Pharmacology of riluzole in acute spinal cord injury

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Object. The aim of this paper was to characterize individual and population pharmacokinetics of enterally administered riluzole in a Phase 1 clinical trial of riluzole as a neuroprotective agent in adults 18–70 years old with acute spinal cord injury (SCI).

Methods. Thirty-five individuals with acute SCI, American Spinal Injury Association Impairment Scale Grades A–C, neurological levels from C-4 to T-12, who were enrolled in the Phase 1 clinical trial sponsored by the North American Clinical Trials Network for Treatment of Spinal Cord Injury, received 50 mg riluzole twice daily for 28 doses. The first dose was administered at a mean of 8.7 ± 2.2 hours postinjury. Trough plasma samples were collected within 1 hour predose, and peak plasma samples were collected 2 hours postdose on Days 3 and 14 of treatment. Riluzole concentrations were quantified by high-performance liquid chromatography assay. The data were analyzed for individual and population pharmacokinetics using basic structural and covariate models. The pharmacokinetic measures studied were the peak concentration (Cmax), trough concentration (Cmin), systemic exposure (AUC0–12), clearance (CL/F), and volume of distribution (V/F) normalized by the bioavailability (F).

Results. The Cmax and AUC0–12 achieved in SCI patients were lower than those in ALS patients on the same dose basis, due to a higher CL and larger V. The pharmacokinetics of riluzole (Cmax, Cmin, AUC0–12, CL, and V) changed during the acute and subacute phases of SCI during the 14 days of therapy. It was consistently observed in patients at all clinical sites that Cmax, Cmin, and AUC0–12 (128.9 ng/ml, 45.6 ng/ml, and 982.0 ng × hr/ml, respectively) were significantly higher on Day 3 than on Day 14 (76.5 ng/ml, 19.1 ng/ml, and 521.0 ng × hr/ml, respectively). These changes resulted from lower CL (49.5 vs 106.2 L/hour) and smaller V (53.7 vs 1297.9/L) on Day 3. No fluid imbalance or cytochrome P 1A2 induction due to concomitant medications was identified during the treatment course to account for such increases in V and CL, respectively. Possible mechanisms underlying these changes are discussed.

Conclusions. This is the first report of clinical pharmacokinetics of riluzole in patients with SCI. The Cmax and AUC0–12 achieved in SCI patients were lower than those in ALS patients on the same dose basis, due to a higher clearance and larger volume of distribution in SCI patients. The finding in SCI patients of an increase in the clearance and distribution of riluzole between the 3rd and 14th days after SCI, with a lower plasma concentration of riluzole on the 14th day, stresses the importance of monitoring changes in drug metabolism after SCI in interpreting the safety and efficacy of therapeutic drugs that are used in clinical trials in SCI. Clinical trial registration no.: NCT00876889. (http://thejns.org/doi/abs/10.3171/2012.5.AOSPINE12112)

Key Words • riluzole • pharmacokinetics • spinal cord injury

Abbreviations used in this paper: ALS = amyotrophic lateral sclerosis; ALT = alanine transaminase; AST = aspartate transaminase; AUC = area under the plasma concentration curve; CL = clearance; Cmax = peak concentration; Cmin = trough concentration; CYP = cytochrome P; GGT = γ-glutamyl transpeptidase; HED = human equivalent dose; HPLC = high-performance liquid chromatography; MVBF = microvascular gastrointestinal blood flow; NACTN = North American Clinical Trials Network; [Na+]i = intracellular sodium; SCI = spinal cord injury; SMA = spinal muscular atrophy; t1/2 = half-life; ULN = upper limit of normal; V = volume of distribution.
abnormalities, ischemia-reperfusion injury, glutamate excitotoxicity, and disturbances in ionic homeostasis, oxidative cell injury, and an extensive inflammatory response.5,41

Clinical guidelines for the management of SCI have been established and widely accepted by physicians treating patients who have sustained SCI.21 These guidelines include stabilization of the vertebrae and cardiopulmonary and metabolic support of the patient. However, beyond supportive care, there are no medical or surgical treatments that have been clearly demonstrated to improve functional outcome after SCI in humans. Clinical trials with methylprednisolone (NASCIS [National Acute Spinal Cord Injury Study] II and III),22 GM-1 ganglioside,17 fampridine (4-aminopyridine),22,23,38 and lithium carbonate40,59 have provided suggestive but equivocal evidence of benefit.

In light of the overwhelming impact of SCI on the individual, new therapeutic interventions are urgently needed. Compelling evidence exists that riluzole, a sodium-channel blocking agent with antiglutamatergic activity, offers promise for improving the outcome of SCI.

Molecular Mechanisms of Riluzole as Potential Neuroprotective Agent

Riluzole (Fig. 1), a benzothiazole anticonvulsant Na+ channel blocker, received FDA approval in 1995 for the treatment of patients with ALS, a progressive neurodegenerative disorder characterized by motor neuron and corticospinal tract degeneration.7,26,28 The standard regimen is fixed oral doses of 50 mg twice daily.

There are potential merits of riluzole, as an Na+ channel blocker, to offer neuroprotective activity in primary immediate (<2 hours) and early acute (<48 hours) injury phases of SCI. Spinal cord injury results in a deleterious accumulation of [Na+], within neurons;52 the resulting membrane depolarization associated with cellular inability to remove [Na+] favors further Na+ influx via noninactivating Na+ channels.

