Animal Study

Pentoxifylline Ameliorates Mechanical Hyperalgesia in a Rat Model of Chemotherapy-Induced Neuropathic Pain

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Disclaimer: Hee Kee Kim and Seon-Hee Hwang contributed equally to this article. There was no external funding in the preparation of this manuscript.

Manuscript received: 08-18-2015
Revised manuscript received: 10-15-2015
Accepted for publication: 11-10-2015

Free full manuscript: www.painphysicianjournal.com

Background: Chemotherapy-induced neuropathic pain is difficult to treat. Pentoxifylline inhibits the production of inflammatory cytokines including tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β).

Objective: The aims of our study were to investigate the analgesic and preventive effects of pentoxifylline on paclitaxel-induced neuropathic pain in rats and to identify its mechanisms of action.

Study Design: Controlled animal study.

Methods: Neuropathic pain was induced with intraperitoneally injected paclitaxel on 4 alternate days in male Sprague-Dawley rats. Pentoxifylline was administered systemically as a single injection and a continuous infusion before or after the injection of paclitaxel. The mechanical threshold for allodynia was measured by using von Frey filaments. Protein levels and localization of inflammatory cytokines were performed by using Western blotting and immunohistochemistry, respectively.

Results: After the rats developed neuropathic pain behavior, a single intraperitoneal injection and continuous infusion of pentoxifylline ameliorated paclitaxel-induced mechanical allodynia. In addition, systemic infusion of pentoxifylline in the early phase of the development of pain behavior delayed the onset of paclitaxel-induced pain behavior. Paclitaxel increased the levels of the catalytic subunit α of protein kinase A, phosphorylated nuclear factor κB, TNF-α, and IL-1β in the lumbar dorsal root ganglia. Pentoxifylline decreased the paclitaxel-induced TNF-α and IL-1β levels. In addition, IL-1β was expressed in neurons and satellite cells in the lumbar dorsal root ganglia after paclitaxel.

Limitations: Although this study was performed in the animal model by well-designed manner, clinical study will be needed to confirm the analgesic effect of pentoxifylline.

Conclusion: Pentoxifylline alleviated chemotherapy-induced neuropathic pain in rats by reducing the levels of inflammatory cytokines in dorsal root ganglia and may be effective chemotherapy-induced neuropathic pain in patients.

Key words: Chemotherapy, chronic pain, inflammatory cytokines, neuropathic pain, paclitaxel, pain behavior, pain treatment, pentoxifylline, phosphodiesterase inhibitor

Pain Physician 2016; 19:E589-E600

Chemotherapeutic drugs such as paclitaxel, cisplatin, oxaliplatin, and vincristine produce peripheral neuropathic pain, which is a dose-limiting side effect (1). Paclitaxel is commonly used to treat breast cancer, cervical cancer, ovarian cancer, and Kaposi sarcoma (2). However, paclitaxel induces peripheral neuropathic pain in hands and feet (3). Currently, no effective medications are available to treat this type of pain (4,5).

Inflammatory cytokines such as tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) have been reported to be involved in the development and main-

tenance of chemotherapy-induced neuropathic pain (6). TNF-α, which is synthesized by several cell types, including activated monocytes, macrophages, B lymphocytes, T lymphocytes, and neurons, induces cytokine production, activates adhesion molecules, stimulates cell growth, and exerts cytotoxic activities against tumor cells, viruses, and nervous tissues (7). IL-1β, which is produced by monocytes, neutrophils, macrophages, keratinocytes, and B lymphocytes, promotes thymocyte proliferation, B lymphocyte proliferation, and substance *P* production (7).

Pentoxifylline (Trental; Sanofi-Aventis, Bridgewater, NJ) has been used to treat intermittent claudication due to peripheral vascular disease of the limbs (8,9). Additionally, pentoxifylline has been shown to have an analgesic effect in various animal models of neuropathic pain (e.g., tibia fracture-induced complex regional pain syndrome, chronic constriction injury, and L5 spinal nerve transection) and inflammatory pain (e.g., acetic acid-induced writhing, carrageenan- or TNF-α-induced hyperalgesia, and zymosan-induced arthritic pain) (10-14). However, no reports have been published on the analgesic effects of pentoxifylline on chemotherapy-induced neuropathic pain.

The aims of our study were to investigate (1) the analgesic effects of pentoxifylline on paclitaxel-induced neuropathic pain (PINP) in an animal model, (2) the preventive effects of pentoxifylline on the development of PINP, and (3) the mechanisms of action of pentoxifylline in dorsal root ganglia (DRGs).

Methods

Experimental Animals

We used adult Sprague–Dawley rats (200 – 350 g; Harlan Sprague Dawley Company, Houston, TX). The animals had free access to food and water and were housed in a room with a normal light-dark cycle (light cycle: 7:00 a.m. – 7:00 p.m.). All animals were habituated for one week before the experiments. The experimental protocol was approved by the institutional animal care and use committees of Beth Israel Deaconess Medical Center (Boston, Massachusetts) and The University of Texas MD Anderson Cancer Center. At the end of the experiment, the rats were euthanized by 100% CO_2 inhalation and exsanguination. After the rats become unconscious upon exposure to 100% CO_2, a thoracotomy was performed to expose the heart. Then we made a deep incision into the heart for exsanguination.

