Exercise training attenuates neuropathic pain and cytokine expression following chronic constriction injury of rat sciatic nerve

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IMPLICATIONS STATEMENT

Our study reported that physical exercise not only ameliorates thermal hyperalgesia and mechanical allodynia but also prevents tumor necrosis factor-α (TNF-α) and interlukin-1β (IL-1β) overexpression in sciatic nerve of CCI (chronic constriction injury) rats. Treadmill and swimming exercise increased heat shock protein 72 (Hsp72) expression of sciatic nerve of CCI rats, which may play an important role in therapy of CCI conditions. The clinical relevance of these effects warrants further investigation.

ABSTRACT

BACKGROUND: The underlying mechanism of exercise on neuropathic pain is not well understood. We investigated whether physical exercise regulates the functional recovery and heat shock protein 72 (Hsp72), tumor necrosis factor-α (TNF-α) and interlukin-1β (IL-1β) expression after chronic constriction injury (CCI) of the sciatic nerve.

METHODS: Male Sprague-Dawley rats were divided into 7 groups: control, sham operated (SO), SO with swimming or treadmill exercise (SOSE or SOTE), chronic constriction injury (CCI), CCI with swimming or treadmill exercise (CCISE or CCITE). We recorded body weight, thermal withdrawal latency and mechanical withdrawal threshold as well as Hsp72, TNF-α and interlukin-1β expression in sciatic nerve.

RESULTS: The body weight in the control and SO groups was heavier than that in the SOSE, SOTE, CCI, CCISE, and CCITE groups. CCI rats with swimming or treadmill exercise showed significant increase in thermal withdrawal latency and mechanical withdrawal threshold when compared with CCI rats without exercise on day 21 after CCI. Both CCISE and CCITE groups demonstrated greater Hsp72 expression and lower TNF-α or IL-1β level than the CCI group in sciatic nerve on day 21 after CCI.

CONCLUSIONS: These results suggest that progressive exercise training decreases peripheral neuropathic pain as well as TNF-α and IL-1β overproduction and increases HSP72 expression following CCI of the sciatic nerve.

KEY WORDS: Exercise; Chronic constriction injury; Tumor necrosis factor-α; Interlukin-1β; Heat shock protein 72; Neuropathic pain

INTRODUCTION

Neuropathic pain hinders the ability of patients to work, walk and sleep, and even their quality of life.[1] Clinically, patients with neuropathic pain following nerve injury often complain of ongoing burning pain as well as pain to light touch.[2] Although many available pharmacotherapies (such as antidepressants, antiepileptics) are effective for neuropathic pain, these drugs produce side effects.[3] We wondered whether non-pharmacotherapies might be treatment options for neuropathic pain.

Recently, there is a growing body of evidence that exercise decreases symptoms of acute pain in humans,[4-6] has numerous beneficial effects on chronic diseases, has an anti-inflammatory effect, and reduces neuropathic pain in rodents.[1,7] Neuropathic pain provokes varying degrees of local inflammatory responses and overexpression of inflammatory cytokines in locally activated macrophages, Schwann cells and other glial cells.[8,9] Furthermore, studies have shown that pro-inflammatory cytokines (e.g., tumor necrosis factor-α, interlukin-1β) induce pain,[10-13] and that treatment with anti-inflammatory cytokines or inhibitors of pro-inflammatory cytokines relieve pain.[14-17] In addition, it has been presumed that exercise pretraining can induce heat shock protein 72 (Hsp72) expression in multiple organs and protect against cerebral ischemia and damage to cerebral neurons in rats.[18] However, only few studies have examined the effects of swimming and treadmill exercise on neuropathic pain,

cytokines and Hsp72.

The aim of this study was to investigate whether exercise training (swimming or treadmill exercise), a non-pharmacotherapy, reduces peripheral neuropathic pain and expression of pro-inflammatory cytokines and increases Hsp72 expression following chronic constriction injury (CCI) of the sciatic nerve in rats. Hyperalgesia, allodynia, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and Hsp72 were evaluated in CCI rats with/without treadmill or swimming exercise.

