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RILUZOLE IMPROVES OUTCOME FOLLOWING ISCHEMIA–REPERFUSION INJURY TO THE SPINAL CORD BY PREVENTING DELAYED PARAPLEGIA

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Abstract—The spinal cord is vulnerable to ischemic injury due to trauma, vascular malformations and correction of thoracic aortic lesions. Riluzole, a sodium channel blocker and anti-glutamate drug has been shown to be neuroprotective in a model of ischemic spinal cord injury, although the effects in clinically relevant ischemia/reperfusion models are unknown. Here, we examine the effect of riluzole following ischemia–reperfusion injury to the spinal cord. Female rats underwent high thoracic aortic balloon occlusion to produce an ischemia/reperfusion injury. Tolerance to ischemia was evaluated by varying the duration of occlusion. Riluzole (8 mg/kg) was injected intraperitoneally 4 h after injury. Locomotor function (Basso, Beattie and Bresnahan (BBB) scale) was assessed at 4 h, 1 day, and 5 days post-ischemia. Spinal cords were extracted and evaluated for neuronal loss using immunohistology (choline acetyltransferase (ChAT) and neuronal nuclei (NeuN)), inflammation (CD11b), astrogliosis (glial fibrillary acidic protein – GFAP) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL). Ischemic injury lasting between 5.5 and 6.75 min resulted in delayed paraplegia, whereas longer ischemia induced immediate paraplegia. When riluzole was administered to rats that underwent 6 min of occlusion, delayed paraplegia was prevented. The BBB score of riluzole-treated rats was 11.14 ± 4.85 compared with 1.86 ± 1.07 in control animals. Riluzole also reduced neuronal loss, infiltration of microglia/macrophages and astrogliosis in the ventral horn and intermediate zone of the gray matter. In addition, riluzole reduced apoptosis of neurons in the dorsal horn of the gray matter. Riluzole has a neuroprotective effect in a rat model of spinal cord injury/reperfusion when administered up to 4 h post-injury, a clinically relevant therapeutic time window.

Key words: spinal cord injury, ischemia, riluzole, drug treatment, ischemia–reperfusion injury.

INTRODUCTION

The spinal cord is extremely vulnerable to ischemic injury caused by a variety of abnormalities such as atherosclerosis, thrombosis, embolism, vasculitis, cardiac arrest, thoracic aortic aneurysm, spinal arteriovenous malformation, and iatrogenic causes such as surgery, angiography, or epidural anesthesia (Millichap et al., 2007; Turkoz et al., 2007; Hobai et al., 2008; Cheng et al., 2009; Dublin et al., 2010). Spinal cord ischemia may complicate thoracoabdominal aneurysm surgery with an incidence of 4–32% and can lead to severe neurological complications including paraplegia (Rosenthal, 1999).

Acute and chronic spinal cord ischemia can result from a number of causes such as nucleus pulposus or fibrocartilaginous embolism (Uppal et al., 2004; Thone et al., 2007; Manara et al., 2010), spinal infection (Almasanu et al., 2005; Giuliani et al., 2010), steroid injections (Ludwig and Burns, 2005; Masson and Bardin, 2009; MacMahon et al., 2010), spinal surgery (Weber et al., 2009) and trauma (Robles, 2007).

Moreover, post-traumatic ischemia is an important element of acute spinal cord injury (Tator and Fehlings, 1991; Beghi et al., 2011). Local vascular alterations and ischemia within the spinal cord are thought to be a key aspect of the secondary injury process (Rivlin and Tator, 1978; Fehlings and Tator, 1988; Tator and Fehlings, 1991; Ng et al., 2011). Ischemia following spinal trauma results in vasospasm, intravascular thrombosis, systemic hypoperfusion, hemorrhage, disruption of vessels, loss of microcirculation, ionic imbalances and glutamate-mediated excitotoxicity. These secondary injury cascade events extend to multiple spinal segments both rostral and caudal to the initial site of injury (Dumont et al., 2001).

Notably, the influx of sodium into cells has been postulated to be an important early event in the pathogenesis of secondary injury process (Fehlings and

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Abbreviations: ANOVA, analysis of variance; ChAT, anti-choline acetyltransferase; GFAP, glial fibrillary acidic protein; NADPH, nicotinamide adenine dinucleotide phosphate; NeuN, neuronal nuclei; PBS, phosphate-buffered saline; SEM, standard error of the mean; TUNEL, transferase-mediated dUTP nick end labeling.

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Agrawal, 1995; Agrawal and Fehlings, 1997; Schwartz and Fehlings, 2001, 2002a; Hains and Waxman, 2007). The influx of sodium can lead to increased levels of intracellular calcium and excessive release of glutamate leading to glutamatergic toxicity, all of which exacerbate the initial injury. A variety of agents such as steroids, barbiturates, superoxide dismutase, prostaglandins and calcium channel blockers have been tested in experimental models of spinal cord ischemia. Experimental and clinical hypothermia has been shown to be effective in reducing ischemic spinal cord injury (Ueno et al., 1994; Cambria et al., 1997a; Cambria et al., 1997b; Sueda et al., 2002). Currently, there are no efficacious pharmacological treatments for spinal cord ischemia. Riluzole, a sodium/glutamate antagonist, approved for use in amyotrophic lateral sclerosis (Miller et al., 2007), is an attractive treatment option for spinal cord ischemia.

