Evaluation of the neuroprotective effects of sodium channel blockers after spinal cord injury: improved behavioral and neuroanatomical recovery with riluzole

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Object. Persistent activation of voltage-sensitive Na⁺ channels is associated with cellular toxicity and may contribute to the degeneration of neural tissue following traumatic brain and spinal cord injury (SCI). Pharmacological blockade of these channels can attenuate secondary pathophysiology and reduce functional deficits acutely.

Methods. To determine the therapeutic effects of Na⁺ channel blockers on long-term tissue sparing and functional neurological recovery after traumatic SCI, the authors injected Wistar rats intraperitoneally with riluzole (5 mg/kg), phenytoin (30 mg/kg), CNS5546A, a novel Na⁺ channel blocker (15 mg/kg), or vehicle (2-HPβCD; 5 mg/kg) 15 minutes after induction of compressive SCI at C7–T1.

Functional neurological recovery of coordinated hindlimb function and strength, assessed 1 week postinjury and weekly thereafter for 6 weeks, was significantly enhanced in animals treated with riluzole compared with the other treatment groups. Seven weeks postinjury the preservation of residual tissue and integrity of descending axons were determined with digital morphometrical and fluorescent histochemical analysis. All three Na⁺ channel blockers significantly enhanced residual tissue area at the injury epicenter compared with control. Riluzole significantly reduced tissue loss in rostrocaudal regions surrounding the epicenter, with overall sparing of gray matter and selective sparing of white matter. Also, counts of red nuclei neurons retrogradely labeled with fluorogold introduced caudal to the injury site were significantly increased in the riluzole group.

Conclusions. Systemic Na⁺ channel blockers, in particular riluzole, can confer significant neuroprotection after in vivo SCI and result in behavioral recovery and sparing of both gray and white matter.

KEY WORDS • spinal cord injury • motor function • riluzole • phenytoin • rat

Spinal cord injury affects approximately 10,000 individuals annually in the United States. More than half of those affected experience complete and/or incomplete paraplegia and tetraplegia. Although current therapeutic methods aimed at mitigating these devastating neurological deficits have provided modest results, the prospects for recovery remain very limited. The answers to improving functional outcome following traumatic SCI may reside with interventions aimed at attenuating pathological alterations to neural tissue that occur subsequent to the initial mechanical injury. These biochemical and physiological alterations constitute a secondary injury that exacerbates the degeneration of residual gray and white matter.

The deregulation of Na⁺ ion homeostasis involving an accumulation of [Na⁺], has been postulated to be a key early event in the pathogenesis of secondary traumatic and ischemic CNS injury. There is strong evidence implicating voltage-sensitive Na⁺ channels in trauma-induced [Na⁺] influx. Moreover, administration of the Na⁺ channel blocker TTX resulted in protection of spinal cord white matter and improved locomotor function in the rat following SCI. However, TTX is very toxic and this precludes its use as a therapeutic intervention for traumatic SCI in humans. Less toxic Na⁺ channel blockers, including the anticonvulsant riluzole, may be effective in the treatment of acute experimental SCI although the effects on long-term outcome remain to be determined. Aside from riluzole’s Na⁺ channel blocking capabilities, it also antagonizes the stimulation of neurotransmitter release by EAAs. Another anticonvulsant, phenytoin, shares riluzole’s basic mechanisms of action, which include Na⁺ channel blockade and glutamate release inhibition. Antagonists to glutamate-release mechanisms have also demonstrated effective amelioration of functional deficits sustained after SCI.
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With this background, we sought to examine the effects of riluzole, phenytoin, and CNS5546A, a novel Na$^+$ channel blocker with NMDA antagonistic activity, on long-term functional neurological recovery and anatomical protection after SCI. We report that all three Na$^+$ channel blockers had a significant effect on tissue preservation at the spinal cord lesion site; however, only treatment with riluzole sufficiently resulted in improved neurological recovery.

