

Establishment and Functional Evaluation of a Rat Model of Spinal Cord Injury

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Abstract: *Objective:* To explore the modified Allen impactor method in establishing a rat model of spinal cord injury, and to preliminarily evaluate the motor function of the forelimbs and hindlimbs of rats. *Methods:* Thirty female SD rats with a body weight of 255 ± 21 g were randomly divided into two groups, namely the sham-operated group and the operated group, with 15 rats in each group. The spinal cord injury SD rat model was established by exposing but not injuring the spinal cord in the sham-operated group, while the SD rat model was established by the modified Allen impactor method in the operated group. The Basso–Beattie–Bresnahan (BBB) rating scale was used to assess the rats' hindlimb motor neurobehavior. A rat model of spinal cord injury was established by the modified Allen impactor method. After the cells were transplanted, the BBB score was used to evaluate the motor function; the changes in the motor function of rats with spinal cord injury were detected. *Results:* The motor function and sensory function of the forelimbs and hindlimbs of the rats showed significant changes after five days. The motor function of the forelimbs and hindlimbs of the rats in the sham-operated group were essentially normal after three days (about 20 points); the sensory function of the rats in the operated group decreased significantly after five days; however, in the sham-operated group, it decreased to 0. The motor function scores of the rats in the operated group at each point of time were significantly lower than those in the sham-operated group ($p < 0.05$), while the forelimb motor function scores were significantly higher than those in the sham-operated group ($p < 0.05$). *Conclusion:* The modified Allen impactor method that was used to establish a rat model of spinal cord injury in this study can significantly reduce the motor function of rats.

Keywords: Spinal cord injury model; Motor function; Sensory function; Modified Allen impactor

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1. Introduction

With the continuous progress and development of human economy and society, the incidence of spinal cord injury is on the rise year by year, easily causing tactile movement disorders of limbs, visceral dysfunctions, and high mortality, morbidity, as well as treatment costs. The cost has caused a certain economic burden to the patients, their families, and the society^[1]. According to the statistics of the World Health Organization, there are about 250,000 to 500,000 spinal cord injury patients around the world every year, and the incidence is still increasing year by year. At present, most researchers are devoted to the study of the pathogenesis, pathology, and treatment of spinal cord injury^[3]. Based on this research, a rat model of spinal cord injury with simple operation, standardization, stability, and reproducibility was established^[4]. The methods of establishing spinal cord injury animal models mainly include blow injury, chemical drug injection, compression, and other methods to establish the former at different degrees^[5,6]. In this study, the

modified Allen method was established to prepare a spinal cord injury model, and two methods were used to preliminarily evaluate the occurrence, development, and change of spinal cord injury in rats; relevant data were obtained to provide new ideas for clinical treatment of patients with spinal cord injury [7].

2. Materials and methods

2.1. Experimental animals

Female Sprague Dawley (SD) rats, with body weight of 255 ± 21 g, were obtained from Chengdu Dashuo Laboratory Animal Co., Ltd.

2.2. Establishment of spinal cord injury model

RIP3, MLKL overexpression, and low expression lentiviral vectors were prepared, and the virus was packaged and infused into the cerebrospinal fluid of female adult SD rats; the rats were randomly divided into a sham-operated group and an operated group [8].

The spinal cord injury model was established by the modified Allen method [9] as follows: anesthetize with 10% chloral hydrate (3 mL/kg) intraperitoneally, fix it on the animal laboratory table, cut the back hair, and routinely sterilize the towel; taking the thoracic vertebra T10 as the center, make a median incision of about 4 cm layer by layer, and peel off the muscles on both sides of the lamina to fully expose the T9-T11 lamina and spinous processes; carefully remove the T9 and T10 lamina, and fully expose the spinal cord at the T10 segment (about 1.0 cm \times 0.6 cm); place a thin gelatin pad on the spinal cord, and drop a 10 g beating stick vertically and naturally from 10 cm away to injure the spinal cord at the area below the gelatin pad; the injury energy should be about 100 gcf (gram cm force); clean, stop the bleeding, suture, and inject 25,000 U/(kg·d) of penicillin intramuscularly for three days to prevent infection [10]. The sham-operated group was only exposed without injury to the spinal cord. In the operated group, the lower limbs fluttered with retraction, and the tail appeared to have spasmodic swing. Behavioral scores were performed at different points of time after modeling.

