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Involvement of increased expression of transient receptor potential melastatin 8 in oxaliplatin-induced cold allodynia in mice

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ABSTRACT

Oxaliplatin is a chemotherapy drug and induces peripheral neuropathy which is aggravated by exposure to cold, the mechanism of which is unclear. In the present study, we investigated in mice whether transient receptor potential melastatin 8 (TRPM8), which is activated by cooling temperature, would be involved in cold allodynia induced by oxaliplatin. Mice were given an intraperitoneal injection of oxaliplatin. Acetone was applied to hind paw for cooling stimulation, and the time spent for licking to the hind paw was measured. The expression of TRPM8 mRNA in dorsal root ganglion was determined by the RT-PCR method. An injection of oxaliplatin induced cold allodynia, which peaked on day 3 after injection and did not disappear even on day 25. Peak cold allodynia was inhibited by capsazepine, a blocker of both TRPM8 and heat-activated TRPV1, but not by 5′-iodoresiniferatoxin, a TRPV1 blocker. Oxaliplatin increased wet-dog shake and jumping behaviors evoked by the TRPM8 agonist icilin. An injection of oxaliplatin increased the expression level of TRPM8 mRNA at day 3 after injection and the expression was decreased to the near-normal level on days 10 and 25. These results suggest that cold allodynia induced by oxaliplatin is at least partly due to the increased expression of TRPM8 in the primary afferents.

Oxaliplatin, a third-generation platinum-based chemotherapy drug, induces serious sensory neurotoxicity, which is aggravated by exposure to cold [16]. The onset of oxaliplatin-induced pain is relatively rapid [16]. The precise mechanisms of oxaliplatin-induced neuropathic pain remain obscure although the involvement of a disturbance of cellular metabolism and the axoplasmatic transport is speculated [16]. In rodents, a single injection of oxaliplatin induces mechanical and cold allodynia [12,9,6]. Controversy exists concerning the induction of heat and mechanical hyperalgesia by oxaliplatin [12,9]. Mechanical allodynia is clearly inhibited by single injections of morphine, lidocaine and gabapentin [13,8]. It is also inhibited by repeated injections of limaprost, clomipramine, magnesium, and calcium [6,13]. Cold allodynia is inhibited by morphine, lidocaine and pregabalin, but the effects of lidocaine and pregabalin are not dependent on doses; the effects are not clear at higher doses [11]. Considering that oxaliplatin-induced sensory symptom is aggravated by cold, it is important to understand the mechanisms of cold allodynia.

Transient receptor potential melastatin 8 (TRPM8) is an ion channel that belongs to the transient receptor potential family [19]. TRPM8 mRNA is expressed in dorsal root ganglion (DRG), but not in other tissues including the brain, spinal cord, heart, skin, muscle [15]. Approximately 20% of the DRG neurons express TRPM8 [10]. TRPM8 is activated by innocuous cool (15–28 °C) and noxious cold (<15 °C) temperature [18]. TRPM8 is also activated by menthol, an ingredient of peppermint that produces a cooling sensation [14]. Studies using TRPM8 null mice have revealed that TRPM8 is required for cold sensation [2,4,5]. The present study investigated whether a single injection of oxaliplatin would induce cold allodynia in mice and whether TRPM8 would be involved in this cold allodynia.

Male C57BL/6 mice (Japan SLC, Shizuoka, Japan), 6 weeks old at the start of the experiment, were used. They were kept under controlled temperature (22 ± 1 °C), humidity (55 ± 10%), and 7:00 am–7:00 pm alternate light–dark cycles. Food and water were freely available. This study was conducted with the approval of the Committee for Animal Experiments at University of Toyama and according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Oxaliplatin (Sigma, St. Louis, MO) and capsazepine (Sigma) were dissolved in 5% glucose and in saline containing 7.5% dimethyl sulphoxide, respectively. 5′-Iodoresiniferatoxin (LC Laboratories, Woburn, MA) was dissolved in saline containing 10% ethanol and 10% Tween 80. Icilin (Cayman Chemical, Ann Arbor, MI) was dissolved in phosphate-buffered saline containing 20% dimethyl sulfoxide. The doses of capsazepine and 5′-iodoresiniferatoxin were chosen from the published literature [24].

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For the assessment of cold allodynia, the mouse was held by the hand and a droplet (50 μl) of acetone, formed on the flat-tip needle of a syringe, was gently touched to the plantar surface of the hind paw. The mouse was immediately put in an acrylic cage with a transparent floor and the behaviors were videotaped from beneath. Measurement of time spent in licking of the plantar region over a 60 s period was made by video playback. Acetone was applied three times at a 10–15 min interval and the average of licking time was calculated. The TRPM8 agonist icilin induces sensations of intense cold in humans [22] and wet-dog shake and jumping behaviors in rodents [20]. For the assessment of responsiveness of TRPM8, mice were given an intraperitoneal injection of icilin, and immediately placed individually under bell jars. Wet-dog shake and jumping behaviors were counted over a 10-min period.

