AWARD NUMBER: W81XWH-10-1-0897

TITLE: Improving the Efficiency and Efficacy of Glibenclamide in Limiting Progressive Hemorrhagic Necrosis Following Traumatic Spinal Cord Injury

PRINCIPAL INVESTIGATOR: Phillip G. Popovich, Ph.D.

CONTRACTING ORGANIZATION: Ohio State University
Columbus, Ohio 43210-1063

REPORT DATE: October 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.
Abstratc
Preclinical work has demonstrated that glibenclamide administration improves outcomes in rat models of spinal cord injury, with the principal mechanism of action being amelioration of post-traumatic hemorrhagic necrosis (PHN). We hypothesize that some but not all patients with spinal cord injury, principally those with incomplete lesions, will respond to glibenclamide therapy. Our goal is to determine whether MRI and circulating biomarkers can be used as early markers of injury that can be used to predict which patients may benefit from glibenclamide treatment. During the first year of this grant acute MRI images (6 and 24 hours post-injury) were collected from (n=36) rats subject to spinal contusion injury. Both the severity and location of injury were changed to create six different experimental groups: (1) Midline (M) severe (50 mm); (2) M moderate (25 mm); (3) M light (12.5 mm); (4) Lateral (L) severe (50 mm); (5) L moderate (25 mm) and (6) L light (12.5 mm). Preliminary analyses of these data reveal that injury location and severity affect the rate and magnitude of secondary hemorrhage. In year 2, we compared the efficacy of glibenclamide in the two most consistent injury models (i.e., groups 2 vs. 5; moderate midline vs. lateral SCI). Data from these studies were recently published and indicate that glibenclamide is beneficial in both models of cervical SCI; however, the magnitude of benefit was greatest when the magnitude and extent of primary hemorrhage was limited during the first 24h (i.e., moderate lateral SCI).
# TABLE OF CONTENTS:

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>Body</td>
<td>2-3</td>
</tr>
<tr>
<td>Key Research Accomplishments</td>
<td>3</td>
</tr>
<tr>
<td>Reportable Outcomes</td>
<td>3</td>
</tr>
<tr>
<td>Conclusion</td>
<td>3</td>
</tr>
<tr>
<td>References</td>
<td>3</td>
</tr>
<tr>
<td>Appendices</td>
<td>4</td>
</tr>
</tbody>
</table>
INTRODUCTION:

The magnitude of acute post-traumatic hemorrhagic necrosis (PHN) is an early prognostic indicator of long-term functional recovery in human spinal cord injury (SCI). Recent preclinical data indicate that PHN can be reduced and functional recovery improved in spinal injured rats using glibenclamide (Glib), an FDA approved anti-diabetic drug that targets SUR1 receptors on endothelia. We recently generated new data suggesting that only a subset of spinal cord injuries will respond to Glib. This may also be true for other therapies that are designed to attenuate PHN. We predict that this is because of spinal cord anisotropy and differences in gray and white matter elasticity. Accordingly, studies in Aim 1 used a rat model of spinal contusion injury to determine whether varying the location and severity of trauma influences the magnitude of primary hemorrhage and the spread of secondary hemorrhage, i.e., PHN. Acute T2 MRI data show that injury location and severity of injury affect the rate and magnitude of PHN. In year 2, a recent publication documents the efficacy of glibenclamide in the context of varying the parameters of primary SCI. Namely, although glibenclamide was efficacious in all SCI rats, the most striking anatomical and functional improvements were observed when primary hemorrhage/edema was localized to the spinal hemi-cord during the first 24 hours. Ongoing experiments continue to explore the utility of using MRI as an acute biomarker for predicting glibenclamide efficacy. Also, optimal protocols are being designed to determine whether serum biomarkers will be useful as a cross-referencing strategy with acute MRI to predict glibenclamide efficacy and outcome after SCI. We predict that only a subset of animals will respond to Glib therapy and that the range of injuries created in our animal models will be analogous to at least two major subclasses of human SCI: (1) those that are neurologically complete within 24 hours post-injury and that are largely untreatable by available methods and (2) those that are incomplete but are at risk to slowly evolve into complete lesions as a result of PHN.