A preliminary report of this work has been published in abstract form. 31

Materials and Methods

Experimental Design

Experiments in this study were performed using a blinded, randomized-balanced design. Briefly, four groups of 15 rats each were randomly and blindly assigned to receive one of three Na$^+$ channel blocking agents or vehicle 15 minutes after induction of compressive SCI at C7–T1. Each animal’s motor recovery was assessed 1 week postoperatively and weekly thereafter for a period of 6 consecutive weeks. Following the completion of behavioral testing the animals were reanesthetized and subjected to a T-6 spinal cord transection and fluorogold implantation. The retrograde tracer was allowed to incubate for 7 days, and thereafter the animals were perfusion fixed while deeply anesthetized with pentobarbital. Histopathological and morphometric analyses were performed as described later. The experimental design is schematically illustrated in Fig. 1.

Spinal Cord Injury Model

All experimental protocols were approved by the animal care committee of The Toronto Western Research Institute in accordance with the policies established in the Guide to the Care and Use of Experimental Animals prepared by the Canadian Council of Animal Care. Halothane anesthesia (1.5%) was induced in 60 adult female rats, each weighing between 225 and 280 g, prior to their undergoing a C6–T1 laminectomy. The spinal cord at C7–T1 was extradurally compressed for 1 minute between the blades of a modified aneurysm clip calibrated to deliver a closing force of 53 g. This degree of injury resulted in severe SCI as described by Fehlings and Tator. 32 Recovery from surgery the animals were placed in a cage with absorbent bedding and given unrestricted access to food and water. They were returned to their housing facility where they were maintained in a 12-hour light/dark cycle at an ambient temperature of 25° to 27°C. Manual expression of their bladders was performed three times daily until a reflex bladder was established.

Drug Preparation and Administration

An independent investigator prepared all experimental compounds to preserve the integrity of the blind experimental design. Drug concentrations were chosen based on the pharmacodynamic and kinetic properties of systemically administered riluzole and phenytoin. 31,23,42,46,93,96 The experimental compound CNS5546A is a novel Na$^+$ channel blocker (50% inhibitory concentration = 0.68 μM) against neuronal Na$^+$ channels by guainidine flux assay) with competitive NMDA antagonistic activity (WF Holt, personal communication, 1997). An effective dose concentration for this drug was chosen based on internal pharmacodynamic and neuroprotective investigations conducted by Cambridge Neuroscience Inc. The Na$^+$ channel blockers were dissolved with 2HP CD, the control compound for this study. Fifteen minutes after induction of SCI, a period in which axonal pathology has not yet fully developed, 21,43 one of the three Na$^+$ channel blockers or 2HP CD was administered intraperipherally using a 25-gauge 5/8 needle in 0.2 M PBS: 140 mM NaCl, 20 mM Na$_2$HPO$_4$, pH 7.4. This was performed in a random and blinded fashion. The PBS was used as a vehicle to ensure that the drug solutions retained their pH characteristics after intraperipheral administration. The volume of PBS vehicle added to the drugs varied depending on weight/volume ratios. In all cases, the injectable volume was 0.6 ml. The concentrations of the drugs were adjusted so that an equal volume of drug was given according to weight (1:1).

Riluzole was initially dissolved in 45% 2HP CD, with 0.2 N HCL to facilitate the drug’s solubility, resulting in a concentration of 14 mg/ml. The final solution was reduced to 5 mg/ml, and 1 N NaOH was added to adjust the pH to 7.4. Phenytoin was dissolved to a concentration of 15 mg/ml with 20% 2HP CD, and 1 N NaOH was added to the solution to adjust the pH to greater than 12. The extremely basic solution was necessary to prevent the compound from precipitating. The drug was delivered at a dose of 30 mg/kg with a final pH of 8.7. The CNS5546A was dissolved in 20% 2HP CD to a concentration of 17 mg/ml and diluted further to a final concentration of 15 mg/ml, pH 7.4. The 2HP CD was dissolved in distilled water at a concentration of 4.5 mg/ml. This concentration corresponded to the maximum concentration used to dissolve one of the Na$^+$ channel blockers (riluzole). The final solution was delivered in PBS vehicle at a dose of 5 mg/kg, pH 7.4.

