Int J Clin Exp Med 2019;12(11):12693-12701
www.ijcem.com /ISSN:1940-5901/IJCEM0099262

Original Article

The effects of interferon-β on neuronal apoptosis and the expressions of BDNF and TrkB in rats with spinal cord injuries

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Received July 5, 2019; Accepted October 3, 2019; Epub November 15, 2019; Published November 30, 2019

Abstract: Objective: This study aimed to explore the effects of interferon (IFN)-β on neuronal apoptosis, and on brain-derived neurotrophic factor (BDNF), and tropomyosin receptor kinase B (TrkB) expressions in rats with spinal cord injuries. Methods: 40 adult female SD rats were equally and randomly divided into a blank group, a model group, a sham operation group, and an IFN-β group. The spinal cord injury rat model was established in the model and IFN-β groups. Only laminectomies were performed without spinal cord injury in the sham operation group. The rats in the control group were fed routinely. The Basso-Beattie-Bresnahan (BBB) score and the modified Tarlov score were used to evaluate the motor function of each group on the 1st, 3rd, and 7th days after the modeling. The Zea-Longa five-grade score was used to evaluate the rats' nerve functions. The expressions of the BDNF and TrkB proteins and the apoptosis-related proteins Bax and Bcl-2 in the rat brains were measured on the 7th day after the modeling. The TUNEL method was used to detect the neuronal apoptosis in each group, and the correlations between the expressions of the BDNF and TrkB proteins and the neuronal apoptosis rate were analyzed. Results: The BBB score, the modified Tarlov score, and the Zea-Longa score in the blank and sham operation groups were higher than the scores in the model and IFN-β groups ($P < 0.05$). The blank and the sham operation groups showed higher BDNF, TrkB, and Bcl2 proteins and lower Bax proteins than the model and IFN-β groups ($P < 0.05$). The IFN-β group exhibited higher BDNF, TrkB, and Bcl2 proteins and lower Bax proteins than the model group ($P < 0.05$). The expressions of the BDNF and TrkB proteins were negatively correlated with the neuronal apoptosis rate ($r = -0.667$, $P < 0.05$; $r = -0.791$, $P < 0.05$). Conclusion: The motor and nerve functions were significantly improved, the expressions of the BDNF and TrkB proteins were up-regulated, and the neuronal apoptosis was inhibited by IFN-β in rats with acute spinal cord injury.

Keywords: Interferon-β, spinal cord injury, neurons, BDNF, TrkB

Introduction

Acute spinal cord injury, a serious central nervous system injury, can lead to motor and sensory dysfunction below the spinal cord injury segment and has a higher disability rate [1, 2]. In recent years, with the development of society and the rapid growth of the transportation industry, the incidence of acute spinal cord injury is also increasing, and it has brought a very heavy burden to families and society [3]. For a long time, the repair of spinal cord injuries has been one of the key issues in medical research. Most previous studies believed that the mechanism of spinal cord injury was secondary necrosis due to ischemia and cellular hypoxia [4, 5]. However, with the sustained development of molecular biology and immunology in recent years, it has gradually become recognized that neuronal apoptosis exerts an important role in secondary spinal cord injury [6]. Therefore, it is now understood that neuronal apoptosis is the main cause of secondary spinal cord injury, so the inhibition of neuronal apoptosis is the key to preventing secondary spinal cord injury [7].

Interferon (IFN)-β is an immunoregulatory factor with an anti-inflammatory regulation function. The role of IFN-β in multiple sclerosis (MS) and antiviral therapy is well known [8]. MS is a disease characterized by demyelination in the white matter of the central nervous system. The key to MS treatment is immune regulation, and

interferon has a more obvious effect [9]. In addition, some studies [10] have indicated that IFN-β could inhibit cell apoptosis under certain conditions. At present, there are few studies on IFN-β as treatment for acute spinal cord injury. However, some studies [11] indicated that IFN-β has a great potential for the treatment of acute spinal cord injury. Therefore, it has been speculated in earlier studies that the immune intervention of IFN-β on the central nervous system could inhibit neuronal apoptosis in acute spinal cord injury.

A spinal cord injury rat model was constructed to explore the effects of IFN-β on neuronal apoptosis in acute spinal cord injury and the expressions of brain-derived neurotrophic factor (BDNF) and tyrosine protein kinase B (TrkB) in spinal cord injury segments, and the therapeutic effect and mechanism of IFN-β on acute spinal cord injury. This study aimed to provide more possible treatment options for acute spinal cord injury.