Paclitaxel-induced Neuropathic Pain

Paclitaxel (Sigma, St. Louis, MO) was dissolved in a vehicle solution (4% dimethyl sulfoxide and 4% Tween 80 in sterile saline) at a concentration of 2 mg/mL just prior to injection and was injected intraperitoneally (i.p.) on days 0, 2, 4, and 6 (cumulative dose 8 mg/kg) to induce painful peripheral neuropathy (15,16). Control animals were injected with the same volume of vehicle without paclitaxel. In a previous report, paclitaxel (2 mg/kg on days 0, 2, 4, 6; total 8 mg/kg) decreased the mechanical threshold in rats. The threshold started to decrease from baseline (18.7 g) on days 6 – 8 and reached its lowest level (0.8 – 1.5 g) on days 12 – 14, which was maintained for at least 2 months (16). In this study, 95.5% of the rats treated with paclitaxel became hyperalgesic.

Measurement of Mechanical Allodynia

To measure mechanical allodynia, we used a behavior test that has been described previously (17). Briefly, rats were placed in a plastic chamber on top of a mesh screen, and the mechanical threshold of the left hind paw was determined by the up-down method (18) using monofilaments (0.45 – 14.45 g). A filament was applied to the most sensitive areas of the plantar surface of the paw. A 50% mechanical threshold value was calculated as $10^{(X + kd)}/10^{-4}$, where X is the value in log grams of the final filament used, k is the tabular value for the pattern of responses, and d is the mean difference between stimuli in log grams. The investigator who conducted the behavior tests did not know which animals received pentoxifylline and which did not until the end of the study. Three out of 67 (4.5%) of the rats that did not develop pain behavior within 2 – 3 weeks of the first paclitaxel injection were removed from the study.

Western Blot Analysis

To examine the levels of signaling molecules, paclitaxel (2 mg/kg administered on days 0, 2, 4, and 6) or vehicle (4% dimethyl sulfoxide and 4% Tween 80 in saline) was i.p. injected, and the L1-6 DRGs were removed on day 14 after the first injection of paclitaxel or vehicle. Subsequently, pentoxifylline was infused for 7 days (days 14 – 20), and the lumbar DRGs were removed on day 20 for Western blotting. The rats were anaesthetized deeply with 4% isoflurane and perfused with cold saline. The L1-6 DRGs were removed and frozen immediately in liquid nitrogen. DRGs were homogenized in RIPA cell lysis buffer with a protease inhibitor, and the supernatants were loaded in 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvi-

nylidene fluoride membranes. Blots were incubated with primary antibody against IL-1β (1:1000; Santa Cruz Biotechnology, Dallas, TX), TNF-α (1:1000; Abcam, San Francisco, CA), phosphorylated nuclear factor kappa B (NFκB) (p-NFκB, 1:1000; Cell Signaling Technology, Danvers, MA), catalytic subunit α of protein kinase A (PKACα, 1:1000; R&D Systems, Minneapolis, MN), and GAPDH (1:1000; Santa Cruz Biotechnology) overnight at 4°. The blots were then incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:5000; GenDepot, Katy, TX) or anti-goat horseradish peroxidase-conjugated secondary antibody (1:5000; GenDepot). The immunoblots were analyzed with a chemiluminescence detection system. The blots were scanned with Spot Advanced and Adobe Photoshop 8.0 (Adobe Inc., San Diego, CA). For equalizing protein loading, GAPDH expression was used as a control. The bad densities were qualified using Image J (NIH, Bethesda, MD). A region of the band was taken and then background was subtracted. The expression of a protein was quantified as the ratio of the expression of that protein over the expression of GAPDH in the same lane. The relative values were calculated by average of expression of a protein of PAC or PTX group divided by that of VEH group.

Behavioral Testing for Sedation

Behavioral testing for sedation was based on 5-point scales of posture (0 = normal, 4 = flaccid atonia) and righting reflexes (0 = rat struggles, 4 = no movement) (19,20). Sedation was assessed immediately after each pain behavior test, and no separate group of rats for sedation testing was therefore necessary.

Experimental Design

This study consisted of 2 parts: (1) assessment of the therapeutic effects of pentoxifylline on PINP, given as a single i.p. injection (50 or 100 mg/kg) or as i.p. infusion (0.96 mg/day for 7 days), and (2) investigation of the preventive effects of pentoxifylline given as i.p. infusions using 2 different paradigms on the development of PINP (Fig. 1).

Assessment of the Therapeutic Effects of Pentoxifylline.

For administration of pentoxifylline as a single i.p.

