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The dynamics of axolemmal disruption in guinea pig spinal cord following compression

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Abstract

Membrane damage has been postulated as a critical factor in mediating axonal degeneration in brain and spinal cord trauma. Despite compelling evidence of membrane disruption as a result of physical insults in both *in vivo* and *in vitro* studies, the dynamics of such damage over the time post injury in *in vivo* studies has not been well documented. Using a well-characterized *in vivo* guinea pig spinal cord compression model and horseradish peroxidase exclusion assay, we have documented significant membrane disruption at 1 hr, 3 days, and 7 days following injury. Furthermore, the membrane damage was found to spread laterally 10 mm beyond the center of original compression site in both rostral and caudal directions. A second-degree polynomial fit of the measured data predicts a bilateral spread of approximately 20–21 mm of membrane disruption from the epicenter of injury over a period of about 20 days. Thus, this study shows that membrane damage exists days, and possibly weeks, after spinal cord trauma in live guinea pigs. This provides the evidence necessary to investigate the role of membrane damage in triggering axonal deterioration in the future. Furthermore, this study has also revealed a long therapeutical window for membrane repair and functional enhancement following traumatic injury in the central nervous system.

Introduction

It is well known from *in vitro* experiments that membrane damage, as a result of physical insult, could result in cellular deterioration leading to cell death (Lucas *et al*., 1985; Shi *et al*., 1989; Xie & Barrett, 1991; Shi & Blight, 1996). Since the environment following acute traumatic spinal cord injury *in vivo* is believed to be hostile for membrane repair (Stokes *et al*., 1983; Young & Koreh, 1986), the extended membrane disruption could play a key role leading to cell death, tissue loss and neurological dysfunction *with survival after injury*. Consistent with this hypothesis, the reestablishment of membrane integrity by membrane repair agents could significantly reduce tissue loss (Shi *et al*., 1999; Shi & Borgens, 1999, 2000; Duerstock & Borgens, 2002) and improve functional recovery in live guinea pig spinal cord injury (Borgens & Shi, 2000; Borgens *et al*., 2002).

It is has been shown repeatedly that controlled mechanical injury produces membrane disruption *in vitro* (Shi *et al*., 1989; Xie & Barrett, 1991; Shi & Borgens, 2000; Shi & Pryor, 2000, 2002), and *in vivo* (Maxwell *et al*., 1993, 1999, 2003; Pettus *et al*., 1994; Pettus & Povlishock, 1996; Povlishock & Pettus, 1996; Shi, 2002). However, it is not well established whether membrane disruption is transient or extended, or a local or a diffusive phenomenon in *in vivo* spinal cord trauma. Consequently, the role of the plasmalemmal lesion in neuronal pathology after traumatic CNS injury has not been firmly established. This hampers the development of effective interventions aiming to reduce cellular destruction & preserve function through membrane repair in such an injury. The current study is designed to examine membrane disruption due to acute compression of the spinal cord in a live guinea pig injury model (Borgens & Shi, 2000; Borgens *et al*., 2002; Shi, 2002). The loss of membrane integrity was investigated as a function of time, 1 hour, 3 days, and 7 days post injury. Furthermore, the spreading of membrane disruption, in reference to the epicenter of the injury, was also studied. This study is expected to provide further insights into the extent of the membrane damage over time and to support a possible role of membrane damage in triggering secondary cellular organelle loss and cell death. Some of the conclusions of the study have appeared in abstract form (Shi, 2002).

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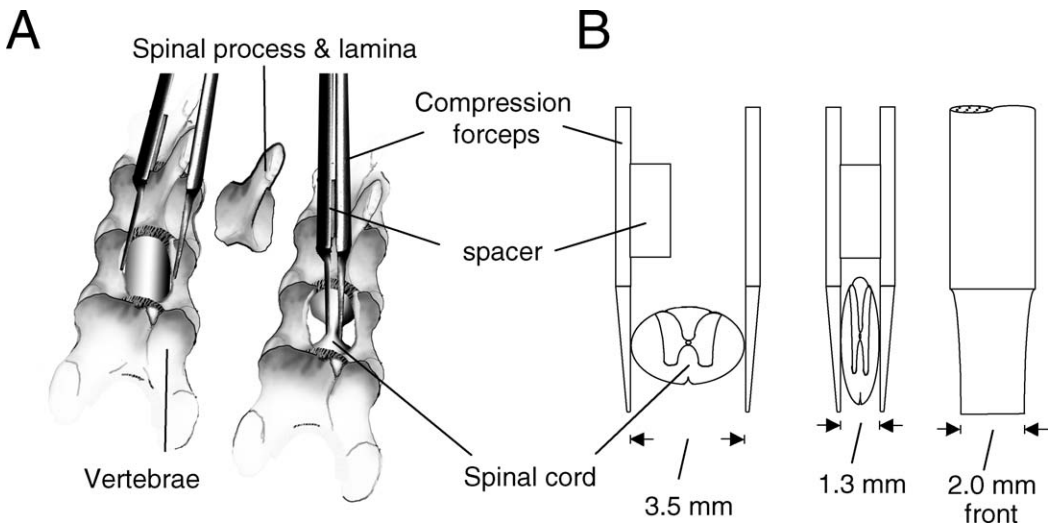

Fig. 1. Schematic drawing of the guinea pig spinal cord compression *in vivo*, the injury device, and its application. (a) After laminectomy, the cord was exposed. A special forceps with a spacer was used to compress the cord. The wound was then closed and the animal was allowed to recover. (b) Schematic showing the tips of the forceps, before and during the crush, in front and side views. As indicated in the drawing, the width of the spacer is 1.3 mm; the width of the forceps is 2 mm. The average width of the cord is 3.5 mm.