The DRGs were isolated from mice that had not been tested for cold allodynia. Total RNA was extracted from pooled DRGs at the level of L4 and 5 by using GenElute™ (Sigma) and mRNAs encoding TRPM8 and glyceraldehyde-3-phosphate dehydrogenase were determined by RT-PCR [1]. The sequences of primers were as follows: TRPM8, 5′-ggctggagatgagattgtgag-3′ (sense) and 5′-gctgaagtgggtggagaaga-3′ (antisense); glyceraldehyde-3-phosphate dehydrogenase, 5′-ccaaggtcatccatgacaac-3′ (sense) and 5′-ttactccttggaggccacgt-3′ (antisense). The expression levels of mRNAs were analyzed with NIH Image software (National Institute of Health, Bethesda, Maryland, USA).

All data are presented as mean ± S.E.M. Results were analyzed with Student's *t*-test, Dunnett's multiple comparisons or Student–Newman–Keuls multiple comparisons; $p < 0.05$ was considered significant.

A recommended intravenous dose of oxaliplatin is 85 mg/m^2 body surface area (package insert of Elplat®, Yakult Honsha Co., Ltd.). If body height and weight are 170 cm and 60 kg, respectively, body surface area is 1.69 m^2, according to Du Bois's formula for calculating the body surface area, the amount is 144 mg (=85 mg/m^2 × 1.69 m^2) and the dose is 2.4 mg/kg (=144 mg/60 kg). Thus, oxaliplatin was administered at an intraperitoneal dose of 3 mg/kg [6]. A single administration of oxaliplatin caused cold allodynia, which was rapidly developed for the initial few days, peaked on day 3 and then gradually decreased by day 14. However, it did not completely disappear even day 25 after administration (Fig. 1).

Since the cold allodynia was most marked on day 3 after administration, the involvement of TRPM8 in the cold allodynia was investigated on this day. Capsazepine (10 and 30 mg/kg, intraperitoneal) attenuated the cold allodynia in a dose-dependent manner, but 5′-iodoresiniferatoxin (0.75 mg/kg, intraperitoneal) did not inhibit it (Fig. 2). In healthy mice, an intraperitoneal injection of icilin (60 mg/kg), a potent TRPM8 agonist, elicited wet-dog shake and jumping behaviors, which peaked 5–6 min after injection and subsided within 10 min (data not shown). The icilin-evoked behaviors were significantly increased in mice treated with oxaliplatin; the number of evoked behaviors was 22.4 ± 1.4 ($n = 10$) and 33.6 ± 1.8 ($n = 8$) in vehicle- and oxaliplatin-treated groups, respectively.

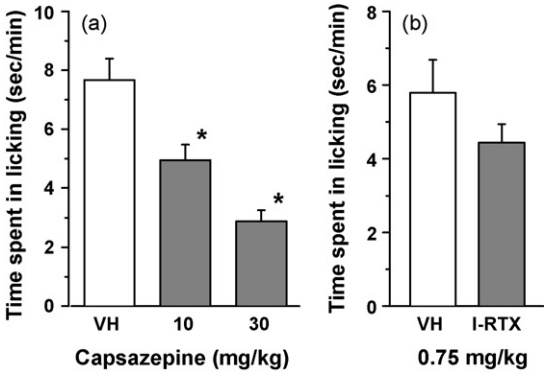

Fig. 2. Effects of capsazepine and 5′-iodoresiniferatoxin (I-RTX) on cold allodynia induced by oxaliplatin. Mice were given an intraperitoneal injection of oxaliplatin (3 mg/kg). Capsazepine, I-RTX and their vehicle (VH) were injected intreaperitoneally on day 3 after oxaliplatin administration and cold allodynia was evaluated 1 h later. Data are presented as mean and S.E.M. ($n = 6$). *$p < 0.05$ vs. vehicle-treated group (Dunnett's multiple comparisons for capsazepine, Student's *t*-test for I-RTX).

Since TRPM8 is expressed in primary sensory neurons [10], we examined the effect of oxaliplatin on the expression of TRPM8 mRNA in the dorsal root ganglia. An injection of oxaliplatin (3 mg/kg, intraperitoneal) significantly increased the expression level of TRPM8 mRNA at day 3 after injection and the expression was decreased to the near-normal level on days 10 and 25 (Fig. 3).

