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ORIGINAL ARTICLE

Analysis of oxytocin and oxytocin receptor expressions after hand therapy treatment in a mouse model of chemotherapy-induced peripheral neuropathy

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Abstract

OBJECTIVES: We aimed to investigate the effect of hand therapy (HT) on oxytocin and oxytocin receptor expression in a chemotherapy-induced peripheral neuropathy (CIPN) model mouse.

METHODS: CIPN model mouse was induced by intraperitoneal injection of paclitaxel (PTX; 4 mg/kg) on days 0, 2, 4 and 6 of the study. HT was performed on the CIPN mice once daily for 14 consecutive days, starting on day 8 after the PTX injection. Following HT,we observed the oxytocin and oxytocin receptor expressions in the skin and dorsal root ganglion (DRG) and assessed the oxytocin in the serum.

RESULTS: Oxytocin expressions in the skin and DRG significantly increased in the PTX + HT group compared to that in the Non-PTX and PTX groups. Additionally, oxytocin receptor expressions in the skin and DRG significantly increased in the PTX + HT group compared to that in the PTX group. Furthermore, the PTX + HT group showed significantly higher serum oxytocin concentration than the Non-PTX and PTX groups.

CONCLUSION: The present study showed that HT reversed PTX-induced suppression of oxytocin receptor expressions and HT increased oxytocin expression locally and its systemic level. Such results connect the gap and previous suggestions that HT improving neurological symptoms are related to oxytocin levels.

INTRODUCTION

Taxane-type anticancer drugs, such as paclitaxel, often used in patients with breast cancer, are also known to cause peripheral neuropathy. Peripheral neuropathy caused by anticancer agents is called chemotherapy-induced peripheral neuropathy (CIPN), which not only significantly reduces the quality of life of patients, but also forces them to reduce or discontinue medication, resulting in inadequate therapeutic effects (Fumagalli *et al.* 2020; Maihöfner *et al.* 2021). However, no precise treatment has been established for CIPN, and effective coping strategies and therapies are currently being sought. A clinical trial indicated

that hand therapy treatment (HT), a non-pharmacological therapy, significantly improved the numbness of CIPN symptoms (Sasaki *et al.* 2020). In addition, we established a model that showed the improvement of thermal hyperalgesia and mechanical allodynia of HT against CIPN (Shinouchi *et al.* 2023). These improving effects of HTmay be attributed to an increase in oxytocin release (Uvnäs-Moberg *et al.* 2014; Li *et al.* 2019). In this study, we applied HT in a CIPN model mouse to investigate whether it has an effect on oxytocin and oxytocin receptor expression in the skin and dorsal root ganglion and serum oxytocin level.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice aged 9 weeks were purchased from CLEA Japan, Inc. (Tokyo, Japan). The mice were habituated to laboratory conditions for 1 week before the commencement of the study. CIPN model mouse was induced by intraperitoneal (i.p.) injection of paclitaxel (PTX; 4 mg/kg) on days 0, 2, 4 and 6 of the study. The PTX (TAXOL®, Bristol-Myers Squibb Company, Tokyo, Japan) was diluted in saline to a concentration of 0.4 mg/mL and injected intraperitoneally at a volume of 10 mL/kg The non-CIPN, mice were injected with an equal volume of saline. The Non-PTX (Non-CIPN model mouse + Non-hand therapy treatment) group, the PTX (CIPN model mouse + Non-hand therapy treatment) group and the PTX + HT (CIPN model mouse + hand therapy treatment) group were comprised of three (Figure 1 and 2) or eight (Figure 3) mice for each group. This study was conducted in accordance with the Animal Experiment Ethics Committee of Showa University Animal Experiment Regulations. (Permit number 23016).

