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Riluzole attenuates neuropathic pain and enhances functional recovery in a rodent model of cervical spondylotic myelopathy

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ABSTRACT

Cervical spondylotic myelopathy (CSM) is the commonest cause of spinal cord impairment worldwide and despite surgical treatment, it is commonly associated with chronic neuropathic pain and neurological impairment. Based on data suggesting a key role of sodium and glutamate mediated cellular injury in models of spinal cord compression, we examined whether riluzole, a sodium channel/glutamate blocker, could improve neurobehavioral outcomes in a rat model of CSM. To produce chronic progressive compression of the cervical spinal cord, we used an established model of graded mechanical cord compromise developed in our laboratory. The chronic (8 weeks) mechanical compression of the cervical spinal cord resulted in persistent mechanical allodynia and thermal hyperalgesia at 8 weeks. Moreover, we found increased expression of phosphorylated NR1 and NR2B in the dorsal horns as well as astrogliosis and increased microglia expression in the dorsal horns after mechanical compression. Following daily systemic administration for 7 weeks after the induction of compression, riluzole (8 mg/kg) significantly attenuated forelimb and hindlimb mechanical allodynia and alleviated thermal hyperalgesia in the tail. Importantly, riluzole led to a decrease in swing phase duration, an increase in hind leg swing speed and an increase paw intensity in gait analysis. Riluzole also decreased the number of phosphorylated NR1 and phosphorylated NR2B positive cells in the dorsal horns and the microglia activation in the dorsal horns. Together, our results indicate that systemic riluzole administration during chronic cervical spinal cord compression is effective at protecting spinal cord tissue, preserving neurobehavioral function and alleviating neuropathic pain, possibly by decreasing NMDA receptor phosphorylation in astrocytes and by eliminating microglia activation. As such, riluzole represents a promising clinical treatment for CSM.

Introduction

Cervical spondylotic myelopathy (CSM) is a chronic progressive disorder in which the cervical spinal cord undergoes chronic compressive injury secondary to degenerative disk disease, ossification of the posterior longitudinal ligament (OPLL) or frank intervertebral disk disruption (Baptiste and Fehlings, 2006; Bohlman and Emery, 1988). CSM is the world's most common cause of spinal cord impairment in adults (Young, 2000). The gold standard of care for CSM is surgical decompression of the spinal cord. While successful surgical intervention can arrest the progression of disease, most patients are left with significant residual neurological impairment, including weakness, numbness, spasticity and neuropathic pain (Kalsi-Ryan et al., 2013). Neuropathic pain is characterized by allodynia and hyperalgesia which initially arise in the primary zone at the site of injury and, over time, may spread to areas that are not directly damaged by the injury (secondary hyperalgesia). Neuropathic pain has also been characterized in animal models of CSM (Karadimas et al., 2013; Lee et al., 2012). Due to its chronic nature and difficulty in management, neuropathic pain has been regarded as one of the most obstinate clinical symptoms of CSM. Importantly, neuropathic pain in CSM patients remains a major clinical problem and a therapeutic challenge because existing treatments are often ineffective and can cause serious side effects.

Glutamate N-methyl-D-aspartate receptors (NMDARs), especially those located in the dorsal horn of the spinal cord, are critically involved

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in nociceptive transmission and synaptic plasticity. Moreover, they have long been considered a target for the treatment of neuropathic pain (Gao et al., 2005; Guo et al., 2002; Miki et al., 2002; Zou et al., 2002). NMDARs are ionotropic channels — heteromers composed of NR1 and one or more of the four NMDA2 (NR2A-D) or two NMDA3 (NR3A and B) subunits (Villmann and Becker, 2007). The NR1 subunit is essential for the function of the NMDA receptor and phosphorylation of the NR1 is a major mechanism of modulating channel activity and trafficking to the neuronal surfaces (Chen and Roche, 2007; Chen et al., 2006). *In vitro* electrophysiologic studies showed that riluzole inhibited NMDA or kainic acid evoked — currents in *Xenopus* oocytes expressing rat NMDA or kainate glutamate receptors (Debono et al., 1993). Additionally, Azbill et al. combining *in vitro* and *in vivo* approaches demonstrated that riluzole increases the glutamate uptake as measured in rat spinal cord synaptosomes (Azbill et al., 2000). In other animal studies, riluzole decreased levels of glutamate in the CSF, possibly by increasing glutamate transporter activity (Bellingham, 2011). Together, this suggests that riluzole has a number of molecular targets involved in the neural mechanism of pain, including SCI-induced pain.

Using a clinically relevant model of gradual chronic compression of the cervical spinal cord developed in our laboratory (Lee et al., 2012), we hypothesized that daily systemic administration of riluzole would decrease neuropathic pain and protect against functional loss during chronic spinal cord compression. Previous studies have shown the efficacy of riluzole in a rat model of SCI (Hama and Sagen, 2011), in a rat model of spasticity induced by cutaneous stimulation and in other pain models (Kitzman, 2009; Munro et al., 2007; Sung et al., 2003). However, riluzole has yet to be tested in models of CSM. In the present paper, we demonstrate for the first time that the sodium–glutamate blocker riluzole attenuates neuropathic pain in a model of CSM.

