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Original Article

A hemi-contusive cervical spinal cord injury model with displacement control in rats

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Abstract: The present study is aimed to develop a hemi-contusive injury model of cervical spinal cord injury (SCI) with displacement control at a higher contusive speed, and demonstrates unilateral cord tissue loss and ipsilateral forelimbs impairment. Ten adult male Sprague-Dawley rats were subjected to the hemi-contusion SCI. 1.5-mm-in-diameter cylinder impactor 22.5° laterally and 1.4 mm off-set was driven to compress unilateral C5 cord 2.2 mm at 600 mm/s. Forelimb functional assessments, including Montoya staircase task, cylinder rearing test and grooming test, were performed before and after injury. At 12 weeks post-injury, spared gray, white matter, and lesion area were calculated on a series of transverse cord sections. Average contusive displacement, speed and compressive force were 2.196±0.003 mm, 598.5±1.4 mm/s and 1.462±0.117 N respectively. The unilateral contusive injury resulted in ipsilateral tissue loss of anterior horn and lateral funiculus, while the ipsilateral dorsal funiculus and dorsal horn and contralateral cord were intact. In epicenter, average lesion area is 1.56±0.30 mm^2, and percentage of white and gray matter area accounted for 40±14% and 41±12% respectively. The ipsilateral forelimb exhibited sustained impairments in ipsilateral forelimb motor function after injury. The present study showed that the hemi-contusive SCI model with displacement control achieved consistent contusive displacement and force at a higher speed. The spinal cord tissue loss was confined within ipsilateral cord. The ipsilateral forelimb exhibited sustained impairments in motor function after injury while contralateral forelimb was intact. Our study provides an alternative method to establish a contusive SCI model for neuroprotective strategies.

Keywords: Behavioral assessment, displacement control, hemi-contusion, spinal cord injury

Introduction

Most human spinal cord injury (SCI) are contusive or compressive as seen in sport injuries and vehicle accidents, and occur with greatest frequency at the cervical level [1]. A lot of efforts have been attempted to develop animal models of cervical contusive SCI [2-4]. Cervical contusion models are typically developed with some modification to a pre-existing thoracic contusion models. Weight drop devices, such as modified Allen's weight drop device and MASCIS/New York University impactor, are used to injury animals either vertical or oblique impact to the cervical spinal cord [2, 5]. The Ohio State University (OSU) Impactor with a displacement limit of 1.5 mm is used to develop spinal cord contusions [6]. The Infinite Horizon Spinal Cord Impactor (Precisions Systems & Instrumentation, Lexington, KY) with a force limit has been used to develop the cervical SCI models with a force ranged from 100 kdyn to 300 kdyn [7-9]. However, all these aforementioned contusion injury occurred at relative lower speed (100-300 mm/s). Previous studies demonstrated the importance of high impact velocity as a variable in models of SCI, because contusion velocity had an effect on the magnitude of injury within the white matter and the amount of neuronal damage in the gray matter [10].

Unilateral cervical SCI model produces forelimb deficits ipsilateral to the side of injury while sparing the function of the forelimb contralateral to the injury, and minimal hindlimb deficits [2, 4, 11-13]. Moreover, this model keeps the descending circuits relatively normal functioning, leads to less complications such as bladder, bowel, or respiratory dysfunction, and significantly reduces the need for chronic intensive care after SCI [2, 14]. Recently, Lee described the systematic development of a cervical unilateral contusion model with an lateral angle of 22.5° and 1.4-mm off-set to the midline using the Infinite Horizon Impactor in rats, and demonstrated additional advantages as the ipsilateral proprioceptive sensory is also largely kept [15]. In the current study, we extended this model using a servo-electromagnetic material testing machine with precise displacement control at higher contusion speed.

This study is aimed to develop a hemi-contusive injury model of cervical spinal cord with displacement control at a higher contusive speed, and demonstrate unilateral cord tissue loss and ipsilateral upper limb impairment after injury.

