Neuroscience Letters 589 (2015) 62–66

Contents lists available at ScienceDirect

Neuroscience Letters

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

Reasearch article

High frequency transcutaneous electrical nerve stimulation with diphenidol administration results in an additive antiallodynic effect in rats following chronic constriction injury

Heng-Teng Lin[a,b], Chong-Chi Chiu[c,d], Jhi-Joung Wang[e], Ching-Hsia Hung[f,*,1], Yu-Wen Chen[e,g,1]

[a] Department of Physical Medicine and Rehabilitation, Madou Sin-Lau Hospital, Tainan, Taiwan
[b] Department of Nursing, Chung Hwa University of Medical Technology, Tainan, Taiwan
[c] Department of General Surgery, Chi Mei Medical Center, Tainan and Liouying, Taiwan
[d] Department of Cosmetic Science, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
[e] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[f] Department of Physical Therapy, National Cheng Kung University, No.1 Ta-Hsueh Road, Tainan, Taiwan
[g] Department of Physical Therapy, China Medical University, Taichung, Taiwan

HIGHLIGHTS

- High frequency TENS and/or diphenidol inhibited mechanical hypersensitivity.
- High frequency TENS or the combination of diphenidol and TENS reduced TNF-α contents.
- TENS decreased the requirement of doses of diphenidol to block mechanical allodynia.

ARTICLE INFO

Article history:
Received 4 December 2014
Received in revised form 7 January 2015
Accepted 9 January 2015
Available online 14 January 2015

Keywords:
Combination
High frequency
Transcutaneous electrical nerve stimulation
Diphenidol
Mechanical allodynia
Tumor necrosis factor-α

ABSTRACT

The impact of coadministration of transcutaneous electrical nerve stimulation (TENS) and diphenidol is not well established. Here we estimated the effects of diphenidol in combination with TENS on mechanical allodynia and tumor necrosis factor-α (TNF-α) expression. Using an animal chronic constriction injury (CCI) model, the rat was estimated for evidence of mechanical sensitivity *via* von Frey hair stimulation and TNF-α expression in the sciatic nerve using the ELISA assay. High frequency (100 Hz) TENS or intraperitoneal injection of diphenidol (2.0 μmol/kg) was applied daily, starting on postoperative day 1 (POD1) and lasting for the next 13 days. We demonstrated that both high frequency TENS and diphenidol groups had an increase in mechanical withdrawal thresholds of 60%. Coadministration of high frequency TENS and diphenidol gives better results of paw withdrawal thresholds in comparison with high frequency TENS alone or diphenidol alone. Both diphenidol and coadministration of high frequency TENS with diphenidol groups showed a significant reduction of the TNF-α level compared with the CCI or HFS group ($P < 0.05$) in the sciatic nerve on POD7, whereas the CCI or high frequency TENS group exhibited a higher TNF-α level than the sham group ($P < 0.05$). Our resulting data revealed that diphenidol alone, high frequency TENS alone, and the combination produced a reduction of neuropathic allodynia. Both diphenidol and the combination of diphenidol with high frequency TENS inhibited TNF-α expression. A moderately effective dose of diphenidol appeared to have an additive effect with high frequency TENS. Therefore, multidisciplinary treatments could be considered for this kind of mechanical allodynia.

Transcutaneous electrical nerve stimulation (TENS) has been known to be an effective and easy analgesic technique to alleviate pain of patients. TENS, either low (2 ~ 10 Hz) or high (80 ~ 100 Hz) frequency of stimulation, is used by percutaneous or surface electrodes positioned ipsilateral, bilaterally to the location of pain or

* Corresponding author. Tel.: +886 6 2353535x5939; fax: +886 6 2370411.
E-mail address: chhung@mail.ncku.edu.tw (C.-H. Hung).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2015.01.026
0304-3940/

contralateral to the location of pain [37]. In addition, the studies reported that daily TENS to contralateral application of exclusively high-frequency prevented the progress of tactile allodynia in rats after chronic constriction injury (CCI) [37,38].

Diphenidol, an antiemetic agent, is widely used in the Latin American to treat the patients with Meniere's disease and labyrinthopathies [23]. Furthermore, diphenidol blocked the voltage-gated sodium, potassium, and calcium channels *in vitro* [19,20] and elicited spinal blockades of motor function and nociception *in vivo* [20]. It has been known that several sodium channel blockers can reduce pain in neuropathic and inflammatory rats [1,15,25,26,41]. Both lidocaine and mexiletine are sodium channel blockers and can attenuate neuropathic pain in patients [11,14]. Recently, we revealed that systemic diphenidol completely suppressed mechanical allodynia evoked by peripheral nerve injury [10].

It has been known that the varying responses of activated inflammatory cytokines overexpression and local inflammation were elicited by neuropathic pain in glial cells and activated macrophages [13,18]. Treatment of nerve injury with anti-inflammatory cytokine or pro-inflammatory inhibitors attenuated pain [33,39], whereas pro-inflammatory cytokines administration (*i.e.*, TNF-α, tumor necrosis factor-α) evoked pain [16,33]. Additionally, the CCI rats displayed increased TNF-α expression of the sciatic nerves [10].

Drugs (*i.e.*, tricyclic antidepressants) for treatment of neuropathic pain may produce side effects [17]. It can be accepted to decrease in dose requirement of drugs for giving a nonpharmacologic intervention of managing neuropathic pain and TNF-α expression. The purpose of this experiment was to examine the impact of the combination of high-frequency TENS and diphenidol in an animal model of neuropathic pain. The TNF-α expression in the sciatic nerve of the CCI rats was assessed as well.

