Electroacupuncture enhances antioxidative signal pathway and attenuates neuropathic pain induced by chemotherapeutic paclitaxel

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Summary

One of the significant limiting complications of paclitaxel is painful peripheral neuropathy during its therapy for several types of cancers. Our recent study showed that impairment of Nrf2-antioxidant response element (Nrf2-ARE) and upregulation of oxidative signals in the dorsal root ganglion (DRG) of rats with treatment of paclitaxel result in neuropathic pain. The purpose of this study was to examine the beneficial role played by electroacupuncture (EA) in modifying neuropathic pain evoked by paclitaxel via Nrf2-ARE and oxidative mechanisms. Behavioral test was performed to determine mechanical and thermal sensitivity in rats. Western Blot analysis and ELISA were used to examine expression of Nrf2-ARE and superoxide dismutases (SOD); and the levels of products of oxidative stress in the DRG. Our data showed that paclitaxel increased mechanical and thermal sensitivity and this was accompanied with impaired Nrf2-ARE and SOD in the DRG and amplified products of oxidative stress (i.e., 8-isoprostaglandin F2α and 8-hydroxy-2′-deoxyguanosine). EA treatment largely restored the levels of Nrf2-ARE/SOD and inhibited products of oxidative stress and thereby attenuated mechanical and thermal hypersensitivity induced by paclitaxel. In conclusion, we revealed specific signaling pathways leading to paclitaxel-evoked neuropathic pain, including impairment of Nrf2-ARE and heightened oxidative signals. We further provided evidence for the role of EA in alleviating paclitaxel-neuropathic pain via these molecular mediators.

Key words: electroacupuncture; paclitaxel; neuropathic pain; Nrf2-ARE; SOD, oxidative stress

Introduction

Paclitaxel is generally used for the treatment of breast, lung and other cancers, but causes a variety of serious side effects, including peripheral neuropathy, leukopenia, joint or muscle pain, vomiting and alopecia (Ghersi *et al.* 2015). Chemotherapy-induced peripheral neuropathy causes severe sensory disturbances that range from mild tingling to spontaneous painful burning paresthesia affecting the sensory nerves to the hands and feet (Dougherty *et al.* 2004) and can persist long after treatment cessation (Tanabe *et al.* 2013) in cancer patients with chemotherapy (Seretny *et al.* 2014). As traditional analgesics generally lack efficacy in treating this condition (Kim *et al.* 2015), it is necessary to have a novel alternative approach for analgesic strategies.

It has been suggested that paclitaxel alters the excitability and survival of neurons via a number of mechanisms, which include disruption of axonal transport, impairment of mitochondrial, amplification of ion channel activity as well as neuronal injury and inflammation (Sisignano *et al.* 2014). In particular, previous studies demonstrated that paclitaxel upregulates multiple transcription factors, enzymes, and cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), playing an important role in regulating neuropathic and inflammatory pain (Cata *et al.* 2008). These inflammatory mediators develop neuropathic pain through activation of the Janus-activated kinase-signal transducer and activator of transcription (JAK/ STAT) transduction pathway as well as activation of mitogen-activated protein kinase (MAPK) pathway (Miller *et al.* 2009). In addition, oxidative stress and reduction of nerve growth factor have been involved in paclitaxel-induced neuropathic pain
(Griffiths and Flatters 2015).

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a transcription factor and as a basic leucine zipper protein it regulates the expression of antioxidant proteins protecting against oxidative damage triggered by injury and inflammation (Moi *et al.* 1994). Numerous drugs that stimulate the Nrf2 pathway were used for treatment of diseases caused by oxidative stress (Gold *et al.* 2012). In a prior study (Miao *et al.* 2018), we suggested the engagement of antioxidative signal in the dorsal root ganglion (DRG) in paclitaxel-induced neuropathic pain. This prior study specifically showed that paclitaxel impaired Nrf2-antioxidant response element (Nrf2-ARE) and superoxide dismutases (SOD, a class of enzymes to catalyze the dismutation of superoxide) in the DRG and amplified products of oxidative stress. Systemic administration of SOD mimetic and antioxidant vitamin C or NADPH oxidase (NOX) inhibitor attenuated mechanical and thermal hypersensitivity induced by paclitaxel. This inhibitory effect was accompanied with decreases of proinflammatory cytokines (PICs) in the DRG.