Materials and method

All animal procedures were approvel by the Committee on the Ethics of Animal Experiments of Southern Medical University (Guangzhou, Guangdong, China). Behavioral assessment was performed at pre-injury, 2 weeks, 4 weeks, 8 weeks and 12 weeks post-injury. After behavioral assessment, all rats were sacrificed at 12 weeks post-injury. All behavioral and histological analyses were performed by persons blinded to the animal injury condition.

Animals and general housing conditions

Adult male Sprague-Dawley rats (300~350 g) were purchased from the laboratory animals center of our institute, and housed in the central animal facility, with room temperature (21°C) and artificial 12-hour light/dark cycle. All animals were given standard rodent food provided by the laboratory animal center of our institute and water ad libitum.

Surgical procedure and C5 hemi-contusion

Rats were put into a box filled with 2% isoflurane for initial anesthetization, then additionally anesthetized via a rodent mask by inhalation with 2% isoflurane carried by a 2:1 of nitrous oxide and oxygen. To alleviate pain, Tramadol was injected (subcutaneously [s.c.]; 2 mg/kg; CSPC Pharma, Heibei, China) and the eyes were lubricated with an eye gel (Liposic, Fabrik GmbH, Berlin, Germany) to prevent desiccation. Skin in the neck region was shaved and disinfected with betadine and 70% ethanol.

Rats were placed in a stereotaxic frame (Ruiwode Instruments, Shengzhen, Guangdong, China) and stretched from the tail with 100 g dead-weight in order to keep the cervical spine straight. A 2-cm dorsal midline skin incision over the upper cervical area was made. Connective and muscle tissue were dissected to expose the posterior vertebral elements from C3 to C7. A unilateral laminectomy was performed to the C5 vertebra to exposure the left cord (**Figure 1A**). A custom designed clamp was mounted along the groove between the transverse processes and facet joint column from C4 to C6, and attached rigidly to the stereotaxic frame. The base of the frame was tilted 22.5° and mounted onto a x-y table which was fixed to the base of a material testing machine (ElectroPuls E1000, Instron, Canton, MA) (**Figure 1B**).

Cylinder impactor tip of 1.5 mm diameter was pointed to midline at C5, then the animal was moved laterally to the left side 1.4 mm via the underneath x-y table. The initial contact position of the impactor was determined by applying a touch force (25 Hz and 0.2 mm) up to 0.01 N. The impactor was driven by the material testing machine to deliver a contusion 2.2 mm to the cord at a speed of 600 mm/s. All biomechanical parameters, including displacement, force and speed, were recorded during the injury. Inertial compensation was made with a blank shot using the same program without animal.

After injury, the clamp was removed from the animal. Underlying muscle layers were sutured, and the skin incision was closed using sterilized disposable skin stapler (PWH-W35, Changzhou Health Microport Medical Device Co., LTD, China). Rats were kept in a temperature-controlled incubator set at 37°C until rats were able to move around freely, and then they were placed back in a recovery cage. To alleviate postoperative pain and prevent dehydration, Tramadol (2 mg/kg) was given twice-daily for an additional 2 days. After 2-days recovery, rats were moved back to regular cages until the end of the study.

