Is the speed of chronic compression an important factor for chronic spinal cord injury rat model?

Hou-Qing Long[1], Guang-Sheng Li[2], Er-Jian Lin[3], Wen-Han Xie[1], Wen-Li Chen[4], Keith Dip-Kei Luk[5]. Yong Hu[5]

[1]Department of Spine Surgery, The First Affiliated Hospital of Sun Yat-Sen University, China

[2]Orthopaedic Research Institute，Department of Orthopaedics, Affiliated Hospital of Guangdong Medical College

[3]Department of Radiology, The First Affiliated Hospital of Sun Yat-Sen University, China

[4]Department of Neurosurgery, The First Affiliated Hospital of Sun Yat-Sen University, China

[5]Department of Orthopaedics and Traumatology, Li Kai Shing Faculty of Medicine, The University of Hong Kong, Pokfulam Hong Kong

Corresponding authors:

Yong Hu

Department of Orthopaedics and Traumatology, The University of Hong Kong, 12 Sandy Bay Road, Pokfulam, Hong Kong

Tel.: +852 29740359; Fax: +852 29740335; E-mail: yhud@ hku.hk.

Houqing Long

Department of Spine Surgery, The First Affiliated Hospital of Sun Yat-Sen University,

Abstract

Objective: To evaluate the effect of expansion speed on chronic compressive spinal cord injury in the rat.

Methods: Thirty-six Sprague-Dawley rats were divided into four groups: a control group, a group receiving compressor in the C5-6 epidural space with instant compression (Group 1), and two other groups receiving water-absorbing polyurethane polymer sheets with two expansion speeds, which reached maximum volume in 2 h (Group 2: fast expansion) or 24 h (Group 3: slow expansion). A C6 laminectomy was performed in the control group. Neurological function, MRI, large motoneuron number in the ventral horn, and myelin staining intensity in the posterior funiculus were evaluated.

Results: In the instant compression group, compression was confirmed on T_2-weighted images by a hypointense signal change in the intramedulla. In the gradual compressive injury groups, large motoneuron number ($p<0.001$), but not myelin staining intensity, was significantly decreased in both the fast and slow expansion groups compared with the instant compression group. However, there was no difference in Basso Beattie Bresnahan score, cord distortion in T_2-weighted image, large motoneuron numbers, or myelin staining between the fast and slow expansion groups.

Conclusion: Instant spinal cord compression caused acute injury. Gradual expansion compression induced reliable pathology and MRI characteristics consistent with chronic compressive spinal cord injury. The speed of expansion is not a significant problem for establishing a reliable model if the chronic compression is induced by gradual expansion.

Keywords: Chronic compressive model; spinal cord injury; compression speed; rat model

1. Introduction

Cervical spondylotic myelopathy (CSM) is one of the most common spinal cord disorders affecting the elderly. However, its pathogenesis and prognosis remain unclear. Because it is difficult to examine these pathological mechanisms clinically, reliable animal models of chronic compression spinal cord injury are indispensable. Although a number of animal models of CSM have been developed, including the use of a balloon catheter [11], screw drilling [4], and dynamic compression [13], these models have a number of deficits including acute or subacute spinal cord injury during the modeling process, which can lead to marked pathological differences compared with the natural history of CSM. Furthermore, some models require repeated operation [4-6, 11], while the pressure applied to the spinal cord does not have a linear effect on pathophysiological findings.

A number of alternative models have been developed to more closely reflect the natural history of CSM. The epidural tumor cell seeding method can cause chronic spinal cord compression while avoiding these shortcomings, although it can cause a local inflammatory reaction, direct damage spinal cord by tumor tissue, systemic side effects, and short survival times [17]. A transgenic twy/twy rat model was recently reported to induce hypertrophy and ossification of the ligamenta flava, resulting in chronic spinal cord compression [18]. This model is more similar to the clinical pathogenesis, although it exhibits low reducibility and the compression segments are inconsistent with common clinical CSM.