Behavioral Assessment

Clinical neurological recovery of motor behavior was assessed using the Ba-Be-Br expanded locomotor rating scale, a rating scale of locomotion has been developed by the Ba-Be-Br scale is an open-field locomotor rating scale that is used to assess quantitatively hindlimb joint movement and motor coordination. Observations for both the right and left hindlimbs are recorded as a score from 0, which reflects a flat hind foot, to 21, which corresponds to normal locomotor performance. Scores are averaged across both the right and left hindlimbs to arrive at a final motor recovery score for each week of testing. Clinical functional assessment of equilibrium and residual strength in upper and lower limbs was evaluated with the IP technique. This task requires an animal to maintain its position for 5 seconds on a moveable plane that can be adjusted by 5° increments to a maximum angle of 90°. The maximum angle at which the animal could maintain its position was recorded weekly and taken to represent its functional ability.

Retrograde Axonal Tracing

The fluorescent tracer fluorogold was dissolved in distilled water to a final concentration of 4% and filter sterilized through a 0.22-μm filter. The technique for placement of the axonal tracer is similar to that described in previous reports from our laboratory. 22,30,32,53,70,85 Following Week 6 of behavioral testing the rats’ spinal cords were completely transected at T-6. Two or three 5 × 5-mm sterile gel foam pledgets saturated with the fluorogold solution were placed between the rostral and caudal surfaces of the severed cords and were allowed to rest on the ventral aspect of the vertebral columns. The pledgets were positioned in a manner that ensured that the rostral surfaces of the severed cords were completely exposed to the fluorogold solution. The transection sites and exposed gel foam were liberally coated with white petroleum jelly to prevent diffusion of the retrograde tracer. The surgical wounds were closed, and the animals were returned to their housing facility for 1 week to allow for retrograde transport of the fluorescent tracer.

One week following fluorogold implantation, the animals underwent a transectional perfusion fixation with 100 ml of 0.2 M PBS followed by 500 ml of 4% buffered (0.2 M PBS) paraformaldehyde, and the entire brain and spinal cord injury site were extracted.

A uniform random number protocol was used to generate a subsample from the larger behavioral population to quantify retrograde tracing and lesion site histopathological outcome measures. A computer program selected four animals from each treatment group. Each treatment-specific subsamples’ behavior was compared (animals per treatment), from both the Ba-Be-Br and IP were compared with those from their larger population (15 animals per treatment) subsequent to random selection. Pearson correlation between the respective subsample and larger sample was performed for each treatment group and resulted in correlation coefficients of $r = 0.50$ to 0.80 with p values $= 0.01$ to 0.000003. Thus, the subsample selection procedure was supported by statistical significance, ensuring adequate representation.

Frozen, serial 40-μm sections were cut in the coronal plane through the brainstem and midbrain. Alternating sections were...
Histopathological and Lesion Site Analysis

Spinal cord tissue was postfixed for 72 hours at 4°C in 10% neutral buffered formalin and was embedded in paraffin. Ten-micrometer axial sections were cut on a microtome through the rostrocaudal boundary of the SCI site and stained with luxol fast blue and hematoxylin and eosin. Morphometrical and volumetrical assessments of the tissue were performed on the same representative sub-sample that had been randomly selected for quantitative retrograde axonal tracing. The area of the cavity and residual tissue (white matter and gray matter) was quantified on a personal computer running Image Pro Plus software and connected to a charge-coupled device camera mounted on a microscope. The injury epicenter and every 10th section (corresponding to every 100 µm of tissue) rostral and caudal to the epicenter were digitized, traced, and false colored according to predefined pixel color values corresponding to histochemical selectivity. Tissue sections displaying the largest proportion of cystic cavity compared with total cross-sectional area were taken to represent the focal point of the injury epicenters. The length of the injury sites was based on the minimum amount of harvested tissue from our representative subsample and was determined by multiplying by 100 µm the number of sections falling within its rostrocaudal boundaries. As a result of this procedure the rostrocaudal boundaries of the injury site and stained with cresyl violet or mounted with Mowiol. Prepared sections of the midbrain and brainstem were quantitatively analyzed for nuclei preservation on the randomly selected representative subsample from each group (four animals per treatment). Sections mounted with Mowiol were viewed under epifluorescence at a magnification of ×100 and the number of fluorogold-labeled neurons in the red nucleus, raphe nuclei, reticular formation, vestibular nuclei, and RVLM were counted.