2.3. Rat hindlimb motor behavior score

The motor function scoring method (Basso, Beattie, and Bresnahan, BBB) was used to observe the rats' hindlimb motor behavior. The BBB rating scale is a 21 point-motor function scale of the hindlimb, studying trunk position and stability, gait, coordination, sex, paw placement, tail position, and other features. It indicates the motor function recovery process of hindlimbs in rats after spinal cord injury. The rats in each group were scored at 1d, 3d, 5d, 7d, 14d, and 28d after the procedure, and the observation time for the scores was 5 minutes [11,12].

The peripheral wall and bottom of the open-field experimental box were black, and the bottom was divided into four quadrants with equal areas. A camera was fixed just above the intersection of the diagonal lines to capture the moving path of the rat, and the data collected were transmitted to the computer for recording and processing. Ten rats were randomly selected from the two groups, and the experimental observation was carried out in an independent, quiet room. Behavioral observation was conducted from 9.00 a.m. to 10.00 a.m. At the beginning of the experiment, each rat was placed in the center of the open field. The camera on the top of the experimental box began to track and record the rat's activity and movement trajectory as soon as it was released. Each rat's activity duration, total distance travelled, and number of upright stands (two front paws were more than 1 cm off the ground or climbing) were all recorded during the 5-minute period. The rats were allowed to roam freely while being scored for domains such as hindlimb movements, posture, tail height, and other factors, in which the average values were taken [13,14].

2.4. Anterior limb placement experiment

The limbs were exposed to three independent stimuli (visual, tactile, and proprioceptive) to assess the integrity of their motor senses. The experiments included visual sub-experiment (frontal and lateral stimuli), tactile sub-experiment, and proprioceptive sub-experiment. The total score of the forelimb placement test ranged from 0 to 10, in which the more severe the functional impairment, the higher the score. The rats in each group were scored on 1d, 3d, 5d, 7d, 14d, and 28d after the procedure, and the observation time was 5 minutes. In order to conduct the forelimb placement experiments on the rats, four investigators were divided into two groups, and double-blind method was used. The average values were then obtained [15-17].

3. Results

3.1. Comparison of BBB scores of hindlimb motor function in rats

It can be seen from **Table 1** that the BBB scores of the hindlimb motor function of the two groups of rats were compared at each point of time (1d, 3d, 5d, 7d, 14d, and 28d). The BBB score of the sham-operated group was significantly higher than that of the operated group ($p < 0.05$); comparing the BBB score of the sham-operated group at different points of time, the BBB score on 1d was significantly lower than that on 3d ($p < 0.05$), while the BBB score on 5d, 7d, 14d, and 28d increased sequentially; the difference between groups was still statistically significant ($p > 0.05$). From the repeated measures analysis of variance, there was a temporal trend in the motor function scores of the hindlimbs of rats at different points of time, and the rate at which the scores change in the operated group was significantly different from that in the sham-operated group ($p < 0.05$) (**Table 2**).

Table 1. BBB score of rats' hindlimb motor function ($\bar{x} \pm s$, $n = 30$)

Postoperative time	Sham-operated group	Operated group
1d	18.85 \pm 0.75*	0.21 \pm 0.35#
3d	19.23 \pm 0.71	0.56 \pm 0.51#
5d	20.53 \pm 0.36	1.23 \pm 0.47#
7d	20.15 \pm 0.75	3.58 \pm 0.72#
14d	20.34 \pm 0.25	7.45 \pm 0.64#
28d	20.48 \pm 0.38	8.20 \pm 0.46#

Note: * indicates the comparison of BBB scores at different points of time in the same group; based on the results of pairwise comparison of Student–Newman–Keuls (SNK) method, the score on 1d is lower than that of 5d, 7d, 14d, and 28d; # represents the comparison with the sham-operated group at the same point of time ($p < 0.05$)

Table 2. Repeated measures analysis of variance for the BBB scores of hindlimb motor function in rats at different points of time

Source of variation	Variance	Type III Sum of squares	Mean square	F value	p value
Group	1	14.49	14.49	6.92	< .0001
Individual error	14	105.56	7.54		
Time	5	240.62	48.12	17.31	< .0001*
Time*Group	5	78.65	15.73	5.66	< .0001*
Time error	65	180.71	2.78		

