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Short Communication

Effects of *Shakuyakukanzoto* on The Exacerbation of Peripheral Neuropathic Pain and The Decreased Grip Strength in Mice Treated with Paclitaxel

Tsugunobu Andoh*, Shinju Fukatsu

Department of Pharmacology and Pathophysiology, College of Pharmacy, Kinjo Gakuin University, Japan.

***Corresponding Author:** Tsugunobu Andoh, Department of Pharmacology and Pathophysiology, College of Pharmacy, Kinjo Gakuin University, 2-1723 Omori, Moriyama-ku, Nagoya, Aichi 463-8521, Japan.

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Abstract

Paclitaxel (PTX) is used for cancer chemotherapy. However, peripheral neuropathy occurs as a side effect, and these symptoms are difficult to control. *Shakuyakukanzoto* (SKT) composed of two herbs (Paeoniae radix (PR) and Glycyrrhizae radix (GR)) is a traditional herbal medicine used for treating muscle cramps. This study demonstrated the effects of SKT and the extracts of each component on the exacerbation of peripheral neuropathic pain and the decreased grip strength in mice treated with PTX. PTX was injected intraperitoneally once daily four times every other day. Water extract (WE) of SKT, PR and GR were orally administered once daily in PTX-treated mice. The pain threshold and grip strength were evaluated using von Frey filaments and a digital grip strength meter, respectively. PTX decreased the pain withdrawal threshold and grip strength. Prophylactic repeated administration of WE-SKT inhibited the decrease in both the pain withdrawal threshold and grip strength. WE-PR, but not WE-GR, inhibited the decrease in the pain withdrawal threshold. However, both WE did not affect the decrease in grip strength. These results suggest that SKT is effective for PTX-induced peripheral neuropathy and the ingredients of both PR and GR play an important role in the control of peripheral neuropathy.

Keywords: Paclitaxel; Mechanical hyperalgesia; Muscle weakness; Shakuyakukanzoto

Introduction

Paclitaxel (PTX) is an anti-cancer drug used to treat solid neoplasms such as ovarian cancer [1]. It has been reported PTX induces serious side effects, such as peripheral neuropathy that includes pain, allodynia, numbness and muscle weakness [2]. However, several treatments that have been attempted (e.g. gabapentin and amifostine) have failed to relieve the neuropathy in patients [3]. Therefore, new therapeutic medicines for regulating neuropathy are needed.

Shakuyakukanzoto (SKT) is a traditional herbal medicine that consists of two components: Paeoniae radix (PR) and Glycyrrhizae radix (GR). It is used to treat muscle pain and spasms, joint pain, and numbness in human patients [4,5]. In rodents, SKT inhibits mechanical allodynia induced by single injection of PTX [6,7]. However, in our preliminary experiments, the animal model prepared by a single injection of PTX did not observe muscle weakness (data not shown), so a single injection

of an anti-cancer drug may not affect muscle or motor neurons. In clinical settings, Bandos et al. reported that 2 years after the start of treatment, more than 40% of participants in their trial said they still experienced numbness and tingling in their hands or feet, and 10% rated their symptoms as severe [8]. Therefore, in the present study, we developed an animal model that showed both long-time peripheral neuropathic pain and muscle weakness in induced by treating repeatedly with PTX, and evaluated the effects of SKT. In addition, pseudoaldosteronism induced by one ingredient of GR (licorice), a component of SKT, is a problematic side effect [9]. Therefore, we also investigated the necessity of GR in SKT on the prevention of PTX-induced peripheral neuropathic pain and muscle weakness.

Methods

Animals

Male C57BL/6NCr mice (6 weeks old; Japan SLC, Ltd., Hamamatsu) were housed in a room with controlled temperature (21–23°C), humidity (45%–65%), and a 12 h light/dark cycle (lights on from 8:00 am to 8:00 pm). Food and water were provided *ad libitum*. All procedures of the animal experiments were approved by the committee for animal experiments of Kinjo Gakuin University (No. 193).

Drugs

PTX (Tokyo Chemical Industry Co., Ltd., Tokyo) was dissolved in vehicle (physiological saline containing 10% Cremophor® EL [Sigma-Aldrich, St. Louis, MO, USA] and 10% ethanol), and the vehicle were administered intraperitoneally once daily 4 times every other day (Day 0, 2, 4, 6) at a volume of 0.1 ml/10g of body weight.

