



Effects of Goshajinkigan, Hachimijiogan, and Rokumigan on Mechanical Allodynia Induced by Paclitaxel in Mice

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ABSTRACT

Peripheral neuropathy is a major dose-limiting side effect of the chemotherapeutic agent paclitaxel. This study examined whether the three related traditional herbal formulations, goshajinkigan (GJG; 牛車腎氣丸 Niú Chē Shèn Qì Wán), hachimijiogan (HJG; 八味地黃丸 Bā Wèi Dì Huáng Wán), and rokumigan (RMG; 六味丸 Liù Wèi Wán), would relieve paclitaxel-induced mechanical allodynia in mice. A single intraperitoneal injection of paclitaxel (5 mg/kg) induced mechanical allodynia, which peaked on day 14 after injection. On day 14 after paclitaxel injection, oral administration of GJG (0.1-1.0 g/kg) produced a significant inhibition of established allodynia, but HJG and RMG did not affect the allodynia. Repeated oral administration of GJG (0.1-1.0 g/kg) starting from the day after paclitaxel injection did not affect allodynia development, but significantly inhibited allodynia exacerbation. Repeated oral administration of HJG produced a slight inhibition of allodynia exacerbation, but that of RMG did not. These results suggest that prophylactic administration of GJG is effective in preventing the exacerbation of paclitaxel-induced allodynia. The herbal medicines *Plantaginis Semen* (車前子 Chē Qián Zǐ) and *Achyranthis Radix* (牛膝 Niú Xī), which are present in GJG but not in HJG, may contribute to the inhibitory action of GJG on the exacerbation of paclitaxel-induced allodynia.

Key words: Goshajinkigan, Hachimijiogan, Mechanical allodynia, Paclitaxel, Rokumigan

INTRODUCTION

Paclitaxel, an anti-microtubule agent originally isolated from the bark of the Pacific yew tree (*Taxus brevifolia*), is widely used to treat solid neoplasms such as ovarian, breast, and lung cancers.^[1,2] However, paclitaxel causes peripheral neuropathy, which is characterized by pain and allodynia, with a stocking-and-glove distribution.^[3] The peripheral neuropathy is a major dose-limiting side effect of paclitaxel. Several

drugs, such as gabapentin and amifostine, were tested and failed to relieve paclitaxel-induced peripheral neuropathy in patients.^[4-6]

Goshajinkigan (GJG; 牛車腎氣丸 Niú Chē Shèn Qì Wán) is a traditional herbal formulation consisting 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 Líng), *Moutan*

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DOI: 10.4103/2225-4110.128906

Cortex (牡丹皮 Mǔ Dān Pí), *Cinnamoni Cortex* (桂皮 Guì Pí), and *Processi Aconiti Radix* (附子 Fù Zǐ)]. GJG is used for the treatment of several neurological symptoms including pain and dysesthesia (unpleasant abnormal sensation). It has been demonstrated to be effective against chemotherapy-induced peripheral neuropathy in cancer patients.^[7,8] Recent studies have shown that GJG does not attenuate the antineoplastic action of chemotherapeutic agents.^[9,10] Excluding *Achyranthis Radix* and *Plantaginis Semen*, hachimijiogan (HJG; 八味地黄丸 Bā Wèi Dì Huáng Wán) consists of the same herbal medicines found in GJG, and rokumigan (RMG; 六味丸 Liù Wèi Wán) consists of all herbal medicines found in HJG, excluding *Cinnamoni Cortex* (桂皮 Guì Pí) and *Processi Aconiti Radix* (附子 Fù Zǐ). There is no clinical evidence of the effects of HJG and RMG on chemotherapy-induced peripheral neuropathy. In this study, we investigated the effects of GJG, HJG, and RMG on paclitaxel-induced mechanical allodynia.

MATERIALS AND METHODS

Animals

Male C57BL/6NCr mice (Japan SLC Ltd, Hamamatsu, Japan) were used. All animals were 6 weeks old at the start of the experiments. They were housed in a room with controlled temperature (21-23°C), humidity (45-65%), and a 12-h light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). Food and water were provided *ad libitum*. Experiments were performed after obtaining approval from the animal care committee of the University of Toyama.