Methods and materials

ANIMAL SURGERY

Adult female guinea pigs (∼350 gm; Hartley strain) were anesthetized with an intramuscular injection of 60 mg/kg ketamine HCL, 0.6 mg/kg acepromazine maleate, and 12 mg/kg xylazine. A 2 cm midline dorsal thoracic incision was made to expose the vertebral column at thoracic level 11 where a one vertebral segment laminectomy was performed with rongeurs, exposing the spinal cord (Fig. 1). The spinal cord was crushed using blunted watchmakers forceps possessing a spacer. The cord was crushed from an original width of approximately 3.5 mm to 1.3 mm in a span of 2 mm along the longitudinal axis of the cord (Fig. 1). The incisions were closed in layers with sutures. A total of 28 guinea pigs were used in this study. The surgical procedures were carried out under protocols approved by the Purdue University Animal Care and Use Committee, in accordance with Federal, State, and University guidelines governing animal use in research.

HORSRADISH PEROXIDASE HISTOCHEMISTRY

At a prescheduled time of sacrifice (1 hour, 3 days, and 7 days post-injury), the guinea pigs were anesthetized as above and perfused with oxygenated, cold Krebs' solution to remove the blood and to lower core temperature. The vertebral column was excised rapidly and the spinal cord was removed from the vertebrae, divided at midline vertically, and immersed in cold Krebs' solution (15°C). The extracted segments of spinal cord strips were transferred to oxygenated Krebs' solution containing 0.015% HRP (type IV, Sigma co. St. Louis MO). After incubation for 1 h at 25°C, the tissue was fixed by immersion in 2.5% glutaraldehyde in phosphate buffer. Transverse sections of the tissue were cut at 30 μm on a vibratome (Electron Microscope Science) and processed with diaminobenzidine to reveal the extent of HRP uptake into damaged axons. The numbers of unsealed axons were counted and normalized by division with the unit area of the sample and expressed as a density (axons/mm^2). In the uninjured control group, animals underwent the same surgical procedure except for the spinal cord crush. For a detailed description of the HRP technique see Shi and Borgens (2000), Shi *et al*. (2000, 2001), and Asano *et al*. (1995).

Figure 2 depicts the locations, along the longitudinal axis of the spinal cord of a guinea pig, where the transverse sections

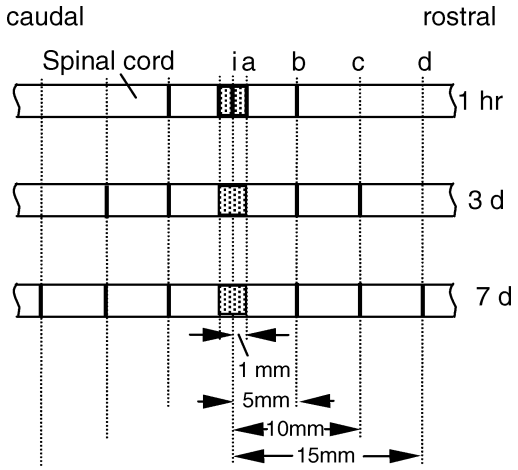

Fig. 2. The location of the original compression site (hatched area) and the cross sections where axonal membrane damage was examined (plane i, a, b, c, d) along the longitudinal axis of the guinea pig spinal cord. One hour after compression, cross sections i, a, and b were examined and analyzed. Three days after compression, cross sections b and c were examined and analyzed. Seven days after compression, cross sections b, c, and d were examined and analyzed. The examination of the density of HRP-labeled axons inside the original compression site was not possible at 3 days and 7 days post injury due to the formation of extensive scar tissue.

were examined for axolemmal integrity using HRP labeling. The determination of membrane integrity was conducted at 1 hour, 3 days, and 7 days following a controlled compression of spinal cord at the T11 spinal segment. The HRP labeling study was performed at both caudal and rostral sides of the injury along the longitudinal axis. Since the density of HRP labeled axons is similar in the rostral and caudal sides of the injury ($P > 0.05$), only the results from one side are presented. The examination of the density of HRP-labeled axons inside the original compression site was not possible at 3 days and 7 days post injury due to the formation of extensive scar tissue.

STATISTICAL ANALYSIS

ANOVA and Tukey-Kramer tests were used in all the data analysis, with a significant level chosen at $P < 0.05$. Averages were expressed as mean $\pm$ standard error of mean (SEM).

