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An efficient device to experimentally model compression injury of mammalian spinal cord☆

Alexander E. Ropper [a,b,1], Xiang Zeng [a,b,1], Jamie E. Anderson [a,b], Dou Yu [a,b], InBo Han [a,b,2], Hariprakash Haragopal [a,b], Yang D. Teng [a,b,c,*]

[a] Department of Neurosurgery, Harvard Medical School/Brigham and Women's Hospital, Boston, MA 02115, USA
[b] Division of SCI Research, Veteran Affairs Boston Healthcare System, Boston, MA 02130, USA
[c] Department of Physical Medicine & Rehabilitation, Harvard Medical School/Spaulding Rehabilitation Hospital, Boston, MA 02114, USA

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ABSTRACT

We report an efficient and effective device to reproducibly model clinically relevant spinal cord injury (SCI) via controlled mechanical compression. In the present study, following skin incision, dorsal laminectomy was performed to expose T10 spinal cord of adult female Sprague–Dawley rats (230–250 g). The vertebral column was suspended and stabilized by Allis clamps at T8 and 12 spinous processes. A metal impounder was then gently loaded onto T10 dura (20, 35 or 50 g × 5 min; n = 7/group), resulting in acute mild, moderate, or severe standing weight compression, respectively. Neurobehavioral outcomes were evaluated using the BBB locomotor scale and inclined plane test for coordinated hindlimb function, and a battery of spinal reflex tests for sensorimotor functions, at 1 day following SCI and weekly thereafter for 7 weeks. Quantitative histopathology was used to assess injury-triggered loss of white matter, gray matter and ventral horn motor neurons. Immunocytochemical levels of glial fibrillary acidic protein (GFAP) and β-amyloid precursor protein (APP) at the cervical and lumbar regions were measured to determine the distal segment impact of T10 compression. The data demonstrates that the standardized protocol generates weight-dependent hindlimb motosensory deficits and neurodegeneration primarily at and near the lesion epicenter. Importantly, there are significantly increased GFAP and APP expressions in spinal cord segments involved in eliciting post-SCI allodynia. Therefore, the described system reliably produces compression trauma in manners partially emulating clinical quasi-static insults to the spinal cord, providing a pragmatic model to investigate pathophysiological events and potential therapeutics for compression SCI.

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1. Introduction

Compression spinal cord injury (SCI) results from malalignment of the spinal column, caused by migration of bone fragments or a herniated disk and/or soft tissue including hematomas, abscesses, and most commonly, benign or malignant neoplasms (e.g., metastatic tumors in the spine narrowing the spinal canal or, less frequently, tumors growing within the spinal cord). The mechanical impact rarely transects the cord completely, even when the functional loss is considered complete (Tator, 1983). The primary consequences of compression injury are the initial parenchyma compression manifesting as a function of the speed, force and volume of tissue deformation/dislocation, which is subsequently complicated by progressive secondary injury processes interacting with very limited endogenous repair responses in the adult mammalian spinal cord (Karadimas et al., 2013; Onifer et al., 2007; Tator and Fehlings, 1991).

Modeling of compression SCI is therefore based on features of different devices designed to mainly emulate the mechanical impact of the spinal canal occlusion onset and progression that usually happen in humans. This line of reasoning has produced compression models that use either clip or balloon to mediate pressure insult to the spinal cord (Poon et al., 2007; Kobrine et al., 1979; Forgione et al., 2014; Dolan and Tator, 1979; Su et al., 2015). However, clip-triggered compression is generally considered to be a combinatorial injury of acute contusion and compression as the clip not only imposes pressure directly to the spinal cord but also introduces shear and even laceration-like force to the cord tissue. Consequently, the clip SCI model does produce direct damage and secondary ischemic events that by principle mimic common pathological outcomes of a large proportion of clinical SCI (Tator

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* Correspondence to: Yang D. Teng, Ph.D., M.D., Departments of Neurosurgery and PM&R, BWH/SRH/Harvard Medical School and Division of SCI Research, VABHS, Boston, MA, USA.
E-mail address: yang_teng@hms.harvard.edu (Y.D. Teng).

[1] A.E.R. and X.Z. contributed equally to the study.

[2] Present addresses: Neurosurgery, CHA Bundang Medical Center, Seongnam, South Korea (I.H.).

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and Fehlings, 1991; Onifer et al., 2007). Although clip-induced compression injury can be calibrated to exert mild, moderate, or severe lesion (Poon et al., 2007), its reproducibility requires sufficient training of the investigator, similar to most contusion SCI models due to the complexity of neurobiology, topology, and biophysics of the spinal cord. By contrast, the balloon-induced compression is used to generate acute trauma as well as chronic injury with the latter closely emulating tumor growth. It also has post care and maintenance benefits due to largely non-penetrating damage to the surrounding structures of the spinal cord. Conversely, the length of the balloon catheter and the inflation volume must be measured and maintained continuously to ensure impact consistency; in addition, the size of the experimental animal needs to be factored in empirically for most cases in order to decide a sufficient balloon volume for attaining a pre-determined injury severity (Kobrine et al., 1979).

For the purpose to create a more efficient device that can effectively model primary outcomes of compression SCI in reproducible, graded, and standardized manners (Poon et al., 2007; Onifer et al., 2007), we designed and manufactured a modeling system and tested its capacity for producing mild, moderate, and severe compression lesions to the rat spinal cord, aiming to apply it as a pragmatic model for investigating mechanisms underlying not only epicenter pathological outcomes but also distal inflammatory responses of compression SCI and for developing targeted therapeutics.

