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Short communication

Involvement of c-Myc-mediated transient receptor potential melastatin 8 expression in oxaliplatin-induced cold allodynia in mice

Shizuka Mizoguchi [a], Tsugunobu Andoh [a,*], Takayuki Yakura [b], Yasushi Kuraishi [c]

[a] Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
[b] Department of Organomolecular Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
[c] Tokyo Medical and Dental University, Tokyo, Japan

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ABSTRACT

Background: Oxaliplatin, a platinum-based chemotherapeutic agent, induces acute cold allodynia and dysesthesia. Cold-sensitive transient receptor potential channels (TRPM8 and TRPA1) have been implicated as candidates to mediate oxaliplatin-induced cold allodynia and hyperalgesia, but precise roles of these channels remain unclear. In this study, we investigated the role of TRPM8 in oxaliplatin-induced cold allodynia.

Methods: Oxaliplatin was injected intraperitoneally in mice. Cold allodynia was evaluated by the acetone test. Expression levels of TRPM8 mRNA and protein were measured using reverse transcription-polymerase chain reaction and Western blotting, respectively.

Results: Oxaliplatin-induced cold allodynia was alleviated by the TRPM8 blockers *N*-(2-aminoethyl)-*N*-[4-(benzyloxy)-3-methoxybenzyl]-*N*′-(1S)-1-(phenyl) ethyl] urea and TC-I 2014. Oxaliplatin increased the expression levels of TRPM8 mRNA and protein in the dorsal root ganglia and plantar skin, respectively. Prophylactic administration of the c-Myc inhibitor 10058-F4 prevented cold allodynia and the increase of TRPM8 mRNA after oxaliplatin injection.

Conclusion: These results suggest that oxaliplatin induces cold allodynia through the increase of c-Myc-mediated TRPM8 expression in primary sensory neurons.

Introduction

Oxaliplatin, a platinum-based chemotherapeutic agent, is used mainly for the treatment of colorectal cancer. Oxaliplatin causes acute and chronic peripheral neuropathies; the former is related to the amount of the single dose and the latter total cumulative dose [1]. Chronic oxaliplatin treatment induces an axonal neuropathy similar to the other platinum-based drugs, while acute neurotoxicity is peculiar to oxaliplatin, mainly consists of cold allodynia and dysesthesia [2]. Acute neurotoxicity develops during or immediately after infusion, peaks in severity at day 3, and then improves [3], in which cold pain threshold is increased from ~12 °C to ~26 °C [4].

In rodents, cold hyperalgesia is observed as early as 2 h after oxaliplatin injection and continues more than 7 days [5], and cold allodynia develops 2 days after oxaliplatin injection, peaks after 3–4 days, and then gradually improves [6,7]. Two members of transient receptor potential (TRP) superfamily, TRPM8 and TRPA1, are present in primary sensory neurons [8], can be activated by cold stimuli in rodents, and their involvement in the acute oxaliplatin neurotoxicity has been investigated. TRPA1 is activated by cold (≤17 °C) stimuli [9]. Deficiency in TRPA1 gene and a TRPA1 blocker inhibits oxaliplatin-induced cold hyperalgesia [5,7]. Deficiency in TRPA1 gene inhibits cold allodynia also [7]. These findings suggest the involvement of TRPA1 in oxaliplatin-induced cold hyperalgesia and allodynia. However, unlike rodent TRPA1, human TRPA1 is not activated by cold stimuli [10], and it is unclear whether TRPA1 plays a role in cold allodynia and dysesthesia in human patients. TRPM8 is activated by cool and cold (25–28 °C or lower) stimuli [9]. Threshold concentration of cold sensation induced by menthol, a TRPM8 agonist, is decreased 5–6 h after oxaliplatin infusion [11]. Oxaliplatin increases expression of TRPM8 mRNA in the dorsal root ganglion (DRG) 3 days after injection [6]. On day 3, after oxaliplatin injection, cold allodynia is inhibited by a nonselective TRPM8 blocker [6]. Taken together, these findings raise the possibility that TRPM8 is involved in cold allodynia. To confirm this, we examined the effects of selective TRPM8 blockers on acute cold allodynia. A transcription factor

* Corresponding author.
E-mail address: andoht@pha.u-toyama.ac.jp (T. Andoh).

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c-Myc is thought to be involved in the expression of TRPM8 gene [12]. Therefore, we also examined whether c-Myc would be involved in oxaliplatin-induced cold allodynia and the increase of TRPM8 expression.

Materials and methods

Animals

Male C57BL/6NCr mice were purchased from Japan SLC (Hamamatsu, Japan) and housed 6 per cage in a room with controlled temperature (21–23 °C), humidity (45–46%), and light (on 07.00–19:00). Food and water were freely available. The animals were 6 weeks old at the start of experiments. This study was conducted with the approval of the Committee for Animal Experiments at the University of Toyama.

