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Effect of paclitaxel on transient receptor potential vanilloid 1 in rat dorsal root ganglion

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ABSTRACT

Peripheral neuropathy is a common adverse effect of paclitaxel treatment. To analyze the contribution of transient receptor potential vanilloid 1 (TRPV1) in the development of paclitaxel-induced thermal hyperalgesia, TRPV1 expression in the rat dorsal root ganglion (DRG) was analyzed after paclitaxel treatment. Behavioral assessment using the tail-flick test showed that intraperitoneal administration of 2 and 4 mg/kg paclitaxel induced thermal hyperalgesia after days 7, 14, and 21. Paclitaxel-induced thermal hyperalgesia after day 14 was significantly inhibited by the TRP antagonist ruthenium red (3 mg/kg, s.c.) and the TRPV1 antagonist capsazepine (30 mg/kg, s.c.). Paclitaxel (2 and 4 mg/kg) treatment increased the expression of TRPV1 mRNA and protein in DRG neurons. Immunohistochemistry showed that paclitaxel (4 mg/kg) treatment increased TRPV1 protein expression in small and medium DRG neurons 14 days after treatment. Antibody double labeling revealed that isolectin B4-positive small DRG neurons co-expressed TRPV1. TRPV1 immunostaining was up-regulated in paw skin day 14 after paclitaxel treatment. Moreover, in situ hybridization histochemistry revealed that most of the TRPV1 mRNA-labeled neurons in the DRG were small or medium in size. These results suggest that paclitaxel treatment increases TRPV1 expression in DRG neurons and may contribute to functional peripheral neuropathic pain.

1. Introduction

The chemotherapeutic drug paclitaxel is widely used to treat several types of malignant tumors. Its major dose-limiting side effect is peripheral sensory neuropathy, which is characterized by painful paresthesia of the hands and feet [13,28]. This pain is often resistant to standard analgesics. Rodent models of paclitaxel-induced neuropathy have been developed to elucidate these pain mechanisms [6,14,27,33]. Pharmacological studies using these models have indicated that the mechanisms underlying mechanical and thermal hyperalgesia behavior after paclitaxel treatment are complex [10,17,37]. Recently, we reported that paclitaxel treatment increases the voltage-dependent calcium channel current in small- and medium-diameter rat dorsal root ganglion (DRG) neurons and upregulates $Ca_v\alpha_2\delta$-1 [18].

Cation channels of the transient receptor potential (TRP) family are widely expressed in the nervous system. Transient receptor potential vanilloid 1 (TRPV1) and its homologue TRPV2, which belong to the TRPV subfamily of the large TRP ion channel superfamily, are critical contributors to normal and pathological pain [33,34]. Moreover, TRPV1 and TRPV2 are expressed in primary sensory neurons in the DRG [1,3,29,31]. TRPV1 can be activated by noxious heat (>43 °C), extracellular acidication, various lipids, and capsaicin; TRPV1 is not observed only in small-diameter DRG neurons but also in medium-diameter DRG neurons [21,36]. TRPV1 also appears to be up-regulated in DRG neurons after peripheral nerve injury [15,16]. Moreover, TRPV1 is expressed by C-fiber nociceptors in the DRG and trigeminal ganglion [8,20]. TRPV1 is up-regulated in DRG neurons after persistent inflammation [18] and is essential for the development of inflammatory heat hyperalgesia [8,13]. Thus, TRPV1 expressed in nociceptors is a receptor for noxious heat and inflammatory molecules.

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Recently, sensitization of TRPV1, TRPV4, and TRP ankyrin 1 (TRPA1) and activation of protease-activated receptor 2 were shown to be involved in paclitaxel-induced peripheral neuropathic pain. In addition, intraperitoneal administration of a TRPV1 antagonist after paclitaxel treatment attenuates thermal but not mechanical hyperalgesia [10]. TRPV1 is the primary receptor involved in chemically and thermally evoked pain sensation [26], but expression of TRPV1 in DRG neurons after paclitaxel treatment has not been studied. In the present study, using a rat model of paclitaxel-induced, painful peripheral neuropathy, we investigated the effects of paclitaxel treatment on TRPV1 expression in the DRG, the mediators of thermal perception, and whether a TRPV1 antagonist or a TRP antagonist alters paclitaxel-induced peripheral thermal neuropathic pain.

2. Materials and methods

2.1. Experimental animals

Male Wistar rats weighing 250 to 300 g were used in the present study. All rats were housed individually in automatically controlled environmental conditions, using a 12-h light–dark cycle (lights on from 08:00 to 20:00) with free access to food and water. All animals were quarantined in centralized animal facilities for at least 7 days after arrival. Each animal was used only once. Experiments were carried out according to the guidelines for animal care and use published by the National Institutes of Health and the committee of Showa Pharmaceutical University.

2.2. Drug administration

Paclitaxel (2 or 4 mg/kg per milliliter, prepared with saline from Taxol or Bristol-Myers-Squibb, 6 mg/mL paclitaxel in Cremophor EL vehicle) or vehicle (Cremophor EL-polyethoxylated castor oil and ethanol, diluted with 2 parts saline to 1 part Cremophor EL) was administered intraperitoneally (i.p.) on 4 alternate days (days 0, 2, 4, and 6; cumulative dose, 8 or 16 mg/kg). Ruthenium red (3 mg/kg, s.c.) or capsazepine (30 mg/kg, s.c.) was administrated 30 min before tail-flick tests.