Materials and methods

Laboratory animals and materials

40 adult female SD rats aged 8 weeks old were selected. The body weight of the rats was (173.55 ± 7.34) g. All the rats were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd., with production license SCXK (Shanghai) 2012-0002.

The rats were fed at a temperature of 20-25°C and a relative humidity of 40%-75%. The rats showed normal circadian rhythm alternation, and were free to eat and drank. Every procedure was approved by the Animal Care and Use Committee of the Guizhou Province Osteological Hospital.

Modeling and grouping

The rats were randomly divided into a blank group (N = 10), a model group (N = 10), a sham operation group (N = 10), and an IFN-β group (N = 10). The rats in the blank group were fed routinely without any treatment. The rats in the model, sham operation, and IFN-β groups were anesthetized with an intraperitoneal injection of 10% chloral hydrate at a dose of 0.4 mL/100 g. After being anesthetized, the rats were sterilized in a supine position. The rats' skin was cut along the midline of the T9-T10 thoracic segment, and their fascia was gradually separated. The spinous process and vertebral plate were exposed through muscle stripping. The follow-up surgery was not carried out on the rats in the sham operation group after this step. A 20 g weight was freely dropped from a height of 5 cm using an Allen device. The T9-T10 thoracic segment was covered with a 3 mm × 6 mm arc metal sheet in the model and IFN-β groups and was struck by the freefalling weight. Successful modeling was marked by a spastic swing, a retractable flutter of both lower limbs and trunk, and the flaccid paralysis of both hind limbs. Then the incision was stitched. The rats in the IFN-β group were injected with IFN-β of $1*10^7$ IU via the tail vein immediately after successful modeling, and IFN-β of $0.5*10^7$ IU was injected into the tail vein again 4 hours after the modeling. The drug intervention was not carried out in the blank, sham operation, or model groups.

Outcome measures

(1) The Basso-Beattie-Bresnahan (BBB) score [12] (0-21 points; 0 point as complete paralysis; 21 points as perfectly normal) and the modified Tarlov score [13] (0-5 points; 0 points as hind limbs without active motion; 5 points as normal walking) were used to evaluate the motor functions of the rats on the 1st, 3rd, and 7th days after the modeling. (2) The Zea-Longa five-grade score [14] was used to evaluate the nerve functions of the rats on the 1st, 3rd, and 7th days after the modeling. Higher scores represent worse nerve functions. (3) Western blot was used to determine the expressions of the BDNF protein, the TrkB protein, and the apoptosis-related proteins Bax and Bcl-2 in the rat brains on the 7th day after the modeling. (4) The TUNEL method was used to measure the neuronal apoptosis in each group on the 7th day after the modeling. (5) The correlations between the expressions of the BDNF and TrkB proteins and the neuronal apoptosis rate were analyzed.

The detection of the BDNF protein, the TrkB protein, and the apoptosis-related proteins Bax and Bcl-2 using Western blot

After the function tests, the rats were anesthetized (10% chloral hydrate), and then they were sacrificed by breaking their cervical vertebra. The blood was emptied. Then the spinal cord tissue of the injured segment was taken and prepared into a tissue homogenate. Then a RIPA tissue lysate was added for a tissue lysis.

The tissue was mixed on ice and centrifuged at 1300 r/min for 10 min. The protein was separated by 10% SDS-PAGE and then transferred onto a PVDF After being stored o membrane. 5% skim milk was added, and then the samples were stored overnight at 4°C. vernight, the primary antibodies BDNF (1:1000), TrkB (1:1000), Bax (1:1000), Bcl2 (1:1000) and β-actin were added to incubate overnight at 4°C. Then a goat anti-rabbit antibody labeled with HRP (secondary antibody, 1:1000) was added to incubate at room temperature for 1 hour. The protein was rinsed with a PBS solution and colored with an ECL developer. The result was expressed by the gray value of the target band.