Materials

Oxaliplatin (Sigma–Aldrich, St. Louis, MO, USA) was dissolved in 5% glucose and injected intraperitoneally. A recommended dose of oxaliplatin is 85 mg/m^2 body surface area [3], which corresponds to 2.4 mg/kg when body height and weight are 170 cm and 60 kg, respectively [13]; therefore, we chose the 3.0 mg/kg dose of oxaliplatin. The selective TRPM8 blocker *N*-(2-aminoethyl)-*N*-[4-(benzyloxy)-3-methoxybenzyl]-*N*′-(1S)-1-(phenyl) ethyl urea hydrochloride (ABMP) was synthesized by T.Y. on the basis of the international patent application information (PCT/EP2007/000192). It was dissolved in saline and injected subcutaneously; the doses were chosen from preliminary experiments (data not shown). Another TRPM8 blocker TC-I 2014 (Tocris Bioscience, Bristol, UK) was dissolved in 20% 2-hdroxypropyl-β-cyclodextrin and administered orally; the doses were chosen from the published literature on its effect on neuropathic cold allodynia [14]. The TRPA1 blocker HC-030031 (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in physiological saline containing 10% dimethyl sulfoxide and 5% Tween-80 and injected intraperitoneally; the doses were chosen from published literature on its effect on oxidant-induced aversive responses [15]. ABMP, TC-I 2014, and HC-030031 were administered on day 3 after single oxaliplatin injection. 5-[(4-Ethylphenyl)methylene]-2-thioxo-4-thiazolidinone (10058-F4) (Sigma–Aldrich), an inhibitor of a transcription factor c-Myc [16], was dissolved in cremophor EL:ethanol:saline (1:1:8) and injected intravenously. 10058-F4 was daily injected immediately after and until day 3, peak time of cold allodynia, because it is rapidly metabolized after administration [16]; the doses were chosen from the published literature [16] and preliminary experiments (data not shown). The volume for administration was 0.1 ml/10 g body weight regardless of the administration route.

Cold allodynia

Cold allodynia of the hind paw was assessed using the acetone test. Mice were placed individually in a plastic cage (110 mm × 180 mm × 150 mm) with a wire mesh bottom. After acclimation of at least 30 min, acetone (10 μL) was applied to the plantar skin, the temperature of which was transiently decreased from 28.7 °C ± 0.1 to 24.5 °C ± 0.2 (7 s after application) in naïve mice (n = 8). Aversive responses during the 30-s period following acetone stimulation were scored as follows: 0, no response; 1, lifting of the hind paw; and 2, flinching or licking of the hind paw; naïve mice showed transient escape response immediately after acetone application, and this response was disregarded. Acetone was applied twice alternately to each hind paw at intervals of more than 60 s, and the average of four trials served as the aversive response score.

Reverse transcription and polymerase chain reaction

After behavioral test on day 3 after oxaliplatin administration, the animals were transcardially perfused with phosphate-buffered saline under sodium pentobarbital (80 mg/kg, intraperitoneal) anesthesia. The bilateral DRGs from L4 to L5 levels and bilateral plantar skin were removed; the plantar skin served for Western blot assay. Total RNA was extracted from DRG samples with TRIzol® regent (Thermo Fisher Sci. Inc., Waltham, MA, USA) and treated with DNAse I (Takara Bio Inc., Shiga, Japan). Reverse transcription-polymerase chain reaction assay for TRPM8 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed as described previously (3). The sequences of primers were as follows: TRPM8, 5′-ggctggagatgagattgtgag-3′ (sense) and 5′-gctgaagtgggtggagaaga-3′ (antisense); GAPDH, 5′-ccaaggtcatccatgacaac-3′ (sense) and 5′-ttactccttggaggccacgt-3′ (antisense). The expression levels of the mRNA were analyzed with the NIH Image software (National Institute of Health, Bethesda, MD, USA).

Western blotting

Protein was extracted from plantar samples with a lysis buffer containing protease and phosphatase inhibitors, separated by electrophoresis using a 7.5% sodium dodecyl sulfate-polyacrylamide gel, and then transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% skim milk solution for 1 h and reacted with anti-TRPM8 (1: 500; Cat No. ab3243, Abcam, Cambridge, MA, USA) or anti-β-actin (1:1000; Cat No. A5316, Sigma–Aldrich) antibody overnight at 4 °C. After washing with Tris-buffered saline containing 0.1% Tween 20, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG antibody (1:5000; GE Healthcare UK Ltd., Buckinghamshire, UK) for 1.5 h at room temperature. Bands were detected by using chemiluminescent reagents (GE Healthcare UK Ltd.) and an X-ray film. The density of protein bands was analyzed with NIH Image software (National Institute of Health).

Data processing

All data are presented as mean and standard error of the mean (SEM). The results were analyzed by Student's *t*-test, or two-way repeated measures analysis of variance (TW-RM ANOVA) or one-way ANOVA followed by Holm–Šídák test; $p < 0.05$ was considered significant.

Results

Effects of TRPM8 and TRPA1 blockers on oxaliplatin-induced cold allodynia

A single intraperitoneal injection of oxaliplatin (3 mg/kg) caused cold allodynia (aversive responses to acetone stimulation). There was an increased tendency of aversive response the day after oxaliplatin injection, significant maximum increases after 3 days, and no longer any increase after 7 days (Fig. 1A). On day 3 after oxaliplatin injection, single administration of the selective TRPM8 blockers ABMP (3 and 30 mg/kg, subcutaneous) and TC-I 2014 (10 and 30 mg/kg, oral) dose-dependently and significantly inhibited cold allodynia, with maximum inhibition 1 and 1.5 h after administration, respectively (Fig. 1B and C). On the other hand, the TRPA1 blocker HC-030031 (30 and 100 mg/kg, intraperitoneal) did not inhibit oxaliplatin-induced cold allodynia (Fig. 1D).