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Establishment of a bio-industry-need hemisection of spinal cord rat model

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Abstract

Spinal cord injury (SCI) is a severe central nervous system disorder, and due to its complex pathophysiology, effective treatment strategies are currently lacking, posing a significant challenge in the field of medicine. While research into the fundamentals of SCI continues to advance and innovate, there exists a gap between basic research and clinical applications, hindering the translation of basic research findings into clinical practice. One of the reasons for this gap is the use of non-standardized animal models for SCI, which leads to inaccuracies in research results and reduces the guidance value of basic research for clinical treatment. This study provides a SCI rat model for R&D of SCI medicine target research and therapeutic strategy designs.

Keywords: Bio-industry; Central nervous system; Hemisection of Spinal Cord; Rat Model; Spinal Cord Injury

1. Introduction

Spinal cord injury (SCI) does not occur alone and it accompanies damage to the spine. The human spine serves two functions as the body's supporting framework and a conduit for the spinal cord (SC) nerves, which are encased in the vertebral canal within the spine. The SC nerves consist of 8 cervical segments, 12 thoracic segments, 5 lumbar segments, and 4-5 sacral segments, and their function is to facilitate communication between the brain and the peripheral nervous system. Signals from the brain are transmitted through SC as a relay station to the muscles of the limbs and trunk, as well as to convey sensory information such as touch, temperature, and more back to the brain to inform it about the body's current state. Additionally, SC nerves transmit information from the autonomic nervous system, which comprises the sympathetic and parasympathetic nervous systems. These two neuro-systems coordinate the functions of the internal organs, including blood pressure and temperature regulation, gastrointestinal and bowel functions, and bladder contraction and urination [1-2].

SCI is an acute injury to SC (including nerve roots). Once SC is damaged, it can result in varying degrees of loss of motor and/or sensory functions, leading to various problems, including motor and sensory impairments, difficulty with urination and bowel movements, respiratory difficulties, sexual dysfunction, skin complications, and autonomic nervous system abnormalities. The causes of SC dysfunction can be broadly categorized into two main types included the traumatic SCI and non-traumatic SCI. In Taiwan, the leading causes of traumatic SCI are motor vehicle accidents, followed by falls from heights, crush injuries, sports-related injuries, and gunshot or stab wounds. These injuries often result in one or more vertebral fractures or dislocations, with fragments of these vertebrae compressing SC and causing

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loss of function. Non-traumatic SCI causes included nerve tumors, vascular malformations, myelitis, and complications of tuberculous spinal lesions. Tumors can lead to swelling that compresses SC nerves then gradually diminish their function [3-6].

The symptoms that arise after SCI vary widely depending on the level and severity of the injury. Clinically, they can be divided into the following categories as limb paralysis and autonomic dysfunction. Damage to SC in the thoracic or lumbar region can impede the transmission of motor and sensory signals to the lower limbs, resulting in complete paralysis that known as complete lower limb paralysis or partial lower limb paralysis. If SCI occurs at a higher cervical level, both the upper and lower limbs may be affected, leading to loss of motor and sensory functions, as well as limited respiratory function and difficulty coughing up phlegm. Since SC nerves control certain aspects of the autonomic nervous system, including heart rate, blood pressure, sweating, bladder and bowel control, and sexual function, SCI often lead to problems such as urinary and fecal incontinence, postural hypotension, and sexual dysfunction [7-9].

For individuals with SCI, it is necessary to receive comprehensive rehabilitation therapy to achieve the ability to live independently. Post-surgical rehabilitation for SCI includes spasticity management, urinary training, pressure sore prevention, and training in the use of wheelchairs, canes, and walkers, as well as occupational therapy [3]. The goal is to enable patients to live independently. In this study, a successful SCI rat model has been established. Therefore, we wish this SCI animal model can be used to medicine research and therapeutic strategy designs.

