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Short communication

Prophylactic administration of an extract from *Plantaginis Semen* and its major component aucubin inhibits mechanical allodynia caused by paclitaxel in mice

Tsugunobu Andoh [a, *], Mitsuru Kato [a], Ryo Kitamura [a], Shizuka Mizoguchi [a], Daisuke Uta [a], Kazufumi Toume [b], Katsuko Komatsu [b], Yasushi Kuraishi [c]

[a] Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
[b] Division of Pharmacognosy, Institutes of Natural Medicine, University of Toyama, Toyama, Japan
[c] Research Administration Division, Tokyo Medical and Dental University, Tokyo, Japan

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ABSTRACT

The chemotherapeutic agent paclitaxel (PTX) causes peripheral neuropathy as a major dose-limiting side effect, and this peripheral neuropathy is difficult to control. Our previous report showed that prophylactic repetitive administration of goshajinkigan (牛車腎氣丸 niú chē shèn qì wán), but not hachimijiogan (八味地黃丸 bā wèi dì huáng wán), which lacks two of the constituents of goshajinkigan, inhibited PTX-induced mechanical allodynia in mice. Thus, the herbal medicines *Plantaginis Semen* (車前子 chē qián zǐ) or *Achyranthis Radix* (牛膝 niú xī) may contribute to the inhibitory action of goshajinkigan on the exacerbation of PTX-induced mechanical allodynia [Andoh et al, J. Tradit. Complement. Med. 2014; 4: 293–297]. Therefore, in this study, we examined whether an extract of *Plantaginis Semen* (EPS) or *Achyranthis Radix* (EAR) would relieve PTX-induced mechanical allodynia in mice. A single intraperitoneal injection of PTX caused mechanical allodynia, which peaked on day 14 after injection. Repetitive oral administration of EPS, but not EAR, starting from the day after PTX injection significantly inhibited the exacerbation of PTX-induced mechanical allodynia. Repetitive intraperitoneal injection of aucubin, one of the main components of EPS, starting from the day after PTX injection also significantly reduced PTX-induced mechanical allodynia. However, repetitive intraperitoneal injection of geniposide acid (a precursor of aucubin) or catalpol (a metabolite of aucubin) did not prevent the exacerbation of mechanical allodynia. These results suggest that prophylactic administration of EPS is effective for preventing the exacerbation of PTX-induced allodynia. Aucubin may contribute to the inhibitory action of EPS on the exacerbation of PTX-induced allodynia.

1. Introduction

Paclitaxel (PTX) is an anti-microtubule agent that is widely indicated to treat solid neoplasms such as ovarian, breast, and lung cancer.[1,2] Approximately 20% of PTX-treated patients develop sensory neuropathy characterized by mechanical allodynia, cold allodynia, spontaneous pain, tingling, and numbness, with a stocking and glove distribution.[3,4] Since several drugs, such as gabapentin and amifostine, that have been used by PTX-treated patients to relive neuropathy have failed,[5–7] new therapeutic drugs are needed.

Goshajinkigan (牛車腎氣丸 niú chē shèn qì wán) is a traditional herbal formulation that consists of 10 herbal medicines [*Rehmanniae Radix* (地黃 dì huáng), *Achyranthis Radix* (牛膝 niú xī), *Corni Fructus* (山茱萸 shān zhū yú), *Dioscoreae Rhizoma* (山藥 shān yào), *Plantaginis Semen* (車前子chē qián zǐ), *Alismatis Rhizoma* (澤瀉 zé xiè), *Poria* (茯苓 fú ling), *Moutan Cortex* (牡丹皮 mǔ dān pí), *Cinnamoni Cortex* (桂皮 guì pí), and *Processi Aconiti Radix* (附子 fù zǐ)]. Goshajinkigan has been shown to attenuate the progression of peripheral neuropathy induced by docetaxel and PTX/carboplatin

* Corresponding author. Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel.: +81 76 434 7511; fax: +81 76 434 5045.
E-mail address: andoht@pha.u-toyama.ac.jp (T. Andoh).

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treatment in cancer patients.[8,9] In mice, repetitive administration of goshajinkigan also inhibits the exacerbation of PTX-induced mechanical allodynia.[10] Interestingly, hachimijiogan (八味地黄丸 bā wèi dì huáng wán), which consists of the same herbal medicines in goshajinkigan, except *Plantaginis Semen* and *Achyranthis Radix*, does not affect the exacerbation of PTX-induced mechanical allodynia.[10] This finding suggests that one of the herbal components (*Plantaginis Semen* or *Achyranthis Radix*) may contribute to the inhibitory action of goshajinkigan on the exacerbation of PTX-induced mechanical allodynia. Therefore, in the present study, we examined whether extracts of *Plantaginis Semen* (EPS) or *Achyranthis Radix* (EAR) could attenuate the exacerbation of PTX-induced mechanical allodynia.

2. Materials and methods

2.1. Animals

Male C57BL/6NCr mice were purchased from Japan SLC Ltd. (Hamamatsu, Japan) and were 6 weeks old at the start of experiments. The mice were housed under controlled temperature (21–23 °C), humidity (45–46%), and light (light from 7:00 AM to 7:00 PM). Food and water were feely available. This study was conducted with the approval of the Committee for Animal Experiments at the University of Toyama and in accordance with the guidelines for investigations of experimental pain in animals published by the International Association for the Study of Pain.

