

Exercise intervention alleviates nerve injury by the suppression of inflammatory mediator expression via the TLR4/NF- κ B signaling pathway

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Abstract. Spinal cord injury (SCI) may cause changes that have damaging effects on sensation and functionality. However, methods for the significant amelioration of SCI-reduced nerve injury are lacking. Previous studies have indicated that reasonable and effective exercise may promote the recovery of injured nerves. Therefore, the aim of the present study was to investigate the ability of exercise to improve recovery following SCI and the underlying mechanism. A rat model was used to evaluate the effects of two different periods of exercise intervention on recovery following SCI. The exercise intervention comprised 15 or 30 min/day passive walking for 30 days. ELISA measurements were used to analysis the plasma levels of inflammatory cytokines. Reverse transcription-quantitative polymerase chain reaction and western blot analyses were performed to examine the levels of proteins and mRNAs associated with nuclear factor (NF)- κ B-related signaling. In addition, histological examination and immunostaining were used to evaluate the neural injury and associated indicators. The results indicated that severe SCI induced a peripheral inflammatory response and increased the expression of inflammatory cytokines. In addition, the SCI-induced nerve injury was associated with increased glial fibrillary acidic protein (GFAP) expression and the upregulation of Toll-like receptor 4 (TLR4)/NF- κ B signaling, which may further aggravate the inflammatory responses induced by SCI. However, the exercise intervention decreased SCI-induced GFAP expression and reduced the activation of the TLR4/NF- κ B signaling pathway compared with that of SCI model rats that did not exercise. Furthermore, the exercise intervention inhibited

the release of inflammatory cytokines into the serum. These results indicate that exercise treatment reduces inflammation and glial activation, and may be beneficial to recovery following SCI.

Introduction

A number of factors contribute to nerve injury-associated diseases (1-3). Among these, spinal cord injury (SCI)-induced nerve damage is a serious threat to health worldwide (4). Following SCI, patients experience continuous and often diverse neurological deficits and disability (5,6). According to the location and severity of SCI, symptoms may vary widely from pain or numbness to paralysis and incontinence (7-10). SCIs may be primary or secondary. Primary SCI involves the mechanical damage of neural elements, including transection or distraction. This damage usually occurs in cases of spinal fracture and/or dislocation, but may also occur in its absence. Penetrating injury of the spinal cord, such as that caused by bullets or weapons or the displacement of bone fragments, may also lead to primary SCI. In addition, in oncology, spinal cord compression may occur as a consequence of metastatic disease. Secondary SCI is mainly caused by arterial rupture, arterial thrombosis and insufficient perfusion (11,12).

Previous studies have indicated that reasonable and effective exercise is able to promote recovery from nerve injury (9,11). Aerobic exercise is regarded as an effective means to reduce fat and weight, and enables patients with obesity and diabetes to quickly improve their health (13,14). In previous studies, researchers have focused on identifying the types of exercise that are the most effective in helping patients who have various diseases, such as diabetes (15,16). To the best of our knowledge, there have been no studies concerning the specific molecular mechanisms underlying the beneficial effects of exercise, and it remains unclear whether exercise ameliorates nerve injury (17,18). Thus, the aim of the present study was to determine whether a defined amount of aerobic exercise is able to ameliorate SCI and the pathogenesis of nerve injury, and investigate the detailed molecular mechanisms by which aerobic exercise promotes recovery from SCI-induced nerve injury.

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Materials and methods

Animals. A total of 24 male Sprague-Dawley rats (6 weeks old; weight, 150–200 g) were purchased from the Shanghai Experimental Animal Center (Shanghai, China) and housed in a temperature and humidity-controlled environment ($25\pm 2^{\circ}\text{C}$, $50\pm 10\%$ humidity) with a standard 12-h light/dark cycle. Water and food were available *ad libitum* in their housing. The study was approved by the ethics committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital (Chengdu, China). All protocols were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Sichuan Academy of Medical Sciences.

The rats were randomly divided into four groups, six in each group: i) Control (CT group); ii) SCI (model group); iii) model with 15 min/day exercise (15 min/day group); iv) model with 30 min/day exercise (30 min/day group). A clinically relevant model of SCI mimicking the impact injury that occurs following traffic and other accidents was established. The rats were anesthetized with 80 mg/kg ketamine (Ketaset; Pfizer, Inc., New York, NY, USA) and 10 mg/kg xylazine (AnaSed; Lloyd Laboratories, Inc. Shenandoah, IA, USA) by intraperitoneal administration, and a laminectomy was performed at T10. Following immobilization of the spine with a spinal stereotactic device, SCI was induced by the method of Perot, which involved dropping a 5-g weight from a height of 8 cm onto an impounder (0.3 cm in diameter) gently placed on the spinal cord (19). Buprenorphine (0.01–0.05 mg/kg) was administered for the treatment of post-operative pain. Following the surgery, the rats were helped to urinate via massage of the bladder twice per day until the micturition reflex was recovered. An intraperitoneal injection of penicillin (200,000 units) was administered twice per day to protect against infection during the first week after surgery. Penicillin was also provided in cases of infection occurrence.

The Basso Beattie Bresnahan (BBB) scoring system (20) was employed to assess the neurological function of the rats using a double-blind method. This scoring system ranges from 0 to 21, where complete paraplegia is scored 0 and normal is scored 21. The scores were determined in a blinded manner by research assistants who were familiar with the BBB scoring system. The rats were allowed to move freely on a wide and flat surface for 2 min. The average score was obtained for each assessment and three assessments for each rat were conducted. The rats in the two treatment groups underwent passive walking rehabilitation training in the roller training machine, which the authors created, with a speed of 5 revolutions/min for 15 or 30 min/day, respectively, with the first training session occurring at 24 h post-injury. Following each 1-min period of movement during the training, the rats were allowed to rest for 2 min. The duration of the training period was 30 days. On day 30, following anesthesia with 5% chloral hydrate (50 mg/kg intraperitoneally; rat body weight at sacrifice, ~ 200 g), the rats were fixed in the supine position and sacrificed by decapitation. A 1-cm section of spinal cord centered on the lesion site, a 1-cm rostral section (starting 0.5 cm rostral from the impact site) and a caudal penumbra section (starting 0.5 cm caudal to the impact site) were taken for all groups for quantitative analysis.

