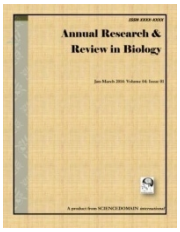

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Ceftriaxone Improves Neuron Protection and Functional Recovery in Rat Model of Spinal Cord Injury

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AB and BHA designed the study. Author AR wrote the first draft of the manuscript and managed the literature searches. Author JT carried out the experiments of the study, wrote and edited all subsequent drafts of the manuscript. Author SM managed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To determine the efficacy of ceftriaxone in improvement of neuron protection and functional recovery of spinal cord injury (SCI) in rat model.
Study Design: This study was designed to evaluate the effect of ceftriaxone on neuron protection in rat model of SCI. Rats were randomly divided into four different experimental groups.
Place and Duration of Study: Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran, between December 2011 through December 2013.

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Methodology: Rats (age, 10 weeks; weight, 165–245 ± 5 g) were randomly divided into four groups of ten (n=40): ceftriaxone before SCI, normal saline before SCI, ceftriaxone after SCI, and normal saline after SCI. SCI was performed on animals under general anesthesia using the weight dropping method. Ceftriaxone was injected intraperitoneally in rats at a dose rate of 200 mg/kg/day for seven days, before and after SCI. Hind limb motor function was assessed using the Basso, Beattie and Bresnahan (BBB) scale. CST axons were traced by injection of biotin dextran amine (BDA), into the sensorimotor cortex.
Result: Our findings showed that ceftriaxone improved functional recovery of SCI in the animal model. Based on the obtained results, there was a statistically significant difference in BBB scores, between groups that received ceftriaxone before and after SCI and control groups. At the same time, significant differences were also observed in axon counting of above mentioned groups.
Conclusion: With attention to increasing demand for innovation of efficient and at the same time cost benefit procedures to improve spinal cord injury, present study seems to be able to open a new way to achieve this goal. No doubt it is still on its experimental model and need further work to validate reliability.

Keywords: Spinal cord injury; ceftriaxone; animal model; axon; recovery.

1. INTRODUCTION

With an approximate rate of 10,000 new cases reported each year, spinal cord injury (SCI) is one of the leading causes of disability in the United States [1-2]. During the last two decades, an obvious evidence for extensive inflammation following SCI has resulted in clinical application of anti-inflammatory agents such as methylprednisolone [3]. However, despite the initial optimism, methylprednisolone has not demonstrated clinical efficacy [4]. Furthermore, a great deal of controversy still exists regarding whether SCI status as an immunologically privileged location limits SCI innate immunity and whether this must be included as a neuroprotective strategy in SCI therapy [5]. Nevertheless, the post-traumatic inflammatory reaction is appropriate to significantly contribute to the secondary injury after SCI. As a result, inflammatory mediators such as cytokines, proteases, and reactive oxygen species can contribute to the activation of apoptosis executioners such as caspases, resulting in neuronal loss that can eventually end in permanent neurological deficit [6]. Following SCI, microglia are activated [7], which may release neurotoxic molecules that can cause additional damage to the neurons nearby [8]. However, microglial involvement in CNS (central nerve system) injury and regeneration is controversial [9]. Several novel important concepts of secondary injury have been recently proposed. Poisonous chemicals released by axons, damaged cells, and blood vessels attack intact neighboring cells. One neurotransmitter, i.e., glutamate, plays a critical role in an excessively disruptive process named excitotoxicity [10]. Glutamate receptors known as AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors play a significant role in oligodendrocyte injury. Glutamate transport is the only known mechanism of extracellular glutamate clearance, and glutamate transporter 1 (GLT-1) is the major glutamate transporter of the mammalian brain. Ceftriaxone has recently been discovered to up-regulate GLT-1 expression in CNS through increasing *GLT-1* transcription [11]. Thus, our study investigated whether pre-treatment with ceftriaxone impedes development of neuronal damage and eventual cell death. In addition, daily intraperitonal treatment with ceftriaxone was investigated for its effect on the improvement of ambulatory ability and prevention of paralysis in rat models of SCI.

