ELSEVIER

CrossMark

The Spine Journal 16 (2016) 1015–1024

THE SPINE JOURNAL

Basic Science

Combining neuroprotective agents: effect of riluzole and magnesium in a rat model of thoracic spinal cord injury

Natália L. Vasconcelos, MSc[a,b], Eduardo D. Gomes, MSc[a,b], Eduarda P. Oliveira, MSc[a,b], Carlos J. Silva, MSc[a,b], Rui Lima, MSc[a,b], Nuno Sousa, MD, PhD[a,b], António J. Salgado, PhD[a,b], Nuno A. Silva, PhD[a,b,*]

[a]*Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus Gualtar, 4710-057 Braga, Portugal*
[b]*ICVS/3B's – PT Government Associate Laboratory, Braga, Guimarães, Portugal*

Received 17 December 2015; revised 23 March 2016; accepted 18 April 2016

Abstract

BACKGROUND CONTEXT: Damage to the spinal cord can result in irreversible impairments or complete loss of motor, sensory, and autonomic functions. Riluzole and magnesium have been widely investigated as neuroprotective agents in animal models of spinal cord injury. As these drugs protect the injured spinal cord through different mechanisms, we aimed to investigate if their neuroprotective efficacy could be cumulative.

PURPOSE: This study aimed to investigate the neuroprotective efficacy of combined administration of riluzole and magnesium chloride in a contusive model of thoracic spinal cord injury.

STUDY DESIGN: An in vivo experiment was set using female Wistar Han rats that underwent a thoracic spinal cord contusion (T8) using a weight drop method. An hour after injury, animals were randomly distributed to receive (1) saline, (2) riluzole (2.50 mg/kg), (3) magnesium chloride (24.18 mg/kg) in a polyethylene glycol formulation, or (4) a combined treatment (riluzole and magnesium). Subsequent treatments were given in four intraperitoneal injections (spaced 12 hours apart).

METHODS: The Basso, Beattie, and Bresnahan locomotor rating scale, an activity box test, and a swimming test were used to evaluate behavioral recovery over a 4-week period. Histologic analysis of the spinal cords was performed to measure the extent and volume of the lesion, axonal preservation, serotonergic and glutamatergic fiber sparing, motor neuron survival, and inflammation.

RESULTS: Our results show that only the riluzole treatment significantly improved behavioral recovery up to 4 weeks after injury when compared with saline controls (6.2±1.8), with animals achieving weight-supported stepping (9.1±1.2). Riluzole also promoted tissue sparing with significant differences achieved from 200 to 600 μm (caudally to the lesion epicenter), and reduced lesion volume, with animals presenting a significantly smaller lesion (3.23±0.26 mm^3) when compared with the saline-treated group (4.74±0.80 mm^3), representing a 32% decrease in lesion volume. Riluzole treatment induced significant axonal preservation, as well as serotonergic fiber sparing, caudally to the injury epicenter.

CONCLUSIONS: Our results suggest that the combined treatment, although simultaneously targeting two excitotoxic-related mechanisms, did not further improve behavioral and histologic outcome when compared with riluzole given alone.

Keywords: Excitotoxicity; Ionic imbalances; Magnesium; Neuroprotection; Riluzole; Spinal cord injury

FDA device/drug status: Not approved for this indication (riluzole); Not approved for this indication (magnesium chloride); Not approved for this indication (polyethylene glycol).

Author disclosures: ***NLV***: Nothing to disclose. ***EDG***: Fellowship Support: Portuguese Foundation for Science and Technology (D), outside the submitted work. ***EPO***: Nothing to disclose. ***CJS***: Nothing to disclose. ***RL***: Nothing to disclose. ***NS***: Nothing to disclose. ***AJS***: Grants: Prémios Santa Casa Neurociências (F); Fellowship Support: Portuguese Foundation for Science and Technology (F), outside the submitted work. ***NAS***: Grants: Prémios Santa Casa Neurociências (F); Fellowship Support: Portuguese Foundation for Science and Technology (F), outside the submitted work.

There are no competing financial interests.

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

* Corresponding author. Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel.: +351 2536044839; fax: +351 253604809.

E-mail address: nunosilva@ecsaude.uminho.pt (N.A. Silva)

http://dx.doi.org/10.1016/j.spinee.2016.04.013
1529-9430/

Introduction

Trauma to the spinal cord can cause severe damage to nervous tissue leading to temporary or permanent changes in the spinal cord's normal motor, sensory, or autonomic function [1]. Following the primary injury, a cascade of cellular and biochemical events causes additional damage to the initial lesion site and further compromises neurologic function [2–6]. Modulation of these deleterious secondary events stands as a promising therapeutic strategy to promote neuroprotection and support functional recovery in spinal cord injury (SCI).

Riluzole and magnesium have been extensively studied as neuroprotective drugs in SCI, with several independent preclinical studies showing that individual administration of these drugs confers behavioral and histologic improvements in cervical and thoracic animal models of SCI.

