Video Article

A Contusion Model of Severe Spinal Cord Injury in Rats

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Abstract

The translational potential of novel treatments should be investigated in severe spinal cord injury (SCI) contusion models. A detailed methodology is described to obtain a consistent model of severe SCI. Use of a stereotactic frame and computer controlled impactor allows for creation of reproducible injury. Hypothermia and urinary tract infection pose significant challenges in the post-operative period. Careful monitoring of animals with daily weight recording and bladder expression allows for early detection of post-operative complications. The functional results of this contusion model are equivalent to transection models. The contusion model can be utilized to evaluate the efficacy of both neuroprotective and neuroregenerative approaches.

Video Link

The video component of this article can be found at http://www.jove.com/video/50111/

Introduction

Choice of appropriate injury model is crucial for preclinical evaluation of novel treatments for spinal cord injury (SCI).[1,2,13] In a recent survey of physicians and scientists in the field of neurotrauma contusion model, as opposed to hemisection or complete transection models, was universally accepted to be clinically relevant.[8] This opinion is based on the observation that majority of spinal cord injury in humans is contusive in nature.[10] The biology of contusion also appears to be different from hemisection or transection models.[11] Iseda, *et al.* compared the effect of intraspinal chondroitinase ABC injection on neuroregeneration separately in hemisection and contusion models.[4] Axonal regeneration was observed in the neuronal bridge in hemisection but not the contusion SCI group. The hemisection or complete transection models also create conditions known to exist in only a very small subset of clinical circumstances. For example, several investigators have employed scaffold-based interventions for implantation in the lesion cavity after hemisection or complete transection to promote regeneration.[6] This approach becomes clinically irrelevant because creation of a cavity within injured spinal cord is impractical and probably unethical.

Variability in functional recovery remains a major challenge for the contusion models.[5,12] This variability can be minimized by the use of computer-controlled impactor and stabilization of spine before impact for uniform force delivery across the spinal cord volume especially the ventrally located motor pathways. It must be noted that plasticity and collateral contribution from surviving axons is the predominant mechanism of recovery after spinal cord injury.[1] Therefore even minor variations in contusion technique may yield significantly different results. To this end we have developed a model of severe spinal cord injury which yields consistent contusion volume and functional recovery comparable with transection models. This model may be utilized for investigating both the neuroprotection and neuroregeneration strategies as a proof of concept for the treatment efficacy.

Protocol

1. Preparation Before Spinal Cord Injury

The surgical instruments required for this procedure are scalpel, pickups with and without teeth, hemostats, self retaining retractors, fine tip rongeurs, needle driver, absorbable sutures, and skin clip applicators. Other surgical supplies required are surgical drapes, sterile sheets for surgical field, gauze sponges, cotton-tip applicators, and metallic foil. Autoclave the surgical instruments and supplies prior to the surgery. Use

the individual set of instruments and supplies for one animal. Clean the surgical area and apparatus (impactor, light source, stereotactic frame, glass bead sterilizers, and heating pads) with alcohol wipes.

Open the surgical drapes and use sterile gloves to drape the surgical field carefully avoiding contamination. Open the individual instruments sterilely and carefully place those in the surgical field. Cover the knobs and handles of the apparatus likely needed during the procedure with the sterile metallic foil. Switch-on the glass bead sterilizer to be ready for use during the procedure.

2. Preparation of the Animals

Bring the rats to the laboratory area few hr before the actual procedure. Administer pain medication at least one hr ahead of the expected procedure (typically buprinorphine 1.5 ml of 0.006 mg/ml subcutaneous). Administer preoperative antibiotics (typically Baytril 4 mg/kg subcutaneous). Anesthetize the rats using 90 mg/kg of Ketamine and wait until there is no toe-pinch response. Palpate the most prominent spinous process in the thoracic spine. This level typically corresponds with T10 spinous process. Mark the location of intended level in relation to T10. In our lab we perform a T10 injury. The following narrative describes the technique of T10 SCI. Shave a 3 cm x 6 cm rectangle longitudinally and centered at T10 level. Clean skin three times with Betadine solution. Apply ophthalmic lubricant on each eye. Transfer the rat in a comfortable position to the surgical field carefully avoiding contamination. Insert a rectal temperature probe to monitor the core temperature and adjust the heating accordingly to keep animal temperature as close to normal (~37.5 °C) as possible. Cover the rat with a surgical drape with a window above the surgical site.