2. MATERIALS AND METHODS

2.1 Animal Models

Prior to initiation of the experiment, permission was obtained from the ethical committee of Tabriz University of Medical Science. Experiments were conducted on female Sprague-Dawley rats (age, 10 weeks; weight, 165–245 ± 5 g) that were procured from the animal colony of the Institute. Animal experiments conform to institutional standards. Rats were anesthetized with a combination of intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg).The animals were randomly divided into four groups of ten, and SCI was performed at T10 using the weight dropping method in which from a 5 cm distance, a 10 g metal rod was dropped on laminectomised area under general anesthesia. Transverse section of rat spinal cord has been shown in Fig. 1. Ceftriaxone was injected at a dose of 200 mg/kg/day in normal saline for seven days before and after injury. Group one included rats receiving ceftriaxone seven days before the injury, group two received normal saline for seven days before the injury, group three received ceftriaxone for seven days after the injury, group four received normal saline for seven days after the injury. A Basso, Beattie and Bresnahan (BBB) score test [12] was performed for six weeks. Two weeks before the end of BBB, biotin dextran amine (BDA) was injected intracerebrally, and tissue staining was performed at the end of the sixth week.

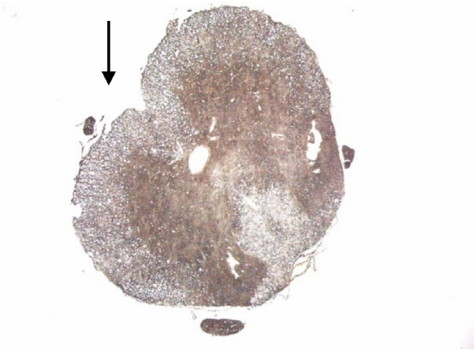

Fig. 1. Transverse section of rat spinal cord (× 10). The lesion site was indicated by arrow

2.2 Drugs

Ceftriaxone, dissolved in sterile endotoxin-free 0.9% normal saline at 60 mg/mL and stored at 4°C. The ceftriaxone solution was injected intraperitoneally in groups one and three. For intraperitonal injections, the 60-µg/µL ceftriaxone stock was diluted with saline to 30 µg/µL and 5 µL of the diluted ceftriaxone was injected at a final dose of 150 µg (227 nmol).

2.3 Locomotor Function Evaluation

Hind limb motor function was assessed based on the BBB scale every week after the injury. The BBB locomotor rating scale is a 21-point scale from 0 (no detectable movement) to 21 (consistent plantar stepping and coordinated gait) [12]. For BBB assessment, rats were allowed to move individually for 5 min on a smooth, nonslip floor in an open field (200 × 100

cm). For each rat, hind limb motor function was scored from 0 to 21 based on the performance of the hind limb by two observers, who were blinded to the groups.

2.4 BDA Detection

Two weeks before the end of BBB, BDA was injected intracerebrally by creating a hole situated 2 mm posterior and 2 mm right to the Bragma using Stereotax, and tissue staining was performed at the end of the sixth week. Sections were washed in PBS and 0.1% Triton X-100, incubated for 1 hour with avidin and biotinylated HRP (Neuro Trace™ BDA-10,000 Neuronal Tracer Kit (N-7167)), washed in PBS, and then reacted with DAB (diamino benzidine) in 50 mM Tris buffer pH 7.6, 0.024% hydrogen peroxide and 0.5% nickel chloride. Cross-sections from the rostral-most block were used to determine the extent of corticospinal (CST) labeling above the lesion and the number of BDA-labeled axon arbors that enter the gray matter of the thoracic spinal cord about ten thoracic vertebra (T10). The axon counted by The Cell software (imaging software for life science microscopy) attached to the light microscopy (Olympus Bx52, Japan).