Riluzole has demonstrated anti-ischemic properties (Pratt et al., 1992; Wahl et al., 1993; Lang-Lazdunski et al., 2000b). The mechanism of action is linked to the blockade of sodium channels, inactivation of voltage-dependent calcium channels, and blockade of sodium-dependent glutamate release (Dessi et al., 1993; Doble, 1996; Azbill et al., 2000; Schwartz and Fehlings, 2002b; Frizzo et al., 2004; Wu et al., 2013). Preclinical studies have demonstrated a role for riluzole in promoting functional recovery after acute spinal cord injury by preventing the aberrant release of sodium and glutamate imbalance (Stutzmann et al., 1996; Schwartz and Fehlings, 2001, 2002b; Chow et al., 2012; Wu et al., 2013). This study examined the effect of riluzole administration 4 h after spinal cord ischemia in a rodent model. This time point represents a realistic, clinically relevant administration point.

EXPERIMENTAL PROCEDURES

Experimental design

All experimental procedures and animal care were approved by the Animal Care Committee at the University Health Network in accordance with the policies of the *Guide to the Care and Use of Experimental Animals*.

Female Sprague–Dawley rats (350–430 g, 12 months old, Taconic Farms) were used in this study. Spinal cord ischemia–perfusion injury was induced using previously described techniques (see below). Rats were randomly assigned to receive different durations of spinal cord ischemia–perfusion injury to test their tolerance of ischemia. All of the animals underwent locomotor assessment to determine the duration that resulted in moderate ischemic injury. Subsequently, duration of ischemia that led to moderate ischemic injury was used to test the neuroprotective efficacy of riluzole. The efficacy of riluzole (8 mg/kg) administrated 4 h after ischemic injury was examined using neuorobehavioural and histopathological techniques.

Spinal cord ischemia–reperfusion model. Briefly, rats were anesthetized with 4% halothane in oxygen and maintained with 2% halothane; body temperature was maintained at 37.0 °C using a homeothermic blanket system (Harvard Apparatus). Rats were placed in supine position and the hair in the surgical area was shaved and prepared for aseptic surgery. Distal arterial blood pressure was measured using a tail arterial catheter connected to a blood pressure transducer and monitor (CyQ BPM302). The left carotid artery was cannulated with a 20-gauge IV catheter for blood sampling.

To induce spinal ischemia, a 2F Fogarty arterial embolectomy catheter (Edwards Lifesciences: Missisauga, Ontario, Canada) was passed through the left femoral artery to the descending thoracic aorta placing the tip at the level of the left subclavian artery (11 cm from site of insertion). Before the insertion of balloon catheter, the femoral artery was distended with a metal dilator (diameter = 1.5 mm) to facilitate placing of the catheter. Subsequent to the insertion of the three catheters and 5 min prior to the inflation of Fogarty balloon, Heparin (200 U, 0.2 ml) was injected into the tail artery. The intra-aortic balloon catheter was inflated with 0.05 ml of saline. An immediate and sustained decrease in distal arterial blood pressure was used to confirm resulting occlusion of aortic blood flow. Systemic hypotension (40 mmHg) was maintained during occlusion by an external blood collecting circuit connected to the carotid artery and positioned at a height of 54 cm (40 mmHg). The temperature of blood collecting circuit was maintained at 37.5 °C with a circulator (Fisher Scientific Isotemp Open-Bath Circulator). Following ischemia, the balloon was deflated, and the blood was re-infused slowly during a 60 s period. Right after blood reinfusion, 4 mg of protamine sulfate was administered subcutaneously to neutralize heparin. Arterial blood pressure was monitored for an additional 10 min post-ischemia for stabilization. After this, the arterial lines were removed, the muscles were sutured with absorbable sutures and skin was closed with wound clips.

Acute postoperative care included rehydration with 5 ml of saline, increasing body temperature with a focused heat lamp and warmed towels, and a subcutaneous injection of buprenorphine hydrochloride (0.05 mg/kg) immediately after surgery to minimize postsurgical pain. Animals were individually housed in standard plastic cages in a room with an ambient temperature of 26 °C. The animals had access to food and drinking water *ad libitum* and their bladders were manually expressed three times per day until they regained normal voiding.

To test the tolerance of rats to spinal cord ischemia, the female rats were assigned to receive occlusion of aortic blood flow for increasing durations from 5 min to 8.5 min with 0.5-min increments. Aortic occlusion for 6 min resulted in a moderate Spinal Cord Injury (SCI). Subsequently, all of the animals in the spinal cord ischemia group underwent 6-min aortic occlusion. A group of rats which underwent catheter insertion without inflation of balloon catheter served as sham control group.

Drug preparation and administration. An independent investigator blinded to group assignment prepared all experimental compounds. Drug concentration was