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A Novel Acute Discogenic Myelopathy Model Using Merocel® Sponge: Comparison With Clip Compression Model in Rats

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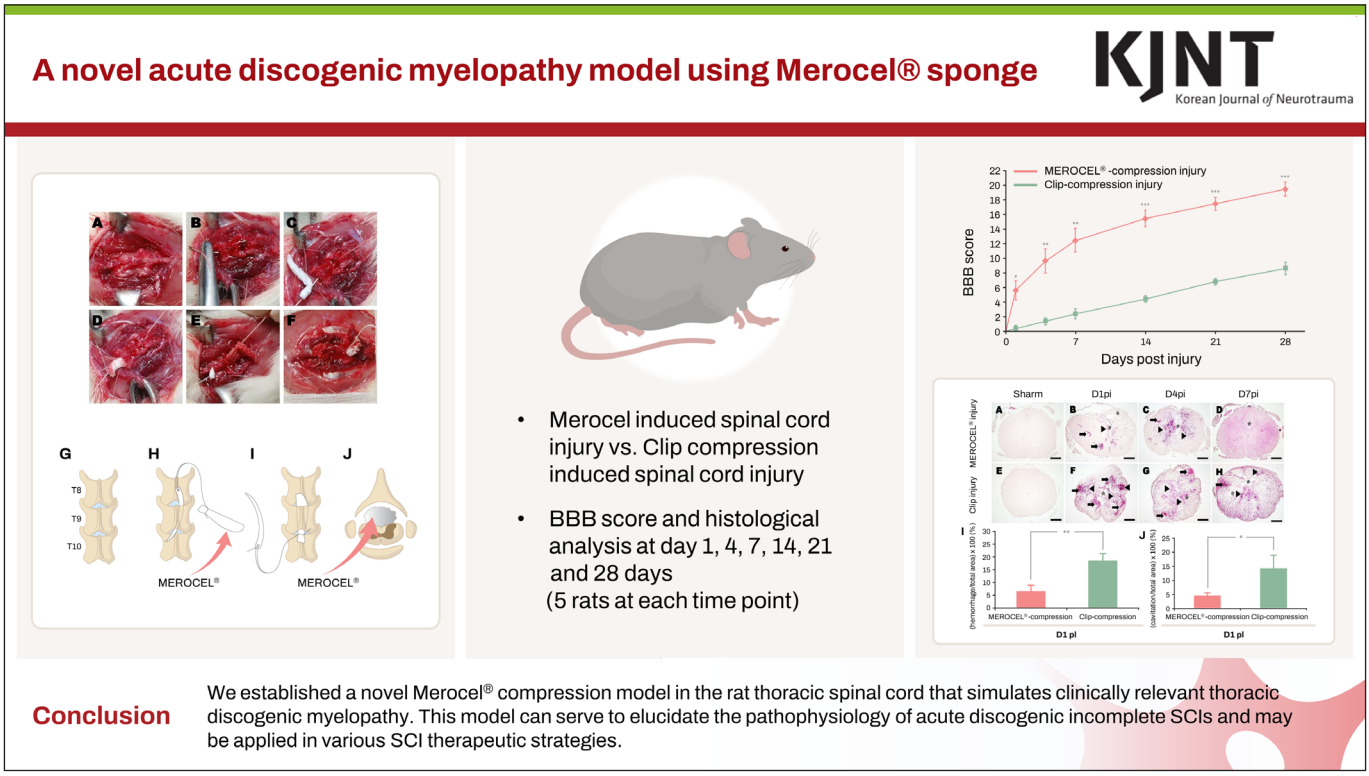
Conflict of Interest
A patent related to this research has been registered at the Korean Intellectual Property Office (Patent number: 10-2053770).

ABSTRACT

Objective: Animal models of spinal cord injuries (SCIs) use rats to simulate human SCIs. Among the various techniques, clips have been used to reproduce the compression-contusion model. However, the mechanism of injury in discogenic incomplete SCI may differ from that in clip injury; however, a model has yet to be established. Previously, we issued a patent (No. 10-2053770) for a rat SCI model using Merocel®, a water-absorbing self-expanding polymer sponge. The objectives of this study were to compare the locomotor and histopathological changes between the Merocel®-compression model (MC group) and clip compression model (clip group).
Methods: This study included 4 groups of rats: MC (n=30), MC-sham (n=5), clip (n=30), and clip-sham (n=5). Locomotor function was evaluated in all groups using the Basso, Beattie, and Bresnahan (BBB) scoring system, 4 weeks after injury. Histopathological analyses included morphology, presence of inflammatory cells, microglial activation, and extent of neuronal damage, which were compared among the groups.
Results: The BBB scores in the MC group were significantly higher than those in the clip group throughout the 4 weeks (p<0.01). Neuropathological changes in the MC group were significantly less severe than those in the clip group. In addition, motor neurons were well preserved in the ventral horn of the MC group but poorly preserved in the ventral horn of the clip group.
Conclusion: The novel MC group can help elucidate the pathophysiology of acute discogenic incomplete SCIs and may be applied in various SCI therapeutic strategies.

Keywords: Animal model; Rat; Spinal cord injury; Spinal cord compression

GRAPHICAL ABSTRACT



INTRODUCTION

Spinal cord injury (SCI) is a critical condition that incapacitates patients. The mechanisms of human SCIs are diverse and complex, leading to the development of various animal models to simulate and reproduce these injuries accordingly.[1,8,14,21,29,31] Rats are commonly used in SCI research due to their relatively low cost and the availability of various types of SCI models.[31] The mechanism of SCI in animal models are classified as contusion, compression, distraction, dislocation, transection or chemical injury.[6] Compression models are particularly helpful in simulating compression and contusion, which are common in human SCIs, and study the effects of therapy.[31] More than half of the compression models use an aneurism clip, as it has a low fatality rate, is cost-effective, and easy to handle.[14,29] The other models use a balloon, calibrated forceps, or straps.[14,29] The balloon-induction method is a simple technique that does damage the surrounding structures.[21] However, a partial laminectomy in the lumbar spine is required to insert catheter and advance the balloon to the thoracic spinal cord.[21] A lumbosacral hiatus could be utilized to introduce a catheter to the spinal canal, but it requires a skin incision at lumbosacral area. In addition, fluoroscopic X-rays are necessary to locate the tip of catheter at the thoracic level. Although calibrated forceps can produce a lateral compression injury by insertion on either side of the spinal cord,[31] a strap can also be placed around the spinal cord to compress it.[6] Among various techniques, the clip-compression model (clip group) simply induces SCIs of varying severity in all regions of the spine, which can imitate spinal cord compression.[6] The injury parameters are calibrated by the closing force of the clip and the duration of compression.[6] However, other factors, such as the velocity of clip closure and the actual force delivered

