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Limited minocycline neuroprotection after balloon-compression spinal cord injury in the rat

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Abstract

Minocycline (MC), a second-generation tetracycline and anti-inflammatory agent reportedly provides neuroprotection following CNS injury. The objective of this study was to examine the neuroprotective effects of short and long-term MC treatment using balloon-compression spinal cord injury (SCI) in the rat. Rats subjected to SCI were treated with MC for 1 day (1DMC group; total dose 180 mg/kg) or 5 days (5DMC group; total dose 450 mg/kg) or placebo. The effects of MC treatment on locomotor recovery (BBB scale) and spinal cord white and gray matter sparing were evaluated for up to 28 days. Morphometric analysis showed that while MC treatment spared spinal cord white and gray matter rostral to the lesion epicenter in both, 1DMC and 5DMC groups, sparing of white and gray matter areas was not observed caudal to the traumatic lesion. In addition, MC treatment had no effect on final locomotor recovery. Limited improvement of spinal cord post-compression consequences raises questions about the neuroprotection efficiency of MC treatment following compression SCI in the rat.

Keywords: Secondary injury; Neurological outcome; Spinal cord white/gray matter

Minocycline is widely used for its neuroprotective effects in animal models of central nervous system injury and several neurodegenerative diseases. Although commonly used as a semisynthetic second-generation tetracycline derivative with antimicrobial action, MC has a broad range of effects affecting CNS injury. It has anti-inflammatory [1,30] and anti-apoptic properties [4,12] that underlie its neuroprotective effects. CNS trauma studies showed that MC decreases secondary tissue injury and neurological deficits following traumatic brain injury [3,16] and improves functional recovery by affecting cytokine expression after clip compression SCI in mice [28]. In rats, MC reduced lesion size and improved functional outcome after contusion SCI [8,12] and dorsal column transection [19]. Improvement of functional recovery and diminishing of traumatic lesion size following SCI is associated with reducing microgliosis and apoptosis [8,19,31] and inhibition of cytochrome c release from mitochondria [22]. MC also had modulating effects on microglia in chronic pain studies using contusion model of spinal cord injury [10]. In tissue culture, MC protects neurons from glutamate excitotoxicity [23]. Thus, it is clear that the action of MC targets the multiple processes involved in secondary tissue injury.

The rat balloon-compression spinal cord injury model, introduced by our lab [26], is useful for studying different aspects of the consequences of post-traumatic SCI [11,18,25]. The aim of this study was to evaluate the potential therapeutic effects of short and long-term MC treatment on time dependent changes in body weight, neurological outcome, and spinal cord white and gray mater sparing following a standardized balloon-compression model of SCI.

In the study, 35 male Wistar rats weighing 300–330 g were used. This study was performed in accordance with the European Communities Council Directive of 24th November 1986 (86/906/EEC) regarding the use of animals in research, Slovak Law for Animal Protection No. 115/1995 and was approved by the Ethics Committee of the Institute of Neurobiology, CE, SAV, Kosice, Slovak Republic. Animals were anesthetized with halothane (1.5–2% halothane in air delivered via face mask) throughout surgery [26]. For induction of the balloon-compression SCI the thoracic spinal column was exposed and a small hole (1.5 mm diameter) was drilled in the Th10 vertebral arch with a dental drill. The periosteal membrane was opened and a 2-French Fogarty catheter (Baxter Healthcare Corporation, Irvine, CA) was inserted into the epidural space and

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advanced cranially so that the center of the balloon rested at the Th8–Th9 segment level of the spinal cord. The Fogarty catheter connected to a 50 μL Hamilton syringe (type 1705) was rapidly inflated with 12.5 μL of distilled water for 5 min, after which the catheter was removed, and the soft tissues and skin were sutured.

The rats were randomly assigned to (i) *placebo group* ($n = 11$): rats subjected to SCI plus intraperitoneal (i.p.) equivolumetric normal saline (NS) administration; (ii) *minocycline groups* ($n = 12$ per group): rats received the first i.p. injection of minocycline hydrochloride (Sigma), 90 mg/kg (3 mg/mL in sterile saline) at 1 h post-SCI followed by one-half MC dose, 45 mg/kg, administered every subsequent 12 h as follows: (A) short-term administration for 1 day (1DMC group, total dose 180 mg/kg); and (B) long-term administration for 5 consecutive days (5DMC group, total dose 450 mg/kg). This treatment protocol was based on those used for spinal cord injury in rat (contusion, transection) where MC neuroprotective effects was observed [19,22]. All animals were allowed to survive for 28 days after SCI.

The neurological score was evaluated using a BBB scale ranging from 0 to 21, where 0 reflects no locomotors activity and 21 reflects a normal performance [2]. Behavioral testing was first done at 24 h after injury and then weekly throughout the 28-day survival period. The body weights of the rats were recorded weekly.