The neuroprotective effects of Na+ channel blockade are likely exerted on neurons and spinal cord axons to reduce intracellular increases in [Na+] and to reverse operation of axonal Na+/Ca2+ exchangers. In addition, Na+ channel blockade may preserve spinal cord white matter by preventing the disruption of the axonal Na+/H+ antiporter system, as shown in tetrodotoxin damage,40 to maintain compound action potentials after acute compression in an ex vivo model of SCI.2 Riluzole is also known to inhibit presynaptic Ca2+-dependent glutamate release.56

Studies have demonstrated that riluzole is neuroprotective and promotes functional neurological recovery in various species of animal models of brain and spinal cord ischemic and traumatic injury.2,4,24,30,45 Other authors have reported that the effects of riluzole are synergistic with those of methylprednisolone, which is the only drug used in routine clinical practices to attempt to attenuate secondary injury effects after SCI.35 In a recent study of prolonged administration of riluzole in Huntington disease, no benefit was found in slowing disease progression, but riluzole was well tolerated. Adverse effects were virtually similar in 357 patients treated with riluzole and in 180 patients receiving placebo. Thirteen patients had elevation of liver enzymes, and 5 patients discontinued treatment due to the elevation.29 Notably, riluzole is without potent neurotoxic and cardiotoxic adverse effects,5 even though potential hepatotoxicity has been noted.29 Therefore, the use of riluzole as a therapy for SCI is potentially feasible. It has been approved by the FDA for ALS36 at a dose of 100 mg/day.

The dose for the present Phase I trial was selected using human data and scaling from animal data (Clinical trial registration no.: NCT00876889). From the human data the most conservative approach based on the FDA-approved dose for patients with ALS was used. In confirmatory dose ranging (50, 100, 200 mg/day) studies of riluzole for ALS,28 a daily dose of 100 mg (50 mg twice daily) of riluzole was confirmed to have the best benefit-risk ratio.

From animal data, the HED for SCI patients was allogrometrically scaled from the animal dose (6 mg/kg twice daily) in SCI female Wistar rats, weighing 250–300 g.43 based on the power equation from FDA Guidance for Industry (2005): HED = Animal Dose (mg/kg) × (animal wt/human wt in kg)0.33 = (6 mg/kg twice daily) × (0.25 kg/70 kg)0.33 = 0.92 mg/kg twice daily = 64.2 mg/70 kg twice daily. The trial dose of 50 mg twice daily is within the HED, 64.2 mg twice daily, scaled from the tolerable dose in rats. Riluzole has been administered for prolonged periods of time to patients with ALS. The duration of riluzole treatment was selected based on current understanding of the duration of sodium- and glutamate-mediated secondary injury after SCI to encompass a period of 14 days after injury.41

Pharmacology of Riluzole—Pharmacokinetics

The pharmacology of riluzole includes the pharmacokinetics (absorption, distribution, metabolism, and excretion) and pharmacodynamics (effects on improving motor and sensory senses, adverse effects in elevating hepatic enzymes) of the agent. This article is focused on the pharmacokinetics of riluzole.

The pharmacokinetics of riluzole have been established in healthy individuals,32,33 young and old, as well as in patients with ALS31,39 and pediatric patients with SMA.1 In humans, riluzole has been administered orally at a dose of 50 mg twice daily, or 50 mg daily in SMA patients.1 The half-life of riluzole is 12 hours. Most drugs reach steady-state plasma concentrations in 4–5 half-lives, and the same is assumed for riluzole at 48–60 hours after the dose.

Riluzole is highly protein bound to serum albumin and lipoproteins (96%), like phenytoin, which poses potential concerns for drug-drug interactions with other concomitant medications that compete for protein binding. In patients taking such concomitant medications, a higher concentration of free riluzole in the plasma, resulting from the competition, will be anticipated to exert a greater therapeutic activity.

Riluzole is metabolized in the liver by an enzyme of the CYP 450 family, which has multiple CYP isozymes. Most of the drug-metabolizing enzymes are in the CYP 1, 2, and 3 families. Riluzole is specifically metabolized by the CYP 1A2 subfamily extensively, with only 2% of the
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Physical-Chemical Properties:

Chemical name: 2-amino-6-(trifluoromethoxy) benzothiazole

Molecular mass: 234.2

Description: Riluzole is a white to slightly yellow powder

Solubility: Riluzole is highly soluble in dimethylformamide, dimethylsulfoxide (DMSO) and methanol, freely soluble in dichloromethane, sparingly soluble in 0.1 N HCl and very slightly soluble in water and in 0.1 N NaOH.

pKa: 3.8

Partition Coefficient: Octanol/Water is about 3000

Log P: 3.5

Melting Point: Between 117°C and 120°C.

Fig. 1. Chemical structure and physical-chemical properties of riluzole.

dose recovered unchanged in the urine. Smoking is known to induce CYP 1A2. In addition, the care of patients with SCI may include the administration of methylprednisolone, which is a substrate and inducer of CYP 3A4 and 2C19, and may indirectly affect the hepatic clearance of riluzole. Therefore, smoking history and other concomitant medications of CYP 1A2 substrates, inhibitors, or inducers may affect riluzole blood concentrations.

The substrates of CYP1A2 include acetaminophen, caffeine, theophylline, and warfarin. The inhibitors include tacrine (Cognex), omeprazole (Prilosec), quinolone antibiotics, erythromycin, and oral contraceptives. Co-administration of riluzole with these drugs can increase riluzole blood concentrations. The inducers, including carbamazepine, phenobarbital, phenytoin, St John’s wort, ritonavir, and smoking, can decrease blood concentrations of riluzole.