2.5 Statistical Analysis

Data was expressed as means ± S.D; statistical computations were calculated using SPSS 10 for windows software (SPSS Inc, Chicago**,** IL, USA). Sample size (n) was n = 10 rats for each group. The results obtained from the four groups were analyzed by ANOVA and Student's t-test and further by least significant difference (LSD). Significant differences were considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

Following pretreatment with ceftriaxone (200 mg/kg/day), the obtained results through hind limb motor function (BBB score),observed by two blind observers, indicated that pretreatment with ceftriaxone (200 mg/kg/day), for seven days every 24 h before SCI, improves motor function of rats, whereas no improvement was observed in rats receiving normal saline (Fig. 2). The recovery rate rose over a period of a single week. As it is shown in Fig. 4, there is a significant recovery rate in ceftriaxone-treated animals (group one) in comparison with control groups (group two) ($P<0.03$), which shows an ascending increasing recovery rate in subsequent weeks (first week 0.3±0.15, second week 2.25±0.67, third week 5.71±1.63, fourth week 5.5±2.20, fifth week 8.3 ± 2.88 and the sixth week 8.16±2.46).

Following treatment with Ceftriaxone (200 mg/kg/day/ip) for seven days on hind limb motor function, which was done after the spinal cord injury, the recovery rate rose over a period of a single week (Fig. 3). As it is shown in Fig. 4, there is a significant recovery rate in ceftriaxone-treated animals (group three) in comparison with control groups (group four) ($P<0.001$), which shows an ascending increasing recovery rate in subsequent weeks (first week 0.6±0.16, second week 1.6±0.41, third week 5.6±1.48, fourth week 8.1±2.12, fifth week 8±2.42 and the sixth week 8.3±2.23).

Following ceftriaxone (200 mg/kg/day/ip) administration for seven days on axonal regeneration, as it is shown in Fig. 5, obtained results showed significant axonal regeneration in experimental and control groups ($P < 0.001$). Our results showed an important role of ceftriaxone for the regulation of axonal growth (Fig. 6).

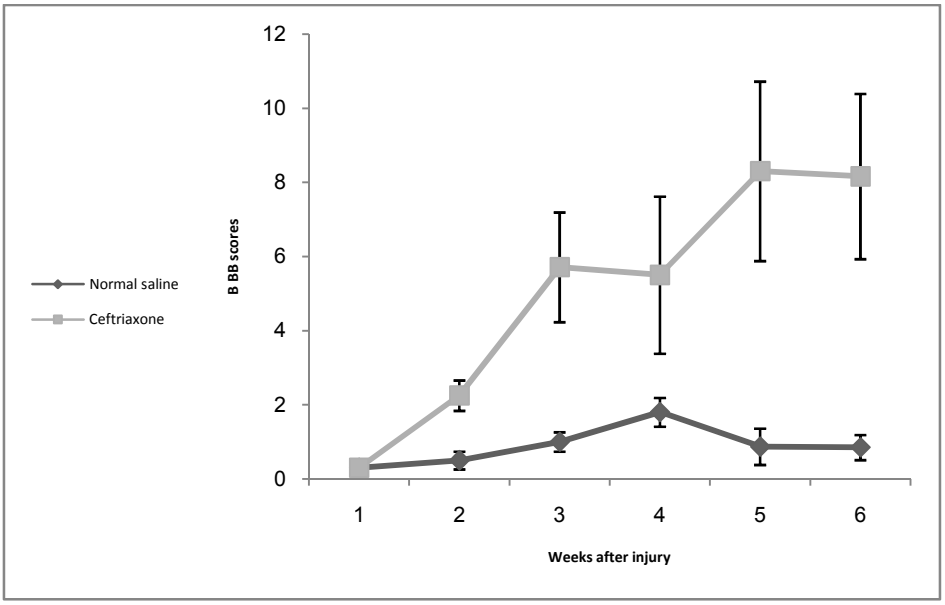

Fig. 2. Hind limb locomotor function as measured by the BBB score before SCI in group 1 (Ceftriaxone) in comparison with group 2 (Normal saline)