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may be variable, is a common limitation of these techniques.[6] Moreover, inducing SCI after laminectomy is different from SCI in human.[1,8,14,21,29,31] Moreover, these techniques may not represent human discogenic incomplete SCI.[6,20,24]

To examine the neuropathological and behavioral changes in discogenic myelopathy, a reliable SCI model that is characterized as partial compression by soft material without laminectomy needs to be developed.[20] Merocel® (Medtronic Xomed, Inc., Jacksonville, FL, USA) is a water-absorbing self-expanding polymer sponge that is used for nose bleeding due to its expansile nature. It is not as hard as metal, and the velocity of compression is self-controlled by absorbing surrounding fluid. Therefore, Merocel® can be used to reproduce SCI by acute disc herniation, and the effect of decompression surgery could be represented by removing Merocel®. This model is characterized by its simplicity, low cost, and reproducibility. The objectives of this study were to show neuropathology and the change in locomotor function in rats with the Merocel®-compression model (MC group) and compare it to the clip group.

MATERIALS AND METHODS

Animals

For this study, 36 male and 34 female Sprague Dawley rats aged 8–10 weeks and weighing between 200–250 g (Daehan Biolink, Cheongju, Korea) were used. The MC group included 15 male and 15 female rats, with subgroups for each time point (days 1, 4, 7, 14, 21, and 28), each consisting of 5 rats. These rats underwent SCI by epidural insertion of Merocel® at thoracic vertebrae 9 (T9). The clip group included 15 male and 15 female rats, with subgroups for each time point (days 1, 4, 7, 14, 21, and 28), each consisting of 5 rats, and these rats underwent SCI induced by clip-compression injury at the same level (T9). Sham surgeries were performed on 3 male and 2 female rats in the MC-sham group and 3 male and 2 female rats in the clip-sham group.

We compared locomotor function, histopathologic changes, including microglial reactions, and the extent of neuronal damage between the MC group and the clip group. All experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals, and the protocols for the care and handling of animals conformed to current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996). Every effort was made to minimize the number of animals and their suffering.

Surgery and postoperative care

Animals were anesthetized with chloral hydrate (Fluka, Buchs, Switzerland; 375 mg/kg body weight, intraperitoneal injection). The back skin was excised along the highest point of the spine, and the paraspinal muscles were stripped off to expose the lamina of T8–T10. In the MC group, a thread of size 3-0 Vicryl was tied to Merocel® (Medtronic Xomed Inc.), and it was passed through the dorsal epidural space guided by a needle. Next, a rectangular parallelepiped Merocel® (1 mm×1 mm×1.7 cm) was introduced into the epidural space by pulling the thread, and the skin was tentatively closed with silk (**FIGURE 1**). In a preliminary experiment, locomotor function was tested after removal of Merocel® at various time points after insertion, and based on the pilot study, Merocel® was removed through T9 laminectomy at 1 hour after insertion to represent therapeutic laminectomy for incomplete SCI. The

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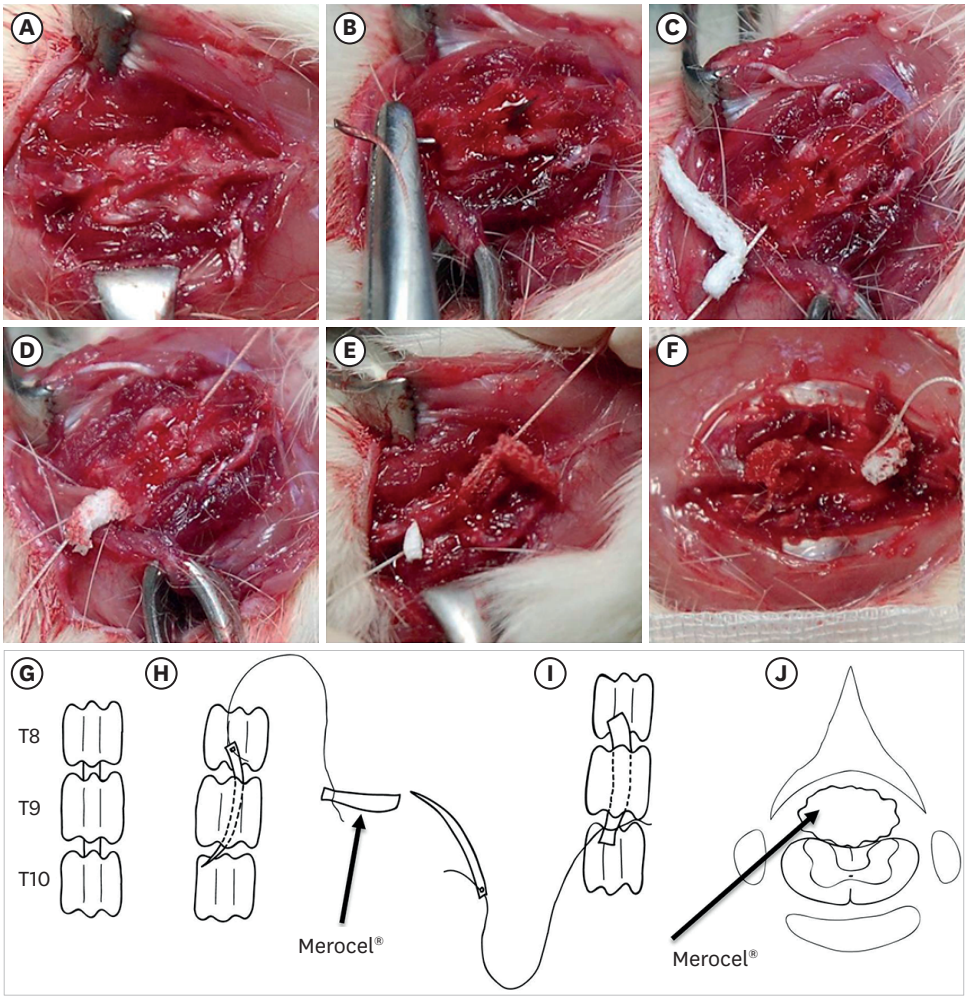

