Neuroscience Letters 643 (2017) 27–31

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Incline treadmill exercise suppresses pain hypersensitivity associated with the modulation of pro-inflammatory cytokines and anti-inflammatory cytokine in rats with peripheral nerve injury

Kun-Ling Tsai[a,1], Po-Ching Huang[a], Li-Kai Wang[b], Ching-Hsia Hung[a,c,1], Yu-Wen Chen[d,e,*]

[a] Department of Physical Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[b] Department of Anesthesiology, Chi Mei Medical Center, Tainan, Taiwan
[c] Institute of Allied Health Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan
[d] Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan
[e] Department of Physical Therapy and Graduate Institute of Rehabilitation Science, College of Health Care, China Medical University, Taichung, Taiwan

HIGHLIGHTS

- Increased incline improved the anti-nociceptive effects of treadmill running.
- Inclined treadmill exercise attenuated levels of sciatic nerve pro-inflammatory cytokines.
- Inclined exercise increased the level of an anti-inflammatory cytokine.

ARTICLE INFO

Article history:
Received 5 December 2016
Received in revised form 30 January 2017
Accepted 8 February 2017
Available online 12 February 2017

Keywords:
Incline treadmill running
Mechanical allodynia
Thermal hyperalgesia
Interleukin-10
Interleukin-6
Tumor necrosis factor-α

ABSTRACT

We aimed to investigate the impact of 3 weeks of incline treadmill exercise (TE) on withdrawal responses elicited by thermal and mechanical stimuli, and on anti-inflammatory cytokine (interleukin-10, IL-10) and pro-inflammatory cytokines (IL-6 and tumor necrosis factor-alpha [TNF-α]) expression in the sciatic nerve of rats underwent chronic constriction injury (CCI). Group 1 received a sham-operation where the sciatic nerve was exposed but not ligated, while Group 2 underwent a sham-operation followed by exercising on an 8%-incline treadmill (TE8). Group 3 underwent only the CCI procedure, and Groups 4 and 5 underwent the CCI procedure followed by exercising on an 0%-incline treadmill (TE0) and TE8, respectively. Mechanical and thermal sensitivity and protein expression of IL-10, IL-6 and TNF-α were evaluated on postoperative days 12 and 26. Among the five groups, Group 5 displayed the least weight gain. Compared with Group 3, Group 5 had smaller decreases in mechanical withdrawal thresholds and heat withdrawal latencies. The CCI rats who received TE at 8% incline showed the downregulation of TNF-α and IL-6 in their sciatic nerves on postoperative days 12 and 26, as was found in the Group 3 rats. TE at 8% incline also prevented the downregulation of IL-10 in their sciatic nerves on postoperative day 12. The results demonstrated that increased incline improves the anti-nociceptive effects of treadmill running. Inclined exercise reduces the levels of pro-inflammatory cytokines and increases the level of an anti-inflammatory cytokine.

Neuropathic pain is caused by peripheral nerve injury, a condition characterized by mechanical allodynia and thermal hyperalgesia [44]. Central and peripheral sensitization is important to the onset and development of neuropathic pain [23,44]. Peripheral sensitization produced pain hypersensitivity occurred around the site of tissue injury and/or inflammation [20,35,46]. However, the therapeutic strategies were commonly ineffective according to their poor response rates [26,30]. Interestingly, physical exercise has been known to attenuate many types of acute and chronic pain, including chronic muscle pain [2], diabetic painful neuropathy [4,5,37], peripheral neuropathic pain [6,24] and persistent postoperative pain [7,10] in rodents.