Previous studies have proposed a chronic compressive cervical spinal cord injury rat model using a water-absorbable polymer that can provide controlled spinal cord compression [5, 7]; the water-absorbing material in these studies produced a gradual expansion to maximum volume in 2 h. This model showed a close similarity

in characteristic features between the progressive neurology deficits and clinical cervical myelopathy. A more recent study reported a similar model with a slower volume expansion [3], where the water-absorbing material was further developed by encasing in a sustained-release membrane to control the expansion speed to produce a gradual expansion to maximum volume in 24 h. Thus, the aim of the present study was to determine the effect of different expansion speeds to create progressive spinal cord compression and to validate the chronic pathology progression.

2. Materials and methods

2.1 Animal models

All experimental procedures were approved by the Research Ethics Committee of the authors' institutes. A total of 36 adult Sprague-Dawley (SD) rats (250–300 g) were allocated to four groups: control group (n=6) with sham surgery, and another three groups with implantation of three different compressors, as follows. Based on the speed of polyurethane polymer sheets, the animals were divided into group 1 (n=6) with instant compression, group 2 (n=12) with gradual compression to the maximum compression ratio in 2 h, and group 3 (n=12) with gradual compression to the maximum compression ratio in 24 h. To make the compressor, 3% agarose gel (Amresco LLC, Solon, OH, USA) was turned into a water-absorbing polymer (14)-3, 6-anhydro-a-L-galactopyranosyl-(1 3)-β-D-galactopyranan, which was dried for 8 h in a low temperature vacuum dryer (Nanjing Hong Gang sheng Machinery Technology Co. Ltd, Nanjing, China). Each compression sheet was cut to a standard size of 1 × 3 × 1 mm. This sheet can absorb liquid in the spinal canal to expand its volume seven-fold in 2 h. To create a progressive compression to expand the maximum volume in 24 h, a sustained-release membrane was coated on the surface of the implant sheet [3]. The sustained-release membrane was made of polyurethane, which was synthesized in the laboratory by isocyanates and polyols (Guangzhou Fischer Chemical Co., Ltd., Guangzhou, China). Next, the polyurethanes were coated on the surface of the implant sheet, and a laser beam was used to create a definite number of microholes to control the expansion speed. A total of 255 microholes of 0.05 mm diameter were created on each surface of the compression sheet. This compression material did not show any inflammatory reaction or tissue granulation after implantation in previous studies [3].

After the animals were successfully anesthetized with 10% ketamine by intraperitoneal injection (1 mL/kg), the C5-C6 lamina was exposed, the ligamentum flavum and partial lamina were removed to access the epidural space, and the compression sheet was implanted into the C5-C6 epidural space on the posterolateral side to induce a compression to the cord. After complete hemostasis, the incision was closed by layers, and the animals were given intramuscular injection of penicillin for infection prevention. After surgery, the animals were housed individually in cages and allowed free access to food and water. In the present study, it was difficult for the rats to survive for more than 24 h after surgical instant compression injury for group 1. In group 1, the instant compressor produced compression immediately after the surgery. In group 2, the fast expansion produced the maximum compression ratio at 2 h after surgery. In group 3, the slow expansion produced the maximum compression ratio at 24 h after surgery. MRI was performed on all rats to verify compression on the cord at 24 h after achieving maximum compression. The rats in the gradual compression groups received a second scan at 1 week after achieving maximum compression. Motor function was assessed at 24 h after achieving maximum compression, followed by daily assessment until sacrifice. The histological and histochemical changes were evaluated after sacrifice.

2.2 Magnetic resonance imaging (MRI)

MRI were obtained using a 1.5 T imaging system (Philips Medical Systems, Netherland B.V., DA Best, The Netherlands). For scanning, each animal was placed in the ventral recumbent position after general anesthesia. Images of the spinal region were acquired in the transverse and sagittal planes. T1-weighted images (500/22 [TR/TE]; section thickness: 2 mm, section gap: 0.1 mm, resolution ratio: $0.27 \times 0.27 \times 2.0\ mm^3$) and T2-weighted images (3000/90 [TR/TE]; section

thickness: 2 mm, section gap: 0.1 mm, resolution ratio: 0.27 × 0.27 × 2.0 mm^3) were obtained at 24 h after achieving maximum compression.