Results

MEMBRANE DAMAGE REVEALED BY HORSERADISH PEROXIDASE LABELING

In uninjured control cords, the HRP labeling was 10 ± 2 axons/mm^2 ($n = 8$). At 1 hour after compression, the density of HRP labeled axons in the epicenter (plane i, Fig. 2) and the edge of the compressed area (plane a, Fig. 2), were 1223 ± 167 axons/mm^2 ($n = 5$), and 335 ± 79 axons/mm^2 ($n = 8$). These two values are significantly higher than that of uninjured control cords ($P < 0.001$, $P < 0.05$ respectively, Fig. 3). However, the number of HRP labeled axons in the cross section 5 mm away from the epicenter of the compression site was 12 ± 8 axons/mm^2 ($n = 6$, plane b, Fig. 2), which is not significantly different than that of the uninjured control ($P > 0.05$, Figs. 2 and 3).

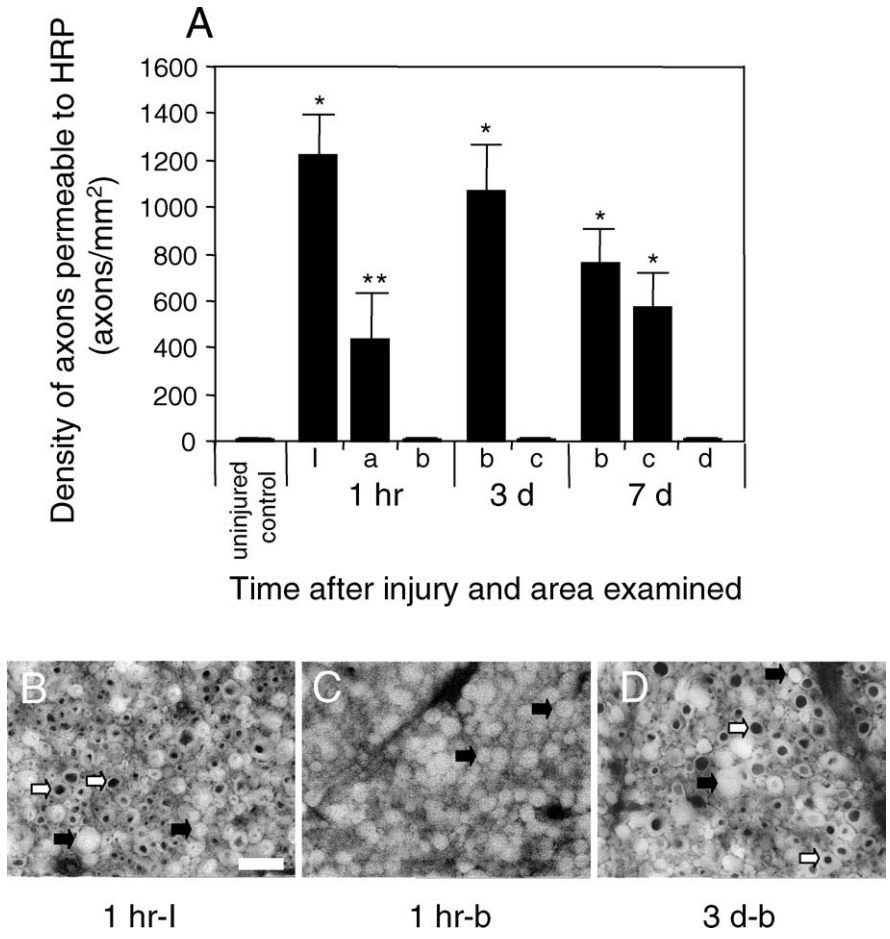

Fig. 3. Photographs of Vibratome sections showing horseradish peroxidase (HRP) labeling and the quantification of the density of axonal permeability to HRP (axons/mm^2) as a function of location along the spinal cord, and the time following compression. (A) Note that HRP labeling increased only at the injury site one hour after injury. However, at three days and seven days after compression, the HRP labeling had spread to neighboring tissues 5–10 mm away from the center of the injury. The number of experiments in each group ranges from 3 to 8. Asterisks indicate significant difference of the density of HRP-labeled axons when compared with that of uninjured control. $^*P < 0.001$, $^{**}P < 0.05$. (B–D) The micrographs from 3 groups represent the location and the time post injury when the HRP uptake was examined: (B) HRP labeling at plane I 1 hr post injury (1 hr-I), (C) HRP labeling at plane b 1 hr post injury (1 hr-b), and (D) HRP labeling at plane b 3 days post injury (3 d-b). Open arrows indicate axons labeled with HRP; filled arrows denote axons that excluded HRP. Scale bar = 10 μm (applies to B–D).

At 3 days after injury, the HRP labeling in the area 5 mm away from the epicenter (plane b, Fig. 2) had risen to 1070 ± 198 axons/mm^2 ($n = 5$), which is significantly higher than uninjured control cords ($P < 0.001$, Fig. 3). However, HRP labeling in the area 10 mm from the center of the injury site was only 15 ± 8 axons/mm^2 ($n = 6$, plane c, Fig. 2), which is not significantly different from the uninjured control ($P > 0.05$, Fig. 3).

At seven days following compression, the HRP labeling in areas 5 and 10 mm from the epicenter of the compression (plane b, c, Fig. 2) were 763 ± 142 axons/mm^2 ($n = 5$) and 573 ± 147 axons/mm^2 ($n = 6$) respectively; both are significantly higher than that of the uninjured control ($P < 0.005$, Fig. 3). However, the examination of membrane integrity at a cross section 15 mm away from the injury center (plane d, Fig. 2) shows that the HPR labeling (15 ± 4 axons/mm^2 $n = 3$) was not significantly higher than that of the uninjured control ($P > 0.05$, Fig. 3).