2.3. Thermal stimulation

Observers blinded to the experimental conditions tested the rats for thermal hyperalgesia at the same time on days 0, 7, 14, and 21 after paclitaxel treatment. In brief, rats were placed in a clear Plexiglas cylinder from which its tail protruded. The intensity of the light with the thermal stimulator (IITC Inc., Woodland Hills, CA) was adjusted at the start of the experiment such that average baseline latencies were approximately 6 s, and a cutoff latency of 15 s was established. The heat was directed to the distal tail (20 mm from the tip). Three trials were done at intervals of 5 min, and 1 score was assigned for each session by averaging the last 2 trials. The latencies were obtained alternately from each tail 5 min apart.

2.4. Quantitative real-time polymerase chain reaction

The rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) on days 7, 14, and 21 after the start of paclitaxel (2 or 4 mg/kg) treatment, and DRGs (L_{4-6}) were analyzed with quantitative real-time polymerase chain reaction (RT-PCR). RNA was purified using TRIzol (Invitrogen, Carlsbad, CA). Total RNA (1 μg) was used for cDNA synthesis with a SuperScript VILO cDNA Synthesis kit (Invitrogen). RT-PCR was performed with an Applied Biosystems StepOne RealTime PCR System (Applied Biosystems, Tokyo, Japan) using EXPRESS SYBR GreenER qPCR SuperMixes and Two-Step qRT-PCR kits (Invitrogen), according to the manufacturers' instructions. The cycling conditions for all primer pairs were as follows: 2 min at 50 °C to incubate uracil DNA glycosylase, 2 min at 95 °C (initiation), followed by a 2-step PCR program of 95 °C for 15 s (denaturation) and 60 °C for 60 s (annealing and extension) for 50 cycles. The TRPV1 and TRPV2 mRNA levels were normalized to the level of β-actin. The primer sequences were as follows: TRPV1, 5′-gcacaatgggcagaatgaca-3′ (forward) and 5′-gctggcattgacaaactgctt-3′ (reverse); TRPV2, 5′-ccgtttcctgctggtctacct-3′ (forward) and 5′-gggcctctctgctcaagctta-3′ (reverse); β-actin, 5′-caggttcatcactatcggcaatg-3′ (forward) and 5′-gagactacaacttacccaggaaggaa-3′ (reverse). These sequences corresponded to the rat TRPV1, TRPV2, or β-actin genes (Sigma–Aldrich, St. Louis, MO). In all cases, the validity of amplification was confirmed by the presence of a single peak in the melting temperature analysis and linear amplification throughout the PCR cycles.

2.5. Western blot analysis

The rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) on day 7, 10, 14, or 21 after the start of paclitaxel (2 or 4 mg/kg) treatment, and DRGs (L_{4-6}) were removed for Western blot analysis. DRG protein (30 μg) was separated through a sodium dodecyl sulphate–polyacrylamide gel (7.5%) and transfered onto nitrocellulose membranes. For Western blotting, anti-TRPV1 antibody (1:200; Alomone Labs, Israel) was used, and anti–β-actin antibody (1:1000; Sigma Aldrich) was used as the loading control. Horseradish peroxidase–labeled rabbit antibody (1:2000) was used as the secondary antibody. Specific bands were detected using enhanced chemiluminescence with the TM Western Blotting Detection kit (GE Healthcare, UK) according to the manufacturer's protocol. The intensities of immunoreactive bands were analyzed with MultiGage version 3 software (Fuji Film, Japan).

2.6. Immunohistochemistry

The rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) on day 14 after the start of paclitaxel (2 or 4 mg/kg) treatment, and DRGs were prepared for immunohistochemistry. Rats were perfused transcardially with 20 mL potassium-free phosphate-buffered saline (K^+-free PBS, pH 7.4) followed by 50 mL 4% paraformaldehyde solution. The DRGs (L_{4-6}) were removed, post-fixed for 3 h, and cryoprotected overnight in 25% sucrose solution. The DRGs were stored at −80 °C until use. DRGs were cut at 10-μm thickness, thaw-mounted on silane-coated glass slides, and air dried overnight at room temperature. DRG sections were incubated with excess blocking buffer containing 2% skim milk in 0.1% Triton X-100 in K^+-free PBS and subsequently reacted overnight at 4 °C with anti-TRPV1 antibodies (1:200; Alomone Labs) in 2% bovine serum albumin/0.1% Triton X-100 in K^+-free PBS. The sections were then incubated in fluorescein isothiocyanate–conjugated anti-rabbit IgG (Sigma–Aldrich, 1:200) for 2 h at room temperature. All sections were treated with Permafluor (Thermo Shandon, Pittsburgh, PA), coverslipped, and evaluated with microscopy.

The immunostained sections were mounted on slides, covered with microslips, and observed with an Olympus laser-scanning confocal microscope (FLUOVIEW BW50, Olympus, Tokyo, Japan) at wavelengths of 488 nm and 568 nm. Optical density (OD) of the stained sites was determined with the National lnstitutes of Health ImageJ 1.46, an open-source Java-based computer program. The OD of TRPV1-positive cells was calculated for day 14 after paclitaxel treatment or cremophor vehicle treatment.