Electroacupuncture (EA) has been used for the clinical treatment of chronic pain caused by various diseases (Kavoussi and Ross 2007, McDonald *et al.* 2013). It has also been documented for the analgesic effects of EA stimulation in animal with peripheral nerve injuries and inflammatory and neuropathic pain (Kim *et al.* 2005, Pittler and Ernst 2008) (DeLeo and Yezierski 2001). Thus, it is likely that EA is beneficial for alternative treatment strategy of neuropathic pain during paclitaxel chemotherapy and this requires studies of the underlying molecular mechanisms by which EA improves paclitaxel-induced neuropathic pain.

Accordingly, in this report we hypothesized that EA intervention restores impairment of Nrf2-ARE and Nrf2-regulated NADPH quinone oxidoreductase-1 (NQO1) in the DRG of rats with injection of paclitaxel. We also hypothesized that EA intervention restores the decreased levels of SOD and inhibits oxidative NOX, and thereby attenuates amplification of 8-

isoprostaglandin F2α (8-iso PGF2α, a product of oxidative stress); and 8-hydroxy-2'-deoxyguanosine (8-OHdG, a key biomarker of protein oxidation) in the DRG of paclitaxel rats. In addition, we hypothesized that EA decreases the levels of PICs (IL-1β, IL-6 and TNF-α) in the DRG of rats with injection of paclitaxel.

Methods

Animal

All animal protocols were in accordance with the guidelines of the International Association for the Study of Pain and approved by the Institutional Animal Care and Use Committee of Jilin University. Adult male Sprague-Dawley rats (200-250 g) were housed in individual cages with free access to food and water and were kept in a temperature-controlled room (25°C) on a 12/12 h light/dark cycle.

A model of neuropathic pain and EA intervention

Paclitaxel (Tocris Biosci) was dissolved in a 40% dimethyl sulfoxide (DMSO) (Smith *et al.* 2004, Chen *et al.* 2011). Neuropathy was induced in rats by intraperitoneal (i.p.) injection of paclitaxel (1 mg/kg/one injection in 0.5 ml of volume) every two days and a total of four injections were given (Smith *et al.* 2004, Chen *et al.* 2011). Control rats received four injections of 40% DMSO (0.5 ml) every two days. Mechanical and thermal sensitivity was tested after paclitaxel injection. Mechanical and thermal hypersensitivity were tested daily after paclitaxel. Mechanical and thermal hypersensitivity were fully developed after four injections of paclitaxel and then six hours of behavioral test was performed as shown in Figure 1A. In separate rats, DRG tissues were removed for western blot analysis and ELISA measurement.

Neiguan and Jianshi acupoints [pericardial meridian (P) 5-6, located on the median nerve] were selected for EA because they represent activation of central regions having inhibitory descending pathways in involvement of somatic and visceral nociceptive responses (Zhou Yi Syuu *et al.* 2006, Lujan *et al.* 2007, Choi *et al.* 2012). The unrestrained EA method was used to allow rats to move freely in the cage during EA stimulation. The rats were briefly restrained in plastic tubes with the hindlimbs extending through two holes. Hook-shaped needles were promptly inserted into the skin and underneath tissues at acupoints. The rats were then released from tubes and allowed to move freely in the cages with the inserted needles. Stainless needles were bilaterally placed at the neiguan and jiangshi acupoint 1.5–2.0 and 2.5–3.0 mm above the wrist (between the ligaments of the flexor carpi radialis and the palmaris longus), respectively. In general, the criterion for accurate needle positioning is relied on the observation of a slight repetitive flexion of the paw during stimulation (Li *et al.* 2006). The stimulation was set at a low current and low frequency (0.5-ms pulses, 2 Hz and 2 mA) by an EA stimulator. This was conducted 20 min/day for five-seven days before each of the experiments. Sham-stimulation was applied to vehicle control rats and paclitaxel rats.

Behavioral test

To quantify the mechanical sensitivity of the hindpaw, rats were placed in individual plastic boxes and allowed to acclimate for > 30 min. Mechanical paw withdrawal threshold (PWT) of rat hindpaw in response to the stimulation of von Frey filaments was determined. A series of calibrated von Frey filaments (ranging from 0.5 to 18.0 g) were applied perpendicularly to the plantar surface of the hindpaw with a sufficient force to bend the filaments or until paw withdrew. In the presence of a response, the filament of next lower force was applied. In the absence of a response, the filament of next greater force was applied. To avoid injury during

tests, the cutoff strength of the von Frey filament was 18 g. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the "up-down" method (Chaplan *et al.* 1994). Each trial was repeated 2 times at approximately 2 min intervals. The mean value was used as the force produced a withdrawal response.