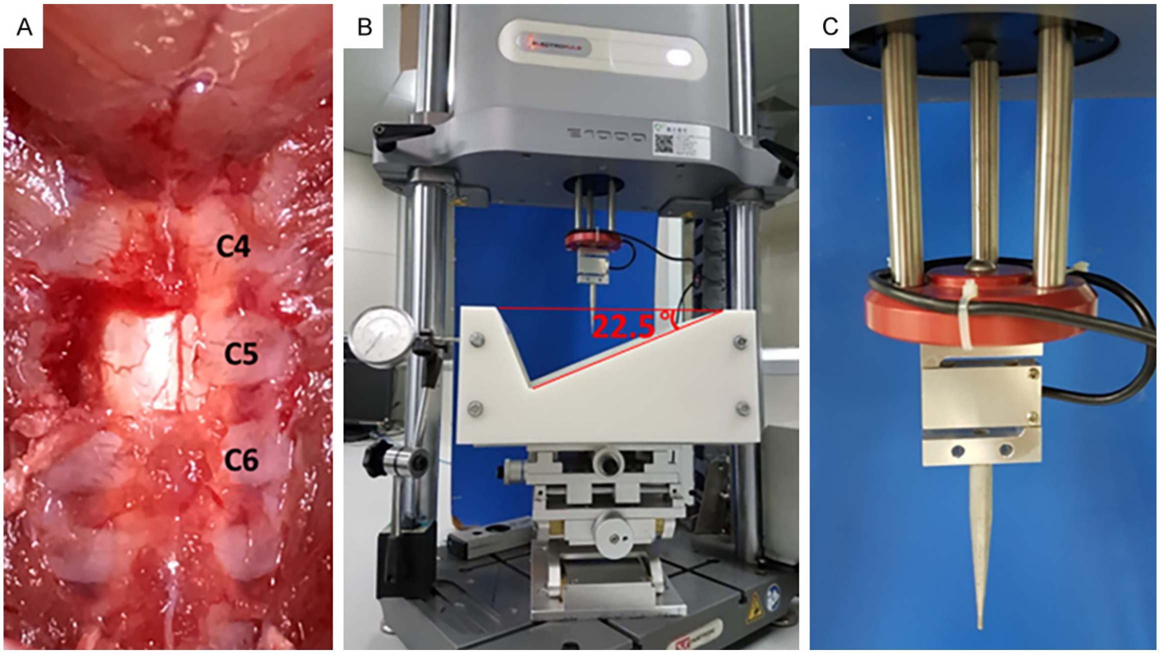

Figure 1. A unilateral laminectomy was performed at C5 (A). Photograph servo-electromagnetic material testing machine with a frame tilted at a 22.5° angle (B) and a truss structure connected with a load cell (C).

Behavioral assessment

Cylinder rearing test was described previously by Schallert [16]. Briefly, the rats were placed in a clear plexi-glass cylinder for 15 min. Two mirrors were placed at a 90° angle behind the cylinder so that the forelimbs movement could be taped at all times. The testing session was videotaped, and forelimb usage was analysed blindly after the test. Frame-by-frame analysis of the forepaws usage during 20 independent rears was performed. During a series of rear, the first paw to contact the wall was scored as initial rearing, and after initial rearing movement rats might continue to contact the wall which score as subsequent rearing. The animal would give a score of one "contralateral" and one "ipsilateral" for total rearing and initial rearing.

Grooming is an innate behavior and is assessed using a scoring system [17]. The rat's head got wet with cool water, was placed in a clear plexi-glass cylinder. Grooming test was recorded with a video camera for 15 minutes. Slow motion video playback was used to score each forelimb independently, and the max score of grooming were recorded for each side.

The staircase reaching tasks are used to assess skilled forelimb reaching. In staircase reaching tasks, rats can reach from a central platform with their forelimbs to retrieve food pellets from six descending steps with shallow wells [18, 19]. Rats were trained for the staircase reaching task using the different colors pellet as described previously [20]. The food pellets (45 mg, catalog F0021; Bioserve) were colored with a gel-based food paste from AmeriColor. Each of the 6 steps of both stairs was filled with 4 colour coded food pellets. Rats were food deprived at 12 hours before task. Animals were placed in the Montoya staircase for a period of 15 minutes. The number of pellets eaten was counted, and maximum step reached and success rates for each step were measured.