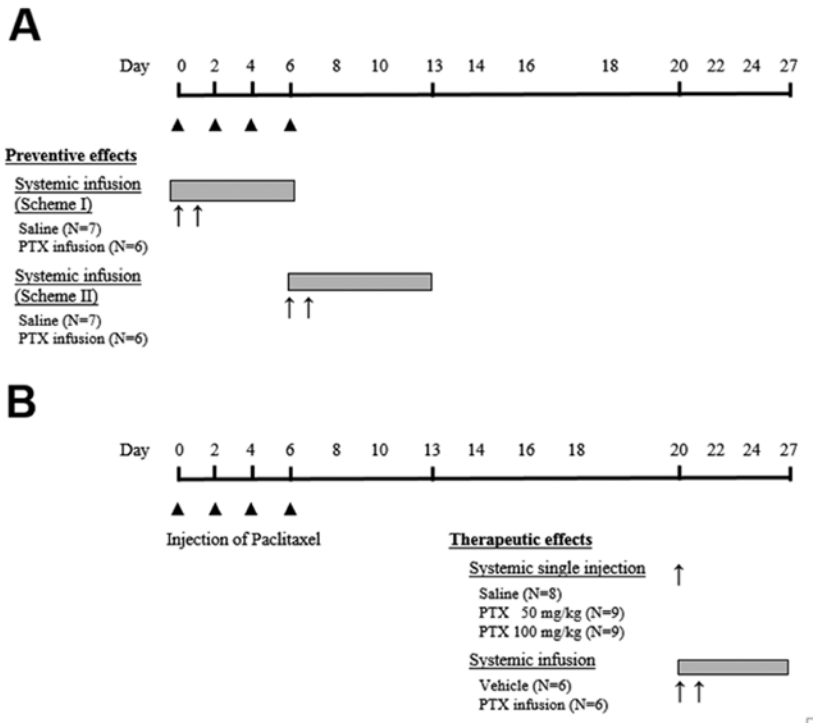

Fig. 1. *Schematic overview of the experimental design. (A) Preventive effects of pentoxifylline including scheme I and II. Infusion of pentoxifylline were done at 2 different points including days 0 – 6 (scheme I) or days 6 – 13 (scheme II). (B) Therapeutic effects of pentoxifylline by single systemic injection or systemic infusion (days 20 – 27).*

injection, on the twentieth day after the first paclitaxel injection, 26 rats were divided into 3 groups. After the rats developed neuropathic pain behavior, they were randomly assigned to one of 2 treatment groups or a control group. Rats in the treatment groups (9 rats per group) received 50 or 100 mg/kg pentoxifylline in 3 mL/kg saline and rats in the control group (8 rats per group) received a single 3-mL/kg injection of saline (Fig. 1).

For administration of pentoxifylline as a systemic i.p. infusion, on the twentieth day after the first paclitaxel injection, the rats that developed neuropathic pain behavior were randomly assigned to either a treatment (pentoxifylline, 6 rats) group or a control (vehicle, 6 rats) group before the insertion of a mini-osmotic pump (Alzet model 2001; Alzet, Cupertino, CA; see below). The rats in the treatment group received an infusion of pentoxifylline at a rate of 1 µl/h for 7 days (0.96 mg/day). Additionally, loading doses of pentoxifylline (100 mg/kg) were administered on days 20 and 21 after the first paclitaxel injection. The rats in the control group received equivalent volumes of saline via the pump and as loading doses (3 mL/kg).

Assessment of the Preventive Effects of Pentoxifylline.

Pentoxifylline (0.96 mg/day) was infused i.p. (Alzet model 2001 mini-osmotic pump) by one of the following methods of administration. In the Paradigm I, a bolus of pentoxifylline was injected i.p. at a dose of 100 mg/kg on days 0 and 1 and continuously infused (i.p.) for 7 days (days 0 through 6). In the Paradigm II, the same dose of pentoxifylline was given as a bolus on days 6 and 7, and continuous infusion was administered on days 6 through 13 (Fig. 1). Mechanical allodynia testing was measured on days 0, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, and 20.

Intraperitoneal Implantation of the Mini-Osmotic Pump

The mini-osmotic pump was implanted i.p. according to the manufacturer's protocol. Briefly, a midline skin and peritoneal wall incision (1 cm) was made in the lower abdomen of rats under isoflurane anesthesia (3 – 4% for induction and 2% for maintenance) in oxygen. Anesthetic depth was assessed by loss of the toe pinch reflex and loss of the blink reflex. The absence of the reflexes in response to stimulation indicated adequate anesthesia for pump implantation. The pump was filled with pentoxifylline or saline and inserted into the peritoneal cavity. The musculoperitoneal layer was sutured with silk sutures, and the skin incision was closed with wound clips. Anesthesia was discontinued, and the animals were allowed to recover from anesthesia.

Immunohistochemical Analyses of IL-1β, NeuN, and GFAP

To examine the localization of IL-1β in DRGs, the L5 DRG was removed on day 14 after the first paclitaxel or vehicle injection. Subsequently, pentoxifylline was infused for 7 days (days 14 – 20), and the lumbar DRGs were removed on day 20 for immunohistochemical experiment.

For immunohistochemical analyses, the animals were deeply anesthetized with 4% isoflurane and transcardially perfused with cold saline followed by cold 4% paraformaldehyde. L5 DRGs were removed, post fixed, cryoprotected in 30% sucrose, cryosectioned to a thickness of 10 µm, and mounted on slides. The sections were incubated with combinations of the following primary antibodies followed by secondary antibodies conjugated with either Alexa Fluor 568 (red) or Alexa Fluor 488 (green). The primary antibodies used were anti-NeuN (neuronal marker, monoclonal anti-mouse, 1:50; GenDepot), anti-glial fibrillary acidic protein (anti-GFAP; satellite cell marker, monoclonal anti-mouse, 1:50; Santa Cruz Biotechnology), and anti-IL-1β (polyclonal anti-rabbit, 1:50; Santa Cruz Biotechnology). The immunostained dorsal root sections were viewed under a Perkin Elmer Vectra multispectral microscope (Caliper Life Sciences, Hopkinton, MA). For analysis of IL-1β colocalization with NeuN or GFAP, DRG sections from 3 rats were double stained. All images were analyzed using InForm 1.2 software.