To determine thermal hyperalgesia, rat paw withdrawal latency (PWL) to a radiant heat was measured. Rats were placed individually in plastic cages on an elevated glass platform and allowed for 30 min acclimation. Each hind paw received three stimuli with a 10 min interval, and the mean of the three withdrawal latencies was defined as PWL. The heat was maintained at a constant intensity. To prevent tissue damage, the cut-off latency was set at 20 s. To prevent experimental bias, all the behavioral tests were performed in a blind manner. In all the tests, the experimenter had no knowledge (blinded) about the treatments that the rats had received.

ELISA measurements

Briefly, DRGs (L4-L6) were removed and total protein of DRG tissues was then extracted by homogenizing sample in ice-cold immunoprecipitation assay buffer. The levels of 8-iso PGF2α and 8-OHdG in the DRGs (L4-L6) were examined using an ELISA assay kits (obtained from Promega Co. and Abcam Co.) according to the provided description. Briefly, polystyrene 96-well microtitel immunoplates were coated with affinity-purified rabbit primary antibodies. Parallel wells were coated with purified rabbit IgG for evaluation of nonspecific signal. After overnight incubation, plates were washed. Then, the diluted samples and 8-iso PGF2α/8-OHdG standard solutions (100pg/ml-100ng/ml) were distributed in each plate. The plates were washed and incubated with anti-8-iso PGF2α/8-OHdG galactosidase. Then, the plates were washed and

incubated with substrate solution. After incubation, the optical density was measured using an ELISA reader.

Western blot analysis

The lysates were centrifuged and the supernatants were collected. After being denatured by heating at 95°C in an SDS sample buffer, the supernatant samples were loaded onto Mini-Protean TGX Precast gels and electrically transferred to a polyvinylidene fluoride membrane. The membrane was then incubated overnight with diluted primary antibodies: rabbit anti-Nrf2 (at 1:250), anti-NQO1 (at 1:500), anti-SOD (at 1:500) and anti-NOX4 (at 1:250). After being washed, the membrane was incubated with horseradish peroxidase-linked anti-rabbit secondary antibody (diluted at 1:500) and visualized for immunoreactivity. All antibodies were obtained from Abcam Co., Santa Cruz Biotech and/or or Antibodies online Com. The membrane was stripped and incubated with anti-β-actin to show equal loading of the protein. The bands recognized by the primary antibody were visualized by exposure of the membrane onto an X-ray film. Then, the film was scanned and the optical densities of primary antibodies and β-actin bands were determined using the Scion Software. Values for densities of immunoreactive bands/β-actin band from the same lane were determined. Each of the values was then normalized to a control sample.

Statistical analysis

All data were analyzed using a two-way analysis of variance for different time courses between two experimental groups. Values were presented as means ± standard deviation of mean. For all analyses, differences were considered significant at $P < 0.05$. All statistical analyses were performed by using SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL).