Histological examination. Spinal cord sections of rats from the four groups were subjected to hematoxylin and eosin (H&E)

staining and examined to detect tissue injury and inflammatory response using light microscopy. Immunohistochemical (IHC) examination was conducted to investigate astrocyte activation using glial fibrillary acidic protein (GFAP) antibody. Immunofluorescence examination of the tissues was also conducted to investigate SCI-induced nerve injury via the detection of proteins associated with nuclear factor (NF)- κ B signaling, including TLR4, MyD88, GFAP and p-I κ B α . Briefly, 8- μ m-thick tissues were fixed with 2.5% glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4) at room temperature for 15 min. The sections were wash twice (4 min each wash) with TBS supplemented by 0.1% saponin (Merck KGaA, Darmstadt, Germany). Endogenous peroxidase activity was blocked by incubating the sections with TBS supplemented by 0.3% H_2O_2 , 0.1% saponin and 0.02% NaN_3 for 30 min at 4°C . The sections were wash three times (3 min each wash) with TBS supplemented by 0.1% saponin. Next, non-specific binding sites were blocked diluted goat serum (1:100; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) in TBS/saponin for 20 min at 37°C . They were then incubated overnight at 4°C , with the following primary antibodies: Rabbit anti-human TLR4 (1:500; cat. no. ab13556), mouse anti-human MyD88 antibody (1:1,000; cat. no. ab107585), rabbit anti-human GFAP polyclonal antibody (1:500; cat. no. ab7260) and rabbit anti-human p-I κ B α polyclonal antibody (1:500; cat. no. ab92700; all Abcam, Cambridge, UK). The sections were wash four times (3 min each wash) with TBS supplemented by 0.1% saponin, then incubated with the following secondary antibodies for 30 min at 37°C : Horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (Ig)G (1:200; cat. no. ab6789) and rabbit anti-mouse IgG (1:200; cat. no. ab6728; both Abcam). The resulting peroxidase activity were revealed by incubating the slides with a 0.5 mg/ml DAB (Beijing Zhongshan Golden Bridge Biotechnology; OriGene Technologies, Inc., Beijing, China) + H_2O_2 prepared in distilled water. The sections were wash four times in TBS (5 min/wash) and counterstained for 1 min at room temperature with hematoxylin. Next, they were dehydrated with sequential washes (1 min each wash) with 75, 80 and 100% ethanol. The sections were analyzed by optical microscopy and Image-Pro Plus (version 5.0) was used to quantify the data.

ELISA measurements. On day 30, following anesthesia with 1.0% pentobarbital sodium (45 mg/kg intraperitoneally), blood was extracted from the eyeball. The inflammatory cytokines interleukin (IL)-1 β (cat. no. MLB00C), IL-6 (cat. no. D6050), IL-18 (cat. no. DBP180) and tumor necrosis factor (TNF)- α (cat. no. MTA00B) in the serum were tested using ELISA kits in accordance with the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA).

Western blot analysis. For western blotting, 100 mg spinal cord tissues from rats in the different groups were lysed using radioimmunoprecipitation assay lysis buffer [150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate and 50 mM Tris-HCl, pH 8.0] to yield a homogenate. The final supernatants were then obtained by centrifugation at 4°C and 12,000 \times g for 15 min. The protein concentration was calculated using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.) with bovine serum

Table I. Primer sequences for reverse transcription-quantitative polymerase chain reaction.

Protein	Direction	Sequence (5'-3')
TLR4	Forward	TGATGTCTGCCTCGCGCCTG
	Reverse	TAGGAACCACCTCCACGCAGGG
MyD88	Forward	GCATGGAACCAGTGGCTGTGAG
	Reverse	GAGGAAGTGGAAATGGGCGGTGT
IkB α	Forward	GCAAAATCCTGACCTGGTGT
	Reverse	GCTCGTCTCTGTGAACTCC
TAK1	Forward	CAACTACAGCCTCTAGCAC
	Reverse	CTTATCATGTCTGCTCGAAG
IKK β	Forward	CCGTGACTGTTGACTACTG
	Reverse	GTCCACTTCGCTCTTCTG

TLR4, Toll-like receptor 4; TAK1, transforming growth factor β 1-activated kinase 1; IKK β , inhibitor of NF- κ B kinase subunit β .

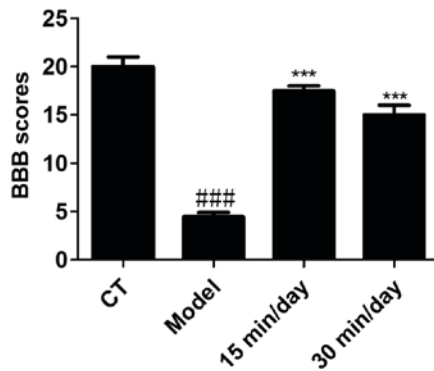


Figure 1. Exercise improves the functional outcomes of rats following spinal cord injury. Exercise training for 15 or 30 min/day was initiated when the BBB score was 1. Following the 30-day training period, the BBB scores of the 15 and 30 min/day exercise groups were significantly higher than those in the CT group. ###P<0.001 vs. the CT group; ***P<0.001 vs. the model group. BBB, Basso Beattie Bresnahan; CT, control.

albumin (Gibco; Thermo Fisher Scientific, Inc.) as a standard. The total protein extract was used for western blot analysis. In this analysis, 40 μ g total protein was loaded per lane and proteins were separated using 10% SDS-PAGE and electrophoretically transferred to a polyvinylidene difluoride membrane (EMD Millipore, Billerica, MA, USA). The membranes were then blocked with 5% non-fat dry milk in Tris-buffered saline (20 mM Tris, pH 7.6, 137 mM NaCl) with 0.1% Tween-20 for 60 min at room temperature, washed and then incubated with primary antibody overnight at 4°C. The membranes were washed again, incubated with secondary antibody at 37°C for 1 h and scanned following the use of the ECL Advanced Western Blotting kit (GE Healthcare Life Sciences, Little Chalfont, UK). The relative expression of protein was analyzed by Quantity One 1-D software (version 4.6.9; Bio-Rad Laboratories, Inc., Hercules, CA, USA) following internal reference calibration. The primary antibodies were as follows (1:1,000): Rabbit anti-NF- κ B (cat. no. ab16502), transforming growth factor β 1-activated kinase 1 (TAK1; cat. no. ab109526), TLR4 (cat.

