Destructive pathological changes in the rat spinal cord due to chronic mechanical compression

Laboratory investigation

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Object. Chronic mechanical compression of the spinal cord, which is commonly caused by degeneration of the spine, impairs motor and sensory functions insidiously and progressively. Yet the exact mechanisms of chronic spinal cord compression (SCC) remain to be elucidated. To study the pathophysiology of this condition, the authors developed a simple animal experimental model that reproduced the clinical course of mechanical compression of the spinal cord.

Methods. A custom-designed compression device was implanted on the exposed spinal cord of female Wistar rats between the T-7 and T-9 vertebrae. A root canal screw attached to a plastic plate was tightened 1 complete turn (1 pitch) every 7 days for 6 weeks. The placement of the compression device and the degree of compression were validated every week using radiography. Furthermore, a motor sensory deficit index was also calculated every week. After 3, 6, 9, or 12 weeks, the compressed T7–9 spinal cords were harvested and examined histologically.

Results. Lateral projection of the thoracic spine showed a progressively increasing rate of mean spinal cord narrowing in the compression group. Motor and sensory deficiencies were observed from Week 3 onward; paralysis was observed in 2 rats at Week 12. Motor deficiency appeared earlier than sensory deficiency. Obvious pathological changes were observed starting at Week 6. The number of neurons in the gray matter of rats with chronic compression of the spinal cord decreased progressively in the 6- and 9-week compression groups. In the white matter, myelin destruction and loss of axons and glia were noted. The number of terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling (TUNEL)–positive neurons increased in the ventral-to-dorsal direction. The number of TUNEL-positive cells increased from Week 6 onward and peaked at Week 9.

Conclusions. This practical model accurately reproduces characteristic features of clinical chronic SCC, including progressive motor and sensory disturbances after a latency and insidious neuronal loss.

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KEY WORDS • animal model • chronic spinal cord compression • neuronal loss • rat • spinal cord

C H R O N I C mechanical compression of the spinal cord, which is commonly caused by spondylosis, disc herniation, and/or calcification/ossification of posterior longitudinal ligaments or yellow ligaments, impairs motor and sensory functions insidiously and progressively. Although the condition of many patients with these lesions has improved after surgical procedures, including after decompression and/or stabilization, some have remained unchanged or have even deteriorated. The exact pathological mechanisms of the injury process remains to be elucidated; circulatory insufficiencies and venous congestion have been postulated as possible mechanisms. Histological examination of the autopsy specimens of the spinal cord showed loss of motor neurons and vacuolar degeneration in the gray matter, as well as demyelination and swelling of the axons (or “spongiform” degeneration). Attempts have been made to reproduce the chronic SCC injury in animal models; however, the characteristic temporal profile, which is the hallmark of the clinical condition, was not adequately reproduced. An appropriate model for the pathophysiology of this condition should exhibit a latency period after induction of compression with insidious onset of neurological dysfunctions, followed by a phase of progressive disturbance. The model should also reproduce histological changes such as loss of motor neurons. The feasi-

Abbreviations used in this paper: SCC = spinal cord compression; SCI = spinal cord injury; TUNEL = terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick-end labeling.
bility of quantitative measurement of neurological alterations and the availability in small animals satisfying these conditions has not been described, and this study was undertaken to establish a practical model using rats.

Materials and Methods

Female Wistar rats, from 10- to 12-weeks old and weighing 350–400 g each, were used in the study. Food and water were provided ad libitum before and after the experiments. The rats were housed in a room maintained at a temperature of 20°C (controlled by a thermostat) and exposed to alternating light and dark periods of 12 hours each. The study was approved by the Shandong University ethical committee for animal research.