3. Surgical Procedure

Begin with an approximate 4 cm incision using a #10 blade centered on the T10 mark. Proceed to patiently dissect fascia and muscle layers away from the T9-T11 spinous processes and laminae. Place retractors to retract muscle and fascia away from the bone. While stabilizing the spinous process of T9 sharply divide the interspinous ligament between T9 and T10 using fine scissors. Similarly stabilize the T10 spinous process and divide the interspinous ligament between T10-T11. Use loop magnification to complete the division of ligaments all the way through the ligamentum flavum (**Figure 1**). The thecal sac is readily apparent once the ligamentum flavum is disconnected. Use fine tip rongeurs to carefully perform piece-meal laminectomy bilaterally at T10. Utmost care is taken to avoid a downward pressure on the thecal sac and inadvertent injury from the rongeur tips. The lamina and spinous process of T10 is completely removed.

Move the animal into position on stabilization platform. Use stabilizing clamps to immobilize the spine by clamping the lateral aspects of the T11 vertebral body followed by the lateral aspects of the T9 vertebral body. Be careful not to compress the rat into the platform with the stabilizing clamps as this would restrict space for respiratory movements and add unwanted stress the animal. After securing the spinal column settings on the computer-controlled impactor are checked. We typically use impactor tip of 3.0 mm at a speed of 4 cm/s with a depth of 2 mm and a dwell time of 0.3 sec. Extend the impactor tip and lower it until it just touches the cord surface. Retract the tip and lower the device 2 mm towards the spinal cord surface. Release piston at 4 cm/sec to cause severe contusion spinal cord injury. Attain hemostasis using just enough pressure to keep the cotton-tip applicator in place, being careful not to create any unnecessary pressure on the cord. Suture muscle and fascia layers with a figure-8 stitch being careful not to pull the too tight using absorbable sutures. Close the skin with a minimum of 2 small staples; up to 4 or 5 staples may be used if parts of the incision remain open after the first 2 or 3 staples.

3. Post-procedure Care

Place rats in a warm environment of about 33-35 °C for 24 hr post-surgery. This entails an incubator (while they are unconscious) and a heated cage space once they begin to move. Once rats are fully awake, administer 5ml of saline, 1.5 ml (0.006 mg/ml) of buprinorphine, and 0.1 ml of Baytril all subcutaneously. Continue with buprinorphine subcutaneously twice a day for first 24-48 hr and Baytril once a day for 7 days. Bladders should be manually expressed three times a day until return of bladder function (<2 ml of urine in early morning expression for 3 consecutive days). Animals should also be checked during this time for infection (blood in urine, whitish color, or foul odor), decreased physical activity or problems with wound healing. Presence of infection should be countered with an increased dosage (or re-initiation) of antibiotics in consultation with local veterinarians. Weigh rats daily beginning the day after surgery to assess their recovery.

Representative Results

Lesion Volume

We have obtained large and consistent lesion volumes by following the technique described above. Using a Luxol fast blue staining a mean lesion volume of 2.04 mm^3 (95% CI 1.9-2.18) (n = 5 animals) was obtained. **Figure 2** shows mean lesion volume with a representative staining using Luxol fast blue through the lesion epicenter.

Functional Scores

The behavioral scores as measured by the Basso, Beattie, Bresnahan (BBB) scale are shown in **Figure 3**. At 12 weeks the rats in control group achieved a mean BBB score of 2.2±1.1 (n = 10 animals).

Other Parameters

Based on our most recent experience with 32 animal surgeries the survival rate of this technique is 93.4%. All the mortalities were related to persistent urinary tract infection (UTI) followed by animal sacrifice to avoid excessive pain and suffering. After finishing the course of antibiotics for 7 days 16.7% animals developed UTI. The mean number of days for detection of UTI post-operative was 14.6±7.6 days. Interestingly the animals attained bladder control on an average 13 days after injury (mean 13.33±3.6 days). Rats who developed UTI took longer to achieve

bladder control as compared to the rats without UTI (Bladder control in rats with UTI - 16±1 days versus 12.8±3.7 days for bladder control in rats without UTI, p = 0.03). In the cohort that attained bladder control within 14 days no subsequent UTI was detected. As shown in **Figure 4**, there was an initial gain in weight after SCI likely from fluid retention. The weight subsequently dropped and stabilized to a lower level (approximately 10-12% lower than baseline) in about 10 days.