Histological analysis

Rats survived for 12 weeks after SCI. Rats were deeply anesthetized with sodium pentobarbital (Euthatal, 80 mg/kg, i.p.) and transcardially perfused with 0.1 M of phosphate-buffered saline followed by ice-cold 4% paraformaldehyde. Immediately after perfusion, lesion site tissue was dissected, and post-fixed overnight and cryoprotected in graded concentrations of sucrose. 10 mm segment of cervical cord including the injury epicenter was sectioned using a cryostat (Leica) at 20 μm thickness in

Table 1. Parameters of hemi-contusion

No.	Displacement (mm)	Speed (mm/s)	Force (N)
1	2.191	599	1.359
2	2.186	598	1.374
3	2.194	598	2.359
4	2.202	599	1.387
5	2.201	602	1.615
6	2.192	587	1.737
7	2.203	599	1.096
8	2.191	599	1.246
9	2.188	602	1.197
10	2.215	602	1.246
Average	2.196±0.003	598.5±1.4	1.462±0.117

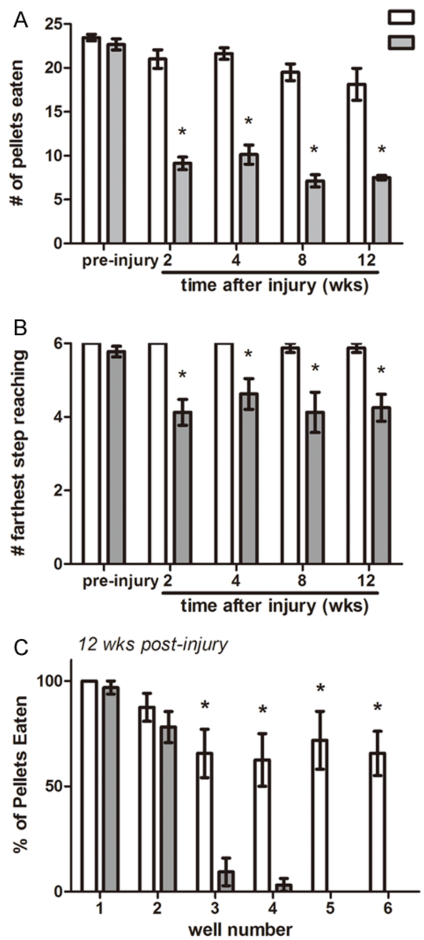

Figure 2. Results of the staircase tasks. Quantification of the number of pellets eaten using the ipsilateral and contralateral forelimb in the staircase tasks was showed (A). Analyses of the tests reveal notable impairments with the ipsilateral forelimb were seen, because of their decreased ability to retrieve pellets. Rats were able to reach the pellets in step 4 to step 5 with the ipsilateral forelimb after injury (B), and failed to reach pellets in step 5 to step 6 at 12 weeks post-injury (C).

the transverse planes. Then the sections were stained with Eriochrome Cyanine (EC). Images were obtained using a Zeiss Axioplan 2 microscope. A customized script for the Northern Eclipse software (Northern Eclipse 6.0, Empix Imaging Inc., Mississauga, ON, Canada) captured and merged images into a complete montage of the tissue sections. The injury epicenter was defined as the section with the largest lesion area. Lesion area, spared white matter and spare gray matter was manually traced using an ImageJ software. The proportion of ipsilateral spared white and gray matter areas on each section were normalized to the contralateral spared white and gray matter areas, respectively. The lesion area was defined as tissue containing abnormal cytoarchitecture based on EC staining. The percentage of lesion area was calculated as a percentage of the area of spinal cord transverse sections.

Statisticial analysis

Statistical analysis was performed using the SPSS v.20 software (SPSS Inc., IL, USA). All data were expressed as the means ± standard error of the mean (SEM). Behavioral assessment and for the ipsilateral, as well as for the contralateral, sides were summed as a measure of total spared tissue. All post-hoc analyses were made using the SNK tests, and $p< 0.05$ was considered to be significant for all tests.

Results

Mechanical parameters

Hemi-contusion SCI was induced in 10 male Sprague-Dawley rats. After SCI, biomechanical data acquired immediately after each impact was collected and analyzed. The average displacement, speed, force were, respectively, 2.196±0.003 mm, 598.5±1.4 mm/s, 1.462±0.117 N (**Table 1**). The errors of displacement readout for each case were within 1%.