2. Material and Methods

2.1. Chemicals and Reagents

Phosphate-buffered saline (PBS; Sigma-Aldrich, Cat. No. P3813), saline (Taiwan Biotech Co., LTD, Cat. No. 100-120-1101), Zoletil 50 (Virbac, Carros, France), rat interleukin 6 (IL-6) ELISA kit (MyBioSource, Cat. No. MBS2021530), rat TNF alpha ELISA kit (MyBioSource, Cat. No. MBS700574), rat interleukin 4 (IL-4) ELISA kit (MyBioSource, Cat. No. MBS2701059), rat interleukin 1 beta (IL-1β) ELISA kit (MyBioSource, Cat. No. MBS2023030), and rat glial fibrillary acidic protein, GFAP ELISA kit (Cusabio, Cat. No. CSB-E08602r) were used in this experiment.

2.2. Experimental Animals and Experimental Design

The male 36 Sprague-Dawley (SD) rats [6 weeks old; body weight (BW) between 180-200 g] with specific pathogen-free conditions were used for this study, were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan). The environment was maintained room temperature (24-27°C) and 60%-70% humidity with a photoperiod of 12-hr light/12-hr dark cycle. The study will begin after a week acclimation. The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 111092 approved by the IACUC ethics committee. The male 36 SD rats were divided respectively the normal control group (n = 18) and the negative control group (n = 18). In the normal control group and the negative control group, three sub-groups were respectively divided according to time of SCI induction (8 hours, 24 hours, and 7 days post SCI induction) and the 6 SD mice were distributed randomly in each sub-group. All SD rats were fed with standard laboratory diet (No. 5053, LabDiet®; PMI Nutrition International, St. Louis, MO, USA) ad libitum during the experimental period. The change of SD rats' BW, the observation of SD rats' behaviors, and cytokine expressions of the collected SD rats' spinal cord tissues were detected, and histopathological examinations of spinal cord tissues were performed.

2.3. Surgery of Spinal Cord

After 6-week-old male SD rats were introduced into the GLP animal facility, they were observed for 3-5 days to ensure that there were no abnormal clinical signs before proceeding with the experiment. The SD rats were anesthetized and continuous monitoring of their respiratory rate. The rats were prone positioned and the limbs were secured. The T7-T13 area of spines were shaved and disinfected with iodine solution. Using a surgical blade, the skin, fat, and muscle above the T8-T11 spines were incised until the spinal columns were visible. If there was minor bleeding affecting visibility, a small amount of sterile saline was applied, and a sterile cotton swab was used to clear the surgery field. With blunt-tipped surgical scissors, the spinal column and adjacent muscle tissues were carefully separated to expose the spinal cord. The vertebral arch was gently clipped off using bone scissors to expose the spinal cord. A 25G needle was used to make a complete transverse cut into the right side of the spinal cord (T9), repeated three times at the same location to ensure complete severance of the right side of the spinal cord. After removing any oozing blood with sterile saline and cotton swabs, the surgical site was sutured in layers, starting with the muscles, then the subcutaneous area, and finally the skin. The area around the wound was disinfected with iodine solution, and broad-spectrum antibiotics

and pain relievers were administered. Later, the surgery SD rats were expected to exhibit right hind leg dragging and an inability to exert control over the limb upon awakening.

2.4. Basso, Beattie, and Bresnahan Locomotor Scale Method

The locomotor functions were tested by using an open field locomotor scale, described by Basso, Beattie and Bresnahan (BBB) from complete paralysis (score 0) to normal locomotion (score 21), to assess recovery after contusion injuries in rats' SC [3].

2.5. Collection of Spinal Cords

After euthanizing the rat with CO_2. Blood was collected via cardiac puncture to remove the majority of blood, ensuring cleanliness for the subsequent dissection. The rats was prone positioned, and a surgical blade was used to make a large incision along the back, with the fur pushed aside to expose a large area of back muscle. Surgical scissors were used to shave the muscles along both sides of the spine, exposing the entire vertebral column. The dissection range extended from C6 to L6 of the spines. The vertebral arches of T8 to T11 were clipped and removed, followed by careful cutting of this spinal segment with surgical scissors. The removed spinal cords were placed in formalin for subsequent analysis. The remaining vertebral arches were also removed to fully expose the spinal cord. The spinal cords were carefully extracted and placed in PBS before being stored in a -80°C.