Drugs

Paclitaxel purchased from Sigma-Aldrich (St Louis, MO, USA) was dissolved in vehicle [physiological saline containing 10% Cremophor® EL (Sigma-Aldrich) and 10% ethanol]. Paclitaxel and the vehicle were administered intraperitoneally; the dose (5 mg/kg) was selected from a published report^[11] and was in accordance with the recommended clinical dose of 210 mg/m² body surface area, which corresponds to a dose of 5.9 mg/kg in a person of 170 cm body height and 60 kg weight. Dried extracts of GJG (Lot. No. 2080107010, 2009), HJG (Lot. No. 2090007010, 2009), and RMG (Lot. No. 2090087010, 2009) were obtained from Tsumura and Co., Ltd (Tokyo, Japan). These dried extracts were dissolved in 5% gum arabic and were administered orally once on day 14 after the paclitaxel injection or once daily from the day after paclitaxel injection is given.

Behavioral experiments

Mechanical allodynia in the hind paw was evaluated using a von Frey filament (North Coast Medical Inc., Morgan Hill, CA, USA), as described.^[11] After an acclimation period of at least 30 min, a fine von Frey filament with a bending force of 0.69 mN was applied perpendicularly against the central part of the plantar hind paw and was held for 1-3 s with it slightly bent. Responses to the stimuli were scored as follows: 0, no reaction; 1, lifting of the hind paw; and 2, licking and flinching of the hind paw. A stimulus of the same intensity was applied three times alternately to each

hind paw at intervals of several seconds, and the average response score served as the allodynia score (the maximum score was 2). When the effects of single administration of dried extracts of traditional formulations were examined on day 14 after paclitaxel injection, mechanical allodynia was evaluated before and after the administration of traditional formulations. When the effects of repeated administrations of the dried extracts were examined, mechanical allodynia evaluation was performed before each administration of traditional formulations.

Statistical analysis

All data are presented as mean ± standard error of the mean. Statistical significance was analyzed using two-way repeated measures analysis of variance (ANOVA) and *post hoc* Holm-Sidak multiple comparison test. A $P < 0.05$ was considered statistically significant. The statistical analyses were performed using SigmaPlot™ graphing and statistical software version 11 (Systat Software, Inc., Chicago, IL, USA).

RESULTS

Paclitaxel-induced mechanical allodynia

A single intraperitoneal injection of paclitaxel (5 mg/kg) caused mechanical allodynia, which became apparent on day 4 after injection, peaked on day 14, and almost subsided by day 21 [Figure 1a; main effect of paclitaxel treatment, $F_{1,10} = 87.676$, $P < 0.001$; interaction between paclitaxel treatment and time, $F_{30,300} = 12.286$, $P < 0.001$ (two-way repeated measures ANOVA)].

Effects of single administration of GJG, HJG, and RMG on paclitaxel-induced mechanical allodynia

Three formulations were administered orally on day 14 after paclitaxel injection. Single administration of GJG (0.1-1.0 g/kg) exerted a relatively short-lasting but significant dose-dependent inhibition on the established mechanical allodynia; two-way repeated measures ANOVA revealed a significant main effect of GJG ($F_{3,20} = 3.611$, $P = 0.031$) [Figure 1b]. Single administration of HJG and RMG did not affect the established mechanical allodynia at doses of 0.1-1.0 g/kg [Figure 1c and d].