METHODS

Animal Preparation

Experiments were performed on 250-300 g male Sprague-Dawley rats (National Laboratory Animal Center, Taipei, Taiwan). The rats were housed in a climate-controlled room maintained at 21℃ with approximately 50% relative humidity in the Animal Center of China Medical University. Lighting was on a 12-hour light-dark cycle (light on at 8:00 a.m.) and food and water were available *ad libitum* up to the time of testing. The experimental protocols were approved to perform this study by the Institutional Animal Care and Use Committee of China Medical University, Taiwan.

Effort was made to minimize discomfort of the animals and reduce the number of experimental animals. All studies were conducted according to IASP ethical guidelines.[19]

Groups and Design

Rats were divided into seven groups: (1) normal rats (control), (2) rats with chronic constriction injury (CCI), (3) rats with CCI combined with swimming exercise treatment (CCISE), (4) rats with CCI combined with treadmill exercise treatment (CCITE), (5) rats sham operated (SO), (6) rats sham operated combined with swimming exercise treatment (SOSE), (7) rats sham operated combined with

treadmill exercise treatment (SOTE). Some rats were considered for the overall behavioral analysis and body weight (*n* = 20, 20, 10, 10, 10, 20, 10 for two control, two SO, SOTE, SOSE, CCITE, two CCI, and CCISE, respectively), while some part of rats were sacrificed for tissue analysis (TNF-alpha) on day 21 after CCI (*n* = 5, 5, 5, for CCITE, CCI, and CCISE, respectively), some part of rats were sacrificed for IL-1beta analysis on day 21 after CCI (*n* = 5, 5, 5, for CCITE, CCI, and CCISE, respectively), and other rats were sacrificed for Hsp72 analysis on day 21 after CCI (*n* = 5, 5, 5, for CCITE, CCI, and CCISE, respectively). Experimenters were blind for rat assignment to different experimental groups. The rats take rest one day after the operation till the start of the training.

Surgery

The animals were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). The skin on the lateral surface of the thigh was incised and an incision was made-through the skin freeing tissue adhesions along about 7 mm of the sciatic nerve just distal to the greater trochanter of the femur bone. The sciatic nerve was exposed through the biceps femoris muscle and then its three terminal branches (the sural, common peroneal and tibia nerves) were identified. Four ligatures (4/0 black silk) were tied loosely around the sciatic nerve as described by Bennett and Xie (1988). The left sciatic nerve was exposed proximal to the sciatic trifurcation and an approximately

7-mm section of the nerve was freed from the adhesive tissue. Loose ligations were tied around the nerve with four silk sutures using 1-mm interligation spacing.[20] Great care was taken to tie the ligatures so that the diameter of the nerve was seen to be just barely constricted. The length of nerve thus affected by ligation was 5-6 mm. The degree of constriction of the sciatic nerve was controlled by ligation and sometimes a small, brief twitch in the muscle surrounding the exposed sciatic nerve was produced. The incision of the muscle, adjacent fascia and skin was closed with 4/0 silk,[20] and then with wound clips and the animal was returned to its cage for recovery. Sham operations involved exposure of the sciatic nerve and its branches with the same procedures but without creating any lesion.

Swimming Exercise

The protocol for swimming exercise training is presented in Table 1. Rats were placed in a plastic container (74 cm [length] x 53 cm [width] x 60 cm [height]) holding approximately 43 cm of water (37±0.5℃). In rare instances, animals need to be mildly stimulated to swim by nudging the nape of their neck with a pen. This protocol can ensure a full session of exercise conditioning. After each exercise session animals were gently dried with a cloth towel.

Treadmill Exercise

The protocol for treadmill exercise training was performed according to

previously described methods.[18,21] The protocol is presented in Table 2. In brief, rats were trained to run on a treadmill (Chanson, CS-5515, Taipei, Taiwan) 5 days a week for 6 weeks. Initially, the rats were acclimated to the training program and ran for 15 minutes at 1.2 km/hr, 0% slope, for 3 days. They ran without electrical stimulation. The duration and intensity of the exercise were increased progressively so that the animals were running for 30 minutes at 1.2 km/hr, 30 minutes at 1.8 km/hr and 60 minutes at 1.8 km/hr after 1, 2 and 3 weeks of training, respectively. The work rate of rats on this training protocol was about 70-75% of their maximal oxygen consumption. If the rats' feet were hurt during the training protocol they were withdrawn from the study.