no. ab22048), IkB α (cat. no. ab32518), MyD88 (cat. no. ab2064), p-NF- κ B (cat. no. ab86299), p-IkB α (cat. no. ab133462), p-IKK β (cat. no. ab59195), inhibitor of NF- κ B kinase subunit β (IKK β ; cat. no. ab178870; all Abcam) and GAPDH (cat. no. 14C10; Cell Signaling Technology, Inc., Danvers, MA, USA). The secondary antibodies were as follows: Horseradish peroxidase-conjugated horse anti-mouse secondary antibodies (cat. no. 7076; 1:1,000; Cell Signaling Technology, Inc.).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR was carried out to calculate RNA expression levels. Total RNA of tissue samples was extracted, frozen and pulverized using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), and cDNA was synthesized from the RNA with oligo (dT) primers using the Advantage RT-for-PCR kit (Clontech Laboratories, Inc., Mountainview, CA, USA). qPCR was performed using SYBR-Green I (Invitrogen; Thermo Fisher Scientific, Inc.) and normalized to GAPDH gene expression. All primer sequences (Table I) used for qPCR were produced by Invitrogen (Thermo Fisher Scientific, Inc.). The cycling conditions were as follows: Pre-denaturation at 95°C for 5 min, and 40 cycles of denaturation at 95°C for 10 sec, annealing at 58°C for 15 sec and extension at 72°C for 20 sec. The copy number was calculated according to the $2^{-\Delta\Delta C_q}$ method described in a previous study (21).

Statistical analysis. Data are expressed as the mean \pm standard error of the mean. The treated tissues and corresponding controls were compared using GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) by one-way analysis of variance with Dunn's least significant difference test. P<0.05 was considered to indicate a statistically significant difference.

Results

Exercise improves the functional outcomes of the rats. The BBB scoring system was employed to assess the neurological functions of the rats in each group. Prior to training, the BBB score of the SCI model rats was 1. As shown in Fig. 1, the BBB scores of the rats that received 15 and 30 min exercise training each day were significantly higher compared with those of the model group. This indicates that effective and moderate exercise helped to improve the functional outcomes of the rats.

Exercise suppresses SCI-stimulated tissue damage. Rats were treated with training exercise for 15 or 30 min/day for 30 days, after which the SCI and tissue inflammatory responses were evaluated. As shown in Fig. 2A and B, the spinal cords of the model and 30 min/day exercise treatment groups appeared clearly different. Also, GFAP was markedly activated in the model group compared with the CT and exercise treatment groups (Fig. 2C). These results indicate that effective and moderate exercise helps to ameliorate SCI and promotes the resolution of inflammation. It also appears that the degree of improvement may be proportional to the duration of exercise. The H&E staining images presented in Fig. 2D further demonstrate that SCI caused tissue damage and induced an inflammatory response, and that exercise was able to suppress the upregulated inflammatory response.

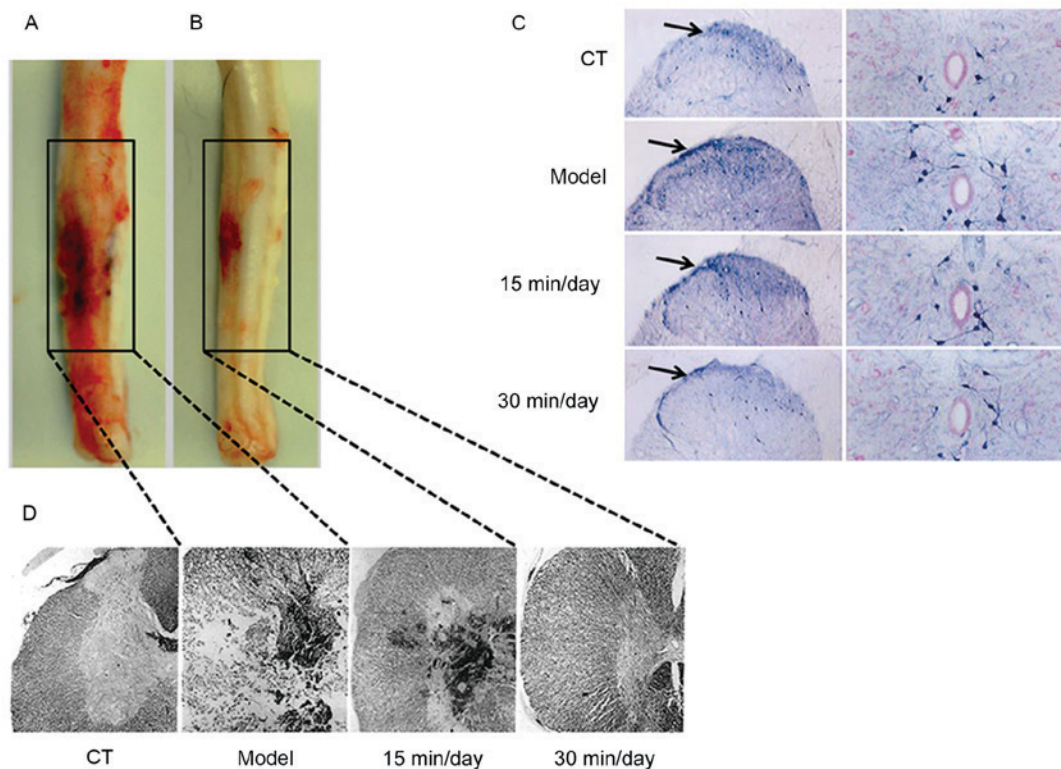


Figure 2. Exercise suppresses SCI-stimulated tissue injury. Following SCI with and without exercise training for 15 or 30 min/day, H&E staining and IHC evaluation of GFAP was conducted to evaluate the SCI and tissue inflammatory responses. Macroscopic examination of (A) SCI model rats and (B) SCI model rats with 15 min/day exercise training treatment. (C) IHC analysis of GFAP. Magnification left, x100; Magnification right, x200. (D) H&E staining for the evaluation of SCI and tissue inflammatory responses. Magnification, x400. SCI, spinal cord injury; H&E, hematoxylin and eosin; IHC, immunohistochemical; GFAP, glial fibrillary acidic protein; CT, control.