BODY:

The data reported below were completed at The Ohio State University and The University of Maryland and correspond with the approved statement of work. Relevant Aims, Tasks and milestones are highlighted.

Aim 1: Compare patterns of acute post-hemorrhagic necrosis, delayed secondary neurodegeneration and chronic functional recovery after varying the location and magnitude of a spinal contusion injury.

Milestone 1: Create optimized T2 MRI protocols that enable an accurate measurement of acute intraspinal hemorrhage in the different models of SCI.

- We have successfully used MRI to visualize gross lesion morphology and, with University of Maryland, we have applied T2-weighted MRI techniques to confirm that glibenclamide reduces acute intraspinal hemorrhage and/or post-traumatic hemorrhagic necrosis (see below). However, at Ohio State, we
continue to perfect the MRI methodology. Even though T2 MRI images have been acquired, we are experiencing difficulties in distinguishing the pathologic significance and boundaries of MRI hypo- and hyper-intensities. Early after injury, these radiologic signatures correlate beautifully with bleeding and edema as predicted (also see Fig. 1 in Simard et al.; appendix). However, at later times post-injury, these correlations are not absolute and subtle differences between injury types make boundary distinctions difficult. Histologic correlations and ex vivo MRI analyses are needed to help inform the true nature of the hyper/hypointensities present in vivo MRI images. Histologic analyses are ongoing and a no-cost extension of existing funds is needed to complete additional MRI studies.

**Milestone 4: Document injury-dependent efficacy of glibenclamide based on T2 MRI**

- Completed. As shown in the attached manuscript (Simard et al., 2013), glibenclamide has a clear effect on reducing intraspinal hemorrhage. Specifically, glibenclamide decreases hemorrhage, as measured in vivo from MRI T2 hypointensities, ~50% by 24h post-injury. These data indicate that acute MRI analyses could have prognostic value in evaluating drug efficacy (e.g., comparing MRI signatures within 24h across treated/non-treated subjects). As described above, ongoing studies are using MRI to determine if changes in injury location differentially affect intraparenchymal hemorrhage at early and chronic post-injury time periods and whether specific types of SCI may be more amenable to glibenclamide therapy than other types (Milestone 4). Future attempts to intervene using glibenclamide will be based on robustness of data from these ongoing experiments.

**KEY RESEARCH ACCOMPLISHMENTS:**

- We published data indicating that MRI can be used to detect dynamic changes in lesion size following glibenclamide therapy.

**REPORTABLE OUTCOMES:**


**CONCLUSION:**

Acute bolus infusion of glibenclamide reduces intraspinal hemorrhage by ~50% within 24h of spinal cord injury. T2-weighted MRI has early prognostic value in predicting neuroprotection following glibenclamide therapy.

**REFERENCES:**


**APPENDICES:**

SUPPORTING DATA: See attached manuscript
MRI evidence that glibenclamide reduces acute lesion expansion in a rat model of spinal cord injury

JM Simard1,2,3, PG Popovich4, O Tsymbalyuk1, J Caridi1, RP Gullapalli5, MJ Kilbourne6 and V Gerzanich1

Study design: Experimental, controlled, animal study.

Objectives: To use non-invasive magnetic resonance imaging (MRI) to corroborate invasive studies showing progressive expansion of a hemorrhagic lesion during the early hours after spinal cord trauma and to assess the effect of glibenclamide, which blocks Sur1-Trpm4 channels implicated in post-traumatic capillary fragmentation, on lesion expansion.

Setting: Baltimore.

Methods: Adult female Long–Evans rats underwent unilateral impact trauma to the spinal cord at C7, which produced ipsilateral but not contralateral primary hemorrhage. In series 1 (six control rats and six administered glibenclamide), hemorrhagic lesion expansion was characterized using MRI at 1 and 24 h after trauma. In series 2, hemorrhagic lesion size was characterized on coronal tissue sections at 15 min (eight rats) and at 24 h after trauma (eight control rats and eight administered glibenclamide).