Results

Mechanical and thermal sensitivity

In this study, we examined PWT and PWL in rats with injection of DMSO (40%) and rats with injection of saline. No significant differences in PWT and PWL were observed between groups. i.e., PWT was 13.8±2.5g in saline group (n=6) *vs.* 13.2±1.9g in DMSO group (n=5) ($P>0.05$, saline *vs.* DMSO); and PWL was 12.8±2.1s in saline group (n=6) *vs.* 12.1±1.7s in DMSO group (n=5) ($P>0.05$, saline *vs.* DMSO). This result suggests that DMSO had minimal effects on pain responses evoked by mechanical and thermal stimulation, and a prior report showed that no significant toxic effects were seen by the similar dose of DMSO (Worthley and Schott 1969).
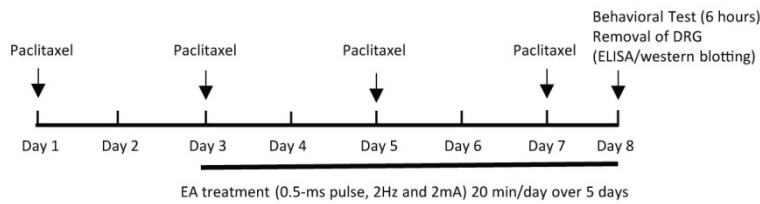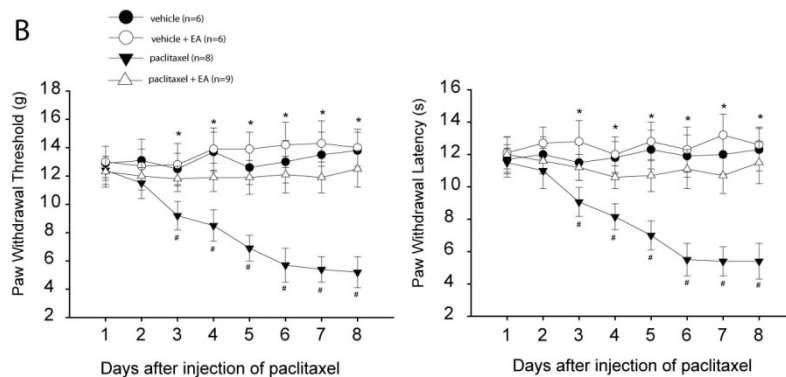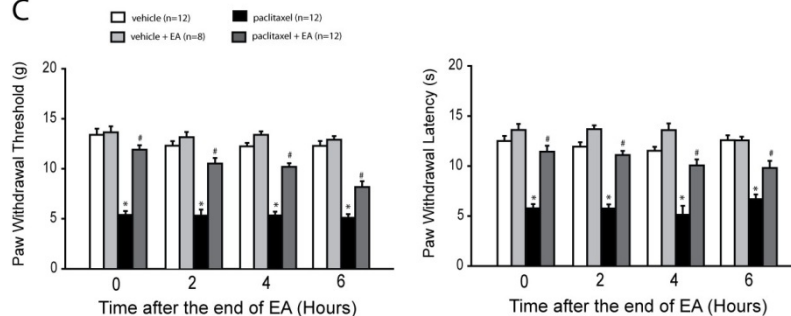

Figure 1

Figure 1. *(A).* A schematic diagram showing the schedule for injection of paclitaxel and EA treatment. Paclitaxel was injected [intraperitoneally (i.p.), 1 mg/kg body weight every other day] (indicated as arrows). An equivalent volume of vehicle was given in control animals. EA was given daily for 5 days. On the 8th day, behavioral tests were performed and in the separate rats the DRGs were removed for ELISA measurements and western blot analysis. In time course experiment, EA was given daily beginning from day 1.

(B). Time courses for mechanical and thermal sensitivity in control rats and paclitaxel rats after EA treatment. Left and right panels indicate the effects of EA on mechanical and thermal sensitivity, respectively. EA increased PWT and PWL during development of mechanical and thermal pain after injection of paclitaxel. *$P < 0.05$, paclitaxel rats *vs.* control rats. #$P < 0.05$, paclitaxel rats *vs.* paclitaxel rats with EA treatment. The number of animals is indicated on the figure. *(C).* Mechanical and thermal sensitivity during six hours of test in control rats and paclitaxel rats after EA treatment. Left panel: Effects of EA on mechanical sensitivity in vehicle control rats and paclitaxel rats. As compared with no EA treatment, EA increased PWT in paclitaxel rats. Right panel: Effects of EA on thermal sensitivity in control rats and paclitaxel rats. EA increased PWL in paclitaxel rats. Note that the increases of PWT and PWL evoked by EA treatment were insignificant in control rats. *$P < 0.05$ *vs.* vehicle

First, we determined time courses for mechanical and thermal sensitivity in control rats and paclitaxel rats by examining PWT and PWL each day after EA treatment. Figure 1B shows that PWT and PWL began to reduce two days after first injection of paclitaxel and they became less with additional paclitaxel injections; EA increased PWT and PWL during development of mechanical and thermal pain after paclitaxel. After four injections of paclitaxel, PWT decreased to a greater degree as compared with vehicle injection ($P < 0.05$, paclitaxel rats *vs.* vehicle control rats; n=12 in each group). A six-hour of behavioral test was performed. Figure 1C (left panel) demonstrates that PWT was increased after EA treatment in paclitaxel rats ($P < 0.05$ *vs.* paclitaxel rats with no EA n=12 in each group). In paclitaxel rats, the inhibitory effects of EA on

mechanical hypersensitivity lasted for several hours after the end of its treatment. In addition,

the increase of PWT evoked by EA was insignificant in vehicle control rats ($P > 0.05$ *vs.* vehicle

control rats with no EA; n=8). Likewise, paclitaxel injection significantly diminished PWL as

compared with vehicle injection. Figure 1C (right panel) further shows that EA significantly

attenuated thermal hypersensitivity during six hours of test in paclitaxel rats ($P < 0.05$ *vs.*

paclitaxel rats with no EA; n=12 in each group). The effects of EA intervention on PWL were

insignificant in control rats ($P > 0.05$ *vs.* vehicle control rats with no treatment; n=8).

