*Short Communication***Pharmacological Characterization of Standard Analgesics on Oxaliplatin-Induced Acute Cold Hypersensitivity in Mice**

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Abstract. Oxaliplatin, a platinum-based chemotherapeutic agent, causes an acute peripheral neuropathy triggered by cold in almost all patients during or within hours after its infusion. We recently reported that a single administration of oxaliplatin induced cold hypersensitivity 2 h after the administration in mice. In this study, we examined whether standard analgesics relieve the oxaliplatin-induced acute cold hypersensitivity. Gabapentin, tramadol, mexiletine, and calcium gluconate significantly inhibited and morphine and milnacipran decreased the acute cold hypersensitivity, while diclofenac and amitriptyline had no effects. These results suggest that gabapentin, tramadol, mexiletine, and calcium gluconate are effective against oxaliplatin-induced acute peripheral neuropathy.

Keywords: oxaliplatin, acute peripheral neuropathy, analgesic

Some chemotherapeutic agents including platinum compounds, taxanes, vinca alkaloids, and bortezomib, cause peripheral neuropathy. However, unlike other chemotherapeutic agents, oxaliplatin, a platinum-based chemotherapeutic agent, induces a peculiar acute peripheral neuropathy, such as paresthesia and dysesthesia, which are often triggered or enhanced by exposure to cold, whereas cumulative and chronic neuropathy induced by oxaliplatin includes pain sensation (1). The acute peripheral neuropathy is induced in almost all patients during or within hours after the infusion. However, an effective pharmacological strategy for its management remains controversial (1, 2).

Many studies in animal models focus on the oxaliplatin-induced chronic and/or subacute painful peripheral

neuropathy that appear several days to several weeks after oxaliplatin administration (3 – 5), while oxaliplatin-induced acute peripheral neuropathy is poorly characterized. Recently, we have reported a mouse model of rapid-onset cold hypersensitivity induced by oxaliplatin (6). When mice are given a single administration of oxaliplatin, cold but not mechanical hypersensitivity is induced within 2 h after the administration, while it is not induced by other chemotherapeutic agents such as cisplatin and paclitaxel. These findings suggest that rapid-onset cold hypersensitivity is representative of the acute peripheral neuropathy characteristic to oxaliplatin in mice. In the present study, we assessed the effects of standard analgesics, such as non-steroidal anti-inflammatory agent (NSAID), opioid analgesics, tricyclic antidepressant, serotonin and noradrenaline reuptake inhibitor (SNRI), calcium channel α_2 - δ ligand, local anesthetic, and calcium gluconate (1, 2), on the oxaliplatin-induced acute cold hypersensitivity in mice.

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This study was carried out in strict accordance with the recommendations in the Guiding Principles for the Care and Use of The Japanese Pharmacological Society. The protocol was approved by the Kyoto University Animal Research Committee. The male C57BL/6J mice aged between 6–8 weeks (Japan SLC, Shizuoka) were housed under constant ambient temperature ($24^{\circ}\text{C} \pm 1^{\circ}\text{C}$), with alternate light–dark cycles. Food and water were freely available.

Oxaliplatin (Wako Pure Chemical Industries, Osaka) was freshly dissolved in 5% glucose solution. Mice received a single intraperitoneal administration of oxaliplatin (5 mg/kg) or vehicle 2 h before behavioral tests (6). Morphine hydrochloride (Takeda Pharmaceutical Co., Osaka); amitriptyline hydrochloride (LKT Laboratories, MN, USA); milnacipran hydrochloride (Santa Cruz Biotechnology, Santa Cruz, CA, USA); tramadol hydrochloride (gift from Nippon Shinyaku, Kyoto); diclofenac sodium salt, gabapentin, and mexiletine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) were freshly dissolved in sterile saline. Morphine and tramadol were administered subcutaneously; milnacipran and mexiletine were administered intraperitoneally 30 min before; and diclofenac, gabapentin, and amitriptyline were administered intraperitoneally 1 h before the behavioral test at a volume of 10 ml/kg. The doses of these analgesics were chosen based on previous reports (3–5, 7–11). Calcium gluconate monohydrate (Wako) was freshly dissolved in 5% glucose solution and was infused intravenously just before oxaliplatin administration (5).