Exercise ameliorates the inflammatory response in the serum and spinal cord. Inflammation-associated cytokines serve an important role in SCI-induced nerve injury and neuroinflammation. Studies have demonstrated that a series of cytokines and inflammation-related signaling pathways may be activated during the occurrence of SCI (22). The ELISA results in Fig. 3A-D illustrate the changes of pro-inflammatory cytokines that occurred in the serum of the rats following SCI. These results demonstrate that SCI significantly increased the serum levels of pro-inflammatory cytokines and promoted their release. However, the 30-min exercise treatment significantly suppressed the pro-inflammatory response compared with that in the model group. Fig. 3E-H shows the corresponding mRNA expression levels of these cytokines in the spinal cord of the different groups. These results indicate that moderate exercise attenuated the SCI-induced changes in RNA levels. The results are consistent with those for the respective proteins. Fig. 3I shows the relative mRNA expression levels of other inflammatory cytokines (interferon- γ , IL-17, IL-10, IL-2, IL-4 and IL-1) in the four groups. The mRNA expression levels of these major cytokines were also significantly downregulated following exercise training compared with those in the model group.

Exercise inhibits SCI-induced glial activation and nerve inflammation. Whether the NF- κ B pathway is involved in the inflammatory process was then investigated using immunofluorescence (Fig. 4). The results in Fig. 4A-C and E show that components of the NF- κ B pathway were significantly

upregulated in the model group compared with the CT group. The immunofluorescence results in Fig. 4D indicate that GFAP positive cells, which serve as a marker of nerve injury (23), were also induced in the SCI model. These results demonstrate that SCI significantly increased GFAP cell activation, and promoted neuroinflammation and nerve injury. However, the training exercise helped to attenuate the damage.

Exercise regulates SCI-induced activation of the NF- κ B inflammatory signaling pathway. Western blot analysis further indicated that SCI significantly induced activation of the NF- κ B-related inflammatory signaling pathway. As shown in Fig. 5, in the model group, the protein expression of TLR4, MyD88, TAK1, p-I κ B α , p-IKK β and p-NF- κ B was significantly upregulated compared with that in the control group. Also, the treatment groups exhibited significantly lower expression levels of these proteins compared with the model group, indicating that reasonable exercise has the ability to attenuate SCI. RT-qPCR analysis was also used to investigate these NF- κ B-associated indicators, and the demonstrated that the mRNA levels of TLR4, MyD88, I κ B α , TAK1 and IKK β were significantly increased in the animals with SCI compared with the CT group (Fig. 6). The results were consistent with the results of western blotting.

Discussion

SCI-induced nerve damage is a serious threat to the health of people worldwide (24-26). SCI is associated with the secretion

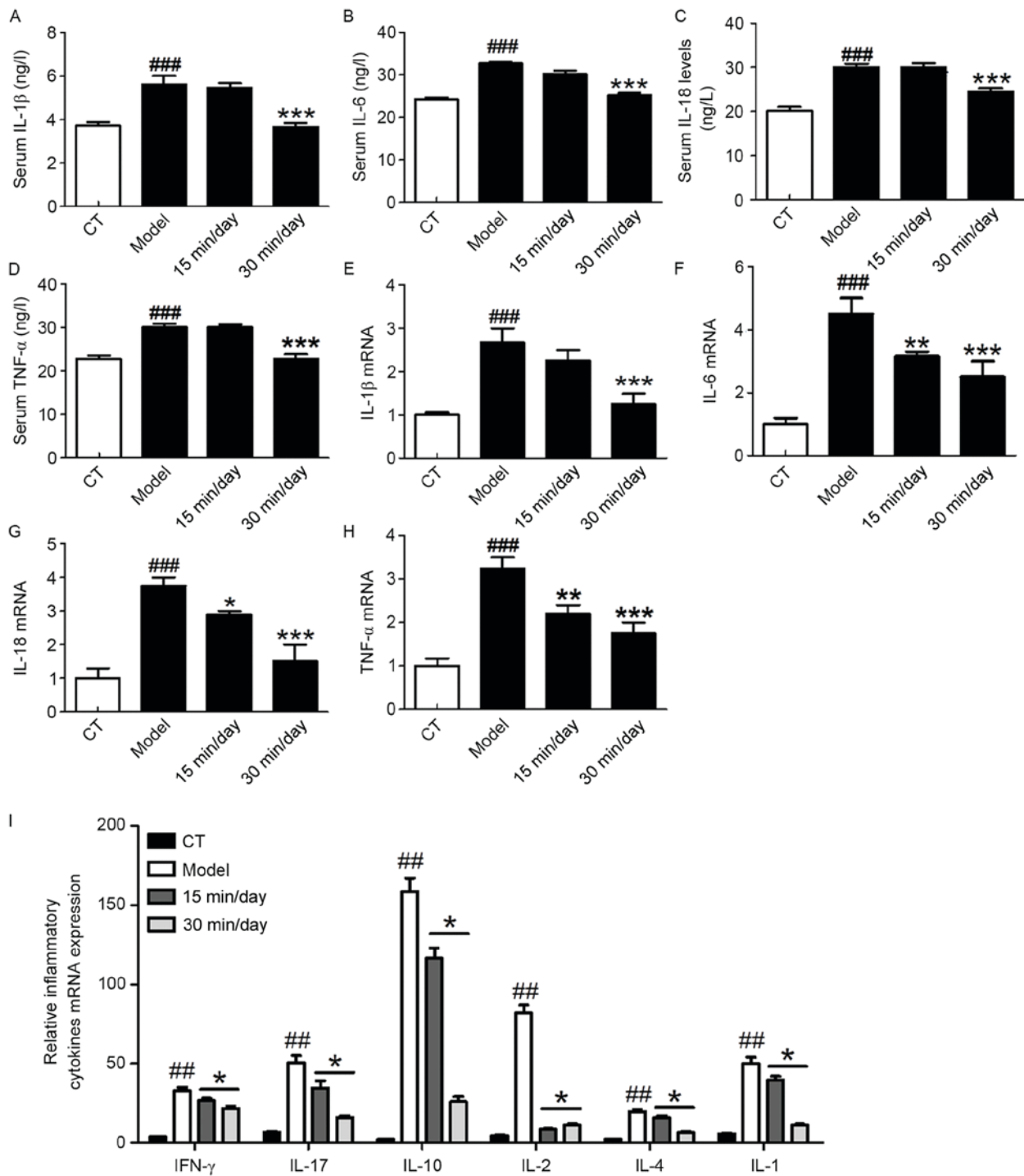


Figure 3. Exercise attenuates the inflammatory responses of peripheral tissues and spinal cord of rats following SCI. ELISA analysis demonstrating the changes in the protein levels of the pro-inflammatory cytokines (A) IL-1 β , (B) IL-6, (C) IL-18 and (D) TNF- α in the serum. RT-qPCR analysis showing the changes in the mRNA expression of (E) IL-1 β , (F) IL-6, (G) IL-18 and (H) TNF- α in the spinal cord. (I) RT-qPCR analysis of the mRNA expression of further inflammatory cytokines in the SCI model. Significance, using one-way analysis of variance with Dunn's least significant difference tests, is indicated. ##P<0.01 and ###P<0.001 vs. the CT group; *P<0.05, **P<0.01 and ***P<0.001 vs. the model group. SCI, spinal cord injury; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