Thermal and Mechanical Sensitivity

We interpret the decrease in heat latency and mechanical threshold as hyperalgesia and allodynia, respectively. The rats were tested for thermal hyperalgesia and mechanical allodynia after a period of at least 3 days of habituation to the testing environment. Unless otherwise specified, behavioral tests were conducted on 1 day before surgery, the day of surgery and on days 1, 3, 7, 14, 21, 28, 35 and 39. All measurements were carried out between 9:00 a.m. and 11:00 a.m. For consistency, one experienced investigator (Dr. Chen) who was blinded to the groups was responsible for behavioral tests.

Thermal hyperalgesia was tested according to the Hargreaves' Method.[22] Briefly, rats were placed individually in a clear plexiglass chamber (22 cm [length] x 22 cm [width] x 13.3 cm [height]), and the animals stood on a glass sheet with the temperature maintained at 30 ±1℃ to decrease the influence of the temperature in different seasons. The lateral plantar surface of the left hind paw of the rat was exposed to a constant intensity radiant heat source (focused beam of light, beam diameter: 0.5 cm, intensity: 20 I.R.) through a transparent perspex surface and a Plantar Analgesia Meter (IITC Life Science Instruments, Woodland Hills, CA). The paw withdrawal latency was recorded. The withdrawal responses evoked by thermal stimulation were determined including foot lifting, shaking, licking and squeaking. Paw movements associated with weight shifting or locomotion were not counted. The heat stimulation was repeated 3 times at intervals of 5 minutes for each test and the mean was calculated. A maximal automatic cut-off latency of 20 seconds was used to prevent tissue damage.

For assessment of mechanical allodynia, rats were placed individually in a clear plexiglass chamber (22 cm [length] x 22 cm [width] x 13.3 cm [height]) and supported by a wire mesh floor (40 cm [width] x 50 cm [length]). An electronic von Frey filament Analgesia Meter (IITC Life Science Instruments, Woodland Hills, CA) was applied at the lateral plantar surface of the left hind paw of the rat. The paw

withdrawal threshold was recorded. The withdrawal responses evoked by mechanical stimulation were determined including foot lifting, shaking, licking and squeaking. Paw movements associated with weight shifting or locomotion were not counted. The mechanical stimulation was repeated 3 times at intervals of 5 minutes for each test and the mean was calculated.

Tissue Preparation

The rats were anesthetized with urethane (1.67 g/kg, i.p.) and sacrificed on day 21 after CCI. Under aseptic conditions, skin was cut to expose the left sciatic nerve, proximal to the trifurcation (about 1 cm), before the four ligatures were removed. The nerve specimen was immediately stored at −80℃ for the protein assay.

Ice cold (4ºC) homogenization buffer was added (300 µl/each sciatic nerve). The homogenization buffer was freshly prepared by adding protease inhibitor (P 8340 cocktail, Sigma-Aldrich, St. Louis, MO) to T-PER™ Tissue Protein Extraction Reagent (Pierce Chemical Co., Rockford, IL) prior to tissue lysis. After adding the buffer, a homogenization probe (Tissue Tearor, Polytron; Biospec Products, Inc., Bartlesville, OK, USA) was applied for 20 seconds on ice at 21,000 rpm. Then the homogenized samples were centrifuged for 40 minutes at a speed of 13,000 rpm at 4°C, stored at −80°C and used subsequently for protein quantification. The protein concentration in the supernatant was quantified using the Lowry protein assay.

Samples were pipetted as duplicates (1 µl/50 µl/well) in a 96-well microtiter plate (Costar). Each plate was inserted into a plate reader (Molecular Device Spec 383, Sunnyvale, CA, USA) to read the optical density of each well at an absorbance of 750 nm. Data were analyzed using Ascent Software (London, UK) for iEMS Reader.

Cytokine Evaluation

The concentrations of TNF-α and IL-1β in the supernatants were determined using the DuoSet® ELISA Development Kit (R&D Systems, Minneapolis, MN). All experimental procedures were performed in accordance with the instructions. Plates were individually inserted into the plate reader for reading optical density using a 450-nm filter. Data were then analyzed using Ascent Software for iEMS Reader and a four-parameter logistics curve-fit. Data were expressed in pg/mg protein of duplicate samples.