THE PREDICTION OF THE OUTCOME OF MEMBRANE DAMAGE FOLLOWING FOCAL COMPRESSION

I have attempted to predict the longitudinal spread of membrane damage along the cord axis, as well as the duration of membrane damage, following a controlled focal compression of spinal cord in live guinea pig. This was accomplished by a polynomial functional fit of the available data.

First, as shown in A of Figure 4, I plotted the highest measured density of HRP-labeled axons against the longitudinal spread of the membrane damage. I assumed the boundaries of membrane damage along the longitudinal axis of the cord at 1 hour, 3 days, and 7 days following compression to be 3.0, 7.5, and 12.5 mm in either caudal or rostral directions. These boundaries were determined by taking the mid point of two transverse sections, one of which has significant membrane damage while the other does not. For example, at 3 days following compression, the HRP labeling is significantly high in the cross section

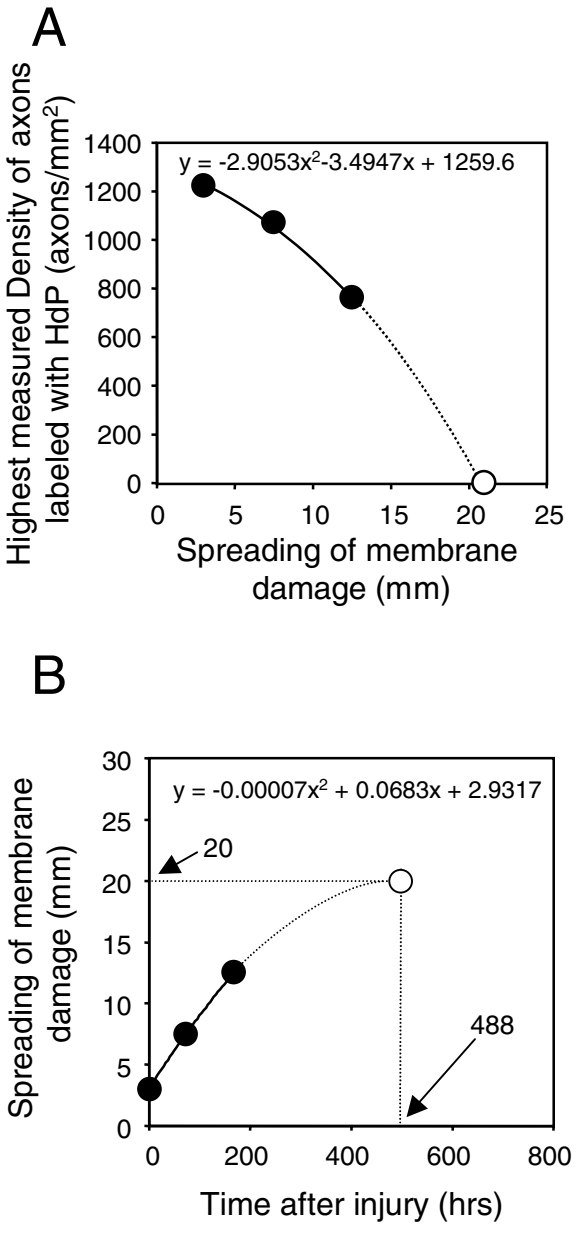

Fig. 4. Mathematical analysis and prediction of membrane damage. A: Fitting and calculation of the maximal axonal membrane damage, revealed by HRP labeling, as a function of the length of cord containing axons with membrane breakdown. The *x*-axis displays the spread of membrane damage and the *y*-axis represents the highest measured density of axons labeled with HRP. A second order polynomial fit of the data generates an equation which predicts the spread of membrane damage to be 21 mm from the center of the original injury site. The lateral margins of the area (extend rostral or caudal) which enclose membrane damage, along the longitudinal axis of the cords, are 3 mm, 7.5 mm, and 12.5 mm at 1 hour, 3 days, and 7 days respectively. The margin of the membrane damage area was determined by taking the mid point of two cross sections where one had significant HRP labeling and the other did not. The filled circles denote the measured data and the open circle represents the calculated data based on the equation. B: A second order polynomial fit of the measured data was performed to estimate the spreading of the membrane damage from the original injury site along the longitudinal axis of the spinal cord as well as the duration of the membrane damage. The *x*-axis displays the time after compression, and the *y*-axis represents the length of the cord containing axons with membrane damage. The method of determining the margin of the membrane damage area was as above. The equation predicts that membrane damage could last 20 days after compression and spread to a final area stretching 20 mm longitudinally (rostal and caudal directions) from the injury site. The filled circles denote the measured data and the open circle represents the calculated data based on the equation.

5 mm lateral from the epicenter of the injury (plane b, Fig. 2), but normal in the cross section 10 mm from the center of the injury (plane c, Fig. 2). Therefore, I chose the plane 7.5 mm lateral from the injury center as the boundary of membrane damage. The second order polynomial fit of the data yields the equation:

$$y = -2.9053x^2 - 3.4947x + 1259.6 \quad (1)$$

y being the highest measured density of HRP-labeled axons within the areas of membrane damage.

x is the distance between the center of the compression site and the location of the boundary of membrane damage.

