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The effect of methylprednisolone intravenous infusion on the expression of ciliary neurotrophic factor in a rat spinal cord injury model

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Abstract

BACKGROUND CONTEXT: Methylprednisolone (MP) infusion after acute spinal cord injury (SCI) remains controversial despite large randomized studies, including the National Acute Spinal Cord Injury Studies (NASCIS).

PURPOSE: To determine the effect of NASCIS protocol MP infusion on the expression of ciliary neurotrophic factor (CNTF), a neuroprotective cytokine, in a rat model after SCI.

STUDY DESIGN: Animal laboratory study.

METHODS: Thirty rats were randomized into an MP infusion group (intravenous [IV]-MP) versus normal saline (NS) control group (IV-NS) after a standardized SCI. Ciliary neurotrophic factor expression was measured by reverse transcription-polymerase chain reaction at 6, 12, 24, 48, and 72 hours post-SCI.

RESULTS: Mean CNTF expression was diminished in the MP group at 12 ($p=.006$) and 24 ($p=.008$) hours postinjury compared with the control group. Expression of CNTF was not significantly different between the groups at 6, 48, and 72 hours post-SCI.

CONCLUSIONS: Standardized MP infusion post-SCI reduces CNTF activation in a rat SCI model. Further study is needed to determine if this effect is seen in human SCIs.

Keywords: Spinal cord injury; Steroid; Cytokine; Ciliary neurotrophic factor

Introduction

Cellular damage after a traumatic spinal cord injury (SCI) occurs in two stages. The initial mechanical disruption is followed by an inflammatory response and cytokine cascade resulting in apoptosis of neurons and their supportive cells [1]. Methylprednisolone (MP) is a potent anti-inflammatory agent. The use of intravenous (IV) MP for patients with acute SCI arose in part from the theoretical benefit that it could inhibit the postinjury inflammatory apoptotic cascade [2–8]. The National Acute Spinal Cord Injury Studies (NASCIS) established a high-dose MP bolus/infusion protocol of 30 mg/kg IV bolus followed by 5.4 mg/kg/h IV infusion for 23 hours initiated within 3 hours of an acute SCI [9]. Although the protocol remains controversial, many emergency centers continue to administer the NASCIS high-dose IV-MP bolus/infusion to patients presenting before 3 hours (24 hours of infusion) or between 3 and 8 hours (48 hours of infusion) of acute SCI [10].

Basic science research has demonstrated that the secondary inflammatory response after acute SCI includes upregulation of several cytokines that have demonstrated neuroprotective and reparative effects [11]. Ciliary neurotrophic factor (CNTF) is one of these protective cytokines [12–17]. Ciliary neurotrophic factor expression and concentration has been shown to increase in the spinal cord in the setting of an acute SCI [18,19]. We hypothesized that administration of a rat dose equivalent of the NASCIS high-dose MP infusion protocol would inhibit the expression of the neuroprotective cytokine CNTF in a rat SCI model.

FDA device/drug status: Not applicable.

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Materials and methods

All animal care and surgical interventions were performed in strict accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Research Council, 1996), and with the approval of The University of North Carolina Institutional Animal Care and Use Committee.

Thirty-three Long-Evans hooded adult rats aged 77±5 days and weighing between 400 and 500 g were subjected to equivalent SCIs using the Multicenter Animal Spinal Cord Injury Study (MASCIS) impactor and protocol [20]. Isoflurane was used for the induction and maintenance of anesthesia. Depth of anesthesia was monitored and adjusted to achieve a tail-pinch response of the intercostal muscles without movement of the extremities. The animals were weighed, and a 50 mg/kg subcutaneous injection of cefazolin was administered for infection prophylaxis. The animals were prepared and draped in sterile fashion, and sterility was maintained throughout each procedure. A dorsal longitudinal incision was made exposing approximately T8–T12. The posterior elements of T10 and T9 (caudal half) were removed with microrongeurs. The rat was suspended from the spinous processes of T8 and T11 and positioned under the impactor. The MASCIS impactor allowed for a precise delivery of a 10 g rod of 25.0 mm to the exposed spinal cord. Optical potentiometers, an electrical circuit between the rod and the animal, and a computer measured the impact velocity, cord compression distance, compression rate, and compression time. Measurement of these parameters provided confirmation of impaction precision. After impaction, the wounds were sutured closed, and the animals were allowed to recover from anesthesia.