Absorption. In SCI there may be reduced bioavailability (F) and prolonged peak time (t max) of oral medications that are commonly prescribed for patients with SCI, such as acetaminophen, theophylline, dantrolene, carbamazepine, 4-aminopyridine, cyclosporine A, and baclofen. The underlying causes are impaired gastric emptying and intestinal motility, as well as reduced MVBF. Moreover, it is also recognized that injury above T-6 induces significant reduction in MVBF to the gastrointestinal tract and liver, more than that in injury below T-6.

Distribution. Distribution implies transporting the drug to tissues and ultimately to cells throughout the bloodstream. This process depends on several factors, including cardiac output, systemic macro- and microcirculation, and drug-protein binding. Population-specific alterations in drug distribution kinetics are unavailable. However, patients with SCI commonly have hypoproteininemia that alters the plasma protein binding of highly

Alterations of Pharmacokinetics of Drugs in SCI

The SCI population is heterogeneous, and possible changes in pharmacokinetics may depend on variables of injury characteristics (intensity, level, and time elapsed after injury), pharmacological properties of the drug, and the route of administration. Based on the knowledge of SCI effects on the pharmacokinetics of drugs reported in the past 26 years (1985–2011), we may anticipate the following alterations of pharmacokinetics of riluzole in patients with acute SCI from that in healthy individuals.

Absorption. In SCI there may be reduced bioavailability (F) and prolonged peak time (t max) of oral medications that are commonly prescribed for patients with SCI, such as acetaminophen, theophylline, dantrolene, carbamazepine, 4-aminopyridine, cyclosporine A, and baclofen. The underlying causes are impaired gastric emptying and intestinal motility, as well as reduced MVBF. Moreover, it is also recognized that injury above T-6 induces significant reduction in MVBF to the gastrointestinal tract and liver, more than that in injury below T-6.

Distribution. Distribution implies transporting the drug to tissues and ultimately to cells throughout the bloodstream. This process depends on several factors, including cardiac output, systemic macro- and microcirculation, and drug-protein binding. Population-specific alterations in drug distribution kinetics are unavailable. However, patients with SCI commonly have hypoproteininemia that alters the plasma protein binding of highly
bound drugs and results in increase of distribution, as known with ketamine, lorazepam, amikacin, and cefotiam, ranging from 20% (amikacin) to 70% (cefotiam).44

Riluzole is a highly plasma protein binding drug (96% bound; fraction unbound = 0.04) and will be sensitive to a change of fraction unbound, since only the free drug molecules are transported to interstitial fluid.

Metabolism. Hepatic clearance related to drug metabolism has been reported to decrease in patients with SCI for phenacetin, methylprednisolone, and cyclosporine A. The underlying causes can be a reduced MVBF in liver, enzyme synthesis, or protein binding singly and in combination.

In SCI, reduction in the MVBF in the liver, spleen, and skeletal muscle occurs in the acute phase of SCI and peaks at approximately 24 hours after the injury. The reduction is more pronounced after a high thoracic complete lesion than a low one. These alterations are likely due to a redirection of blood flow to maintain an adequate perfusion of the brain and heart.12

The decrease of hepatic blood flow due to SCI will reduce the hepatic metabolism clearance (ClH) of drugs with high hepatic extraction ratios (high E = ClH/Q ≥ 0.7), such as phenacetin, methylprednisolone, and cyclosporine A. In contrast, biotransformation of low-extraction drugs (E ≤ 0.3), such as most nonsteroidal antiinflammatory drugs, does not depend on liver blood flow but on liver intrinsic enzymatic activity (CLR). The ClH of drugs with an intermediate hepatic extraction ratio (0.7 > E > 0.3), such as riluzole whose E = 0.67, will be affected by all 3 factors—hepatic blood flow, liver intrinsic enzyme activity, and fraction unbound.

Elimination (Excretion). Decreased renal clearance (ClR) and prolonged half-time have been reported with amikacin, cefotiam, doxycycline, ketamine, diclofenac, vancomycin, and lorazepam, due to a decrease in renal function. Riluzole is excreted unchanged in urine at only 2% of the dose, and urinary excretion may not be significantly affected by SCI.

Methods

Riluzole Phase 1 Trial

A Phase 1 trial of riluzole was conducted as a multisite, single-arm active treatment pilot study with an enrollment goal of 36 patients. The primary aim of the trial was to obtain data on safety and pharmacokinetics of riluzole in patients who had sustained an acute traumatic SCI.

Patient Recruitment for Riluzole Clinical Phase 1 Trial at NACTN. A total of 36 patients with SCI were enrolled at 6 sites; 35 patients completed the full dosing regimen. Inclusion criteria were as follows: patient age from 18 to 70 years; written informed consent from the patient; no other life-threatening injury; SCI injury level in the region of C-4 to T-12; ASIA Impairment Scale Grade A, B, or C; no cognitive impairment that would preclude obtaining informed consent (including moderate or severe traumatic brain injury); and dosing time within 12 hours of injury. Exclusion criteria were as follows: hypersensitivity to riluzole or any of its components; inability to receive riluzole orally or via a nasogastric tube; history of liver or kidney disease (for example, hepatitis A, B, or C, or cirrhosis); a recent history of regular substance abuse (illicit drugs or alcohol); unconsciousness; penetrating SCI; pregnancy as established by urine pregnancy test; current involvement in another SCI research study; presence of a mental disorder or other illness, which in the view of the site investigator, would preclude accurate evaluation; inability to commit to the follow-up schedule; is a prisoner; and inability to converse, read, or write English at the elementary school level.