2.3 Behavior analysis

Severity of paralysis due to spinal cord compression was evaluated in terms of motor function by using the Basso Beattie Bresnahan (BBB) score [16]. For the control group and group 1, the BBB scores were evaluated at 24 h after surgery. For groups 2 and 3, the BBB scores were evaluated at 24 h and 1 week after achieving maximum compression, respectively. The evaluation was performed using a double-blind method, and the average scores in each group were calculated.

2.4 Histological and histochemical evaluations

After BBB evaluation and MRI, rats in the instant compression group were sacrificed at 24 h after surgery, while rats in the gradual compression groups were sacrificed at 24 h and 1 week after achieving maximum compression, respectively. Animals were euthanized with an overdose of 40 mg/kg of intravenous sodium pentobarbital, and the rats were perfused with 50 mL heparin-saline through the ascending aorta, followed by 300 mL formalin-picric solution (4% formaldehyde, 0.4% picric acid in 0.16 mol/L phosphate buffer, pH 7.4). Unabridged cervical spinal cords were carefully harvested and fixed with 4% phosphate buffer liquid in formaldehyde solution for another 72 h, and the cords were embedded in paraffin. Transverse and sagittal sections (8 μm) were then stained with hematoxylin-eosin (H&E) and luxol fast blue (LFB).

2.5 Statistical analysis

All histology images were obtained by light microscopy. The quantity of the anterior horn motor neurons and LFB staining intensity of the spinal cord were analyzed and compared by IPP software (Image Pro Plus 6.0, Media Cybernetics, Rockville, MD,

USA). Differences between the groups were compared using variance analysis (SPSS 13; IBM Co., Chicago, IL, USA). A value of $p<0.05$ was considered statistically significant.

3. Results

3.1 MRI results

The spinal cord was compressed, swollen, and displaced at 24 h post compression, without a difference in visual inspection of the compression ratio between the three groups (Fig. 1A-D). In the instant compression group, MR results showed a low signal intensity on T1-weighted images and high signal intensity on T2-weighted images suggestive of intramedullary hemorrhage (Fig. 1B1-2). In the gradual compression groups, MR results showed compression of the dorsal side of the spinal cord on T2-weighted images, but no evidence of spinal cord edema or hemorrhage (Fig. 1C1-2). At 1 week after surgery, the compression ratio of the cord remained consistent, without evidence of spinal cord edema, hemorrhage, or myelomalacia (Fig. 1D1-2).

3.2 BBB score

In the instant compression group, paralysis appeared after surgery, with an instant drop in the BBB to 10±1.4 at 4 h and to 6.0±1.8 at 24 h (Table 1). By contrast, BBB scores in the gradual compression groups showed a small decrease after surgery, and then plateaued at approximately 17 from 2 days to 1 week after insertion (Table 1). In the gradual compression groups, the front and rear limbs of the animals moved in coordination, with the tails hanging constantly. There was a significant difference in BBB scores in the gradual compression groups compared with the instant compression group, but no difference between the fast and slow compression groups.

2.3 H&E staining

Subdural and intramedullary hemorrhage and edema were observed at 24 h after compression in the instant compression group, especially in the posterior horn; large motoneurons characterized by a spindle-shape, posterior funiculus neural fiber tract

edema, and rupture were observed in sagittal slices, which were not found in the gradual compression groups. In the instant compression group, the lesion sites showed subdural hematoma, spinal cord central canal deformation, intramedullary hemorrhage focus liquefaction, and a significant decrease in neuron counts compared with the control group (Table 1); some neurons showed an elongated spindle-shape, cytoplasm loss, and karyoplast dissolution. Posterior funiculus fibers of the white matter were damaged, characterized by acute demyelination (Fig. 2).