Results: MRI (T2 hypodensity) showed that lesions expanded 2.3 ± 0.33-fold ($P<0.001$) during the first 24 h in control rats, but only 1.2 ± 0.07-fold ($P>0.05$) in glibenclamide-treated rats. Measuring the areas of hemorrhagic contusion on tissue sections at the epicenter showed that lesions expanded 2.2 ± 0.12-fold ($P<0.001$) during the first 24 h in control rats, but only 1.1 ± 0.05-fold ($P>0.05$) in glibenclamide-treated rats. Glibenclamide treatment was associated with significantly better neurological function (unilateral BBB scores) at 24 h in both the ipsilateral (median scores, 9 vs 0; $P<0.001$) and contralateral (median scores, 12 vs 2; $P<0.001$) hindlimbs.

Conclusion: MRI is an accurate non-invasive imaging biomarker of lesion expansion and is a sensitive measure of the ability of glibenclamide to reduce lesion expansion.

Keywords: spinal cord injury; glibenclamide; riluzole; Sur1-Trpm4 channel; MRI; progressive hemorrhagic necrosis

INTRODUCTION

Spinal cord injury (SCI) remains one of the foremost unsolved challenges in medicine. Worldwide, the incidence of SCI ranges from 10 to 83 per million people per year, with half of these patients suffering a complete lesion and one-third becoming tetraplegic.1 At present, little can be done to undo or repair the initial damage to spinal cord tissues, but great hope lies in reducing secondary injury processes triggered by the primary injury that increase the damage and worsen clinical outcome.

Histological studies on animal models of SCI have shown that early expansion of a hemorrhagic contusion is a common feature following trauma to the spinal cord. During the hours after a blunt impact, a dynamic process ensues wherein a hemorrhagic contusion slowly enlarges, resulting in the progressive autodestruction of spinal cord tissues.2–4 Discrete petechial hemorrhages appear, first around the site of injury and then in more distant areas.5 As petechial hemorrhages form and coalesce, the lesion gradually expands, with a characteristic region of hemorrhage that ‘caps’ the advancing front of the lesion. A small hemorrhagic lesion that initially involves primarily the capillary-rich gray matter doubles in size during the first 24 h after injury. The advancing hemorrhage results from delayed progressive catastrophic failure of the structural integrity of capillaries, a phenomenon termed ‘progressive hemorrhagic necrosis’.2–4

Progressive hemorrhagic necrosis has been linked to de novo upregulation of sulfonylurea receptor 1 (Sur1)—transient receptor potential melastatin 4 (Trpm4) channels (a.k.a., Sur1-regulated NCx, ATP channels)6 in microvessels.4,7,8 Sur1-Trpm4 channels have been shown to be responsible for the necrotic death of endothelial cells that results in delayed fragmentation of capillaries and formation of petechial hemorrhages. Gene suppression as well as pharmacological blockade of Sur1-Trpm4 channels prevents progressive hemorrhagic necrosis, reduces lesion size and significantly improves neurological function in rodent models of SCI.7–10

To date, the magnitude and time course of lesion expansion due to progressive hemorrhagic necrosis have been characterized in rat models by measuring the amount of extravasated blood in the spinal cords of animals that were killed at different times after trauma.4 Although an important measure of injury, quantifying the amount of extravasated blood may not accurately reflect the actual volume of compromised tissue, as this technique cannot distinguish between an
increase in the number of erythrocytes within a given volume of tissue versus an actual increase in volume of contused tissue affected by hemorrhage and edema. We hypothesized that magnetic resonance imaging (MRI) could be used to assess early lesion expansion non-invasively and to independently corroborate the existence of early lesion expansion due to progressive hemorrhagic necrosis after spinal cord trauma. Here, we used serial MRI scans obtained at 1 and 24 h after trauma to characterize lesion expansion, and we validated our MRI measurements by comparing them with measurements based on coronal tissue sections in a different group of rats with the same injury. In these experiments, we also examined the effect of glibenclamide on lesion expansion and on neurological function.

MATERIALS AND METHODS

Ethics statement

We certify that all applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research. Animal experiments were performed under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland, Baltimore, and in accordance with the relevant guidelines and regulations as stipulated in the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering. In accordance with ‘good laboratory practice’, different investigators blinded to injury-group conducted behavioral tests and analyzed the data.