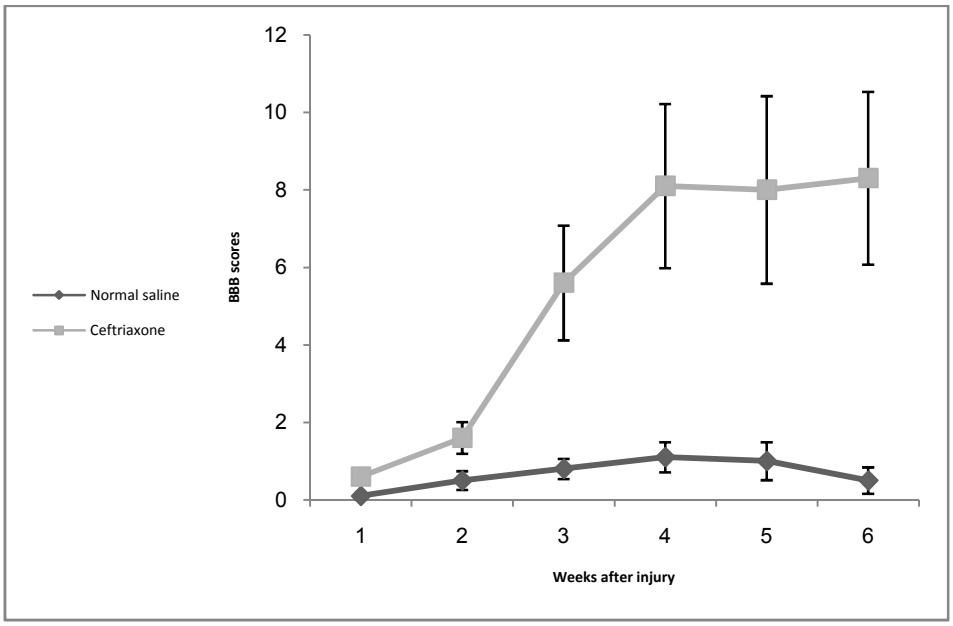

Fig. 3. Hind limb locomotor function as measured by the BBB score before SCI in group 3 (Ceftriaxone) in comparison with group 4 (Normal saline)

