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Spinal cord injury with unilateral versus bilateral primary hemorrhage — Effects of glibenclamide

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Abstract

In spinal cord injury (SCI), block of Sur1-regulated NC_{Ca}-ATP channels by glibenclamide protects penumbral capillaries from delayed fragmentation, resulting in reduced secondary hemorrhage, smaller lesions and better neurological function. All published experiments demonstrating a beneficial effect of glibenclamide in rat models of SCI have used a cervical hemicord impact calibrated to produce primary hemorrhage located exclusively ipsilateral to the site of impact. Here, we tested the hypothesis that glibenclamide also would be protective in a model with more extensive, bilateral primary hemorrhage. We studied the effect of glibenclamide in 2 rat cervical hemicord contusion models with identical impact force (10 g, 25 mm), one with the impactor positioned laterally to yield unilateral primary hemorrhage (UPH), and the other with the impactor positioned more medially, yielding larger, bilateral primary hemorrhages (BPH) and 6-week lesion volumes that were 45% larger. Functional outcome measures included: modified (unilateral) Basso, Beattie, and Bresnahan scores, angled plane performance, and rearing times. In the UPH model, the effects of glibenclamide were similar to previous observations, including a functional benefit as early as 24 h after injury and 6-week lesion volumes that were 57% smaller than controls. In the BPH model, glibenclamide exerted a significant benefit over controls, but the functional benefit was smaller than in the UPH model and 6-week lesion volumes were 33% smaller than controls. We conclude that glibenclamide is beneficial in different models of cervical SCI, with the magnitude of the benefit depending on the magnitude and extent of primary hemorrhage.

Keywords

Spinal cord injury; Hemorrhage; Glibenclamide

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Introduction

In spinal cord injury (SCI), inhibition of Sur1-regulated $\text{NC}_{\text{Ca-ATP}}$ channels by gene suppression in *Abcc8*^{-/-} mice or by antisense oligodeoxynucleotide directed against *Abcc8* mRNA, as well as pharmacological blockade of Sur1 using glibenclamide or repaglinide, protects penumbral capillaries from delayed fragmentation (Simard et al., 2007; Simard et al., 2010). As a result, the formation of secondary petechial hemorrhages in the penumbra is prevented, the total amount of hemorrhage that accumulates during the first 12 h after impact is halved, lesion volumes measured at 1 and 6 weeks are reduced 2–3-fold, and various measures of neurological function assessed out to 6 weeks are significantly improved compared to controls (Simard et al., 2007; Simard et al., 2010). Independent replication of some of these findings has been reported (Popovich et al., 2011).

To date, published experiments demonstrating a beneficial effect of glibenclamide after SCI in rats have used a cervical hemicord impact in which care was taken to obtain a primary hemorrhage exclusively ipsilateral to the site of impact. This model was used because it emphasizes the distinction between primary and secondary hemorrhage, and it shows the influence of secondary hemorrhage on outcome (Simard et al., 2010). 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 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.

Many spinal cord injuries, both clinical and experimental, involve bilateral primary injury that yields bilateral primary hemorrhage. Neurological dysfunction due to secondary hemorrhage is more difficult to detect with bilateral compared to unilateral primary injury. When the primary hemorrhage already occupies most of a spinal segment, expansion laterally of secondary hemorrhage may be limited or mute. Rostro-caudal expansion would still occur but, depending on the spinal cord level involved, this may be difficult to detect neurologically.

The question arose whether glibenclamide would have a beneficial effect in an injury model characterized by bilateral primary hemorrhage. Here, we examined this question by studying the effect of glibenclamide in two rat cervical hemicord impact models: in the first model, the impact force and location were identical to those previously described, which results exclusively in unilateral primary hemorrhage (UPH); in the second model, the same impact force was applied more medially, resulting in bilateral primary hemorrhage (BPH) and lesion volumes at 6 weeks that were 45% larger than in the UPH model. In brief, the effects of glibenclamide in the UPH model were similar to previous observations, including a significant neurological benefit that was apparent as early as 24 h after injury, and lesion volumes at 6 weeks that were 57% smaller than in controls. In the BPH model, glibenclamide was beneficial, but the neurological benefit and the reduction in lesion volume (33% smaller than in controls) were less than in the UPH model.