Forelimb motor function

Montoya staircase task, grooming test and cylinder rearing test were adopted to evaluate the motor function after injury as they were commonly used to test the forelimb function (**Figure 2**). In the preoperative baseline staircase testing, all animals were able to reach, grasp and eat approximately 80% pellets from the first to

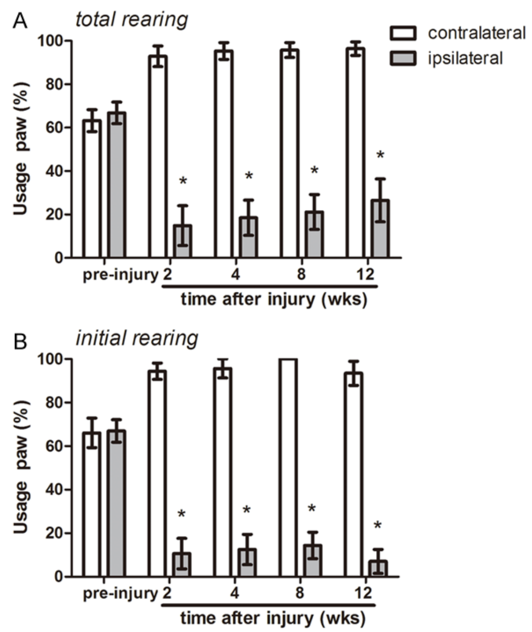

Figure 3. Results of cylinder rearing test. Before injury, rats almost equally use both paws for spontaneous vertical exploration in cylinder rearing test. There was a significant decrease in independent usage of ipsilateral paw after injury. Rats showed recovery for total rearing at 12 weeks post-injury (A), but not for initial rearing (B).

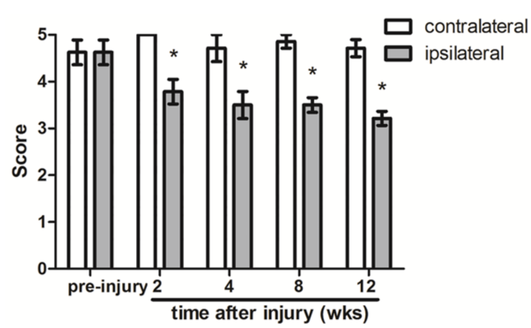

Figure 4. Grooming scores before and after C5 hemi-contusion. We observed a significant decreased range of ipsilateral forelimb motion compared to contralateral forelimb.

the sixth step without difference between sides. After hemi-contusion, the ipsilateral forelimb grasped and ate about 28%~40% of pellets, while the contralateral forelimb slightly less pellets, with significant difference in number of pellets eaten between sides after injury (**Figure 2A**). The pellets in lower steps are more difficult for rats to grasp than those in higher steps on the staircase. After SCI, rats could use ipsilateral forelimb to grasp and eat the pellets in step 4 to step 5, while the contralateral forelimb to reached the step as far as pre-injury (**Figure 2B**). No differences of the success rates for eaten pellets from the first two steps were observed between sides. The success rates of ipsilateral side were dramatically reduced in the step 3 to step 6. None pellet of on the step 5 and step 6 was reached by the ipsilateral paw (**Figure 2C**).

The cylinder rearing test was used to evaluate spontaneous forelimb usage for rodents. Before injury, all rats used both paws 80% simultaneously for the majority of weight support in the cylinder. There was no significant difference between sides before injury. An obvious less usage of the ipsilateral paw after injury was observed in total rearing, while slightly more usage of the contralateral paw as compensation to the injury (**Figure 3A**). After injury, usage of the ipsilateral paw for total rearing was increased from 14% in 2 weeks post-injury to 26% in 12 weeks post-injury. However, no recovery was showed in the initial rearing of ipsilateral paw (**Figure 3B**). Furthermore, there was no significant difference in usage of the ipsilateral paw between time points after injury.