* Corresponding author at: Department of Physical Therapy, College of Health Care, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan.
E-mail address: cywhwok@mail.cmu.edu.tw (Y.-W. Chen).
[1] Kun-Ling Tsai and Ching-Hsia Hung contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2017.02.021
0304-3940/

Increasing evidence suggests that cytokine signalling and neuroinflammation play an important role in triggering neuropathic pain [26,28,36,38]. Furthermore, pro-inflammatory and anti-inflammatory cytokines are associated with neuropathic pain [26,38,45]. Interestingly, the therapeutic administration of interleukin-10 (IL-10), which was effective for therapizing neuropathic pain, produced a downregulation of voltage-dependent Na^+ channels in dorsal root ganglion neurons of rats [40]. Following this further, our previous study [6] indicated that treadmill exercise (0% incline) in the early phase of interventions reduced thermal hyperalgesia, but not mechanical allodynia. We proposed that treadmill was inclined as opposed to flat and had a greater effect. Therefore, we aimed to (a) investigate if increased "dose" of training exercise (14–16 m/min with 8% incline grade for 30 min) has a stronger effect on pain modulation than the effects previously shown by the group with TE without incline, and (b) investigate possible mechanisms in terms of locally produces anti-inflammatory and pro-inflammatory cytokine expression of the sciatic nerve in a constricted-based (chronic constriction injury, CCI) animal model of neuropathic pain.

The investigative protocols were approved *via* the Experimental Animal Committee of the National Cheng Kung University (Tainan, Taiwan) and were conducted according to the IASP ethical guidelines [47]. Experiments were performed on male Sprague-Dawley rats (National Laboratory Animal Center, Taipei, Taiwan) weighing 285–335 g. Animals were housed in the animal housing facility of the same University with a 12-h light-dark cycle (light on at 6:00 a.m.), a climate-controlled room maintained at 22 °C and the approximately 50% relative humidity. Food and water were available *ad libitum* to them until the time of experiments.

Seventy rats were blindly divided into 5 groups. Group 1, rats underwent a sham-operation where the sciatic nerve was exposed but not ligated. Group 2, rats underwent a sham-operation followed by exercising on an 8%-incline treadmill (TE8). Group 3, rats underwent CCI surgery but received no other intervention. Group 4, rats underwent the CCI procedure followed by exercising on a 0%-incline treadmill (TE0). Group 5, rats underwent the CCI procedure followed by TE8. Beginning on postoperative day 6 (POD 6) and continuing daily for the next 3 weeks, Groups 2, 4 and 5 had TE alone for 30 min. In the same direction, the onset of neuropathic pain occurred in rats three days after CCI surgery and remained for up to one month [6]. Animals were assessed twice for heat withdrawal latencies, mechanical withdrawal thresholds and body weight on the day before surgery, and the two measurements were averaged to obtain a single baseline. Subsequently, Groups 1–5 were assessed on PODs 5, 12, 19 and 26. The IL-10, IL-6 and tumor necrosis factor-alpha [TNF-α] levels in the sciatic nerve was detected on PODs 12 and 26.

After animals were anesthetized with pentobarbital sodium (45 mg/kg, i.p.), four ligatures, surrounded by four chromic gut sutures, were tied around the right sciatic nerve as described by Bennett & Xie [3]. After the CCI procedure, the skin incision was closed with wound clips, and animals were returned to their cages for recovery. An experienced experimenter, blinded to which group the rat belonged, handled all the neurobehavioral evaluations. After a period of 5–7 days of adapting to experimental environment and investigator, rats were assessed for mechanical withdrawal thresholds and thermal withdrawal latencies.

To measure mechanical withdrawal thresholds, rats were placed individually in a clear, plexiglass conditioning chamber on a wire-mesh floor. In brief, a von Frey hair filament (Anesthesiometer, Somedic AB, Sweden) was used to probe the lateral-plantar area of the right hindpaw for 3 s. The paw withdrawal threshold (gram) was testified by performing the next thicker von Frey hair filament, which always elicited paw withdrawal [9,12]. Additionally, thermal withdrawal latencies were evaluated according to the Hargreaves' Method [14]. In brief, the lateral plantar surface of the right hindpaw was exposed to a constant intensity of radiant thermal source by the Hargreaves' plantar apparatus (Ugo Basile, Comerio, Italy). The cut-off times were set at 20 s to prevent tissue from damage [8]. The paw withdrawal latency (sec) was constructed when the rat withdrew its right hindpaw.