Subjects and experimental series

These experiments were conducted on new series of animals distinct from those previously reported from this laboratory. Thirty-six female Long–Evans rats (250–300 gm; Harlan, Indianapolis, IN, USA) were used in two experimental series (Table 1). In series 1, 12 rats underwent an SCI (see below), six were administered glibenclamide (see below) and six served as untreated controls; these 12 rats were studied using MRI at 1 and 24 h after trauma, after which they were euthanized. In series 2, 24 rats underwent an SCI; eight were euthanized 15 min; of the remaining 16 rats, eight served as untreated controls and eight were administered glibenclamide; these 16 rats were assessed for locomotor function at 24 h, after which they were euthanized to obtain tissue sections for measurements of the hemorrhagic lesion area at the epicenter.

Sample size calculation

Previous experiments with the model used here suggested that an effect size (Cohen’s d) of 2.27 (means, 2 vs 1) was achievable. Prior experiments with the model used here suggested that an effect size of 2.27 (means, 2 vs 1) was achievable. Sample size calculation for a 2-sample comparison (z = 0.05; 2-tailed), an effect size of 2.27, and a desired power of 90% indicated a minimum sample size of six per group.

Table 1 Experimental series

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
</tr>
</tbody>
</table>
| **Series 1**  
(n=–6) |        |      |      |
| Group 1    | Control | MRI | MRI euthanize |
| Group 2    | Glibenclamide | MRI | MRI euthanize |
| **Series 2**  
(n=–8) |        |      |      |
| Group 1    | Control | Euthanize section spinal cord |        |
| Group 2    | Control | mBBB score euthanize | Section spinal cord |
| Group 3    | Glibenclamide | mBBB score euthanize | Section spinal cord |

Abbreviations: mBBB, modified (unilateral) Basso, Beattie and Bresnahan; n, number of rats
Magnetic resonance imaging
In series 1, all rats were imaged 1 and 24 h after trauma; four control rats also were imaged 6 h after trauma. The delay of 1 h in obtaining the ‘baseline’ MRI was necessitated by logistical considerations, principally the distance between the site where injuries were produced and the site where MRIs were obtained. MRI was performed on a 3.0 Tesla Siemens Trio MR scanner equipped with 18 receiver channels and high performance gradients (200 mT/m ms⁻¹). An eight-channel wrist coil was used to image the rats. Anesthetized (Ketamine, 60 mg kg⁻¹ plus Xylazine, 7.5 mg kg⁻¹, IP) animals were placed in the supine position with the ‘sweet spot’ of the coil centered on the cervical spine of the rat. The depth of anesthesia was monitored continuously during the imaging session.

The imaging sequences consisted of localizers in the three orthogonal planes. Following localization, T1-weighted magnetization prepared rapid acquisition gradient echo (MP-RAGE) images were acquired in the axial plane with adequate coverage of the injury using 64 slices over a field of view of 53 mm × 62 mm and an interpolated pixel resolution of 448 × 512. The imaging parameters were as follows: inversion time TI = 900 ms and TE/TR 4 ms/1900 ms for an acquisition time of 3 min and 53 s using an acceleration factor of 2. T2-weighted 3D spin-echo images were then acquired in the sagittal and the axial plane. For both planes, the imaging parameters were as follows: TE/TR = 62 ms/512 ms, 64 slices, slice thickness 0.3 mm over a field of view of 45 mm x 90 mm at an interpolated pixel resolution of 312 × 640 for a total acquisition time of 5 min and 24 s using parallel imaging with an acceleration factor of 2.

All images were reconstructed in three planes to visualize the extent of the lesion. The hemorrhagic portion of the contusion was identified as a hypointense region (due to the presence of hemoglobin) within the cord on T2-weighted images. These regions were demarcated on each slice, and the total volume of the lesion was determined.