Effects of prophylactic administration of GJG, HJG, and RMG on paclitaxel-induced mechanical allodynia

In this series of experiments, three formulations were administered daily from the day after paclitaxel injection. Repeated administration of GJG (0.1-1 g/kg) did not affect the initial development of mechanical allodynia induced by paclitaxel, but significantly inhibited the exacerbation of allodynia from day 9 after paclitaxel injection, in comparison with the vehicle, although no clear dose-dependency was observed [Figure 2a; interaction between paclitaxel treatment and time, $F_{42,252} = 1.889$, $P = 0.002$ (two-way repeated measures ANOVA)]. Similarly, repeated administration of HJG (0.1-1 g/kg) did not affect the initial development of mechanical allodynia, but the highest dose of HJG (1 g/kg) significantly inhibited allodynia on day 14 after the injection, in comparison with the vehicle [Figure 2b; interaction between paclitaxel treatment and time, $F_{42,266} = 1.504$, $P = 0.03$ (two-way repeated measures ANOVA)]. Repeated ad-

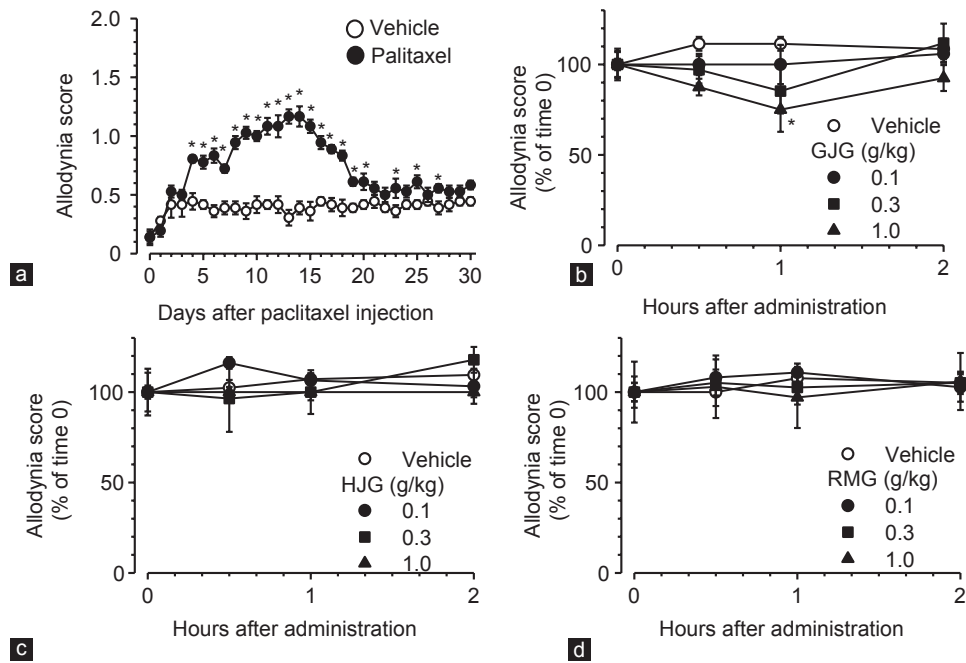


Figure 1. Effects of single administration of goshajinkigan (GJG), hachimijiogan (HJG), and rokumigan (RMG) on the established mechanical allodynia after paclitaxel injection. (a) Development of mechanical allodynia after a single injection of paclitaxel. Mice were injected intraperitoneally with paclitaxel (5 mg/kg) or vehicle on day 0. GJG (b), HJG (c), RMG (d), or vehicle (5% gum arabic) was orally administered on day 14 after paclitaxel injection. Data are presented as mean and standard error of the mean ($n = 5-6$). * $P < 0.05$ compared to vehicle (Holm-Sidak multiple comparisons)

ministration of RMG (0.1-1 g/kg) did not affect the mechanical allodynia induced by paclitaxel [Figure 2c].