FIGURE 1. Surgical procedure (A-F) and schematic illustration (G-J). The skin was excised at the level of T8–T10 (A), and a 1-mm²-thick and 1.7-cm-long piece of Merocel® was inserted through the epidural space (B-F). Then, 1 hour after insertion, the Merocel® sponge was removed through a laminectomy, and the muscles and skin layers were closed.

muscles were closed with 2-0 Vicryl, and skin layers were closed with 3-0 nylon. In the MC-sham group, only a needle with silk was passed through, the skin was tentatively closed with silk, T9 laminectomy was performed 1 hour later, and the muscle and skin were closed with the same method as in the MC group.

In the clip group, laminectomy was performed at T9 and 10 after the same anesthesia and exposure of T8, 9, and 10 laminas. Immediately following laminectomy, the spinal cord was compressed with a vascular clip (Stoelting, Wood Dale, IL, USA) for 1 minute that was applied vertically to the exposed spinal cord at an occlusion pressure of 15–20 g.[12,23,32,33] After removal of the clip, the muscles were closed with 2-0 Vicryl, and the skin layers were closed with 3-0 nylon. In the clip-sham group, laminectomy at T8, T9 and T10 was performed after anesthesia, and the muscle and skin were closed with the same method as in the clip group without clip injury to the spinal cord.

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After surgery, rats in both MC group and clip group were fed in a position where their front paws were hanging on the cage due to bilateral lower extremity weakness, and were given free access to food. Difficult urination was detected in 5 rats in the clip group, and bladder emptying was facilitated by massaging their lower belly once a day before the behavior test. Behavior tests were performed by 2 independent researchers at 1, 4, 7, 14, 21 and 28 days postoperatively. Morphological analysis, histological examination, and immunohistochemistry were performed on all rats at 1, 4, and 7 days after euthanasia.

Behavioral tests

To evaluate the locomotor outcomes in rats' hind limbs, we utilized the Basso, Beattie, and Bresnahan (BBB) score, which was evaluated by 2 independent researchers using a double-blind method. The average scores were used to compare the severity of hindlimb paralysis between the MC and clip groups.

Histological examinations

The spinal cord tissue was prepared after euthanization with CO_2 gas. The dead animals were fixed by transcardial perfusion with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2) for 24 hours. T8–T10 regions of spinal cords were collected and fixed in 10% neutral buffered formalin for 48 hours. Cross-sections of the cords at the epi-center of the SCI (core lesion), 3 mm cranial, and 3 mm caudal points from the epicenter were examined. Sections of spinal column (5-μm thickness) were stained with hematoxylin and eosin (H&E), cleared, and mounted with Canada balsam. To examine the myelin damage in the spinal cords after injury, luxol fast blue (LFB, Acros, Vernon Hills, IL, USA; 0.1% in 95% ethanol with 10% acetic acid) stain was applied to the deparaffinized sections at 56°C for 24 hours, followed by rinsing in 95% ethanol and then distilled water; they were differentiated in 0.05% lithium carbonate, rinsed in 70% ethanol, dehydrated in 99% ethanol, cleared with xylene, and cover-slipped.

Immunohistochemistry

To assess the early response in spinal cords, we compared microglial reactions in the MC group and clip group at 4 and 7 days post-injury, as the microglial reaction is prominent within the first week.[19,23,32]. Additionally, we investigated the primary sensory fiber and motor neurons using specific markers, including calcitonin gene-related peptide (CGRP) and choline acetyltransferase (ChAT), respectively. Sections (5 μm) of paraffin wax-embedded tissues were deparaffinized and heated in citrate buffer (0.01 M, pH 6.0) in a microwave oven for 3 minutes. The tissues were then incubated in 0.3% hydrogen peroxide in methyl alcohol for 20 minutes to block endogenous peroxidase activity. After 3 washes with PBS, the sections were incubated with a matching blocking serum (10% normal goat or rabbit serum, Vectastain ABC Elite kit; Vector Laboratories, Inc., Newark, CA, USA) diluted in PBS and then incubated with rabbit anti-ionized calcium-binding protein-1 (Iba-1; 1:800; Wako Pure Chemical Industries, Ltd., Osaka, Japan), a marker for activated microglia and macrophages, CGRP (1:20,000; Sigma-Aldrich, St. Louis, MO, USA), a marker for the primary sensory fibers, and goat anti-ChAT (1:3,000; Millipore, Darmstadt, Germany), a marker for the motor neurons, for 1 hour at room temperature. After 3 more washes in PBS, the sections were incubated with an appropriate biotinylated secondary antibody (1:200 dilution; Vector Laboratories, Inc.) for 45 minutes at room temperature. After 3 additional washes in PBS, the sections were incubated with avidin-biotin peroxidase complex (Vector Laboratories, Inc.), prepared according to the manufacturer's protocol, for 45 minutes at room temperature. The peroxidase reaction was developed using a diaminobenzidine substrate kit (Vector Laboratories, Inc.) prepared according to the manufacturer's instructions.

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Semiquantitative analysis

For semiquantitative evaluations, sections were subjected to H&E staining, LFB staining (visualize white matter), CGRP staining, ChAT staining, and Iba-1 immunostaining (n=3 per group) and photographed at ×4 magnification using a digital camera (Olympus DP72; Olympus Corp., Tokyo, Japan) attached to a light microscope (Olympus BX53/U-LH 100HG; Olympus Corp.); staining was semiquantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The areas of hemorrhage and cavitation per total spinal cord area were compared between the MC and clip groups. The percentage of spared white matter per total spinal cord area was calculated by determining the area of blue pixels divided by the total area, multiplied by 100%. The activity of microglia was represented as Iba-1-positive area/total spinal cord area.