Statistical Analyses

Data were summarized as means with standard errors of the means for the behavioral testing and as means with standard deviations for Western blotting. The data were analyzed using the SigmaStat program (Systat Software, San Jose, CA) and 2-way repeated-measures analyses of variance with one repeated factor (time), followed by Tukey post hoc test for behavioral testing and the Mann-Whitney U test for Western blotting. In all cases, $P < 0.05$ was considered statistically significant. The study design was based on the use of parallel groups and investigator blinding.

Results

Pentoxifylline Did Not Produce Sedation

All rats treated with pentoxifylline or vehicle had

a score of 0 on both the posture scale and the righting reflex scale, indicating that the rats were not sedated.

Pentoxifylline Had an Analgesic Effect on PINP

In a preliminary study, we used pentoxifylline at doses of 10, 50, 100, and 300 mg/kg. The 100-mg/kg dose of pentoxifylline increased the mechanical threshold to more than 10 g, which is a relatively normal threshold of rats and did not produce side effects such as sedation. We therefore used 100 mg/kg as the maximal dose in this study. In addition, the dose of 10 mg/kg had only weak analgesic effects. Therefore, doses of 50 and 100 mg/kg were chosen for the single i.p. injection.

Pentoxifylline significantly increased the mechanical threshold by single injection at doses of 50 and 100 mg/kg in rats (Fig. 2A). The 100-mg/kg dose significantly increased the mechanical threshold for 1.5 hours post injection ($P < 0.001$). Further, there was a significant difference between the treatment group (PTX 100) and the control group (saline) at 0.5 – 1.5 hours post treatment.

Intraperitoneal infusion, pentoxifylline (for 7 days starting on day 20) (Fig. 2B) significantly increased the mechanical threshold starting on day 21, and this value remained significantly higher than that of the control group for a total of 8 days. These data indicate that the treatment produced prolonged analgesia without sedation.

Pentoxifylline Delayed the Development of PINP

The early treatment (starting on day 0) did not affect the development of pain behavior (Fig. 3A). In contrast, the treatment beginning on day 6 significantly delayed the development of pain behavior in the

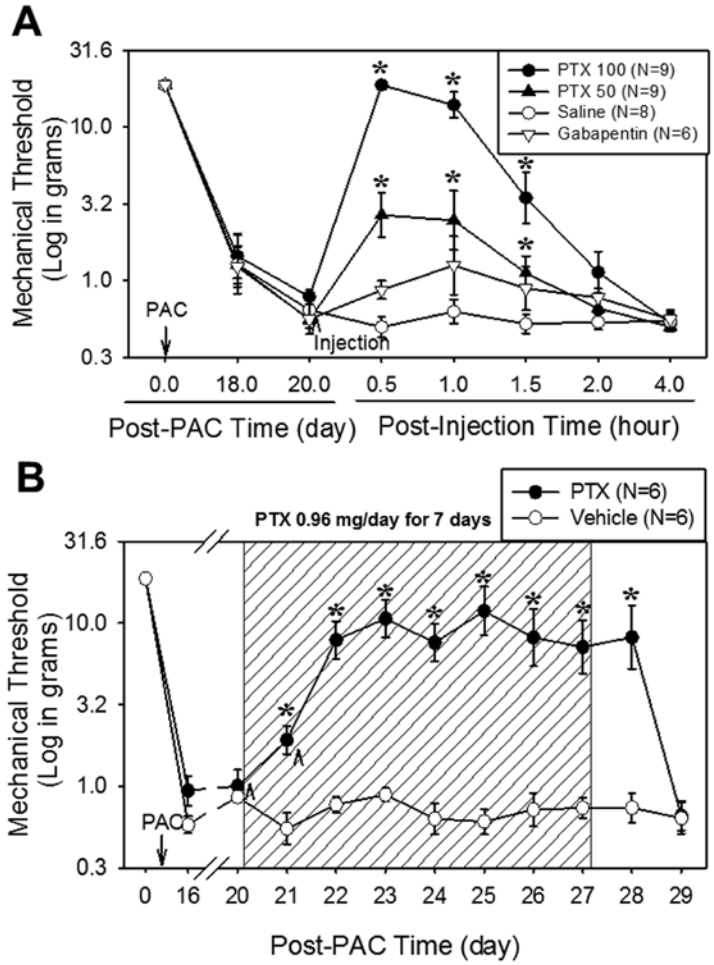

Fig. 2. *Analgesic effects of a single systemic injection (A) or systemic infusion (B) of pentoxifylline (PTX) on PINP in rats. Paclitaxel (PAC, 2 mg/kg) was injected i.p. on 4 alternate days (days 0, 2, 4, and 6), and the mechanical threshold was measured. (A) On the twentieth day after the first paclitaxel injection, 26 rats were divided into 3 groups, which received an i.p. injection of saline or 50 or 100 mg/kg of pentoxifylline (3 mL/kg). Note the normal or almost normal mechanical threshold after the injection of 100 mg/kg of pentoxifylline. (B) On the twentieth day after the first paclitaxel injection, 12 rats were divided into 2 groups. In one group, the rats received an i.p. infusion of pentoxifylline (0.96 mg/day) for 7 days (hatched box) in addition to i.p. injections of 100 mg/kg of pentoxifylline on days 20 and 21. In the second group, the rats received saline (vehicle) instead of pentoxifylline. The systemic infusion of pentoxifylline significantly increased the mechanical threshold starting on day 21, and the threshold remained significantly higher than the threshold in the control group at total of for 8 days. The data are means with standard errors of the means. The asterisks indicate values that are significantly different ($P < 0.05$) from the corresponding values for the control group as determined by a 2-way repeated-measures analysis of variance with one repeated factor (time) followed by the Tukey post hoc test.*