DISCUSSION

Single oral administration of GJG (0.1-1 g/kg) exerted a dose-dependent inhibition on the established mechanical allodynia after paclitaxel injection, whereas the GJG-related herbal formulations HJG and RMG did not affect the established allodynia. The recommended clinical daily dose of GJG granules is 7.5 g, which contains 4.5 g of dried GJG extract. Given that the body weight is 60 kg, the dose of the dried GJG extract is 0.075 g/kg. Thus, the antiallodynic dose (1 g/kg) in mice with paclitaxel-induced neuropathy was 13 times higher than the recommended clinical dose. Recently, we have demonstrated that single oral administration of GJG (0.3 and 1.0 g/kg) inhibits the established mechanical allodynia after the injection of oxaliplatin, a platinum chemotherapeutic agent.^[12] The inhibitory effect of 1 g/kg GJG on paclitaxel-induced allodynia (present study) was less than that of 0.3 g/kg GJG on oxaliplatin-induced allodynia.^[12] The inhibitory action of GJG on oxaliplatin-induced allodynia is at least in part mediated by the activation of the descending noradrenergic and serotonergic systems,^[12] and in our preliminary experiment, single administration of milnacipran, a serotonin–noradrenaline reuptake inhibitor, markedly inhibited the established allodynia after oxaliplatin injection (data not shown). In contrast, single administration of milnacipran does not affect the established allodynia after paclitaxel injection.^[13] These findings, taken together, raise the possibility that a low efficacy of GJG in paclitaxel-induced allodynia is due to the

decreased activity of the descending noradrenergic and serotonergic systems. It is possible that paclitaxel decreases the activity of the descending noradrenergic and/or serotonergic systems.

Prophylactic repeated administration of GJG inhibited paclitaxel-induced mechanical allodynia. GJG consists of the following 10 herbal medicines: *Rehmanniae Radix* (地黃 *Dì Huáng*), *Corni Fructus* (山茱萸 *Shān Zhū Yú*), *Dioscoreae Rhizoma* (山藥 *Shān Yào*), *Alismatis Rhizoma* (澤瀉 *Zé Xiè*), *Poria* (茯苓 *Fú Líng*), *Moutan Cortex* (牡丹皮 *Mǔ Dān Pí*), *Cinnamoni Cortex* (桂皮 *Guì Pí*), *Processi Aconiti Radix* (附子 *Fù Zǐ*), *Achyranthis Radix* (牛膝 *Niú Xī*), and *Plantaginis Semen* (車前子 *Chē Qián Zǐ*); HJG consists of the first 8 of these herbal medicines and RMG consists of the first 6 of these medicines. The results showed that HJG had only a slight effect at the highest dose tested and RMG did not have a prophylactic effect, suggesting that *Plantaginis Semen* and/or *Achyranthis Radix* play a role in the prophylactic activity of GJG. The underlying mechanisms are unclear, and allodynia-preventing substances have not yet been isolated from the extracts of these two herbal medicines. However, one possibility is that the activity of the scavengers of reactive oxygen species is involved in the antiallodynic activity. Although a single administration is ineffective, prophylactic administration of the reactive oxygen species scavenger N-tert-butyl- α -phenyl-nitrone suppresses paclitaxel-induced mechanical allodynia.^[14] Since *Plantaginis Semen* and *Achyranthis Radix* have antioxidant activity,^[15-17] this action may be involved in the prevention of an exacerbation of paclitaxel-induced allodynia. Another possibility is the improvement of decreased blood flow. Paclitaxel, but not vincristine, decreases peripheral blood flow.^[11] The prostaglandin E1 analog limaprost, which counteracts this reduction in blood