Methods

Subjects and experimental series

All experimental procedures were approved by the University of Maryland Institutional Animal Care and Use Committee. In accordance with “good laboratory practice”, different investigators blinded to injury-group conducted behavioral tests and analyses of data.

Fifty-eight female Long–Evans rats (250–300 g; Harlan, Indianapolis, IN) were used in 2 experimental series, the first to determine the pattern of primary and secondary hemorrhage in each model, and the second to study the effect of glibenclamide in each model. In series 1, 24 rats underwent SCI (see below), 12 with the impact delivered “laterally” (UPH model), and 12 with the impact delivered “medially” (BPH model). For rats in each impact group, 4 were euthanized at 15 min, 4 were administered vehicle, 4 were administered glibenclamide (see below) and the 8 rats were euthanized at 24 h. In series 2, 34 rats underwent SCI, 16 with the UPH model and 18 with the BPH model. For rats in each group, half received vehicle and half received glibenclamide. Rats in this series were maintained for 6 weeks after injury, during which they underwent serial measurements of locomotor function, and after which they were euthanized to measure lesion volumes.

Rat models of SCI

We used two rat models of unilateral cervical SCI that were slightly modified from the original description (Soblosky et al., 2001), one of which (the UPH model) was reported by us previously (Simard et al., 2007; Simard et al., 2010).

Female Long–Evans rats (~11 weeks; 250–300 g; Harlan) were anesthetized (Ketamine, 60 mg/kg plus Xylazine, 7.5 mg/kg, IP) and the head was mounted in a stereotaxic apparatus (Stoelting, Wood Dale, IL). Using aseptic technique and magnification (Zeiss operating microscope with co-axial lighting), the spine was exposed dorsally from C3 to T3 via a 3-cm midline incision and subperiosteal dissection of the paraspinal muscles. The ear bars were removed and the animal was repositioned so that it was supported from the spinous process of T2 (Spinal Adaptor, Stoelting) and the snout. On the left side, the entire lamina of C7 and the dorsal half of the pedicle of C7 were removed using a 1.9-mm diamond burr (#HP801-019, DiamondBurs.Net) and a high-speed drill, with care taken to avoid mechanical and thermal injury to the underlying dura and spinal cord. For the UPH model, a unilateral laminectomy was used as described whereas, for the BPH model, the laminectomy was extended to include the contralateral side. The dura was exposed by sharply removing the interlaminar ligaments rostral and caudal to the lamina of C7 and any remaining ligamentum flavum. The guide tube was angled 5° medially and was positioned using the manipulator arm of the stereo-taxic apparatus. The impactor (1.55-mm tip diameter, 57 mm length, 1.01 g) (Soblosky et al., 2001) was gently lowered down the guide tube and was positioned on the dura at the desired location. The impactor was then activated when struck by a 10 g weight released from a height of 25 mm inside the guide tube (velocity, 0.7 m/s).

For both models, the impactor was carefully positioned on the dura mater near the left C8 nerve root, with the caudal edge of the impactor in line with the caudal edge of the C8 nerve root at the axilla (Fig. 1). For the UPH model, the medial edge of the impactor was positioned 0.3 mm medial to the dorsolateral sulcus, as previously (Simard et al., 2007; Simard et al., 2010). For the BPH model, the lateral edge of the impactor was positioned 0.3 mm lateral to the dorsolateral sulcus, which placed the medial edge of the impactor at the midline. With the UPH model, the impact caused an immediate, forceful flexion of the trunk and 70–90° flexion of the ipsilateral knee, which had been extended before impact. With the BPH model, flexion of the trunk and of the ipsilateral knee was less pronounced.

After injury, the surgical site was flushed with 1 ml of normal saline (NS), the musculature was replaced over the spine and the skin incision was stapled. Within 5–10 min of injury, all rats were administered a “treatment”, either vehicle or glibenclamide (see below). Rats were nursed on a heating pad to maintain rectal temperature ~37 °C, and were given 10 ml of glucose-free NS subcutaneously. Food and water were placed within easy reach to ensure that the rats were able to eat and drink without assistance. Bladder function was assessed 2–

3 times daily by observing for urination and, if needed, manual emptying of the bladder was carried out using the Credé maneuver.