Data analysis
T2-hypodensity volumes at 1 versus 24 h were analyzed using Student’s paired t-test. Areas of hemorrhagic contusion on tissue sections at the epicenter in three groups of rats (15 min untreated, 24-h vehicle, 24-h glibenclamide) were analyzed using ANOVA, with Bonferroni post hoc comparisons. Modified (unilateral) BBB scores in two groups of rats (vehicle vs glibenclamide) were analyzed using the Mann–Whitney U-test.

RESULTS
In series 1, we evaluated the expansion of the hemorrhagic contusion using serial MRI scans obtained at 1 and 24 h after trauma, with the T2 hypodensity being taken as a measure of extravasated blood.16–19 In control rats, a small T2 hypodensity was evident at the first examination, obtained 1 h after trauma (Figure 1a). Subsequent imaging obtained at 6 h (n = 4) and 24 h (n = 6) confirmed progressive enlargement of the hemorrhagic lesion (Figure 1a). In six control rats, the volume (mean ± s.e.) of the T2 hypodensity at 1 h after trauma was 1.52 ± 0.37 μl and, after 24 h, the volume increased 2.3-fold to 3.55 ± 0.87 μl (P < 0.01) (Figure 1b). By contrast, in six rats treated with glibenclamide 5 min after trauma, the volume of the T2 hypodensity at 1 h was 0.51 ± 0.21 μl and, after 24 h, the volume increased 1.2-fold to 0.59 ± 0.24 μl (P = 0.43) (Figure 1b).

In series 2, we evaluated the size of the hemorrhagic contusion using images of tissue sections taken through the epicenter of injury at 15 min and 24 h. We considered the hemorrhagic area observed at 15 min to represent the primary hemorrhage due to the trauma and the hemorrhagic area observed at 24 h to represent the primary hemorrhage plus the secondary hemorrhage, the latter attributable to progressive hemorrhagic necrosis.

At 15 min, a contusion was apparent that was confined largely to the ipsilateral hemicord, mostly the gray matter (Figure 2a, upper). In control rats, the hemorrhagic contusion at 24 h invariably involved a larger area, typically extending to the contralateral side (Figure 2a, middle). By contrast, in rats administered glibenclamide 5 min after trauma, the hemorrhagic contusion at 24 h typically occupied an area comparable to that observed at 15 min (Figure 2a, lower). Quantification of the hemorrhagic area showed that, in control rats, lesions were 2.2 ± 0.12-fold larger at 24 h compared with that at 15 min. By contrast, in glibenclamide-treated rats, lesions were 1.1 ± 0.05-fold larger at 24 h compared with that at 15 min (Figure 2b).

Commensurate with the favorable effect of glibenclamide on hemorrhagic lesion area, assessment of modified (unilateral) BBB scores at 24 h showed that glibenclamide was associated with significantly better neurological function. Median scores were 0 vs 9 (P < 0.001) for the ipsilateral hindlimb and 2 vs 12 (P < 0.001) for the contralateral hindlimb, for the control vs glibenclamide groups, respectively (Figure 2c).

DISCUSSION
The principal finding of the present study is that, based on measurements of MRI T2-lesion volume and measurements of hemorrhagic lesion area, there is a 2- to 2.5-fold increase in the hemorrhagic contusion that takes place during the first 24 h after blunt impact trauma to the spinal cord, in good agreement with previous measurements based on tissue hemoglobin.4 Previous histological and MRI studies in rats have characterized spinal cord lesions at various times after injury, but relatively few have examined temporal and spatial characteristics of lesion progression during the first hours after injury. To our knowledge, the earliest study...
Spinal Cord

4

793.7

312

amount of extravasated blood in tissues from the epicenter during the

235

using serial MRI scans in humans with cervical SCI.21 The importance
to the spinal cord. Recently, lesion expansion also was demonstrated

246

significant lesion expansion during the early hours after blunt impact

studies using different methods establish conclusively the existence of

471

epicenter of injury 15 min (top) and 24 h (middle and bottom) after impact,
in control rats (top and middle), and in a glibenclamide-treated rat

480

percentiles; line, median; small square, mean.