Morphometric analysis was performed on sections from spinal cords perfused with NS followed by 4% paraformaldehyde on postoperative day 28 [25,26]. Briefly, from each spinal cord the entire 2-cm segment centered on the lesion was cut, and a series of 20 sections (5 μm thick) was collected with 1 mm distance between individual sections following the rules of systemic random sampling [9]. Luxol Fast Blue and Cresyl Violet stained sections were captured by digital camera and high-resolution images were used to trace the areas of spared spinal cord white and gray matter, respectively, and the areas were measured using image analysis software (ImageJ). Statistical analysis was performed on 11 lesion-centered sections from each spinal cord.

In individual animals, body weight was compared at appropriate survival time points by the unpaired Student's *t*-test. BBB were averaged across hind limbs, and intergroup differences analyzed using the nonparametric Kruskall–Wallis and Mann–Whitney *U*-tests. Morphometric measurements were used to construct plots of consecutive cross-sectional areas of the spared tissue at the lesion epicenter and at the individual levels of spinal cord rostral and caudal to the lesion epicenter. The differences at each level were analyzed using Kruskall–Wallis and Mann–Whitney *U*-tests. All values are expressed as means ± S.E.M. Differences between groups were considered statistically significant if $P < 0.05$.

Spinal cord injury produced by balloon-compression was followed by body weight loss in all, placebo and MC treated animals (Fig. 1). The body weight of all of the animals decreased continuously during the first week after SCI reaching a minimum weight on day 7. Thereafter, a gradual increase in body weight was observed that continued until sacrifice at 28-day.

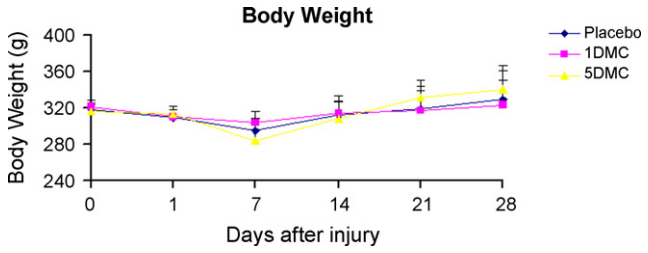

Fig. 1. Effect of MC treatment on body weight. No statistically significant differences in body weight changes were observed between placebo and MC treated animals surviving 28 days after balloon-compression spinal cord injury. Placebo (normal saline); 1DMC (1 day MC treatment); 5DMC (5 days MC treatment). Data points represent the group means ± S.E.M.

Significant differences in body weight between groups were not observed.

Immediately after balloon-compression SCI complete paraplegia occurred in all animals. BBB open field-testing revealed progressive neurological recovery from day 1 postinsult to the end of the survival period (Fig. 2). Locomotor scores between placebo and 1DMC group were not statistically significant different. Comparison of BBB changes between placebo and 5DMC groups revealed significant worsening in motor outcome in 5DMC group at day 14 and 21 postinsult. However, by day 28 after SCI, these differences between placebo and MC treated animals were not significant.

Morphometric analysis of the cross-sectional areas revealed differences in the rostro-caudal distribution of spared tissue between placebo and MC treated groups (Fig. 3). 1DMC and 5DMC treatment increased sparing of the spinal cord gray and white matter, which was significant in rostral direction beginning 2 mm from the lesion epicenter. However, both short and long-term MC administration did not reduce spinal cord white and gray matter damage caudal to the lesion epicenter.

This is the first study investigating the effects of MC following balloon-compression SCI in the rat. We showed, that short and long-term MC treatment had neuroprotective effects on the spinal cord rostral but not caudal to the epicenter of injury; improvement of motor outcome was not detected. These results are partly in agreement with previous observation of con-

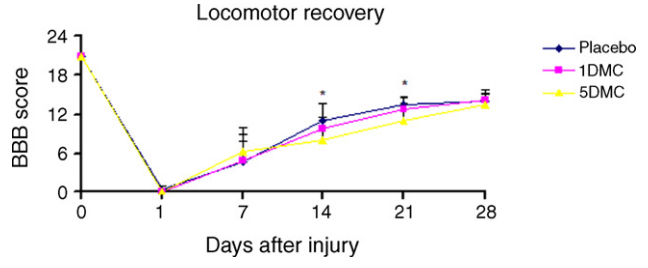

Fig. 2. Effect of MC treatment on locomotor recovery (BBB scores). Data comparison of placebo and 1DMC group (total dose 180 mg/kg) showed no statistically significant differences in motor outcome between the groups. Assessment of placebo vs. 5DMC group (total dose 450 mg/kg) revealed statistically significant worsening of motor outcome in 5DMC group on day 14 and 21 post-injury. Asterisks indicate that the means are significantly different from the placebo group (Student's *t*-test). Data points represent the group means ± S.E.M. *(5DMC) denotes statistical significant differences ($P < 0.05$).