By solving this formula, I have determined that when y (the density of damaged axons) $= 10$ axons/mm^2, $x = 21$ mm. The value of $y = 10$ was chosen because this was the background level of membrane damage in uninjured cords. This equation predicts that the membrane damage could spread longitudinally for approximately 21 mm from the center of the injury site following compression using our protocol.

Next, I estimated the duration of membrane damage following a controlled compression of live guinea pig spinal cord. The method of determining the longitudinal boundary of membrane damage was the same as that mentioned above. I plotted the time after injury against the spreading of membrane damage. As it shows in Figure 4B, a second order polynomial fit of the data points gives the equation:

$$y = -0.00007x^2 + 0.0683x + 2.9317 \quad (2)$$

y being the distance between the center of the compression site and the location of the boundary of membrane damage.

x is the time (hrs) following compression.

I reasoned that the time when y reaches the maximum value is the moment that the spread of membrane damage ceases. Therefore, I used the method of derivative to calculate the maximal value of y, which is the final length of the membrane damage area. The value of x when y reaches the maximum value gives the duration during which membrane damage continues to spread following initial compression. Based on the equation mentioned above (Eq. (2)), I have determined that when x is 488 hrs, or approximately 20 days, y reaches its maximum value, which is 20 mm. The lateral spreading of membrane damage determined by Eq. (2) (20 mm) is quite similar to the value attained through Eq. (1) (21 mm). This indicates that under the current compression protocol, membrane damage should continue to spread longitudinally for 20–21 mm from the epicenter of the crush and last for approximately 20 days post injury (see Fig. 4).

Discussion

THE DYNAMICS AND OUTCOMES OF MEMBRANE DAMAGE FOLLOWING FOCAL COMPRESSION

Membrane damage has been suggested as a pivotal factor in the neuropathology of axonal deterioration and cell death in brain and spinal cord injury (Shi & Blight, 1996; Fitzpatrick *et al*., 1998; Shi & Pryor, 2002). In support of this hypothesis, axolemmal disruption as a result of physical insult has been well documented in *in vitro* experiments (Shi *et al*., 1989, 2000; Xie & Barrett, 1991; Shi & Borgens, 2000; Shi & Pryor, 2002), and to a lesser extent in *in vivo* studies. For example, membrane damage following CNS trauma has been documented based on dye-exclusion assay (Pettus *et al*., 1994; Povlishock & Pettus, 1996), distribution of membrane pump activity (Maxwell *et al*., 1995), and quantitative freeze-fracture (Maxwell *et al*., 1999). Despite the strong evidence of the existence of such lesions, the dynamics and the temporal and spatial characteristics of axolemmal disruption following physical insults has not been well described.

In this study, I have substantiated the observation we made earlier based on the HRP-exclusion test and have clearly shown that significant membrane damage exists at one hour, 3 days, and 7 days following a controlled compression of spinal cord *in vivo* (Shi, 2002). In addition, the length of the cord segment with axonal membrane damage had expanded by 10 times, from 2 mm of the original crushing site to 20 mm at 7 days post compression. The long-lasting and diffusive membrane damage, either by direct observation or mathematical calculation, is consistent with the hypothesis that membrane damage may play a pivotal role in the triggering of cellular destruction, tissue loss, and functional deficits in traumatic brain and spinal cord injury.

According to our calculations using the equations derived from the measured data, I have estimated that the membrane damage following a controlled compression could persist for about 20 days and spread about 20–21 mm from the original compression site (Fig. 5). This study is the first report to not only document the presence of axonal membrane damage, but also attempt to demonstrate the spreading of such a lesion outside the original compression site over time *in vivo*.

Based on measured data, the rate of the spreading of membrane damage is quite fast in the first 7 days without significant decline, which is averaged to be approximately 1.6 mm/day. These findings further indicate that an early and timely intervention is important in the management of acute spinal cord injury. In effect, the earlier the axonal degeneration is halted, the smaller the amount of axonal segment is lost. This in turn minimizes the distance required for functional reconnection via regeneration. Since the rate of peripheral nerve regeneration is approximately 1 mm/day and the rate of regeneration of CNS axons is far slower (Fawcett