Hand therapy treatment method

The Sophia method of HT was performed under isoflurane anesthesia (initiation, 5%; maintenance, 2%), based on a previous report (Shinouchi *et al.* 2023). We carried out the HT procedure while wearing surgical gloves. In brief, 1)the right index finger and middle finger pressed on the sole of the hind paw and the left index finger pressed on its instep for 10 seconds. Then, 2) the instep of the hind paw was supported with the left index finger and rubbed its sole three times in a circular motion with the right index finger in the order of heel → arch → toetip (3 sets). Then3)Macadamia Nut OiL (GREEN FLASK Co., Ltd., Tokyo, Japan) was applied all over the index finger, and4)the instep of the hind paw was supported with the left index finger and rubbed up from the toetip to the heel and rubbed down from the heel to the toetip with the right index finger (3 round trips). Then, 5) the same procedure as above in 2) was performed while 6) supporting the malleolus medialis or lateralis of the hind paw with one index finger, and rubbing the malleolus lateralis or medialis of the hind paw three times in a circular motion with one index finger (3 sets). Then, 7) the procedure was performed in the order of 4) → 2) → 4). Finally, 8) the right index finger and the middle finger pressed on the sole of the hind paw and the left index finger on its instep and make a pulling out motion from the heel to the toetip with the top and bottom fingers. Steps 1 to 8 were performed on one hind paw for 7 min and 30 seconds each. The hind paw was rubbed at a speed of 5 cm/s and a force within the range of 0.7 to 0.8 N.

Tissue sampling

On day 21 after the PTX treatment, the skin on the instep of the hind limb and the DRG were removed and fixed in 10% formalin neutral buffer (pH 7.4; Fujifilm Wako Pure Chemicals Corporation, Osaka, Japan) for 24 h. The harvested tissues were then embedded in paraffin blocks and thin sections at 3 microns were attached to glass slides Formalin-fixed, paraffin-embedded sections (3-μm) were stained with hematoxylin and eosin (HE; Sakura Finetek Japan Co., Ltd., Tokyo, Japan). The sections were observed under an all-in-one microscope BZ-X810 (Keyence Corporation, Osaka, Japan) to characterize any histological changes.

Immunohistochemical (IHC) staining

Formalin-fixed, paraffin-embedded sections (3-μm) were dewaxed with xylene and ethanol. Then, 0.3% of hydrogen peroxide was added dropwise to the sections and placed in an incubator at room temperature for 5 min. After being washed with Tris-Buffered Saline (TBS), Protein Block serum-free (X0909, Agilent Technologies, Inc., CA, USA) was added dropwise to the sections and placed in an incubator at room temperature for 5 min to quench endogenous peroxide activity. The primary antibodies, anti-oxytocin receptor antibody rabbit polyclonal ab217212 (1:300, Abcam, Tokyo, Japan), and anti-oxytocin antibody goat polyclonal Ls-C112933 (1:100, Funakoshi, Tokyo, Japan), diluted with the antibody diluent with background reducing component (Agilent Technologies, Inc., CA, USA) buffer added to the sections and placed in an incubator at room temperature for 1 h. After being washed several times with TBS, hoechst33342 solution (1:100, DOJINDO, Kumamoto, Japan) for staining nuclear and HRP-conjugated secondary antibodies, alexa fluor 555 goat anti-rabbit IgG (1:100, Invitrogen, Tokyo, Japan) for anti-oxytocin receptor antibody and polyclonal rabbit anti-goat Immunoglobulins/FITC (1:400, Dako, Tokyo, Japan) for anti-oxytocin antibody were added to the sections and placed in an incubator at room temperature for 30 min. The sections were then washed and covered using Fluoromount (Cosmo Bio Co., LTD., Tokyo, Japan), observed with an all-in-one microscope BZ-X810 (Keyence Corporation, Osaka, Japan), and quantified as brightness per unit area.