499

plots showing modified (unilateral) BBB scores for the ipsilateral and

509

plots showing modified (unilateral) BBB scores for the ipsilateral and

510

contralateral hindlimbs at 24 h, for the two groups of rats depicted in (b,

520

24 h Veh and 24 h Glib); box, 25th and 75th percentiles; × , 1st and 99th

percentiles; line, median; small square, mean.

575

addressing this question (weight drop; midline, lower thoracic/upper

lumbar) reported that, on H&E-stained sections, intramedullary hemorrhages involved an aggregate of 11% of the spinal cord area

at the level of maximal bleeding immediately after trauma and that

584

this increased 2.5-fold to 28% after 8 h.20 In our previous study

(weight drop; lateral C7), we reported a twofold increase in the

593

amount of extravasated blood in tissues from the epicenter during the

first 12 h after trauma.4 In an MRI study (0.5 mm compression for

30 msc; T7), the T2-lesion volume was found to expand ~1.5-fold

598

over 5.5 h.16 Together with the present study, these various animal

studies using different methods establish conclusively the existence of

significant lesion expansion during the early hours after blunt impact

to the spinal cord. Recently, lesion expansion also was demonstrated
using serial MRI scans in humans with cervical SCI.21 The importance

of these observations lies in the hope that, if early lesion expansion
can be halted, patients with acute SCI may suffer the least possible

secondary injury.

The model of cervical hemicord impact that we used here is
particularly well suited to examining the expansion of the primary
injury to the contralateral side. In this model, care is taken to obtain a
primary hemorrhage exclusively ipsilateral to the site of impact. This
model emphasizes the distinction between primary and secondary
hemorrhage, and it shows the influence of secondary hemorrhage on outcome.8 When primary hemorrhage is located exclusively
unilaterally, subsequent spread of the hemorrhagic lesion to the
contralateral side during the ensuing hours—by definition, secondary
hemorrhage—is readily discerned both histologically and functionally.
Moreover, if the primary hemorrhage is confined to one side and if
secondary expansion of the hemorrhagic lesion to the contralateral
side is prevented, the magnitude and importance of secondary

hemorrhage is readily appreciated. By contrast, with bilateral primary
hemorrhage, neurological dysfunction due to secondary hemorrhage is
more difficult to detect. When the primary hemorrhage already
occupies most of a spinal segment, expansion laterally of secondary
hemorrhage may be limited or functionally mute. Rostro-caudal
expansion would still occur but, depending on the spinal cord level
involved, for example, cervical vs thoracic, this may be difficult to
detect neurologically.

An important aspect of the present study is that it confirmed
previous observations that early lesion expansion can be prevented by
blocking either of the two subunits of the Sur1-Trpm4 channel.6
Lesion expansion is prevented by blocking Sur1, either pharma-

cologically with glibenclamide or repaglinide,4 or by gene suppression
with antisense oligodeoxynucleotide or gene deletion of Abcc8,8 or by
blocking Trpm4, either pharmacologically with flufenamic acid7 or
riluzole,9 or by gene suppression with antisense oligodeoxynucleotide
or gene deletion of Trpm4.7

In the present study examining acute outcomes at 24 h, in three
other series from our laboratory examining outcomes at 1 or 6
weeks,4,8,10 and in one series from an independent laboratory,22

glibenclamide treatment beginning shortly after trauma was found
to be highly effective in reducing lesion size and improving

neurological function. In a 6th series of rats with outcomes evaluated at 6 weeks, treatment at the clinically more relevant time

of 3 h after trauma also was found to be highly beneficial.9 As might
be expected, the magnitude of the benefit observed with glibenclamide
depends on the magnitude of the primary injury,10 but all studies to
date examining functional outcome and lesion size at 6 weeks have
demonstrated a significant treatment effect, regardless of the initial
severity.8–10

In the 6 series to date with glibenclamide, drug was delivered by
constant subcutaneous infusion. From a pharmacokinetic perspective,
cutaneous delivery of glibenclamide is highly effective for maintaining
steady plasma levels, is superior to enteral administration and appears
to be equivalent to intravenous (IV) administration.23 Constant

subcutaneous infusion of glibenclamide was used in the preclinical
studies as a convenient alternative to constant IV infusion, as is used
with injectable glibenclamide (RP-1127) in clinical trials for other
CNS indications (ClinicalTrials.gov identifiers: NCT01454154;
NCT01268683; NCT01794182). In the animal studies, no clinically
relevant hypoglycemia or other toxicity has been detected with
infusions of 200 ng h−1 4,12,13 or 400 ng h−1.24 In a Phase I trial of
RP-1127 in 16 healthy volunteers (ClinicalTrials.gov identifier:
NCT01132703), a 3-day IV infusion (125 µg/h) produced no
clinically significant hypoglycemia or other serious adverse event
(S. Jacobson, personal communication).