Statistical analysis

All values are presented as the means ± standard errors of the means. The results were analyzed using one-way analysis of variance followed by the Student–Newman–Keuls post hoc test for multiple comparisons. The *p*-values <0.05 were considered to indicate statistical significance.

RESULTS

Locomotor outcomes

FIGURE 2 shows the BBB score after surgery in the MC group and clip group. The BBB scores in the MC group were significantly higher than those in the clip group on days 1, 7, and 28 ($p<0.01$, $p<0.01$, and $p<0.001$, respectively). Bladder emptying was required in 5 rats in the clip group but in no rats in the MC group.

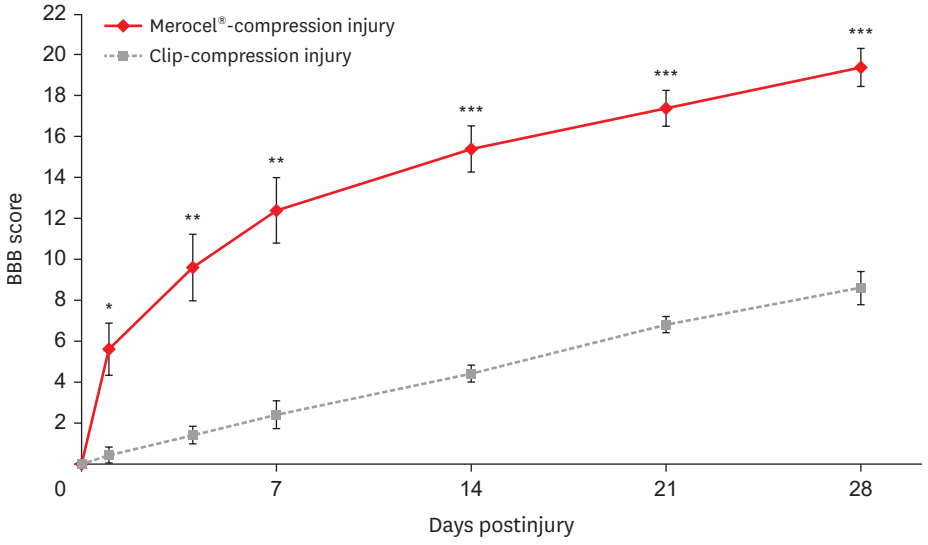

FIGURE 2. Locomotor outcomes by BBB score (n=5/each day). On day 1, the BBB scores were less than 7 in both groups, whereas on day 7, the BBB score in the Merocel®-compression group was significantly higher than that in the clip-compression group. At 28 days postinjury, the BBB score of the Merocel®-compression group remained higher than that of the clip-compression group, showing that the former rats exhibited more rapid locomotor recovery than did those with clip-compression injuries.
BBC: Basso, Beattie, and Bresnahan.
*$p<0.05$, **$p<0.01$, ***$p<0.001$, vs. clip-compression injury.

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Histological findings

In the sham control groups (**FIGURE 3A & E**), there was no abnormal change in the core region of the spinal cord. The MC group showed hemorrhage, inflammatory cells, and cavitations on day 1, with inflammatory cells showing crowding and increased size and number of cavities on day 4, and increased density of cells throughout the spinal cord with increased size and number of cavities on day 7. In contrast, the clip group showed prominent hemorrhage, inflammatory cells, and cavitations from day 1, with accumulations of round-type inflammatory cells, such as activated microglia, and evident cavitations on day 4, and centralized changes on day 7. The hemorrhagic area/total spinal cord area was compared between the MC group and clip group, and the hemorrhagic area and cavitation area per total spinal cord area were significantly lower in the MC group than in the clip group on day 1 (p<0.01 and p<0.05, respectively).

To evaluate the percentage of spared white matter in the spinal cord after surgery, we performed LFB staining in the cranial, core, and caudal regions on day 4 (**FIGURE 4**). LFB staining showed that the core regions of the clip group had more severe myelin destruction in the white matter