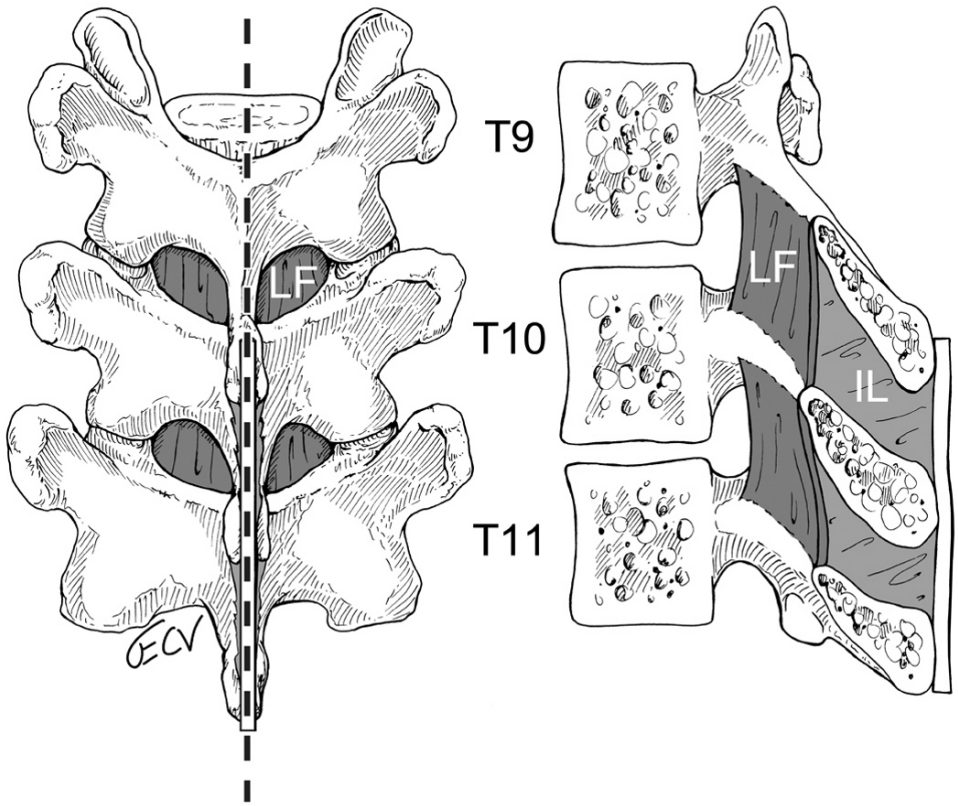

Figure 1. Vertebral anatomy of rat from the posterior approach (left) demonstrating the spinous processes and laminae. The position of ligamentum flavum (LF) is depicted in the space between the laminae. Mid-saggital section (right) showing the relative position of the ligamentum flavum and the interspinous ligament in relation to the spinal cord.

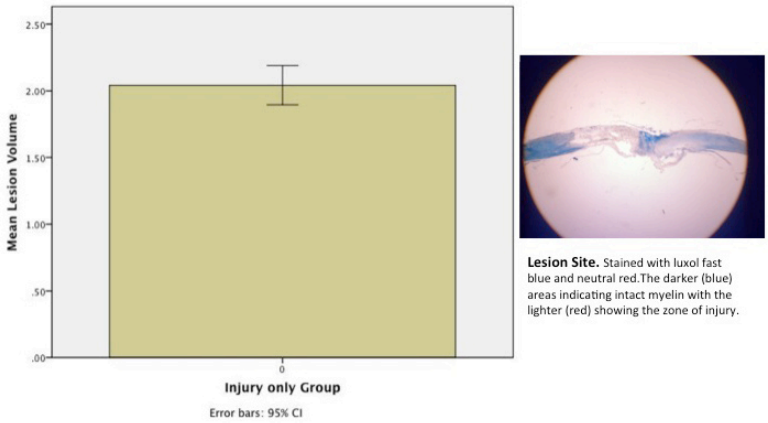

Figure 2. Bar graph showing lesion volume (in mm^3, Y axis) in animals receiving severe spinal cord injury (left). A representative longitudinal section through injury epicenter after Luxol fast blue staining (right).