2.6. Histopathological Examination of Spinal Cords

After the SD rats were sacrificed with CO_2, the spinal cords of rats were taken out and fixed with 10% formalin for 48 hours, and then the spinal cords were immersed in decalcification solution for 28 days under the decalcification step. After the decalcification was completed, the spinal cords were embed the tissue in paraffin, make a paraffin section (paraffin section), and cut into three sections each with a thickness of 5 μm. Then, the sections were stained for tissues (including HE staining and Masson's trichrome staining), and the average morphological changes of the spinal cord tissues were observed under a microscope by a senior pathologic veterinarian.

2.7. Cytokine Assay

The SD rats' spinal cords were homogenized in PBS, and after homogenization, the supernatant was collected by centrifugation at 4°C and 12,000 ×g. The supernatant was then storage at -80°C. The rats' spinal cord extract were used with enzyme-linked immunosorbent assay (ELISA) assay kits to detect inflammatory factors, including IL-1β, IL-4, IL-6, TNF-α, and GFAP according to the manufacturer's instructions.

2.8. Statistical Analysis

SPSS (Statistical package for the social sciences) statistical software (version 28.0) were used for statistical analysis. Measurement data were expressed as mean ± standard deviation (SD). All comparisons were made by one-way ANOVA (Analysis of Variance). All significant differences are reported at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

3. Results

3.1. Locomotor Activity of SD Rats During the Experiment

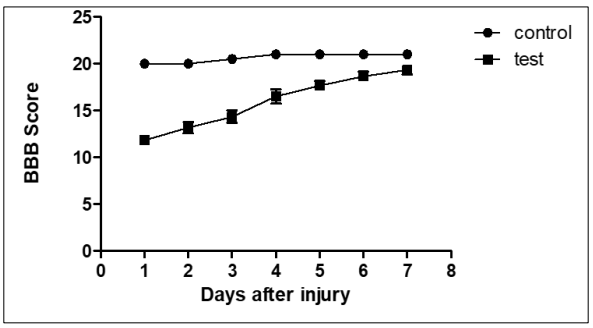

Figure 1 Locomotor activity of rats during the experiment. According to the Basso, Beattie, and Bresnahan locomotor scale (BBB scale) method, rats in both the control and test groups were scored for 7 days post-surgery. By the 7th day post-surgery, the mean score for the control group (n = 18) was 21, while for the test group (n = 18) was 19.33. Data presented mean ± SD.

According to the BBB scale method, rats in both the control and test groups were scored for 7 days post-surgery. On the first day, the mean score for the control group was 20, while for the test group it was 11.83. Subsequently, the scores gradually recovered to approach those of the control group. By the 7th day post-surgery, the mean score for the control group was 21, while for the test group it was 19.33 (Fig. 1).

3.2. Cytokine Expressions of SD Rats' SC Samples

According to the standard operating procedure provided by the ELISA kit, spinal cord samples were subjected to ELISA analysis. The results showed no difference in the expression of IL-4 and TNF-α between the control and test groups within 24 hours post-surgery. However, the expression of IL-4 and TNF-α at 7th days post-surgery were significantly higher in the test group compared to the control group ($p < 0.05$-$p < 0.01$) (Fig. 2A, C). Additionally, the expression levels of IL-6, GFAP, and IL-1β did not differ between the two groups at any time point of the experiment (Fig. 2B, D, E).