Custom-Designed Compression Device

The compression device consisted of a double-folded “H”-shaped stainless-steel plate 1.0 mm thick, a plastic cannula 3 mm in diameter with a screw thread in the middle, and a root canal screw 1 mm in diameter with 0.4 mm of pitch (used in the stomatology department). During the surgical procedure, the distance between the middle of the laminae of T-8 to the spinous processes of T-7 and T-9 was measured. Based on these measurements, the H-shaped stainless-steel plate was then adjusted to fit the spinal cord between T-7 and T-9 (Fig. 1).

Rat Model of Chronic SCC

Anesthesia was induced using an intraperitoneal injection of pentobarbital (40 mg/kg) and all surgical procedures were performed under aseptic conditions. For surgical preparation, the back area of each rat was shaved and disinfected with povidone-iodine solution. A longitudinal incision was then made on the back, centered on the spinous processes of the T6–10 vertebrae. The paravertebral muscles were stripped from the spinous processes and laminae. The spinous process and the middle part of the laminae of T-8 were resected. The dura underneath was separated from the laminae carefully without causing the cerebrospinal fluid to leak. Spinal cord compression was induced by placing a plastic plate (2 mm in height, 1.5 mm in width, and 0.5 mm thick) horizontally on the spinal cord at T-8. The compression device was then placed on the laminae of T-8 and fixed between T-7 and T-9. Compression on the spinal cord was ensured by turning the screw to tighten the plastic plate (Fig. 1). Subsequently, the surgical area was sutured in layers. Chronic compression on the spinal cord was maintained by turning the root canal screw 1 pitch (1 complete turn) every 7 days for 6 weeks.

Experimental Groups

After rats with an intraoperative SCI were excluded, a total of 49 rats were used in the present study. In the control groups, 17 rats underwent sham surgery without implantation of the compression device. For histological studies, 3 rats each were killed at 3, 6, 9, or 12 weeks after surgery (12 rats total). For neurobehavioral studies, rats were evaluated at the same time point after surgery, and were chosen at random (8 rats at each time point). In the 4 compression groups, rats underwent implantation of the compression device and were also killed at 3, 6, 9, or 12 weeks after surgery (8 rats per group).

Postoperatively, the rats were kept under a heating lamp until they regained consciousness. No pre- and postoperative antibiotics were given. All animals were housed separately.

Radiographic Imaging

Radiographic films were obtained every week to ascertain the location of the compression device and to evaluate the degree of SCC.

Evaluation of Neurobehavioral Outcome

Serial assessments of motor and sensory functions in the hind limbs of rats were performed every week, using the scale of LeMay.
Histopathological Study

The rats were killed using an overdose of pentobarbital; they were perfused through the heart with 200 ml of phosphate-buffered saline followed by 200 ml of 4% paraformaldehyde in a 0.1 mol/L phosphate buffer. The thoracic spine was removed en bloc immediately after perfusion and stored in 4% paraformaldehyde for 3 days. Samples were taken at the T-8 level and embedded in paraffin before preparing 4 \( \mu \)m transverse sections. The sections were stained with H & E and examined for the density of large-sized neurons in the gray matter. To count the neurons without redundancy and disregard, we chose a slice thickness of 4 \( \mu \)m and a gap interval of > 8 \( \mu \)m. Klüver–Barrera Luxol fast-blue staining was also performed to evaluate demyelination in the white matter.

Staining With TUNEL

The fragmentation of DNA was detected by the TUNEL method using the ApopTag plus in situ apoptosis detection kit (Boehringer Mannheim). After undergoing deparaffinization and hydration, sections were treated with 20 \( \mu \)g/ml of proteinase K in 0.1 mol/L of Tris buffer (pH 8) at room temperature for 15 minutes to strip nuclei of tissue sections. All procedures were performed according to the instructions of the manufacturer. The reaction with terminal deoxynucleotidyl transferase was terminated by washing the sections with stop-wash buffer for 30 minutes at room temperature. The reaction product was enhanced by developing it with 3,3'-diaminobenzidine tetrachloride and counterstained using hematoxylin. The number of cells labeled by TUNEL staining were counted by 3 people, including a pathologist not participating in this study. Cells with nuclei clearly stained using the TUNEL method that contained apoptotic bodies were considered to be apoptotic. Neurons with very faint nuclear TUNEL staining and without apoptotic bodies were considered to be necrotic.

