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The regulatory effect of electro-acupuncture on the expression of NMDA receptors in a SCI rat model

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ABSTRACT

Background: In early spinal cord injury (SCI), glutamate receptors, including *N*-methyl-D-aspartate (NMDA) receptors (NMDARs), are over-stimulated by excessively released glutamate. The enhanced activity of NMDARs may cause cell death by overloading calcium (Ca^{2+}) into cells based on their high permeability to Ca^{2+}. Studies in SCI animals have shown that treatment with electro-acupuncture (EA) is able to reduce cell death and to improve functional recovery. One possible mechanism of this neuroprotective effect is that EA has regulatory effect on NMDARs.

Aims: To test whether EA could protect the spinal cord after SCI by decreasing the expression levels of NR1 and NR2A.

Main methods: We conducted EA treatment on a rat SCI model produced with a New York University (NYU) Impactor and measured hindlimb locomotor function by Basso, Beattie and Bresnahan Locomotor Rating Scale (BBB Scale). The expression of NR1 and NR2, the subunits of NMDARs, in the injured spinal cord was measured by Immunofluorescence stainings, western blot and real-time quantitative PCR (RT-qPCR).

Key finding: Our results showed that two days after the SCI the expression of NR1 and NR2 were dramatically enhanced at both protein and mNRA levels, which were significantly reduced by EA treatment at two specific acupoints, Dazhui (DU14) and Mingmen (DU4).

Significance: EA is a potential therapeutic method for treating early SCI in human.

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1. Introduction

Spinal cord injury (SCI) is a devastating event that leads to physical, psychological, and social impact on individuals, family, and society [1]. In early SCI, two different phases are involved in the damages of spinal cord. The acute phase causes immediate hemorrhage and rapid cell death due to mechanical impact, and the secondary phase causes further tissue loss and dysfunction due to multiple degenerative processes [2,3]. Despite the significant improvement in the management for the acute SCI in recent years, there remain no effective medications to improve neurological outcomes from the secondary injury due to the complexity of its pathogeneses. Indeed, microcirculation failure [4], oxidative stress [5,6], inflammatory responses [7,8], and glutamate induced excitotoxicity [9,10] are all reported to be involved in the secondary phase of SCI.

N-methyl-D-aspartate receptors (NMDARs), one of three ionotropic glutamate receptors, may play a critical role in cell death in early SCI. Due to the initial mechanics [11,12] and the secondary injuries [13,14], excessive glutamate, an excitatory neurotransmitter, is released into extracellular space, which may over-stimulate NMDRs and result in calcium (Ca^{2+}) overload in neurons through NMDARs due to their high permeability to Ca^{2+}. The overloaded Ca^{2+} may last from hours to weeks after the initial SCI [15] and trigger many pathological changes, including cell death [16–18]. Therefore, controlling the activity of NMDARs is likely a very efficient strategy in SCI therapy. However, concerns exist on the side effects with the use of NMDARs' antagonists [19]. Therefore, continuous effort should be made to find new therapy that is efficient and safe for SCI patients.

Corticosteroids have been used in the treatment of central nervous system (CNS) trauma initially with the purpose of reducing edema. Studies in animal models of SCI have shown that the glucocorticoid, methylprednisolone (MP), can prevent lipid peroxidation [20,21], help maintain blood flow [22], and prevent lactacidosis in CNS tissues [23], leading to long-term functional improvement [5]. MP is proposed to inhibit the inflammatory cascades contributing to secondary spinal cord damage after SCI [24], and MP was a first choice for treating acute SCI worldwide [25].

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Acupuncture is a therapeutic method used in traditional Chinese medicine. Research has demonstrated the beneficial effects of acupuncture on certain pathogenic conditions, including glutamate induced excitotoxicity. In a global ischemia rat model, manual acupuncture (MA) was reported to reduce extracellular glutamate level [26]. The regulatory effect of MA or electro-acupuncture (EA) on NMDARs at protein, mRNA and phosphorylation levels was also reported in animal models with certain medical conditions. Sun et al. indicated both manual acupuncture and EA interventions could improve the learning-memory ability in morphine withdrawal rats, which was probably partially related to their effects in up-regulating the expression of NR2B in the amygdala (AMG) [27]. Li et al. suggested that EA had antidepressive and anxiolytic effects on rats with neuropathic pain and that this might be associated with restoring the phosphorylation of NR1 in the hippocampus [28]. Tian et al. indicated that the EA-mediated attenuation of chronic visceral hypersensitivity was correlated with the down-regulation of NMDA receptors phosphorylation at the spinal level [29]. We thus hypothesized a protective role of EA for early SCI by regulating the expression of NMDARs at translational and transcriptional levels.

2. Materials and methods

2.1. Animal and experimental groups

All experimental procedures, including the surgical injury of spinal cord, were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University. Ninety-six rats (Sprague-Dawley, male, 150 to 180 g, about 6 weeks of age) were randomly assigned into four groups. The sham group ($n = 24$) received only a laminectomy. The remaining three groups underwent the SCI at the T10 spinal segment. The control group ($n = 24$) received no treatment following the SCI, the EA group ($n = 24$) received EA treatment at DU14 and DU4 acupoints, and the methylprednisolone (MP) group ($n = 24$) received MP treatment. All animals were housed in individual cages with free access to food and water. Room temperature was set at 25 $\pm$ 3 °C.