The male Sprague–Dawley rats (200–250 g) were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and housed within the laboratory animal facility of the National Cheng Kung University, with a 12 h light/dark cycle with lights on at 6:00 AM, room temperature (24 C), and controlled humidity (∼50% relative humidity). The investigative methods and procedures of the experiment were agreed *via* the Institutional Animal Care and Use Committee of the National Cheng Kung University. Diphenidol hydrochloride was obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO) and was dissolved in normal saline as solution prior to intraperitoneal injections.

The animals were under 2% isoflurane anesthesia. High frequency TENS (100 Hz) by the self-adhesive surface electrodes was administered to the animals using the TENS machine (Trio 300, Ito Co., Tokyo, Japan) when the stimulator setting was at almost continuous stimulation *via* without any preprogrammed options [7,9]. The intensity of TENS stimulation (30–40 μA passed by 45 mm and 5 mm electrodes) was kept at 80% of that enough to elicit an obvious muscle contraction. The pulse duration was set at 100 μs for 20 min [34]. The positions of two surface electrodes were on the denuded, presumably uninvolved, skin overlying the right paraspinal musculature between several lumbar vertebras (L1–L6) [40].

The experimenters were blind for animal assignments to five groups. The 1st group, the CCI animals ($n = 8$) received constrictive sciatic nerve injury. The 2nd group, the sham-operated animals underwent the same procedure with the exception of the ligation of the sciatic nerve ($n = 8$). The 3rd group, HFS rats ($n = 8$) received CCI and high frequency TENS by two surface electrodes positioned on the skin overlying the dorsal region of the right paraspinal musculature. The 4th group, diphenidol rats ($n = 8$) received intraperitoneal injection of diphenidol 2.0 μmol/kg after CCI, and the fifth group, HFS + diphenidol rats was treated high-frequency TENS and diphenidol 2.0 μmol/kg after CCI ($n = 8$).

On day 1 after CCI, animals received either intraperitoneal injection of diphenidol 2.0 μmol/kg or high frequency TENS for 20 min once per day and then daily for the next 13 days. In our previous experiment, marked paw mechanical hypersensitivity in rats started 1 day after animals had been received CCI and lasted for up to 30 days [4]. To get the baseline value of the mechanical withdrawal threshold, the rat was assessed twice for the mechanical withdrawal threshold before the actual day of surgery and on the day of the operation, and the two values were averaged. Then the animals were assessed again on 3, 7, 11, and 14 days after the operation. The procedure of CCI, which caused marked mechanical allodynia, was observed on postoperative day 10 (POD10) as previously described [2]. The last TENS or diphenidol treatment occurred 14 days after CCI. On PODs 7 (30 rats; $n = 6$ per group for tissue analysis) and 14 (40 rats; $n = 8$ per group for the behavior testing), the total of 70 animals were employed in this study.

The animals were under 3% isoflurane anesthesia to operate the rat model of CCI. In brief, peripheral nerve damage was produced in the rat through placing four loosely constrictive ligatures around the sciatic nerve [2]. At the end of the study the sciatic nerve of every rat receiving CCI was checked again to confirm the integrity of the sutures [4,10].

For the evaluation of the mechanical withdrawal thresholds, the rats were placed individually in a clear plexiglass chamber (22 cm [length] × 22 cm [width] × 13.3 cm [height]) and supported by a wire mesh floor (40 cm [width] × 50 cm [length]). Behavioral assessments were performed from 8 a.m. to 11 a.m. A trained experimenter, who did not know the treatment groups, took charge of neurobehavioral examinations to keep the experiment consistency. The electronic von Frey filament (IITC Life Science Instruments, Woodland Hills, CA) was tested to apply to the lateral aspect of the plantar surface of the hind paw while the paw withdrawal threshold was recorded [6,8].

The rats were under urethane (1.67 g/kg, i.p.) anesthesia and sacrificed to obtain the affected sciatic nerve (about 1 cm). The protein concentration in the supernatant was determined by the Lowry protein assay. These investigative protocols were achieved according to the instructions. TNF-α concentration in the supernatant was quantified by the DuoSet® ELISA Development Kit (R&D Systems, Minneapolis, MN). Each plate was separately pushed into the plate reader to read optical density using a 450-nm filter [3,5]. The parameters were analyzed by Ascent Software (London, UK) for iEMS Reader (Molecular Device Spec 383, Sunnyvale, CA, USA) and then it was expressed in pg/mg protein of duplicate specimens.

The data are recorded as the mean ± S.E.M. of N observations unless noted otherwise. Evaluation for statistical significance between multiple investigative groups was analyzed *via* one-way or two-way analysis of variance (ANOVA) with a Tukey–Kramer multiple comparison *post hoc* tests. A statistical software, SPSS for Windows (version 17.0, SPSS, Inc Chicago, IL, USA), was run, and the difference between group was considered to be significant at values of $P < 0.05$.

The CCI rats on POD7 showed a decrease in the degree (maximal 45%) of the mechanical withdrawal threshold in comparison with the baseline value (Fig. 1). Compared with the sham rats, CCI rats demonstrated a marked decrease in the mechanical withdrawal thresholds (Fig. 1, $P < 0.05$, 2-way repeated measures ANOVA). Daily application of high frequency TENS, diphenidol, or the combination attenuated CCI-evoked lowered paw withdrawal thresholds from day 3 to day 14 (Fig. 1, $P < 0.05$, 2-way repeated measures ANOVA). Moreover, in HFS + Diphenidol and sham groups there was no predominant difference in the mechanical nociceptive thresholds (Fig. 1, $P > 0.05$, 2-way repeated measures ANOVA). The effect of the combination (HFS + Diphenidol) is better than each along (HFS or Diphenidol) on paw withdrawal thresholds from day 3 to day 14 (Fig. 1, $P < 0.05$, 2-way repeated measures ANOVA).