Dried water extract (WE)-SKT (Lot. No. 2200068010, 2021), WE-PR (Lot. No. 2191001010, 2021) and WE-GR (Lot. No. 2191013010, 2021) were obtained from Tsumura & Co., Ltd. (Tokyo). These dried extracts were dissolved in 5% gum arabic (Wako Pure Chemical Inc., Osaka) and administered orally once a day after a behavioral evaluation. When PTX (or the vehicle) was injected, WE-SKT, WE-PR, WE-GR, or their vehicle was administered orally 1 h after PTX (or the vehicle) injection at a volume of 0.1 ml/10 g of body weight.

Behavioral evaluation

Mice were placed individually in a plastic cage (8 cm ×10 cm × 18 cm) with a wire mesh bottom, and the pain threshold was evaluated using an Aesthesio® Precision Tactile Sensory Evaluator (Muromachi Kikai Co., Ltd., Tokyo). The filaments were pressed perpendicularly against the plantar hind paw of the freely moving mouse. The threshold was determined in an up-down testing paradigm. Grip strength was evaluated using a digital grip strength meter (GPM-101B/V, Melquest Ltd., Toyama).

Statistical analyses

All data are represented as the mean ± standard error of the mean. The statistical significance of differences between groups was analyzed using a one-way analysis of variance (ANOVA) or two-way repeated measures ANOVA followed by a post hoc Holm–Šidák multiple comparisons test. p values of <0.05 were considered to indicate statistical significance.

Results

PTX-induced mechanical hyperalgesia and reduction in the grip strength

In the present study, we used PTX at doses of 2 and 8 mg/kg based on the human dose-converted report of Toma et al [10].

Intraperitoneal PTX induced mechanical hyperalgesia from the day after the first dose (Figure 1a). PTX-induced mechanical hyperalgesia induced by PTX at doses of 2 and 8 mg/kg was peaked from day 6 and day 3, respectively (Fig. 1a). Although PTX (2 mg/kg)-induced mechanical hyperalgesia began to recover from day 12 after the first dose PTX (8 mg/kg)-induced mechanical hyperalgesia was observed even at 14 days from the first dose (Figure 1a).

Intraperitoneal PTX induced a reduction in the grip strength from day 2 after the first dose (Figure 1b). PTX (2 and 8 mg/kg)-induced reduction in the grip strength was peaked from day 4 (Figure 1b). Although PTX (2 mg/kg)-induced reduction of grip strength began to recover from day 10 after the first dose, PTX (8 mg/kg)-induced reduction in the grip strength was observed even 14 days from the first dose (Figure 1b).

Subsequent experiments used PTX (8 mg/kg), which markedly induced both mechanical hyperalgesia and reduction in the grip strength.

