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Full paper

Oxaliplatin treatment changes the function of sensory nerves in rats

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ABSTRACT

Oxaliplatin (L-OHP) is a platinum-based chemotherapy drug, used in standard treatment of colorectal cancer. L-OHP frequently causes acute peripheral neuropathies. These adverse effects limit cancer therapy with L-OHP. The present study was designed to reveal the changes in sensory nerve function in L-OHP-injected rats. Mechanical static allodynia, dynamic allodynia, and cold allodynia were evaluated using the von Frey test, brush test, and acetone test, respectively. Sensory nerve fiber responsiveness was measured using a Neurometer. The fifth lumbar ventral root was sectioned to record multi-unit efferent discharges. Single intraperitoneal administration of L-OHP induced mechanical static allodynia, dynamic allodynia, and cold allodynia in Wistar/ST rats. The thresholds for paw withdrawal induced by 2000 Hz (Aβ-fiber) and 5 Hz (C-fiber), but not 250 Hz (Aδ-fiber) sine-wave electrical stimulation were reduced in L-OHP-treated rats. Multi-unit efferent discharges were increased by mechanical stimulation using a von Frey filament applied to the plantar surface of the hindpaw. The discharges during and after stimulation were increased in the L-OHP-treated rats. Cold stimulation, but not brush stimulation, increased the discharges in L-OHP-treated rats. These results suggest that sensitization of Aβ- and C-fibers, but not Aδ-fibers, contributes to the development of L-OHP-induced mechanical and cold allodynia.

1. Introduction

Oxaliplatin (L-OHP) is a third-generation platinum agent (1) that binds to DNA and prevents the DNA replication required for mitosis (2). Although it is especially effective in the treatment of colorectal cancer (3), L-OHP causes peripheral neuropathy in many patients (4), which is the most frequent dose-limiting neurotoxicity of L-OHP (5). L-OHP exhibits two types of neurotoxicity (3). One is acute cold-hypersensitivity, which is a characteristic toxicity of L-OHP, and the other is chronic neuropathy, which is common to platinum-containing drugs, caused by the accumulation of platinum in the dorsal root ganglion (DRG) (6). Previous reports have shown an increase in ion-channel (7) and TRPA1 (8) mRNA expression in the DRG after L-OHP treatment. However, the mechanisms underlying L-OHP-induced acute neuropathy still remain unclear. Previous studies have mostly focused on the contribution of sensory receptors; only a few studies have focused on the functional differences in the peripheral nerve. The hypersensitivity induced by L-OHP may result from aberrant changes not only in the sensory reception system but also in the conduction and transduction pain systems.

Primary afferent nerve fibers have been classified into three major classes: unmyelinated C, myelinated thin Aδ, and myelinated Aβ fibers. The sensory C-fibers and Aδ-fibers conduct noxious chemical, mechanical, or thermal stimuli, which in turn cause nociceptive responses. The stimulation of Aβ-fibers is thought to cause mostly innocuous tactile sensations. Therefore, the functions of primary afferent fibers need to be evaluated individually. The Neurometer can selectively activate sensory nerve fibers using sine-wave pulses of different frequencies without affecting nociceptors. It has been reported that frequencies of 5, 250, and 2000 Hz activate C-, Aδ-, and Aβ-fibers, respectively. This stimulus-dependent selective activation of a specific type of primary afferent fiber has been confirmed by electrophysiological (9), pharmacological, and immunohistochemical (10,11) procedures.

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In light of the established importance of the spinal cord in the study of pain mechanisms and analgesics (12,13), several *in vivo* and *in vitro* electrophysiological methods have been developed and used to evaluate spinal cord function in nociceptive transmission. At the spinal level, nociceptive signals are transmitted from the dorsal horn to the motoneurons in the ventral horn, and withdrawal responses are induced through motoneurons to avoid noxious stimuli (14). In a previous study we recorded the discharges from the ventral root induced by mechanical stimulation of the ipsilateral hindpaw plantar surface, and these responses consisted of during- and after-discharges (15). Application of noxious mechanical stimuli to the corresponding hindpaw evoked spinal ventral root after-discharges (6,7). These after-discharges are significantly related to the above-mentioned mechanical hyperalgesia, because these phenomena are prolonged after cessation of the noxious stimuli (5,16). Such after-discharges are also observed in the spinal dorsal horn neurons (16,17) and are simultaneously recorded from motor units after high intensity transcutaneous stimulation (18,19). Since ventral root after-discharges are enhanced in neuropathic pain models, L-OHP treatment might affect ventral root firing after mechanical stimulation.

In this study, we examined the contribution of the various peripheral fibers in the acute phase of L-OHP-induced neuropathy using behavioral and electrophysiological methods. Therapies and prophylaxis for L-OHP-induced neuropathy, such as calcium-magnesium infusions (16,17), pregabalin (18), and Kampo medicines (19), have been reported but the findings are inconclusive (20). It is expected that elucidation of the changes in sensory fibers and the contribution of sensory fibers to neuropathy will be useful for the development of new therapies and prevention of L-OHP neuropathy.

