Journal of Pharmacological Sciences xxx (2017) 1–6

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Pregabalin reduces cisplatin-induced mechanical allodynia in rats

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ARTICLE INFO

Article history:
Received 10 February 2017
Received in revised form
15 May 2017
Accepted 6 June 2017
Available online xxx

Keywords:
Cisplatin
Pregabalin
Allodynia
Rat

ABSTRACT

Although cisplatin (CDDP) is a key drug in cancer chemotherapy, CDDP-induced peripheral neuropathy is a dose-limiting factor. We previously reported that CDDP-induced peripheral neuropathy, which progressed from allodynia to hypoalgesia, was ameliorated by the administration of CDDP to rats at a specific time. However, mechanical allodynia cannot be prevented therapeutically. Pregabalin (PGN) is used to suppress neuropathic pain from herpes zoster and diabetes. Therefore, we investigated the effects of PGN on CDDP-induced mechanical allodynia in rats.

CDDP (4 mg/kg) was administered intravenously to male Sprague–Dawley rats at 5:00 once a week for 2 weeks, while saline was given to the control group. PGN (10 mg/kg/day) was administered orally twice a day at 8:00 and 20:00, and distilled water was given to the control group. The von Frey and hot-plate tests were performed to assess CDDP-induced peripheral neuropathy.

Withdrawal thresholds were significantly greater than those in with the CDDP alone group when PGN was administered before and after the onset of CDDP-induced mechanical allodynia. Furthermore, CDDP-induced mechanical allodynia was suppressed by the administration of PGN only.

These results demonstrate that PGN effectively ameliorates CDDP-induced mechanical allodynia during the administration of PGN.

1. Introduction

Cisplatin, *cis*-diamminedichloro-platinum (CDDP), is an anticancer drug that is used to treat non-small cell lung, head and neck, ovarian, and breast cancers.[1–4] However, the development of peripheral neuropathy has been reported in cancer patients treated with CDDP.[5] CDDP-induced peripheral neuropathy presents as hyperalgesia and allodynia in the early stage and as hypoalgesia in the advanced stage.[6–8] Although previous studies have attempted to prevent or ameliorate peripheral neuropathy, clinical trials have not yet reported successful findings.[9–12]

Nephropathy, vomiting, and peripheral neuropathy caused by CDDP, which are dose-limiting factors, were previously shown to be improved by chronopharmacology, which is defined as the administration of medication in accordance with biological rhythms in order to optimize therapeutic outcomes and/or control adverse effects.[13–16] The development of hypoalgesia, which is a severe form of CDDP-induced peripheral neuropathy, in rats was previously shown to be delayed or inhibited more in the 5:00-treated group than in the 17:00-treated group[16]; however, mechanical allodynia was not inhibited in the 5:00-treated group in that study. Since allodynia significantly reduces the quality of life (QOL) of patients, difficulties are associated with the continuation of cancer chemotherapy with CDDP.[17]

Pregabalin (PGN) is administered to patients with neuropathic pain from herpes zoster, diabetes, and fibromyalgia.[18–20] The mechanism responsible for the anti-neuropathic effects of PGN has been identified as binding to the voltage-dependent calcium channel α2δ1 subunit.[21,22] Previous animal studies reported that PGN reduced mechanical allodynia induced by paclitaxel, vincristine, and bortezomib.[23–25] PGN also reduced oxaliplatin-induced cold allodynia in rats.[26] Thus, we speculated that PGN may suppress CDDP-induced mechanical allodynia.

In the present study, we investigated the effects of PGN on CDDP-induced mechanical allodynia in rats as well as its influence on the anti-tumor activity of CDDP in an *in vitro* study.

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Peer review under responsibility of Japanese Pharmacological Society.

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http://dx.doi.org/10.1016/j.jphs.2017.06.003

Please cite this article in press as: Seto Y, et al., Pregabalin reduces cisplatin-induced mechanical allodynia in rats, Journal of Pharmacological Sciences (2017), http://dx.doi.org/10.1016/j.jphs.2017.06.003

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats (6 weeks old) were purchased from Japan SLC, Ltd. (Japan). Rats were housed 2 to 3 per cage under standardized light–dark cycle conditions (lights on and off at 7:00 and 19:00, respectively) at a room temperature of 23–25 °C and humidity of 50–70% with free access to food and water. All rats were kept under these conditions for one week until used in experiments. Experiments were performed after formal approval by the Committee for Animal Experiments at the University of Toyama.