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research.

2.2. Spinal cord injury

Moderate SCI was induced using a New York University Impactor (NYU) [7,30]. The rats were anesthetized *via* intraperitoneal injection of 5% chloral hydrate (500 mg/kg), and a laminectomy was performed at the T10 level (Fig. 1), in which the cord was exposed without disrupting the dura. The spinous processes of T9 and T11 were then clamped to stabilize the spine, and the exposed dorsal surface of the cord was subjected to contusion injury (10 g × 25 mm) using the NYU Impactor with the exception of the sham group. The SCI was evidenced by observing spinal cord ischemia and edema around the wound, tail sway reflex, flicking of both body and legs, and appearance of sluggish paralysis. The wound was then sutured and covered with cotton soaked in normal saline (NS) to avoid direct air exposure of the injured cord. Postoperative care included manual urinary bladder emptying per 12 h and the administration of cefazolin sodium (50 mg/kg, i.p.) for preventing bacterial infection.

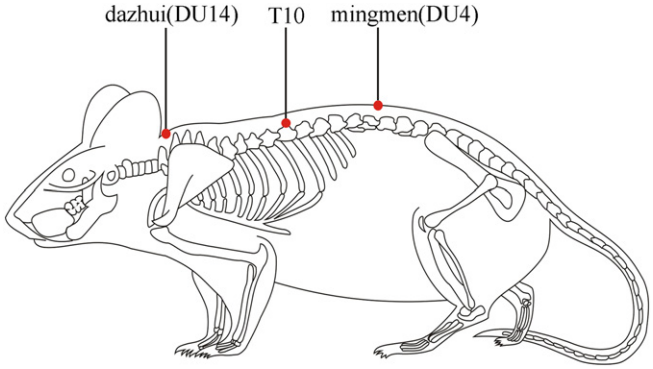

Fig. 1. Schematic diagram indicating the locations of SCI and EA.

2.3. BBB locomotor rating scale

An open field evaluation of locomotor recovery after SCI was conducted using the BBB locomotor rating scale [31]. In SCI after 6 to 12 h, the white matter axons had not changed significantly, and effective treatment in this period could reverse the situation of partial spinal cord injury. According to the characteristics of pathological changes in the acute SCI, early treatment should be performed within 24 h after trauma, and treatment within 6 h post-trauma was regarded as the golden period treatment [32]. In this study, the SD rats were observed in 48 h after spinal cord injury. Therefore, we selected the BBB Scale of rats before SCI and 6, 24, and 48 h after. So we selected the BBB Scale of rats before SCI and 6, 24, and 48 h after. Scoring was performed by three trained technicians who were blinded to treatments, and took the average values. If the left and right lower limb scores were different, took the average.

2.4. EA and MP treatments

The DU14 is located on the posterior midline and in the depression below the spinous process of the seventh cervical vertebra in prone position. DU4 is located on the posterior midline and in the depression below the spinous progress of the second lumber vertebra in prone position (Fig. 1). Rats were kept in an immobilization apparatus designed by our laboratory (Chinese Patent Application Number: 201110021482.5, State Intellectual Property Office) without anesthesia. The system was designed to be both comfortable for the experimental rats and convenient for EA treatment (Fig. 2).

The first acupuncture treatment was applied to the acupoints 30 min after the surgery. Stainless-steel needles of 0.20 mm in diameter were inserted to a depth of 6–8 mm at each acupoint. In the EA group, the needles were connected to an EA apparatus (HANS-200E, Jisheng Medical Instruments) and the acupoints were continuously stimulated at frequency of 2 Hz with current intensities between 0.6 and 0.8 mA for 30 min [33]. The second and third EA treatments were administrated at 12 h and 24 h post-surgery.

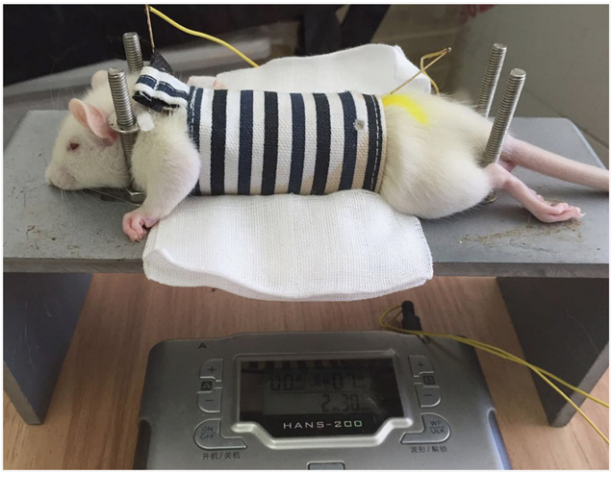

Fig. 2. Experimental settings During treatment, rat was hold in an immobilizing apparatus and two acupoints, DU14 and DU4, were connected to the EA device HANS-200E.

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