Treatments

Treatment consisted of administering a loading dose IP plus implanting a mini-osmotic pump for continuous 1-week long subcutaneous delivery of either vehicle or glibenclamide. A fresh stock solution of glibenclamide, different from the stock solutions previously used (Simard et al., 2007; Simard et al., 2010; Popovich et al., 2011), was prepared by placing 25 mg glibenclamide (#G2539; meets USP testing; Sigma, St. Louis, MO) into 10 ml dimethyl sulfoxide (DMSO). The solution to be loaded into the miniosmotic pumps (Alzet 2001, 1.0 μ l/h; Alzet Corp., Cupertino, CA) was made by taking 2.3 ml unbuffered NS, adding 4 μ l of 10 N NaOH, then adding 200 μ l stock solution, in that order to prevent precipitation of drug. The solution to be used for the loading dose was made by adding 4 μ l of stock solution to 1 ml NS. For vehicle controls, solutions were made with DMSO and NS as above, but glibenclamide was omitted.

After loading, the mini-osmotic pumps were primed overnight in NS at 37 °C with the outlet of the pump connected to a length of PE-60 tubing that extended above the level of the priming solution, to prevent H⁺ ions from entering the pump chamber.

Loaded and primed mini-osmotic pumps were implanted subcutaneously after completing surgery to induce SCI; pumps containing drug delivered 200 ng/h of glibenclamide for 7 days. A few minutes after surgery, the loading dose was administered IP, with the volume of the loading dose in microliters equal to the weight of the rat in grams (syringes with glibenclamide delivered 10 μ g/kg IP). Infusion of 200 ng/h of glibenclamide yields plasma concentrations ~5 ng/ml (Simard, unpublished observation) and has a minimal effect on serum glucose (Simard et al., 2006; Simard et al., 2007; Simard et al., 2009).

Treatment coding and allocation

The syringes and mini-osmotic pumps used to deliver the “treatments” were prepared by one investigator, after which they were coded by a separate investigator so that the surgeon performing the SCI and administering the treatment was unaware of their content (“blinded”).

Treatment allocation was determined daily by the same investigator who coded the syringes and pumps, but who otherwise did not participate in the experiment. On each day, 4 treatment sets were prepared, each consisting of a syringe with the loading dose and a mini-osmotic pump with the infusion solution; 2 sets contained vehicle and 2 sets contained glibenclamide. The order for administering vehicle or glibenclamide in 4 successive rats with SCI was determined by coin toss.

Measurements of primary and secondary hemorrhage

At the designated time after injury (15 min for primary hemorrhage; 24 h for primary plus secondary hemorrhage), the rat was anesthetized with sodium pentobarbital (100 mg/kg), a thoracotomy was performed, and the rat was transcardially perfused with NS (50 ml) at a pressure of 100 cm H₂O. The spinal cord was harvested, sectioned coronally through the epicenter of injury, and the face of the epicenter was imaged at high resolution using a flat-bed scanner.

Locomotor function

Basso, Beattie and Bresnahan (BBB) scores were determined as described (Basso et al., 1995), except that modifications were introduced to allow more accurate assessment of the

functional asymmetry associated with hemicord injury. The scoring sheet shown in Fig. 1 of the original paper (Basso et al., 1995) provides for scoring of the right and left hindlimbs separately, with the final score for the animal being based on the two scores. Here, because we were studying hemicord injuries, we maintained the two scores separately, which we refer to as modified BBB (mBBB) scores. The method for scoring up to and including scores of 11 is identical for mBBB and BBB. For mBBB scoring of coordination, forelimb–hindlimb coordination was operationally defined as a one-to-one correspondence between forelimb and *ipsilateral* hindlimb steps on each side; “alternation in hindlimb stepping”, which is taken into account in BBB scoring, was ignored for mBBB scoring. For scores above 19, both right and left sides were credited for “tail consistently up” and for “trunk stability”.

Tests to determine the angle of failure on up-angled and downangled planes (Rivlin and Tator, 1977) were performed on a wooden surface (unfinished Luan plywood) with the plane position raised from horizontal at $\sim 5^\circ/\text{s}$. The better of two consecutive tests was taken as the final measure.

To measure rearing times, rats were videotaped while in a translucent cylinder (19×20 cm) and spontaneous rearing was quantified as the number of seconds spent with both front paws elevated above shoulder-height during a 3-minute period of observation (Simard et al., 2007). In separate experiments, assessment of rearing behavior in sham-lesioned rats showed that performance waned after 2–3 weeks, presumably because the rats became habituated to the test chamber.