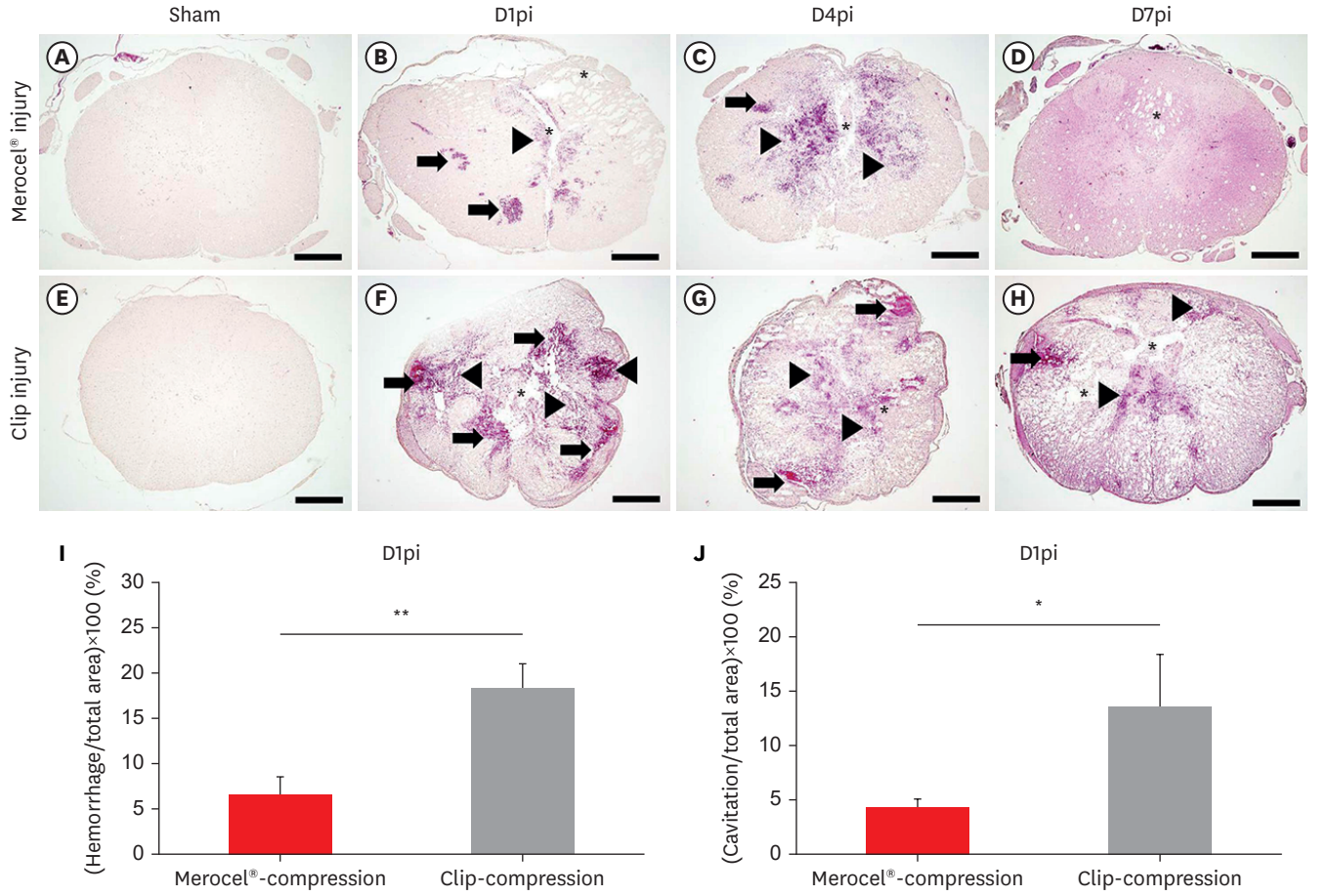

FIGURE 3. Histological findings of spinal cords with Merocel®-compression (A-D) and clip-compression injuries (E-H) by hematoxylin and eosin staining. (A & E) Sham; (B & F) D1pi; (C & G) D4pi; (D & H) D7pi, respectively. The neuropathological outcomes, including hemorrhage (arrows), infiltration of inflammatory cells (arrowheads), and cavity formation (asterisks), in the spinal cords of rats in the Merocel®-induced SCI group were milder than those of rats in the clip-compression group. The bar graphs reflect semiquantitative analyses of the hemorrhage areas (I) and cavitation areas (J). These areas were significantly greater in the clip-compression SCI group than in the Merocel®-compression group on D1pi. Scale bars=100 μm.
Sham: sham control, D1pi: day 1 postinjury, D4pi: day 4 postinjury, D7pi: day 7 postinjury, SCI: spinal cord injury.
*p<0.05, **p<0.01, vs. clip-compression injury.

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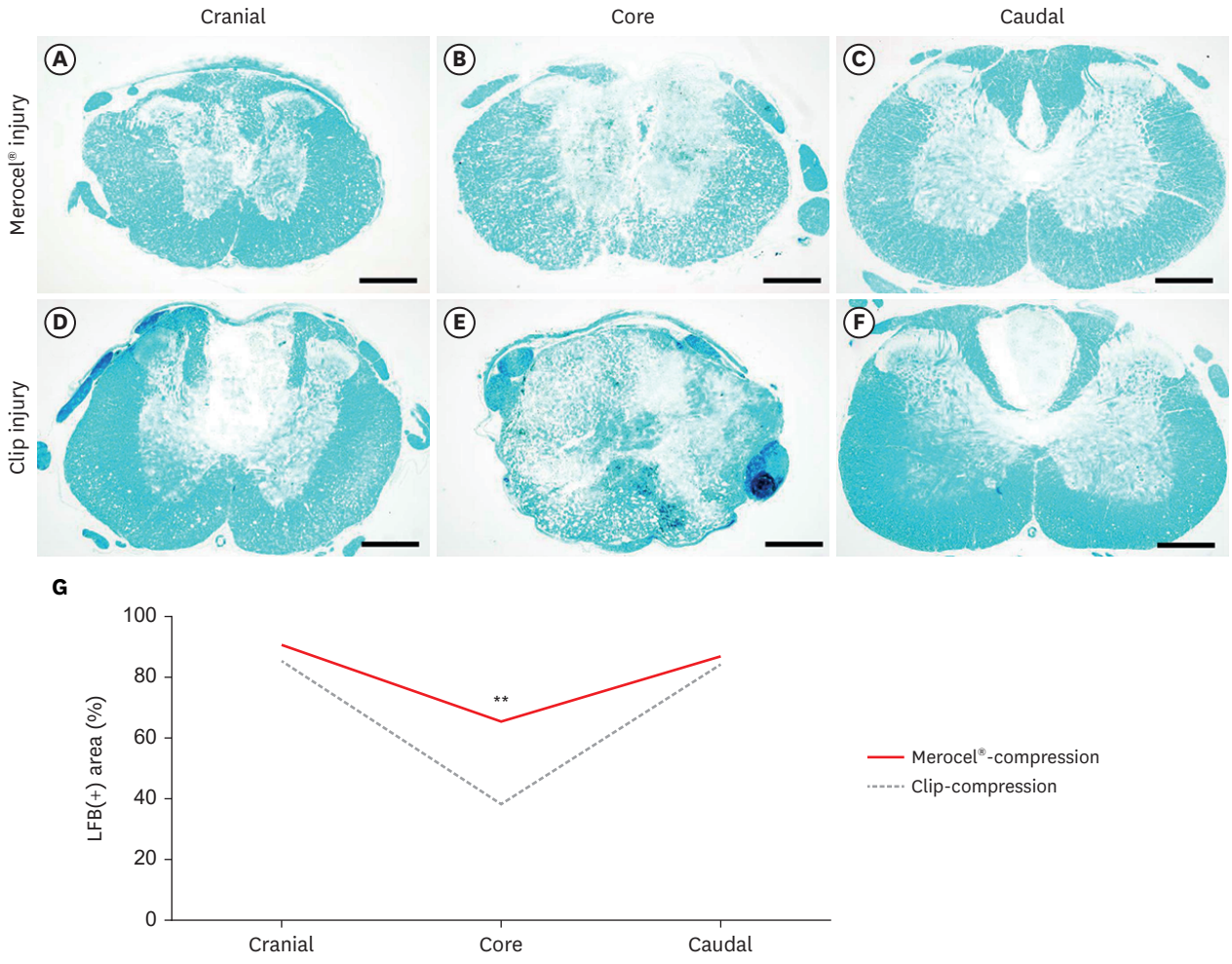

FIGURE 4. Spared white matter in the cranial, core, and caudal regions of the spinal cord with Merocel®-insertion (A-C) and clip-compression injuries (D-F) on day 4 postinjury. The cranial and caudal regions in the spinal cords of the Merocel®-compression (A & C) and clip-compression groups (D & F) were similar histologically. However, the core regions of the clip-compression-injured spinal cords exhibited more severe loss of white matter myelin (E) than did those of the Merocel®-compression group (B). The bar graph (G) indicates the percentage of spared white matter. LFB staining. Scale bars=100 μm.
LFB: luxol fast blue.
**p<0.01, vs. clip-compression injury.

than did the core regions of the MC group, and the percentage of white matter in the core region was significantly higher in the MC group than in the clip group (p<0.01).