In the gradual compression groups, the polyurethane material showed expansion in the cross section, and the C5-6 spinal cord was compressed and swollen. In contrast to the instant compression group, there was no evidence of subdural or intramedullary hemorrhage in the gradual compression groups at 24 h after surgery, although spinal cord central canal expansion and deformation were observed. Furthermore, there was no evidence of tissue edema, venous congestion, reduced number of neurons, or spindle-shaped neurons at 24 h after surgery (Table 1). At 1 week after surgery in the gradual compression groups, the number of motor neurons in the anterior horn was significantly reduced, the visual cortical cells showed cytoplasmic reduction and nuclear pyknosis, the number of neuronal synapses was decreased, fewer corneal nerve bundles were observed, and there were scattered areas of lower density of myelin sheath (Fig. 2).

2.4 LFB staining

In the instant compression group, the arrangement of neural fibers was disordered, there was axonal degeneration, and the density of myelin staining was reduced. In the gradual compression groups, the structure of the spinal cord was normal at 24 h after surgery, and myelin staining and the nerve fibers of the posterior funiculus

maintained their integrity. At 1 week after surgery in the gradual compression groups, there was evidence of deformation of the posterior horn and the posterior funiculus, the anterior horn was mildly deformed, the number of decussating fibers in the gray matter were reduced, vacuolar degeneration was observed around axons, and there was a significant decrease in the thickness and blue staining density of the myelin sheath (Fig. 3). However, there was no difference in LFB staining between the fast and slow compression groups.

3 Discussion

Development of appropriate experimental chronic spinal cord injury animal models is crucial for investigating the pathophysiological mechanisms of CSM [10]. In previous studies, chronic compression spinal cord injury rat models were developed using a modified water absorption polyurethane material with different expansion speeds [3, 5, 15]. In the present study, we examined whether the speed of expansion was important in the development of a chronic spinal cord injury by comparing the results from spinal cord compression models with instant compression (e.g., acute spinal cord injury [1]) and gradual compression with two different expansion speeds. Different properties of pressure-induction materials can produce different patterns of spinal cord injury [3, 5, 15]. For example, instant compression produces a direct compression on the spinal cord, with evidence of acute histological spinal cord injury [15]. In the present study, we found that instant compression caused hemorrhage and edema around the central canal and the gray matter, myelomalacia, neuron death, and nerve fiber damage.

By using a water-absorbing polymer, the expansion of the compressor can produce gradual compression on the spinal cord, leading to an efficient model of chronic compression spinal cord injury. In the present study, the spinal cord showed slight pathological edema after 24 h of compression, while H&E staining and MRI showed no evidence of intramedullary hemorrhage or abnormal neural function. The polyurethane tablets expanded slowly to their maximum volume by 24 h after insertion, inducing obvious spinal cord compression, venous congestion, and central canal expansion deformation, but no intramedullary hemorrhage. No abnormalities were observed in the epidural space or the subarachnoid and intramedullary regions by visual observation of MRI. With continuous compression of the spinal cord and

venous congestion, there was aggravation of spinal cord edema and ischemia, and a decrease in the vertebral canal volume. At 1 week after surgery, there was reduced spinal cord edema, but evidence of intramedullary vacuolization, marked neuronal loss, obvious demyelination of nerve fibers, and lack of limb coordination. These pathological changes are very similar to the early stage of CSM [2, 9, 12]. The abnormal MRI signal changes and the pathology and neuronal dysfunction characteristic of acute spinal cord injury were not found in the chronic compression group. The polyurethane implants form a continuous compression that leads to neuronal loss and demyelination, similar to chronic compressive spinal cord injury [6, 8], which results in reduced spinal cord neural function.

In agreement with previous findings, the chronic rat model using water-absorbing polymer exhibits the following characteristics: (a) the operation is simple and avoids an anterior approach operation or other trauma that can damage the animal, and the survival and success rates of the model are high [3, 5]; (b) the compression sites range from the C5-C6 levels, which is the common compression segment in clinical CSM [7]; (c) the model can be adapted to monitor neural function and electrophysiological and radiological evaluation *in vivo* [3]; and (d) the chronic progressive spinal cord compression process is performed by modification of a water absorption polyurethane material [3, 5], and the slow expansion generates a linear pressure and maintains a stable volume for a long time after saturation, consistent with the natural history of CSM [14]. Therefore, this model may be useful for studying CSM pathogenesis and early intervention therapies.