of pro-inflammatory cytokines, including IL-1 β , IL-6 and TNF- α (27). While an inflammatory response is necessary to clear debris from the site of injury, if it becomes out of control, an enlargement of the initial lesion results, with additional axonal damage, contusion, compression or stretch injury, and demyelination with concomitant exacerbation of neurological function loss (28). Congenital lesions and tumor compression

may also cause SCI, disc herniation and ischemia (29-32). The initial physical injury of SCI causes progressive damage, which spreads from the epicenter of injury. The initial physical injury causes tissue necrosis, neuronal disruption and vascular damage, and progressive damage may ensue, including oxidative stress, necrosis, inflammation, ischemia and apoptotic response (12,24). However, pharmacotherapies

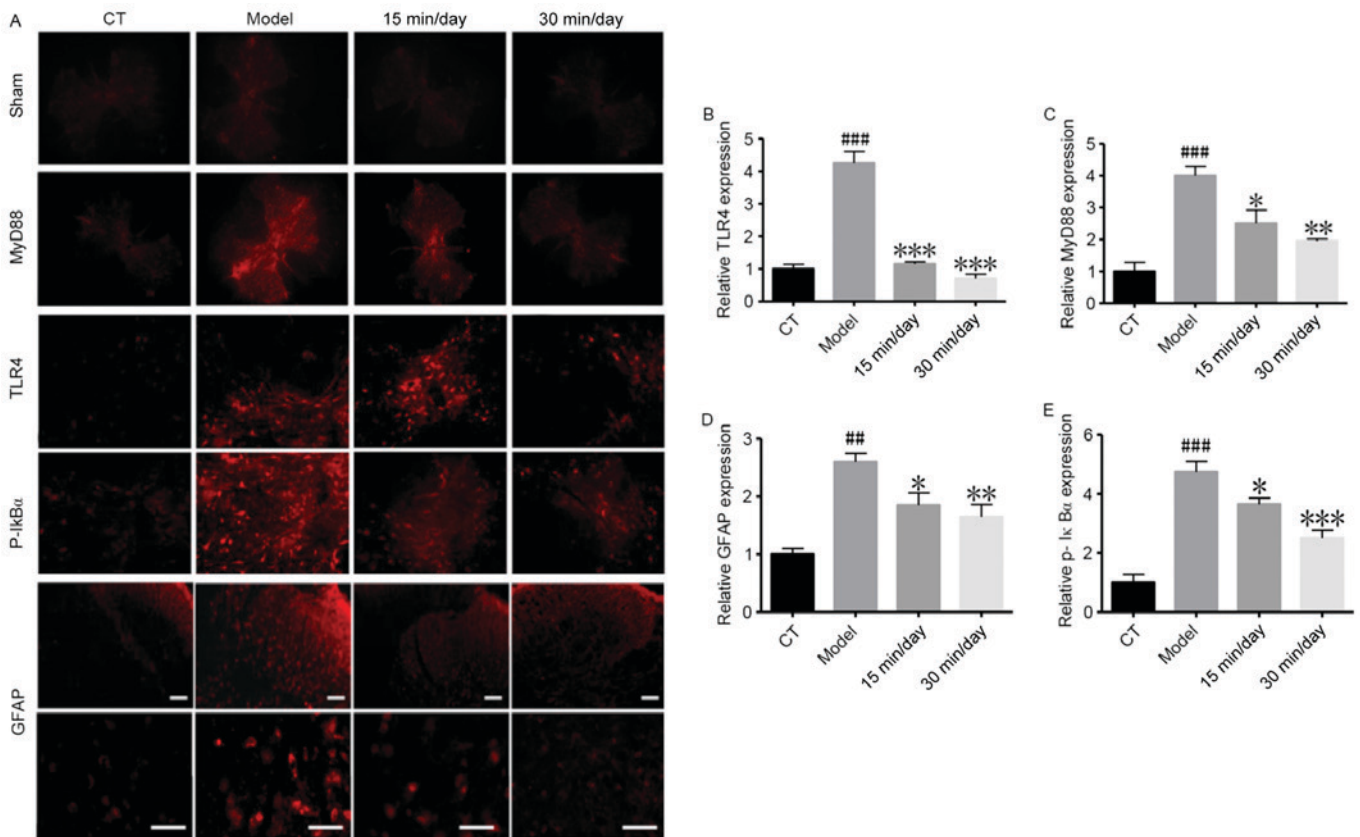


Figure 4. Exercise inhibits SCI-induced glial activation and neural inflammation. Immunofluorescence assays were used to analyse GFAP activation in glial cells and nuclear factor- κ B-associated indicators following SCI. (A) Representative immunofluorescence staining images. Magnification, $\times 400$. Quantified results showing the relative (B) TLR4, (C) MyD88, (D) GFAP and (E) p-I κ B α protein expression. Data of all groups are normalized to the control. Significance, using one-way analysis of variance with Dunn's least significant difference tests, is indicated. ## $P < 0.01$ and ### $P < 0.001$ vs. the CT group; * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$ vs. the model group. SCI, spinal cord injury; GFAP, glial fibrillary acidic protein; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response gene 88; p-I κ B α , phosphorylated inhibitor of κ B; CT, control. Scale bar, 100 μ m.

for the amelioration of neuronal injury and enhancement of regeneration are limited.