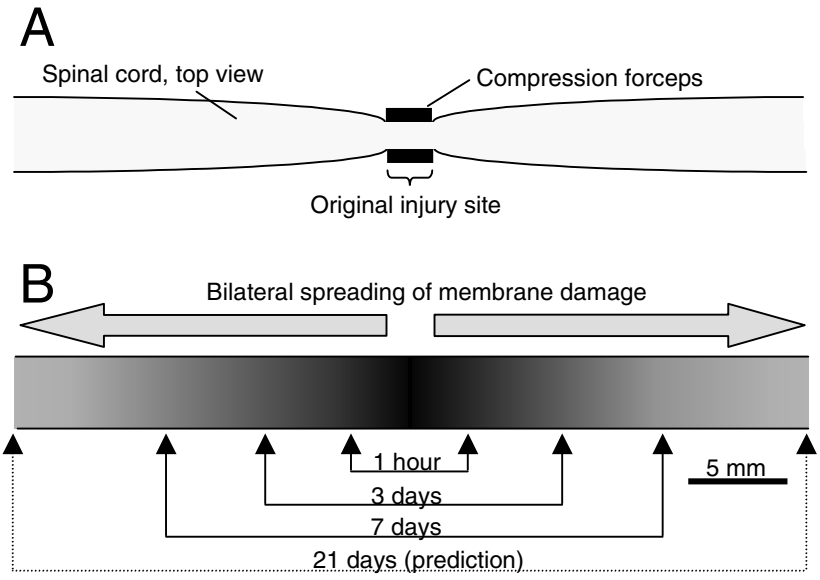

Fig. 5. Schematic drawings depicting the spreading of membrane damage following focal spinal cord compression. A: Depicts the dimension of the original compression site. B: Shows the bilateral spread of membrane damage at various post injury times. The length of the area enclosing membrane damage at 1 hr, 3 days, and 7 days was derived from the measured data, while the same parameter at 7 days was predicted based on the equation generated by a second degree polynomial fit of the measured data. Note the maximal length of membrane damage is indicated to be about 21 mm from the epicenter of the injury, while duration of the membrane damage is about 20 days.

& Keynes, 1990; Schwab *et al*., 1993), it is reasonable to state that the attenuation of axonal degeneration is at least equally, if not more important than, the promotion of axonal regeneration. On the other hand, the fact that membrane damage lasts days (measurements) and even weeks (calculation) after initial injury also indicates that membrane repair is not just a treatment for acute injury (within hours after injury), but also suitable for the sub acute stage (days and weeks after injury).

THE MECHANISM OF EXTENDED MEMBRANE DAMAGE FOLLOWING COMPRESSION

Though extensive membrane damage is documented in the current study, the mechanism of this persistent and extended axolemmal disruption is not clear. In particular, it is not known whether the extended membrane damage is a direct result of primary injury, or a secondary phenomenon mediated by factors arising secondary to the primary insult. In the direct primary injury mechanism the extended membrane damage is a result of continuous axonal degeneration from the original compression site, which is perhaps due to the failure of membrane resealing (Meiri *et al*., 1981; Roederer *et al*., 1983; Emery *et al*., 1987; Strautman *et al*., 1990). In the secondary injury mechanism, the extended membrane damage distant from the original compression site is mainly mediated by secondary factors, such as membrane-damaging toxin(s) produced after the initial trauma.

It has been shown that the *in vivo* environment is conducive for extended degeneration since it is hostile for membrane resealing in mammalian spinal cord trauma (Stokes *et al*., 1983; Young & Koreh, 1986). On the other hand, we have found a significant increase of acrolein, a lipid peroxidation byproduct that is known to disrupt plasma membranes (Shi *et al*., 2002), at the cord segment 10 mm away from the compression site 1 day after compression (Luo *et al*., 2003). Therefore, it is possible that both direct and indirect mechanisms contribute to the extended membrane damage.

THE LIMITATIONS OF MATHEMATICAL CALCULATIONS

There are several limitations to the simple mathematical model used in the current study for the purpose of estimating the duration and length of membrane damage. First, the prediction of duration and length of membrane damage, based on the model, is unique to our particular experimental protocol and cannot be applied to other injuries with different conditions. For example, I compressed the cord from a width of 3.5 to 1.3 mm, an approximately 63% compression. In addition, the original span of the compression site was 2 mm and the compression was maintained for 15 seconds. A variation of any one of these parameters could potentially change the biomechanics of spinal cord compression and result in different levels of structural damage.

Second, even though the spread of membrane damage was found to be nearly symmetric along the

longitudinal axis of the traumatized cord, this may not be the case if the original compression site is close to either end of the cord, such as at the cervical or lower lumber segments. In addition, this simple model does not take into consideration many other important factors, such as the temperature (Yawo & Kuno, 1985; Shi & Pryor, 2000), oxygen level (Ransom *et al*., 1990; Peasley & Shi, 2002), and pH (Huang & Young, 1994; Kim *et al*., 1996), which could also potentially influence the outcome of the injury.

In this study, I assume the boundaries of membrane damage along the longitudinal axis to be in the middle of two transverse sections, one of which has significant membrane damage while the other does not. In order to examine the degree to which a small deviation of the boundary off the midline would influence the outcome of our estimation, I performed the same calculation assuming the boundary to be at the 30% or 70% of the distance from the proximal section with significant membrane disruption. When 30% was chosen, the calculated final length of the area containing membrane damage was 32 mm and the duration was 42 days. When 70% was assumed, the values were 19 mm and 18 days respectively. These calculations indicate that even a modest deviation of the boundaries will not dramatically change our estimate of the spread and duration of membrane damage.

MEMBRANE REPAIR AS A TARGET FOR EFFECTIVE THERAPEUTIC INTERVENTION

Based on the above mentioned experimental observations and the predictions based on mathematic calculation, it is reasonable to speculate that membrane damage could play a critical role in mediating axonal degeneration, neuronal cell death, tissue loss and functional deficits following spinal cord trauma. The cellular deterioration following membrane disruption could result primary from calcium influx. In addition, it has been demonstrated that sodium influx could also indirectly promote influx of calcium through reverse operation of the Na^+/Ca^{2+} exchanger (Styse *et al*., 1991, 1992: Waxman *et al*., 1991). Reduction of Na^+ entry can significantly reduce tissue damage in various injuries (Stys *et al*., 1992; Teng & Wrathall, 1997). Therefore, membrane damage can trigger cellular deterioration by elevating intracellular calcium through various mechanisms (Lucas *et al*., 1997) and repairing the membrane breach constitutes a reasonable means to prevent cell death.