Statistical Analysis

The Ba-Be-Br and IP scores were analyzed with two-factor (main effects: treatment and time) ANOVA. The Student-Newman-Keuls multiple range test was used to examine multiple comparisons between the different levels of treatment and time. Weekly differences in behavioral outcome were assessed post hoc by using the Fisher least-significant difference test.

Cell count data from the red nucleus and brainstem nuclei were each analyzed with univariate ANOVA and Student-Newman-Keuls post-hoc analysis between different treatment groups. Morphometrical data involving analyses of cross-sectional residual tissue area along the length of the SCI site and cross-sectional cavity area at the injury epicenter were analyzed with two-factor (main effects: treatment and distance) ANOVA and Student-Newman-Keuls test as post-hoc analyses. In addition, two-factor (main effects: tissue components/cavity and distance) ANOVA with post-hoc Student-Newman-Keuls testing was applied to white matter, gray matter, and cavity volumes at discrete sites, corresponding to 500-µm increments surrounding the injury epicenter. Differences between treatment effects on the two residual tissue components and cavity volumes surrounding the injury epicenter were analyzed with one-factor ANOVA and Student-Newman-Keuls testing post-hoc analysis. All results are expressed as the mean ± SEM and considered significant at p < 0.05.

Sources of Supplies and Equipment

The experimental compound, CNS5546A, was obtained from Cambridge Neuroscience Inc., Cambridge, MA. The 2HPβCD and Riluzole were purchased from RBI, Natick, MA. The sterilization filter was supplied by Millipore, Bedford, MA. The 25-gauge needle was obtained from Becton Dickinson, Franklin Lakes, NJ. The fluorogold tracer was obtained from Fluorochrome, Inc., Denver, CO. The animal-selection software was supplied by Jandel Scientific, Chicago, IL. The epifluorescence microscope (Eclipse E800) and the charge-coupled device camera were purchased from Nikon Canada Inc., Mississauga, Canada. The Image Pro Plus software came from Media Cybernetics, Silver Spring, MD.

Results

Effects of Na+ Channel Blockers on Functional Neurological Recovery After Compressive SCI

Immediately postoperatively all animals were uniformly paraplegic with progressive, partial recovery of hindlimb function seen over several weeks. Two-factor
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ANOV A revealed a statistically significant main effect of treatment and time for the Ba-Be-Br (p = 0.000115 and p = 3.65⁻¹², respectively) and IP (p = 4.05⁻⁷ and p = 1.84⁻³⁵, respectively) over the entire 6-week testing period. Post-hoc multivariate parametric analyses of the different levels of treatment indicated higher mean Ba-Be-Br and IP scores for riluzole- compared with phenytoin- and CNS5546A-treated animals (p < 0.05).

Within-week comparisons demonstrated that animals treated with riluzole consistently presented with higher mean Ba-Be-Br scores at each time trial (Fig. 2A). Riluzole treatment improved locomotor function from extensive single joint movement at Week 1 to consistent weight-supported stepping with marginal coordination at Weeks 5–6; 8.3–8.7° respectively) and from control animals at Weeks 2 to 4 and 6 (9.6–11.9° ± 1.8°) compared with those of phenytoin (4.9–6.6° ± 1.8–1.9°) or CNS5546A at Week 6 (7.8 ± 1.6°) (p < 0.05).