Detection of spinal neuronal apoptosis by TUNEL

First, the spinal cord tissue was paraffin-embedded, sectioned, dewaxed, and dewatered. Then 20 ug/ml protease K was added. The tissue protein was digested for 20 min at 37°C and rinsed with distilled water four times. After the rinsing, PBS containing 2% hydrogen peroxide was added to the protein and it was incubated at room temperature for 5 min. The protein was then rinsed with PBS. After it was rinsed, two drops of TdT enzyme buffer were added to the sections, and the reaction was performed at room temperature for 5 min. Then 50 ul of TUNEL reaction solution was added to and the sections were incubated at 37°C for 30 min. After the sections were washed, the reaction was terminated by adding 0.2 ml of termination solution. Then the protein was stained with hematoxylin again. Finally, the protein was stained with DAB developer, and observed and counted under a lighted microscope. Apoptosis rate (%) = Number of apoptotic cells/Number of total cells × 100%.

Statistical analysis

SPSS 19.0 software (Biz Insight (Beijing) Information Technology Co., Ltd.) was used in this study. The measurement data were expressed as the mean ± standard deviation. One-way ANOVA was used for comparisons among groups, and an LSD/t test was used for comparisons between two groups. Repeated measurement analyses of variance were performed for a multipoint data comparison, and the Pearson method was used for the correlation analysis. $P < 0.05$ indicates that there was a significant statistical difference.

Results

Comparisons of the BBB scores at different time points among the four groups

The BBB scores in blank group were 21 points, 21 points and 21 points on the 1st, 3rd, and 7th days after the modeling. The BBB scores in the sham operation group were 21 points, 21 points and 21 points on the 1st, 3rd, and 7th days after the modeling. The BBB scores in the model group were (0.11 ± 0.02) points, (1.13 ± 0.26) points, and (3.05 ± 0.57) points on the 1st, 3rd, and 7th day after the modeling. The BBB scores in the IFN-β group were (0.12 ± 0.02) points, (2.13 ± 0.65) points, and (4.69 ± 1.01) points on the 1st, 3rd, and 7th days after the modeling. The BBB scores of blank and sham operation groups were higher than those of the model and IFN-β groups on the 1st, 3rd, and 7th days after the modeling ($P < 0.05$). The BBB scores of the model and the IFN-β groups were increased gradually on the 1st, 3rd, and 7th days after the modeling. There was no obvious difference between the model and IFN-β groups on the 1st day after the modeling ($P > 0.05$). The BBB score of IFN-β group was higher than the score of model group on the 3rd and 7th days after the modeling ($P < 0.05$) (**Figure 1**).

A comparison of the modified Tarlov scores at different time points among the four groups

The modified Tarlov scores in the blank group were 5 points, 5 points, and 5 points on the 1st, 3rd, and 7th days after the modeling. The modified Tarlov scores in the sham operation group were 5 points, 5 points, and 5 points on the 1st, 3rd, and 7th days after the modeling. The modified Tarlov scores in the model group were (0.09 ± 0.01) points, (0.18 ± 0.03) points, and (0.71 ± 0.13) points on the 1st, 3rd and 7th days after the modeling. The modified Tarlov scores in the IFN-β group were (0.10 ± 0.01) points, (0.47 ± 0.08) points, and (1.49 ± 0.14) points on the 1st, 3rd, and 7th days after the modeling. The modified Tarlov scores of the blank and sham operation groups were higher than those of the model group and the IFN-β group on the 1st, 3rd, and 7th days after the modeling ($P < 0.05$). The modified Tarlov scores of the model and IFN-β groups were increased gradually on the 1st, 3rd, and 7th days after the modeling. There was no clear difference between the model and IFN-β groups on the 1st

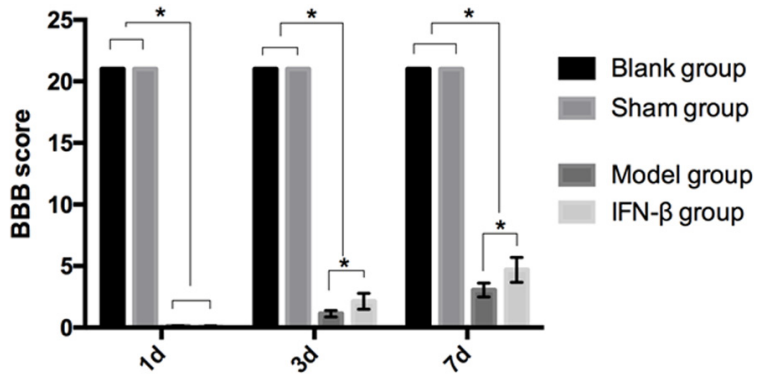

Figure 1. Comparison of the BBB scores at different time points among the four groups. The BBB scores of the blank and sham operation groups were higher than those of the model and IFN-β groups on the 1st, 3rd, and 7th days after the modeling ($P < 0.05$). The BBB scores of the model and IFN-β groups were increased gradually on the 1st, 3rd, and 7th days after the modeling. There was no observable difference between the model and IFN-β groups on the 1st day after the modeling ($P > 0.05$). The BBB score of the IFN-β group was higher than the score of the model group on the 3rd and 7th days after the modeling ($P < 0.05$). *$P < 0.05$.