Microglial reaction and infiltration of inflammatory cells

Microglial activation and infiltration of inflammatory cells were evaluated by performing immunohistochemistry for Iba-1 and calculated the percentage of Iba-1-positive area/total spinal cord area. No significant difference was observed between the MC-sham group (4.90%±0.42%, **FIGURE 5A**) and the clip-sham group (5.64%±1.08%, **FIGURE 5D**) (p<0.05). Iba-1-positive microglial cells were detected in all regions, including the white matter and gray matter, but there were no inflammatory cells. On day 4, both the MC group (12.71%±2.31%,**FIGURE 5B**) and the clip group (15.66%±4.88%, **FIGURE 5E**) showed the presence of ramified microglial cells and many inflammatory cells in the core region (p<0.05). However, on day 7, the Iba-1-reactivity and Iba-1-positive area in the core region were significantly lower in the MC group (14.00%±1.79%,**FIGURE 5C**) than in the clip group (24.85%±5.19%) (p<0.05, **FIGURE 5F**).

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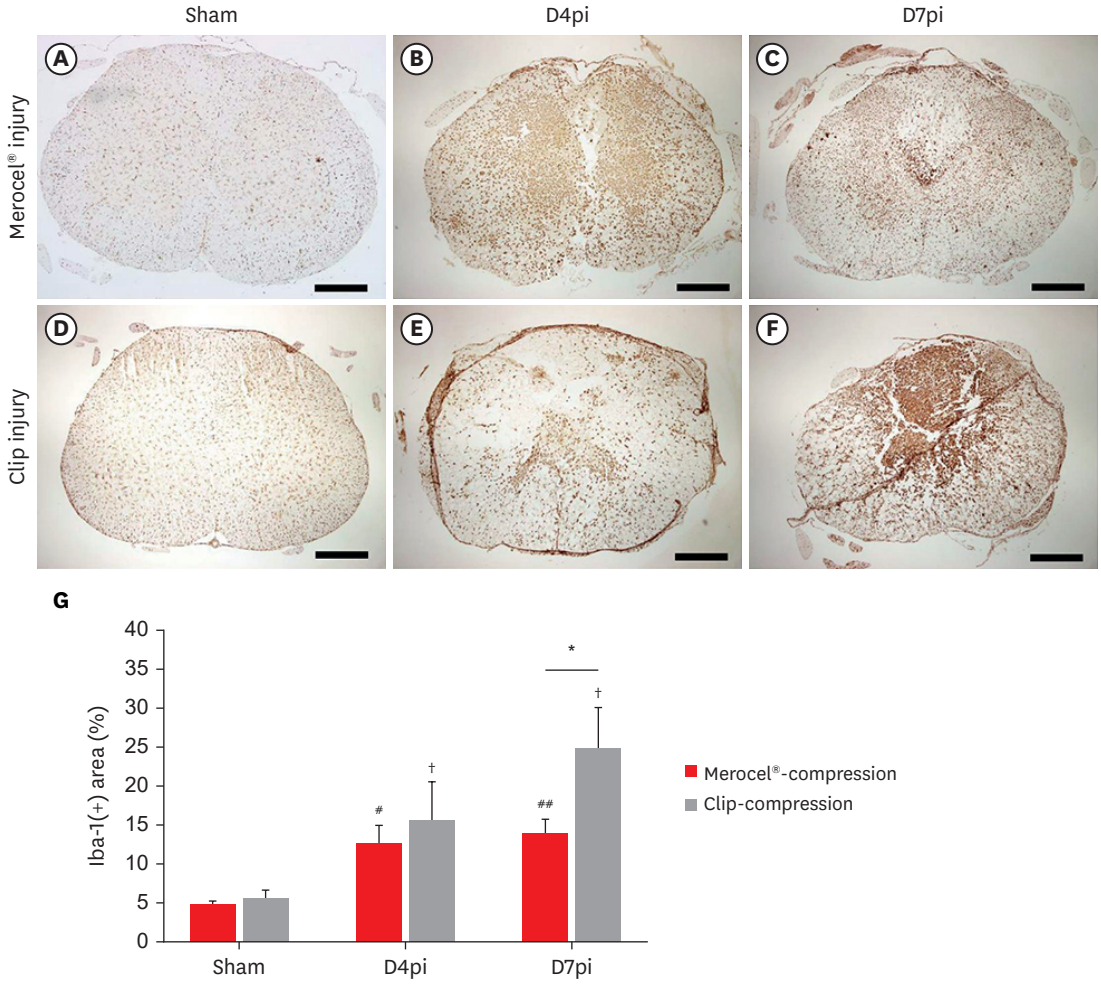

FIGURE 5. Iba-1 immunostaining in the core regions of Merocel® (A-C) and clip-compression injuries (D-F). (A & D) Sham; (B & E) D4pi; (C & F) D7pi, respectively. In both the Merocel®-compression Sham (A) and clip-compression Sham (D), Iba-1-positive microglial cells were evident in all regions, including the white matter and gray matter; inflammatory cells were absent. On day 4, ramified microglial cells and many inflammatory cells were evident in the core region of both the Merocel®-compression SCI (B) and clip-compression groups (E). On day 7, the Iba-1 reactivity and Iba-1-positive area of the core region were significantly lower in the Merocel®-compression group than in the clip-compression group. (G) Bar graphs show semiquantitative analysis of the percentages of the Iba-1-positive area. Scale bars=100 μm.
Iba-1: ionized calcium-binding protein-1, Sham: sham control, D4pi: day 4 postinjury, D7pi: day 7 postinjury, SCI: spinal cord injury.
*p<0.05, vs. Merocel®-induced SCI on D7pi; †p<0.05, vs. clip-compression Sham; #p<0.05, ##p<0.01, vs. Merocel®-insertion Sham.