Individuals who survive SCI are likely to suffer from medical complications such as chronic pain, bladder and bowel dysfunction, and increased susceptibility to respiratory and heart problems, and their successful recovery is dependent upon the daily treatment of these chronic diseases (33). Numerous researchers consider that reasonable exercise may help patients with SCI to recover, by improving their health and restoring normal physiological functions (34,35). Therefore, the current study aimed to investigate whether exercise is able to ameliorate SCI.

The results of the present study indicated that SCI caused nerve damage and systemic inflammation, and that moderate exercise had the ability to ameliorate SCI through the regulation of NF- κ B signaling pathways and inhibition of nerve injury. The IHC and western blot analyses demonstrated that inflammation of the spinal cord was induced by SCI, and exercise was able to suppress this. The stimulation of peripheral inflammation by SCI was also examined in the present study. The increased serum cytokine levels detected by ELISA indicated that SCI caused a peripheral inflammatory response, this was also suppressed by exercise. NF- κ B regulates the expression of genes associated with apoptosis, viral replication, tumorigenesis, inflammation and autoimmune disease (36). NF- κ B activation is considered to be part of the stress response,

as it is induced by a variety of stimuli, including growth factors, cytokines, lymphokines, ultraviolet, pharmacological agents and stress (37). The TLR4/NF- κ B pathway is one of the most important signal transduction pathways in the initiation and development of inflammation (37). The aforementioned stimuli also activate the phosphorylation, ubiquitination and degradation of I κ B, which leads to the translocation of NF- κ B to the nucleus where it binds to the consensus sequence of target genes and activates their transcription (38). The phosphorylation of I κ B is mediated by an I κ B kinase complex comprising IKK1/IKK α , IKK2/IKK β and IKK3/IKK γ (39). In the present study, the expression of these TLR4/NF- κ B pathway components in the spinal cord tissues of the rats was investigated using western blotting and RT-qPCR. The analysis indicated that SCI increased inflammatory gene transcription and protein expression, and significantly activated the TLR4/NF- κ B signaling pathway. By contrast, exercise training suppressed protein phosphorylation in this pathway, and attenuated the SCI-induced inflammatory response.

In summary, the results of the present study indicate that SCI stimulates inflammatory responses by increasing the activation of glial cells and TLR4/NF- κ B signaling pathways. However, exercise training may alleviate SCI-induced nerve injury by suppressing the expression of inflammatory mediators via TLR4/NF- κ B signaling, and thus be beneficial to recovery following SCI.

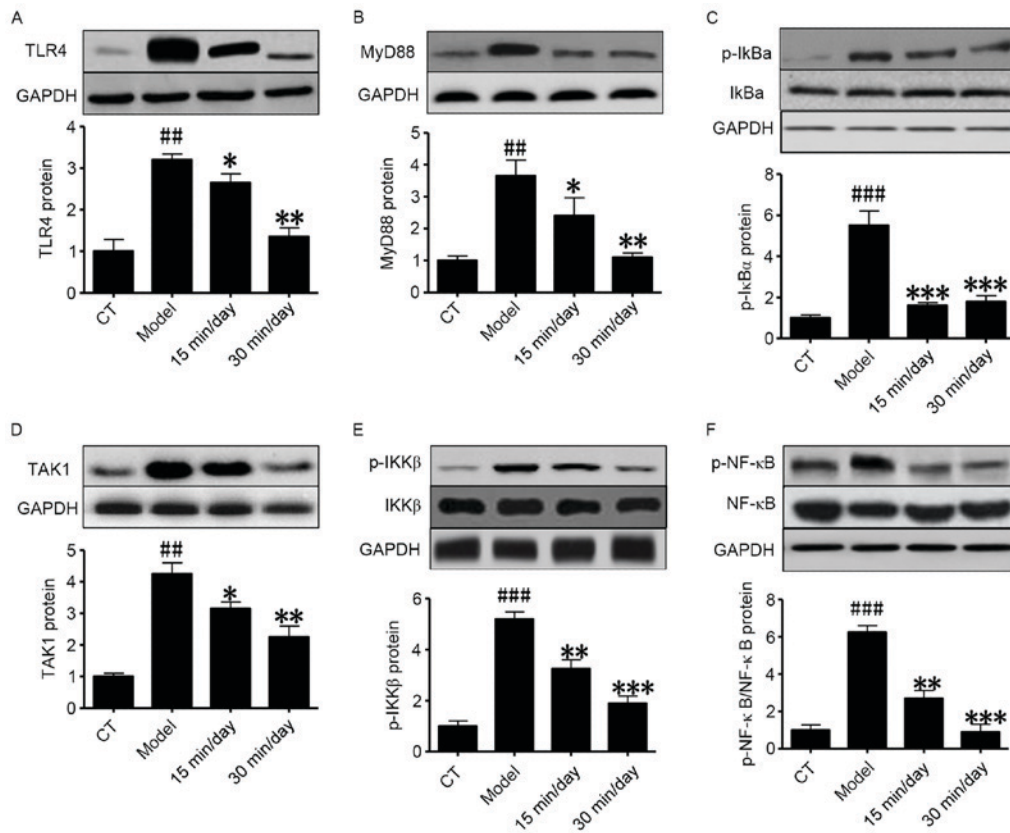


Figure 5. Exercise inhibits SCI via the inhibition of NF-κB pathways. Western blot analysis of the expression of NF-κB pathway-associated proteins in a rat model of SCI. Relative protein expression of (A) TLR4, (B) MyD88, (C) p-IκBα, (D) TAK1, (E) p-IKKβ and (F) p-NF-κB. Significance, using one-way analysis of variance with Dunn's least significant difference tests, is indicated. $^{##}P<0.01$ and $^{###}P<0.001$ vs. the CT group; $^{*}P<0.05$; $^{**}P<0.01$ and $^{***}P<0.001$ vs. the model group. SCI, spinal cord injury; NF-κB, nuclear factor-κB; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response gene 88; p-IκBα, phosphorylated inhibitor of κB; TAK1, transforming growth factor β1-activated kinase 1; p-IKKβ, phosphorylated inhibitor of NF-κB kinase subunit β; CT, control.