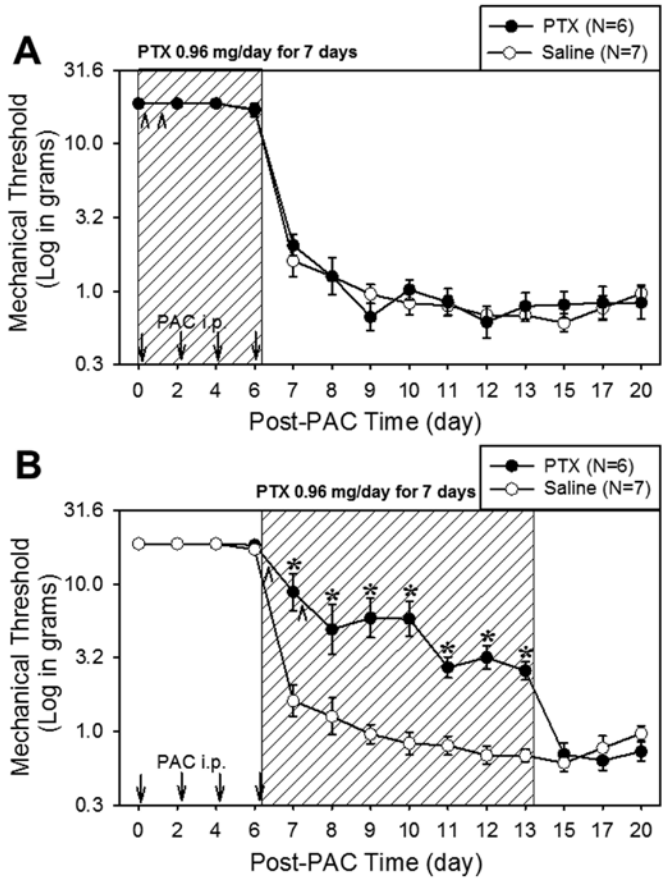

Fig. 3. Preventive effects of pentoxifylline (PTX) on PINP in rats. Paclitaxel (PAC, 2 mg/kg) was injected i.p. on 4 alternate days (days 0, 2, 4, and 6) and the mechanical threshold was measured. (A) On the day of the first paclitaxel injection, 13 rats were divided into 2 groups. The rats in the treatment group, received an i.p. infusion of pentoxifylline (0.96 mg/day) for 7 days (hatched box) in combination with an i.p. injection of pentoxifylline (100 mg/kg) on days 0 and 1 (arrowheads) of the infusion period. The rats in the control group received equivalent amounts of saline. The systemic infusion of pentoxifylline did not affect the development of PINP. (B) On the day of the last paclitaxel injection, another 13 rats were divided into 2 groups. The rats in the treatment group received an i.p. infusion of pentoxifylline (0.96 mg/day) for 7 days (hatched box) in combination with i.p. injections of pentoxifylline (100 mg/kg) on days 6 and 7 (arrowheads). The rats in the control group received equivalent amounts of saline. The systemic infusion of pentoxifylline significantly delayed the development of PINP for 8 days. The data are means with standard errors of the means. The asterisks indicate values that are significantly different ($P < 0.05$) from the corresponding values for the control group as determined by a 2-way repeated-measures analysis of variance with one repeated factor (time) followed by the Tukey post hoc test.

Paradigm II (Fig. 1A, 3B). These data indicate that pentoxifylline delayed the onset of PINP when given during the development phase of mechanical hyperalgesia.

Paclitaxel Raised the Levels of NFκB, TNF-α, and IL-1β in DRGs, and Pentoxifylline Subsequently Decreased Them

Paclitaxel significantly increased the levels of PKACα (1.6 times), p-NFκB (1.5 times), TNF-α (2.3 times), and IL-1β (2.6 times) in the lumbar DRGs compared to those in the vehicle control group (Fig. 4). Subsequently, pentoxifylline decreased the paclitaxel-induced PKACα, p-NFκB, TNF-α, and IL-1β levels in the DRGs (Fig. 4A-E).

IL-1β Was Colocalized in Both Neurons and Satellite Cells in DRGs

IL-1β was expressed in the L5 DRG in both vehicle- and paclitaxel-injected rats (Fig. 5A and B). In addition, IL-1β was co-expressed in NeuN-positive neurons and GFAP-positive satellite cells in the DRG (Fig. 5C and D). Paclitaxel increased the density of IL-1β in DRGs compared to the vehicle (Fig. 5A and B). Most importantly, pentoxifylline decreased the paclitaxel-induced IL-1β density in the DRGs (Fig. 5E and F).

Discussion

This study investigated the analgesic effect of pentoxifylline in rats with PINP. Pentoxifylline, administered as a combination of a single systemic injection and continuous systemic infusion, produced analgesia by inhibiting PKACα, p-NFκB, TNF-α, and IL-1β in DRGs. In addition, pentoxifylline delayed the onset of PINP when given during the development phase of mechanical hyperalgesia. Thus, our results suggest that pentoxifylline has potential for treatment of chemotherapy-induced neuropathic pain.