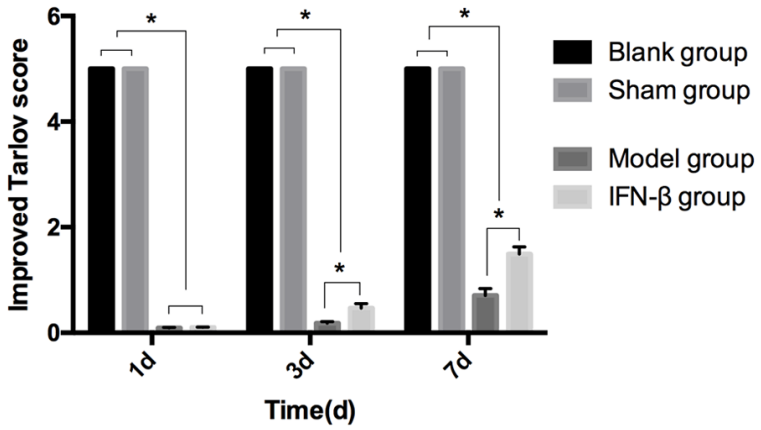

Figure 2. Comparison of the modified Tarlov scores at different time points among the four groups. The Modified Tarlov scores of the blank and sham operation groups were higher than those of the model and IFN-β groups on the 1st, 3rd, and 7th days after the modeling ($P < 0.05$). The modified Tarlov scores of the model and IFN-β groups were increased gradually on the 1st, 3rd, and 7th days after the modeling. There was no observable difference between the model and IFN-β groups on the 1st day after the modeling ($P > 0.05$). The modified Tarlov score of the IFN-β group was higher than the score of the model group on the 3rd and 7th days after the modeling ($P < 0.05$). Note: *$P < 0.05$.

day after the modeling ($P > 0.05$). The modified Tarlov score of the IFN-β group was higher than the model group score on the 3rd and 7th days after the modeling ($P < 0.05$) (**Figure 2**).

Comparisons of the Zea-Longa scores at different time points among the four groups

The Zea-Longa scores in the blank group were (0.17 ± 0.03) points, (0.18 ± 0.02) points, and (0.18 ± 0.03) points on the 1st, 3rd and 7th days after the modeling. The Zea-Longa scores in the sham operation group were (0.17 ± 0.03) points, (0.18 ± 0.02) points, and (0.19 ± 0.03) points on the 1st, 3rd, and 7th days after the modeling. The Zea-Longa scores in the model group were (3.47 ± 0.58) points, (2.99 ± 0.52) points, and (1.87 ± 0.31) points on the 1st, 3rd, and 7th days after the modeling. The Zea-Longa scores in the IFN-β group were (3.34 ± 0.51) points, (2.08 ± 0.21) points, and (0.96 ± 0.15) points on the 1st, 3rd, and 7th days after the modeling. The Zea-Longa scores of the blank and sham operation groups were higher than those of the model and IFN-β groups on the 1st, 3rd, and 7th days after the modeling ($P < 0.05$). The Zea-Longa scores of the model and IFN-β groups were decreased gradually on the 1st, 3rd, and 7th days after the modeling. There was no observable difference between the model and IFN-β groups on the 1st day after the modeling ($P > 0.05$). The Zea-Longa score of the IFN-β group was lower than the score of the model group on the 3rd and 7th days after the modeling ($P < 0.05$) (**Figure 3**).