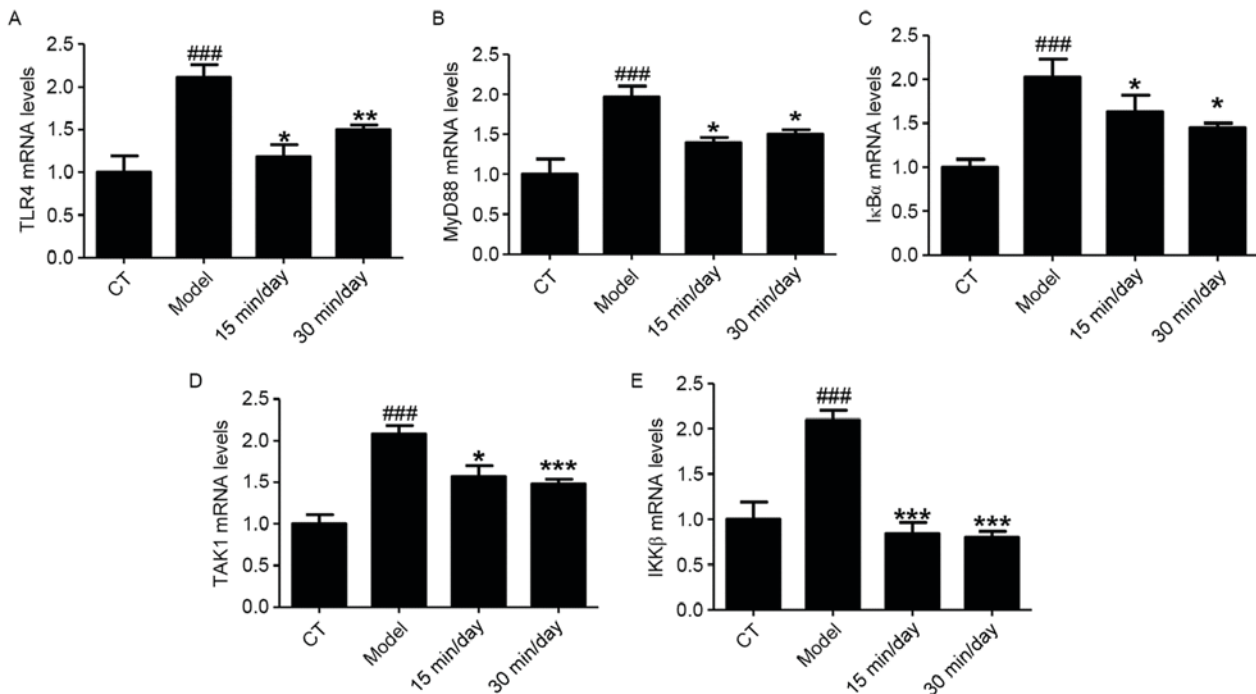


Figure 6. Exercise suppresses SCI by regulating NF-κB pathways at the mRNA level. Reverse transcription-quantitative polymerase chain reaction analysis was used to test the mRNA levels of NF-κB related-indicators in a rat model of SCI. Relative mRNA expression of (A) TLR4, (B) MyD88, (C) IκBα, (D) TAK1 mRNA and (E) IKKβ. Data of all groups are normalized to the control. Significance, using one-way ANOVA with Dunn's least significant difference tests, is indicated. $^{###}P<0.001$ vs. the CT group; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. the model group. SCI, spinal cord injury; NF-κB, nuclear factor-κB; TLR4, Toll-like receptor 4; MyD88, myeloid differentiation primary response gene 88; IκBα, inhibitor of κB; TAK1, transforming growth factor β1-activated kinase 1; IKKβ, inhibitor of NF-κB kinase subunit β; CT, control.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