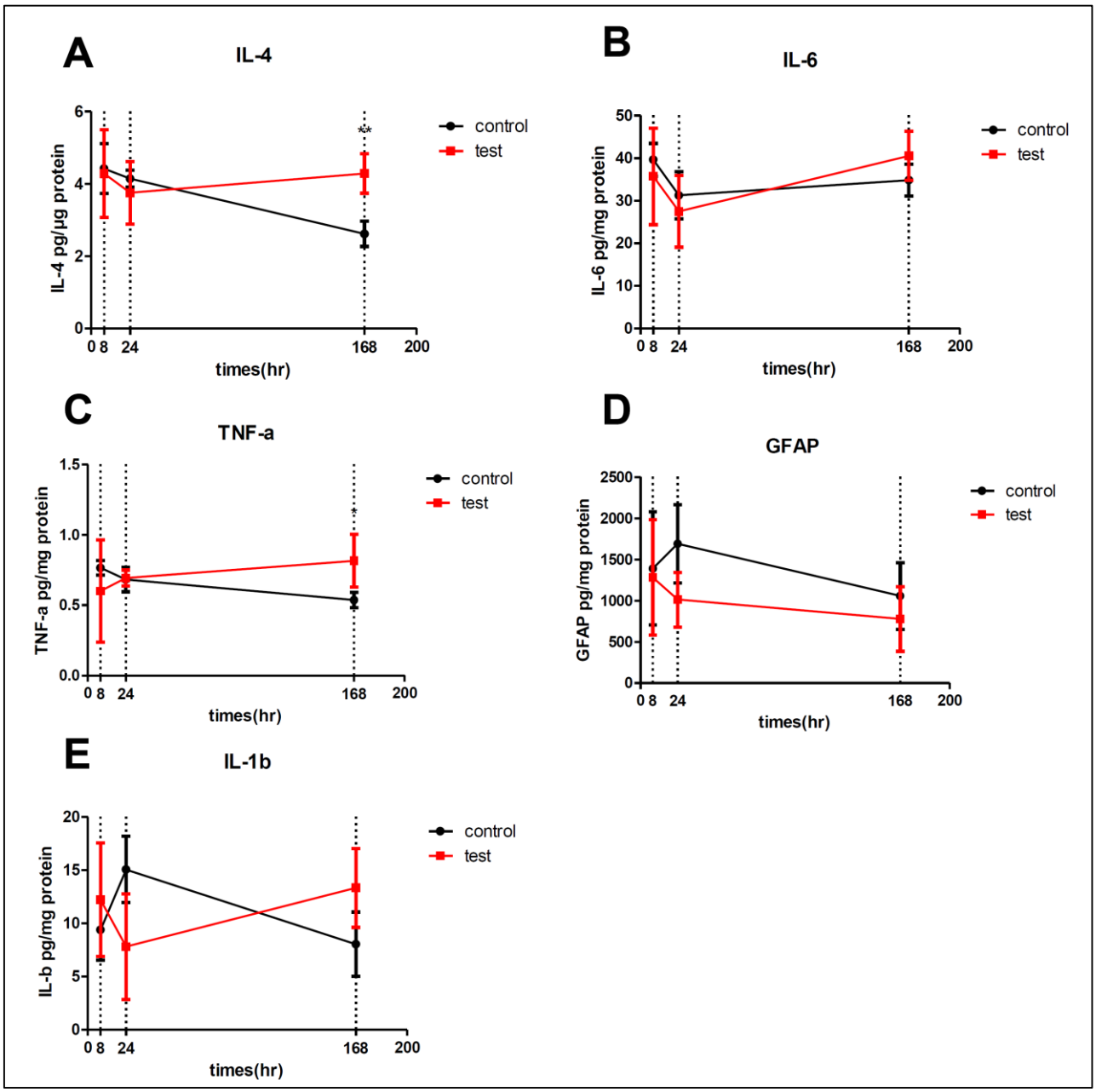

Figure 2 Cytokine expressions of SD rats' SC samples. The expression of IL-4 and TNF-α at 7th days post-surgery were significantly higher in the test group compared to the control group. The expression levels of IL-6, GFAP, and IL-1β did not differ between the two groups (n = 18 per group) at any time point of the experiment. (A) IL-4, (B) IL-6, (C) TNF-α, (D) GFAP, (E) IL-1β. Data presented mean ± SD. $^{*}p < 0.05$; $^{**}p < 0.01$.