Treatment With Riluzole (Rilutek). Patients received riluzole (Rilutek, Sanofi-Aventis, supplied by the hospital pharmacy at each center) 50 mg by oral or nasogastric administration every 12 hours, starting within 12 hours of injury for 28 doses. On the 3rd and 14th days, plasma samples were collected 1 hour predose and 1 or 2 hours postdose for trough and peak concentrations, respectively. All other treatments were per standard of care. Five patients received concomitant methylprednisolone.

Pharmacokinetic Evaluation

Specific Aims. The specific aims of the pharmacokinetic evaluation were to determine the individual peak and trough concentrations of riluzole on Day 3 and Day 14 and to derive individual pharmacokinetic parameters of half-life (t1/2), systemic exposure (AUC0–12), volume of distribution (V/F), and clearance (CL/F) normalized by bioavailability (F), with 2 concentration-time data and population pharmacokinetics using NONMEM, version 7.2.0 (ICON Development Solutions) for basic structural and covariate models.

Plasma Sampling. Plasma blank control (5 ml) and 2 plasma samples for peak and trough concentrations on both Days 3 and 14 were collected by centrifugation of blood samples immediately at 2700 G for 10 minutes, then stored at ~80°C (or at least as low as −20°C) prior to the shipment with dry ice to the Pharmacology Center of NACTN at the University of Houston, College of Pharmacy at the Texas Medical Center. The blood samples were labeled to conceal patient identity.

Plasma samples (instead of serum samples) were collected, because it has been established that riluzole concentrations in plasma and serum are comparable at a concentration less than 500 ng/ml.4 With a standard drug regimen of 50 mg twice daily, riluzole serum concentrations are in the range of 20–250 ng/ml. The plasma samples retaining clotting factors will have less variability than serum samples.

HPLC Assay of Riluzole Plasma Concentrations. The HPLC chromatogram demonstrated that the riluzole peak with retention time of 9.0 minutes was well resolved from methylprednisolone (6.1 minutes), acetaminophen (2.1 minutes), and other potential concomitant medications (Fig. 2). The detailed descriptions of the HPLC assay...
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development and validation, as well as plasma sample preparation, are listed in Appendix 1.

Individual Pharmacokinetic Analysis. Individual pharmacokinetics were evaluated using 2 concentration-time data on each day (Day 3 and Day 14) to obtain the elimination rate constants (k), then using the standard pharmacokinetic equations (Appendix 2) to estimate other parameters of clearance (CL/F) and volume of distribution (V_F) normalized by the bioavailability (F). AUC_0–12 and AUC_0–∞ were calculated using the trapezoidal rule.

Population Pharmacokinetic Analysis. The model development, selection, and evaluation are described in detail in Appendix 3.

Plasma Protein Binding. To evaluate the extent of free (unbound) riluzole in human plasma, ultrafiltration was used. Centrifree YM-30 devices (Millipore Ireland Ltd.) were used. One-milliliter human plasma samples were added into the ultrafiltration device and centrifuged at 1000 G with a fixed angle rotor.

Monitoring of Potential Hepatotoxicity and Metabolic Status. Blood for monitoring the levels of liver enzymes was drawn on admission and on Days 3, 7, 10, and 14. The enzymes monitored were alanine transaminase, aspartate transaminase, GGT, and alkaline phosphatase. Albumin and bilirubin were also monitored, as were the hemogram, platelets, electrolytes, glucose, creatinine, prothrombin time, and International Normalized Ratio.

Results

Patient Demographics

Thirty-six patients with SCI were enrolled between April 12, 2010, and June 20, 2011, to receive 50 mg riluzole twice daily for 28 doses. The first dose was administered at 8.7 ± 2.2 hours postinjury. Thirty-five patients completed the 2-week regimen. Riluzole administration was stopped in 1 patient on Day 7 because the patient had a moderate elevation of GGT on that day. This patient had received a large number of medications, and a definite relationship of the elevated GGT to riluzole could not be established. Blood tests at 6 months revealed normal liver enzymes. The basic demographics of the patients with plasma samples available and evaluable for Day 3, Day 14, and both days are summarized in Table 1. The mean patient age (± SD) was 39.4 ± 18.3 years (range 18–69 years), with a mean body weight of 83.0 ± 16.9 kg and a mean height of 68.7 ± 4.2 in. Among the 35 patients receiving 28 doses of riluzole, 6 were female. One-third of the patients were smokers. The ASIA Impairment Scale grades were A (in 52.8% of patients), B (in 25%), and C (in 22.2%). The levels of neurological injury were C4–8 (in 77.8% of patients), T1–6 (in 13.9%), or T7–12 (in 8.3%).

Distinct Alteration of Riluzole Pharmacokinetics in Patients With SCI During the 2-Week Period Postinjury

The plasma profiles of riluzole on Days 3 and 14 were constructed for individual patients as represented by those of 1 patient (Fig. 3). The C_max and trough concentration C_min were derived from the quantified samples. The C_max (mean ± SE) achieved with the 50 mg twice daily dose varied significantly among patients: 128.8 ± 13.8 ng/ml (range 23.9–409.2 ng/ml) (n = 33) on Day 3 and 76.2 ± 13.7 ng/ml (8.5–317.0 ng/ml) (n = 32) on Day 14. The C_min was of large intersubject variability as well: 45.6 ± 6.8 ng/ml (8.4–183.8 ng/ml) on Day 3 and 19.1 ± 2.5 ng/ml (2.8–61.2 ng/ml) on Day 14. The declines of C_max and C_min on Day 14 from those of Day 3 were significant by nonparametric test (p < 0.05), and they were consistently observed in individual patients from all clinical sites. The
extents of reduction were 68.6% and 56.5% for \( C_{\text{max}} \) and \( C_{\text{min}} \), respectively.