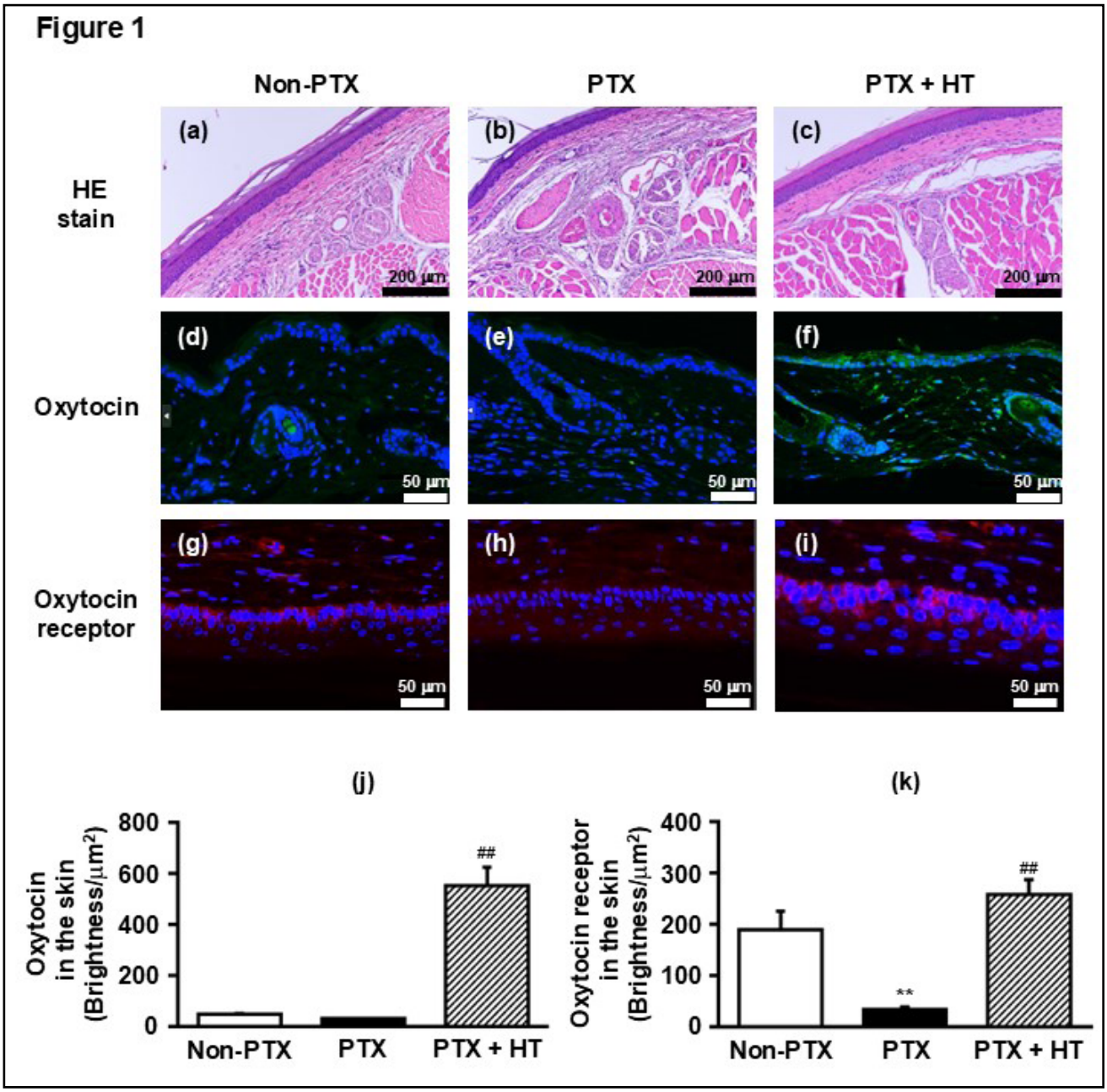

Fig. 1. Oxytocin and oxytocin receptor expression in the skin of the CIPN model mouse. HT was performed on the CIPN mice once daily for 14 consecutive days, starting on day 8 after the PTX injection. Expression of oxytocin and oxytocin receptor by the IHC staining were performed on day 21 after the PTX treatment. Representative images of HE staining (a, b, and c) and IHC staining of oxytocin (d, e, and f) and oxytocin receptor (g, h, and i) **in the skin** are shown. The green fluorescence indicates the expression of oxytocin. The red fluorescence indicates the expression of oxytocin receptor. The nuclei indicate blue fluorescence. Analysis of oxytocin (j) and oxytocin receptor (k) expression in the skin was quantified as brightness per unit area. The data are represented as the mean ± SEM (n = 3). **P < 0.01 vs Non-PTX group; ##P < 0.01 vs PTX group by one-way ANOVA followed by post hoc Bonferroni test. Magnifications: (a, b, and c) are 200×; (d, e, and f) are 400×; (g, h, and i) are 400×.