Riluzole-treated animals showed higher mean IP scores at each test week (Fig. 2B). The riluzole-treated animals’ IP scores were significantly greater than those of the phenytoin-treated and control animals at Week 1 (24.6° ± 2.9° compared with 15.4° ± 2.6° and 14.6° ± 3.4°, respectively) and from control animals at Weeks 2 to 4 and 6 (36.9–46.5° ± 2.9–3.6°, 50.4° ± 3.7° compared with 23.9–37.3° ± 3.1–3.9°, and 41.5° ± 3.4°, respectively) (p < 0.05). Treatment with phenytoin and CNS5546A also resulted in higher mean IP scores compared with controls across the 6-week trial; however, statistical significance was achieved only in Week 2 (30.0–34.6° ± 2–3 compared with 23.9° ± 3.9°, p < 0.05). Moreover, both phenytoin and CNS5546A showed similar rates of improvement in IP scores compared with the other two treatment groups in the first 4 weeks and thereafter reached a plateau.

Effects of Na⁺ Channel Blockers on the Integrity of Descending Axons After Compressive SCI

The survival of descending motor tract axons was assessed in a subsample of randomly selected animals from each treatment group as described previously (four animals per treatment). Fluorogold-labeled neurons in the magnocellular and parvocellular red nucleus; raphe magnus, obscurus, and pallidus; reticular formation (nucleus giganto-cellaris and paragigantocellularis, and caudal pontine nuclei); vestibular nuclei (lateral, medial, spinal, and superior); and RVLM (caudal aspect) were counted following spinal cord transection and fluorogold implantation distal to the injury site.

Univariate parametric ANOVAs were performed on total neuron counts, revealing a statistically significant difference between treatments in the red nucleus (p = 0.02) but not in the raphe nuclei (p = 0.74), reticular formation (p = 0.63), vestibular nuclei (p = 0.94), or RVLM (p = 0.54). Post-hoc Student-Newman-Keuls tests indicated that riluzole-treated animals had significantly higher mean neuron counts in the red nucleus (p < 0.05) and that riluzole produced a trend toward enhancement of survival of neurons within all brainstem nuclei when compared with the other treatments. A summary of the mean neuron counts is presented in Table 1. Quantitative analysis of surviving red nucleus neurons indicated differences in cellular character-
istics among the four treatment groups, including cellular
diameter, dendritic arborization, and extent of fluorogold
filling. A univariate parametric ANOVA revealed statisti-
cally significant different mean cell diameters between
treatment groups ($p = 0.002$). Riluzole-treated animals pre-
sented with the largest mean cellular diameter ($26.06 \pm 0.926 \mu m$)
compared with phenytoin-treated ($18.42 \pm 0.926 \mu m$), CNS5546A-
treated ($16.16 \pm 0.926 \mu m$), and control animals ($18.73 \pm 0.926 \mu m$), ($p < 0.05$). Representative examples of labeled neurons in the
red nucleus are shown in Fig. 3.

Effects of Na+ Channel Blockers on the Preservation of
Spinal Cord Tissue After Compressive SCI

Morphometrical assessments were performed to quantify
the dimensions of the cavity and the degree of tissue
preservation in the representative subsamples (four animals
per treatment). There was a significant difference in
preserved residual tissue between treatment groups ($p = 3.43 \times 10^{-17}$; two-factor ANOVA) and distances from in-
jury epicenters ($p = 1.71 \times 10^{-109}$; two-factor ANOVA)
with riluzole treatment, resulting in the greatest preserva-
tion of residual tissue compared with phenytoin,
CNS5546A, or control ($p < 0.05$). Based on these obser-
vations we performed an analysis on proportional residual
cross-sectional tissue and cavity areas at the epicenter in
addition to discrete-interval (0.5 mm rostral and caudal)
analyses on proportional cross-sectional residual tissue
areas surrounding the epicenter.

The injury epicenter, which represented the width of the
clip, encompassed 1 mm of tissue surrounding the sec-
tions with the greatest cavity/tissue ratio, which was taken
to represent the focal point of the injury epicenters. In this