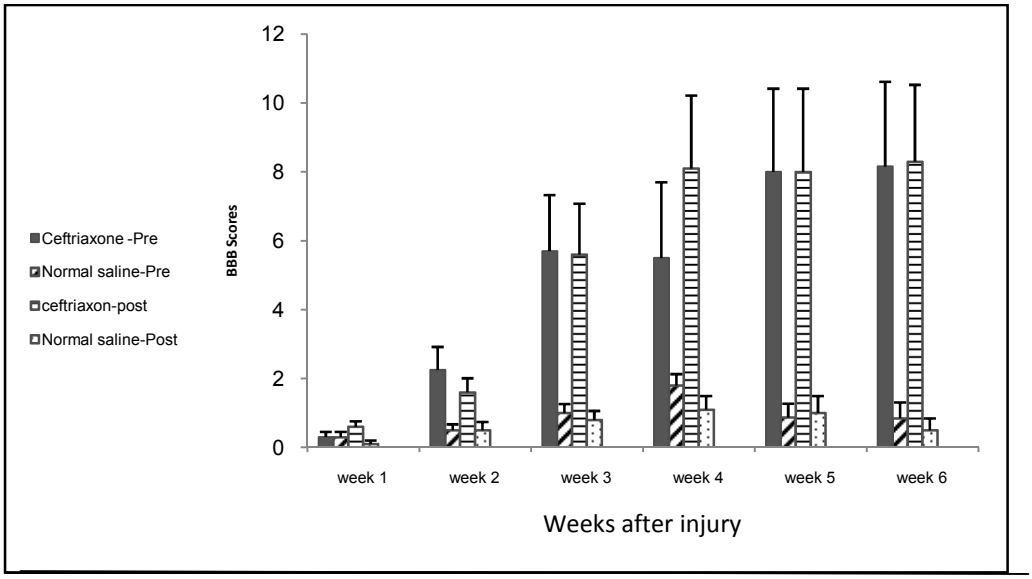

Fig. 4. Repeated measurements between groups

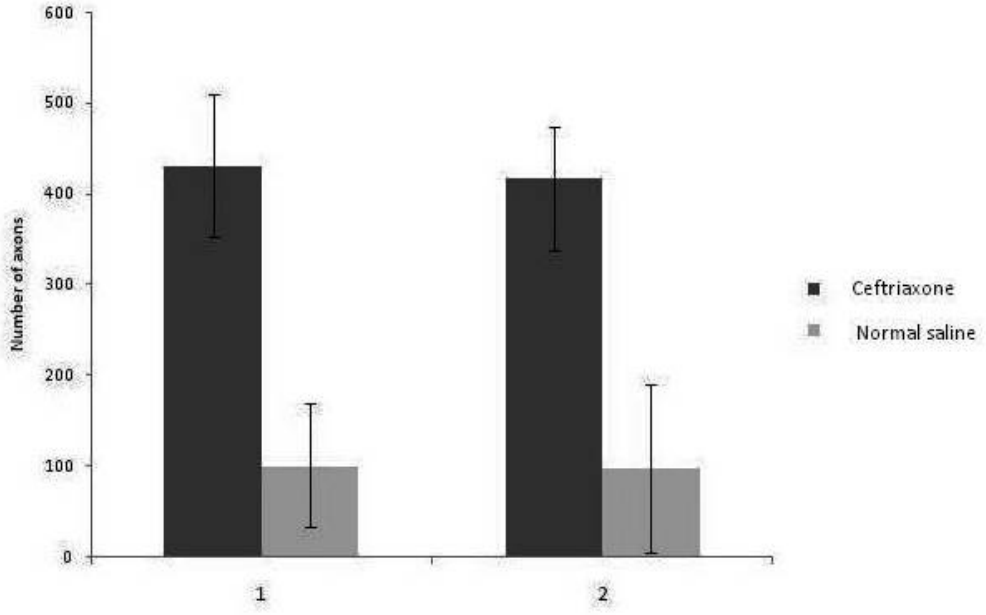

Fig. 5. Number of axons in different experimental groups before (1) and after (2) SCI. As shown in figure there is significant differences between treated and untreated group before and after SCI

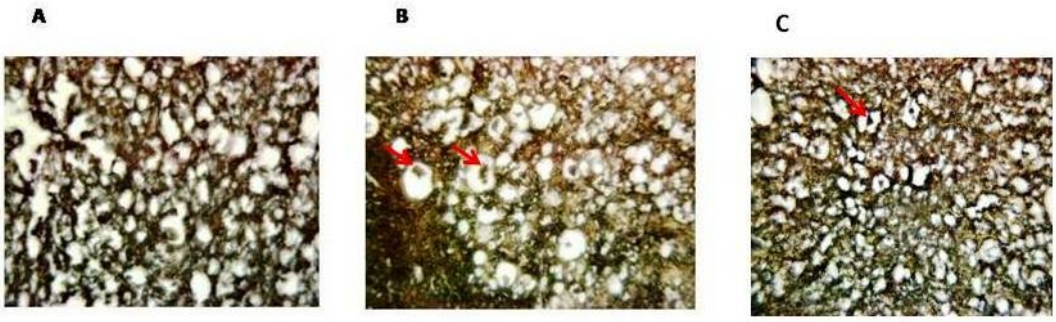

Fig. 6. Distribution and density of CST axons (indicated by red arrows) by light microscopy (× 40) in a PBS injected control (A), ceftriaxone treated before SCI (B) and ceftriaxone treated after SCI (C)