Measurement of serum oxytocin levels

On day 21 after the PTX treatment, blood was collected from the abdominal aorta under anesthesia and placed in Bloodsepar, (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) centrifuged for 15 min at 800 g, then serum was separated and stored. Oxytocin concentrations were measured by ELISA using an oxytocin enzyme immunoassay Kit (ARBOR ASSAYS., Michigan, USA).

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated using a one-way analysis of variance (ANOVA), followed by post hoc Bonferroni test. $p < 0.05$ was considered statistically significant.

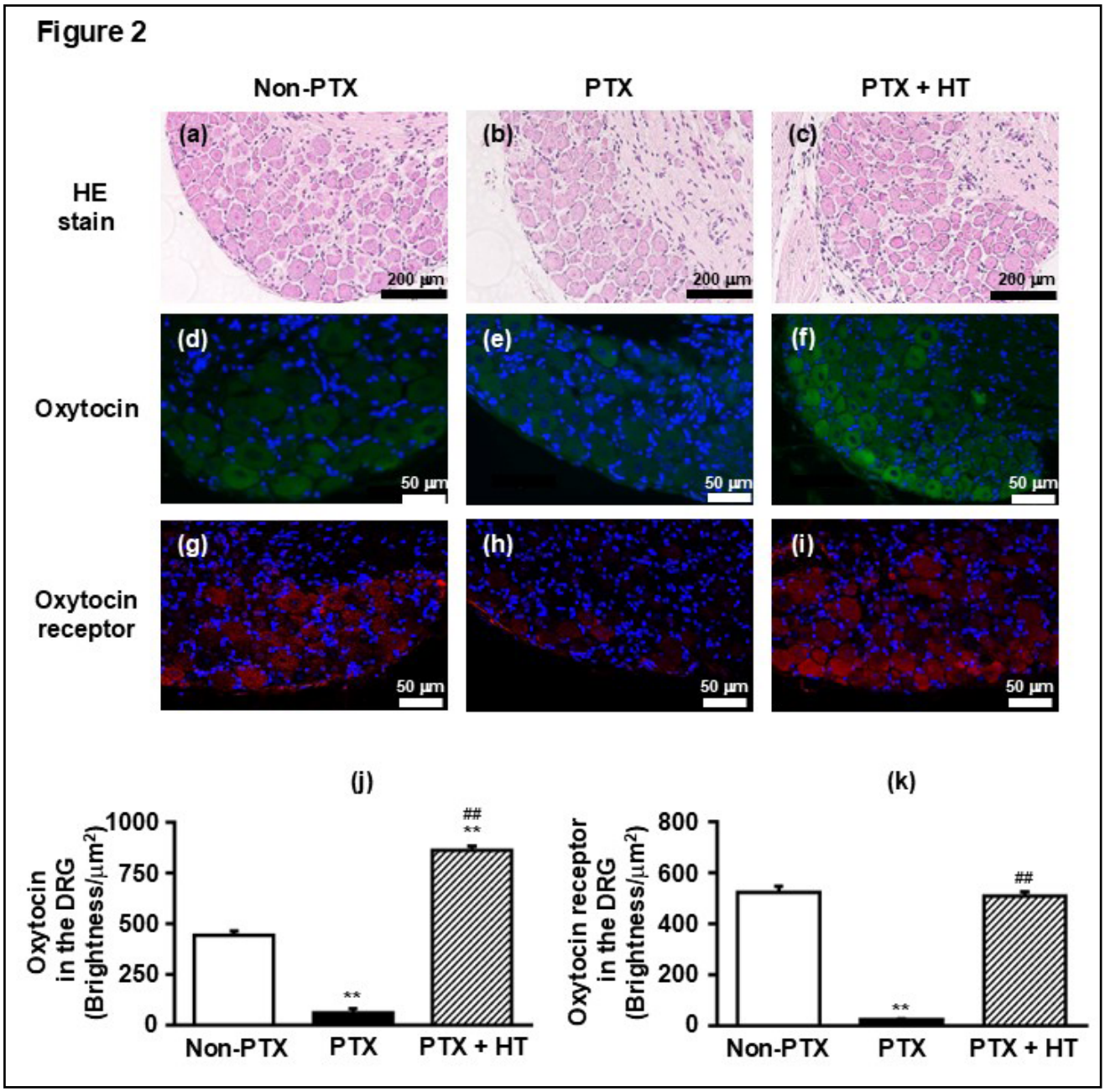

Fig. 2. Oxytocin and oxytocin receptor expression in the DRG of the CIPN model mouse. HT was performed on the CIPN mice once daily for 14 consecutive days, starting on day 8 after the PTX injection. Expression of oxytocin and oxytocin receptor by the IHC staining were performed on day 21 after the PTX treatment. Representative images of HE staining (a, b, and c) and IHC staining of oxytocin (d, e, and f) and oxytocin receptor (g, h, and i) **in the DRG** are shown. The green fluorescence indicates the expression of oxytocin. The red fluorescence indicates the expression of oxytocin receptor. The nuclei indicate blue fluorescence. Analysis of oxytocin (j) and oxytocin receptor (k) expression in the DRG was quantified as brightness per unit area. The data are represented