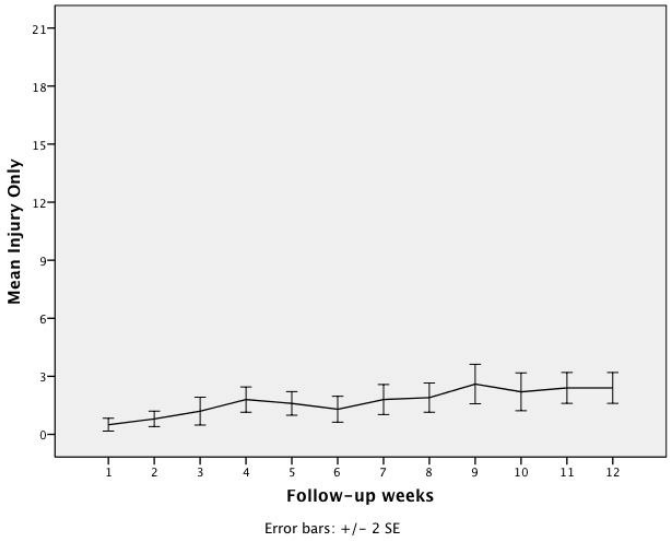

Figure 3. Mean BBB (Y axis) score of the rats over the course of 3 month follow-up (X axis) demonstrating minimal recovery typical of severe SCI.

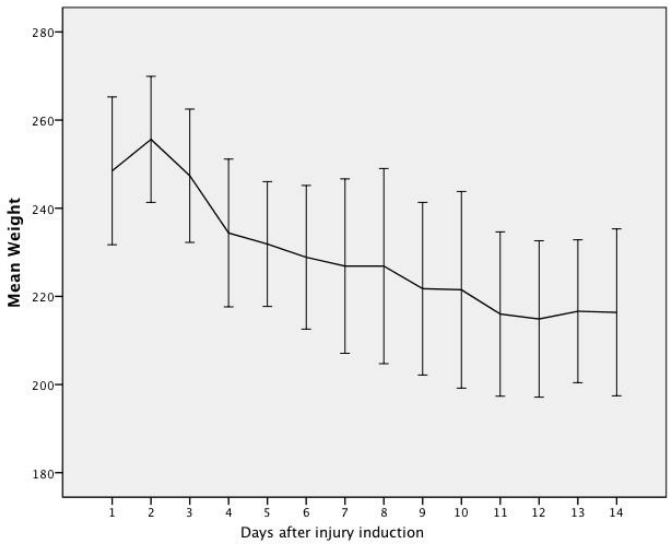

Figure 4. Line diagram showing variation in daily weight (in g, Y axis) of rats over 2 weeks duration (X axis) after induction of spinal cord injury.

Discussion

Several novel treatments have recently shown early promise in the field of SCI research.[3] Careful evaluation of these treatments is essential in clinically relevant model of SCI to select strategies with maximum translational potential. A scheme of grading was recently developed to evaluate the strength of preclinical studies.[9] This scheme emphasized the importance of utilizing contusion model of severe SCI. Here we describe such a contusion model of severe SCI with consistent lesion volumes and functional scores resembling those of transection models. This model may be utilized as a 'proof of concept' to establish treatment efficacy both for neuroprotection and neuroregeneration strategies.

The generation of uniform contusive SCI remains challenging. For reproducibility, it is essential that the injury be performed as uniformly as possible. The targeted level of the spinal cord must be identified consistently from animal to animal. Removal of the bone during laminectomy should be done carefully to ensure no bone fragments are left in the spinal canal; these could cause compression injury and introduce unwanted variations in injury mechanisms and recovery potential. We have adopted several steps to ensure uniformity including size of impactor, rigid stabilization of spine on a frame with mounted stabilizing arms, using a uniform impact speed and depth of impaction. It is important that the rat be positioned comfortably when the stabilizing forceps are clamped to the vertebral bodies. Any variations in the spinal alignment or excess stress during the clamping may alter the biomechanics of impact. We used a 2.5 mm impactor since the average size of rat spinal cord in our region of interest is 2.5-3 mm. The contact time or dwell was uniformly set at 0.3 sec in our experiments. However in our observation and reports from other labs, for a severe model of SCI the depth of impact is the most crucial parameter. In the experiments described in this paper the

impact was delivered at a constant depth of 2 mm. Other factors affecting uniformity include personnel training, clear visualization of the tissue, and impactor tip to ensure direct impact in the cord tissue (and not the bony structures).