Our results showed that the cervical cord presented acute injury after instant compression without gradual expansion. However, there were no differences in neurological deterioration in rats after chronic compression using fast expansion or

slow expansion methods. Production of a water-absorbing polymer with fast expansion (i.e., to the maximum volume in 2 h) was relatively easier than the polymer with a slower expansion speed (i.e., maximum volume in 24 h). The later compressor required a sustained-release membrane coating on the surface of the fast compressor to control the water transmission through the membrane. In addition, the insertion of the polymer requires careful surgical placement to avoid damage to the surface membrane.

There are some limitations to the present study. The main purpose of the present study was to confirm the effect of expansion speed on the chronic model. The larger experiments are required to confirm the reduction in numbers of neurons, glial scar formation, and demyelination in our chronic compressive spinal cord injury model. In addition, longitudinal studies of the temporal pattern longer than 1 week are required to investigate the long-term pathological changes after compressive spinal cord injury.

In conclusion, we confirmed the pathology changes, imaging characteristics, and neurological dysfunction of our chronic compressive spinal cord injury model. Instant compression produced acute spinal cord injury without chronic neurology degeneration. By contrast, a reliable chronic compressive spinal cord injury model was created using a gradually expanding compressor that approached its maximum compression at 2 h or later. Our model may be useful for the design of future studies examining the pathological mechanisms of CSM.