Cold sensitivity was assessed with the hot/cold-plate analgesimeter (Ugo Basile, Milan, Italy) as previously described (6). Briefly, mice were individually placed on a cold plate maintained at 5°C . Escape behaviors were graded with a score of 0 = no response, 1 = moderate effort to avoid cold, and 2 = vigorous effort to escape cold. The sum of the scores recorded within a 60-s period was calculated.

The data are presented as means $\pm$ S.E.M. Statistical significance was calculated by one-way analyses of variance (ANOVA), followed by the Tukey-Kramer *post-hoc* test. In all cases, differences of $P < 0.05$ were considered statistically significant.

Consistent with our previous study (6), the score of cold escape behaviors was significantly increased 2 h after a single intraperitoneal administration of oxaliplatin, compared with the vehicle-treated group (Fig. 1: A–I), suggesting that oxaliplatin produces rapid-onset cold hypersensitivity.

An NSAID, diclofenac (25 and 50 mg/kg) had no effect on the oxaliplatin-induced cold hypersensitivity (Fig. 1A). NSAIDs are representative broad-spectrum analgesics especially for nociceptive pain, while they

are ineffective on neuropathic pain. Although NSAIDs are initially used for the management of chemotherapy-induced painful peripheral neuropathy (1), acute peripheral neuropathy is unlikely to include inflammatory components. The present results suggest that NSAIDs are ineffective on oxaliplatin-induced acute peripheral neuropathy.

The strong opioid analgesic morphine (5 and 10 mg/kg) tended to inhibit the cold hypersensitivity (Fig. 1B). It is reported that morphine (1–4 mg/kg) dose-dependently inhibits the chronic and subacute cold allodynia induced by oxaliplatin in rats (3, 4). However, the present results suggest that morphine is less efficacious against oxaliplatin-induced acute peripheral neuropathy, which may include an aspect of A-fibers-mediated sensations other than pain (12).

A calcium channel $\alpha_2\text{-}\delta$ ligand, gabapentin (10 and 30 mg/kg), dose-dependently decreased the cold hypersensitivity, and a significant difference was observed at 30 mg/kg (Fig. 1C). Gabapentin is often used as a first-choice drug for the management of neuropathic pain, as well as pregabalin (13). Pregabalin reduces the severity of oxaliplatin-induced peripheral neuropathy in patients, although gabapentin has not well proven its efficacy (1). In animal models, gabapentin and pregabalin reduce oxaliplatin-induced chronic and/or subacute peripheral neuropathy (3, 4, 7).

The tricyclic antidepressant amitriptyline (5 and 10 mg/kg) had no effect (Fig. 1D), while the SNRI milnacipran (10 and 30 mg/kg) tended to inhibit the cold hypersensitivity (Fig. 1E). Tricyclic antidepressants and SNRIs are widely used as first-choice drugs for the management of neuropathic pain (13). Both antidepressants inhibit neuropathic pain by blocking noradrenaline and serotonin reuptake. However, their efficacy on the oxaliplatin-induced peripheral neuropathy is controversial (1). In animal models, repeated administration of amitriptyline reduces oxaliplatin-induced chronic mechanical allodynia (8). It is considered that disinhibition and imbalance of the descending serotonergic and noradrenergic pain inhibitory pathways contribute to neuropathic pain. However, oxaliplatin-induced acute peripheral neuropathy is unlikely to be mediated through such disinhibition and imbalance, which may result in no and weak efficacy of these antidepressants. On the other hand, the anti-allodynic effect of milnacipran is more potent than that of amitriptyline on cold allodynia in a neuropathic pain model rats (9), consistent with the present results.

Tramadol (10 and 20 mg/kg) dose-dependently decreased the cold hypersensitivity, and a significant difference was observed at 20 mg/kg (Fig. 1F). It is clinically reported that tramadol/acetaminophen combination is