The systemic exposures of riluzole from the treatment, \( \text{AUC}_{0-12} \) (truncated for each dosing interval of 12 hours) were calculated from individual plasma profiles using the trapezoidal rule. The mean \( \text{AUC}_{0-12} (\pm \text{SE}) \) was 982.0 \( \pm \) 111.2 ng \( \times \) hr/ml and 521.0 \( \pm \) 87.3 ng \( \times \) hr/ml for Day 3 and Day 14, respectively (Table 2), and exhibited the same trend of decline in \( C_{\text{max}} \) and \( C_{\text{min}} \) for Day 14 from Day 3 on the same dose basis (Fig. 4).

The pharmacokinetic parameters of clearance (CL/F), volume of distribution (V/F), and biological half-life (\( t_{1/2} \)) were derived using standard pharmacokinetic equations in Appendix 2 and compared with those from the final population pharmacokinetics model.

The population pharmacokinetics model was best represented by a 1-compartment first-order absorption and elimination model that included interindividual and intraindividual variability. The parameter estimates given by this model are summarized in Table 3. The absorption constant (\( k_a \)) proved to be difficult to estimate. Fixing \( k_a \) to values from 0.5 to 100/hour did not affect the estimates of the other parameters and the fit of model, indicating that those values are equally probable, based on the available data. Therefore, \( k_a \) was fixed to 5/hour.

The basic 1-compartment pharmacokinetic model that incorporated interindividual and intraindividual variability was retained for covariate model building. The covariates that were introduced into the clearance model did not significantly improve the fit of the basic model (objective function values > 3.8, \( p < 0.05 \)). The tested co-

**TABLE 1: Demographics of patients**

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<td>39.44 ( \pm ) 18.34</td>
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<td>18–69</td>
</tr>
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<td>T7–12</td>
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</tbody>
</table>

* Values represent the number of patients (%) unless specified otherwise.

The t1/2 remained as 10.6–11.9 hours on Day 3 and Day 14 (Table 2). The higher \( C_{\text{max}} \), \( C_{\text{min}} \), and \( \text{AUC}_{0-12} \) observed on Day 3 as compared with those on Day 14 resulted from the lower CL and smaller V on Day 3.

In comparing the pharmacokinetic parameters of riluzole in SCI patients with those in healthy volunteers (Table 2), the \( C_{\text{max}} \) on Day 3 and Day 14, as well as \( \text{AUC}_{0-12} \) on Day 14, was lower than those in healthy volunteers. However, it may be difficult to determine the cause of this difference due to the different doses given (9 doses32 and 20 doses33 in healthy volunteers versus a maximum of 6 doses on Day 3 and 28 doses on Day 14 in the present trial).

**Individual and Population Pharmacokinetic Parameters**

The individual pharmacokinetic parameters were estimated using 2 concentration-time data on each day (Day 3 and Day 14) to obtain the elimination rate constant and other parameters using the equations described in Appendix 2 and compared with those from the final population pharmacokinetics model.

The population pharmacokinetics model was best represented by a 1-compartment first-order absorption and elimination model that included interindividual and intraindividual variability. The parameter estimates given by this model are summarized in Table 3. The absorption constant (\( k_a \)) proved to be difficult to estimate. Fixing \( k_a \) to values from 0.5 to 100/hour did not affect the estimates of the other parameters and the fit of model, indicating that those values are equally probable, based on the available data. Therefore, \( k_a \) was fixed to 5/hour.

The basic 1-compartment pharmacokinetic model that incorporated interindividual and intraindividual variability was retained for covariate model building. The covariates that were introduced into the clearance model did not significantly improve the fit of the basic model (objective function values > 3.8, \( p < 0.05 \)). The tested co-

**Fig. 3.** Typical pharmacokinetic profiles of riluzole in human plasma samples for Days 3 and 14. The riluzole dose of 50 mg was given at 0 time. Plasma concentrations of riluzole are shown in a blood sample taken 2 hours after dose (peak value) and in a blood sample taken at 12 hours after dose, prior to the next dose (trough value). Open symbols denote the calculated values, and solid symbols denote the measured values.
The results of the riluzole population pharmacokinetics model evaluation revealed that the final model provided a reliable description of the data with good precision of parameter estimates. The stratified nonparametric bootstrap procedure resulted in 95% CIs for population pharmacokinetics parameter estimates, which are presented in Table 3.

The diagnostic plots from the fit of the final model are presented for both Day 3 and Day 14 (Fig. 6), which confirmed that the current sampling schedule (2 blood samples for peak and trough concentrations, respectively) was adequate to characterize the pharmacokinetics of riluzole for future clinical trial in patients with SCI.

### Plasma Protein Binding

Retrospectively, the plasma samples in 10 patients were reassayed for free fractions (fractions unbound) of riluzole. The fractions unbound were comparable between Day 3 and Day 14 (mean ± SE 6.18% ± 0.42% and 9.57% ± 1.09%, respectively) and could not account for the significantly larger V on Day 14.

### Safety Data

**Medical Complications.** The incidence and types of medical complications in the Phase 1 study group were similar to those in a comparable group of patients who were matched for demographic and injury characteristics, whose clinical courses were recorded in the NACTN Registry of SCI patients admitted to the NACTN clinical centers. In the present study, the number of complications by organ/system within 30 days of admission were as follows: 16 pulmonary complications (11 patients); 19 infections including pneumonia (14 patients); 5 cardiovascular complications (5 patients); 5 gastrointestinal complications (4 patients); 5 skin complications (4 patients); 9 hematological complications (7 patients); 6 psychiatric complications (5 patients); and 5 neurological complications (5 patients).