as the mean ± SEM (n = 3). **P < 0.01 vs Non-PTX group; ##P < 0.01 vs PTX group by one-way ANOVA followed by post hoc Bonferroni test. Magnifications: (a, b, and c) are 200×; (d, e, and f) are 400×; (g, h, and i) are 400×.

RESULT

Oxytocin and oxytocin receptor expressions in the skin

The effect of HT on the oxytocin and oxytocin receptor expressions in the skin on day 21 is shown in Figure 1. Oxytocin expressions in the skin significantly increased in the PTX + HT group ($p < 0.01$, 551.5 ± 72.9 Brightness/μm²) compared to that in the Non-PTX group (49.2 ± 2.9 Brightness/μm²) and PTX group (31.4 ± 1.1 Brightness/μm²). Oxytocin receptor expressions in the skin significantly decreased in the PTX group ($p < 0.01$, 31.6 ± 5.6 Brightness/μm²) compared to that in the Non-PTX group (219.4 ± 28.7 Brightness/μm²). Oxytocin receptor expressions in the skin significantly increased in the PTX + HT group ($p < 0.01$, 257.2 ± 29.7 Brightness/μm²) compared to that in the PTX group.

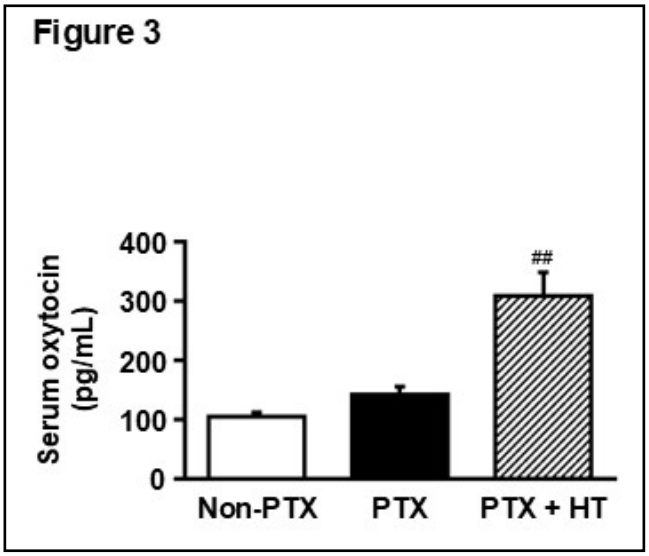

Fig. 3. Changes in serum oxytocin in the CIPN model mouse. HT was performed on the CIPN mice once daily for 14 consecutive days, starting on day 8 after the PTX injection. The measurement of serum oxytocin levels was performed on day 21 after the PTX treatment. The data are represented as the mean ± SEM (n = 8). ##P < 0.01 vs PTX group by one-way ANOVA followed by post hoc Bonferroni test.