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Fig1

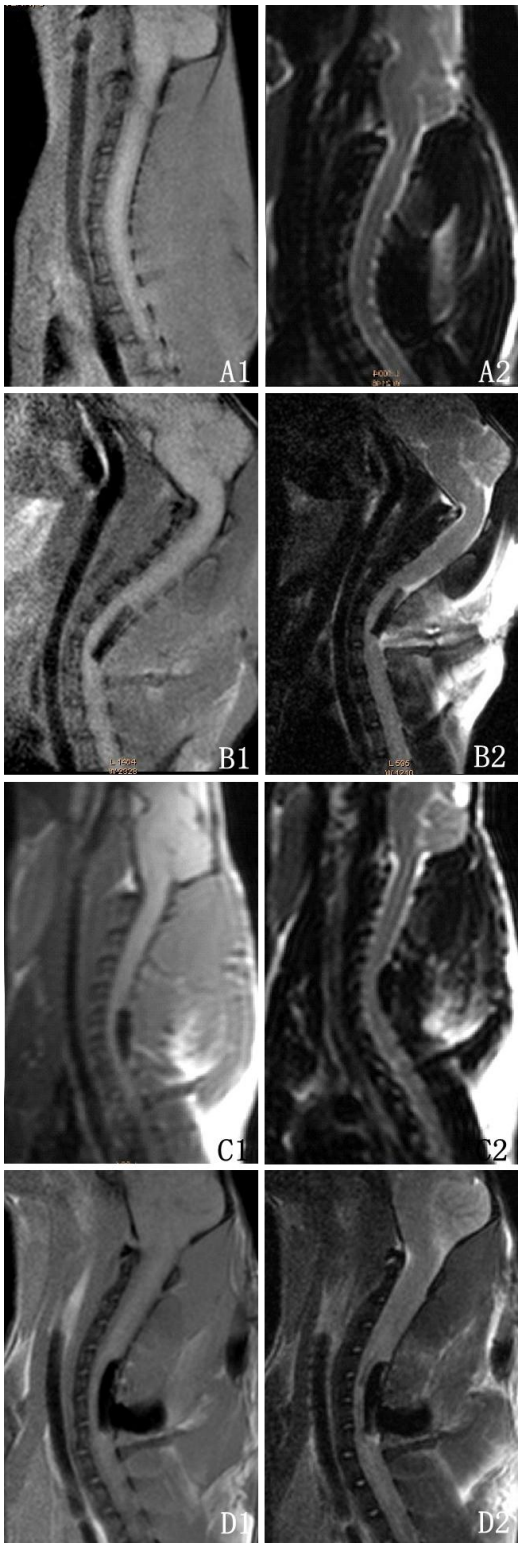

Fig1 T1、T2-weighted images of cervical spinal cord in normal rats(A1,A2); 24h post acute compression intramedullary medium-high signal intensity and edema in the Surrounding tissues, as well as low signal polyurethane tablet in the dorsal side of spinal cord(B1,B2); no spinal cord hemorrhage on T1(C1) or T2(C2)-weighted images at 24h post chronic compression;spinal cord was steadily compressed, uneven signals were caught at the compression sites, no hemorrhage was found at 1w post chronic compression(D1,D2).

Fig2

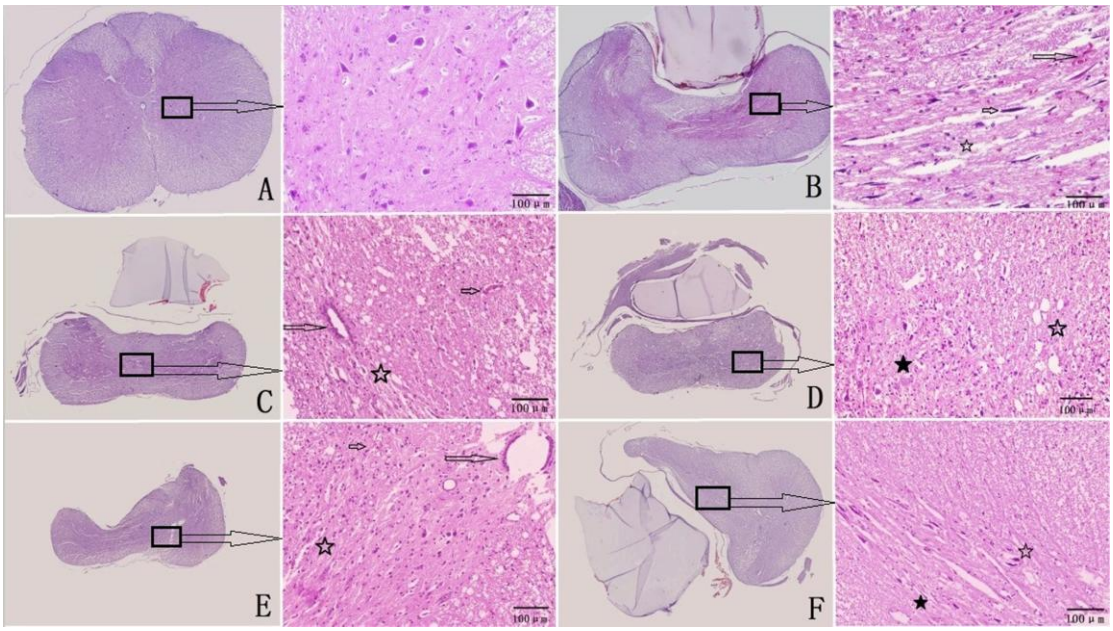

Fig 2.The histology features of each group by HE stain.
(A)Normal Cord. (B)Subdural andintramedullary hemorrhage as well as edema, especially in the posterior horn(long arrow); large motoneurons were characterized by a spindle-shape(short arrow), posterior funiculus neural fiber tract edema(☆) for Group 1. (C)Central canal expansion deformation(long arrow), venous congestion(short arrow), and neurons number reduction(☆) can be seen at 24h without intramedullary hemorrhage or edema for Group 2. (D)Anterior horn motor neuron number and synapse decrease(★)，nerve fiber layer and myelin sheath were sparse with vacuolated cord，reduced cytoplasm, atrophied nucleus and glial scar formation(☆) at 1w after surgery for Group 2. (E) The histology features of Group 3 were same as (C) at 24h. (F)The histology features of Group 3 were same as (D) at 1w.