### Table 2: Pharmacokinetic parameters of riluzole in spinal cord–injured patients and healthy volunteers*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spinal Cord–Injured Patients Individual Estimation</th>
<th>Healthy Volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3 (n = 32)</td>
<td>Day 14 (n = 27)</td>
</tr>
<tr>
<td>dose</td>
<td>50 BID</td>
<td>50 BID</td>
</tr>
<tr>
<td>sex (M/F)</td>
<td>28/4</td>
<td>23/4</td>
</tr>
<tr>
<td>mean age (yrs)</td>
<td>41.15 ± 3.17</td>
<td>39.63 ± 3.22</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>128.86 ± 14.03 (10.9%)</td>
<td>76.46 ± 15.04 (19.7%)§</td>
</tr>
<tr>
<td>AUC0–12 (ng × hr/ml)</td>
<td>982.03 ± 111.18 (11.3%)</td>
<td>521.01 ± 87.32 (16.6%)§</td>
</tr>
<tr>
<td>AUC0–∞(ng × hr/ml)</td>
<td>2101.99 ± 441.09 (21.0%)</td>
<td>807.83 ± 111.26 (13.8%)§</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>49.47 ± 7.77 (15.7%)</td>
<td>106.20 ± 19.80 (18.6%)§</td>
</tr>
<tr>
<td>V_F (L)</td>
<td>557.06 ± 73.80 (13.2%)</td>
<td>1287.88 ± 218.92 (16.9%)§</td>
</tr>
<tr>
<td>k (hr⁻¹)</td>
<td>0.095 ± 0.009 (9.3%)</td>
<td>0.101 ± 0.010 (9.7%)</td>
</tr>
<tr>
<td>t₁/₂ (hr)</td>
<td>11.91 ± 2.18 (18.3%)</td>
<td>10.61 ± 2.23 (21.0%)</td>
</tr>
</tbody>
</table>

* Mean values are ± SE; when this construction is followed by a value in parentheses, the value is the relative standard error (the standard error divided by the mean). Abbreviations: AUC0–∞= the area under the curve (calculated by Dose/[CL/F]); BID = twice daily; CL/F = apparent oral clearance; k = elimination rate constant; RSE = relative standard error (the standard error divided by the mean and expressed as a percentage); V_F = apparent volume of distribution.

† Based on data from Le Liboux et al.33
‡ Based on data from Le Liboux et al.32
§ Statistical difference between Day 3 and Day 14 using the nonparametric test (sign), p < 0.05.
Liver Enzyme and Bilirubin Elevations. An elevation of ALT, AST, or GGT, singly or in combination, occurred on one or more of the 4 days on which liver enzyme levels were monitored. Elevations were classified as mild (> ULN to 2.5 times ULN), moderate (> 2.5 to 5 times ULN), or severe (> 5 to 20 times ULN). The highest level obtained was used to classify the severity of elevation for each patient. For each of these enzymes, mild and moderate elevations occurred in the following proportions: ALT: mild 42%, moderate 28%; AST: mild 44%, moderate 19%; GGT: mild 36%, moderate 14%; ALP: mild 14%, moderate 3%. A severe elevation of ALT (6 times the ULN) occurred in 1 patient, of AST (5.5 times the ULN) in another, and of GGT (7 times the ULN) in a third patient; all returned to normal at the 3- and 6-month follow-up examinations. The levels fluctuated over the 14-day course in individual patients; even if they were elevated on 1 of the days of monitoring, the levels did not necessarily continue to increase.

There was mild elevation of bilirubin in 4 patients and moderate elevation in 1 patient. No patient had an elevated bilirubin on Day 14. No relationship was found between elevation of enzymes and AUC<sub>0–12/kg</sub>.

Discussion

Riluzole pharmacokinetics in SCI was distinguished from those in ALS, as well as SMA in children. The C<sub>max</sub> and AUC<sub>0–∞</sub> in SCI patients on the same dose basis did not achieve the comparable levels as in ALS patients, but were lower (128.8 ng/L and 827.8 ng x hr/ml on Day 3, and 76.5 ng/ml and 337.8 ng x hr/ml on Day 14) compared with those in patients with ALS (231 ng/ml and 3409 ng x hr/ml) and SMA (371 ng/ml and 2257 ng x hr/ml). The decreased bioavailability (F) in SCI may be due to reduced GI absorption. The apparent clearance (CL/F) and volume of distribution (V<sub>F</sub>) in the SCI population, 60.4–148 L/hour and 663–2080 L, were substantially higher than those in the ALS population (25.9 L/hour and 361 L) and SMA patients (22.2 L/hour and 299 L) (Table 4).

The difference of CL between Day 3 and Day 14 post-SCI may result from the following potential causes: 1) Impaired hepatic metabolic clearance shortly after the early acute phase (≤ 48 hours) on Day 3, due to decreased hepatic microvascular blood flow and hepatocyte gene expression. Riluzole is an intermediate hepatic extraction drug whose hepatic metabolism would be decreased by lower hepatic blood flow, similar to methylprednisolone and cyclosporine A. 2) Concomitant medications that are CYP 1A2 substrates, inducers, or inhibitors would affect the metabolism of riluzole by CYP 1A2. However, it was unlikely that any significant drug-drug interaction was accountable for the observed CL difference. After screening the medication charts of the patients, 21 medications were identified, namely, acetaminophen, fentanyl, oxycodone, Percocet, gabapentin, methylprednisolone, morphine, aspirin, tramadol, pregabalin, lorazepam, diphenhydramine, propofol, methadone, hydromorphone, ibuprofen, lidocaine, MS Contin, meperidine, Norco, and Vicodin. Nevertheless, among the first 6 medications that were used by more than 5 patients, only acetaminophen is a known substrate of CYP 1A2. Acetaminophen was used by 6 patients on both Day 3 and Day 14.