Statistical Analysis

Statistical analyses of measured physiological data were performed by one-way analysis of variance. All physiological data are expressed as means ± standard errors of the means. Neurological scores were analyzed using Kruskal–Wallis tests, followed by the Mann–Whitney U-test when significant differences were found. Differences were considered statistically significant at a probability value < 0.05. All analyses were performed using SPSS statistical software version 11.5 (SPSS, Inc.).

Results

Postoperatively, the rats were in good, healthy condition and did not develop any infections. Depending on the time and degree of compression, a graded outcome was evident from neurological tests, radiography, and light microscopic examination as described below.

Radiographic Observations

The spinal cords were progressively compressed. Lateral projections of the thoracic spine were obtained to ascertain the location of the compression device and to evaluate the degree of SCC (Fig. 2). The mean spinal cord narrowing rate was 16.39% after 1 week of compression, 25.71% after 2 weeks, 37.14% after 3 weeks, 48.57% at 4 weeks, 68.85% at 5 weeks, and 81.97% after 6 weeks (Fig. 3).

TABLE 1

<table>
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<tr>
<th>Function Evaluated</th>
<th>Score</th>
<th>Features</th>
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<td>walking w/ lower extremities</td>
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<td>normal</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>toes flat under body when walking but ataxia is present</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>knuckle walking</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>movements in lower extremities but unable to knuckle walk</td>
</tr>
<tr>
<td>pain sensation</td>
<td>4</td>
<td>no movement, drags lower extremities</td>
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<tr>
<td></td>
<td>0</td>
<td>normal, withdrawal to toe pinch</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>squeals to toe pinch but does not withdraw</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>no reaction to toe pinch</td>
</tr>
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</table>

* Originally created by LeMay et al., 1987, and modified by Lang-Lazdunski et al., 2000.

Neurobehavioral Outcome

All sham-operated rats had a normal postoperative neurological outcome (score of 0). Rats with compression (operated rats) had graded motor and sensory function injury depending on the time and degree of compression. In the 3-week compression group, rats suffered from mild motor injury. In the 6-week compression group, rats demonstrated...
mild motor and mild sensory injury. In the 9- and 12-week compression groups, rats showed severe motor and mild sensory injury. Two rats in the 12-week compression group (25%) were observed to have paralysis. A significant difference was detected between the control groups and the compression groups \( (p < 0.05) \). Comparison of the 3-, 6-, and 9-week groups showed statistically significant differences among them \( (p < 0.05) \). But there was no significant difference between the 9- and 12-week groups \( (p > 0.05; \) Table 2).

**Histopathological Observation**

Hematoxylin and eosin and Luxol fast blue staining were used to analyze histopathological change in the spinal cords. The extent of compression damage was proportional to the neurological score. In the 3-week compression group, the spinal cords were observed to have mild pathological changes. In 6-, 9-, and 12-week compression groups, a boomerang-shaped spinal cord was observed at the site of compression. In the gray matter, neurons were flattened, small, and decreased in number. The number of neurons in the gray matter of chronically compressed spinal cords decreased progressively in the 6- and 9-week compression groups in comparison with the control group; 41.2% of neurons were lost in the 6-week compression group, and 61.5% of neurons were lost in the 9-week compression group (Fig. 4). Chromatolysis, both central and peripheral, was observed in the remaining neurons. Sinusoidal dilation of veins and small cavitations of the gray matter was also observed. In the white matter, myelin damage and loss of axons and glia were noted, as was status spongiosis. Cavity formation and myelin ovoids were observed in the anterior, lateral, and posterior columns. Myelin ovoids were believed to result from phagocytosis of degenerating axons and the myelin sheath. Sham-operated rats had normal spinal cords.