The active range of motion of each forelimb in the grooming test reveals the forelimb function. Before injury, average score of ipsilateral and contralateral paws were up to 4.6 and 4.6, that mean both paws can contact back of the ears. There were significant decreases in score of ipsilateral paw after injury. There were no significant differences for the ipsilateral score in each time point after SCI. Almost all rats performed normal grooming with the contralateral paw after injury (**Figure 4**).

Lesions and tissue spare

The hemi-contusion lesions in the present study were similar to those reported the unilateral cervical contusion injuries [2, 15]. The injury resulted in substantial damage to the ipsilateral spinal cord. Tissue damage was observed from the spinal cord transverse sections rostral and causal 1600 μm to the epicenter (**Figure 5A**). At the epicenter, the average lesion area on the transverse spinal cord section was 1.56±0.30 mm^2, the percentages of average white matter and gray matter area were 39.58±13.85% and 40.75±12.36% of the con-

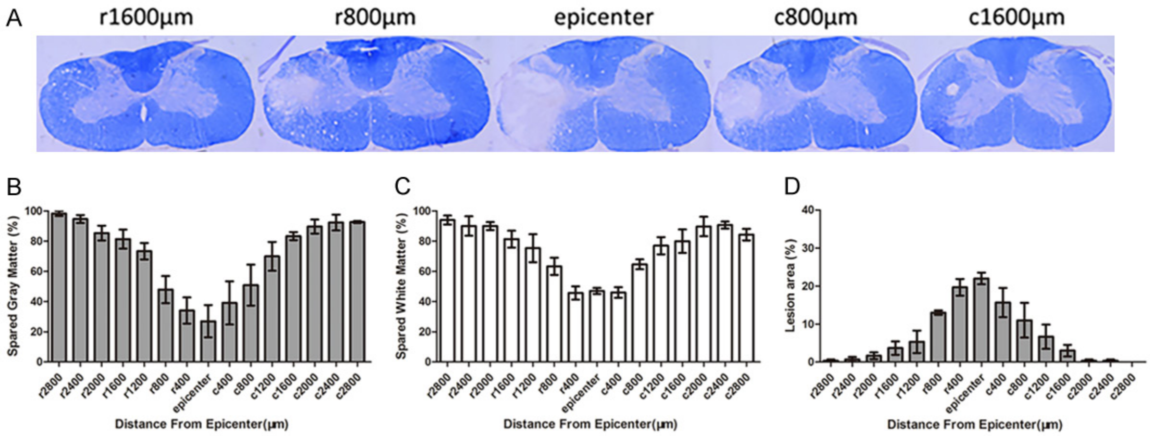

Figure 5. The histology feature of hemi-contusion SCI. There are representative images of spinal cord cross-section from rostral 1600 μm to caudal 1600 μm by EC stain (A). At 12 weeks post-injury, EC stained sections showed that the percentage of average gray matter and white matter area are 27±15%, 47±3% of hemicord contralateral area (B and C), the average lesion area encompassed 22±2% of transverse spinal cord (D) in epicenter.

tralateral hemicord area. The most severe damage was at the epicenter, with loss of the anterior horns of gray matter and lateral funiculus of white matter. However, the ipsilateral dorsal funiculus and horn were mainly undamaged in most cases, as well as the entire contralateral cord (**Figure 5B-D**).

Discussion

In this study, we developed a hemi-contusion injury model of cervical spinal cord using servo-electromagnetic material testing machine with a displacement of 2.2 mm at a speed of 600 mm/sec in rats. Compared to previous study of unilateral hemi-contusion model, the present study generated greater contusion displacement (2.2 mm vs 1.5 mm) at higher contusion speed (600 mm/sec vs 120 mm/sec), and was supposed to lead to severer contusion injury. The current model resulted in sustained motor function impairment in the ipsilateral forelimb, including rearing, grooming and grasping deficits, and disruptured the cord parenchymal largely to the ipsilateral side, including the lateral funiculus and the v around the epicenter.

