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Involvement of mast cells and proteinase-activated receptor 2 in oxaliplatin-induced mechanical allodynia in mice

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ABSTRACT

The chemotherapeutic agent oxaliplatin induces neuropathic pain, a dose-limiting side effect, but the underlying mechanisms are not fully understood. Here, we show the potential involvement of cutaneous mast cells in oxaliplatin-induced mechanical allodynia in mice. A single intraperitoneal injection of oxaliplatin induced mechanical allodynia, which peaked on day 10 after injection. Oxaliplatin-induced mechanical allodynia was almost completely prevented by congenital mast cell deficiency. The numbers of total and degranulated mast cells was significantly increased in the skin after oxaliplatin administration. Repetitive topical application of the mast cell stabilizer azelastine hydrochloride inhibited mechanical allodynia and the degranulation of mast cells without affecting the number of mast cells in oxaliplatin-treated mice. The serine protease inhibitor camostat mesilate and the proteinase-activated receptor 2 (PAR2) antagonist FSLLRY-NH_2 significantly inhibited oxaliplatin-induced mechanical allodynia. However, it was not inhibited by the H_1 histamine receptor antagonist terfenadine. Single oxaliplatin administration increased the activity of cutaneous serine proteases, which was attenuated by camostat and mast cell deficiency. Depletion of the capsaicin-sensitive primary afferents by neonatal capsaicin treatment almost completely prevented oxaliplatin-induced mechanical allodynia, the increase in the number of mast cells, and the activity of cutaneous serine proteases. These results suggest that serine protease(s) released from mast cells and PAR2 are involved in oxaliplatin-induced mechanical allodynia. Therefore, oxaliplatin may indirectly affect the functions of mast cells through its action on capsaicin-sensitive primary afferents.

1. Introduction

Oxaliplatin is a platinum-based chemotherapeutic agent that is mainly used for the treatment of colorectal cancer. However, it causes dose-limiting side effects such as pain and dysesthesia, which are thought to be mainly attributable to peripheral neuropathy [1,2]. Although altered cellular metabolism and axoplasmic transport, and increased expression of the transient receptor potential melastatin 8 are speculated to be involved [1,3], the underlying mechanisms of oxaliplatin-induced pain and dysesthesia are not completely understood.

The clinical use of oxaliplatin was reported to induce mast cell-mediated anaphylactic reactions such as respiratory and cutaneous symptoms [4]. Mast cells are immune cells characterized by an abundance of secretory granules that contain numerous inflammatory mediators such as histamine, tryptase, and ATP [5]. Mast cell mediators are also involved in the induction of pain [6,7]. For example, the proteinase-activated receptor 2 (PAR2), which can be activated by mast-cell tryptase [8], participates in hyperalgesia [9–11]. On the basis of these findings, the present study investigated whether mast cells and their mediators are involved in oxaliplatin-induced mechanical allodynia in mice.

Here, we show that oxaliplatin increases the number of mast cells and their degranulation in the skin and that mast cell deficiency and topical application of a mast cell-stabilizing drug inhibit oxaliplatin-induced mechanical allodynia. We also show that oxaliplatin increases serine protease activity in the skin and that inhibitors of serine protease or its receptor PAR2 suppress oxaliplatin-induced mechanical allodynia. Regarding the primary site of action of oxaliplatin, we show that neonatal capsaicin treatment prevents the increase in the number of mast cells and mast cell degranulation and serine protease activity after oxaliplatin administration.

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2. Materials and methods

2.1. Animals

Male C57BL/6NCr mice were used in all of the experiments except for one series, which used male mast-cell deficient mice (WBB6F1 W/W^v) and their normal littermates (WBB6F1 +/+) (Supplemental data-1). The percentage of mast cells in the skin of WBB6F1 W/W^v mice is lower than that in the skin of WBB6F1 +/+ mice early after birth, decreasing to less than 1% in mice older than 50 days of age [12]. All the mice were purchased from Japan SLC (Shizuoka, Japan) and were 6 weeks old at the start of the experiments. The mice were housed 4–7 per cage in a room with controlled temperature (21–23 °C), humidity (45–65%), and light cycle (lights on from 07:00 to 19:00). Food and water were available ab libitum. The animal experimental procedures were approved by the Committee for Animal Experiments at the University of Toyama and were conducted in accordance with the guidelines of the Japanese Pharmacological Society.

2.2. Drugs

Oxaliplatin (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in 5% glucose and was administered intraperitoneally in a volume of 0.1 mL per 10 g body weight using a 26-gauge needle. Azelsatine hydrochloride (Sigma–Aldrich) was dissolved in 100% ethanol and was applied twice a day in a volume of 50 μL per paw. Terfenadine (Sigma–Aldrich) was dissolved in 0.5% sodium carboxymethyl cellulose (Wako Pure Chemical Industries, Osaka Japan) and camostat mesilate (Wako Pure Chemical Industries) was dissolved in tap water. They were administered orally in a volume of 0.1 mL per 10 g body weight using a feeding needle. FSLLRY-NH_2 was synthesized and identified using the peptide synthesizer PSSM-8 (Shimazu Co., Kyoto, Japan) and a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (Autoflex T1, Bruker Daltonics, Billerica, MA, USA). LSFYRL-NH_2 was synthesized by BEX Co., Ltd. (Tokyo, Japan). The peptides were dissolved in physiological saline and were administered into the plantar hind paw in a volume of 20 μL per site using a 29-gauge needle. The doses of terfenadine [13], camostat mesilate [14], and FSLLRY-NH_2 [15] were selected from that reported in the specified published literature.