**Staining With TUNEL**

In the 3-week compression group, spinal cords showed

<table>
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<th>Group</th>
<th>Control</th>
<th>3-Week</th>
<th>6-Week</th>
<th>9-Week</th>
<th>12-Week</th>
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\* Number listed below each group represents the number of rats in that group with the corresponding MDSI score on the left. Abbreviation: MDSI = motor sensory deficit index.
little evidence of TUNEL staining, but in the 6-, 9-, and 12-week compression groups, spinal cords demonstrated TUNEL-positive neurons scattered within gray matter. The number of TUNEL-positive neurons increased in the ventral-to-dorsal direction. The number of TUNEL-positive cells increased starting at Week 6, reached a peak at Week 9, and decreased thereafter (Fig. 5). Spinal cords from sham-operated rats showed no evidence of TUNEL staining.

**Discussion**

Mechanical compression injury causes extensive pathological changes in the spinal cord and compromises neural function. Acute SCI results in destruction of neural cells as well as the blood–brain barrier, which subsequently leads to extensive tissue injury and damage to ascending and descending axonal tracts. This primary trauma could cause secondary tissue reactions, activation of astrocytes, or invasion of foreign cells from the periphery. It could also induce various pathological events and release of inflammatory mediators that are believed to negatively influence spinal cord function. Axonal injury could also lead to degeneration of interneurons and motor neurons caudal to the site of injury and degeneration of sensory neurons above the injured site. The combination of primary and secondary insults results in progressive cellular death of both neuronal and nonneuronal cells. Only a few studies have investigated the pathological events in the spinal cord in experimental conditions associated with chronic compression.

In previous studies, various methods have been undertaken to induce SCC. These include transplantation of tumor cells, placement of screws and gradual tightening, the implantation of an expanding sheet, and a combination of vascular ligation plus screw compression.

Manabe and colleagues transplanted fibrosarcoma cells epidurally in the rat spinal cord. The growth of the epidural tumor was rapid and paralysis developed within 13–18 days, a course not representative of chronic myelopathy. A similar attempt was made in rats using Walker 256 tumor or carcinoma cells implanted in the thoracic epidural or prevertebral space, and paralysis developed in 10–20 days. In rabbits, Ikeda and associates studied circulatory disturbances in the spinal cord after transplantation of epidermal carcinoma cells. These investigators reported changes in the white matter, such as edema, necrosis, and hemorrhage, with relative sparing of the gray matter; the onset of paraparesis was 18 days. These tumor models utilized metastatic tumors, which can be irregular in shape, and therefore the degree and shape of compression varied according to the tumor model. The disease course in these models that used tumor implantation was too rapid to represent the characteristic time course of the clinical condition in humans. In addition, the tumor itself could cause tissue damage.

Kim and coworkers implanted an expanding polymer sheet into the cervical epidural space in rats, and reported that motor dysfunction was observed from Week 17 onward. Kasahara et al. implanted an expanding sheet into the thoracic epidural space but did not observe limb paralysis, even after 15 weeks of compression. They did report neuronal losses that decreased progressively in the 6- and 9-week compression groups.

In an attempt to reproduce the temporal profile of human chronic SCC, Shinomiya and associates developed a cat model in which screws were placed in the vertebral body. Mild obstruction of the spinal canal did not cause paraparesis initially but did result in gait disturbance with delayed onset and progression over several months. Histological studies showed alterations in the gray matter and a decrease in the number of neurons. In a similar attempt, Tanaka placed 3 screws in the C4–6 vertebral bodies in cats, gradually tightening them until the canal was compromised by 50%. A delayed paralysis developed 7 months after this procedure. Microangiography showed thinning and meandering of vessels in the spinal cord. Al-Mefty and colleagues placed a Teflon screw anteriorly in dogs and tightened it until canal constriction reached 35% or until somatosensory-evoked potential was impaired. After 60 weeks, animals developed delayed myelopathy with gait disturbance. Histologically, loss of motor neurons with vac-
volution was observed, with little white matter changes such as demyelination.