Primary sensory fibers and motor neurons

In the MC group, CGRP-positive fibers were observed in laminae I and II of the dorsal horn (**FIGURE 6A & B**). The immunohistochemical reactivity of CGRP fibers was lower in the clip group (**FIGURE 6C & D**) on day 7. Furthermore, many ChAT-positive motor neurons were evident in the ventral horn in the MC group (**FIGURE 6E & F**), whereas only a few ChAT-positive motor neurons were present in the clip group (**FIGURE 6G & H**). The CGRP-positive area/total spinal cord area was significantly larger in the MC group (37.47%±1.36%) than in the clip group (25.11%±4.46%) (p<0.05, **FIGURE 6I**). The number of ChAT-positive motor neurons in the MC group (24±4.7) was also significantly greater than that in the clip group (5±1.7) (p<0.05, **FIGURE 6J**).

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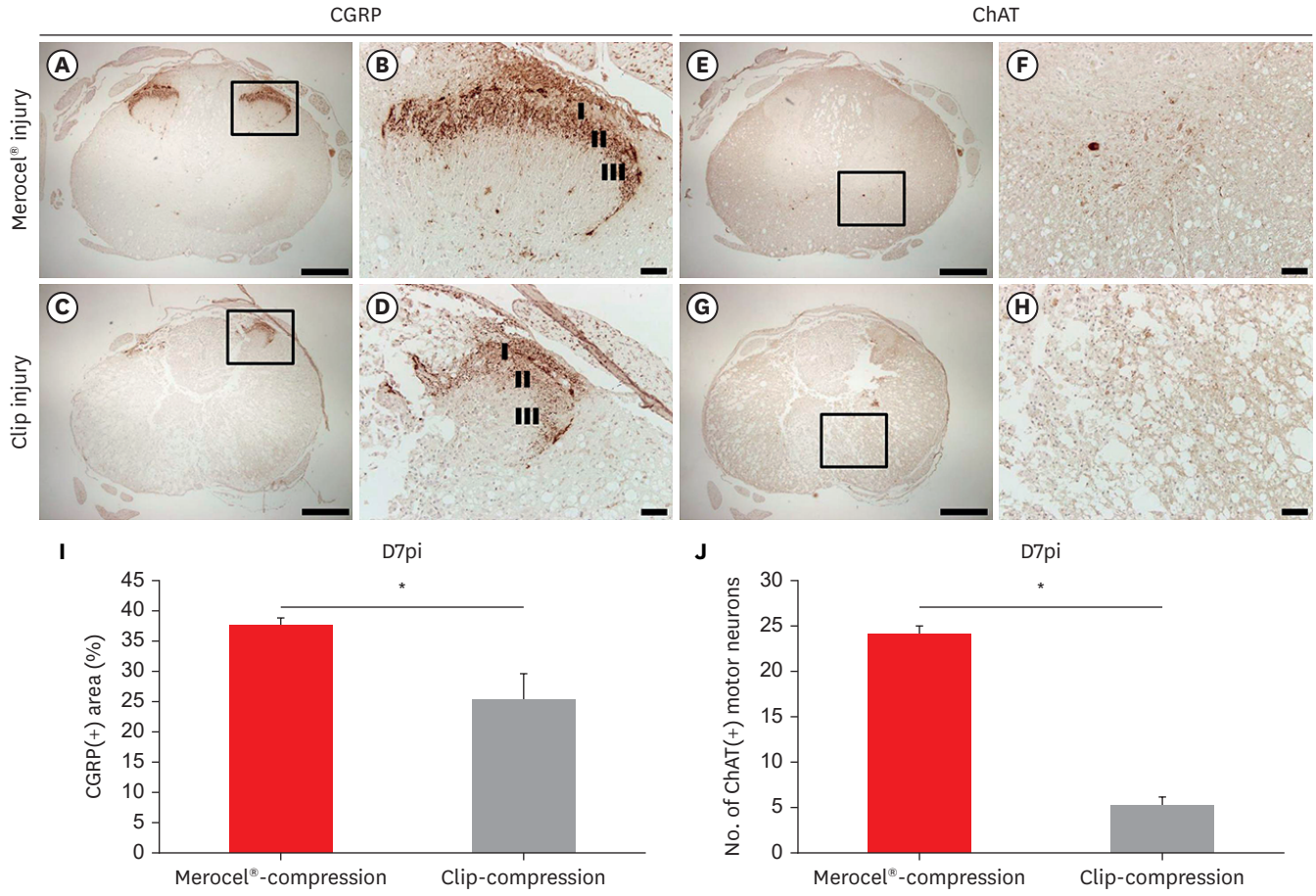

FIGURE 6. Primary sensory fibers (CGRP-stained) and motor neurons (ChAT-stained) in the core region of the spinal cord 7 days after induction of Merocel®-compression and clip-compression injuries. CGRP-positive fibers (A-D) are shown in the dorsal horns, and ChAT-positive motor neurons (E-H) are shown in the ventral horns of spinal cords with Merocel®-compression (A & E) and clip-compression SCI (C & G). The immunoreactivities of both CGRP and ChAT in the Merocel®-compression SCI rats were higher than those in rats with clip-compression injuries. Squares indicate the regions shown at higher-magnification views of (B & F) Merocel®-compression and (D & H) clip-compression SCI sections prepared on D7pi. The bar graphs show that the CGRP-positive areas (I) and the number of ChAT-positive motor neurons (J) in the Merocel®-compression group were significantly greater than those in the clip-compression group. Scale bars: (A & C) 100 μm; (B & D) 50 μm.
CGRP: calcitonin gene-related peptide, SCI: spinal cord injury, ChAT: choline acetyltransferase, D7pi: day 7 postinjury, I–III: laminae I–III.
*p<0.05, vs. clip-compression injury.