Fig. 1. Effects of analgesics on oxaliplatin-induced acute cold hypersensitivity. Mice were given intraperitoneal administration of oxaliplatin (5 mg/kg). Cold plate tests were performed 2 h after oxaliplatin administration. Morphine (5 and 10 mg/kg, $n = 6-8$), tramadol (10 and 20 mg/kg, $n = 5-8$), milnacipran (10 and 30 mg/kg, $n = 4-8$), mexiletine (10 and 30 mg/kg, $n = 5-8$), and combination of morphine (5 mg/kg) + milnacipran (30 mg/kg, $n = 3-6$) were administered subcutaneously or intraperitoneally 30 min before the tests. Diclofenac (25 and 50 mg/kg, $n = 5-9$), gabapentin (10 and 30 mg/kg, $n = 4-9$), and amitriptyline (5 and 10 mg/kg, $n = 5-9$) were administered intraperitoneally 60 min before the tests. Calcium gluconate (0.5 mmol/kg) was administered intravenously before oxaliplatin administration ($n = 3-4$). Data are presented as the means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$: compared to the group treated with vehicle alone instead of oxaliplatin. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ (one-way ANOVA, followed by the Tukey-Kramer *post-hoc* test).

effective in the management of oxaliplatin-induced painful neuropathy (14). Tramadol exhibits an analgesic effect through both μ -opioid receptor agonistic activity and antidepressant-like enhancement of descending serotonergic and noradrenergic pain inhibitory pathway by blocking monoamine reuptake. Dual analgesic mechanisms of tramadol, acting both complementarily and synergistically, are suggested to contribute to its efficacy against neuropathic pain (10). However, the combination of morphine (5 mg/kg) and milnacipran (30 mg/kg) tended to inhibit the cold hypersensitivity,

while the efficacy was weak, compared with tramadol (Fig. 1G). Therefore, dual action of opioids and antidepressants may only partly contribute to the efficacy against oxaliplatin-induced acute peripheral neuropathy. The efficacious mechanism of tramadol remains unclear, but it may be due to the inhibitory effect of tramadol on voltage-gated Na^+ channels (15).

The local anesthetic, mexiletine (10 and 30 mg/kg) dose-dependently decreased the cold hypersensitivity, and a significant difference was observed at 30 mg/kg (Fig. 1H). Mexiletine blocks voltage-gated Na^+ channels,

and it is clinically used for painful diabetic neuropathy (13). An aspect of the oxaliplatin-induced peripheral neuropathy is mediated through voltage-gated Na^+ channels in A-fibers (12). In animal models, the oxaliplatin-induced chronic cold allodynia is inhibited by mexiletine (11). Taken together, mexiletine is a potential treatment option for oxaliplatin-induced acute neuropathy.

Calcium gluconate (0.5 mmol/kg) significantly inhibited the cold hypersensitivity (Fig. 1I). Infusion of calcium and magnesium has been clinically used for prevention and management of oxaliplatin-induced peripheral neuropathy, although a recent meta-analysis does not support them (3). In animal models, calcium reduces oxaliplatin-induced chronic and/or subacute cold hyperalgesia but not mechanical allodynia (4, 5). It is hypothesized that chelation of calcium by an oxaliplatin metabolite, oxalate, induces functional impairment of voltage-gated Na^+ channels, resulting in hyperexcitability of sensory neurons. Therefore, calcium infusion could enhance the closing rate of Na^+ channels, which may decrease the neuronal hyperexcitability induced by oxaliplatin (12).

The present results show the efficacy of Ca^{2+} channel $\alpha_2\text{-}\delta$ ligands, tramadol, Na^+ -channel blockers, and calcium, rather than opioid analgesics, antidepressants, and NSAIDs, on the oxaliplatin-induced acute cold hypersensitivity. We previously reported that oxaliplatin-induced acute cold hypersensitivity is caused by the enhanced responsiveness of transient receptor potential ankyrin 1 (TRPA1) expressed in primary sensory neurons (6). Furthermore, it includes an aspect of A-fibers-mediated sensations (12). The present results suggest that the analgesics that have an ability to directly suppress the hyperexcitability of sensory neurons, including gabapentin, local anesthetic, and calcium, have higher efficacy than the centrally-acting analgesics activating the descending pain inhibitory pathway, including antidepressants, as well as opioids and gabapentin. The drug sensitivity of these analgesics further supports the possibility that oxaliplatin-induced cold hypersensitivity observed in mice may represent cold-triggered paresthesia and dysesthesia as clinical symptoms of oxaliplatin-induced acute peripheral neuropathy, rather than pain. The present study provides evidence for the treatment of cold-triggered acute peripheral neuropathy induced by oxaliplatin.

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Conflicts of Interest

The authors indicated no potential conflicts of interest.

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