2.2. Preparation of drugs

CDDP, supplied by Nippon Kayaku Co., Ltd. (Tokyo, Japan), was dissolved in saline. Its final concentration was 2 mg/mL (4 mg/kg) in each dosing group. CDDP was intravenously (i.v.) administered at 2 mL/kg to rats. PGN, supplied by Pfizer Inc., was dissolved in distilled water. Its final concentration was 5 mg/mL (5 mg/kg) in each dosing group. PGN was perorally (p.o.) administered at 1 mL/kg to rats. The doses of CDDP and PGN, which showed the effect of PGN on chemotherapy induced peripheral neuropathy were referenced by previous studies in rats.[16,25–27]

2.3. Experiment I: Influence of PGN on the cytotoxicity of CDDP in tumor cells

The rat breast carcinoma cell line, Walker 256 was obtained from the Cell Resource Center for Biomedical Research, Tohoku University (Miyagi, Japan). Walker 256 cells were maintained in RPMI 1640 supplemented 10% fetal bovine serum at 37 °C in a humidified 5% CO_2 atmosphere. Cells were seeded at 2×10^3 cells on each well of a 96-well plate and then incubated for approximately 24 h. CDDP (1, 5, 10, 20, 30, or 40 μM) and/or PGN (1, 5, 10, 50, or 100 μM) was added to cell media for 24 h. The MTT assay was then performed using the MTT Cell Count Kit (Nacalai Tesque, Inc., Japan).

2.4. Experiment II: Influence of PGN on the toxicity of CDDP in rats

CDDP or saline was i.v. administered to rats (n = 8) at 5:00 once a week for 2 weeks (days 0 and 7). PGN or distilled water was p.o. administered twice a day (8:00 and 20:00) from days −1 to 14. Blood samples were obtained from the tail vein on day 14 in order to measure leukocyte counts and blood urea nitrogen (BUN) concentrations, with leukocyte counts being measured immediately. Blood samples were centrifuged at 3000× *g* at 15 °C for 10 min, and serum was then frozen at −80 °C until assays were performed. BUN concentrations were measured using a manufactured kit (Wako Pure Chemical Industries, Ltd., Japan).

2.5. Experiment III: Therapeutic effects of PGN on CDDP-induced mechanical allodynia

CDDP or saline was i.v. administered to rats (n = 8) at 5:00 once a week for 2 weeks (days 0 and 7). PGN or distilled water was p.o. administered twice a day (8:00 and 20:00) from days 7–14. Withdrawal thresholds were measured on days 3, 6, 10, and 13 using the von Frey test. Withdrawal latency by a heat stimulation was assessed on day 14 using the hot-plate test.

2.6. Experiment IV: Relevance between effect on CDDP-induced mechanical allodynia and intermittent administration of PGN

CDDP or saline was i.v. administered to rats (n = 8) at 5:00 once a week for 2 weeks (days 0 and 7). PGN or distilled water was p.o. administered twice a day (8:00 and 20:00) from days −1 to 10 and from days 18–21. Withdrawal thresholds were measured on days 3, 6, 10, 13, 17, and 20 using the von Frey test. Withdrawal latency by a heat stimulation was assessed on day 21 using the hot-plate test.

2.7. von Frey test

We employed a previously described method.[28] Mechanical allodynia was assessed using a Touch-test sensory evaluator (Muromachi Kikai Co., Ltd., Japan) between 12:00 and 14:00 on each measuring day. Each rat was placed in a plastic cage with a wire mesh floor and allowed to acclimate for 10 min before measuring hind paw mechanical thresholds. Filaments, with bending forces that ranged between 1 and 60 g, were applied to the middle of the plantar surface of the right hind paw and held for 5 s. The withdrawal threshold of the right hind paw was assessed by increasing the stimulus strength from the 1-gram filament until paw withdrawal occurred.

2.8. Hot-plate test

Thermal hypoalgesia was assessed using a hot plate analgesia meter (Muromachi Kikai Co., Ltd., Japan) between 12:00 on 14:00 on each measuring day. Each rat was placed in a plastic cage with a wire mesh floor and allowed to acclimate for 10 min before measuring hind paw thermal thresholds. A hot plate was preheated and maintained at a temperature of 50 ± 0.5 °C. The time for the first sign of nociception, paw licking, flinching, or a jumping response to avoid heat was recorded. A cut-off period of 60 s was maintained to avoid damage to the hind paws.[29]

2.9. Statistical analysis

All values were shown as means with the standard error (S.E.M.). A one-way analysis of variance (ANOVA) was used for multiple comparisons, and Scheffé's test was employed for comparisons between two groups. Dunnett's test was used for data obtained from the MTT assay for comparisons with the CDDP 1 μM group. *P* values of less than 0.05 were considered significant.

3. Results

3.1. Experiment I: Influence of PGN on the cytotoxicity of CDDP in Walker 256 cells

CDDP induced dose-dependent decreases in cell viability ($P < 0.01$, respectively, Fig. 1a), whereas PGN had no effect (Fig. 1b). CDDP and PGN did not exert synergistic cytotoxic effects in Walker 256 cells (Fig. 1c).

3.2. Experiment II: Influence of PGN on CDDP-induced bone marrow suppression and nephropathy

Leukocyte counts were significantly lower, while BUN levels were significantly higher in the CDDP-treated groups than in the control group on day 14 ($P < 0.05$ and $P < 0.01$, respectively, Fig. 2). No significant differences were observed in leukocyte counts (Control: 10,988/μL, PGN alone: 10,913/μL) or BUN levels (Control: 17.8 mg/dL, PGN alone: 20.8 mg/dL) between the control and PGN

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