Lesion volume

At 6 weeks after SCI, the rats were deeply anesthetized, perfusion-fixed with 10% neutral buffered formalin, and the spinal cords were harvested. Serial paraffin sections were stained with hematoxylin and eosin (H&E). Lesion areas (in mm^2) were measured on serial sections every 250 μm ; lesion volumes (in mm^3) were calculated as the sum of the areas multiplied by 0.25.

Data analysis

Like BBB scores (Scheff et al., 2002; Ferguson et al., 2004; Barros Filho and Molina, 2008), mBBB scores are derived from an ordinal scale and are not normally distributed. Therefore, we analyzed mBBB scores using a nonparametric (distribution-free) procedure based on ranks (Conover and Iman, 1981). mBBB scores were rank-transformed as described (Conover and Iman, 1981) prior to the application of parametric statistical analysis. Rearing times (Kilbourne et al., 2009) and angled plane performance are normally distributed. Serial measurements of the *ranks* of mBBB scores, the angles, and the rearing times were analyzed using a 2-way mixed-model repeated measures ANOVA, with Tukey post-hoc comparisons. Lesion volumes were analyzed using an ANOVA.

Results

Ipsilateral vs. bilateral primary hemorrhage models

We studied the effect of an impact force (10 g, 25 mm, 0.7 m/s) applied to the left lower cervical hemicord, delivered in two different locations, one with the impact directed “laterally”, as used previously in this laboratory, and the other with the impact directed “medially” (see Methods).

Initial contusion

The initial contusion produced by the impact was observed as it developed within 1–2 min of impact, visualizing it through the intact, translucent dura under the operating microscope.

With the “lateral” impact, a hemorrhagic contusion was evident that involved the lateral surface of the cord extending up to the dorsolateral sulcus, but not more medially and never contralaterally (Fig. 1C).

With the “medial” impact, a hemorrhagic contusion was evident that approximated the size and shape of the impactor on the ipsilateral dorsal surface of the cord, and that had rostral and caudal expansion of bleeding evident within the ipsilateral sulcus, and also had a short strip of hemorrhage evident within the contralateral sulcus (Fig. 1D).

Primary hemorrhage at 15 min

The hemorrhage present within the spinal cord 15 min after impact was considered to be the primary hemorrhage. The primary hemorrhage was assessed by imaging a coronal section of the spinal cord through the epicenter that was prepared after removing intra-vascular contents by perfusion.

With the “lateral” impact, the primary hemorrhage was limited to the ipsilateral cord, including the dorsolateral funiculus and dorsal horn, with variable extension into the ventral horn (Fig. 2A). Subsequent experiments that utilized the “lateral” impact site are designated as the unilateral primary hemorrhage (UPH) model.

With the “medial” impact, the primary hemorrhage was invariably located both ipsilaterally and contralaterally in gray matter and white matter (Fig. 2B). Subsequent experiments that utilized the “medial” impact site are designated as the bilateral primary hemorrhage (BPH) model.

Effects of glibenclamide

Primary plus secondary hemorrhage at 24 h—After SCI, progressive hemorrhagic necrosis continues to expand the hemorrhagic lesion for ~12 h after impact, after which it reaches steady-state (Zhang et al., 1996; Guth et al., 1999; Simard et al., 2007). Thus, the hemorrhage present 24 h after impact was considered to consist of both primary plus secondary hemorrhage. The primary plus secondary hemorrhage was assessed by imaging a coronal section of the spinal cord through the epicenter that was prepared after removing intravascular contents by perfusion.

With the UPH model in vehicle treated rats, hemorrhage at 24 h typically extended through much of the ipsilateral cord and well into the contralateral gray and white matter (Fig. 2C), representing significant progression compared to the primary hemorrhage evident at 15 min (Fig. 2A). In glibenclamide-treated rats, there was no progression of hemorrhage at 24 h compared to 15 min; the hemorrhage was confined to the ipsilateral spinal cord (Fig. 2E). These observations are consistent with glibenclamide halting progressive hemorrhagic necrosis (Simard et al., 2007; Simard et al., 2010).

With the BPH model in vehicle treated rats, hemorrhage at 24 h typically extended through much of the ipsilateral cord and well into the contralateral gray and white matter (Fig. 2D), similar to observations at 15 min. In glibenclamide-treated rats, hemorrhage at 24 h was located bilaterally, with the pattern of hemorrhage largely indistinguishable from vehicle-treated rats (Fig. 2F). The apparent absence of an effect of glibenclamide was attributed to

the presence of bilateral primary hemorrhage in this model that precluded or masked any potential reduction in contralateral spread of hemorrhage.