3.3. Histopathologic Examination of SC Samples of SD Rats

The histopathologic examination of SC samples of SD rats was performed at the time points of the experiment (Figs. 3-4). Five evaluated items were monitored.

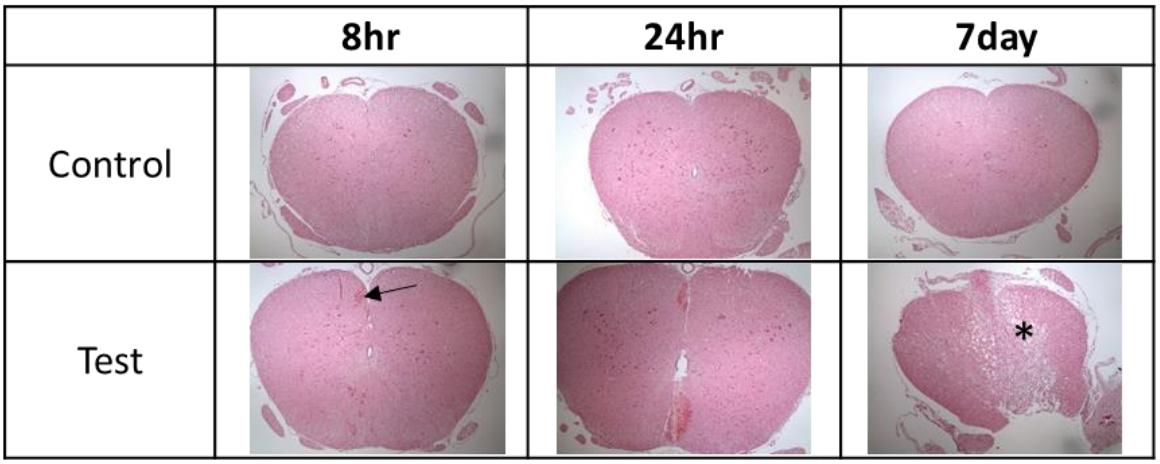

Figure 3 Histological changes in spinal cord hemisection injury at different time points post-injury in all groups. Black arrows indicate areas of hemorrhage lesions, and black asterisks indicate areas of demyelination lesions. H&E stain, magnification: 40×

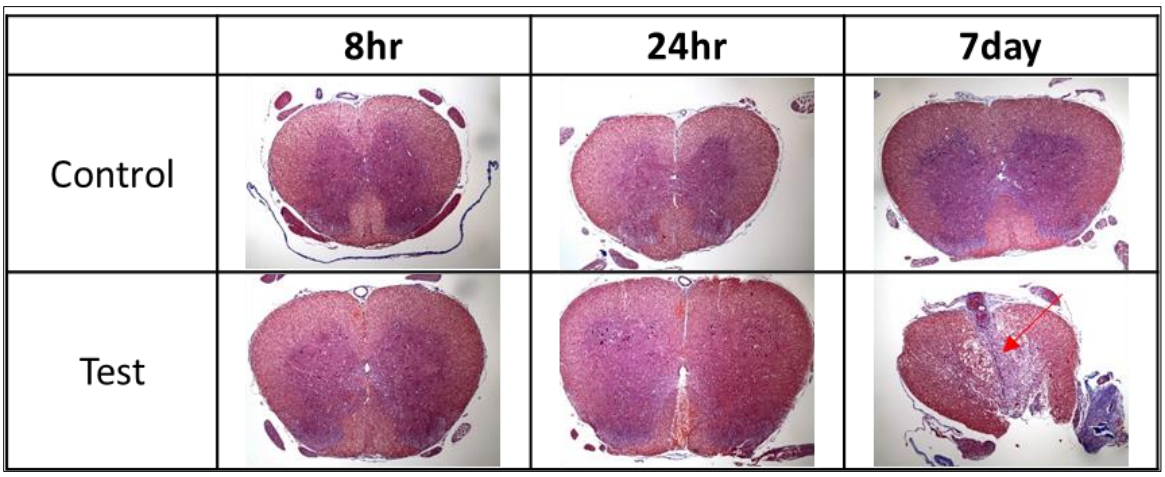

Figure 4 Histological changes in spinal cord hemisection injury at different time points post-injury in all groups. Red arrows indicate areas of fibrotic lesions. Masson's Trichrome stain, magnification: 40×

