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Protective effects of phosphatidylcholine on oxaliplatin-induced neuropathy in rats

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ABSTRACT

Aims: The present study was designed to investigate the therapeutic potential of phosphatidylcholine (PC) on oxaliplatin-induced peripheral neuropathy.
Main methods: Male Sprague–Dawley rats were randomly divided into three groups: the control group, the oxaliplatin group (4 mg/kg, twice per week for 4 weeks) and the oxaliplatin + PC (300 mg/kg) group. To evaluate the effect of PC, we examined the thermal nociceptive threshold changes in oxaliplatin-induced peripheral neuropathy by conducting paw pressure, hot-plate and tail-flick tests. Additional experiments on the degree of oxidative stress in the sciatic nerves were performed by measuring the level of MDA, total glutathione (GSH), glutathione peroxidase (GPx) activity and superoxide dismutase (SOD) activity. We also used histopathological and immunohistochemical methods to observe neuronal damage and glial activation.
Key findings: PC attenuated oxidative stress by increasing antioxidant levels. In histopathological evaluation, the PC administrated group maintained normal morphologic appearance of sciatic nerves, similar to the control group. In spinal cords, however, no significant difference between the oxaliplatin-alone group and the oxaliplatin + PC group was observed. In the immunohistochemical evaluation, PC administration ameliorated oxaliplatin-induced microglial activation.
Significance: It is suggested that PC has a therapeutic potential against oxaliplatin-induced peripheral neuropathy due to its antioxidant property and modulation of microglial activities.

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1. Introduction

Oxaliplatin is a third-generation platinum-based antineoplastic agent, which is commonly used in treating advanced colorectal cancer, and as adjuvant therapy in several types of cancer [12]. Although oxaliplatin has less ototoxicity and nephrotoxicity than other platinum-based chemotherapeutic agents, it causes acute and chronic peripheral neurotoxicity [1,13]. Acute neuropathy can be observed in almost all patients. This neuropathy occurs within hours of injection, and can be resolved within days [4,18]. On the other hand, chronic neuropathy is observed in 10–15% of patients after cumulative injection of oxaliplatin, which cannot be resolved easily [10,16]. It is one of the main reasons that patients do not continue their cancer treatments; therefore, it is important to protect cancer patients from chemotherapy-induced neuropathic pain.

Developing effective treatments to attenuate peripheral neuropathy is difficult because knowledge about the mechanism of oxaliplatin-induced neuropathy is still insufficient [13]. Many studies suggest that oxidative stress associated with oxaliplatin is a direct cause of neuropathy. It is generally known that chemotherapeutic agents generate reactive oxygen species (ROS) to induce apoptosis in cancer cells [11]. However, ROS also affects normal cells and tissues and may be associated with neurotoxicity. In particular, peripheral nerves can be physically damaged by demyelination, mitochondrial dysfunction, inflammation, and apoptosis [26]. Thus, levels of glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and activities of mitochondrial enzymes are good biomarkers for determining neuropathy. A recent study suggests that the spinal cord and its subpopulation are directly

Abbreviations: PC, phosphatidylcholine; GSH, total glutathione; GPx, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; PMSF, phenylmethylsulfonyl fluoride; TBA, thiobarbituric acid; GSSG, glutathione disulfide; GFAP, glial fibrillary acidic protein.

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damaged by oxaliplatin, and oxidative stress is the major reason for this neuronal damage [7].

To ameliorate oxaliplatin-induced neuropathy, various treatments have been suggested including acetyl-L-carnitine, vitamin E, vitamin C, glutathione, and amifostine [2,3,15]. Among these treatments, antioxidants (glutathione, N-acetylcysteine, and vitamin E) are known for their neuron-protecting actions, alleviating functional impairments of neurons [6,8,9,22]. However, their abilities to relieve pain have proven to be insufficient. There are several reasons that can explain this weakness: 1) irreversibility of established oxidative damage; 2) radical specificity of antioxidants; and 3) interference with oxidation-reduction signaling pathways [17].

Phosphatidylcholine (PC, 1,2-diacyl-sn-glycero-3 phosphocholine) is a major component of biological membranes and several studies suggest that PC has antioxidant effects and prevents lipid peroxidation [23,27]. In our previous study, the treatment with PC resulted in a significant attenuation on the increase in serum levels of TNF-α and IL-6, pro-inflammatory cytokines in lipopolysaccharide-induced acute inflammation in multiple organ injury, suggesting that PC may be a functional material for its use as an anti-inflammatory agent [20]. In addition, decreases in choline level are associated with oxidative damage, resulting in cellular injury and necrosis [30]. Therefore, we evaluated the protective effect of PC in rats by recording the thermal nociceptive threshold changes in oxaliplatin-induced peripheral neuropathy by conducting paw pressure, hot-plate and tail-flick tests. Then, we examined quantity of MDA, total GSH, glutathione peroxidase (GPx) activity and superoxide dismutase activity to determine the oxidative stress level. We used histopathological and immunohistochemical methods to observe neuronal damage and glial activation in sciatic nerves and lumbar spinal cords.