Note: * The testing for spherical symmetry shows $p < .0001$; hence, the p value after correction for degrees of freedom is used

3.2. Comparison of sensory function scores in rats

It can be seen from **Table 3** that the sensory function score of rats in the sham-operated group on 1d was significantly higher than that on 3d ($p < 0.05$), and the sensory function scores of the rats in the sham-operated group on 5d, 7d, 14d, and 28d were not significantly different ($p > 0.05$); the sensory function scores of rats in the sham-operated group on 1d, 3d, 5d, 7d, 14d, and 28d were significantly lower than those in the operated group ($p < 0.05$). Repeated measures analysis of variance showed that there was a time trend in the sensory function scores of rats at different points of time, and the rate at which the scores change in the operated group was significantly different from that in the sham-operated group ($p < 0.05$) (**Table 4**).

Table 3. Sensory function scores of rats ($\bar{x} \pm s$, $n = 30$)

Postoperative time	Sham-operated group	Operated group
1d	1.05 ± 0.35*	9.61 ± 0.38 [#]
3d	0.33 ± 0.51	9.41 ± 0.38 [#]
5d	0	8.32 ± 0.63 [#]
7d	0	6.88 ± 0.62 [#]
14d	0	4.75 ± 0.47 [#]
28d	0	2.65 ± 0.63 [#]

Note: * indicates the comparison of sensory function scores at different points of time in the same group; based on the results of pairwise comparison of SNK method, the score on 1d was higher than that on 3d, 5d, 7d, 14d, and 28d; [#] represents the comparison with the sham-operated group at the same point of time ($p < 0.05$)

Table 4. Repeated measures analysis of variance for sensory function scores in rats at different points of time

Source of variation	Variance	Type III Sum of squares	Mean square	F value	P value
Group	1	33.83	33.83	6.23	<.0001
Individual error	14	76.02	5.43		
Time	5	204.65	40.93	12.44	<.0001*
Time*group	5	117.45	23.49	7.14	<.0001*
Time error	65	213.85	3.29		

4. Discussion

None of the experimental rats in this study died. As shown in **Table 1**, the rats in the non-spinal cord injury group had good mental state and voluntary activity, normal diet, bowel movements, and hindlimb motor function, as well as a high BBB score of about 20. The rats in the operated group had several symptoms, including hindlimb weakness, gait disorder, and different heights of upturned tail ^[18]. The scores of the operated group changed significantly on 5d, 7d, and 14d, but the change was less noticeable after 2 weeks ^[19].

The rats in the two groups were scored in the forelimb placement test at the same point of time; the operated group scored significantly higher than the sham-operated group ($p < 0.05$). As shown in **Table 2**, the vision, touch, and proprioception of the sham-operated group were essentially normal, and the scores tended to 0, which indicated that the forelimb movement was normal ^[20]. In the operated group, the forelimb placement response was delayed for more than 2 seconds. Significant changes occurred after 3d, 5d, 7d, and 14d, and the motor function of the forelimbs recovered steadily.

At present, the four main methods for preparing SCI animal models, commonly used in animal experimental research at home and abroad, include impact injury model, compression injury model, cutting or suction experimental model, and traction SCI model [21]. In this study, the BBB score of all rats before the experiment was 21 points, but the BBB score of the rats in the spinal cord injury group was 0.21 points after modeling. A rat model of spinal cord injury can be replicated by employing a spinal cord impactor [22]. The findings revealed that the motor function of the forelimbs and hindlimbs of the rats in the sham-operated group was marginally diminished in the first three days. However, it was essentially close to normal, indicating that the reduction may be related to the pain caused by surgical trauma or other adverse stimuli [23]; on the other hand, the motor function of the operated group was significantly lower ($p < 0.05$), indicating that the spinal cord injury had a significant impact on the motor function of the hindlimbs and forelimbs of the rats; without treatment, the function of the hindlimbs of the rats in the operated group had different degrees of recovery over time, which may be related to the regression of local inflammation in the injured spinal cord, the functional reorganization of the tissue structure, the sprouting of the injured axon stump or residual axon collaterals, the restart of the latent pathway, the synapse, and the changes in efficiency [24].

5. Conclusion

The self-made modified Allen impactor can successfully replicate a rat model of spinal cord injury, which is stable, reliable, and easy-to-operate.

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Disclosure statement

The authors declare no conflict of interest.

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