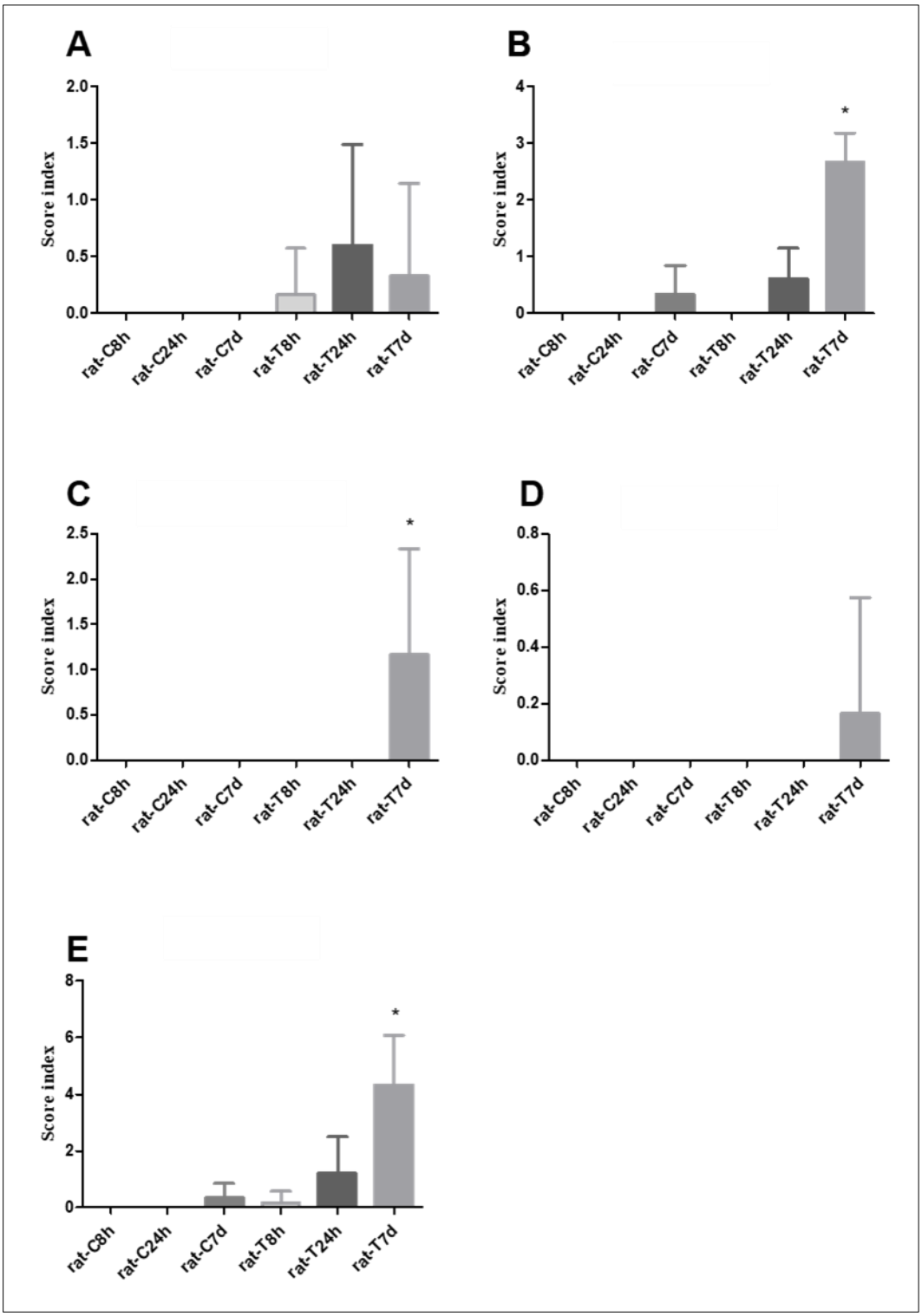

Figure 5 Severity of tissue lesions in different experimental groups. (A) Severity of hemorrhagic lesions; (B) Demyelination severity; (C) Neuronal loss or degeneration; (D) Fibrosis severity; (E) Comprehensive assessment scores. $^{*}p < 0.05$ indicated that the statistically significant differences between the test group and the control group at the same time point of the experiment

4. Discussion

SCI represents a significant challenge in the medical field due to its complex pathophysiology and the lack of effective treatment strategies. Despite ongoing advancements in SCI research, there remains a gap between basic research

findings and their clinical application, primarily attributed to the use of non-standardized animal models for SCI. These models often lead to research inaccuracies, limiting the translational value of basic research for clinical treatments. This study aims to address this gap by providing a standardized SCI rat model for researching SCI medicine targets and designing therapeutic strategies. The BBB scoring method was utilized and modified for rats undergoing free exploration in an open field. Locomotion was observed for four minutes, with each rat observed three times at three different time points post-surgery: 8 hours, 24 hours, and 7 days after the procedure [10-13].

In cases of accidents, such as car crashes or traumatic incidents, first responders should be vigilant for potential spinal cord injuries. Proper handling techniques and the use of assistive devices to stabilize the head, neck, and body are crucial to prevent further injury. Injured individuals should be promptly transported to a specialized spinal cord injury center or an experienced medical facility for treatment. Various physical and neurological examinations, along with imaging techniques such as X-rays, myelography, computed tomography scans, and magnetic resonance imaging, are utilized to assess the location and severity of the spinal cord injury and identify associated injuries, such as traumatic brain injury or limb fractures [14-16].