Locomotor function—mBBB scores for each hindlimb, performance on an angled plane, and spontaneous rearing were measured serially over the course of 6 weeks in rats from both the UPH and the BPH models that were administered either vehicle or glibenclamide.

With the UPH model, glibenclamide-treated rats fared significantly better than vehicle-treated rats in both ipsilateral and contralateral lower extremity function (Figs. 3A,B). mBBB scores contralateral to the injury were highly different 24 h after injury in glibenclamide-treated rats compared to vehicle-treated rats. Scores were consistently better with glibenclamide but, by 6 weeks, vehicle-treated rats had recovered contralateral function almost to the level of the glibenclamide-treated rats. mBBB scores ipsilateral to the injury also were highly different 24 h after injury in glibenclamide-treated rats compared to vehicle-treated rats, with ipsilateral mBBB scores showing persistent discrimination throughout the 6 weeks of testing.

With the BPH model, glibenclamide-treated rats also fared significantly better than vehicle-treated rats in both ipsilateral and contralateral lower extremity function, but the treatment effect was smaller (Figs. 3C,D). mBBB scores contralateral to the injury were similar 24 h after injury in glibenclamide-treated rats compared to vehicle-treated rats. Scores were consistently better with glibenclamide, but by 6 weeks vehicle-treated rats had recovered contralateral function to the level of the glibenclamide-treated rats. mBBB scores ipsilateral to the injury showed persistent discrimination throughout the 6 weeks of testing.

The ability to maintain position on an angled plane is a complex task that assesses the coordinated function of all 4 limbs and is therefore an excellent test of cervical spinal cord function. With both the UPH and the BPH models, glibenclamide-treated rats fared significantly better than vehicle-treated rats in both the up-angled and down-angled tests (Figs. 4A,B). The effect of treatment was evident as early as 24 h after injury, and was sustained for the duration of testing.

We quantified vertical exploratory behavior (rearing), a complex exercise that requires balance, trunk stability, bilateral hind limb dexterity, and strength, and at least unilateral forelimb dexterity and strength, which together are excellent markers of cervical spinal cord function.

With the UPH model, glibenclamide-treated rats fared significantly better than vehicle-treated rats. The effect of treatment was evident as early as 24 h after injury, and was sustained for the duration of testing (Fig. 5A).

With the BPH model, glibenclamide-treated rats also fared significantly better than vehicle-treated rats. However, the effect of treatment became evident only 2 weeks after injury, after which it was sustained for the duration of testing (Fig. 5B). The performance achieved in both vehicle and glibenclamide-treated rats from the BPH model was less than that in comparable groups from the UPH model.

Lesion volume—Lesion volumes were measured after completing the locomotor function tests at 6 weeks. In vehicle-treated rats, 6-week lesion volumes were 45% larger in the BPH model compared to the UPH model (Fig. 6). In both models, glibenclamide exerted a significant effect on tissue preservation, but the magnitude of protection was greater with the UPH model compared to the BPH model: with the UPH model, 6-week lesion volumes with

glibenclamide were 57% smaller than in controls; with the BPH model, 6-week lesion volumes with glibenclamide were 33% smaller than in controls (Fig. 6).

Discussion

The principal finding of the present study is that glibenclamide is an effective treatment for SCI, with the magnitude of the benefit depending on the magnitude of primary hemorrhagic insult. With both models, one with unilateral primary hemorrhage and the other with bilateral primary hemorrhage, glibenclamide afforded significant protection in terms of neurological function and preservation of spinal cord tissues. The effect of treatment in the UPH model was similar to our previous observations (Simard et al., 2007; Simard et al., 2010). In addition, here we show that an injury model characterized by bilateral primary hemorrhage also is influenced favorably, albeit the benefit at 6 weeks in terms of function and lesion volume was less than when the primary hemorrhagic injury was unilateral.