All the animals were procured from the supplier (Charles River Laboratories, Wilmington, MA, USA) with indwelling internal jugular catheters. On completion of the SCI, three rats were sacrificed immediately to serve as a baseline control. The remaining 30 rats were randomized to either an experimental group that received the rat dose equivalent of the NASCIS MP-IV infusion or a control group that received an equivalent volume of IV normal saline (NS). The initial bolus dose was administered 10 minutes after impaction through an infusion harness attached to the internal jugular catheter. The infusion harness was then attached to a spring catheter and a swivel that allowed the animal to have freedom of movement to obtain food and water in its enclosure while maintaining the continuous IV infusion. Each animal underwent manual bladder expression every 8 hours until sacrifice. Three control and three experimental animals were sacrificed at 6, 12, 24, 48, and 72 hours after injury. Guillotine decapitation immediately after isoflurane induction was used for the sacrifice, and the spinal cords were quickly procured in a cooled environment. A 1-cm section of spinal cord centered on the injury hematoma was obtained (Fig. 1) and placed immediately in RNAlater (Qiagen, Valencia, CA, USA) solution cooled with dry ice. The samples were then delivered to a blinded investigator (RDG) who extracted the mRNA and performed reverse transcription-polymerase chain reaction for the expression of CNTF and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase for each sample.

The relative expression of CNTF for each sample was quantified by normalizing the data to the expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase as per a protocol described by Shintani et al. [21]. The mean relative expression of CNTF was calculated for each time point in the control and experimental groups. The two-tailed Student *t* test was used to determine the statistical significance between the means of CNTF expression at each time point between control (IV-NS) and experimental (IV-MP) groups.

Results

All spinal cord impaction measurements were within 3% of the expected as measured and recorded by the MASCIS impactor. One rat randomized to the experimental group/6-hour time point died about 45 minutes after the surgery, probably from hypothermia when the heating element in the postprocedure recovery cage malfunctioned. A second rat in the control group/48-hour time point developed a wound infection and was euthanized. Data from these animals were not used.

At 12 hours after injury, the mean relative expression of CNTF in the control (IV-NS) group increased more than

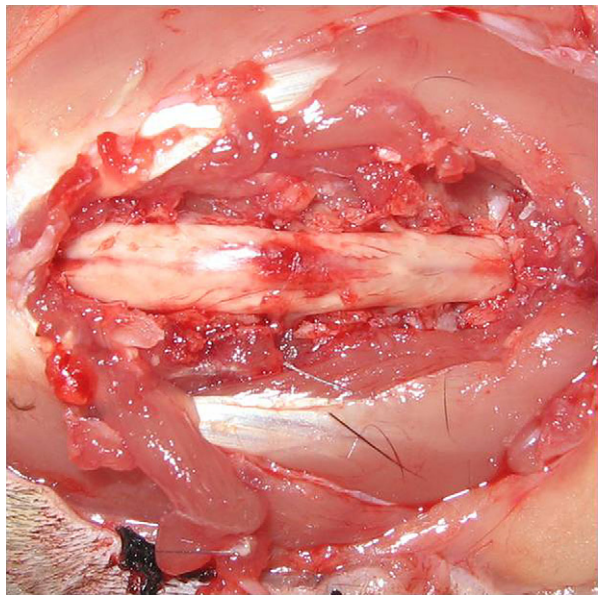

Fig. 1. A 1-cm section of spinal cord centered on the hematoma was obtained by rapid laminectomy in a cooled environment and placed into RNAlater solution.