Cancer is one of the leading causes of pain and suffering in the US (21). As cancer treatments improve and the average life expectancy increases, pain associated with cancer and/or cancer treatment (chemotherapy,

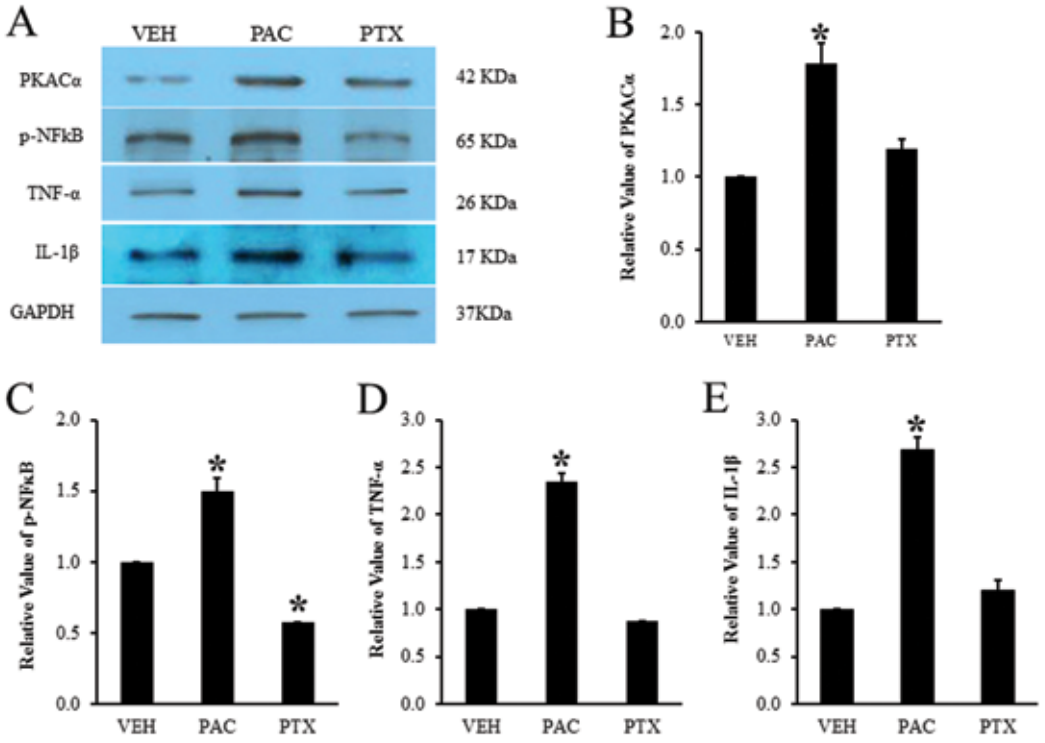

Fig. 4. *Paclitaxel increased the levels of PKACα, p-NFκB, TNF-α, and IL-1β in rat DRGs. (A) Western blot showing the expression of PKACα, p-NFκB, TNF-α, and IL-1β in DRGs after an injection of vehicle (VEH; 4% dimethylsulfoxide and 4% tween 80 in saline) or paclitaxel (PAC). Pentoxifylline (PTX) was infused for 7 days after the paclitaxel injection. (B-E) Quantification of PKACα, p-NFκB, TNF-α, and IL-1β in DRGs. The data are means with standard deviations for 3 rats. The asterisks indicate values that are significantly different ($P < 0.05$) from the values for the vehicle group as determined by the Mann-Whitney U test.*

radiation, and/or surgery) will continue to frequently affect physical and psychosocial functioning and the overall quality of life in cancer patients. It has been reported that the symptoms of chemotherapy-induced neuropathic pain are predominantly sensory and may occur at any time during the course of chemotherapy and even after termination of chemotherapy. Neuropathy and pain could further increase in frequency and/or severity with concurrent chemotherapies or coexisting diseases such as diabetes (22). Chemotherapy-induced neuropathy is a dose-limiting side effect in cancer patients that significantly reduces the quality of life in cancer survivors (23-26). Commonly used analgesic drugs such as nonsteroidal anti-inflammatory agents, opioids, anticonvulsants, antidepressants, and sodium channel blockers show little or no analgesic effects in PINP models (27). Recently, Wolf et al (5) reviewed the analgesic effects of glutamine, glutathione, N-acetylcysteine, oxcarbazepine, and xaliproden and concluded that those drugs did not prevent chemotherapy-induced neuropathy.

Patients who undergo chemotherapy develop pain behaviors such as mechanical allodynia, thermal hyperalgesia, and cold allodynia, with mechanical allodynia/hyperalgesia, being the most common complaint (28,29). Similarly, more than 90% of rats treated with paclitaxel develop neuropathic pain behaviors such as mechanical allodynia and hyperalgesia in their hind paws, but not many develop thermal hyperalgesia according to our preliminary findings. We therefore chose mechanical hyperalgesia/allodynia as the more reliable and clinically relevant measure. Mechanical hyperalgesia or allodynia is a common clinical symptom in patients with chemotherapy-induced painful peripheral neuropathy (28,29) and is more common than thermal hyperalgesia/allodynia in cancer patients