In SCI, the magnitude of the primary injury plays a leading role in determining outcome. Several distinct factors associated with the primary injury may be independent predictors of outcome, including physical aspects of the force generating the injury (magnitude, velocity, depth, etcetera) as well as immediate biological aspects of the response to injury (number of axons severed, amount of blood extravasated, etcetera). Among these various factors, the amount of primary hemorrhage is exceedingly important, given the role of blood in determining the magnitude of the subsequent free radical and inflammatory responses. In the models studied here, differences in apparent injury severity were not due to the magnitude of the force applied, which was identical in both cases, but were due to the specific location of the impact, which determined whether the primary hemorrhage would be unilateral or bilateral. By adjusting the location of the impact, we were able to obtain either a unilateral or a bilateral primary hemorrhage, thereby separating the biophysics of injury from the biological response to injury. In SCI research, the “severity” of a contusive injury is generally equated with impact force or tissue strain because, in any given study, the impact is typically delivered to a single location, e.g., midline, low thoracic. The data presented here suggest that, because of the anisotropy of spinal cord tissues, the same force can generate large differences in the severity of neurological injury, with severity correlating with the amount of primary hemorrhage.

SCI is normally complicated by progressive hemorrhagic necrosis, a secondary injury process that results in expansion of the hemorrhagic lesion (Balentine, 1978; Simard et al., 2007). When SCI is associated initially with a unilateral primary hemorrhage, the process of progressive hemorrhagic necrosis spreads to involve the contralateral side, resulting in a bilateral hemorrhagic lesion. Halting progressive hemorrhagic necrosis by blocking Sur1-regulated NC_{Ca}-ATP channels with glibenclamide results in hemorrhagic lesions that remain largely unilateral, significantly preserving both tissue and function (Simard et al., 2007). With an injury involving bilateral primary hemorrhage, the opportunity to reduce lesion expansion is restricted largely to rostro-caudal expansion, which yields a less dramatic benefit in terms of tissue sparing and neurological function. Notably, with the UPH model, an effect of glibenclamide was evident as early as 24 h after injury, whereas 1 week or more was required to observe a beneficial effect of glibenclamide with the BPH model. This latter observation recapitulates and expands upon a similar observation recently reported for glibenclamide (Popovich et al., 2011).

The physical properties of gray matter and white matter of the spinal cord are highly different. Compared to white matter, gray matter has 4–5 times greater capillary density (Ireland et al., 1981), is less tolerant to compression before failure of the blood–spinal cord barrier (Maikos and Shreiber, 2007), and is more fragile and ruptures at lower strains

(Ichihara et al., 2001). These features make gray matter the dominant source of primary hemorrhage following contusion injury. In addition, gray matter and white matter respond differently to injury dynamics, especially impact velocity. Whereas impact velocity has relatively little effect on the volume of primary hemorrhage in gray matter, this is not the case for white matter, where primary hemorrhage volume as well as primary axonal disruption are significantly greater with high velocity impact compared to low velocity impact (Sparrey et al., 2008).

With lower cervical cord injury, the consequences of injury to gray matter versus white matter are very different, with white matter injury having a greater impact on several aspects of neurological function. Injury (primary or secondary) to white matter interrupts ascending and descending fiber tracts, resulting in sensorimotor dysfunction below the level of injury involving trunk, lower extremities and bladder, whereas injury to gray matter results in limited segmental neuronal dysfunction which, by itself, may be tolerable. However, primary injury to gray matter results in hemorrhage, which is one of the most important triggers of secondary injury to white matter, greatly exacerbating and expanding any primary white matter injury. At present, little can be done to ameliorate primary white matter damage, but an important goal of acute treatment is to minimize secondary injury to white matter, to best preserve distal function. Because low velocity impact is less likely to produce primary white matter damage, but is still highly effective at inducing primary gray matter injury with hemorrhage (Sparrey et al., 2008), low velocity impacts may be more useful than high velocity impacts for studying secondary white matter injury and its treatment.

In SCI research, some workers have used parametric statistical methods to analyze raw BBB scores even though this approach is technically incorrect, due to the fact that BBB scores are ordinal in nature. Relying on “the robustness of ANOVA to handle violations of the normality assumption” (Scheff et al., 2002) is less than ideal, and collapsing the scale to force it to be more normal (Ferguson et al., 2004) clouds differences at the upper end of the scale. Here, we used rank transformation prior to the application of standard parametric methods (Conover and Iman, 1981). Apart from being technically appropriate, this method of analysis can yield statistically significant differences when scores are close; even if scores are no more than one point apart, consistent superiority in ranks can yield statistical significance. This approach is of clinical relevance, reflecting for example, the experience in human SCI trials on methylprednisolone where it was argued, rightly, that one additional cervical spinal level could be of great clinical importance to patients.