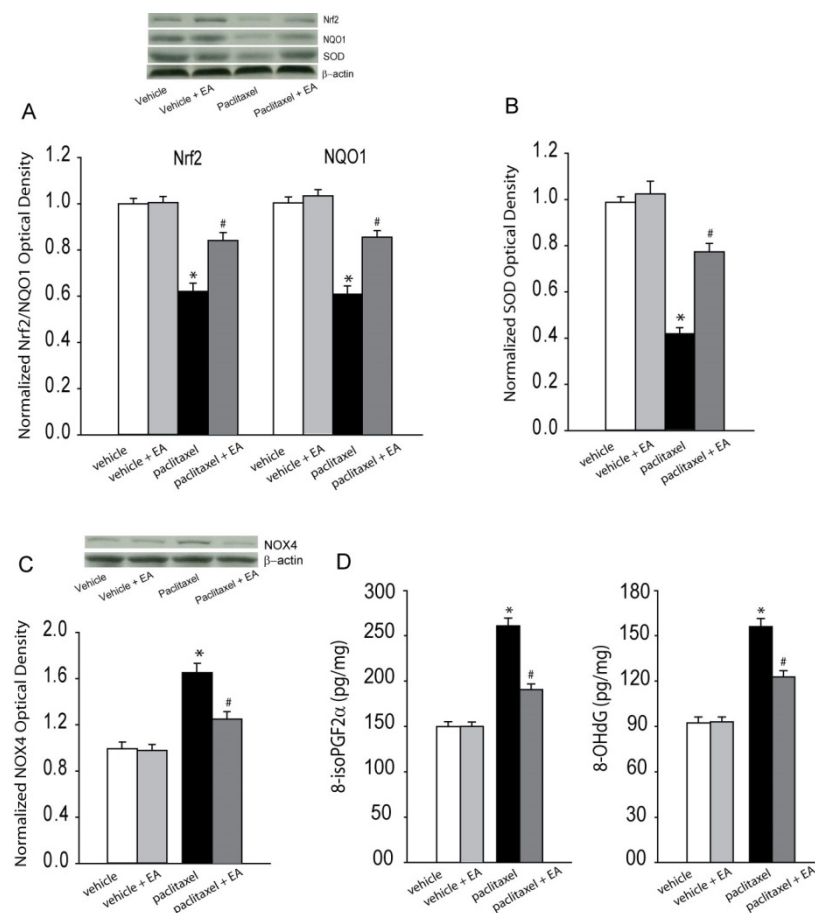*Expression of Nrf2-ARE and oxidative stress signal pathways*

Figure 2

Figure 2. Effects of paclitaxel on signal pathways of oxidative stress. *(A&B)*. Protein expression levels of Nrf2-ARE and SOD. Top panel and bottom panel are representative bands and averaged data: Nrf2/NQO1 and SOD were decreased in the DRG of paclitaxel rats. *P<0.05, paclitaxel rats *vs.* vehicle control rats. #$P < 0.05$ *vs.* paclitaxel rats with no EA. n=6-10 in each group. *(C).* Representative bands and averaged data, showing that protein expression levels of NOX4 were amplified by paclitaxel. *P<0.05, paclitaxel rats *vs.* vehicle controls. #$P < 0.05$ *vs.* paclitaxel rats with no EA. n=6-10 in each group. (D), Systemic administration of paclitaxel amplified the levels of oxidative products 8-iso PGF2α and 8-OHdG in the DRG. *$P < 0.05$ *vs.* vehicle control rats. #$P < 0.05$ *vs.* paclitaxel rats with no EA. n=12 in each group of control rats and paclitaxel rats; n=15