Hsp72 Analysis

Protein samples (30 µg/lane) were separated using 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) at a constant voltage of 75 V.[18] Electrophoresed proteins were transferred to a polyvinylidene difluoride (PVDF) membrane with a 0.45 µm pore size (Millipore, Bedford, MA,) using a transfer apparatus (Bio-Rad, Hercules, CA, USA). The PVDF membrane was incubated in 5% milk in TBS buffer. The membrane was blocked in TBS (20 mM Tris, 500 mM NaCl, and 0.1% Tween 20, pH

7.5) containing 5% skim milk (Difco, Detroit, MI) for 1 hour. Mouse monoclonal anti-Hsp72 primary antibody (SPA 810; StressGen Biotechnologies, Victoria, British Columbia, Canada) was diluted to 1:500 in antibody binding buffer overnight at 4℃. The membrane was then washed three times with TBS (10 minutes per wash) and incubated for 1 hour with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and diluted 500-fold in TBS buffer at 4℃. The membrane was washed in TBS buffer for 10 minutes three times.

Immunodetection for Hsp72 was performed using the enhanced chemiluminescence ECL Western blotting luminal reagent (Santa Cruz Biotechnology) and then the membrane was quantified using a Fujifilm LAS-3000 chemiluminescence detection system (Tokyo, Japan).

Statistical Analysis

The results are presented as mean ± SEM. The differences between data related to body weight (Fig. 1), paw withdrawal latency (Figs. 2 and 3) and paw withdrawal threshold (Figs. 2 and 3) were analyzed by 2-way analysis of variance (ANOVA) of repeated measures, followed by Tukey–Kramer post *hoc* comparison. Exercise treatment was the between-subjects factor and time was the repeated measure. The differences in TNF-α (Fig. 4), IL-1β (Fig. 4), and Hsp72 (Fig. 5) were determined

using 1-way ANOVA followed by post *hoc* Tukey's test for multiple comparisons. Statistical calculations were performed using SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL). Differences were considered significant at $p< 0.05$.

RESULTS

Body Weight

The change in body weight after surgery is shown in Figure 1. ANOVA for repeated measures (including control, SO, SOSE, SOTE, CCI, CCISE and CCITE groups) showed significant main effects for groups ($F_{4,45}$ = 22.13; P < 0.0001), time ($F_{9,405}$ = 10.31; P < 0.0001) and significant interaction ($F_{36,405}$ = 5.89; P < 0.0001). Post *hoc* comparisons demonstrated significant differences between control (or SO) and SOTE (or CCI, CCITE) groups, and between control (or SO) and SOSE (or CCI, CCISE) groups (P < 0.05, Tukey–Kramer), respectively. There was no significant difference between the SO and control groups (Fig. 1A and 1B). The CCISE (Fig. 1B) and CCITE (Fig. 1A) groups had no significant change in body weight when compared with the CCI group, respectively. Furthermore, the animals' grooming, sleep-wake cycles and social interaction in the cage were not obviously affected (data not shown).

Thermal and Mechanical Sensitivity

ANOVA of repeated measures (including control, SO, SOSE, SOTE, CCI, CCISE and CCITE groups) for thermal withdrawal latency and mechanical withdrawal threshold demonstrated significant main effect for groups ($F_{4,45}$ = 58.88; P < 0.0001), time ($F_{9,405}$ = 41.58; P < 0.0001) and significant interaction ($F_{36,405}$ = 8.74;

$P < 0.0001$) in Figures 2 and 3. Post-*hoc* comparisons showed no significant differences between control, SO and SOSE (or SOTE) ($P > 0.05$, Tukey–Kramer). We indicate these decreases in heat latency and mechanical threshold as hyperalgesia and allodynia, respectively.

In SO and CCI (or CCITE) groups (Fig. 2A), the significant difference in thermal withdrawal latency was maintained from day 3 to day 21 ($P < 0.05$, Tukey–Kramer). Post *hoc* comparisons demonstrated significant differences between CCITE and CCI groups from day 3 to day 21 ($P < 0.05$, Tukey–Kramer). In CCI group comparing with SO group (Fig. 3A), the significant difference in thermal withdrawal latency was maintained from day 3 to day 28 ($P < 0.05$, Tukey–Kramer), while in CCISE group only from day 7 to day 14. Post *hoc* comparisons demonstrated significant differences between CCISE and CCI groups from day 3 to day 21 ($P < 0.05$, Tukey–Kramer).