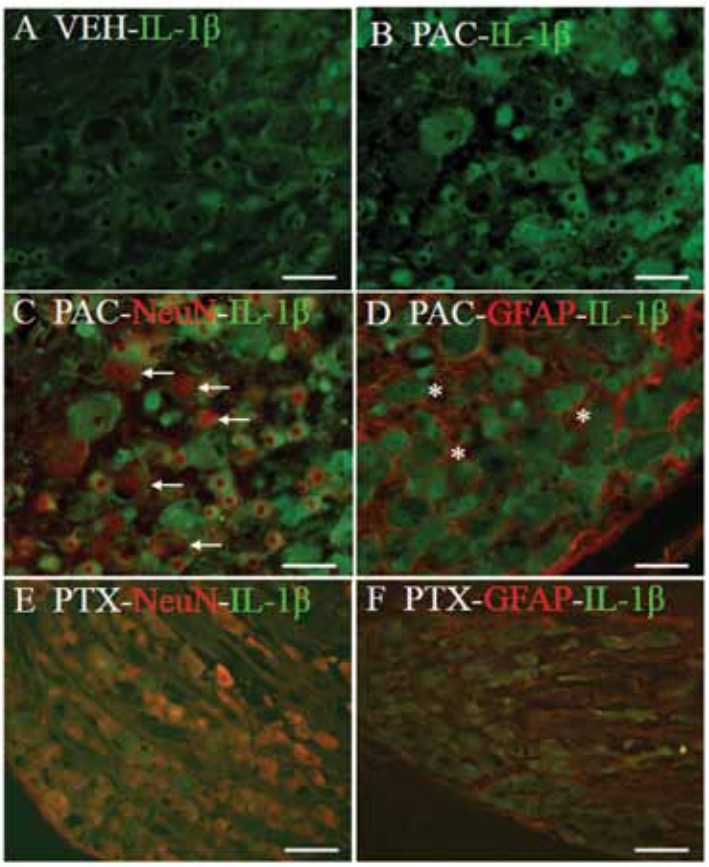

Fig. 5. *Colocalization of IL-1β, NeuN, and GFAP in DRGs. (A) IL-1β (green, Alexa Fluor 488) in the L5 DRG of vehicle (VEH; 4% dimethylsulfoxide and 4% tween 80 in saline)-injected rats. (B-D) NeuN (red, Alexa Fluor 568) and IL-1β (green) in DRGs of paclitaxel-injected rats. Stars and arrows indicate satellite cells and neurons, respectively. (E, F) NeuN (red, Alexa Fluor 568) and IL-1β (green) in DRGs of paclitaxel-injected rats treated by pentoxifylline. Scale bars, 100 μm.*

in our clinics. Cold allodynia is also frequent in patients with PINP; however, for the current study, we chose to use mechanical allodynia because prior animal and human studies for PINP have shown this parameter was reduced (1,15).

In this study, we have shown that pentoxifylline produced an analgesic effect on PINP. Pentoxifylline increases secondary messengers such as cyclic AMP (cAMP) and cyclic GMP (cGMP) by inhibiting phosphodiesterases (PDEs) 1-5 (10,30). PDEs degrade the phosphodiester bond of cAMP and cGMP and thereby terminate the action of those molecules. At least 11 PDEs have been sequenced and characterized for substrate specificity to cAMP, cGMP, or both (31). PDEs 1, 2, 3, 10, and 11 hydrolyze both cAMP and cGMP; PDEs 5, 6, and 9 hydrolyze only cGMP; and PDEs 4, 7, and 8 strictly hydrolyze cAMP (31). In addition to increasing cAMP and cGMP, pentoxifylline decreases the production of inflammatory cytokines, chemotaxis, and cytotoxic effects in immune cells (basophils, eosinophils, neutrophils, monocytes, macrophages, and T lymphocytes) (32-34). In the present study, pentoxifylline produced analgesic effects on PINP likely by inhibiting inflammatory cytokines (Fig. 2-4).

Paclitaxel produced pain behaviors in rats in our study. This drug is accumulated in DRGs and cannot penetrate the blood-brain barrier (35). It may cause damage to the sciatic nerve and DRGs during the de-

velopment of pain behaviors. Paclitaxel also causes accumulation of macrophages in DRGs and an increase in calcium channel subunits (36-38). In addition, Nishida et al (39) reported that paclitaxel increased the expression of inflammatory and/or immune response-related genes in DRGs, including genes encoding phospholipase A2, chemokine ligand 21b, complement components 1 and 3, and matrix metalloproteinase 3. Paclitaxel increases the production of TNF-α, IL-1, and IL-6. A local injection into the sciatic nerve and an intrathecal injection of TNF-α can induce pain through the p75 TNF-α receptor 2. The pain behaviors we observed in the present study were due to the increases in the levels of inflammatory cytokines in DRGs.