We first examine the protein levels of Nrf2 and Nrf2-regulated NQO1 in the DRG. Figure 2A shows that the protein expression levels of Nrf2 and Nrf2-regulated NQO1 were decreased in paclitaxel rats (*P<0.05, paclitaxel rats *vs.* control rats; n=6-10). In the similar way, Figure 2B further shows that paclitaxel led to downregulation of SOD expression (*P<0.05, paclitaxel rats *vs.* controls rats; n=6-8). It is noted that downregulation of Nrf2, NQO1 and SOD in paclitaxel rats were considerably restored after EA treatment as shown in Figure 1A-B (*P<0.05, paclitaxel rats with EA *vs.* paclitaxel rats with no EA; n=6-10). In contrast, NOX4 was amplified by paclitaxel and EA significantly attenuated increases of NOX4 as shown in Figure 2C (*P<0.05, paclitaxel rats with EA *vs.* paclitaxel rats with no EA; n=6-10 in each group).

In addition, we examined products of oxidative stress in the DRG of control rats and paclitaxel rats as shown in Figure 2D. 8-iso PGF2α and 8-OHdG were increased in the DRG of paclitaxel rats (*$P <0.05$ *vs.* control rats, n=12 in each group). Note that EA intervention significantly attenuated amplification of 8-iso PGF2α and 8-OHdG induced by paclitaxel (*$P <0.05$, paclitaxel rats with EA/n=15 *vs.* paclitaxel rats with no EA/n=12).

Levels of PICs

Figure 3. Levels of PICs in control rat and paclitaxel rats. Paclitaxel increased IL-1β, IL-6 and TNF-α as compared with vehicle control rats. EA treatment significantly attenuated amplifications of PICs evoked by paclitaxel. *$P < 0.05$ *vs.* control rats and paclitaxel rats with EA treatment. #$P < 0.05$ *vs.* paclitaxel rats with no EA. n= 12 in vehicle control; n=10 in vehicle control + EA; n=12 in paclitaxel; n=15 in paclitaxel + EA.
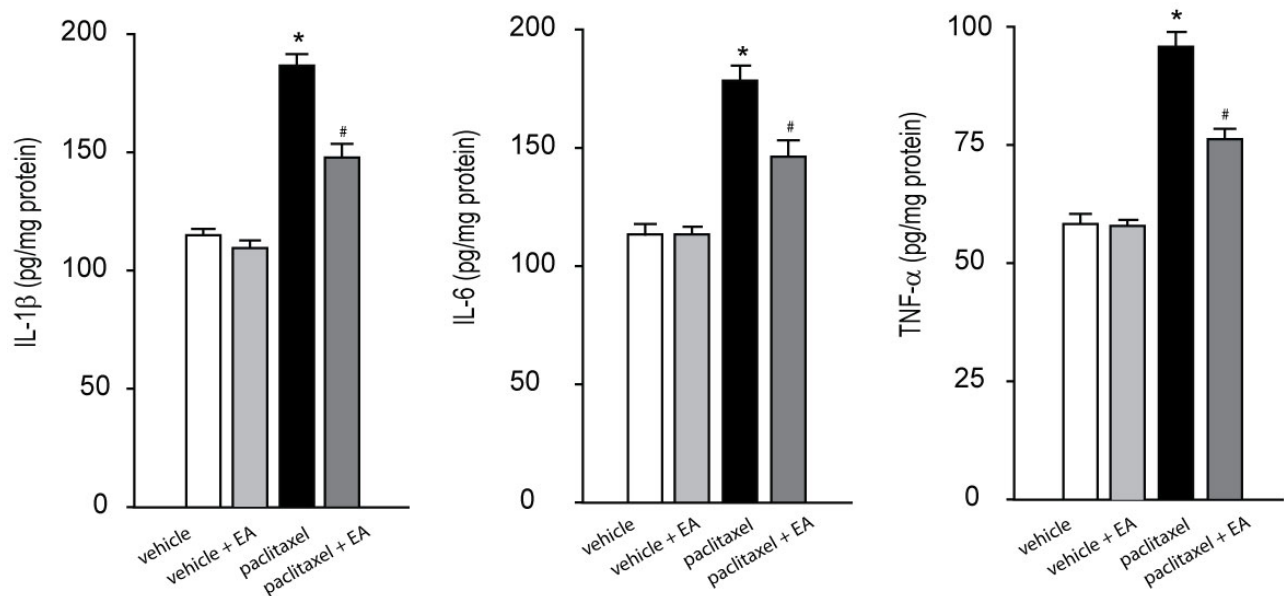