Post-*hoc* comparisons showed significant differences in mechanical withdrawal threshold between CCI and SO groups from day 1 to day 35, between CCITE and SO groups from day 1 to day 28, and between CCITE and CCI groups from day 21 to day 35 (Fig. 2B; $P < 0.05$, Tukey–Kramer). In SO and CCI (or CCISE) groups (Fig. 3B), the significant difference in mechanical withdrawal threshold was maintained from day 1 to day 35 ($P < 0.05$, Tukey–Kramer). Furthermore, CCI group was again

significantly different from CCISE group from the day 14 to day 21 showing an increase in mechanical withdrawal threshold ($P < 0.05$, Tukey–Kramer).

Furthermore, on day 21 after CCI, data showed a significant increase in thermal withdrawal latency and mechanical withdrawal threshold in the CCITE or CCISE group when compared with the CCI group. Therefore, we selected day 21 after CCI to evaluate TNF-α, IL-1β and Hsp72 expression in sciatic nerve. There was no significant difference in thermal withdrawal latency and mechanical withdrawal threshold between CCITE (or CCISE) and CCI groups after exercise training for 39 days (day 39 after CCI).

Cytokine Expression

Figure 4A and 4B depict the levels of TNF-α and IL-1β in sciatic nerve of CCI, CCISE and CCITE rats after exercise training for 21 days (day 21 after CCI). The expression of TNF-α was decreased in the CCITE group (59.4±3.2 pg/mg protein, $p < 0.05$) or CCISE (60.9±2.9 pg/mg protein, $p < 0.05$) group compared with the CCI group (75.3±2.1 pg/mg protein) on day 21 after CCI (Fig. 4A). The expression of IL-1β was decreased in the CCITE group (69.3±8.8 pg/mg protein, $p < 0.01$) and CCISE group (92.3±24.0 pg/mg protein, $P < 0.05$), compared with the CCI group (162.9±19.8 pg/mg protein) on day 21 after CCI, as shown in Figure 4B.

Hsp72 Expression

Figure 5 showed the expression of Hsp72 in sciatic nerve after CCI in three different groups. It can be seen that the Hsp72 level in sciatic nerve was significantly increased 3.2-folds in the CCITE group ($P < 0.01$) and 2.1-folds in the CCISE group ($P < 0.05$) on day 21 after CCI when compared with the CCI group (Fig. 5).

DISCUSSION

The main finding of this study is that swimming and treadmill exercises appeared to retard peripheral neuropathic pain following chronic constriction injury of the sciatic nerve in rats. Rats following chronic constriction injury with swimming or treadmill training had decreased tumor necrosis factor-α and interlukin-1β expression and increased levels of heat shock protein 72 in sciatic nerve when compared with rats following chronic constriction injury without exercise training.

Effects of Exercise on Thermal and Mechanical Sensitivity

Physical exercise is often recommended to patients who have chronic pain. For instance, it has been showed that treadmill and swimming exercises were found to ameliorate spinal cord injury-induced allodynia and restore normal sensation after spinal cord contusion in rats.[23] Bement and Sluka's study demonstrated that low-intensity exercise reversed mechanical hyperalgesia in a chronic muscle pain rat model through the activation of opioid receptors.[24] This study also indicated that the CCI rats in the swimming or treadmill exercise group had attenuated thermal hyperalgesia and mechanical allodynia on day 21 after CCI when compared with the CCI rats not in exercise groups (Figs. 2 and 3). Our results are in agreement with those of previous studies, which reported that swimming exercise attenuated behavioral hypersensitivity in formalin- and nerve injury-induced animal models of

persistent pain.[1] However, moderate-intensity exercise training cannot treat but can significantly decrease deep and cutaneous tissue mechanical hypersensitivity induced via acidic saline injection.[25] In our study, the degree of reduction in decreased thermal withdrawal latencies (maximal 30%) and mechanical von Frey thresholds (< 50%) by exercise was quite small and demonstrates the relevance of the findings in relation to neuropathic pain that is still present. Our study is consistent with the findings of animal studies, in that exercise does not completely reverse the painful condition.[25]

In this study, SOSE, SOTE, CCI, CCISE or CCITE rats showed decreases in body weight compared with control or SO rats. We speculate that rats suffered from stress (e.g., CCI) and exercise, and therefore a similar time course of body weight in the SOSE, SOTE, CCI, CCISE or CCITE rats was found. Weight reduction is one of the health benefits of regular exercise that should be emphasized and reinforced by every mental health professional to their patients.[26]

Our previous study demonstrated that progressive exercise training for at least 3 weeks induces Hsp72 overexpression in many vital organs and attenuates overproduction of tissue cytokines, including TNF-alpha, and arterial hypotension during heatstroke.[27] This present study also showed that a 3-week (21-day) of exercise training decreases heat hyperalgesia and mechanical allodynia of rats following CCI (Figs. 2 and 3). Therefore, we tested sciatic nerve Hsp72, IL-1beta and TNF-alpha

expression on day 21 after CCI.

