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Short communication

Polaprezinc reduces paclitaxel-induced peripheral neuropathy in rats without affecting anti-tumor activity

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ABSTRACT

Paclitaxel, an anticancer drug, frequently causes painful peripheral neuropathy. In this study, we investigated the preventive effect of polaprezinc on paclitaxel-induced peripheral neuropathy in rats. Polaprezinc (3 mg/kg, p.o., once daily) inhibited the development of mechanical allodynia induced by paclitaxel (4 mg/kg, i.p., on days 1, 3, 5 and 7) and suppressed the paclitaxel-induced increase in macrophage migration in dorsal root ganglion cells. In addition, polaprezinc did not affect the anti-tumor activity of paclitaxel in cultured cell lines or tumor-bearing mice. These results suggest a clinical indication for polaprezinc in the prevention of paclitaxel-induced neuropathy.

Paclitaxel, an anticancer drug, is widely used for the treatment of many types of solid tumor. However, it frequently causes peripheral neuropathy. Since this neurotoxicity is a dose-limiting side effect, and preventive strategies have not yet been established, it remains a major clinical limitation of paclitaxel chemotherapy (1,2).

Paclitaxel induces the expression of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) in rat dorsal root ganglion (DRG) (3). Intrathecal IL-1 receptor antagonist and plasmid DNA encoding interleukin-10 (IL-10), an anti-inflammatory cytokine, reverse paclitaxel-induced mechanical allodynia in rats (3). Though the mechanisms of paclitaxel-induced peripheral neuropathy have yet to be fully elucidated, inflammatory cytokines may be involved in the pathogenesis of this side effect. In general, inflammatory cytokines are produced and released by activated macrophages, and macrophage migration is caused by inflammatory mediators including chemokines (4).

Polaprezinc, a zinc complex of L-carnosine that protects the gastric mucosa, is widely used for the treatment of gastric ulcer (5). Zinc has been shown to down-regulate proinflammatory cytokine-induced nuclear factor-kappa B (NF-κB) activation (6). Recently, zinc supplementation has been reported to alleviate peripheral neuropathy in diabetic rats (7). However, the effect of polaprezinc on paclitaxel-induced peripheral neuropathy remains unknown. In this study, we investigated the preventive effect of polaprezinc on paclitaxel-induced mechanical allodynia in rats. Moreover, we examined the effects of paclitaxel and polaprezinc on macrophage migration in rat DRG cells. Finally, we investigated the effect of polaprezinc on the anti-tumor activity of paclitaxel in cultured cell lines and tumor-bearing mice.

Male Sprague-Dawley rats weighing 200–250 g (Kyudo Co., Tosu) and male C57/BL6 mice weighing 15–20 g (Kyudo Co.) were used in this study. Rats and mice were housed in groups of four to five per cage with lights on from 07:00 AM to 07:00 PM. Animals had free access to food and water in their home cages. All experiments were approved by the Experimental Animal Care and Use Committee of Kyushu University, and were conducted in accordance with National Institutes of Health guidelines as well as the International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues guidelines for animal research (8).

Paclitaxel (Taxol®; 6 mg/mL in Cremophor EL/ethanol 1:1) was obtained from Bristol-Myers Squibb (Tokyo). Polaprezinc was obtained from ZERIA Pharmaceutical Co., Ltd (Tokyo) and was suspended in 0.1% carboxymethyl cellulose-Na. Paclitaxel (4 mg/kg) or vehicle (50% Cremophor EL/ethanol) was administered intraperitoneally (i.p.) in volumes of 1 mL/kg, four times over 1 week (on days 1, 3, 5, and 7). The dose of paclitaxel was chosen based on a

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previous report (9). Polaprezinc (3 mg/kg) was administrated orally (p.o.) once daily for 7 days. The dose of polaprezinc was calculated from the clinical dose.

Mechanical allodynia was assessed using the von Frey test, prior to the first drug administration and on days 2, 4, 6, and 8. On days 2, 4, and 6, the test was performed before drug administration. Each rat was placed in a clear plastic box (20 × 17 × 13 cm) with a wire mesh floor and allowed to habituate for 30 min prior to testing. Von Frey filaments (The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, UK) ranging from 1 to 15 g bending force were applied to the midplantar skin of each hind paw, with each application held for 6 s. The paw withdrawal threshold was determined by a modified up-down method (10).