FT conceived the study. YS and JQL conducted the experiments. FT, YS and JQL analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Siriphorn A, Dunham KA, Chompoopong S and Floyd CL: Postinjury administration of 17 β -estradiol induces protection in the gray and white matter with associated functional recovery after cervical spinal cord injury in male rats. *J Comp Neurol* 520: 2630-2646, 2012.
- Petrinovic MM, Hourez R, Aloy EM, Dewarrat G, Gall D, Weinmann O, Gaudias J, Bachmann LC, Schiffmann SN, Vogt KE and Schwab ME: Neuronal Nogo-A negatively regulates dendritic morphology and synaptic transmission in the cerebellum. *Proc Natl Acad Sci USA* 110: 1083-1088, 2013.
- Wang F, Xing S, He M, Hou Q, Chen S, Zou X, Pei Z and Zeng J: Nogo-A is associated with secondary degeneration of substantia nigra in hypertensive rats with focal cortical infarction. *Brain Res* 1469: 153-163, 2012.
- Zhu WW, Ma XL, Guo AL, Zhao HY and Luo HH: Neuroprotective effects of NEP1-40 and fasudil on Nogo-A expression in neonatal rats with hypoxic-ischemic brain damage. *Genet Mol Res* 10: 2987-2995, 2011.
- Kim JH, Choi KH, Jang YJ, Bae SS, Shin BC, Choi BT and Shin HK: Electroacupuncture acutely improves cerebral blood flow and attenuates moderate ischemic injury via an endothelial mechanism in mice. *PLoS One* 8: e56736, 2013.
- Kim MW, Chung YC, Jung HC, Park MS, Han YM, Chung YA, Maeng LS, Park SI, Lim J, Im WS, *et al*: Electroacupuncture enhances motor recovery performance with brain-derived neurotrophic factor expression in rats with cerebral infarction. *Acupunct Med* 30: 222-226, 2012.
- Tan F, Wan S, Wu H, Huo Q, Wang J, Chen W, Fang M, Liu X, Wang X and Sun J: Expression of neurocan mRNA and ultrastructure of brain tissue after cerebral ischemia and reperfusion in stroke-prone renovascular hypertensive rats treated by electroacupuncture. *Neural Regen Res* 6: 2834-2838, 2011.
- Hwang L, Choi IY, Kim SE, Ko IG, Shin MS, Kim CJ, Kim SH, Jin JJ, Chung JY and Yi JW: Dexmedetomidine ameliorates intracerebral hemorrhage-induced memory impairment by inhibiting apoptosis and enhancing brain-derived neurotrophic factor expression in the rat hippocampus. *Int J Mol Med* 31: 1047-1056, 2013.
- Sung YH, Kim SC, Hong HP, Park CY, Shin MS, Kim CJ, Seo JH, Kim DY, Kim DJ and Cho HJ: Treadmill exercise ameliorates dopaminergic neuronal loss through suppressing microglial activation in Parkinson's disease mice. *Life Sci* 91: 1309-1316, 2012.
- Donnelly DJ and Popovich PG: Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Exp Neurol* 209: 378-388, 2008.
- Anwar MA, Al Shehabi TS and Eid AH: Inflammogenesis of secondary spinal cord injury. *Front Cell Neurosci* 10: 98, 2016.
- Dominguez E, Mauborgne A, Mallet J, Desclaux M and Pohl M: SOCS3-mediated blockade of JAK/STAT3 signaling pathway reveals its major contribution to spinal cord neuroinflammation and mechanical allodynia after peripheral nerve injury. *J Neurosci* 30: 5754-5766, 2010.
- Swift DL, Johannsen NM, Lavie CJ, Earnest CP and Church TS: The role of exercise and physical activity in weight loss and maintenance. *Prog Cardiovasc Dis* 56: 441-447, 2014.
- Foster-Schubert KE, Alfano CM, Duggan CR, Xiao L, Campbell KL, Kong A, Bain CE, Wang CY, Blackburn GL and McTiernan A: Effect of diet and exercise, alone or combined, on weight and body composition in overweight-to-obese postmenopausal women. *Obesity (Silver Spring)* 20: 1628-1638, 2012.
- Kujala UM: Benefits of exercise therapy for chronic diseases. *Br J Sports Med* 40: 3-4, 2006.
- Lu Y, Xu J, Zhao W and Han HR: Measuring self-care in persons with Type 2 diabetes: A systematic review. *Eval Health Prof* 39: 131-184, 2016.
- Tajirina N, Yasuhara T, Shingo T, Kondo A, Yuan W, Kadota T, Wang F, Baba T, Tayra JT, Morimoto T, *et al*: Exercise exerts neuroprotective effects on Parkinson's disease model of rats. *Brain Res* 1310: 200-207, 2010.
- Hutchinson KJ, Gómez-Pinilla F, Crowe MJ, Ying Z and Basso DM: Three exercise paradigms differentially improve sensory recovery after spinal cord contusion in rats. *Brain* 127: 1403-1414, 2004.
- Perot PL Jr, Lee WA, Hsu CY, Hogan EL, Cox RD and Gross AJ: Therapeutic model for experimental spinal cord injury in the rat: I. Mortality and motor deficit. *Cent Nerv Syst Trauma* 4: 149-159, 1987.
- Basso DM, Beattie MS, Bresnahan JC, Anderson DK, Faden AI, Gruner JA, Holford TR, Hsu CY, Noble LJ, Nockels R, *et al*: MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. *Multicenter Animal Spinal Cord Injury Study. J Neurotrauma* 13: 343-359, 1996.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) method. *Methods* 25: 402-408, 2001.
- Gaudet AD and Popovich PG: Extracellular matrix regulation of inflammation in the healthy and injured spinal cord. *Exp Neurol* 258: 24-34, 2014.
- Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J and Ji RR: Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. *Brain Behav Immun* 21: 642-651, 2007.
- Guerrero AR, Uchida K, Nakajima H, Watanabe S, Nakamura M, Johnson WE and Baba H: Blockade of interleukin-6 signaling inhibits the classic pathway and promotes an alternative pathway of macrophage activation after spinal cord injury in mice. *J Neuroinflammation* 9: 40, 2012.
- Einstein O and Ben-Hur T: The changing face of neural stem cell therapy in neurological diseases. *Arch Neurol* 65: 452-456, 2008.
- Kuh SU, Cho YE, Yoon DH, Kim KN and Ha Y: Functional recovery after human umbilical cord blood cells transplantation with brain-derived neurotrophic factor into the spinal cord injured rat. *Acta Neurochir (Wien)* 147: 985-992, 2005.

27. Carlson NG, Wieggl WA, Chen J, Bacchi A, Rogers SW and Gahring LC: Inflammatory cytokines IL-1 alpha, IL-1 beta, IL-6, and TNF-alpha impart neuroprotection to an excitotoxin through distinct pathways. *J Immunol* 163: 3963-3968, 1999.
28. Jones TB, McDaniel EE and Popovich PG: Inflammatory-mediated injury and repair in the traumatically injured spinal cord. *Curr Pharm Des* 11: 1223-1236, 2005.
29. López-Vales R, Forés J, Verdú E and Navarro X: Acute and delayed transplantation of olfactory ensheathing cells promote partial recovery after complete transection of the spinal cord. *Neurobiol Dis* 21: 57-68, 2006.
30. Zhao ZM, Li HJ, Liu HY, Lu SH, Yang RC, Zhang QJ and Han ZC: Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. *Cell Transplant* 13: 113-122, 2004.
31. Mansilla E, Marin GH, Sturla F, Drago HE, Gil MA, Salas E, Gardiner MC, Piccinelli G, Bossi S, Salas E, *et al*: Human mesenchymal stem cells are tolerized by mice and improve skin and spinal cord injuries. *Transplant Proc* 37: 292-294, 2005.
32. Pal R, Gopinath C, Rao NM, Banerjee P, Krishnamoorthy V, Venkataramana NK and Totey S: Functional recovery after transplantation of bone marrow-derived human mesenchymal stromal cells in a rat model of spinal cord injury. *Cytotherapy* 12: 792-806, 2010.
33. Chen HY and Boore JRP: Considering the physiological and psychological consequences of spinal cord injury. *Br J Neurosci* 1: 225-232, 2006.
34. Driver HS and Taylor SR: Exercise and sleep. *Sleep Med Rev* 4: 387-402, 2000.
35. Gorman PH, Scott W, York H, Theyagaraj M, Price-Miller N, McQuaid J, Eyvazzadeh M, Ivey FM and Macko RF: Robotically assisted treadmill exercise training for improving peak fitness in chronic motor incomplete spinal cord injury: A randomized controlled trial. *J Spinal Cord Med* 39: 32-44, 2016.
36. Waris G and Ahsan H: Reactive oxygen species: Role in the development of cancer and various chronic conditions. *J Carcinog* 5: 14, 2006.
37. Li QT and Verma IM: NF-kappaB regulation in the immune system. *Nat Rev Immunol* 2: 725-734, 2002.
38. Janssen-Heininger YM, Poynter ME and Baeuerle PA: Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. *Free Radic Biol Med* 28: 1317-1327, 2000.
39. Zandi E, Rothwarf DM, Delhase M, Hayakawa M and Karin M: The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. *Cell* 19: 243-252, 1997.



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