Material and methods

Animal care

A total of 41 female Sprague–Dawley rats (weight 300–400 g, average weight 349 g; Charles River Laboratories, Wilmington, MA) were used for the experiments. All experimental protocols of this study were approved by the animal care committee of the University Health Network to ensure an ethical study, in accordance with the policies established in the guide on the care and use of experimental animals prepared by the Canadian Council of Animal Care.

Drug preparation and administration

The experimental design involved random allocation of treatment. Drug concentration was chosen based on the pharmacodynamic and kinetic properties of systemically administered riluzole (R116, Sigma, St. Louis MO, USA). Riluzole was initially dissolved in the media, 30% 2-hydroxypropyl-β-cyclodextrin solution (HBC, Sigma, H107), resulting in a concentration of 8 mg/ml. The final dosage is 8 mg/kg, diluted with saline to final volume of 1 ml. The HBC solution without riluzole was used as control. To investigate the neuroprotective effects of riluzole on chronic cervical spinal cord compression, we started daily intraperitoneal administration of 8 mg/kg of riluzole as treatment or HBC solution as control after the onset of cord compression (1 week post-initial surgery) and we terminated the treatment at 8 weeks post-surgery.

Surgical procedures & experimental groups

Chronic compression

For the purpose of this study we exploited a clinically relevant model of CSM which has been recently characterized by our laboratory (Lee et al., 2012). Briefly, under halothane anesthesia (1–2%) and a 1:1 mixture of O_2/N_2O, the surgical area was shaved and disinfected with 70% ethanol and betadine. A midline incision was made at the cervical area (C2–T2), and the skin and superficial muscles were retracted. The rats underwent a C6–C7 laminectomy and the rod of the chronic compression device (CCD) was inserted into the C2 and the T2 spinous processes. A threaded screw with an extradural plate fixed to the tip was advanced through the CCD rod. To ensure the stability of the CCD we followed the procedure described by Lee et al. The screw was advanced very precisely 0.2 mm (one half turn) using a microscope at an oblique angle. The sham-operated animals underwent identical surgical procedure but without having cord compression. The surgical wounds were sutured, and the animals were given post-operative analgesia and saline (0.9%; 5 ml) to prevent dehydration. Animals were allowed to recover and housed in standard rat cages with absorbent bedding at a temperature of 26 °C. In addition, the animals received Clavamox (amoxicillin and clavulanic acid). Gradual mild chronic compression starting 1 week post-surgery was achieved by advancement of the screw by 0.4 mm (one turn) weekly up to 3 weeks.

Experimental groups and treatments

At first week post-operatively, all injured rats were divided blindly and randomly into three experimental groups: 1) sham (no compression, $n = 6$), (2) control group (HBC in saline injection, $n = 18$), and (3) riluzole group (riluzole injection, $n = 17$). A summary of the experimental protocol is depicted in Fig. 1.

Micro-computed tomography

The extent of the compression was quantitatively evaluated using micro-computed tomography (micro-CT; GE Locus Ultra MicroCT at the STTARR facility, University Health Network). This system offers 150 μm^3 resolution, scan times as low as 1 s, and maximum transaxial and longitudinal fields of view of 14 and 10 cm, respectively. Under isoflurane anesthesia, micro-CT was used to image the spinal canal at 4 weeks post-surgery. Based on the acquired mid-sagittal images from micro-CT, the Compression Ratio was calculated using the following formula (Fehlings et al., 1999): Compression Ratio (%) $= \{1 - 2c / (a + b)\} \times 100$, where 'c' is the anteroposterior canal diameter at the level of maximum compression, 'a' is the anteroposterior canal diameter at the nearest normal level above the site of compression, and 'b' is the anteroposterior canal diameter at the nearest normal level below the level of the site of the compression. The distances were calculated using MicroView software (2D and 3D image view 2.1.2., GE Healthcare, Little Chalfont, U.K.).

Neurobehavioral assessments

The effect of riluzole on neuropathic pain during chronic compression of the cervical spinal cord was characterized using assessments of mechanical allodynia and thermal hyperalgesia. To measure functional deficits, automated computerized gait assessment was done using the CatWalk system (Hamers et al., 2001; Koopmans et al., 2005). Importantly, the assessment of outcomes was performed by two blinded observers.

Assessment of mechanical allodynia by von Frey filament testing

Cutaneous sensitivity to innocuous mechanical stimulation of both forepaws and hindpaws was assessed weekly in all rats using a series of filaments of varying thicknesses (von Frey filaments). Filaments were applied to the mid-plantar surface of forepaws and hindpaws, one paw at a time. We used 14 Touched-Test von Frey filaments, number 5–16 (North Coast Medical, Inc., CA, USA) with a regularly calibrated stiffness corresponding to 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0, 10, 15, and 26 g. Probing was only performed when the animal's four paws were in contact with the floor. Each probe was applied to the foot until it was bent. A minimum of three withdrawals of the tested paw out of five filament applications was considered a positive response. Filaments were applied in ascending order, and the smallest filament that elicited a positive response was considered the threshold stimulus.