Fig3

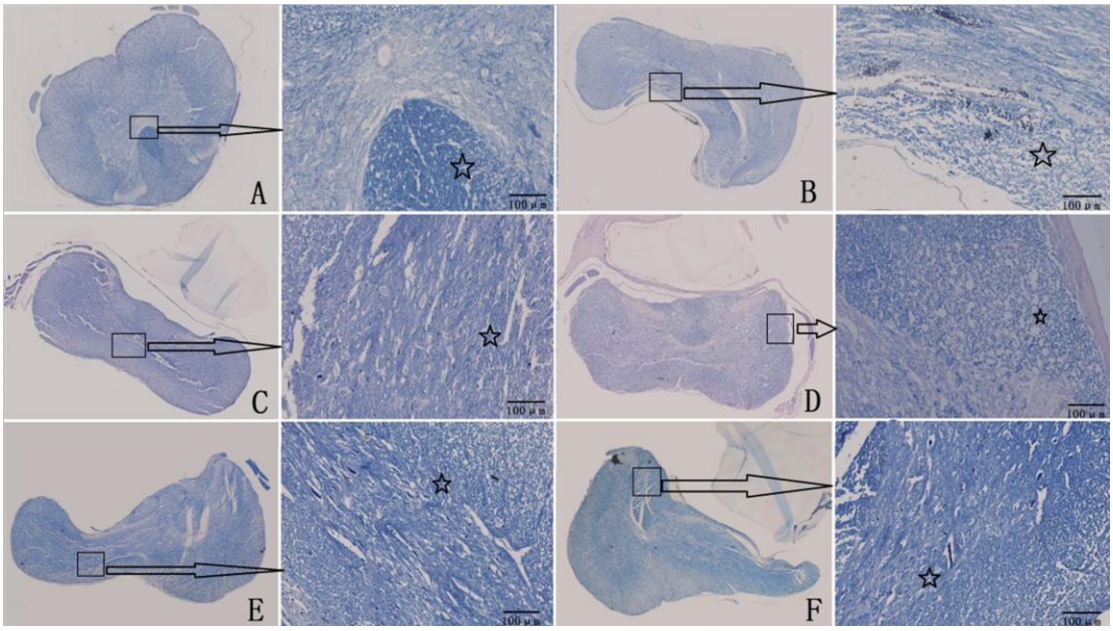

Fig 3. The histochemical features by Luxol fast blue (LFB) stain.
(A) Normal Cord. (B) Group1: significant deformation, structural disorder in posterior funiculus, and some nerve fibers fracture;myelin destruction(☆). (C) Group 2: vacuolation of myelin , sparse blue stain, and more nerve fibers fracture were observed at 24h after surgery(☆). (D) Group 2: 1W after chronic compression blue stained myelin reduced significantly, myelin destruction, axonal degeneration while the vacuolization increased(☆). (E) The neuropathological features of Group 3 were same as (C) at 24h. (F)The neuropathological features of Group 3 were same as (D) at 1w.

Table1

Table 1 Quantity of the motor neurons of anterior horn of spinal cord, LFB staining intensity and the BBB score

Group	Control	Group 1	Group 2		Group 3	
time	24h	24h	24h	1w	24h	1w
motor neurons	21.0±2.4	3.5±1.3 [b,c]	16.±1.8 [a]	3.3±1.0[d]	15.6±1.3 [a]	3.2±1.2 [d]
Staining intensity	90±4	80±4	77±3	24±2 [a,d]	78±3	23±3 [a,d]
BBB score	21±0.7	6.0±1.8 [b,c]	15.7±0.8[a]	17.1±1.3	15.5±0.9 [a]	16.9±1.1

a. Significant difference in comparison with group 1, *P<0.05*; b. Significant difference in comparison with group 2, *P<0.05;* c. Significant difference in comparison with group 3, *P<0.05*

d. Significant difference in comparison between 24 Hours and 1 week after surgery