Exercise Prevents the Increase in Sciatic TNF-α and IL-1β

The appearance of cytokines in plasma or in the tissues (e.g., nerves, muscles, bones) has been reported in work-related musculoskeletal disorders in humans[28] and in animal models.[29] However, the relationships among cytokines, pain, and exercises have not been analyzed. Our results showed that treadmill or swimming exercise training attenuated TNF-α and IL-1β expression on day 21 after CCI. This evidence may provide a reasonable explanation for why exercise training can partly alleviate neuropathic pain following CCI in rats.

Evidence has been presented that neuropathic pain consequent to peripheral nerve injury is associated with local inflammation and overexpression of inflammatory cytokines.[30] It is well known that CCI induces axons to become hypersensitive and enhances retrograde transmission to cell bodies in the dorsal root ganglia (DRG) and spinal cord with subsequent release of some mediators. These mediators were able to activate the microglia cells via specific receptors and induce phosphorylation of p38 mitogen-activated protein kinase in spinal cord, where they may alter gene expression of the neurons.[31-33] Moreover, the hyperactive microglia result in the release of bioactive substances, including cytokines, prostaglandin E2 and excitatory amino acids (such as glutamate and aspartate) that alter the responses of

dorsal horn cells and maintain the neuropathic pain states.[8,34]

The cellular and molecular changes in DRG are primarily involved in neuropathic pain induction, including the signaling for ED-1+ macrophage invasion into DRG.[35] In addition, there is a growing body of evidence that cytokines contribute to both induction and maintenance of neuropathic pain derived from changes in DRG, including the activity of the primary sensory neurons and their satellite glial cells (SGC). For example, there is a significant down-regulation of substance P (SP) and calcitonin gene-related peptide (CGRP), primary afferent neurotransmitters, in the dorsal horn while there is an up-regulation of galanin (GAL) and neuropeptide Y (NPY) in DRG neurons in the neuropathic models.[36] Furthermore, unilateral CCI of the sciatic nerve-induced bilateral elevation of transcription 3 (STAT3) and interleukin-6 (IL-6) in SGC, but increased specific membrane-bound IL-6 receptor was found in SGC only in ipsilateral L4–L5 DRG.[37]

Effects of Exercise on Hsp72

Voluntary exercise for 7 days upregulates the small heat shock protein Hsp27 in the hippocampus,[38] and forced long-term exercise in mice has been reported to increase heat-shock protein/cognate 70 (Hsp/C 70).[27] Our previous study demonstrated that a 3-week, but not a 1- or 2-week, exercise training regimen conferred significant protection against the hyperthermia, decreased cardiac output,

arterial hypotension, and increased serum or tissue levels of TNF-α, and improved survival during heatstroke. A 3-week exercise training treatment is able to maintain a high level of HSP72 in several vital organs for only 3 to 4 days,[27] and a more than 3-fold overexpression of Hsp72 in the nucleus tractus solitarii (NTS) may play an important role in protecting against hemodynamic dysfunction during heatstroke onset.[18] In this study, we noted that swimming or treadmill exercise retarded mechanical allodynia and heat hyperaglesia and significantly increased Hsp72 expression on day 21 after CCI. Therefore, HSP72 expression in the sciatic nerve after 3 weeks of progressive exercise may be the factor that both decreased pro-inflammatory cytokines and improved mechanical allodynia and heat hyperaglesia throughout the sciatic nerve injury period.