After perfusion fixation of rats anesthetized with isoflurane (Escain®, Pfizer Japan Inc., Tokyo) by 4% paraformaldehyde (PFA), lumbar L4-6 DRG were removed on day 2, 4, or 8 and immersed in 4% PFA followed sequentially by 10%, 20%, and 30% sucrose buffer. DRG frozen blocks were prepared using optimal cutting temperature compound (Sakura Finetek Japan Co., Ltd., Tokyo). DRG frozen sections (thickness: 10 μm) were cut on a cryostat, fixed using 4% PFA for 15 min, and blocked in 5% skim milk for 1 h before overnight incubation at 4 °C with anti-CD68 antibody (GeneTex, Irvine, CA, USA). DRG sections were then incubated for 2 h with Alexa Fluor® 488-conjugated anti-mouse IgG (H+L), F (ab′) 2 fragment, mounted in 80% glycerol, and viewed under a fluorescence microscope (Keyence Co., Ltd, Osaka). Macrophage migration was calculated by dividing the number of macrophage (CD68-positive cells) by DRG cell area measured using image analysis software (Image J 1.45).

A549 cells (Japanese Collection of Research Bioresources Cell Bank, Osaka), Walker 256 cells (WRC256, American Type Culture Collection, Walkersville, MD, USA), and Lewis lung carcinoma (LLC) cells (RIKEN BioResource Center, Ibaraki) were used in this study. All cell lines were cultured according to a previous report (11).

A549 and Walker 256 cells were seeded at a density of 4×10^4 cells/cm^2 onto 24-well plates and used for experiments on the following day. Cells were exposed to Cremophor EL + ethanol, paclitaxel (100 ng/mL) or polaprezinc (10, 30, or 100 mM) for 6, 12, 24, 48, or 72 h. The drugs were dissolved in medium prior to exposure. Cell viability was assessed by the mitochondrial activity that reduced WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium, monosodium salt) to formazan (11).

LLC cells (1.5×10^6 cells per mouse in 50 μL serum-free medium) were implanted subcutaneously in the chests of C57/BL6 mice. Three days after implantation, administration of drugs was initiated. Paclitaxel (6 mg/kg, i.p.) and polaprezinc (3 mg/kg, p.o.) were injected once daily for 12 days. The resulting tumor volumes were calculated as follows: volume (mm^3) = 1/2 × length (mm) × width (mm)^2.

Values were expressed as the mean ± S.E.M. Results were analyzed by Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post-hoc test to determine differences among the groups (StatView; Abacus Concepts, Berkeley, CA, USA). A *P* value of less than 0.05 was considered statistically significant.

Paclitaxel (4 mg/kg, i.p.) significantly reduced the paw withdrawal threshold compared with vehicle on days 4, 6, and 8 ($P < 0.05$, Fig. 1). Repeated administration of polaprezinc (3 mg/kg, p.o.) significantly inhibited the paclitaxel-induced reduction in the withdrawal threshold ($P < 0.05$).

Macrophages increased around DRG cells after paclitaxel administration (Fig. 2A). Macrophage migration significantly increased on day 8 ($P < 0.01$, Fig. 2B). Polaprezinc completely suppressed the paclitaxel-induced increase in macrophage migration ($P < 0.01$).

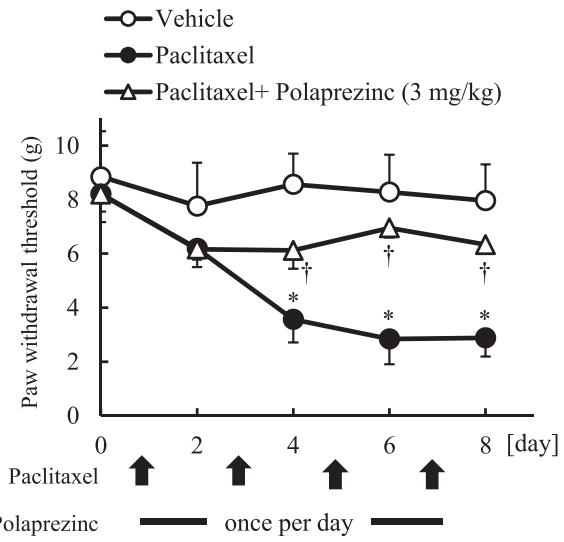

Fig. 1. Effect of polaprezinc on paclitaxel-induced mechanical allodynia by the von Frey test in rats. Paclitaxel (4 mg/kg) was administered i.p. four times (on days 1, 3, 5, and 7). Polaprezinc (3 mg/kg) was administered p.o. once daily for 7 days. The von Frey test was performed prior to the first drug administration (day 0) and on days 2, 4, 6, and 8. Values are expressed as the mean ± S.E.M. of 5–7 animals. $^*P < 0.05$ compared with vehicle. $^\dagger P < 0.05$ compared with paclitaxel alone.