Riluzole is a sodium channel blocker agent that exerts neuroprotection in SCI mainly through blocking of persistent sodium currents, inhibition of presynaptic glutamate release, as well as increased glutamate uptake (Fig. 1) [7–10]. Several preclinical studies have evaluated the systemic administration of riluzole in acute animal models of SCI reporting improvements in locomotor function and neural tissue preservation, reduced apoptosis and inflammation, and improved axonal conduction [11–14]. A phase I safety trial of riluzole in acute cervical SCI has already established the absence of serious adverse effects and determined that its administration may have a beneficial effect in motor outcome (clinical trial registration no.: NCT00876889) [15].

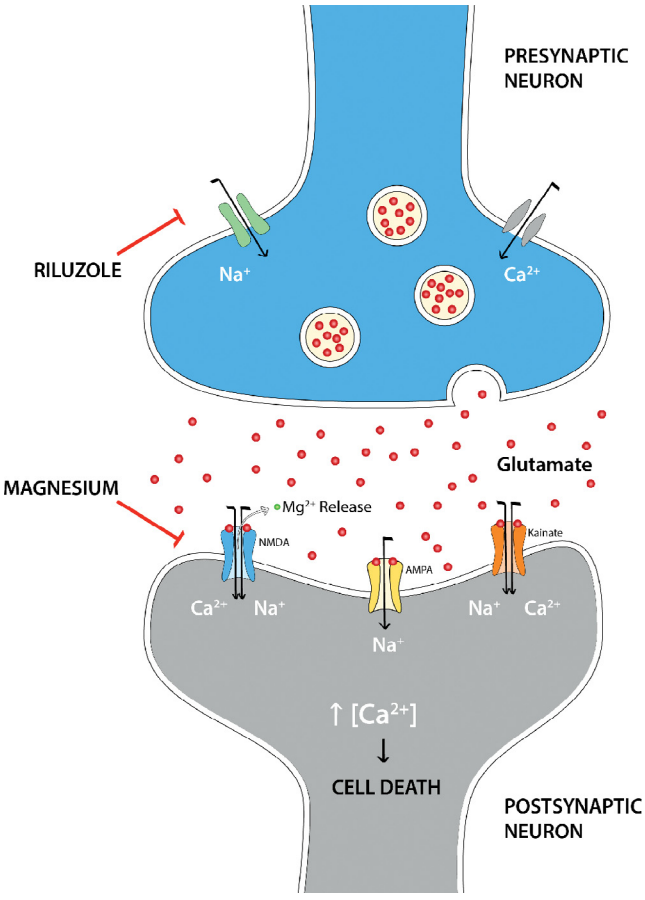

Fig. 1. Ionic imbalance and glutamate excitotoxicity following SCI lead to neuronal and glial death, further aggravating the initial damage. The neuroprotective effects of riluzole result mainly from the blockade of sodium channels while magnesium blocks NMDA receptors. NMDA, N-methyl-D-aspartate; AMPA, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Ca^{2+}, calcium; Na^{+}, sodium. Adapted from Reference [15].

Another possible route to modulate secondary injury is through the use of magnesium (Fig. 1). Magnesium acts as an N-methyl-D-aspartate (NMDA) receptor blocker preventing excitotoxicity caused by excessive activation of this glutamate receptor. Because glutamate levels increase rapidly after traumatic SCI while magnesium levels decrease, magnesium administration allows for the restoration of magnesium levels and modulation of excitotoxic events. Ditor and colleagues [16] combined magnesium in a polyethylene glycol (PEG) formulation, a widely used excipient, allowing a low dose treatment (tolerated by humans [17]) that improved tissue neuroprotection, increased locomotor recovery, and reduced mechanical allodynia. Subsequent studies using different animal models of SCI further validated magnesium as a potential therapeutic strategy [18,19]. Similar to riluzole, the strong preclinical evidence toward increased functional and histologic recovery led this treatment to clinical trials in SCI [15].

Combining drugs may lead to improved efficacy over single-drug treatments as drug interactions may occur contributing to additive or synergistic effects that further promote neuroprotection. Herein, we aimed to investigate the efficacy of both individual and combined administration of riluzole and magnesium chloride as these therapeutic agents putatively confer neuroprotection and functional recovery after SCI through different mechanisms of action. To do that, we used an animal model of thoracic spinal cord contusion. Improvement of behavior and histologic outcome after SCI was assessed as a measure of treatment efficacy.

Methods

Spinal cord injury model

All procedures were carried out in accordance with the European Union Directive 2010/63/EU and Portuguese National Authority for animal experimentation, Direção Geral de Veterinária (ID: DGV9457), guidelines on animal care and experimentation.

Nineteen, in-house–bred, female Wistar Han rats (14 weeks old, weighing 210–260 g) were used for the study. Animals were kept under standard laboratory conditions (12-hour light:12-hour dark cycles, 22°C, relative humidity of 55%, and ad libitum access to standard food and water) and housed in pairs. Animal handling was carried out 3 days before surgery.

General anesthesia was induced by an intraperitoneal (IP) injection of a ketamine (100 mg/mL, Imalgene; Merial, Duluth, GA, USA) and medetomidine hydrochloride (1 mg/mL,