Figure 3

In additional experiments, we examined the effects of EA on upregulated PICs induced by paclitaxel in the DRG. Figure 3 shows that IL-1β, IL-6 and TNF-α were significantly increased in paclitaxel rats (n=12; *P*<0.05 *vs.* vehicle control rats/n=12) as compared with vehicle control rats. Furthermore, EA intervention (n=15) significantly attenuated amplifications of PICs evoked by paclitaxel. Insignificant difference in the levels of PICs in the DRG was observed between vehicle control rats with EA and vehicle control rats with no treatment (*P*>0.05, EA treatment/n=12 *vs.* no EA treatment/n=10).

Discussion

One of the most common and distressing symptoms suffered by patients with progression of cancer is pain (Hanna *et al.* 2013). Cancer pain mainly arises from a tumor compressing or infiltrating tissue; from nerve and other changes caused by a hormone imbalance or immune response; and/or from treatments and diagnostic procedures (Pasetto *et al.* 2006, Hanna *et al.* 2013). It should be noted that chemotherapy and radiotherapy produce painful conditions persisting long after treatment has ended (Hoskin 2008, Portenoy 2011, Hanna *et al.* 2013). As a result, how to effectively manage cancer pain related to these therapies becomes am important issue for treatment and management of cancer patients in clinics. Nonetheless, treatment options for these abnormal sensations have been restricted, partly due to a poor understanding of the underlying mechanisms responsible for neuropathic pain induced by chemotherapy. Also, there are lacking of effective drugs applied to patients; and costs and side effects of drugs affect a treatment strategy of neuropathic pain in patients with chemotherapy. Alternative treatment strategy of neuropathic pain is noteworthy during chemotherapy. Thus, in this report we examined the beneficial role play by EA intervention in modifying neuropathic pain induced by chemotherapeutic paclitaxel.

Prior studies have shown that injection of paclitaxel produces neuropathic pain in rats including mechanical hyperalgesia and thermal hypersensitivity several days after initiation of the chemotherapy regimen in rats (Chen *et al.* 2011). The signs of mechanical hyperalgesia and thermal hypersensitivity lasted for several weeks after administration of paclitaxel (Chen *et al.* 2011). Thus, in this study we employed this well-established rat model to examine the mechanisms leading to neuropathic pain induced by paclitaxel. Using the same intervention, in

our current study we observed that PWT declined to evoke mechanical withdrawal and PWL

decreased to induce thermal withdrawal after injection of paclitaxel.

In addition, results of our recent study demonstrated that the expression of Nrf2-ARE

signal and SOD expression were downregulated in the DRG of paclitaxel rats as compared with

control rats (Miao *et al.* 2018). The expression of NOX4, the levels of oxidative products 8-iso

PGF2α/8-OHdG and PICs such as IL-1β, IL-6 and TNF-α were amplified in the DRG of

paclitaxel rats. In this prior study (Miao *et al.* 2018), we further demonstrated that PICs were

decreased and mechanical and thermal hypersensitivity were attenuated in paclitaxel rats after

inhibition of NOX4 and systemic supplying of SOD and antioxidant vitamin C. Notably, results

of current study showed that EA intervention 1) restored impairment of Nrf2-ARE and SOD in

the DRG of paclitaxel rats; 2) inhibited amplification of NOX4 and 8-iso PGF2α/8-OHdG and

PICs, thereby alleviating neuropathic pain induced by paclitaxel.

The role of oxidative stress in chemotherapy-induced peripheral neuropathy has been

studied. As a non-specific reactive oxygen species (ROS) scavenger, N-tert-butyl-α-

phenylnitrone (PBN) was reported to inhibit the development of paclitaxel-induced mechanical

hypersensitivity (Fidanboylu *et al.* 2011). Also, tempol inhibited the development and

maintenance of paclitaxel-induced mechanical hypersensitivity, but was ineffective on cold

allodynia (Fidanboylu *et al.* 2011, Kim *et al.* 2017). In addition, the activity of different

antioxidant enzymes in the DRG and peripheral sensory nerves were examined during the time

course of paclitaxel-induced painful neuropathy. Enhanced activity of mitochondrial and cellular

endogenous antioxidant enzymes in the DRG and peripheral nerves was observed, however this

was inadequate and delayed in its onset leading to excessive ROS in peripheral sensory axons

(Duggett *et al.* 2016). Others have demonstrated an impaired mitochondrial antioxidant response

after paclitaxel (Janes *et al.* 2013). In our current study, we demonstrated that expression of SOD was decreased in the DRG of paclitaxel rats and EA largely restored impaired SOD and attenuated paclitaxel-induced mechanical and thermal hypersensitivity. These data provided evidence for the role of EA in modifying mitochondrial ROS in the development of paclitaxel-induced peripheral neuropathy.