Contusive SCI models have been widely used to understand the pathology of humans SCI [21, 22]. The contusion devices can be categoried into 3 types according to the control modes. The weight-drop devices, such as the Allen's impactor and MASCIS impactor, drop freely a weight from a height and apply certain level of energy to the cord, are considered as a mode of "energy control". These devices are simple in nature and easily used, but the impactor rod may bounce on the spinal cord after initial drop, resulting in multiple impacts [23, 24]. The Infinite Horizon Impactor is classified as a "force control" device as the contusion force is limited during injury. In theory, the force control mode is difficult as force as an external signal in a close-loop control, particularly conducting at high-speed like the Infinite Horizon Impact [15, 21]. In addition, the impact rod of the Infinite Horizon Impact is slender and may sway off the target location. The OSU impactor and the present system are considered as "displacement control" device. The contusion displacement is programable, and suitable for a contusion at a higher speed. The present system in a servo-electromagnetic material testing machine is equipped a linear motor to drive the impact rod, which decreases sway significantly during contusion.

For the displacement-control contusion, determination of the contact position of the impact tip to the cord surface is critical for a consistent and comparable contusion injury. The spinal cord is viscoelastic material and can't sustain a compression with a constant force. The present study adopted the method of the OSU impactor to determine the contact position as a vibrating rod compressed the cord up to a small amount of force, such as 0.01 N in the present study. Contusion depth is another key paramenter in the displacement control contu-

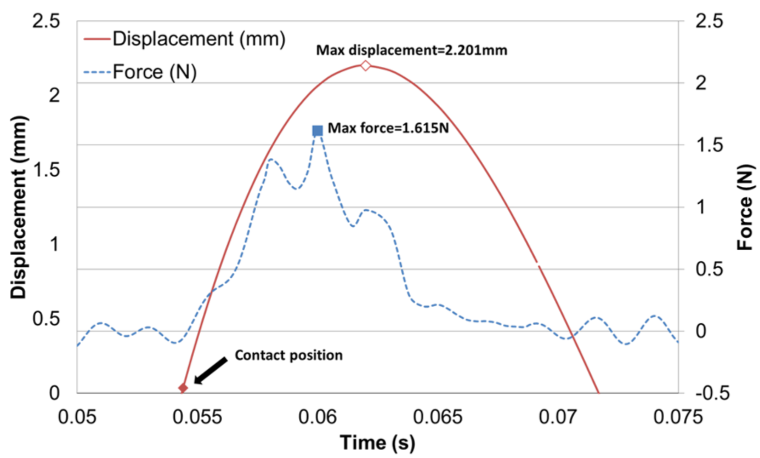

Figure 6. The contusive displacement and force during the C5 hemi-contusion. The contact position of the impactor to the cord was determined by a touch force reached 0.01 N. Note of that the maxium contusive force occurred before the maximum displacement, and the inertial force was compensated with the force of a blank shot.

sion. For large experimental animals, such as non-human primates and procine, MRI images are helpful to determine depths for the cord parenchymal and cerebrospinal fluid thickness [10]. CT images are used to determine the contusion depth in small animals. Based on the CT images and pilot tests, the present study found that contusion displacement of 2.4 mm is the maximal depth in rats. So, the constusion displacement of 2.2 mm should lead to a severe SCI.

A fine contusion SCI model should consider possible movement of the cord in the spinal canal during the injury, particularly for a hemi-contusion and in the cervical spine as the cord is slipped away to render to the occupation of impactor. One technique is to aim the impactor across midline of the cord to compensate the lateral slippery during hemi-contusion injury. However, the amount of accruing over is dependent on the contusion speed, diameter of the impactor and spinal levels, etc. Another technique is to aim the impactor perpendicular to the cord as cross-section of the cord is close to ellipse [15]. The present study adopted the set-up of the previous study by rotating the animal 22.5° laterally and off-set the impactor 1.4 mm from the midline of the cord. This set-up should reduce the cord lateral movement during the contusion and keep the dorsal column intact.