The difference of V between Day 3 and Day 14 may have the following potential causes: 1) Fluid imbalance during the first 14 days. However, no apparent net gain in body fluid on Day 14 was recognized, based on patients’ fluid intake and output records. 2) Decreased protein binding of riluzole that would result in volume of distribution (V<sub>F</sub>) increase on Day 14. Riluzole is 96% bound to plasma proteins, mainly to albumin and lipoproteins over the clinical concentration range. Retrospectively, the plasma samples obtained in 10 patients were reassayed for free fractions of riluzole. The fractions unbound were comparable between Day 3 and Day 14 (mean ± SE) 6.18% ± 0.42% and 9.57% ± 1.09%, respectively) and could not account for the significantly larger V on Day 14.

The individual and population pharmacokinetic

![Fig. 5. Spaghetti plots of clearance (CL/F) (left) and volume of distribution (V<sub>F</sub>) (right) on Day 3 and Day 14. Twenty-four patients had both Day 3 and Day 14 data available. Symbols without lines connecting Days 3 and 14 have values only for Day 3 or Day 14.](image-url)
## TABLE 3: Comparison of population and individual estimated pharmacokinetic parameters of riluzole for Day 3 and Day 14*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 3</th>
<th>Day 14</th>
<th>Day 3</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE (n = 33)</td>
<td>Bootstrap 95% CI (n = 33)</td>
<td>Mean ± SE (n = 32)</td>
<td>Bootstrap 95% CI (n = 32)</td>
</tr>
<tr>
<td>C_{max} (ng/ml)</td>
<td>128.86 ± 14.03 (10.9%)</td>
<td>76.46 ± 14.03 (10.9%)</td>
<td>128.86 ± 14.03 (10.9%)</td>
<td>76.46 ± 14.03 (10.9%)</td>
</tr>
<tr>
<td>AUC_{max} (ng × hr/ml)</td>
<td>827.81 ± 337.84 (44.1%)</td>
<td>2101.99 ± 441.09 (21.0%)</td>
<td>827.81 ± 337.84 (44.1%)</td>
<td>2101.99 ± 441.09 (21.0%)</td>
</tr>
<tr>
<td>CL/F (L/hr)</td>
<td>60.4 ± 6.24 (12.3%)</td>
<td>59.4 (47.4 to 71.4)</td>
<td>148 ± 25.5 (17.2%)</td>
<td>133 (95.4 to 171)</td>
</tr>
<tr>
<td>V_F (L)</td>
<td>563 ± 391 (17.3%)</td>
<td>553 (339 to 715)</td>
<td>2080 ± 947 (45.5%)</td>
<td>1650 (523 to 2780)</td>
</tr>
<tr>
<td>ka (hr⁻¹)</td>
<td>0.095</td>
<td>0.071</td>
<td>0.095</td>
<td>0.071</td>
</tr>
<tr>
<td>τ (hr)</td>
<td>7.29</td>
<td>7.96</td>
<td>7.29</td>
<td>7.96</td>
</tr>
<tr>
<td>ωCL (%)</td>
<td>20.0 ± 18.3% (40.6%)</td>
<td>14.5% (1.56 to 27.4%)</td>
<td>41.7 ± 29.6% (56.6%)</td>
<td>33.9% (2.93 to 64.9%)</td>
</tr>
<tr>
<td>ωV (%)</td>
<td>0.13 ± 0.11% (79.1%)</td>
<td>0.16% (0.009 to 0.3%)</td>
<td>0.02 ± 0.01% (37.2%)</td>
<td>0.006% (0.002 to 0.01%)</td>
</tr>
<tr>
<td>α-proportional error (%)</td>
<td>11.8 ± 2.27% (19.2%)</td>
<td>14.4% (8.68 to 20.1%)</td>
<td>20.6 ± 5.19% (25.2%)</td>
<td>18.2% (11.8 to 24.6%)</td>
</tr>
<tr>
<td>α-additive error (ng/ml)</td>
<td>13.8 ± 11.9 (86.2%)</td>
<td>30.1 (−63.4 to 124)</td>
<td>2.43 ± 3.24 (133%)</td>
<td>2.57 (−5.68 to 10.8)</td>
</tr>
</tbody>
</table>

* Mean values are ± SE; when this construction is followed by a value in parentheses, the value is the relative standard error (the standard error divided by the mean and expressed as a percentage). Abbreviations: ka = absorption rate constant; ω = residual variability; σ = interindividual variability.
† Statistical difference between Day 3 and Day 14 using a nonparametric test (sign), p < 0.05.
‡ Fixed parameter.

### HPLC Quantification

#### Sample Preparation for HPLC Quantification

Two hundred microliters of human plasma was mixed with 10 μl of a solution of 5-MOP (10 g/ml), an internal standard. After the addition of 1 ml acetonitrile, the mixture was vortexed and then centrifuged. Of the clear organic layer, about 1 ml was evaporated by rotary evaporation. The residue was reconstituted with 1 ml of 50% methanol in water. A 200 μl aliquot of the sample was used for HPLC analysis.