The rats should be constantly observed during the procedure for necessary vital signs most importantly core temperature and breathing. Hypothermia is the leading cause of mortality during and immediately after anesthesia administration. Monitoring of core temperature with rectal probe and appropriate heating pads will avoid this complication. If the breathing becomes irregular or the animal ceases to breath, the procedure should be immediately stopped until breathing returns to baseline. Excessive pain and or inadvertent over dosage of pain and anesthetic medication may also result in breathing problems. The rats should be carefully monitored post-procedure. Their weights should be measured accurately and a loss of >20% from baseline weight should prompt an investigation into food and water intake, urinary tract infection, skin breakdown, post-SCI ileus, etc. Early consultation with veterinary staff for evaluation and treatment is crucial in such situations. As discussed earlier, rats who have not regained bladder control by day 14 should be started back on antibiotics to avoid a potentially fatal urinary tract infection.

Spinal cord injury can be induced by either a displacement method or constant force method.[5,12] The technique described in this paper is displacement method where force is delivered to fixed depth within the spinal cord tissue. The use of a computer-controlled impactor allows for a level of control that is not attainable by the other common methods of experimental SCI. The clip compression technique does not allow for a blunt, contusive force with immediate release, while the NYU impactor depends on gravity to determine the force of contusion. These techniques although reproducible, don't allow the simulation of conditions encountered during a motor vehicle accident, the most common cause of acute SCI in humans. Previous studies have shown that injury severity is directly correlated with depth of impact than the velocity of impact.[7] Therefore this model allows for a severe SCI to be routinely performed with the potential to modify the severity through a greater range of combinations of force and displacement.

Disclosures

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References

1. Blesch, A. & Tuszynski, M.H. Spinal cord injury: plasticity, regeneration and the challenge of translational drug development. *Trends Neurosci.* **32**, 41-7 (2009).
2. Dobkin, B.H. Curiosity and cure: translational research strategies for neural repair-mediated rehabilitation. *Dev. Neurobiol.* **67**, 1133-47 (2007).
3. Fehlings, M.G., Cadotte, D.W., & Fehlings, L.N. A series of systematic reviews on the treatment of acute spinal cord injury: a foundation for best medical practice. *J. Neurotrauma.* **28**, 1329-33 (2011).
4. Iseda, T., Okuda, T., Kane-Goldsmith, N., *et al.* Single, high-dose intraspinal injection of chondroitinase reduces glycosaminoglycans in injured spinal cord and promotes corticospinal axonal regrowth after hemisection but not contusion. *J. Neurotrauma.* **25**, 334-49 (2008).
5. Khan, T., Havey, R.M., Sayers, S.T., *et al.* Animal models of spinal cord contusion injuries. *Laboratory Animal Science.* **49**, 161-72 (1999).
6. Kim, B.G., Kang, Y.M., Phi, J.H., *et al.* Implantation of polymer scaffolds seeded with neural stem cells in a canine spinal cord injury model. *Cytotherapy.* **12**, 841-5 (2010).
7. Kim, J.H., Tu, T.W., Bayly, P.V., *et al.* Impact speed does not determine severity of spinal cord injury in mice with fixed impact displacement. *Journal of Neurotrauma.* **26**, 1395-404 (2009).
8. Kwon, B.K., Hillyer, J., & Tetzlaff, W. Translational research in spinal cord injury: a survey of opinion from the SCI community. *J. Neurotrauma.* **27**, 21-33 (2010).
9. Kwon, B.K., Okon, E.B., Tsai, E., *et al.* A grading system to evaluate objectively the strength of pre-clinical data of acute neuroprotective therapies for clinical translation in spinal cord injury. *J. Neurotrauma.* **28**, 1525-43 (2011).
10. Norenberg, M.D., Smith, J., & Marcillo, A. The pathology of human spinal cord injury: defining the problems. *J. Neurotrauma.* **21**, 429-40 (2004).
11. Siegenthaler, M.M., Tu, M.K., & Keirstead, H.S. The extent of myelin pathology differs following contusion and transection spinal cord injury. *J. Neurotrauma.* **24**, 1631-46 (2007).
12. Talac, R., Friedman, J.A., Moore, M.J., *et al.* Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies. *Biomaterials.* **25**, 1505-10 (2004).
13. Tator, C.H. Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. *Neurosurgery.* **59**, 957-82, discussion 82-7, (2006).