In agreement with our results, exercise has been shown to have beneficial effects on an anti-inflammatory effect and neuropathic pain resolution.[1,7] Several studies demonstrated that exercise-induced modulation of heat shock factor-1 (HSF-1, an HSP's transcription factor) aggregation, subsequently affects expression of Hsp72 in multiple organs or neurons of rats.[18,27,38,39] In addition, the effect of treatment with BRX-220 (co-inducer of HSPs) on the expression of Hsp70 leads to either slowly developing analgesic actions or enhancement of recovery processes in rat following L5 spinal nerve ligation.[40] Moreover, it has been proven that the increase in the

expression of HSPs can decrease the production of the pro-inflammatory cytokines.[41] Our results revealed that swimming or treadmill exercise training significantly promoted Hsp72 expression and ameliorated the CCI-induced expression of pro-inflammatory cytokines (TNF-α and IL-1β) in sciatic nerve of rats. Furthermore, we also demonstrated that swimming or treadmill exercise training decreases CCI induced neuropathic pain. Taken together, we suggest that the protective effect of Hsp72 observed in this study was induced by the exercise-mediated aggregation of HSF-1, consequently promoting Hsp72 generation in the CCI rat. This proposed mechanism needs future investigation.

Although we did not provide direct evidence of the mechanism of Hsp72 which attenuated pro-inflammatory cytokine expression in this study, accumulated evidence shows that HSPs can decrease the production of the pro-inflammatory cytokines.[41] However, we did note that the observations in this study on thermal hyperalgesia, mechanical allodynia, TNF-α, IL-1β, and Hsp72 are, at present, merely coincident.

Our study demonstrated that swimming and treadmill exercises increase heat shock protein 72 expression in sciatic nerve of chronic constriction injury rats and ameliorate thermal hyperalgesia, mechanical allodynia, and the expressions of tumor necrosis factor-α and interlukin-1β in sciatic nerve of chronic constriction injury rats.

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FIGURE LEGENDS

Fig. 1. Body weight change with treadmill (A) and swimming (B) exercises in control, SO, SOTE, SOSE, CCITE, CCI and CCISE rats. Data are presented as mean ± SEM for 10 rats per group. (SO: sham operation; SOTE: sham operation with treadmill exercise training; SOSE: sham operation with swimming exercise training; CCI: chronic constriction injury; CCITE: chronic constriction injury with treadmill exercise training; CCISE: chronic constriction injury with swimming exercise training)

Fig. 2. Time courses of thermal withdrawal latency (A) and mechanical withdrawal threshold (B) in control, SO, SOTE, CCI and CCITE rats. (see Fig. 1 for abbreviations). The thermal withdrawal latency (s) and mechanical threshold (g) to heat and mechanical stimulation were not significantly different between the SO or SOTE group compared with the control group. Data are presented as mean ± SEM for 10 rats per group. The asterisk (*) indicates $P < 0.05$ when the CCITE group was compared with the CCI group; the symbol (+) indicates $P < 0.05$ when the CCI or CCITE group was compared with the SO group (2-way ANOVA of repeated measures followed by post *hoc* Tukey–Kramer test).

Fig. 3. Time courses of thermal withdrawal latency (A) and mechanical withdrawal threshold (B) in control, SO, SOSE, CCI and CCISE rats. (see Fig. 1 for abbreviations). The paw withdrawal latency (s) and threshold (g) to heat and

mechanical stimulation were not significantly different between the SO or SOSE group compared with the control group. Data are presented as mean ± SEM for 10 rats per group. The asterisk (*) indicates $P < 0.05$ when the CCITE group was compared with the CCI group; the symbol (+) indicates $P < 0.05$ when the CCI or CCITE group was compared with the SO group (2-way ANOVA of repeated measures followed by post *hoc* Tukey–Kramer test).

Fig. 4. The levels of TNF-α (A) and IL-1β (B) on day 21 after CCI in sciatic nerve in different groups of rats: CCI, CCITE and CCISE (see Fig. 1 for abbreviations). The values are presented as mean ± SEM for 5 rats per group. Symbols (*,**) indicate $P < 0.05$ and $P < 0.01$, respectively when the CCITE or CCISE group was compared with the CCI group (1-way ANOVA followed by post hoc Tukey's test).

Fig. 5. The levels of Hsp72 in sciatic nerve on day 21 after CCI in different groups of rats: CCI, CCITE and CCISE (see Fig. 1 for abbreviations). The values are presented as mean ± SEM for 5 rats per group. Symbols (*,**) indicate $P < 0.05$ and $P < 0.01$, respectively, when the CCISE or CCITE group was compared to the CCI group (1-way ANOVA followed by post hoc Tukey's test).