Our model has several distinctive advantages over the previously reported models. The use of rats, instead of cats or dogs, allows quantitative measurement of motor function using standard methods. The supply of rats is stable and homogenous, and handling and maintenance are facile. There are fewer ethical concerns or issues, and the financial cost is much smaller. Use of the double-folded, H-shaped stainless-steel plate and plastic cannula makes dorsal compression stable. Use of a plastic plate epidurally avoids screw damage to the spinal cord directly. This model allows for easy control of the degree of compression. The surgical preparation is simple and can be performed within 30 minutes after some practice. These features make our model suitable for future therapeutic trials as well.

Some investigators have reported pathological features of degenerative spinal cord changes in patients with cervical spondylotic myelopathy. Wilkinson reported that degenerative changes were observed in this condition especially in the lateral and posterior columns at the site of compression. In the gray matter, neurons were damaged and decreased in number. Mair and Druckman reported that segments cephalad to the most damaged segment displayed ascending degeneration in the periphery of the lateral columns and in the dorsal columns. In the segments caudal to the lesion, descending degeneration was present in the lateral columns.

Our method applies compression to the dorsal aspect of the spinal cord. In clinical situations, the location of the cause of the pathology is ventral in cases involving discs, spondylosis, and ossification of the posterior longitudinal ligament; dorsal in cases involving hypertrophied yellow ligaments; or both in cases of developmental stenosis. Myelopathy usually starts some time after the spinal cord becomes constricted in the canal. In this model, the spinal cord is actually constrained along the entire circumference, and the diminished motor neuron pool indicates that the compression is sufficient to alter the parenchymal milieu entirely, including that of the ventral horn.

This rat model reproduces the characteristic course and features of clinical SCC, and induces disturbance of motor and sensory functions, which was noted as the decrease in these functions becomes apparent 9 weeks after induction of compression and progressed thereafter. Acute disturbance suggestive of SCI was not observed. The number of neurons began decreasing 3 weeks after the operation and neuronal loss decreased progressively in the 6- and 9-week compression groups. In a clinicopathological study of poliomyelitis, a loss of motor neurons as much as 40% did not cause apparent weakness, but a decrease in the neuron count as low as 10–40% was associated with mild weakness. Presumably, sprouting and reinnervation by the remaining neurons compensated for the loss of degenerating neurons. In a study of motor neuron disease, collateral reinnervation very efficiently compensated for the loss of motor units (up to 50%). The findings in these studies indicate that in chronic conditions, the decrease in the number of motor neurons preceded the onset of the disturbance of motor function.

The exact mechanisms involved in chronic and progressive neuronal loss associated with SCC have not been elucidated. Vascular alterations and circulatory disturbances have been suggested in clinical studies. Kasahara and associates studied the relationship between neuronal loss and expression of neurotrophic factors in an animal model of SCC. Fehlings and colleagues reported that TUNEL-positive cells and oligodendrocytes exhibiting caspase-3 antibody activity were observed in postmortem cervical spinal cords from 8 patients with cervical spondylotic myelopathy, but not in 4 spinal cords from healthy controls. The results of the current study suggest that spinal cord cell apoptosis may participate in the expression of profound and irreversible motor paresis caused by destructive pathological spinal cord changes during chronic mechanical compression.

Conclusions

We developed an accurate animal model to study chronic compression of the spinal cord. For pathophysiological research of chronic SCC, this model satisfies the following characteristics of the clinical condition: 1) absence of acute neurological deficit after induction of SCC; 2) insidious and delayed onset of symptoms; and 3) progression once the process becomes symptomatic. We believe this model of chronic SCC will benefit the investigation of alterations occurring in the cellular environment with this condition and will help the design of future treatments for this common and debilitating disease.

References

Pathophysiological study of chronic SCC


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