The present hemi-contusion SCI model was modified the model developed by Lee instead of using displacement control for the contusion injury. The present model achieved an excellent control to the contusion displacement and speed, as well as the contusion forces, limiting heterogeneous outcome in neurological disorders. The advantage of using displacement control is to achieve higher constusion speed, because a large part of human's SCI caused by motor vehicle is high energy and high speed in etiologic character [1]. The contusion speed is an important factor to SCI severity [10]. A contusion model with high speed seems closer to clinical SCI scenario. The another advantage is easy to achieve the SCI severity by adjusting the contusion displacement. Lee et al reported the hemi-contusion injury using force control at 150 kdyn achieved actual contusion displacement of 1.5 mm at an average speed of 120 mm/sec. However, severer SCI may account for obstruction using force-control hemi-contusion model as the present study was observed a nonlinear relationship between the contusion displacement and force (**Figure 6**).

The present hemi-contusion SCI model presented distinctive unilateral behavioral deficits. Montoya staircase task, cylinder rearing test and grooming test have been used to evaluate both the fine and gross components of the overall forelimb functions [25]. We found most of these motor functions were abolished, and the usage of the injured forelimb dramatically reduced after injury. A significant reduction in the number of pellets eaten in the staircase pellet test with the ipsilateral forepaw, and the deficits in ipsilateral forelimb usage or contact were observed in the cylinder rearing test and the grooming test. Despite the present model led to a stable forelimb deficit throughout the entire experiment, the deficit was lesser compared to the hemi-contusion injury with force control [2]. The difference between two models in techniques may contribute to the discrepancy in function assessment.

The present hemi-contusion SCI model showed unilateral tissue damage to the cord in histological analysis. Severe damage to ipsilateral

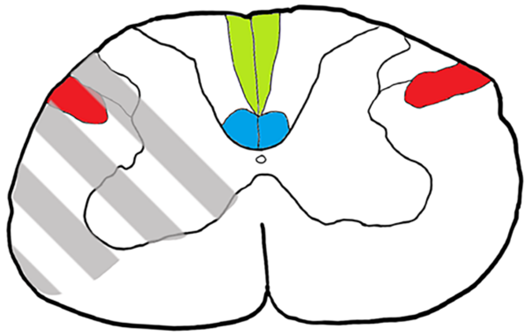

Figure 7. Histological effect of our hemi-contusion model demonstrates the ipsilateral white matter and gray matter damage with extensive damage of the lateral funiculus (shadow area) which include rubrospinal tracts (red area), but complete sparing of the dorsal column, which contains the main contingent of ascending sensory axons (green area) and corticospinal tract (blue area).

anterior horn and lateral funiculus was observed in the present study using an oblique hemi-contusion cervical SCI model. This hemi-contusion model was first developed by Lee and his colleague, who declared his model was aimed to injure the corticospinal and rubrospinal tracts of the ipsilateral side so that the deficit of motor function was confined to the ipsilateral forelimb. Compared to the hemi-contusion injury with force control [15], the spare of white matter and gray matter is larger in the present model. Most tissue of anterior funiculus was undamage in the present study, while anterior funiculus and posterior horn were within the lesion area in the previous study [15]. We speculated the different contusion injury devices contributed to the discrepancy. A branch of corticospinal tract is located in the dorsal column in rats, and different to human being [26]. Accordingly, we consider that motor functional deficit of ipsilateral forelimb is caused by the completely destroying the anterior horn neurons (**Figure 7**).

In summary, the present study showed that the hemi-contusive SCI model with displacement control achieved consistent contusive displacement and force at a higher speed. The spinal cord tissue loss was confined within the ipsilateral. The ipsilateral forelimb exhibited sustained impairments in motor function after injury while the contralateral forelimb was mostly intact. The present study provides an alternative method to establish a clinically relevant SCI model for neuroprotective strategies.

Acknowledgements

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Disclosure of conflict of interest

None.

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