Oxytocin and oxytocin receptor expressions in the DRG

The effect of HT on the oxytocin and oxytocin receptor expressions in the DRG on day 21 is shown in Figure 2. Oxytocin expressions in the DRG significantly decreased in the PTX group ($p < 0.01$, 61.1 ± 19.4 Brightness/μm^2) compared to that in the Non-PTX group (443.3 ± 19.4 Brightness/μm^2). Oxytocin expressions in the DRG significantly increased in the PTX + HT group ($p < 0.01$, 861.9 ± 18.3 Brightness/μm^2) compared to that in the Non-PTX group and PTX group. Oxytocin receptor expressions in the DRG significantly decreased in the PTX group ($p < 0.01$, 24.7 ± 2.5 Brightness/μm^2) compared to that in the Non-PTX group (523.1 ± 23.6 Brightness/μm^2). Oxytocin receptor expressions in the DRG significantly increased in the PTX + HT group ($p < 0.05$, 508.2 ± 18.0 Brightness/μm^2) compared to that in the PTX group.

Serum oxytocin

The effect of HT on the serum oxytocin on day 21 shown in Figure 3. The PTX + HT group ($p < 0.01$, 309.2 ± 38.9 pg/mL) showed significantly higher serum oxytocin concentration than the Non-PTX group (105.8 ± 7.0) and PTX group (142.60 ± 12.9 pg/mL).

DISCUSSION

The results of this study showed that oxytocin and oxytocin receptor expressions were decreased in the skin and DRG of the PTX group compared to the Non-PTX group, and HT significantly reversed the PTX effect on oxytocin and oxytocin receptor expressions. Since it has been shown that oxytocin promoted epidermal turnover (Hayre, 2020), and HT promoted the proliferation of the epidermal layer and improved neurotransmission sensitivity to physical stimuli, thereby reducing abnormal excitation, the present results suggest that the increase in oxytocin by HT contributes to improving CIPN-inducing symptoms.

The DRG processes sensory information through various interneurons that convert stimuli entering the skin into electrical signals to transmit pain and tactile sensations (Noguri *et al.* 2022; Cho *et al.* 2019, Denda *et al.* 2012). It was hypothesized that oxytocin expression in the DRG excites inhibitory interneurons, thereby suppressing pain signaling. It has also been reported that sensations such as numbness and pain caused by CIPN involve pathways through the DRG (Nguyen *et al.* 2021). Therefore, it was suggested that stimulation with HT may affect oxytocin expression by responding through the DRG-mediated pathway and not through the pituitary gland. Although the present study showed that serum oxytocin levels after HT in PTX model mice were higher than those in the Non-PTX and PTX groups, HT also increased oxytocin expressions in skin and DRG. It has been postulated that oxytocin synthesized by cutaneous stimulation of HT from the skin may be instantly transmitted to the posterior pituitary gland and secreted from nerve endings into the blood, which increases serum expression (Jahangard *et al.* 2020).

The actions of oxytocin such as pain relief, analgesia, reduction of anxiety and empathy toward others have been shown to involve its activity in the brain (Denda *et al.* 2012; Vittner *et al.* 2018; Carter *et al.* 2020; Kendrick *et al.* 2018; de Jong *et al.* 2021; Goh *et al.* 2021). Previously, it has been reported that injured neurons could be regenerated through mechanical stimulation such as massage (Shibasaki *et al.* 2010). In addition, clinical trials have also reported that HT involving finger massage improved mild to moderate numbness in the fingers of patients with CIPN caused by taxane-typed anticancer drugs (Sasaki *et al.* 2020). Furthermore, the high oxytocin gene expression in the subcortical and olfactory regions suggests that HT may promote oxytocin synthesis (Gimpl *et al.* 2001; Xin *et al.* 2017; Quintana *et al.* 2019). Thus, the present study confirms the latter suggestions, and adds an evidence that HT improving mild to moderate neuron-related disease symptoms could be attributed to increase in oxytocin expression locally and systemically.

CONCLUSIONS

The present study showed that HT reversed PTX-induced suppression of oxytocin receptor expressions and HT increased oxytocin level systemically. Such results connect the gap and previous suggestions that HT improving neurological symptoms are related to oxytocin expression locally and oxytocin systemic levels.

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