In support of recognizing membrane damage as an important target for effective treatment, we have recently shown that polyethylene glycol (PEG), a hydrophilic polymer, can repair membrane damage and reduce tissue loss and enhance functional recovery following a severe spinal cord compression in live guinea pigs (Borgens & Shi, 2000; Borgens *et al*., 2002). What makes that study particularly interesting is that PEG can still bring about significant structural improvement and functional recovery even when applied at 7 to 8 hours after the initial injury (Borgens & Shi, 2000; Borgens *et al*., 2002). In that study, we hypothesized that PEG-mediated functional recovery in *in vivo* spinal cord trauma is due to membrane repair, even though the evidence of delayed membrane damage was not available. The existence of delayed membrane damage demonstrated in the current study is clearly in favor of our hypothesis. Furthermore, considering that membrane damage is still actively evolving days and possibly weeks after the original injury, I suggest that membrane repairing agents such as PEG could be applicable to injuries during this extended post-injury period. I believe the current study certainly widens the window of opportunity for effective treatment, especially for the use of membrane repairing agents.

Acknowledgments

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References

ASANO, T., SHI, R. & BLIGHT, A. R. (1995) Horseradish peroxidase used to examine the distribution of axonal damage in spinal cord compression injury *in vitro*. *Journal of Neurotrauma* **12**, 993 (abstract).

BORGENS, R. B. & SHI, R. (2000) Immediate recovery from spinal cord injury through molecular repair of nerve membranes with polyethylene glycol. *FASEB Journal* **14**, 27–35.

BORGENS, R. B., SHI, R. & BOHNERT, D. (2002) Behavioral recovery from spinal cord injury following delayed application of polyethylene glycol. *Journal of Experimental Biology* **205**, 1–12.

DUERSTOCK, B. S. & BORGENS, R. B. (2002) Three-dimentional morphometry of spinal cord injury follow polyethylene glycol treatment. *Journal of Experimental Biology* **205**, 13–24.

EMERY, D. G., LUCAS, J. H. & GROSS, G. W. (1987) The sequence of ultrastructural changes in cultured neurons after dendrite transection. *Experimental Brain Research* **67**, 41–45.

FAWCETT, J. W. & KEYNES, R. J. (1990) Peripheral nerve regeneration. *Annual Review of Neuroscience* **13**, 43–60.

FITZPATRICK, M. O., MAXWELL, W. L. & GRAHAM, D. I. (1998) The role of the axolemma in the initiation of traumatically induced axonal injury (editorial).

Journal of Neurology, Neurosurgery & Psychiatry **64**, 285–287.

HUANG, P. P. & YOUNG, W. (1994) The effects of arterial blood gas values on lesion volumes in a graded rat spinal cord contusion model. *Journal of Neurotrauma* **11**, 547–562.

KIM, H., KOEHLER, R. C., HURN, P. D., HALL, E. D. & TRAYSTMAN, R. J. (1996) Amelioration of impaired cerebral metabolism after severe acidotic ischemia by tririlazad postteatment in dogs. *Stroke* **27**, 114–121.

LUCAS, J. H., EMERY, D. G. & ROSENBERG, L. J. (1997) Physical injury of neurons: Important roles for sodium and chloride ions. *The Neuroscientist* **3**, 89–101.

LUCAS, J. H., GROSS, G. W., EMERY, D. G. & GARDNER, C. R. (1985) Neuronal survival or death after dendrite transection close to the perikaryon: Correlation with electrophysiologic, morphologic, and ultrastructural changes. *Central Nervous System Trauma* **2**, 231–255.

LUO, J., UCHIDA, K. & SHI, R. (2003) Lipid peroxidation products, acrolein and 4-hydroxynonenal, are increased following traumatic spinal cord injury in guinea pig (abstract). 33rd Society for Neuroscience annual meeting neurobiology of lipid sessions. *Neurobiology of Lipids* **2**, 3.

MAXWELL, W. L., DOMLEO, A., MCCOLL, G., JAFARI, S. S. & GRAHAM, D. I. (2003) Post-acute alterations in the axonal cytoskeleton after traumatic axonal injury. *Journal of Neurotrauma* **20**, 151–168.

MAXWELL, W. L., KOSANLAVIT, R., MCCREATH, B. J., REID, O. & GRAHAM, D. I. (1999) Freeze-fracture and cytochemical evidence for structural and functional alteration in the axolemma and myelin sheath of adult guinea pig optic nerve fibers after stretch injury. *Journal of Neurotrauma* **16**, 273–284.

MAXWELL, W. L., MCCREATH, B. J., GRAHAM, D. I. & GENNARELLI, T. A. (1995) Cytochemical evidence for redistribution of membrane pump calcium-ATPase and ecto-Ca-ATPase activity, and calcium influx in myelinated nerve fibers of the optic nerve after stretch injury. *Journal of Neurocytology* **24**, 925–942.