### TABLE 1

Neuron counts after retrograde labeling with fluorogold

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Red Nucleus (range)</th>
<th>Raphe Nuclei (range)</th>
<th>Reticular Formation (range)</th>
<th>Vestibular Nuclei (range)</th>
<th>RVLM (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>riluzole</td>
<td>$1442.5 \pm 299.7$</td>
<td>$576.8 \pm 129.2$</td>
<td>$1819.3 \pm 281.2$</td>
<td>$591.5 \pm 240.6$</td>
<td>$86.8 \pm 25.8$</td>
</tr>
<tr>
<td>phenytoin</td>
<td>$396.3 \pm 116.5$</td>
<td>$408.5 \pm 226.5$</td>
<td>$1026.8 \pm 780.3$</td>
<td>$487.3 \pm 442.6$</td>
<td>$40.0 \pm 31.5$</td>
</tr>
<tr>
<td>CNS5546A</td>
<td>$526.3 \pm 109.8$</td>
<td>$338.5 \pm 115.4$</td>
<td>$985.5 \pm 517.9$</td>
<td>$348.8 \pm 262.2$</td>
<td>$37.5 \pm 16.5$</td>
</tr>
<tr>
<td>control</td>
<td>$733.8 \pm 273.5$</td>
<td>$467.8 \pm 115.4$</td>
<td>$1359.5 \pm 228.0$</td>
<td>$402.0 \pm 171.8$</td>
<td>$82.5 \pm 42.6$</td>
</tr>
</tbody>
</table>

* $p < 0.05$ compared with other groups.

**FIG. 3.** Fluorescence micrographs showing retrogradely fluorogold labeled neurons in the red nucleus 7 weeks after a
severe spinal cord compression injury. Red arrowhead indicates the large-diameter cells, typifying magnocellular neu-
rons, preferentially spared in the riluzole group compared with the other treatments.
region, the lesion consisted of a central cavitation with a loose network of nonneuronal cells surrounded by an incomplete subpial rim of white matter, with the occasional presence of the most peripheral elements of gray matter (Fig. 4). Univariate ANOVAs indicated significant differences in residual tissue (p = 1.86 × 10^{-11}) and cavity (p = 2.38 × 10^{-9}) among treatments at the epicenter. Riluzole treatment significantly increased residual tissue and decreased cavity areas compared with the other treatments (p < 0.05) (Fig. 5). In addition, phenytoin and CNS5546A treatment resulted in significant enhancement of residual tissue and reduction of cavity areas compared with the control (p < 0.05). Significant tissue sparing by riluzole was also evidenced 1 mm and 1.5 mm caudal to the epicenter compared with the control (p < 0.05). Similarly, CNS5546A treatment resulted in a significantly greater proportion of residual tissue area 1 mm caudal to the epicenter compared with the control (p < 0.05).

Spinal cord sections were further analyzed using volumetric techniques. Normalized white matter, gray matter, and cavity volumes were assessed at discrete increments (1 mm, 1.5 mm, and 2 mm) both rostral and caudal to the injury epicenter. The proportional volumes of the three components at each increment are illustrated in Fig. 6.

An overall significant difference between the components at each increment was found (p < 0.0001; two-factor ANOVA). Post-hoc analysis indicated that the volume of white matter was significantly greater than that of both gray matter and cavity; in addition, gray matter volume was increased compared with that of the cavity (p < 0.05). When the effects of treatment on the components’ volumes were assessed (two-factor ANOVA), overall significantly greater gray matter and reduced cavity volumes emerged (p = 7.84 × 10^{-11} and p = 6.20 × 10^{-7}, respectively), with riluzole having the greatest effect on both volumes over all other treatments (p < 0.05). Moreover, significant interactions among tissue and cavity volumes emerged between increments (p = 2.40 × 10^{-5}) and drug treatments (p = 1.02 × 10^{-13}). Because of the aforementioned findings, a series of univariate ANOVAs with post-hoc testing were applied to the normalized data at specific rostrocaudal increments to ascertain the nature of these interactions. Although an overall treatment effect on white matter volume did not emerge, significance was achieved (p ≤ 0.05) at specific rostrocaudal sites (1.5 mm and 2 mm rostral and 2 mm caudal to the epicenter). At 1.5 mm rostral to the epicenter, riluzole significantly increased white matter volume compared with CNS5546A and control treatments (p < 0.05); however, at 2 mm rostral both riluzole and phenytoin significantly increased volumes compared with the control treatment (p < 0.05) (Fig. 6B and C). At the most distal caudal increment (Fig. 6F) the greatest white matter volume was associated with CNS5546A treatment, which was significantly larger than that of riluzole (p < 0.05). Differences in proportional gray matter volumes as a result of drug effects were seen at every rostrocaudal increment (p ≥ 0.02). Riluzole had a marked effect on gray matter volumes at 1 mm rostral, 1.5 mm and 2 mm caudal, and 1 mm caudal compared with all other treatments or the control alone, respectively (p <
Conversely, control treatment significantly increased gray matter volumes over riluzole, phenytoin, and CNS5546A treatments at 1.5 mm and 2 mm rostral, 1 mm and 2 mm rostral, and 1 mm rostral, respectively (p < 0.05) (Fig. 6A–F). With regard to cavity volume, significant treatment effects were seen at all caudal increments (p ≤ 0.005). Riluzole had a marked effect on reduced cavity volumes when compared with phenytoin (p
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Discussion