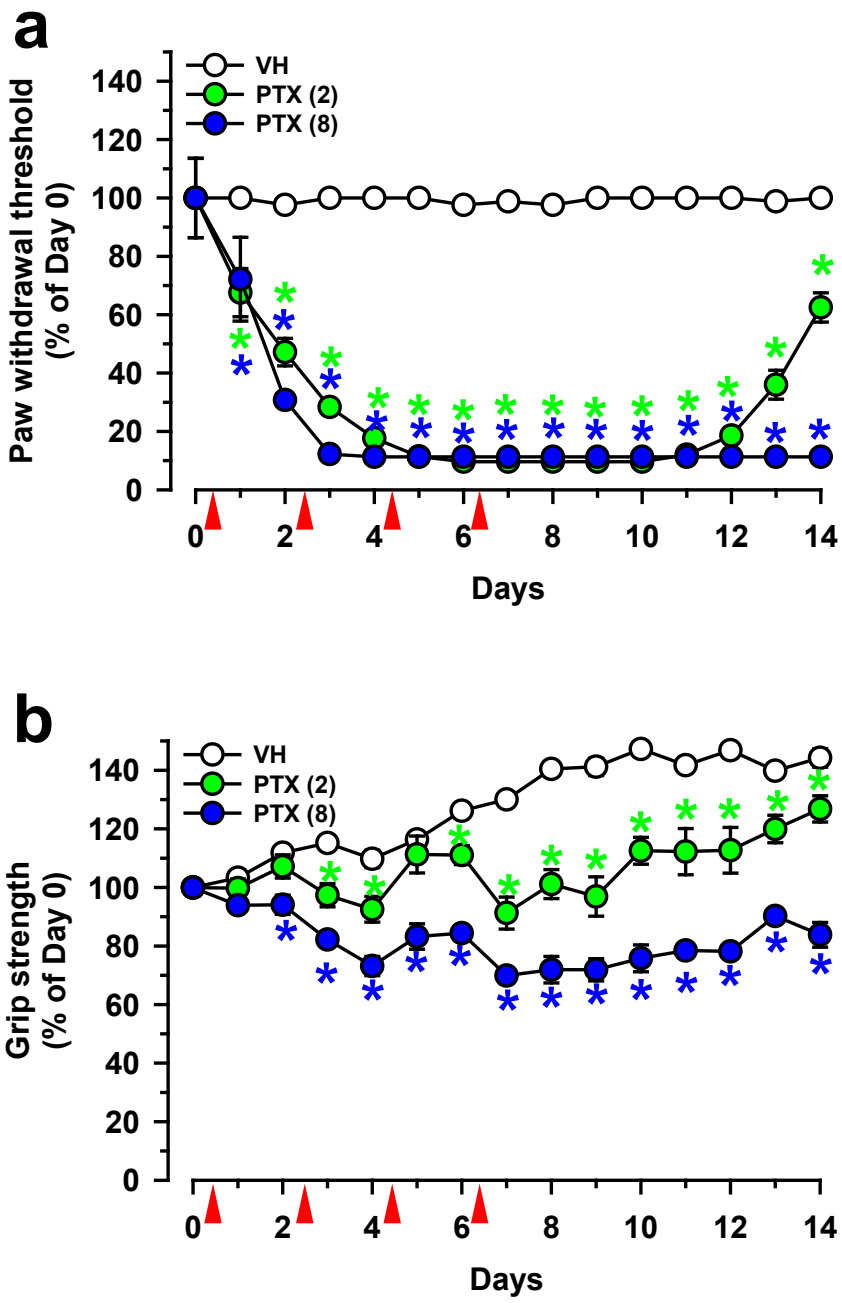

Figure 1: Development of mechanical hyperalgesia (a) and reduction in the grip strength (b) in paclitaxel (PTX)-treated mice. At day 0, 2, 4 and 6 (red arrow head), PTX (2 or 8 mg/kg) or vehicle (VH) was injected intraperitoneally to mice. Data are presented as the mean and standard error of the mean (n = 6). **(a)** Main effect of PTX treatment, $F_{5,14} = 45.140$ ($p < 0.001$). Interaction between PTX treatment and time, $F_{28,140} = 21.601$ ($p < 0.001$). * Indicates $p < 0.05$ when compared with VH (Holm-Šidák test). **(b)** Main effect of PTX treatment, $F_{5,14} = 29.371$ ($p < 0.001$). Interaction between PTX treatment and time, $F_{28,140} = 22.117$ ($p < 0.001$). * Indicates $p < 0.05$ when compared with VH (Holm-Šidák test).

Effects of WE-SKT, WE-PR and WE-GR on PTX-induced mechanical hyperalgesia

Prophylactic repeated oral administration of WE-SKT (0.3 and 1 g/kg) significantly inhibited PTX-induced mechanical allodynia, compared with the oral vehicle-administered group (Figure 2a). Although not completely, WE-PR (1 g/kg) also significantly inhibited PTX-induced mechanical hyperalgesia (Figure 2b). However, WE-GR did not affect PTX-induced mechanical hyperalgesia (Figure 2c).