2.2. Drugs

PTX (Sigma, St. Louis, MO, USA) was dissolved in vehicle (physiological saline containing 10% Cremophore EL® [Sigma] and 10% ethanol) and administered intraperitoneally at a dose of 5 mg/kg in a volume of 0.1 mL/10 g of body weight. The dose calculations for PTX were based on the recommended clinical doses.[11] Dried water extracts of *Plantaginis Semen* (EPS: Lot. No. 2111049010) and *Achyranthis Radix* (EAR: Lot. No. 2101066010) were obtained from Tsumura and Co. Ltd. (Tokyo, Japan) and were dissolved in 5% gum arabic. Aucubin, geniposide acid, and catalpol were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were dissolved in saline. The extracts and agents were administered orally and intraperitoneally, respectively, in a volume of 0.1 mL/10 g of body weight once daily from the day after PTX injection.

2.3. Behavioral experiments

Mechanical allodynia was evaluated using a fine von Frey filament with a bending force of 0.69 mN (North Coast Medical Inc., Morgan Hill, CA, USA).[11] The mice were placed individually in an acrylic cage (11 cm × 18 cm × 15 cm) with a wire mesh bottom. After an acclimation period of at least 30 min, the von Frey filament was pressed perpendicularly against the central part of the plantar hind paw of freely moving mice and was held there for 1–3 s by slight buckling. Responses to the stimulus were scored as follows: 0, no reaction; 1, lifting of the hind paw; and 2, licking and flinching of the hind paw. A stimulation of same intensity was applied three times to each hind paw at intervals of several seconds, and the average value of six trials was used as the response score (the maximum score was 2).

2.4. Statistical analysis

All data are presented as the mean and standard error of the mean. Statistical significance was analyzed using Mann–Whitney rank sum test or Kruskal–Wallis one way analysis of variance on ranks followed by Dunn's multiple comparisons (comparisons with a control), and a p value less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of prophylactic administration of EPS and EAR on PTX-induced mechanical allodynia

A single intraperitoneal injection of PTX (5 mg/kg) induced mechanical allodynia, which peaked on day 14 and almost subsided by day 39 (Fig. 1A). When administered once daily from the day after PTX injection, EPS (0.3 g/kg, oral) significantly inhibited the exacerbation of allodynia from day 5 (Fig. 1B). A lower dose (0.1 g/kg, oral) of PTX had a tendency to inhibit allodynia from day 9, with a significant inhibition observed on day 10 (Fig, 1B). The lowest dose tested (0.03 g/kg, oral) had a tendency to inhibit allodynia from day 10 (Fig. 1B). On the other hand, daily oral administration of EAR (0.03–0.3 g/kg) did not affect the mechanical allodynia induced by PTX (Fig. 1C). In addition, repetitive administration of EPS and EAR did not cause diarrhea and sedation.

3.2. Effects of prophylactic administration of aucubin, geniposide acid, and catalpol on PTX-induced mechanical allodynia

Aucubin is one of the main components of *Plantaginis Semen*. When administered once daily from the day after PTX injection aucubin (50 mg/kg, intraperitoneal) significantly inhibited the exacerbation of allodynia from day 6 (Fig. 2A). A lower dose (15 mg/kg, intraperitoneal) had a tendency to inhibit allodynia from day 6, with a significant inhibition observed on days 12 and 13 (Fig, 2A). No significant inhibition was observed at the lowest dose tested (5 mg/kg, intraperitoneal) during the experimental period (Fig. 2A). On the other hand, daily intraperitoneal administration of geniposide acid (50 mg/kg, a precursor of aucubin) and catalpol (50 mg/kg, a metabolite of aucubin) did not affect the mechanical allodynia induced by PTX (Fig. 2B). In addition, repetitive administration of aucubin, geniposide acid, and catalpol did not cause diarrhea and sedation.

4. Discussion

Prophylactic repetitive administration of EPS, but not EAR, inhibited the exacerbation of PTX-induced mechanical allodynia, suggesting that EPS is an effective herbal medicine for inhibiting PTX-induced mechanical allodynia. We investigated the anti-allodynic effect of the components of EPS. In this study, we examined the effects of a water extract of *Plantaginis Semen*, and iridoid glycosides, such as aucubin and catalpol, are more efficiently extracted from the plant matrix by the water-based method than by the methanol-based method.[12] Aucubin is one of the main components of *Plantaginis Semen*.[13] Thus, the antiallodynic effect of aucubin, its precursor geniposide acid, and its metabolite catalpol were also tested. Prophylactic repetitive administration of aucubin attenuated the exacerbation of PTX-induced mechanical allodynia. However, geniposide acid and catalpol did not attenuate PTX-induced mechanical allodynia. Taken together, these results suggest that aucubin plays an important role in the inhibition of exacerbation of PTX-induced mechanical allodynia.

The mechanisms underlying aucubin- and EPS-mediated inhibition of PTX-induced mechanical allodynia are still unclear. A single oral dose of goshajinkigan has been shown to slightly inhibit established mechanical allodynia after PTX injection.[10] In our preliminary experiments, a single intraperitoneal injection of aucubin did not affect the established mechanical allodynia induced by PTX