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● *Original Contribution*

ANALGESIC EFFECTS OF TRANSCUTANEOUS ULTRASOUND NERVE STIMULATION IN A RAT MODEL OF OXALIPLATIN-INDUCED MECHANICAL HYPERALGESIA AND COLD ALLODYNIA

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Abstract—This study investigated the effects and underlying mechanisms of therapeutic ultrasound (TUS) in a rat model of oxaliplatin-induced peripheral neuropathy. Animals received a total of eight injections with oxaliplatin (4 mg/kg), administered at 3-d intervals. TUS intervention (1 MHz, 0.5 W/cm^2) started on the fifth oxaliplatin administration and continued for 10 consecutive d. Sensory behavioral examinations, protein levels of transient receptor potential channels (TRPM8 and TRPV1) in dorsal root ganglia (DRG) and substance P (SP) in spinal dorsal horn were examined. Results indicated that TUS can reduce mechanical and cold hyper-responsive behaviors caused by repeated administration of oxaliplatin. Oxaliplatin-related increases in protein levels of TRPM8 in DRG and SP in the dorsal horn were also reduced after TUS. Taken together, the results revealed beneficial effects of TUS on oxaliplatin-induced mechanical hyperalgesia and cold allodynia and suggested involvement of TUS biochemicals in suppressing TRPM8 in DRG and SP in spinal cords. (E-mail: sherrie@mail.cmu.edu.tw) © **2017 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Therapeutic ultrasound, Oxaliplatin-induced peripheral neuropathy, Mechanical hyperalgesia, Cold allodynia, Substance P, Transient receptor potential channels.**

INTRODUCTION

Oxaliplatin is a third-generation platinum-based antineoplastic agent, which commonly causes acute and chronic peripheral neurotoxicity (Ventzel et al. 2016; Zedan et al. 2014). Acute neuropathy occurring within hours of oxaliplatin injection and spontaneously resolving within days can be observed in almost all patients. However, after cumulative injections of oxaliplatin, this neuropathy became persistent (Ventzel et al. 2016). Oxaliplatin-induced peripheral neuropathy (OIPN) causing paresthesia, allodynia and pain is often one of the main reasons patients discontinue their cancer treatments (Hopkins et al. 2016; Tofthagen et al. 2011). Therefore, it is important to develop strategies to protect patients from OIPN-induced sensory impairment.

Oxaliplatin causes damage to cell bodies, inhibition of neurite outgrowth, alterations in nuclear morphology and selective atrophy of subpopulations of DRG neurons (Alcindor and Beauger 2011; Argyriou et al. 2008; Jamieson et al. 2005). In addition to morphologic and functional changes in DRG cells, the prolonged activation of transient receptor potential (TRP) channels) could affect sensory nerve cells, thereby playing a role in the development of thermal allodynia (Di Cesare Mannelli et al. 2013; Schulze et al. 2011). Sensitization induced by oxaliplatin in peripheral nerves may then lead to hyperexcitability in the dorsal column of spinal cord by increasing the release of substance P (SP) (Chen et al. 2015a; Ling et al. 2007b).

Moreover, as research continues into the role of pharmacologic agents for treatment and/or prevention of OIPN, interest in non-pharmacologic treatment options is increasing. Therapeutic ultrasound (TUS), a form of acoustic energy, exerts its effect on cells and tissues *via* both thermal and non-thermal mechanisms. The

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thermal effect occurs when acoustic waves penetrate the tissue and produce molecular vibration, which results in heat production and facilitates pain relief (Zhang et al. 2016). The non-thermal effect of TUS therapy includes cavitation, media motion and standing waves, which might elicit anti-inflammatory and tissue-stimulating effects (Moura Junior Mde et al. 2014; Tsai et al. 2011). TUS, which can stimulate nerve tissue to enhance nerve function and promote regeneration, has frequently and effectively been used for management of neuropathic pain (Akhlaghi et al. 2012; Chang et al. 2014; Chen et al. 2015b; Ozkan et al. 2015; Raso et al. 2005). In view of these beneficial effects, we examined the animal sensory behaviors, protein levels of transient receptor potential cation channel subfamily M member 8 (TRPM8) and subfamily V member 1 (TRPV1) of DRG and SP in spinal cords to validate the efficacy of TUS on alleviation of characteristic allodynia signs *via* a rat model during repeated administration of oxaliplatin.