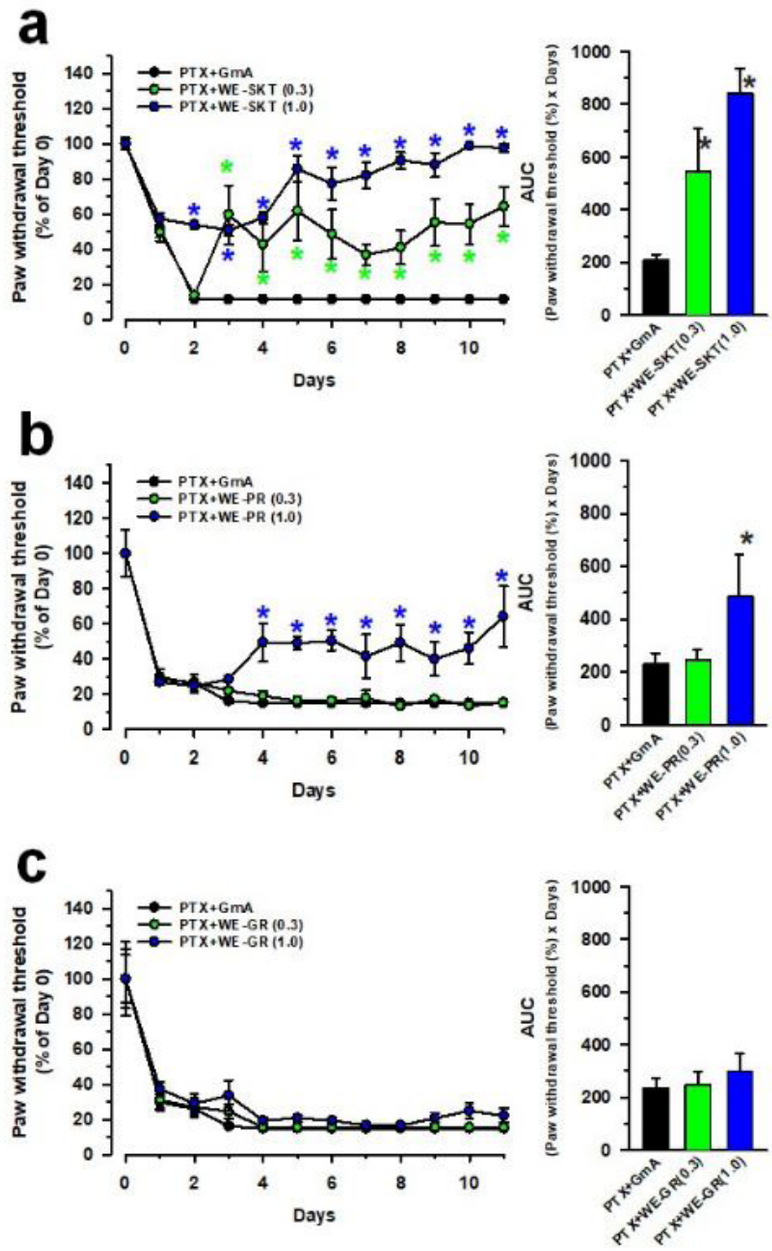

Figure 2: Effects of water extract (WE) of *shakuyakukanzoto* (SKT), *paeoniae radix* (PR) or *glycyrrhizae radix* (GR) on paclitaxel (PTX)-induced mechanical hyperalgesia in mice. At day 0, 2, 4 and 6 (red arrow head), PTX (8 mg/kg) or vehicle (VH) was injected intraperitoneally to mice. Gum Arabic (a-c, vehicle, 5% GmA), WE-SKT (a: 0.3 and 1 g/kg), WE-PR (b: 0.3 and 1 g/kg) or WE-GR (b: 0.3 and 1 g/kg) was administered orally once a day after the behavioral evaluation. When injected intraperitoneally PTX, GmA or WE of herbal medicines (WE-SKT, WE-PR, WE-GR) was administered orally 1 h after PTX injection. **(a)** Effects of WE-SKT on PTX-induced mechanical allodynia in mice. The left and right panels show the time-course of the paw withdrawal threshold and the area under the curve (AUC), respectively. Data are presented as the mean and standard error of the mean (n = 6). (Left panel) Main effect of PTX+WE-SKT treatment, $F_{5,11} = 22.244$ ($p < 0.001$). Interaction between PTX+WE-SKT treatment and time, $F_{22,110} = 6.262$ ($p < 0.001$). * Indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). (Right panel) * indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). **(b)** Effects of WE-PR on PTX-induced mechanical allodynia in mice. The left and right panels show the time-course of the paw withdrawal threshold and AUC, respectively. Data are presented as the mean and standard error of the mean (n = 6). (Left panel) Main effect of PTX+ WE-PR treatment, $F_{5,11} = 92.308$ ($p < 0.001$). Interaction between PTX+WE-PR treatment and time, $F_{22,110} = 4.136$ ($p < 0.001$). * Indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). (Right panel) * indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). **(c)** Effects of WE-GR on PTX-induced mechanical allodynia in mice. The left and right panels show the time-course of the paw withdrawal threshold and AUC, respectively. Data are presented as the mean and standard error of the mean (n = 6).