The expressions of the BDNF and TrkB proteins in the four groups after 7 days of modeling

The expressions of BDNF and TrkB in the blank group were (1.03 ± 0.07) and (1.07 ± 0.09) after 7 days of modeling, respectively. The expressions of BDNF and TrkB in the sham operation group were (0.98 ± 0.08) and (1.01 ± 0.07) after 7 days of modeling, respectively. The expressions of BDNF and TrkB in the model group were (0.19 ± 0.03) and (0.20 ± 0.04) after 7 days of modeling, respectively. The expressions of BDNF and TrkB in the IFN-β group were (0.57

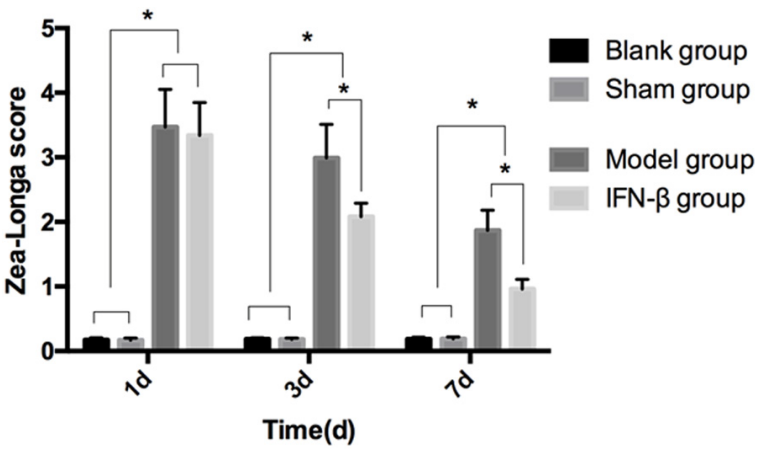

Figure 3. Comparisons of the Zea-Longa scores at different time points among the four groups. The Zea-Longa scores of the blank and sham operation groups were higher than those of the model and IFN-β groups on the 1st, 3rd and 7th days after the modeling ($P < 0.05$). The Zea-Longa scores of the model and IFN-β groups were decreased gradually on the 1st, 3rd, and 7th days after the modeling. There was no obvious difference between the model and IFN-β groups on the 1st day after the modeling ($P > 0.05$). The Zea-Longa score of the IFN-β group was lower than the score of the model group on the 3rd and 7th days after the modeling ($P < 0.05$). *$P < 0.05$.

± 0.09) and (0.62 ± 0.10) after 7 days of modeling, respectively. There were no significant differences in the expressions of BDNF and TrkB between the blank and sham operation groups ($P > 0.05$). The expressions of the BDNF and TrkB proteins in the blank sham operation groups were higher than those in the model and IFN-β groups ($P < 0.05$), and the expressions of the BDNF and TrkB proteins in the IFN-β group were higher than those in the model group ($P < 0.05$) (**Table 1** and **Figure 4**).

The expressions of the Bax and Bcl2 proteins in the four groups after 7 days of modeling

The expressions of Bax and Bcl2 in the blank group were (0.33 ± 0.05) and (1.03 ± 0.11) after 7 days of modeling, respectively. The expressions of Bax and Bcl2 in the sham operation group were (0.34 ± 0.04) and (1.02 ± 0.09) after 7 days of modeling, respectively. The expressions of Bax and Bcl2 in the model group were (0.52 ± 0.13) and (0.43 ± 0.09) after 7 days of modeling, respectively. The expressions of Bax and Bcl2 in the IFN-β group were (0.46 ± 0.09) and (0.77 ± 0.10) after 7 days of modeling, respectively. There were no significant differences in the expressions of Bax and Bcl2 between the blank and sham operation groups ($P > 0.05$). The expression of the Bax protein in the blank and sham operation groups was lower than the expression in the model and IFN-β groups, and the expression of the Bcl2 protein in the blank and sham operation groups was higher than the expression in the model and IFN-β groups ($P < 0.05$). The expression of the Bax protein in the IFN-β group was lower its was in the model group, and the expression of the Bcl2 protein in the IFN-β group was higher than it was in model group ($P < 0.05$) (**Table 2** and **Figure 5**).

Comparison of the neuronal apoptosis rate in the four groups

The neuronal apoptosis rates in the blank, sham operation, model and IFN-β groups were (0.12 ± 0.01)%, (0.11 ± 0.01)%, (21.34 ± 1.27)%, and (11.46 ± 1.05)% respectively after 7 days of modeling. There was no remarkable difference in the neuronal apoptosis rates between the blank and sham operation groups ($P > 0.05$). The neuronal apoptosis rate in the blank and sham operation groups was lower than it was in the model and IFN-β groups, and the neuronal apoptosis rate in the IFN-β group was lower than it was in the model group ($P < 0.05$) (**Figure 6**).

The correlation analysis between the expressions of the BDNF and TrkB proteins and the neuronal apoptosis rate

The expressions of the BDNF and TrkB proteins were negatively correlated with the neuronal apoptosis rate ($r = -0.667$, $P < 0.05$; $r = -0.791$, $P < 0.05$) (**Figures 7** and **8**).