In the present study, we attempted to achieve the aims of the study by focusing on the secondary injury phase of SCI. This phase of SCI has its roots in some auto destructive events such as reactive oxygen species-induced lipid peroxidation [13], caspase-3 activation [14-15], and glutamate production. Results of previous studies in animals suggest that compounds that can protect cells from excess glutamate can limit SC destruction [16]. It is also believed that the injury cascade of neurodestructive events will extend when secondary injury increases owing to delayed treatment [3]. The first scientifically grounded pharmacological treatment for SCI dates back to the 1990s. Although results of a clinical study showed that a high dose of the steroid methylprednisolone could decrease disability when administered within 8h of trauma [17], treatment with a high dose of methylprednisolone was later reported to be associated with complications, including wound infection and increased frequency of gastric bleeding, thereby methylprednisolone treatment remains controversial in many countries [18-20]. Furthermore, although treatment with this drug might result in the reduction of swelling, inflammation, glutamate release, and free-radical accumulation, its specific mechanism of action remains unclear [19]. In a similar study, experimental drugs including monosialoganglioside sodium (GM-1 ganglioside), naloxone, and tirilazad were tested in multicentre clinical trials, but the desired results were not achieved [21]. However, significant improvement in functional recovery (BBB) of SCI was reported in another study in which minocycline was administered early (0.5–24h) [22]. The results of another similar study suggested that drugs that impede AMPA-type glutamate receptors turn out to be efficacious in keeping lesions and disability to a minimum level [23]. Specific AMPA-receptor antagonists have also been tested in patients with SCI in recent years [16]. A large number of studies assert that glutamate and its structural analogues could have both short and long-term poisonous impacts on cortical and motor neurons [24-26]. The exposure of neurons to abnormally high concentrations of glutamate results from defective clearance of glutamate from the extracellular space [16]. Glutamate neurotransmission is greatly regulated, mainly via glutamate transporters. The glutamate transporter GLT-1 is principally responsible for glutamate clearance in the spinal cord. Downregulation of GLT-1 can happen in activated astrocytes, and is associated with increased extracellular glutamate and neuroexcitation. During other conditions, astrocyte activation occurs subsequent spinal cord destruction. Recently glutamate transporters have emerged as a potential therapeutic target in a wide range of acute and chronic neurological disorders, owing to their novel mode of action. The modulation of GLT-1, a main glutamate transporter, has been revealed to have neuroprotection in different models of ischemic injury and motoneuron degeneration [27]. Therefore, an attempt was made to explore its neuroprotective potential in spinal cord injury

using ceftriaxone, a GLT-1 modulator. In the present study pre-treatment or (early after SCI) with ceftriaxone (200 mg/kg. i.p) for seven days reulted significant difference in BBB scores between groups that received ceftriaxone before and after SCI compared with the control group. Furthermore, in the present study data indicates that pre-treatment before SCI with ceftriaxone (200 mg/kg. i.p) for seven days causes axons regenerating in experimental groups. In present study it was shown that pre-treatment with intraperitoneal ceftriaxone impedes development of neuronal damage and eventual cell death and improves motor functions. In addition, daily intraperitonal treatment with ceftriaxone also improves ambulatory ability and prevention of paralysis in rat models of SCI and improves motor functions. High serum concentration of ceftriaxone causes higher penetration through the inflamed blood-brain barrier. Higher antibiotic penetration correlated with the extent of systemic inflammatory response. Ceftriaxone increases both brain expression of GLT-1 and its biochemical and functional activity *In vivo* and is neuroprotective *In vitro* in models of ischemic injury and motor neuron degeneration, based in part on protection from glutamate toxicity [28]. These results probably are related to the effect of ceftriaxone that upregulates GLT-1 expression, hence it could prevent development of neuronal damage and prevention of paralysis.

4. CONCLUSION

In conclusion our study show that pre-treatment with intraperitoneal ceftriaxone impedes development of neuronal damage and eventual cell death and improves motor functions in rat model of SCI. In addition, daily intraperitonal treatment with ceftriaxone also improvement of ambulatory ability and prevention of paralysis in rat models of SCI and improves motor functions.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethics committee of Tabriz University of Medical Sciences.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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