Surgical intervention may be necessary to realign dislocated vertebrae or maintain spinal stability. High-dose steroids are often administered in the acute phase to aid nerve recovery. The goals of surgery include protecting the spinal cord from further injury, realigning dislocated vertebrae promptly, and maintaining spinal stability. Surgical approaches may involve decompression or fusion considerations, including anterior or posterior spinal surgery, vertebral body resection, laminoplasty, intervertebral fusion surgery, and posterior lateral fusion surgery. Modern spinal surgery frequently employs metal internal fixation devices to provide immediate stability to the spine, improving surgical outcomes and simplifying postoperative care. Following surgery, protective measures such as cervical collars or standard braces are utilized based on the location of the injury and the strength of internal fixation. Typically, three months of protective immobilization is necessary to allow for initial fracture healing, bone fusion, and the restoration of sufficient spinal stability for resuming normal activities while protecting the spinal cord [17-19].

5. Conclusion

In this study, we have successfully established a SCI rat model. According to all results, According to the scoring results of the BBB scale, rats with spinal cord hemisection injury gradually regained motor function postoperatively, approaching the motor capabilities of the control group rats by the 7th day. There was a significant increase in the expression levels of IL-4 and TNF-α in the spinal cord of the test group at 7th days post-surgery compared to the control group. The histopathological interpretation results, spinal cord hemisection surgery did not readily induce intraspinal hemorrhage. However, at 7th days post-surgery, rats with spinal cord injury exhibited significant differences from the control group in terms of demyelination lesions and neuronal loss or degeneration. Therefore, the comprehensive assessment indicates that the lesion severity in the test group rats was significantly greater than that in the control group at 7th days post-surgery.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute inspected all animal experiments and this study comply with the guidelines of protocol IACUC 111092 approved by the IACUC ethics committee.

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