Discussion

IFN-β is a cytokine with and immunoregulatory effect and can reduce cell apoptosis under certain conditions. It has been applied in MS [15, 16]. Some studies [17-19] have shown that although there are many mechanisms for secondary injury in acute spinal cord injury, the immune system activation exerts a key role. The inflammatory reactions and a series of lipid peroxidation reactions are caused by an aggregation of inflammatory cells after the injury,

Table 1. The expressions of the BDNF and TrkB proteins in the four groups after 7 days of modeling

Protein	Blank group n = 10	Sham group n = 10	Model group n = 10	IFN-β group n = 10	F	P
BDNF	1.03 ± 0.07*	0.98 ± 0.08*	0.19 ± 0.03	0.57 ± 0.09#	304.8	< 0.001
TrkB	1.07 ± 0.09*	1.01 ± 0.07*	0.20 ± 0.04	0.62 ± 0.10#	263.9	< 0.001

*, # indicates compared with the Model group; P < 0.05.

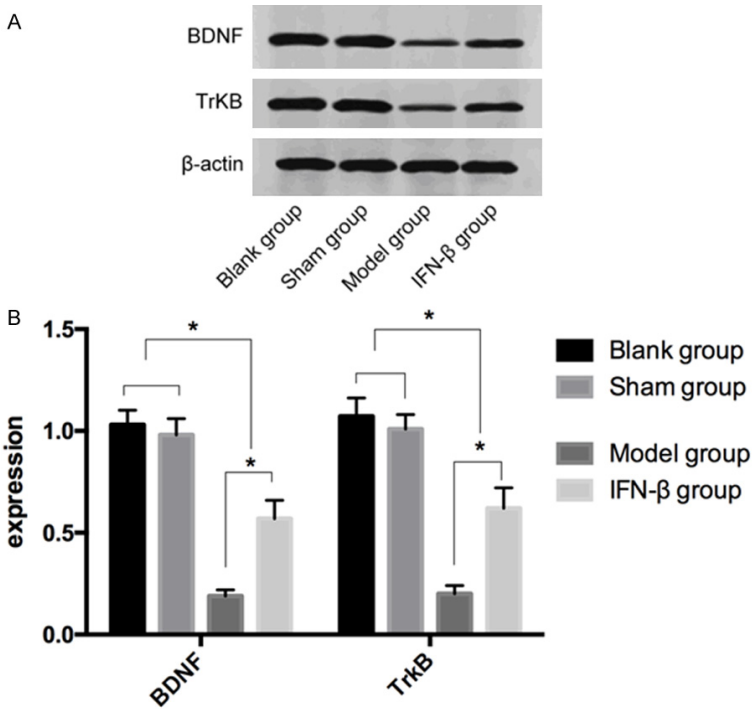

Figure 4. The expressions of the BDNF and TrkB proteins in the four groups after 7 days of modeling. There was no significant difference in the expressions of BDNF and TrkB between the blank and sham operation groups (P > 0.05). The expressions of the BDNF and TrkB proteins in the blank and sham operation groups were higher than those in the model and IFN-β groups (P < 0.05), and the expressions of the BDNF and TrkB proteins in the IFN-β group were higher than those in the model group (P < 0.05).

which ultimately leads to neuron injury. Therefore, it is speculated that IFN-β might have a certain effect on acute spinal cord injury. In our study, IFN-β intervention was performed on rats with acute spinal cord injuries. It was found that the BBB score, the modified Tarlov score, and the Zea-Longa scores of the blank and sham operation groups were higher than those of the model and IFN-β groups, and the scores of each item in the IFN-β group were markedly improved compared with those in the model group on the 3rd and 7th days after the modeling. At the same time, it was found that the scores of each item in the model group was gradually improved without drug intervention, suggesting that IFN-β intervention can effectively promote the recovery of motor and nerve functions in rats with acute spinal cord injury. Previous studies [20] have revealed that even if spinal cord injury is not treated, the nerve function could partially recover by itself, which is the reason why the scores of the model group were improved in our experiment. As an important neurotrophic factor in the spinal cord, BDNF plays a very important role in the growth, differentiation, and normal physiological function of the maintenance of neurons, and it can also resist the stimulation of spinal cord by injury, thus playing a protective role on neurons [21, 22]. TrkB, as a receptor of BDNF, can bind to BDNF to stimulate the transduction of various signaling pathways and promote the recovery of nerve function [23]. The BDNF and TrkB proteins of the spinal cord tissues in each group were also detected. The results suggest that the expressions of the BDNF and TrkB proteins in the blank and sham operation groups were higher than those in the model and IFN-β groups, and the expressions of the BDNF and TrkB proteins in the IFN-β group were higher than those in the model group, indicating that the expressions of the BDNF and TrkB proteins in spinal cord tissue could be down-regulated in rats with acute spinal cord injury. It was suggested that IFN-β could promote the recovery of the BDNF and TrkB protein expressions in rats with acute spinal cord injury, thereby promoting the recovery of nerve function. It has been reported that the expression of the BDNF protein in the injured spinal cord was down-regulated [23]. As the spinal cord has a partial self-recovery function, the expression of