2.3. Neonatal capsaicin treatment

To deplete capsaicin-sensitive sensory neurons, capsaicin was dissolved in 10% ethanol and 10% Tween 80 in saline and was injected subcutaneously into mice at a dose of 50 mg/kg body weight, twice on day 2 and 5 after birth [16]. To verify the depletion of the capsaicin-sensitive primary afferents, one drop (10 μL) of 0.1% capsaicin was applied to one cornea and the number of wiping movements performed by the treated mice in 30 s was counted as described previously [17]. We excluded neonatally capsaicin-treated mice that showed a wiping frequency similar to that of neonatally vehicle-treated mice.

2.4. Behavioral tests

Mechanical allodynia of the hind paws was assessed by punctate stimulation with a von Frey filament (North Coast Medical Inc., Morgan Hill, CA, USA) with a bending force of 0.69 mN (innocuous stimulation) [18]. The mice were placed individually in an acrylic cage (11 × 18 × 15 cm) with a wire mesh bottom for at least 30 min for acclimation. Subsequently, the von Frey filament was pressed perpendicularly against the central part of the plantar hind paw of the freely moving mice and was held there for 1–3 s with slight buckling. The responses of the hind paw to the stimulation were ranked as follows: 0—no response; 1—lifting of the hind paw; and 2—flinching or licking of the hind paw. The stimulation was applied 6 times to each hind paw at intervals of several seconds, and the average score was used as the response score. All behavioral experiments were carried out in a blinded fashion.

2.5. Determination of serine protease activity

The plantar skin was isolated on day 10 after the oxaliplatin injection; camostat was administered 1.5 h before isolation. The serine protease activity was measured as described previously [15]. Briefly, the skin sample was homogenized and sonicated in 10 mM Tris pH 6.1 containing 2 M NaCl. After centrifugation (700 × *g* for 5 min at 4 °C), the protein concentration in the supernatant was determined using a protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Five microliters of the supernatant (2 μg protein/μL) was added to 45 μL of solution A (0.06 M Tris at pH 7.8 containing 0.4% dimethyl sulfoxide and 30 μg/mL heparin). The cocktail (50 μL) was reacted with 50 μL of the enzyme substrate N-*p*-Tosyl-Gly-Pro-Arg-*p*-nitroanilide (480 μg/mL, Sigma–Aldrich) in solution A at 37 °C for 1 h; the substrate is acted on by serine proteases such as tryptase [19], kallikrein 14 [20], thrombin [21], and trypsin [22]. The *p*-nitroanilide released was determined colorimetrically at 405 nm.

2.6. Toluidine blue staining

On day 10 after the oxaliplatin injection, the mice were anesthetized with intraperitoneally administered sodium pentobarbital (80 mg/kg body weight, Sigma–Aldrich) and were transcardially perfused with phosphate-buffered saline following by perfusion with 4% paraformaldehyde. The skin of the central region of the plantar hind paw was isolated, postfixed with 4% paraformaldehyde for 4 h, and immersed in 30% sucrose solution for 4 days. The tissue was embedded in Tissue-Tek® O.C.T. compound (Sakura Fineteck Co., Ltd., Tokyo, Japan) and was kept at −80 °C until use. The frozen samples were sectioned at 20-μm thickness with a cryostat (Leica, Wetzlar, Germany). After three washing steps in phosphate-buffered saline, the sections (3–6 sections per animal) were stained with 0.1% toluidine blue and were washed with tap water. For dehydration, the slides were immersed sequentially in 50%, 70%, 80%, and 90% ethanol for 1 min each, following by 100% ethanol and xylene immersions for 10 min each. The sections were mounted with Canada balsam and were observed using a light microscope (BX-61, Olympus, Osaka, Japan) with a charge-coupled device camera (DP70, Olympus). The staining and counting of mast cells were performed in a blinded fashion. Typical examples of non-degranulated and granulated mast cells are shown in Supplemental data-2.

2.7. Data processing

The data represent the means ± S.E.M. unless otherwise indicated. Statistical significance was determined using the Student's *t*-test (two groups), or one- or two-way analysis of variance (ANOVA) or two-way repeated measures ANOVA followed by a post hoc Holm–Šidák test (three or more groups). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Oxaliplatin-induced mechanical allodynia

A single intraperitoneal injection of oxaliplatin (1–10 mg/kg body weight) induced mechanical allodynia in the C57BL/6NCr mice. The time-courses were similar among the doses tested