DISCUSSION

The objectives of this study were to show the neuropathology and change in locomotor function of rats with the MC group and to compare those with the clip group. We found that SCI induced by Merocel® expansion resulted in better locomotor function and less severe histological changes, including hemorrhage, inflammatory cell recruitment, and spared white matter in the core region, compared to SCI induced by clip compression.[10] These results suggest that the Merocel® model simulates incomplete SCI and may represent therapeutic decompressive laminectomy for discogenic incomplete SCI.

On the other hand, the clip group, intended to induce complete SCI with a compressive power of 25 g for 1 minute, aims to replicate SCIs caused by blunt trauma (such as motor vehicle crashes), where the spinal cord is contused or compressed by an object or displaced bone, disc, or other tissue. Therefore, previous animal studies used blunt trauma injury patterns such as contusion or compression in 62% of studies.[10,31] The severity of injury was

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complete in 52% and incomplete in 48% of studies.[31] Compression models are helpful to simulate compression and contusion, which are common in human SCIs, and to study the effects of therapy.[31]

Poon et al.[28] reported a correlation between clip force and locomotor function as well as histological changes in the spinal cord. The main pathological changes in clip-compression injuries can be categorized into 3 stages: (1) the early inflammatory stage, (2) the clearing stage, and (3) the reactive gliosis stage.[32] However, the clip group has limitations. Clinical symptoms and histopathological changes may greatly depend on the operative technique, such as the clip position, any trauma induced during clip insertion, and the velocity of clip closing.[5,12,20,28] In addition, trauma is not the only cause of SCI, and disc herniation is another cause of SCI.[24,25] The severity and secondary changes associated with discogenic SCI may be milder than those of traumatic SCI, with SCI occurring in the presence of intact lamina in humans. Therefore, the clip group may not caused by disc herniation, as it occurs in the presence of intact lamina.[6,20,24,25] Balloon compression model may simulate SCI caused by disc herniation, as it occurs in the presence of intact lamina.[21] However, the balloon compression model has limitations, as a partial laminectomy was necessary to insert the catheter at the lumbar spine, and fluoroscopic X-ray was required to locate the tip of the catheter at the thoracic level, which may affect the reproducibility of the injury. Those may be limitations of balloon compression model.[21] In this regard, we aimed to develop an SCI model representing SCI by disc herniation using Merocel®, which is a biocompatible synthetic sponge that expands in volume when hydrated. By implanting a piece of Merocel® under the spinal lamina in the epidural space, sponge self-expands and compresses the spinal cord. The self-expandable nature of Merocel® may decrease operator variability, which may occur during clip application. In a preliminary experiment, we found that installation of a Merocel® sponge for 1 hour induced reproducible incomplete SCI and a recovery model confirmed by pathological changes and locomotor function.

The main neuropathological changes of the core region in the MC group were hemorrhage on day 1, infiltration of inflammatory cells on day 4, and increased cell density on day 7. Hemorrhage increased the pressure on the dura mater of the spinal cord, leading to exacerbation of edema and axonal destruction during the early inflammatory stage.[9] These sequential changes trigger functional problems in the lower motor system.[33] Furthermore, the core regions of Merocel®-compressed spinal cords exhibited less white matter myelin destruction than was noted in our previous studies.[2,15,16,23] The preservation of descending fibers reflected in axon sparing paralleled the functional locomotor recovery seen after injury.[30,34,35] CGRP, consisting of 37 amino acids, is a neuropeptide translated from calcitonin mRNA.[3] CGRP is distributed throughout the central and peripheral nervous system, including primary sensory fibers, motor neurons, and dorsal root ganglions.[13] In the rat SCI model, induced by clip compression and transection, CGRP in the dorsal horn is drastically reduced after injury.[17,18] However, after Merocel®-induced injury, CGRP-positive fibers of the dorsal horn were retained in SCI rats to a much greater extent than those after clip-compression injury. ChAT is a representative marker of motor neurons, including those of the spinal cord, and is reduced in expression after SCI.[27,28] In the present study, we found that ChAT-positive motor neurons were well preserved in the ventral horn of the MC group but poorly preserved in that of the clip group.

During the clearing and reactive gliosis stages (less than 2 weeks postinjury), the innate immune response, mounted by monocytes, macrophages, and activated microglia, creates an

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inflammatory environment[4,22] and induces significant inflammatory responses.[11] However, an excessive inflammatory response after SCI can delay recovery.[7] In the present study, the level of activated microglia/macrophages in the core regions of the MC group was lower than those of the clip group. Our new SCI model, induced by Merocel®, was associated with lower numbers of activated macrophages/microglia and only a moderate inflammatory response at the site of injury.

The MC group may be used to induce SCI by spinal intervertebral disc herniation. However, the intervertebral disc was located ventral to the spinal cord, and Merocel® was inserted dorsal to the spinal cord. The discrepancy comes from the differing locations of the corticospinal tract in rats versus humans.[26] In humans, the corticospinal tract, which controls voluntary movement, is located in the lateral funiculus of the spinal cord, while it is located in the dorsal funiculus in rats.[26] As the direction of human disc herniation is anterior-to-posterior, the posterior-to-anterior direction of Merocel®-induced compression injuries in rats may partly mirror human discogenic myelopathy.

This study has a limitation. In the MC group, the extent of spinal cord compression seems to be determined by the equilibrium between cord elasticity and the expansion force of Merocel® within the spinal canal. In a preliminary experiment, we found that intravertebral insertion of Merocel® resulted in nearly complete replacement of the spinal canal within 7 days of injury. The extent of SCI was determined by the expansion power of Merocel®, elasticity of the spinal cord and the area of injury. Spinal cord tissue is soft and viscoelastic in nature; it is difficult to obtain accurate modulus and stiffness values. To establish a constant and steady model, those data need to be assessed in further study.

CONCLUSION

We established a novel MC group in the rat thoracic spinal cord that simulates clinically relevant thoracic discogenic incomplete SCI. The protocol of this model is simple and reliable with appropriate reproducibility and little variability. This model can serve as an alternative tool for understanding the pathophysiology of acute incomplete SCIs and can be applied in future experimental studies dealing with various SCI therapeutic strategies.

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