2. Materials and methods

2.1. Chemicals

Oxaliplatin (5 mg/ml) was purchased from the Sanofi-Aventis pharmaceutical company. Phosphatidylcholine was purchased from Lipoid GmbH (Phospholipon90G). MDA, SOD activity, total GSH and GPx activity assay kits were purchased from Biovision Inc. (San Francisco, CA, USA). All other essential chemicals were purchased from Sigma-Aldrich chemical Co. (St. Louis, MO, USA).

2.2. Animals

Male Sprague–Dawley rats, 5 weeks old and weighing about 180 g, were purchased from Samtako Biotechnology (Osan, Republic of Korea). Animals were housed in standard laboratory conditions (22 ± 2 °C, light:dark cycle of 12:12 h), with food (Purina, Republic of Korea) and tap water ad libitum for 1 week before starting the experiment. The animal experiments were approved by the Institutional Animal Care and Use Committee of Chung-Ang University, in accordance with the guidelines for the Care and Use of Laboratory Animals in Seoul, Republic of Korea.

2.3. Treatments

To determine the proper dosage of PC, we conducted preliminary experiments in which rats were orally administered several dosages of PC (200 mg, 300 mg or 500 mg/kg) and injected intraperitoneally with 4 mg/kg of oxaliplatin [1,31]. As a result, the PC dosage 300 mg/kg was chosen because it showed the most desired effect against oxaliplatin-induced neuronal damage and thermal & mechanical hypersensitivity.

Rats were divided into three groups (n = 6 for each group): the control group was injected intraperitoneally with 0.9% saline twice a week for 4 weeks and orally administered distilled water five times a week for 4 weeks. The oxaliplatin group was injected intraperitoneally with oxaliplatin (4 mg/kg) twice a week for 4 weeks and orally administered distilled water. The oxaliplatin (4 mg/kg) + PC group was injected intraperitoneally with oxaliplatin twice a week for 4 weeks and orally administered PC (300 mg/kg) five times a week for 4 weeks. PC was suspended in distilled water (100 mg/ml).

2.4. Behavioral tests

All behavioral tests were performed on days 0, 7, 14, 21, and 28. Each test was conducted by the same researcher to minimize variation.

2.5. Paw pressure test

Mechanical nociceptive threshold was assessed by using an analgesiometer (Ugo Basile, Varese, Italy). Briefly, rats were lightly restrained and mechanical pressure was applied to a hind paw of the rat. Pressure was constantly increased until withdrawal reflex was observed, then operators read the scale and recorded the force. The cut-off pressure was 180 g.

2.6. Hot-plate test

The thermal nociceptive threshold was assessed using the hot-plate method. Rats were placed inside an acryl pipe with a hot-plate floor. The hot plate's temperature was kept constant at 49–50 °C. Once the pain-reflex behavior was observed, operators recorded the time (seconds). The cut-off time of the latency to pain-reflex behavior was 60 s.

2.7. Tail-flick test

The thermal nociceptive threshold was measured in rats using a tail-flick unit (Ugo Basile, Varese, Italy). Briefly, rats were lightly restrained and infrared heat stimulus was applied to the tail. When the animal flicked its tail due to thermal stimulus, a sensor detected it and automatically recorded the reaction time. The cut-off time of the latency to tail-flick was 10 s.

2.8. Sample collection

After the final behavioral tests (28 days after starting the experiment), the animals were anesthetized with ethyl ether. The right sciatic nerve was isolated and washed with 0.9% saline, then homogenized in lysis buffer containing a protease inhibitor and PMSF. After homogenization, the homogenate was incubated on ice for 1 h then sonicated. The obtained material was centrifuged (13,000 *g* for 15 min at 4 °C) and stored at −70 °C, pending biochemical analysis. The left sciatic nerve was excised and fixed in 10% neutral formalin for histopathological examination.

2.9. Biochemical measurements

2.9.1. Lipid peroxidation

Lipid peroxidation was determined by measuring MDA production using the lipid peroxidation assay kit (K739-100, Biovision). Briefly, sciatic nerve tissue homogenate was diluted with MDA lysis buffer and the total sample volume was 200 μl. A 600 μl of thiobarbituric acid (TBA) reagent was added to each sample and incubated at 95 °C for 60 min. The tube was cooled to room temperature in an ice bath for 10 min. After cooling, 300 μl of n-butanol was added and centrifuged (3 min at 16,000 *g*). n-Butanol was removed and the MDA–TBA adduct was placed into a 96-well plate and absorbance was measured at 532 nm. MDA content was calculated with MDA standards.

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