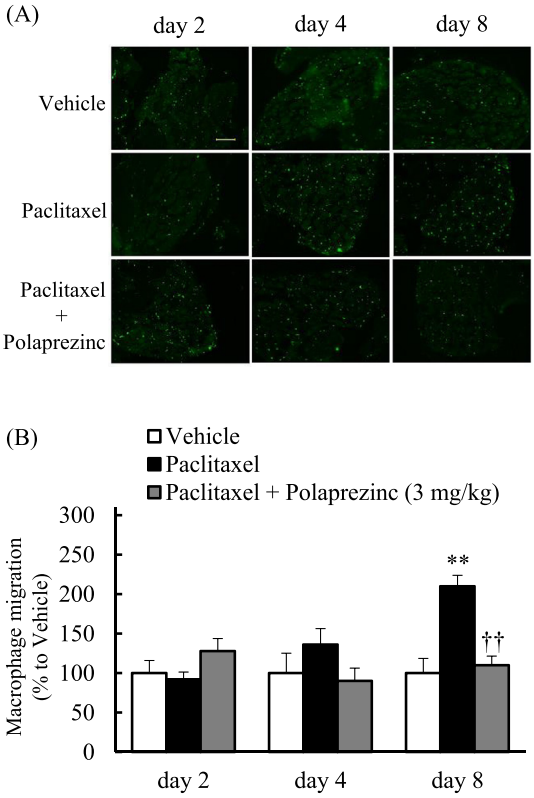

Fig. 2. Effects of paclitaxel and polaprezinc on macrophage migration in rat DRG cells. Paclitaxel (4 mg/kg) was administered i.p. four times (on days 1, 3, 5, and 7). Polaprezinc (3 mg/kg) was administered p.o. once daily for 7 days. On day 2, 4, or 8, lumbar L4-6 DRG were harvested and samples were immunostained with anti-CD68 antibody for imaging of macrophages as green fluorescence areas. (A) Original magnification ×200 for all images; scale bar, 100 μm. (B) Macrophage migration was calculated by dividing the number of macrophage (CD68-positive cells) by DRG cell area. Results are expressed as the mean ± S.E.M. of 6–11 experiments. $^{**}P < 0.01$ compared with vehicle, $^{\dagger\dagger}P < 0.01$ compared with paclitaxel alone.