#### HPLC Conditions

The HPLC assay was performed using a Waters system equipped with a Symmetry C18 guard column (2.1 × 15 mm, 3.5 μm) followed by a Symmetry C18 column (3.0 × 10 mm, 3.5 μm) and eluted at the flow rate of 0.8 ml/min. The mobile phase of acetonitrile:water:methanol (50:49:1, by vol) with a flow rate of 0.3 ml/min was used. Detection was at 263 nm. The assay was linear from 7.8 to 1000 ng/ml, with a lower limit of quantification of 7.8 ng/ml. The mean recoveries of riluzole from human plasma samples ranged from 72% to 85%. The accuracy and precision were within 7% of the mean.

#### Appendix 1: Validation of HPLC Assay for Riluzole

The HPLC assay developed was validated for the quantification of riluzole in small volumes of plasma or serum using a Waters system equipped with a Symmetry C18 column (2.1 × 15 mm, 3.5 μm) and eluted at the flow rate of 0.8 ml/min. The mobile phase of acetonitrile:water:methanol (50:49:1, by vol) with a flow rate of 0.3 ml/min was used. Detection was at 263 nm. The assay was linear from 7.8 to 1000 ng/ml, with a lower limit of quantification of 7.8 ng/ml. The mean recoveries of riluzole from human plasma samples ranged from 72% to 85%. The accuracy and precision were within 7% of the mean.
following standard pharmacokinetic equations: \( t_{1/2} = \frac{0.693}{k} \) and \( \text{AUC}_{0-\infty} = \text{AUC}_{0-t} + C_t/k \), where \( C_t \) was the trough concentration calculated from the last sampling time: \( \text{CL/F} = \frac{\text{Dose}}{\text{AUC}_{0-\infty}} \) and \( \text{V}_F = \frac{\text{CL}}{k} \).

Appendix 3: Population Pharmacokinetic Analysis

The population pharmacokinetic analysis for repeated measures was conducted via nonlinear mixed-effects modeling using software of NONMEM version 7.2.0. The population pharmacokinetic software is capable of analyzing clinical data with sparse samples, deviated sampling time, and/or missing samples. Riluzole plasma concentration-time data were fitted by 1 compartment structural pharmacokinetic models with first-order absorption and elimination, taking intraindividual variability (\( \sigma \)) and interindividual variability (\( \omega \)) into consideration in building the model. Riluzole concentration observations that were below the analytical assay quantification limit or any values that were otherwise missing were excluded from the analysis. Model selection was guided by various goodness-of-fit criteria, including diagnostic scatter plots, plausibility of parameter estimates, and precision of parameter estimates.

Covariate regression modeling was also attempted with covariates of demographic factors (age, sex, and body weight), laboratory parameters (creatinine clearance), and smoking status. The covariate model was developed using forward addition and backward elimination approaches. The final population pharmacokinetic model was evaluated using a stratified nonparametric bootstrap and a predictive check.

Disclosure

This study was supported by Telemedicine and Advanced

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TABLE 4: Comparison of population pharmacokinetic parameters in acute spinal cord–injured patients versus patients with ALS and SMA*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PopPK in SCI Patients</th>
<th>ALS</th>
<th>SMA†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 14</td>
<td></td>
</tr>
<tr>
<td>dose 50 BID</td>
<td>50 BID</td>
<td>50 BID</td>
<td>50 BID</td>
</tr>
<tr>
<td>no. of patients</td>
<td>33</td>
<td>32</td>
<td>169‡/179§</td>
</tr>
<tr>
<td>sex (M/F)</td>
<td>28/5</td>
<td>26/6</td>
<td></td>
</tr>
<tr>
<td>age (yrs)</td>
<td>41.15 ± 3.17</td>
<td>39.63 ± 3.22</td>
<td>55.0 ± 0.9§</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng/ml)</td>
<td>128.86 ± 14.03 (10.9%)¶</td>
<td>76.46 ± 15.04 (19.7%)¶</td>
<td>231 ± 5‡</td>
</tr>
<tr>
<td>( \text{AUC}_{0-\infty} ) (ng × hr/ml)</td>
<td>827.81</td>
<td>337.84</td>
<td>3409 ± 220 (70 kg);‡</td>
</tr>
<tr>
<td>( \text{CL/F} ) (L/hr)</td>
<td>60.4 ± 6.24 (10.3%)‡</td>
<td>148 ± 25.5 (17.2%)</td>
<td>25.9 ± 14.72; 51.4 (7.2%)§</td>
</tr>
<tr>
<td>( \text{V}_F ) (L)</td>
<td>663 ± 103 (16.3%)‡</td>
<td>2080 ± 947 (45.5%)</td>
<td>361 (10.1%)§</td>
</tr>
<tr>
<td>( t_{1/2} ) (hr)</td>
<td>7.29</td>
<td>9.76</td>
<td>4.93</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (hr)</td>
<td>1 (1–5) (N)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All mean values are ± SE. Abbreviations: C = parameters calculated by compartmental model; N = parameters calculated by noncompartmental model; PopPK = population pharmacokinetics; QD = once daily; \( T_{\text{max}} \) = peak time (the time to reach the peak concentration, \( C_{\text{max}} \) of the plasma profile).
† Data obtained from Abbara et al., 2011.
‡ Data obtained from Groeneveld et al., 2003.
§ Data obtained from Bruno et al., 1997.
¶ From individual estimation.
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Author contributions to the study and manuscript preparation include the following. Conception and design: Chow, Grossman. Acquisition of data: Toups, Aarabi, Harrop, Shaffrey, Johnson, Boakye, Frankowski, Fehlings. Analysis and interpretation of data: Chow, Teng. Drafting the article: Chow, Teng. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Chow. Statistical analysis: Chow, Teng.

References


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