In the present study, we have demonstrated significant amelioration of traumatic SCI sequelae with systemically administered riluzole compared with other selective Na\(^+\) channel blockers (phenytoin and CNS5546A). A single bolus of riluzole administered intraperitoneally 15 minutes postinsult effectively reduced the extent of locomotor and equilibrium-related deficits characteristic of a severe cervical SCI.\(^{32}\) These functional neurological recovery indices were supported by histopathological evidence indicating significant long-term tissue sparing at and around the site of mechanical spinal cord compression; significant increase in the number and size of retrogradely labeled neurons in the red nucleus; and a trend to increase neuron counts in other motor tract–associated brainstem nuclei.

Previous findings in rodent models of CNS ischemia and trauma demonstrated riluzole-induced attenuation of...
neuropathological consequences including reduced cortical infarct volume, neuropathological consequences including reduced cortical infarct volume,39,87 and histological and behavioral abnormalities.52,74,88

White matter protection by riluzole has been also demonstrated in an acute in vivo model of compressive SCI. Stutzmann and colleagues15 showed reduced lesion volume with notable white matter protection, recovery of neuronal somatosensory evoked potentials, and qualitatively assessed functional improvement with low-dose riluzole (2 mg/kg) administered twice daily for 10 consecutive days following trauma. Our findings expand riluzole’s therapeutic effectiveness to include long-term tissue sparing at and around the site of spinal cord compression. Previous studies have established a relationship between white matter sparing and chronic hindlimb function following traumatic SCI.10,11,54,84 Moreover, with fluorescent retrograde tracing we found greater neuron counts and preferential sparing of large-diameter cells in the red nucleus and a trend toward increasing counts of neurons in the raphe, reticular, and vestibular nuclei in rats treated with riluzole. In reports from our laboratory strong associations have been described between the persistence of axons originating from these nuclei and residual hindlimb locomotor function in rats after SCI.15 In addition, preservation of large-diameter cells typifying the magnocellular portion of the red nucleus strongly correlates with functional neurological recovery following SCI.33,88 Although the role of magnocellular rubrospinal projections in human motor activity are not well known, in the rat these projections are understood to contribute to localized motor activity involving flexor muscles and distal segments.56 Thus, our findings demonstrate that riluzole effectively preserves the integrity of descending axons associated with locomotor behavior in the rat and are directly translatable to the observed behavioral recovery.

Involvement of Na+ Channels in the Pathophysiology of Neural Injury

Neurotrauma is associated with a primary mechanical injury resulting in focal destruction of neural tissue at the site of insult, followed by a secondary injury involving sequential pathological alterations to residual tissue.10,11,32,65