2. Materials and methods

All of the experimental protocols used in the present study were approved by the Animal Care and Use Committee of Nagoya City University, and carried out in accordance with the guidelines of the Japanese Pharmacological Society.

2.1. Behavioral tests

Wistar/ST male rats (6–8 weeks old, SLC, Shizuoka, Japan) were used. Each behavioral test was performed before L-OHP administration (dissolved in 5% glucose, 10 ml/kg i.p.) and 1 and 3 days after administration. Rats were placed in suspended cages with wire-mesh floors. Stimuli were applied alternately three times to the left hindpaw and average scores were generated from the three scores for each rat.

2.2. Assessment of static allodynia

Static allodynia was assessed by measuring hindpaw withdrawal responses to von Frey filaments (Semmes–Weinstein monofilaments, Stoelting, Wood Dale, IL), ranging from 2 to 60 g (2, 4, 6, 8.5, 10, 15, 26, 60 g). The 50% likelihood of a paw withdrawal response (50% threshold) was determined using the up–down method (30). Testing was initiated with the 8.5 g filament, and each filament was applied perpendicular to the plantar surface of the hindpaw with sufficient force to cause a slight bending of the filaments, for about 3 s. In case of a positive response (lifting of the hindpaw), the next weaker filament was used. In case of a negative response (absence of a hindpaw withdrawal response), the next stronger filament was used. This paradigm was continued until four measurements had been obtained after an initial change in behavior, or until four consecutive positive (score of 1.5 g) or five negative scores (score of 80 g) had been obtained. The resulting scores were used to calculate the 50% threshold (31).

2.3. Assessment of dynamic allodynia

Dynamic allodynia was assessed by lightly stroking the plantar surface of the hindpaw with a paintbrush for 3 s. The allodynic response was ranked as follows according to (32): 0, no response or moving the stimulated paw aside; 1, lifting of the stimulated paw toward the abdomen; 2, flinching or licking of the stimulated paw.

2.4. Assessment of cold allodynia

Cold allodynia was assessed with the acetone drop method. A drop (50 μl) of acetone was placed at the lateral side of the plantar hindpaw. Responses evoked by vaporization of acetone were graded with the following 4-point scale (33,34): 0, no response; 1, quick withdrawal, flick, or stamp of the paw; 2, flicking of the hindpaw; 3, repeated flicking of the hindpaw with licking or biting of the lateral side of the hindpaw.

2.5. Electrical stimulation-induced paw withdrawal test

Three days after L-OHP administration rats were gently fixed in hammocks without anesthesia. A pair of ball-shaped electrodes (2 mm in diameter) was fastened to the left plantar surface and instep of the rats. Transcutaneous nerve stimuli using each of the three sine-wave frequencies (5, 250, and 2000 Hz) were applied using the Neurometer CPT/LAB (Neurotron Inc. Baltimore, MD, USA). The minimum intensity (microampere) at which each rat withdrew its paw and/or vocalized was defined as the stimulus threshold. Stimuli were applied at 2 min intervals. Means of three measurements were calculated.

2.6. Measurement of ventral root discharges

After the behavioral study, ventral root discharges were recorded using similar protocols to those reported in our previous studies (15,35). In brief, rats were anesthetized with α-chloralose (150 mg/kg, i.p.), and cannulae were inserted into the trachea for artificial respiration. The spinal cord was transected at the first cervical segmental level. A dorsal laminectomy was performed in the lumbo-sacral region of each rat. Both the ventral and dorsal roots below the sixth lumbar segment were cut distally at their points of exit from the vertebral column. The left fifth lumbar segmental (L5) ventral root was sectioned for recording and the ipsilateral L5 dorsal root was left intact to receive peripheral signals. The entire exposed surgical area was covered with liquid paraffin that was maintained at 36 ± 0.5 °C by radiant heat. Rectal temperature was maintained at 36 ± 0.5 °C.

The left plantar surface of the hindpaw was stimulated using von Frey filaments, paintbrush, and acetone. The ventral root discharges occurring during the 3 s of von Frey filament stimulation were defined as 'during-discharges' and those occurring up to 60 s after the stimulation as 'after-discharges'. The ventral root discharges induced by brush stimuli were evaluated by lightly stroking the plantar surface of the rat's left hindpaw with a paintbrush (brush) for 3 s. The ventral root discharges induced by cold stimuli were evaluated for 30 s after the application of 50 μl acetone onto the plantar surface. These responses were normalized by subtraction of the spontaneous activity measured before application of the stimuli. A pair of Ag–AgCl wire electrodes was used for recording. Motoneuronal multi-unit firing from the left L5 whole ventral root was recorded on a digital recorder (sampling rate: 48 kHz, R-44, Roland, Shizuoka, Japan). The signals were amplified and analyzed