METHODS

Experimental design

Twenty-four animals were randomly divided into four groups: oxaliplatin and TUS treatment (n = 6, Oxa-TUS); oxaliplatin and sham TUS (n = 6, Oxa-sTUS); vehicle-only injection and TUS treatment (n = 6, Veh-TUS); and vehicle-only injection and sham TUS (n = 6, Veh-sTUS). Each animal received a total of eight injections of either oxaliplatin or vehicle, administered at 3-d intervals (days 1, 4, 7, 10, 13, 16, 19 and 22). TUS or sham-TUS intervention was started 4 h after the fifth oxaliplatin/vehicle administration and then continued for 10 consecutive d (one session per day, days 13–22). Sensory behavioral examinations were assessed on the day before the first administration of oxaliplatin/vehicle (day 0, Pre-oxa) and before (day 12, Pre-treat) and after (day 24, Post-treat) all TUS/sTUS treatments. Animals were sacrificed for immunoassays 4 h after completion of final assessments (day 24). Figure 1 is a schematic of the experimental design.

Animals

Adult male Sprague-Dawley rats (BioLASCO, Taiwan) weighing 200–250 g were maintained in the animal facility under an artificial 12-h light–dark cycle. Animals had access to food and water *ad libitum*. All experimental procedures were performed in accordance with the ethical guidelines of the International Association for Study of Pain in Animals (Zimmermann 1983), and were approved by the China Medical University Committee on Animal Care and Use. All rats were

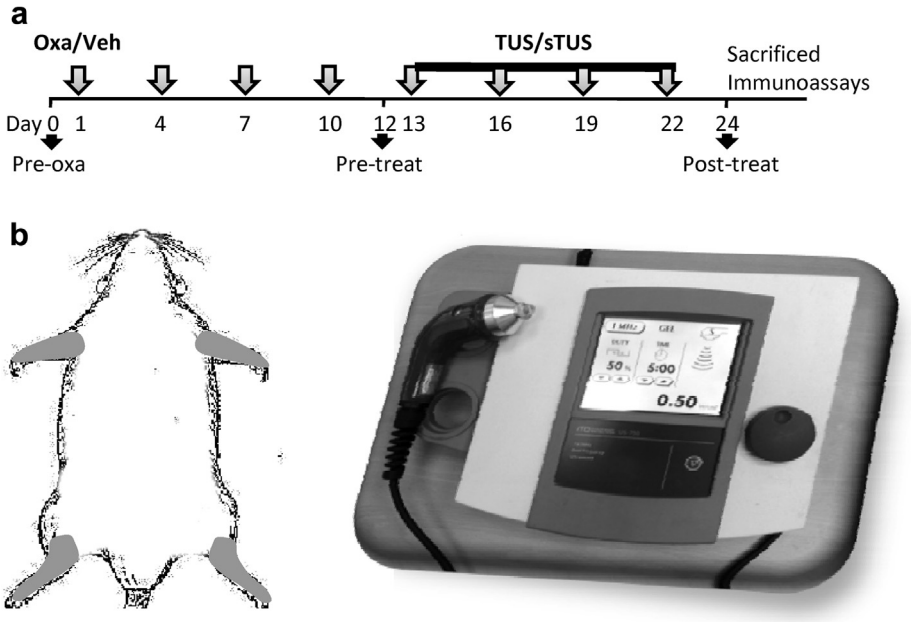

Fig. 1. (a) Overview of experimental design. Rats received a total of eight doses of oxaliplatin (Oxa) or its respective vehicle (Veh) (*open arrows*) injected at 3-d intervals (days 1, 4, 7, 10, 13, 16, 19 and 22). Measurements including sensory behaviors and TRPV1 and TRPM8 channel agonist-evoked nocifensive responses were evaluated on days (*solid arrows*) before oxaliplatin or vehicle administration (Pre-oxa, Days 0), before treatments (Pre-treat, day 12) and after completion of all treatments (Post-treat, day 24). Therapeutic ultrasound (TUS) or sham TUS (sTUS) treatments were started 4 h after the fifth oxaliplatin/vehicle administration and continued for 10 consecutive d (one session/d, days 13–22). On the day after final assessments (day 24), animals were sacrificed for immunoassays (immunohistochemistry and Western blotting). (b) Illustration of TUS device and its application area (in gray) over the rat's extremities through the transcutaneous method.