Effects of WE-SKT, WE-PR and WE-GR on PTX-induced reduction in the grip strength

Prophylactic repeated oral administration of WE-SKT (0.3 and 1 g/kg) significantly inhibited PTX-induced reduction in the grip strength, compared with the oral vehicle-administered group (Figure 3a). However, WE-PR (0.3 and 1 g/kg) and WE-GR (0.3 and 1 g/kg) did not affect PTX-induced reduction in the grip strength (Figure 3b,c).

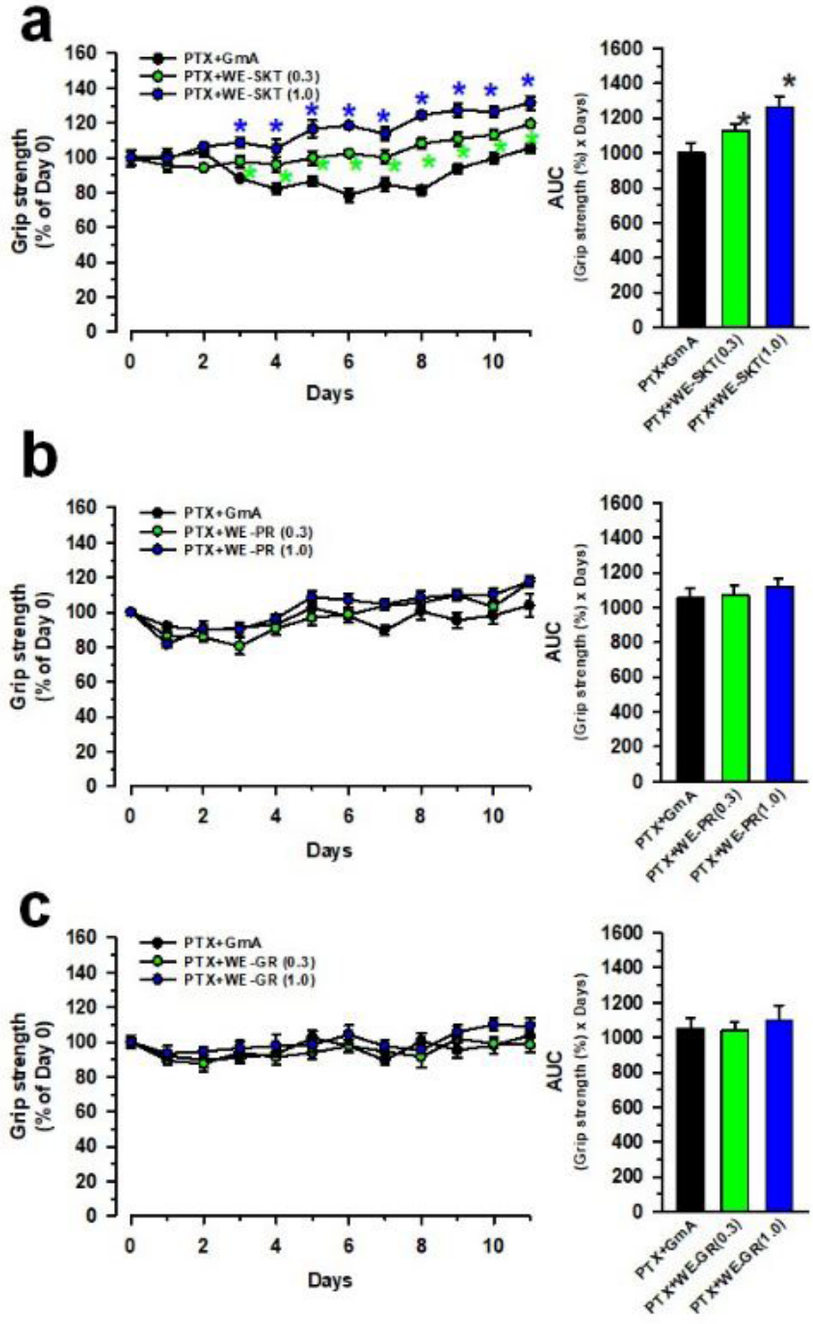

Figure 3: Effects of water extract (WE) of *shakuyakukanzoto* (SKT), *paeoniae radix* (PR) or *glycyrrhizae radix* (GR) on the paclitaxel (PTX)-induced reduction in the grip strength in mice. At days 0, 2, 4 and 6 (red arrow head), PTX (8 mg/kg) or vehicle (VH) was injected intraperitoneally to mice. Gum Arabic (a-c, vehicle, 5% GmA), WE-SKT (a: 0.3 and 1 g/kg), WE-PR (b: 0.3 and 1 g/kg) or WE-GR (b: 0.3 and 1 g/kg) was administered orally once a day after the behavioral evaluation. When PTX was injected intraperitoneally, GmA or WE of herbal medicines (WE-SKT, WE-PR, WE-GR) was administered orally 1 h after PTX injection. **(a)** Effects of WE-SKT on the PTX-induced reduction in the grip strength in mice. The left and right panels show the time-course of the grip strength and the area under the curve (AUC), respectively. Data are presented as the mean and standard error of the mean (n = 6). (Left panel) Main effect of PTX+WE-SKT treatment, $F_{5,11} = 14.658$ ($p < 0.001$). Interaction between PTX+WE-SKT treatment and time, $F_{22,110} = 8.825$ ($p < 0.001$). * Indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). (Right panel) * indicates $p < 0.05$ when compared with PTX+GmA (Holm-Šidák test). **(b)** Effects of WE-PR on PTX-induced reduction in the grip strength in mice. The left and right panels show the time-course of the grip strength and AUC, respectively. Data are presented as the mean and standard error of the mean (n = 6). **(c)** Effects of WE-GR on PTX-induced reduction in the grip strength in mice. The left and right panels show the time-course of the grip strength and AUC, respectively. Data are presented as the mean and standard error of the mean (n = 6).

Discussion

Intraperitoneal injection of PTX (especially, at 8 mg.kg) once daily four times every other day elicited long-term mechanical hyperalgesia and a reduction in the grip strength. This finding in mice is the first of its kind. Although there have been no reports on the reduction in the grip strength, the time course of PTX (2 and 8 mg/kg)-induced mechanical hyperalgesia is similar to that in Toma's report [10]. Since this animal model showed symptoms of peripheral neuropathy in PTX-treated patients [2], it is expected to be useful for drug evaluations and elucidating the developmental mechanism.