In summary, we report that glibenclamide is an effective treatment for spinal cord injuries associated with various amounts of primary hemorrhage. Previous work using the UPH model reported strong efficacy in two different series of rats (Simard et al., 2007; Simard et al., 2010), with the present study representing the third independent series in which a beneficial effect of glibenclamide is shown in this model. In the present study, we now show that glibenclamide treatment also favorably influences outcome in an injury model characterized by bilateral primary hemorrhage. We conclude that glibenclamide is beneficial in models of different injury severity, with the magnitude of the benefit depending on the magnitude of primary hemorrhagic insult.

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Fig. 1.

Impact locations and initial contusions for the UPH and BPH models. (A,B) Low (A) and high (B) magnification photomicrographs of the surgical exposure used for cervical hemicord contusion injury; after removing the spinous processes from C5 to T1, the entire left and part of the right lamina of C7 and the interlaminar ligaments and ligamentum flavum at C7, the left hemicord at C7 is visualized through the translucent dura; the anatomical details visualized include the laminae (L) of cervical (C) and thoracic (T) vertebral levels C5 through T1, the left facette joint between C6 and C7, the C8 nerve root exiting the spinal canal caudal to the left pedicle (P) of C7, and the dorsolateral sulcus of the spinal cord, from which emerges the rootlets of the dorsal roots; note the metallic clamp on the spinous process of T2 in (A); the 1.55 mm diameter impactor used to produce contusion injury is directed at an angle of 5° medially and is positioned to strike the spinal cord in the area denoted by the green or purple circles for the UPH and BPH models, respectively; adapted from Simard et al., 2010. (C,D) Intraoperative photomicrographs showing contusions on the surface of the spinal cord evident 2 min after impact, for the UPH model (C) and for the BPH model (D); the contralateral hemorrhage evident at the surface in the BPH model is indicated by arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Patterns of primary and secondary hemorrhage and effect of glibenclamide. (A)–(F) Representative coronal sections of perfusion-cleared but otherwise unprocessed spinal cords through the epicenter of injury 15 min (A,B) and 24 h (C–F) after impact, in vehicle-treated rats from the UPH (C) and BPH (D) models, and in glibenclamide-treated rats from the UPH (E) and BPH (F) models. The data shown are representative of 4 rats per group.

Fig. 3. Effect of glibenclamide on mBBB scores. (A)–(D) Serial measurements of mBBB scores for the ipsilateral hindlimb (A,B) and for the contralateral hindlimb (C,D) in vehicle-treated (empty squares) and glibenclamide-treated (filled circles) rats from the UPH (A,C) and BPH (B,D) models; 8–9 rats per group; data are shown as mean $\pm$ S.E.; statistical significance was determined using the Conover method; *, $P < 0.05$; the abscissa gives time in days (d) or weeks (w).

Fig. 4.

Effect of glibenclamide on angled plane performance. (A,B) Serial measurements of the angle of failure (mean±S.E.) for up-angled and down-angled tests in vehicle-treated (empty squares) and glibenclamide-treated (filled circles) rats from the UPH (A) and BPH (B) models; 8–9 rats per group; *, $P<0.05$; **, $P<0.01$; the abscissa gives time in days (d) or weeks (w).

Fig. 5. Effect of glibenclamide on spontaneous rearing. (A,B) Serial measurements of the time (mean±S.E.) spent in spontaneous rearing in vehicle-treated (empty squares) and glibenclamide-treated (filled circles) rats from the UPH (A) and BPH (B) models; 8–9 rats per group; *, $P < 0.05$; **, $P < 0.01$; the abscissa gives time in days (d) or weeks (w).

Fig. 6. Effect of glibenclamide on lesion volumes. (A,B) Representative H&E-stained sections from the epicenter in vehicle-treated rats from the UPH (A) and BPH (B) models. (C,D) Lesion areas as a function of distance from the epicenter in vehicle-treated (empty squares) and glibenclamide-treated (filled circles) rats from the UPH (C) and BPH (D) models. (E,F) Lesion volumes in vehicle-treated and glibenclamide-treated rats from the UPH (E) and BPH (F) models; 8–9 rats per group; **, $P < 0.01$; ***, $P < 0.001$.