Table 2. The expression of the Bax and Bcl2 proteins in the four groups after 7 days of modeling

Protein	Blank group n = 10	Sham group n = 10	Model group n = 10	IFN-β group n = 10	F	P
bax	0.33 ± 0.05*	0.34 ± 0.04*	0.62 ± 0.13	0.46 ± 0.09#	25.14	< 0.001
bcl2	1.03 ± 0.11*	1.02 ± 0.09*	0.43 ± 0.09	0.77 ± 0.10#	83.02	< 0.001

*, # indicates compared with the Model group; P < 0.05.

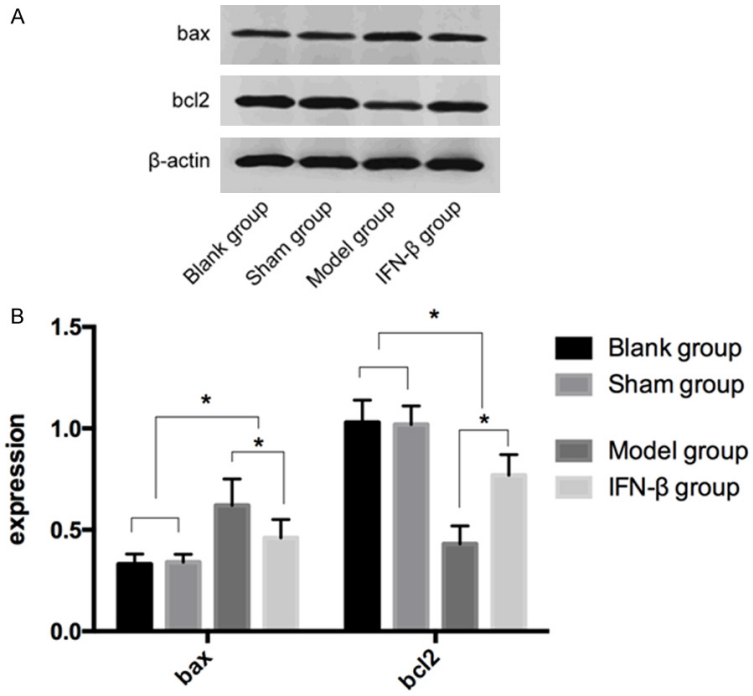

Figure 5. The Expressions of the Bax and Bcl2 proteins in the four groups after 7 days of modeling. There were no significant differences in the expressions of Bax and Bcl2 between the blank and sham operation groups (P > 0.05). The expression of the Bax protein in the blank and sham operation groups was lower than it was in the model and IFN-β groups, and the expression of the Bcl2 protein in the blank and sham operation groups was higher than it was in the model and IFN-β groups (P < 0.05). The expression of the Bax protein in the IFN-β group was lower than it was in the model group, and the expression of the Bcl2 protein in the IFN-β group was higher than it was in the model group (P < 0.05). Note: *indicates P < 0.05.

the BDNF protein would be increased to promote the partial recovery of nerve function. However, due to the problem of sample size, it is impossible to monitor the expression of the BDNF protein at multiple time points, which will be further improved on in subsequent experiments.