A single injection of oxaliplatin (3 mg/kg) induced cold allodynia in mice. It peaked day 3 after injection and then gradually decreased, but it did not completely disappear even on day 25. The time-course of cold allodynia was roughly similar to that observed in rats [12,13]. In contrast, after a single injection of oxaliplatin (3 mg/kg) in mice, mechanical allodynia is gradually increased, peaks on day 10, and almost subsides by day 25 [6]. Differences in time-course between cold and mechanical allodynia suggest that

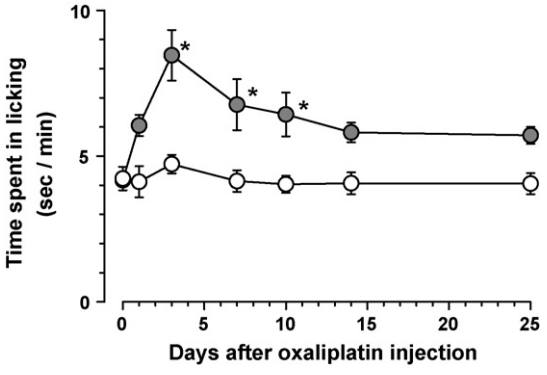

Fig. 1. Cold allodynia after single injection of oxaliplatin in mice. A droplet of acetone was gently applied to the plantar region of the hind paw as cool stimulus and time spent in licking of the treated paw was measured. It was repeated three times at a 10–15 min interval and the average was calculated. Oxaliplatin (3 mg/kg) was injected intraperitoneally. Closed and open circles indicate oxaliplatin- and vehicle-treated groups, respectively. Data are presented as mean and S.E.M. ($n = 6$). *$p < 0.05$ vs. vehicle-treated group (Student–Newman–Keuls multiple comparisons).

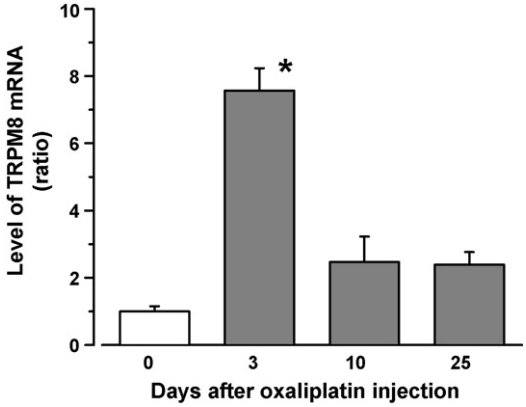

Fig. 3. Effect of oxaliplatin on the expression of TRPM8 mRNA in the dorsal root ganglia. Mice were given an intraperitoneal injection of oxaliplatin (3 mg/kg). The expression levels of mRNAs of TRPM8 and glyceraldehyde-3-phosphate dehydrogenase were determined by RT-PCR and the ratio of the former to the latter was normalized to that of day 0. Data are presented as means and S.E.M. ($n = 4$–5). *$p < 0.05$ vs. untreated group (Dunnett's multiple comparisons).

mechanisms of cold allodynia is different from those of mechanical allodynia.

TRPM8 is involved in the induction of hypersensitivity to cold stimuli [2,4,5]. Capsazepine, a blocker of both TRPM8 and TRPV1 channels [23], inhibited oxaliplatin-induced cold allodynia. 5′-Iodoresiniferatoxin, a selective TRPV1 blocker [21], did not attenuate the allodynia. It has been shown to have a partial TRPV1 agonistic activity and to cause hypothermia [17]. However, it neither aggravated nor inhibited cold allodynia, suggesting that TRPV1 antagonsitic and partial agonistic actions do not affect cold allodynia. Thus, the results taken together suggest that TRPM8 plays a role in cold allodynia caused by oxaliplatin.

Oxaliplatin increased the expression of TRPM8 mRNA in the dorsal root ganglia; the effect peaked day 3 after injection, when cold allodynia also peaked, suggesting that increase in cold allodynia is at least partly due to the increased expression of TRPM8 in primary afferents. To determine whether increased expression of TRPM8 in the dorsal root ganglia resulted in the increase of response to TRPM8 stimulation, we examined the effect of oxaliplatin on the behavioral response to icilin. An injection of icilin caused wet-dog shake and jumping behaviors, which was significantly increased in mice that were given oxaliplatin. Icilin-elicited wet-dog shake and jumping behaviors are absent in mice deficient in TRPM8 [4], suggesting that these behaviors are TRPM8-mediated responses. Therefore, it is suggested that increased expression of TRPM8 results in the increase of responses to cooling stimulation.

Oxaliplatin induced cold and mechanical allodynia in rodents [present experiment, 2, 23]. Electrophysiological study showed that oxaliplatin enhanced the response of the saphenous nerve to mechanical [7] and cold stimuli [Gauchan et al., unpublished observation]. Innocuous and noxious mechanical stimulation evokes firing in cold-sensitive wide dynamic range neurons in the spinal cord [3]. It is suggested that oxaliplatin affects cold- and mechanosensitive neurons. On the other hand, oxaliplatin does not cause pain-like response to innocuous 42 °C stimulation [12]. The mechanisms of modality-specific action of oxaliplatin remain unclear, which should be elucidated for the understanding of the mechanisms of oxaliplatin-induced neuropathy.

In conclusion, single administration of oxaliplatin caused cold allodynia in mice, which may be at least partly due to the increased of TRPM8 in the peripheral terminals of sensory neurons. Therefore, TRPM8 may be an important target for the alleviation of oxaliplatin-induced cold allodynia.

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