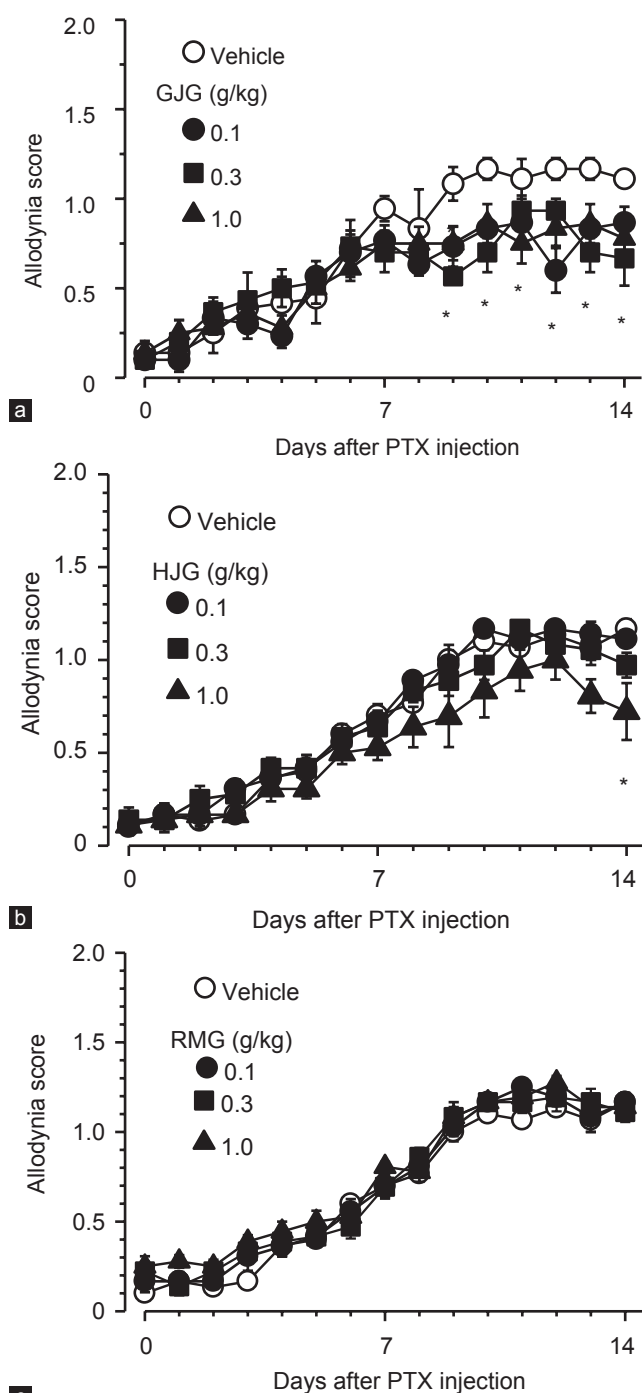


Figure 2. Effects of prophylactic administration of goshajinkigan (GJG), hachimijiogan (HJG), and rokumigan (RMG) on paclitaxel-induced mechanical allodynia. Paclitaxel (5 mg/kg) was injected intraperitoneally in mice, and GJG (a), HJG (b), RMG (c), or vehicle (5% gum arabic) was administered orally once daily from the day after the paclitaxel injection. Data are presented as mean and standard error of the mean ($n = 5-6$). * $P < 0.05$ compared to vehicle (Holm-Sidak multiple comparisons)

flow without affecting normal blood flow,^[18] exerts prophylactic inhibition on allodynia induced by paclitaxel, but not by vincristine.^[11] In this study, prophylactic administration of GJG prevented mechanical allodynia induced by paclitaxel. In contrast, in our

preliminary experiments, prophylactic administration of GJG did not prevent vincristine-induced allodynia. GJG and its constituent *Achyranthis Radix* invigorate blood circulation.^[15,17,19] Taken together, these findings raise the possibility that the prevention of the decrease in peripheral blood flow contributes to the prophylactic activity of GJG.

Prophylactic administration of HJG, but not RMG, showed slight antiallodynic action at the highest dose tested in paclitaxel-treated mice. Thus, it is possible that *Cinnamoni Cortex* and *Processi Aconiti Radix* also have prophylactic antiallodynic activity. Similar to *Achyranthis Radix*, *Cinnamoni Cortex* and *Processi Aconiti Radix* invigorate blood circulation.^[20,21] However, the prophylactic antiallodynic activity of HJG was weaker than that of GJG. Thus, although *Cinnamoni Cortex* and *Processi Aconiti Radix* may have some prophylactic antiallodynic activity, *Plantaginis Semen* and *Achyranthis Radix* may play important roles in the antiallodynic action of GJG.

CONCLUSION

Prophylactic administration of GJG prevents an exacerbation of paclitaxel-induced mechanical allodynia. Since GJG also has acute antiallodynic activity, it may be useful for the prevention of paclitaxel-induced peripheral neuropathy. Although the underlying mechanisms remain unclear, *Plantaginis Semen* and/or *Achyranthis Radix* may contribute to the antiallodynic activity of GJG.

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