The deregulation of Na⁺ homeostasis is a key secondary injury mechanism, the pathogenesis of which is marked by increasing intracellular shifts beginning within 5 minutes of spinal cord trauma and progressing over a 60-minute interval.46 Trauma-induced accumulation of [Na⁺]i triggers a biochemical cascade involving 1) stimulation of intracellular phospholipase activity,1,37 2) activation of Ca²⁺ influx and a cytotoxic edema,64 and 5) excitotoxic cell death.82 These deleterious events, downstream of Na⁺ influx, are associated with acute and indirect neurotoxicity involved in secondary gray matter and white matter degeneration.1,15,21,46,62,70 It follows that voltage-sensitive Na⁺ channels are an important therapeutic target for the treatment of secondary CNS injury. This assumption relies on the following findings: 1) distinct rat brain Na⁺ channel subtypes are uniquely distributed in neuronal cell bodies, fiber tracts and axons, and glial cells;20,34,39,69 2) voltage-sensitive Na⁺ channels mediate Na⁺-induced cytotoxicity related to secondary CNS injury;35,61,63,73,83 3) CNS trauma models demonstrating effective attenuation of posttraumatic axonal dysfunction in vitro, and neuroprotective anatomical and functional efficacy in vivo with potent Na⁺ channel neurotoxins;25,70,84,90,94 and 4) attenuation of acute CNS pathophysiology with therapeutic Na⁺ channel blockers such as local anesthetics,24,41,42 antiarrhythmics,75,71 and anticonvulsants.86,99

Although neuroprotective pharmacological compounds described as Na⁺ channel blockers share similar pharmacodynamic properties, including neurotoxin–receptor site 2 affinity and suppression of Na⁺ currents, their distinct interactions with different channel subtypes and states produces different pharmacological profiles and dissimilar neuroprotective potencies.13,16,71 Pharmacokinetic suitability is also important given the short therapeutic window allotted by secondary injury processes following traumatic SCI.17 Although a pharmacokinetic profile for the experimental compound CNS5546A has yet to be determined, riluzole and phenytoin are understood to have similar properties.15,36 Pharmacodynamic comparisons between riluzole and phenytoin indicate that the former more effectively inhibits persistent Na⁺ channel activation by batrachotoxin25,44,61 and voltage-evoked Na⁺ currents and guanidine uptake in the rat cortical brain slice.16,33 In addition, riluzole has a unique ability to inhibit both resting and inactivated TTX-sensitive and TTX-resistant Na⁺ channels, which could facilitate neuroprotection by enhancing cellular resistance to sustained, acute trauma-induced depolarizing conditions.72

Riluzole’s neuroprotective effects have been also ascribed to antagonism of neurotransmitter release by EAAs,76 which is attributed to its therapeutic effectiveness in amyotrophic lateral sclerosis, a disorder characterized by neuronal cell death.5,66,67 Thus, inhibition of excitotoxicity could explain our results given riluzole relatively spared gray matter to a greater extent than white matter. Although, riluzole has no affinity for any known sites of ionotropic or metabotropic glutamate receptors and its ability to inhibit NMDA or α-amino-3-hydroxy 5-methyl-4-isoxazole propionate/kainic acid evoked EAA release is significant only at concentrations that are on the order of several magnitudes greater than that administered in this study.1,12 These properties suggest that a primary inhibition of excitotoxicity cannot explain our observed neuroprotective effects.

Alternatively, modulation of other molecular targets affected by [Na⁺], including Na⁺-K⁺-adenosine triphosphase, Na⁺-H⁺ and Na⁺-Ca²⁺ exchangers, Na⁺-dependent glutamate and anion transporters, or glutamate and GABA uptake mechanisms would, however, be a rational neuroprotective strategy.1,40,42,44,68,70 Of importance, riluzole’s neuroprotective effectiveness has been also attributed to its ability to modulate the Na⁺ dependency of these metabolic transporters and exchangers.86 In addition, riluzole has been shown to stimulate the production of trophic activities in spinal astrocyte culture monolayers.57 These aforementioned properties of riluzole may be relevant to understanding the mechanism by which this drug exerted both functional and neuroanatomical protection in the present study.

Conclusions

Our findings confirm the hypothesis that voltage-sensi-
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tive Na⁺ channel antagonism is an effective neuroprotective strategy for reducing long-term functional and neuroanatomical deficits sustained after traumatic SCI. Moreover, the Na⁺ channel blocker riluzole effectively mitigated chronic histopathology and improved behavioral recovery in rats after SCI. Riluzole should be considered an important therapeutic candidate for this form of CNS trauma.

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