Rats were trained to run on the treadmill (treadmill exerciser T510, Diagnostic & Research Instruments, Singa, Taiwan) daily for three weeks, beginning on POD 6 and continuing until POD 26. The exercise protocol was set the intensity of 14–16 m/min with/without 8% incline grade for 30 min [42]. When necessary, some rats were gently prodded in its hind legs to encourage them to run for compliance.

After animals were anesthetized with urethane (1.67 g/kg, i.p.), thirty rats were killed on POD 12, while another thirty rats were killed on POD 26. Before the 4 ligatures were removed, the right sciatic nerve, proximal to the trifurcation (about 1 cm), was cut. The tissues were immediately stored at −80 °C for next assay.

After adding homogenization buffer (4 °C), tissue samples were homogenized and centrifuged for preparing the protein assay. The protein concentrations (supernatant) were analyzed by the Lowry protein assay. Each plate was inserted into a plate reader (Molecular Device Spec 383, Sunnyvale, CA, USA) in order to read the optical density of each well at an absorbance of 750 nm [11,27]. The experimental protocols were practiced according to the manufacturer's recommended procedures. The concentrations of IL-10, IL-6 and TNF-α in the supernatant were detected using the DuoSet® ELISA Development Kit (R&D Systems, Minneapolis, MN, USA) [16,17].

Data are shown as mean ± S.E.M. of N observations unless noted otherwise and were analyzed by one-way or two-way analysis of variance (ANOVA) of repeated measures. Alpha = 0.05 was indicated as the significance threshold for ANOVA and was also noted as the threshold for evaluating post *hoc* significance (post Bonferroni correction). The statistical analysis was used Statistical Package for the Social Sciences (SPSS for Windows, version 17.0; SPSS, Inc., Chicago, IL, USA).

Groups 1–5 showed significantly weight gain over time (Fig. 1A). The rate of weight gain slowed down in sham + TE8 (Group 2), CCI-only (Group 3), or CCI + TE0 (Group 4) group compared with the sham-CCI (Group 1) group on PODs 19 and 26 (all $p < 0.01$, Fig. 1A). The rate of weight gain slowed down significantly in the CCI treated with TE8 (Group 5) rats compared with Groups 1, 2, 3 and 4 (all $p < 0.01$, Fig. 1A).

Mechanical withdrawal thresholds and heat withdrawal latencies in sham, sham + TE8, CCI, CCI + TE0 and CCI + TE8 groups (Fig. 1B and C) were analyzed using two-way repeated measures ANOVA and displayed significant main effect for groups ($F_{4,35} = 31.66$, $p < 0.0001$; $F_{4,35} = 41.86$, $p < 0.0001$), time ($F_{4,140} = 51.26$, $p < 0.0001$; $F_{4,140} = 42.58$, $p < 0.0001$) and significant interactions ($F_{16,140} = 6.66$, $p < 0.0001$; $F_{16,140} = 7.82$, $p < 0.0001$), respectively. The post-*hoc* comparisons exhibited significant differences among sham, sham + TE8, CCI, CCI + TE0 and CCI + TE8 groups ($p < 0.01$, Fig. 1B and C).

Compared with the CCI-operated rats (Group 3), the sham-CCI (Group 1), sham + TE8 (Group 2) and CCI + TE8 (Group 5) rats on PODs 12 and 26 showed a significant increase in the IL-10 level (all $p < 0.05$, Fig. 2A). The CCI + TE0 (Group 4) and CCI + TE8 (Group 5) rats exhibited an increase (all $p < 0.05$) in the IL-10 level on POD 12 compared with the sham-CCI (Group 1) rats, whereas only CCI + TE8 (Group 5) rats displayed an increase ($p < 0.05$, Fig. 2A) in the IL-10 level on POD 26.

Furthermore, IL-6 and TNF-α expression in the sciatic nerve significantly increased in the CCI-only rats (Group 3) (all $p < 0.001$) and the CCI + TE0 rats (Group 4) (all $p < 0.001$) when compared with the sham-CCI rats (Group 1) or the sham-TE8 rats (Group 2) on PODs 12 and 26 (Fig. 2B and C). By comparison, the CCI rats undergoing