosphate; ANOVA, analysis of variance; BBB, Basso–Beattie–Bresnahan; CNS, central nervous system; ELISA, enzyme-linked immunosorbent assay; LIF, leukemia inhibitory factor; OFS, Oscillating field stimulation; OLs, Oligodendrocytes; OPCs, oligodendrocyte precursor cells; SCI, spinal cord injury; tcMMEPs, transcranial magnetic motor-evoked potentials.

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EXPERIMENTAL PROCEDURES

Study design

All animal care and surgical interventions were approved by the ethics board of the second hospital of AnHui Medical University. Efforts were made to control the number of animals used and the amount of distress. A total of 60 female Sprague–Dawley rats obtained from AnHui Medical University were used. Rats were approximately 8 weeks old and weighed between 220 and 240 g. Rats were randomly divided into three groups. Group 1 was the sham group; group 2 was the SCI group, which experienced the implanted stimulator alone rather than the oscillation of the electric field; and group 3 was the OFS group. The rats in the OFS group all received the oscillating electric field as the intervention.

The following items were examined: (1) immunofluorescence staining of the spinal cord on the 4th, 7th, 10th, and 14th days after the surgery; (2) protein quantification in the spinal cord by ELISA 4, 6, and 8 weeks after surgery; and (3) the Basso–Beattie–Bresnahan (BBB) score and transcranial magnetic motor-evoked potentials (tcMMEPs) of each rat every week after the surgery.

Animal surgery

The rats were anesthetized with Nembutal (50 mg/kg, i.p.). Once an appropriate level of anesthesia was achieved, the skin above and below the injury site was shaved and disinfected. The rats received a dorsal laminectomy at the level of the T9–10 thoracic vertebrae to expose the spinal cord, and then an injury was produced by allowing the 10-g NYU impactor (New York University, USA) to drop from a height of 5 cm. Following injury of the spinal cord, the two electrodes of the OFS stimulating device (Institute of Electrical Engineering, CHN, Bejing, China) were implanted close to the lamina and spinous at the level of the T8 and T11 vertebrae. The two stimulator electrodes were sutured close to the lamina and spinous at the level of T8 and T11 vertebrae with 3–0 silk thread. The OFS stimulating device was placed in vitro. The sham group received only a laminectomy. Group 2 and group 3 both experienced the implanted stimulator, but only group 3 received electrical stimulation as an intervention.

The stimulator electrodes were made of an alloy of platinum and iridium, and it's size was 0.3 mm in diameter and 2 cm in length. The power of the stimulator electrodes was supplied by a 3.0-V battery and the output current of the stimulator electrodes was 40 μA. The electric field intensity between the two stimulator electrodes was 400 μV/mm and the polarity of the electric field alternated every 15 min. The power supply was an inductive system, and the rats were stimulated when they returned to a conscious state after surgery (Fig. 1). The power supply was interrupted when the rats were sacrificed.

To prevent infection, the rats were treated with 10^5 units/kg penicillin G once daily after surgery for 3 days. Bladders were squeezed twice daily (morning and evening) until the rats had empty bladders at the times of squeezing.

Immunofluorescence and microscopy

Spinal cord sections were cut with a freezing microtome at 10 μm thick from the lesion epicenter to the caudal side (CM1850, Leica Microsciences, Mannheim, Germany). The sections were then fixed in ice-cold 4% paraformaldehyde (catalog number 30525, Sigma, St Louis, MO, USA) for 15 min, permeabilized with 1% Triton X-100 (Sigma, St Louis, MO, USA) and blocked in 10% BAS (Biosharp, CHN, Bejing, China) for 1 h. The sections were then incubated with primary antibodies (MBP (1:200; ab40390) and Galc (1:200; ab83752)) overnight at 4 °C. After washing, the sections were incubated with Alexa Fluor 594 donkey anti-sheep IgG (1:500; catalog number: ab150180, Abcam, Cambridge, Massachusetts, USA), Alexa Fluor 488 goat anti-rabbit IgG (1:500; catalog number: ab150077, Abcam, Cambridge, Massachusetts, USA), and DyLight 649 goat anti-mouse IgG (1:500; catalog number: E032610, Abbkine, CA, USA) for 1 h in the dark at room temperature. The sections were examined with an Olympus FV-1000 confocal microscope, and the images were analyzed with Image-5 (Carl Zeiss, Jena, Germany). Cell counts were calculated within a 1-mm diameter region in the DG, and the mean number of cells was evaluated for 10 sections. The number of positively stained cells labeled with Galc and MBP were calculated.

ELISA assessment of ATP and LIF

ELISA was performed to detect the expression of adenosine triphosphate (ATP) and leukemia inhibitory factor (LIF). A 1.5-cm segment of the spinal cord (0.5 cm above the injury site and 1.0 cm below the injury site) was extracted and weighed. The supernatant of the extracted spinal cord homogenate was collected, and protein concentrations were detected with the BCA protein assay (R&D Systems, Minneapolis, USA). After protein concentrations were calculated with the BCA protein assay, the expression of LIF and ATP in the extracted spinal cord was detected by the ELISA assay.

Behavioral assessment of locomotion

Every week after surgery, open-field locomotor behavior was evaluated in each animal using the BBB locomotor rating scale (Basso and Nichelli, 1995) to investigate the effectiveness of the recovery of neurological function after SCI. The mean BBB scores were assessed and calculated by two blinded investigators.

Electrophysiology

The procedures used to measure tcMMEP responses for the electrophysiological assessment of each rat have been described in detail in previous papers (Beaumont et al., 2006; Cao et al., 2010). Briefly, tcMMEPs were examined preoperatively and every week postoperatively. The tcMMEP system consisted of a pair of Magstim 200

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