In addition to antioxidants and/or free radical scavengers, it is also interesting to determine the effects of decreasing ROS generation on paclitaxel-induced mechanical and thermal hypersensitivity. One source of ROS production is the enzyme family of NOX. The rodent genome encodes four genes that contain the catalytic NOX subunit, namely NOX1, NOX2, NOX3, and NOX4 (Altenhofer *et al.* 2012). This electron-transferring subunit is constitutively inactive in resting cells and generates ROS only upon activation, e.g. after noxious stimuli (Salvemini *et al.* 2011). While NOX2 activation is predominantly associated with innate immunity mediated host defense and NOX1 with blood pressure control and related vascular mechanisms (Gavazzi *et al.* 2006, Lam *et al.* 2010), NOX4 was shown highly expressed under ischemic conditions in the central nervous system, e.g. ischemic stroke (Suzuki *et al.* 2012). Evidence has also identified activation of NOX4 as a causative factor that contributes to inflammatory or neuropathic pain role in the peripheral nervous system (Kallenborn-Gerhardt *et al.* 2012). Thus, in the current study we further determined the effects of EA intervention on expression levels of NOX4 in the DRG of paclitaxel rats. We observed that NOX4 was upregulated after administration of paclitaxel and EA attenuated upregulation of NOX4 as well as increases of oxidative 8-iso PGF2α/8-OHdG.

Furthermore, paclitaxel was reported to increase the level of IL-1β, IL-6 and TNF-α in the DRG of rats thereby inducing pain behaviors (Kim *et al.* 2016, Kim *et al.* 2017). Consistent

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with these findings, in the current study we observed that paclitaxel amplified IL-1β, IL-6 and TNF-α in the DRG. Interestingly, EA attenuated increases in IL-1β, IL-6 and TNF-α after paclitaxel and attenuated mechanical and thermal hypersensitivity. Results of the present report suggest that downregulation of IL-1β, IL-6 and TNF-α in the DRG is involved in the inhibitory effects of EA on mechanical pain and thermal hypersensitivity in paclitaxel-induced neuropathy.

It should be noted that Cochrane reviews of acupuncture for a wide range of pain conditions have been published (Lee and Ernst 2011), suggesting that acupuncture is effective for some but not all types of pain. There have also been many clinical trials in humans testing the effectiveness of EA for relieving neuropathic pain. A recent Cochrane review has indicated that there is insufficient evidence to support or refute the use of acupuncture for neuropathic pain whereas more studies are still ongoing (Ju *et al.* 2017).

Study limitations: First, one concern is that we used the separated group of rats to perform western blot/ELISA experiments and behavioral experiments and the status of those animals might be different with respect to the development of neuropathic pain. It should be noted that those rats received the same treatment of paclitaxel as well as EA which was likely to cause minimal differences in pain response in both groups. Second, in the current study neuropathic pain began two days after the first injection of paclitaxel and with treatment of EA PWT and PWL were increased, suggesting that EA had inhibitory effects on mechanical and thermal pain during development of neuropathy. Our results also showed that neuropathic pain was fully developed in paclitaxel rats on the 8th day and PWT and PWL remained at higher levels in paclitaxel rats with EA while a six-hour of test was performed. However, a study limitation is that later time points showing the duration of the EA effects were not included.

Conclusions

In the DRG of paclitaxel rats, Nrf2-ARE signal and SOD expression are downregulated and oxidative products and PICs are amplified, leading to mechanical pain and thermal hypersensitivity. EA intervention has a significant inhibitory effect on neuropathic pain induced by paclitaxel, accompanied with restoring the levels of Nrf2-ARE and SOD as well as inhibiting oxidative products and PICs. Nonetheless, our data demonstrated the beneficial role of EA in modifying neuropathic pain induced by paclitaxel. Clarification of the underlying molecular mechanisms for analgesia of EA would also open an opportunity to combine EA with medications to manage and control pain induced by chemotherapy as a potential clinical implication.

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