Our findings showed that paclitaxel increased the levels of PKACα, p-NFκB, TNF-α, and IL-1β in DRGs. We added 2 publications (39,40) about changes of gene expression of neuronal ion channels and macrophage in the dorsal root ganglia of paclitaxel-treated rats. We will perform proteomics and phosphoproteomics in the future. The increase in PKACα likely promoted the change from NFκB to p-NFκB (active form), which in turn increased TNF-α and IL-1β in DRGs, thereby inducing pain behaviors. Our findings also showed that pentoxifylline inhibited the elevated PKACα, p-NFκB, TNF-α, and IL-1β levels in the DRGs. Liu et al (12) reported that pentoxifylline significantly inhibited the activation of NFκB and reduced the production of TNF-α and IL-1β in the brain in rats. In the present study, pentoxifylline significantly decreased the p-NFκB and slightly decreased the TNF-α compared to the vehicle group. It is an exciting find that brings up further question for future investigation. Pentoxifylline seems to differentially suppress the expression of p-NFκB even in normal cells but not the expression of TNF- α or IL-1β. The NFκB pathway is involved in many physiological and pathological functions, which means the activity of NFκB was increased or decreased by many factors (41). The p-NFκB, an active form of NFκB, was either increased by inflammatory modulators (TNF-α, IL-1β, lipopolysaccharide) and neuromodulators (nerve growth factor, free radicals) or decreased by anti-inflammatory cytokines (IL-10) and anti-oxidants (41). Pentoxifylline can increase the level of IL-10 and decrease the level of free radicals. Therefore, pentoxifylline may decrease the p-NFκB level than the vehicle group. For TNF-α level, pentoxifylline group slightly decreased the TNF-α level in the DRGs compared to the vehicle group. This decrease of TNF-α may be involved in the decrease of NFκB level and free radical levels by pentoxifylline.

We selected IL-1β for the immunohistochemical experiments because it is the potent cytokine for pain behavior in the peripheral tissues. In the present study, paclitaxel increased the density of IL-1β in the DRG compared to that of vehicle-injected rats. IL-1β is released from immune cells (monocytes and macrophage) and nonimmune cells (fibroblasts and endothelial cells). It is also expressed in nociceptive DRG neurons (42). IL-1 receptor antagonist attenuated the cytokine-induced inflammatory hyperalgesia model and nerve injury pain model (43,44). Therefore, paclitaxel may produce the pain behavior by increasing IL-1β in DRGs. On the other hand, pentoxifylline decreased IL-1β level in the L5 DRG compared to paclitaxel-injected rats. IL-1β can produce hyperalgesia following intraperitoneal and intraplantar injection (43,45). It also increases the production of substance P and prostaglandin E2 in neurons and glial cells (46,47). In the present study, IL-1β was expressed in both satellite cells and neurons in the DRGs. Satellite cells are tightly surrounded by sensory neurons in the somata and are associated with the transport of various molecules, including glutamate, ATP, and cytokines. Satellite cells also modulate sensory transmission, including nociception (48). The activation of satellite cells by inflammatory cytokines releases calcium-dependent ATP and calcitonin gene-related peptide and produces nitric oxide and action potentials (48). Therefore, we speculate that pentoxifylline decreased IL-1β level in DRGs and then may decrease the activation of satellite cells and decrease the production of substance P and prostaglandin E2 in neurons.

Interestingly, pentoxifylline delayed the development of pain behaviors when it was administered on days 6 – 13 after the first injection of paclitaxel and not when it was administered on days 0 – 6. Pentoxifylline is known to inhibit both glial activation and NFκB, and induces a decrease in TNF-α and IL-1β production by microglia (30). Therefore, activation of microglia and induction of inflammatory cytokines may have been involved in the development of pain behaviors during days 6 – 13 after the first injection of paclitaxel.

We tested gabapentin, one of the most (if not the most) widely used medication for chemotherapy-induced neuropathic pain, in our rat model of PINP as a comparison. We chose a dose of 50 mg/kg, which is a relatively high dose that does not induce sedation in rats. In contrast to pentoxifylline, gabapentin did not significantly increase the mechanical threshold (Fig. 2A).

In the present study, the analgesic effect of pentoxifylline lasted for 1.5 hours and one day after a single

injection and 7-day infusion, respectively. That means, the effect of pentoxifylline lasted only for a relatively short period of time. Thus, we wanted to investigate the effect of chronic application of the drug to prolong its efficacy.

Pentoxifylline has been used for many years to treat many diseases, especially intermittent claudication or ischemia associated with peripheral vascular disease. Several reports have indicated that pentoxifylline decreases radiation-induced side effects and decreases the proliferation of melanoma cells (49-52). Pentoxifylline also has a number of adverse side effects (including hypotension), which are important to consider in using pentoxifylline to treat pain.

Limitations

Some of the limitations of the present study include: a) This study was performed in an animal model of PINP. Thus, clinical study must be performed to evaluate if indeed pentoxifylline has an analgesic effect in humans. b) The present study was performed in a small number of rats in each group; and higher numbers of rats are needed to increase the reliability. c) PINP was produced using one chemotherapy agent, namely paclitaxel. However, it will be interesting to see if pentoxifylline has similar anti PINP effect when other chemotherapy agents are used to induce PINP.

Conclusion

This study showed that systemic administration of pentoxifylline ameliorated neuropathic pain behavior in a rat model of PINP by inhibiting PKACα, p-NFκB, TNF-α, and IL-1β in DRGs without inducing sedation and systemic infusion of pentoxifylline in the early phase of the development of pain behavior delayed the onset of paclitaxel-induced pain behavior. We conclude that pentoxifylline may be effective for chemotherapy-induced neuropathic pain in cancer patients.

Acknowledgments

The authors thank Arthur Gelmis (Department of Scientific Publications, The University of Texas MD Anderson Cancer Center) for editorial assistance. This work was supported by grants from Beth Israel Deaconess Medical Center, Harvard Medical School (Boston, Massachusetts), Helen Buchanan & Stanley Joseph Seeger Research Endowment and Peggy and Avinash Ahuja Center of Excellence at The University of Texas MD Anderson Cancer Center.

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