A recent comprehensive review25 shows how difficult it can be to
translate preclinical trials on acute pharmacotherapeutic neuro-

protection to clinical trials in SCI. Among numerous challenges,
consideration must be given to the accumulated preclinical data on
any proposed therapeutic agent as well as the effect of that agent on
surrogate end points and functional outcome. The work reported here
shows that early MRI may be a useful imaging biomarker of lesion

expansion, a phenomenon that is prominent in human SCI,21 and

that may serve as a useful proxy for treatment efficacy.

Riluzole has been found to be efficacious in preclinical models of
SCI,26,27 may have a beneficial effect on motor outcome in cervical

SCI, as recently reported in a small open-label Phase I clinical trial,28

and is planned for study in a Phase II clinical trial of acute SCI
(ClinicalTrials.gov identifier, NCT01397518). A recent preclinical
study using a rat model of SCI, which was so severe as to have

Figure 2 Progressive expansion of hemorrhagic contusion assessed from
tissue sections at the epicenter. (a) Representative coronal tissue sections
of perfusion-cleaned but otherwise unprocessed spinal cords through the
epicenter of injury 15 min (top) and 24 h (middle and bottom) after impact,
in control rats (top and middle), and in a glibenclamide-treated rat
(bottom); the data shown are representative of eight rats per group. (b):
Fold-change in areas of hemorrhage at the epicenter in the three groups of
rats depicted in (a); mean ± s.e.; eight rats per group; ***P<0.001. (c) Box
plots showing modified (unilateral) BBB scores for the ipsilateral and
contralateral hindlimbs at 24 h, for the two groups of rats depicted in (b,
24 h Veh and 24 h Glib); box, 25th and 75th percentiles; × , 1st and 99th
percentiles; line, median; small square, mean.
attendant mortality, compared treatment with riluzole (2.5 mg·kg⁻¹ IP every 12 h x 1 week) vs glibenclamide (200 mg·h⁻¹ continuous subcutaneous infusion – 1 week), starting 3 h after trauma. This study found that glibenclamide is superior to riluzole in terms of both toxicity and efficacy. Riluzole exhibits a peculiar, dose-limiting CNS toxicity in the context of CNS trauma that is absent in non-injured controls: in SCI, mortality rates of 0, 8 and 70% were observed with 4, 6 and 8 mg·kg⁻¹ IP every 12 h, respectively. Riluzole is a pleiotropic drug with inhibitory effects on sodium channels, glutamatergic pathways and Trpm4. However, the near-identity of the phenotypes observed with riluzole compared with gene suppression of Sur1 as well as gene suppression of Trpm4 suggests that a major effect of riluzole in SCI is via inhibition of the Sur1–Trpm4 channel. Glibenclamide appears to be a safer choice than riluzole for targeting the Sur1–Trpm4 channel in acute CNS injury.

The present study has important shortcomings. First, it was not designed as an efficacy study, but rather to demonstrate the concept that MRI could be used to measure progressive lesion expansion in a rat model of SCI. Thus, the number of subjects studied was small, although the specific number was determined by a power analysis. The most important shortcoming was that the first MRI was obtained 1 h after trauma, whereas treatment with glibenclamide was administered 5 min after trauma. The long delay in obtaining the MRI was necessitated by logistical considerations, principally the distance between the site where injuries were produced and the site where MRIs were obtained. We believe that this problem with study design is the reason for the apparently smaller ‘baseline’ lesions in glibenclamide–10-h treatment window in a clinically relevant model of stroke. Transl Stroke Res 2012; 3: 286–295.