2. Materials and methods

2.1. Animal care

Adult female Sprague Dawley (SD) rats (230–250 g) for this study were purchased from Charles River Laboratories (Wilmington, MA), housed in a facility with a diurnal 12 hour light cycle and ambient temperature (i.e., 22–25 °C), and had free access to food and water. Following compression lesion, rats received buprenorphine (0.06 mg/kg, s.c., t.i.d.). Manual bladder expression of urine was performed twice daily until a reflex bladder was established. For the injuries described, this was usually achieved in days 3–5 postoperatively, much shorter than in rats with contusion SCI (Teng et al., 1999; Cheriyan et al., 2014). Other postoperative care procedures were given as per established formulas that included housing the rats in pairs to reduce isolation-induced stress, adequate hydration with Ringer's Lactate solution (Abbott Laboratories, Chicago, IL) in days 0–5 post injury (p.i.; 5–10 ml/rat/day × 5, s.c.), maintaining body temperature with warm water circulating pads, and daily change of highly absorbent bedding in 0–5 days p.i., as previously described (Teng et al., 2004). No prophylactic antibiotics were prescribed. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Harvard Medical School.

2.2. Experimental protocol

The experiments were performed according to a randomized block design. Experimental group size was decided on the basis of power analysis of outcome measure data from lower thoracic SCI studies previously published (Teng et al., 1998; Konya et al., 2008). On the basis of these analyses, with seven rats per group, there is an 87% probability of detecting an effect of 50% or more in ventral horn neuronal sparing at a spinal level 3 mm caudal to the injury epicenter, offering sufficient statistical power to assess post-SCI pathology (Teng et al., 1998; Konya et al., 2008). All rats survived the entire study.

2.3. Standing weight compression spinal cord injury

2.3.1. Device

The instrument was designed by the principal investigator Dr. Teng in productive collaboration with his postdoctoral fellows Dr. D. Yu and Dr. A. Ropper and manufactured by the Machine Shop of Harvard School of Engineering and Applied Sciences. To mimic quasi-static compression to the spinal cord, a stainless steel impounder carrying pre-determined weight (i.e., 20, 35, or 50 g) was loaded to the spinal cord to produce mild, moderate, and severe compression, respectively. The injury impounder has a whole piece design, which simplifies cleaning, sterilization, and application procedures. The impounder has an impacting rod (end surface: r = 1 mm; area = 3.14 mm^2) centrally anchored under the weight disc (r = 1 cm; area = 3.14 cm^2; Fig. 1A). After suspending and stabilizing the spine with two pairs of Allis clamps supported by the stereotactic frame of the device (Fig. 1B, C), the impounder tip was carefully loaded upon the spinal cord dorsal surface via releasing the weight disc by fine-tuning a micromanipulator (Model: 80563; Prior Scientific Inc., Rockland, MA; Fig. 1D) to deliver a predetermined severity of focal compression (Yu et al., 2013). The compression rate resulting from the impact of each pre-determined standing impounder load was tested in 4% paraformaldehyde mildly fixed thoracic spinal cord in order to confirm the quasi-static characteristics of the compression insult (see Results).

2.3.2. SCI modeling

After dermal preparation and precise positioning of an anesthetized rat (mixture of 75 mg/kg ketamine and 10 mg/kg xylazine, i.p.) along the X, Y, and Z coordinates to best expose the T10 segment (Fig. 1C), a mid-line skin incision was made in the lower thoracic area (T8-T12). Subcutaneous and periosteal soft tissue and muscle dissections were performed to expose the dorsal lamina before T10 laminectomy was performed. Hemostatic procedure comprised applications of Gelfoam® (Pfizer Inc., MA) and saline irrigation. The T8 and T12 spinal spinous processes were secured by angulated Allis clamps attached to the stereotaxic frame (Fig. 1C). The levels of the clamps were adjusted to maintain T10 spinal cord horizontal (i.e., parallel to the frame baseboard). Impounder rod of 20 g, 35 g, or 50 g was gently placed onto the exposed dorsal dura surface via the micromanipulator (Fig. 1D) to deliver a 5 min standing of the rod to produce mild, moderate, or severe compression injury, respectively. Rats undergoing laminectomy only served as surgical control. After removing the impounder, a small square of Surgifoam® (Ethicon US, LLC, Somerville, NJ) was placed over the injury site. The overlaying muscle was reapproximated with suture (4-0 Vicryl coated absorbable sterile surgical suture; Ethicon, Johnson & Johnson, USA) and skin stapled with Reflex® 9 mm wound clips (Cellpoint Scientific Inc., MD). Please see postoperative procedures detailed in Animal Care.

2.4. Coordinated hindlimb function assays

Rats were behaviorally tested for functional deficits starting 1 day after surgery then once per week for 7 weeks.

BBB open-field locomotion score: The general locomotor ability of the rats was assessed using the BBB locomotor rating scale per our established expertise (Basso et al., 1995; Teng et al., 1999; Konya et al., 2008). For this test, rats were placed individually in an open, flat area staged by a piece of rubber pad with a patterned surface design to ensure consistent traction quality. Each locomotion evaluation session was done by two observers blind to the group assignment, and a final score was recorded after a mutual agreement was reached. The BBB scale ranges from 0 to 21 where 0 indicates complete paralysis and 21 normal function, which factors in parameters including joint movement, weight-bearing stepping ability, front and hind limb coordination, trunk stability, paw posture, and tail position (Basso et al., 1995; Teng et al., 2004). The BBB system was designed as a parametric statistic to provide a continuous measure of overall hindlimb function that is reversely correlated to lower thoracic injury severity (Basso et al., 1995).

Inclined plane test: Rats were placed individually on a 40 × 30 cm board covered with a rubber mat that had a fine groove-patterned surface (Teng et al., 2004; Konya et al., 2008). Since the rats endured a