Some studies [24] have shown that cell apoptosis is an important part of secondary spinal cord injury. The death of nerve cells after spinal cord injury is not caused by external forces, but by neuronal apoptosis. Therefore, the neuronal apoptosis rate and the apoptosis-related proteins in the injured spinal cord were also detected. The results suggest that the expression of the Bax protein and the neuronal apoptosis rate in the blank and sham operation groups were lower than those in the model and IFN-β groups, and the expression of the Bcl2 protein in the blank and sham operation groups was higher than it was in the model and IFN-β groups. The expression of the Bax protein and the neuronal apoptosis rate in the IFN-β group were lower than they were in the model group, and the expression of Bcl2 protein in the IFN-β group was higher than it was in the model group. It was suggested that IFN-β can inhibit neuronal apoptosis by regulating the apoptotic protein Bax and the anti-apoptotic protein Bcl2. Previous studies [25] have shown that IFN-β can inhibit neuronal apoptosis in rat hippocampal cultures to some extent, which could confirm our conclusion from another angle. However, there are relatively few studies about the role of IFN-β on acute spinal cord injury. Therefore, more experiments are needed to confirm our conclusions.

In conclusion, the motor and nerve functions are significantly improved, the expressions of the BDNF and TrkB proteins are up-regulated, and neuronal apoptosis is inhibited by IFN-β in rats with acute spinal cord injury. However, there are some deficiencies in this study. First, due to the problem of sample size, a dynamic monitoring of the expressions of the BDNF and TrkB proteins in rat spinal cord tissues was not carried out. Furthermore, the mechanism of

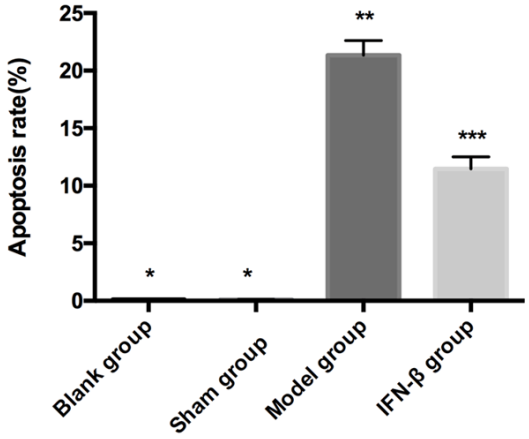

Figure 6. Comparison of the neuronal apoptosis rates in the four groups. The neuronal apoptosis rate in the blank *and sham operation groups was lower than it* was in the model and IFN-β groups, but the neuronal apoptosis rate in the IFN-β group was lower than it was in the model group ($P < 0.05$). Note: *Compared with ** and ***$P < 0.05$. **Compared with ***$P < 0.05$.

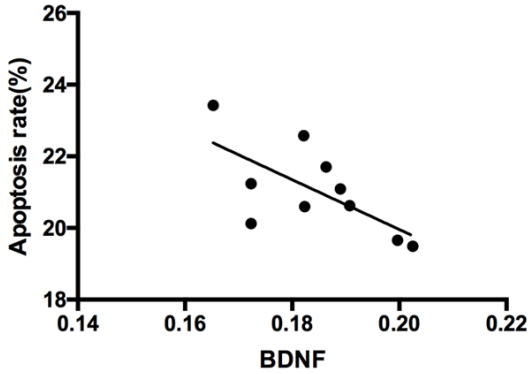

Figure 7. Correlation analysis between the expression of the BDNF protein and the neuronal apoptosis rate. The expression of the BDNF protein was negatively correlated with the neuronal apoptosis rate ($r = -0.667$, $P < 0.05$).

IFN-β inhibiting neuronal apoptosis by regulating the expressions of the apoptosis-related proteins Bax and Bcl2 is still unclear, which makes the conclusion inaccurate. In the follow-up experiments, we will further explore these problems.

Disclosure of conflict of interest

None.

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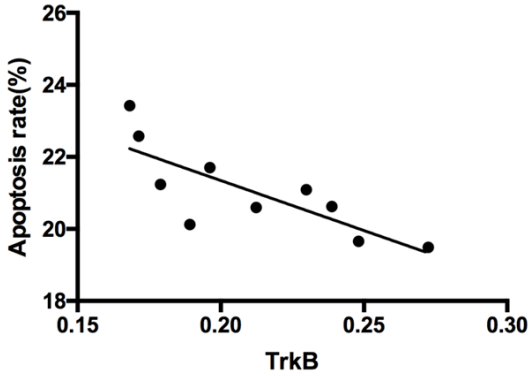

Figure 8. Correlation analysis between the expression of the TrkB protein and the neuronal apoptosis rate. The expression of the TrkB protein was negatively correlated with the neuronal apoptosis rate ($r = -0.791$, $P < 0.05$).

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