MAXWELL, W. L., WATT, C., GRAHAM, D. I. & GENNARELLI, T. A. (1993) Ultrastructural evidence of axonal shearing as a result of lateral acceleration of the head in non-human primates. *Acta Neuropathologica* **86**, 136–144.

MEIRI, H., SPIRA, M. E. & PARNAS, I. (1981) Membrane conductance and action potetial of a regenerating axon tip. *Science* **211**, 709–712.

PEASLEY, M. A. & SHI, R. (2002) Resistance of isolated mammalian spinal cord white matter to oxygen-glucose deprivation. *American Journal of Physiology Cell Physiology* **283**, C980–C989.

PETTUS, E. H. & POVLISHOCK, J. T. (1996) Characterization of a distinct set of intra-axonal ultrastructural changes associated with traumatically induced alteration in axolemmal permeability. *Brain Research* **722**, 1–11.

PETTUS, E. H., CHRISTMAN, C. W., GIEBEL, M. L. & POVLISHOCK, J. T. (1994) Traumatically induced altered membrane permeability: Its relationship to traumatically induced reactive axonal change. *Journal of Neurotrauma* **11**, 507–522.

POVLISHOCK, J. T. & PETTUS, E. H. (1996) Traumatically induced axonal damage: Evidence for enduring changes in axolemmal permeability with associated cytoskeletal change. *Acta Neurochirurgica—Supplementum* **66**, 81–86.

RANSOM, B. R., STYS, P. K. & WAXMAN, S. G. (1990) The pathophysiology of anoxic injury in central nervous system white matter. *Stroke* **21**, 52–57.

ROEDERER, E., GOLDBERG, N. H. & COHEN, M. J. (1983) Modification of retrograde degeneration in transected spinal axons of the lamprey by applied DC current. *Journal of Neuroscience* **3**, 153–160.

SCHWAB, M. E., KAPFHAMMER, J. P. & BANDTLOW, C. E. (1993) Inhibitors of neurite growth. *Annual Review of Neuroscience* **16**, 565–595.

SHI, R. (2002) *In vivo* evidence of membrane damage following compression of adult guinea pig spinal cord. *Journal of Neurotrauma* **19**, 1394.

SHI, R. & BLIGHT, A. R. (1996) Compression injury of mammalian spinal cord *in vitro* and the dynamics of action potential conduction failure. *Journal of Neurophysiology* **76**, 1572–1580.

SHI, R. & BORGENS, R. B. (1999) Acute repair of crushed guinea pig spinal cord by polyethylene glycol. *Journal of Neurophysiology* **81**, 2406–2414.

SHI, R. & BORGENS, R. B. (2000) Anatomic repair of nerve membrane in crushed mammalian spinal cord with Polyethylene Glycol. *Journal of Neurocytology* **29**, 633–644.

SHI, R. & PRYOR, J. D. (2000) Temperature dependence of membrane sealing following transection in mammalian spinal cord axons. *Neuroscince* **98**, 157–166.

SHI, R. & PRYOR, J. D. (2002) Pathological changes of isolated spinal cord axons in response to mechanical stretch. *Neuroscience* **110**, 765–777.

SHI, R., ASANO, T., VINING, N. C. & BLIGHT, A. R. (2000) Control of membrane sealing in injured mammalian spinal cord axons. *Journal of Neurophysiology* **84**, 1763–1769.

SHI, R., BORGENS, R. B. & BLIGHT, A. R. (1999) Functional reconnection of severed mammalian spinal cord axons with polyethylene glycol. *Journal of Neurotrauma* **16**, 727–738.

SHI, R., LUO, J. & PEASLEY, M. A. (2002) Acrolein inflicts axonal membrane disruption and conduction loss in isolated guinea pig spinal cord. *Neuroscience* **115**, 337–340.

SHI, R., LUCAS, J. H., WOLF, A. & GROSS, G. W. (1989) Calcium antagonists fail to protect mammalian spinal neurons after physical injury. *Journal of Neurotrauma* **6**, 261–276; discussion 77–78.

SHI, R., QIAO, X., EMERSON, N. & MALCOM, A. (2001) Dimethylsulfoxide enhances CNS neuronal plasma membrane resealing after injury in low temperature or low calcium. *Journal of Neurocytology* **30**, 829–839.

STOKES, B. T., FOX, P. & HOLLINDEN, G. (1983) Extracellular calcium activity in the injured spinal cord. *Experimental Neurology* **80**, 561–572.

STRAUTMAN, A. F., CORK, R. J. & ROBINSON, K. R. (1990) The distribution of free calcium in transected spinal axons and its modulation by applied

electrical fields. *Journal of Neuroscience* **10**, 3564–3575.

XIE, X. & BARRETT, J. N. (1991) Membrane resealing in cultured rat septal neurons after neurite transection: Evidence for enhancement by Ca^{2+}-triggered protease activity and cytoskeletal disassembly. *Journal of Neuroscience* **11**, 3257–3267.

YAWO, H. & KUNO, M. (1985) Calcium dependence of membrane sealing at the cut end of the cockroach giant axon. *Journal of Neuroscience* **5**, 1626–1632.

YOUNG, W. & KOREH, I. (1986) Potassium and calcium changes in injured spinal cords. *Brain Research* **365**, 42–53.