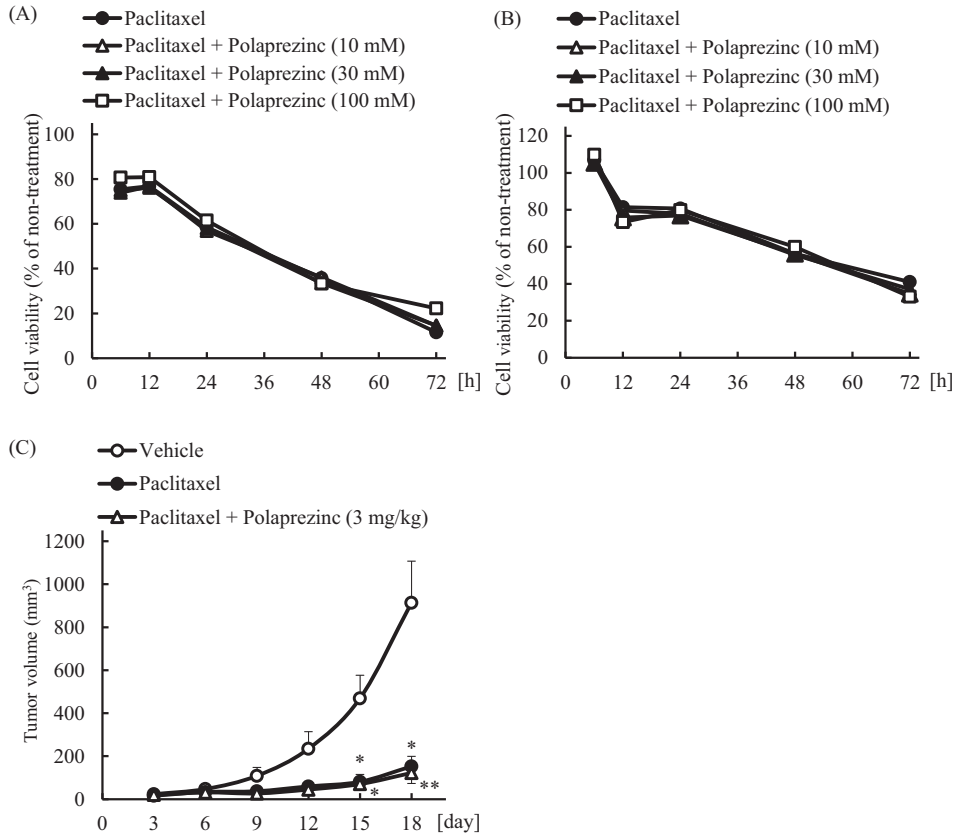

Fig. 3. Effect of polaprezinc on the anti-tumor activity of paclitaxel. (A) A549 and (B) Walker 256 cells were incubated with paclitaxel (100 ng/mL) for 6, 12, 24, 48, or 72 h in the presence or absence of various concentrations of polaprezinc. Cell viability was measured by WST-8 assay. Results are expressed as percentages of control group viability (n = 3). (C) LLC cell-implanted mice were treated with paclitaxel (6 mg/kg, i.p.) and polaprezinc (3 mg/kg, p.o.) once daily for 12 days. Results are expressed as the mean ± S.E.M. of 5–7 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with vehicle.

Paclitaxel exposure (100 ng/mL) caused a time-dependent decrease in cell viability in cultured A549 and Walker 256 cells (Fig. 3A and 3B). Treatment with polaprezinc (10, 30, or 100 mM) had no effect on paclitaxel-induced inhibition of tumor growth. In tumor cell-implanted mice, paclitaxel (6 mg/kg, i.p.) significantly inhibited the increase in tumor volume compared with vehicle at days 15 and 18 ($P < 0.05$, Fig. 3C). Treatment with polaprezinc (3 mg/kg, p.o.) had no effect on paclitaxel-induced inhibition of tumor growth.

In this study, repeated administration of polaprezinc inhibited the development of paclitaxel-induced mechanical allodynia. Therefore, polaprezinc may be beneficial for the prevention of paclitaxel-induced peripheral neuropathy.

Furthermore, we found that paclitaxel increased macrophage migration in rat DRG cells. The macrophage migration is caused by inflammatory mediators, including chemokines (4). Paclitaxel has been reported to induce the expression of proinflammatory cytokines, including IL-1 and TNF, in rat DRG (3). Therefore, the paclitaxel-induced increase in macrophage migration may be caused by chemokines or inflammatory cytokines induced by paclitaxel. On day 4, paclitaxel induced mechanical allodynia but not macrophage migration. These results suggest that macrophage migration is not a trigger of paclitaxel-induced painful neuropathy.

We also found that polaprezinc suppressed the paclitaxel-induced increase in macrophage migration. Since zinc down-regulates proinflammatory cytokine-induced NF-κB activation (6), it may also suppress inflammatory cytokines induced by paclitaxel in rat DRG. Also, intrathecal IL-1 receptor antagonist and plasmid DNA encoding IL-10 have been reported to reverse paclitaxel-induced mechanical allodynia in rats (3), suggesting that inflammatory cytokines are involved in paclitaxel-induced neuropathy. Recently, zinc supplementation has been reported to alleviate peripheral neuropathy in diabetic rats (7). Zinc suppresses inflammatory cytokines via the induction of zinc finger protein A20, which A20 binds to TNF-receptor-associated factors (TRAFs), inhibits Iκ kinase-α (IKK-α)/NF-κB, and suppresses the activation of NF-κB (6). Moreover, chemokines, such as CCL3 and CX3CL-1, have been reported to be involved in the paclitaxel-induced peripheral neuropathy (12–14). These chemokines are also controlled by NF-κB. Therefore, these chemokines may be suppressed by zinc. Taken together, the induction of A20 and the inhibition of NF-κB signaling induced by zinc in polaprezinc may inhibit neuronal anti-inflammatory reactions and the development of paclitaxel-induced mechanical allodynia.

Our results also show that polaprezinc had no effect on paclitaxel-induced cytotoxicity in tumor cells or on the anti-tumor effect of paclitaxel in tumor cell-implanted mice.

In conclusion, our results suggest that polaprezinc reduces paclitaxel-induced peripheral neuropathy without affecting the anti-tumor activity of paclitaxel.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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