The exacerbation of PTX-induced mechanical hyperalgesia was inhibited by WE-SKT. In addition, WE-PR, but not WE-GR, attenuated the exacerbation. Hidaka et al. reported that the extract of PR, but not that of GR, inhibits acute PTX-induced mechanical allodynia in mice [7]. Our previous report has been shown that prophylactic repeated local application of paeoniflorin (PF), a major ingredient of PR, inhibits mechanical allodynia and peripheral nerve firing in mice given a single injection of PTX [11]. However, glycyrrhizic acid (GA), a major ingredient of GR, does not affect PTX-induced mechanical allodynia (data not shown). These findings suggest that the ingredients of PR (especially, PF), rather than the ingredients of GR, contribute to the inhibitory action of SKT on PTX-induced mechanical hyperalgesia.

In addition to peripheral dysesthesia (e.g. pain, allodynia and numbness), PTX-treated patients also show muscle weakness [2]. In the present study, we found for the first time that mice treated with PTX once daily 4 times every other day exhibited a reduction in the grip strength. However, whether this PTX-induced reduction in the grip strength was due to muscle weakness, muscle pain, muscle stiffness or motor neuron disfunction in mice remains unclear. Further studies will be needed to clarify this point. In addition. WE-SKT, but not WE-PR or WE-GR, inhibited the exacerbation of the PTX-induced reduction in the grip strength. PF and GA are known to suppress the influx of Ca^{2+} into skeletal muscle cells and to promote the outflow of K^{+} from cells, respectively [12,13]. GA alone does not relax skeletal muscle but rather enhances skeletal muscle relaxation induced by PF [13]. Taken together, our findings suggest that the ingredients of both PR and GR are needed to achieve the inhibitory effect of WE-SKT on the exacerbation of the PTX-induced reduction in the grip strength.

Conclusion

SKT prevents the exacerbation of mechanical hyperalgesia and reduction in the grip strength induced by PTX. Although the underlying mechanisms remain unclear, both PR and GR may contribute to the inhibitory action of SKT in PTX-induced peripheral neuropathy.

Role of Funding Source

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Ethics Committee Approval

Animal studies were approved by the committee for animal experiments of Kinjo Gakuin University (No. 193).

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