論　文　要　約

論 文 題 目　Iridoids showing anti-allodynic activity from Plantaginis Semen and Viticis Fructus

課程・専攻名　博士後期課程・薬科学専攻

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Chemotherapy-induced peripheral neuropathy (CIPN) is one of the frequent adverse effects of commonly used chemotherapeutic agents like the paclitaxel (PTX), oxaliplatin, and vincristine. Typical symptoms of CIPN are seen in patients such as mechanical allodynia (pain from innocuous stimuli such as light touch), hyperalgesia, numbness, and tingling primarily in hands and feet. These symptoms may lead to dose reduction or termination of chemotherapy, potentially impacting on treatment efficacy. Further, CIPN may persist even after the chemotherapy completion, leading to long-term influence on the quality-of-life in patients. However, the associated mechanism is not completely elucidated and currently available therapeutic drugs are not sufficiently effective to manage CIPN in clinical. Thus, there is a critical need for the discovery of novel agents for treatment of this devastating side effect.

Goshajinkigan (GJG), a Kampo (traditional Japanese medicine) formula used for the treatment of sensitivity to cold, pain, numbness, reduced micturition, nocturnal enuresis, edema, and lumbago, has been shown to be effective for the treatment of CIPN based on observational studies in clinical. Remarkable activity of GJG against PTX-induced allodynia in mice models has been reported. The research on the effects of GJG and its related formulae (Hachimijiogan and Rokumijiogan) on PTX-induced mechanical allodynia in mice showed that only GJG was effective in inhibition of PTX-induced allodynia. Furthermore, the activity study of Plantaginis Semen and Achyranthis Radix, which are included in GJG but not in other two formulae, indicated that Plantaginis Semen contributed to the inhibitory activity of GJG on the exacerbation of PTX-induced allodynia. This study aimed to identify active compounds responsible for the anti-allodynic activity of Plantaginis Semen, as well as search for active compounds showing potential benefit for the management of CIPN from other crude drugs.

1　Search for anti-allodynic compounds from Plantaginis Semen [1]

To elucidate the anti-allodynic compound in Plantaginis Semen, the boiled water extract of Plantaginis Semen was subjected to activity-guided isolation using PTX-induced mechanical allodynia mice model. From the active fraction separated by HP-21 and ODS MPLC column chromatography, four iridoids aucubin (**1**), geniposidic acid (**2**), pedicularis-lactone (**3**), and iridolactone (**4**) were purified and identified by comparison of MS and NMR data with those in the literature (Fig. 1). To investigate the anti-allodynic activity of these compounds, commercial **1** and isolated **2** were used. While crude fractions (**crude 3** and **crude 4**) primarily containing **3**

and **4**, respectively, were used for animal experiment, because of their low content in Plantaginis Semen.

A single intraperitoneal administration of PTX (5 mg/mL) resulted in an increasing allodynia score in mice, peaking on day 14. Oral administration of **1** (30 and 100 mg/kg) and **crude 3** (100 mg/kg) attenuated PTX-induced mechanical allodynia in mice significantly, while oral administration of **2** (75 mg/kg) and **crude 4** (100 mg/kg) didn't exhibit this activity (Fig. 2A-C). The major compound was identified to be **3** according to the ^{1}H-NMR spectrum of **crude 3** (Fig. 3A). To clarify the quantitative level of **3** in **crude 3**, the quantitative ^{1}H-NMR (qHNMR) as a simple and acute quantitative method was applied to the purity assessment and DSS-d_6 was used as internal standard. The H-7 of **1** was selected for quantification due to its clear baseline separation (Fig. 3A). As a result, the purity of **3** in **crude 3** was estimated to be 13.4±0.3%.

Thus, Compound **1** was identified as an active compound, and **3** was proposed to be one of active compounds responsible for the anti-allodynic effect of Plantaginis Semen. However, further confirmatory studies for the anti-allodynic activity using purified **3** is necessary.

2 Iridoids isolated from Viticis Fructus inhibit paclitaxel-induced mechanical allodynia in mice [2]

Viticis Fructus, dried fruits of *Vitex rotundifolia* L.f. or V. *trifolia* L., was selected to isolate **3** after comparison of the signal intensity of **3** in the methanol extracts of Plantaginis Semen, Viticis Fructus, and Scrophulariae Radix at the same amount. Large scale extraction of Viticis Fructus followed by diverse column chromatography, six iridoids (**1, 3**-**7**) and one phenolic compound (**8**) were isolated and identified by comparison of MS and NMR data with those in the literature (Fig. 1). Among them, pedicularis-lactone (**3**), viteoid I (**5**), viteoid II (**6**), and iridolactone (**4**) had the same molecular formula ($C_9H_{12}O_4$, MW: 184.2) and anti-allodynic activities of compounds **3, 5, 6** were evaluated in the mouse model of PTX-induced mechanical allodynia. Prior to activity evaluation, the purities of **3**, **5**, and **6** were estimated to be 67.15%, 92.12%, and 86.72%, respectively, by qHNMR (Fig. 3B-D).

Daily oral administration of **3** (10.1 mg/kg), **5** (13.8 mg/kg), and **6** (13.0 mg/kg) starting the day after PTX injection significantly inhibited PTX-induced allodynia when compared with the vehicle-treated group. The statistical significance of the anti-allodynic activities of **3**, **5**, and **6** were observed starting on day 3, 7, and 5 after PTX injection, respectively (Fig. 2D). And a significant anti-allodynia activity of **3**, **5**, and **6** was observed at a lower dose and with earlier onset than **1**. These results suggested that these three iridoids have more acute and stronger effect than **1**. Furthermore, pre-treatment with **3**, **5**, or **6** did not inhibit the PTX-enhanced expression of CHOP, a known endoplasmic reticulum (ER) stress marker in LY-PPB6. This result indicated that the underlying mechanisms of anti-allodynic activities of **3**, **5**, and **6** were different from **1**.

3 Quantification of four anti-allodynic iridoids in Viticis Fructus

Four iridoids **1**, **3**, **5**, and **6** showing anti-allodynic activity were obtained from Viticis Fructus

in present study. Investigation of quantitative levels of four anti-allodynic iridoids in Viticis Fructus was conducted by LC-UV/MS. The commercial **1** and isolated **3**, **5**, and **6** were used as standards and their purities were determined by qHNMR. After optimization of the sample preparation and chromatography conditions, ultrasonication-assisted methanol extracts were analyzed using reversed phase HPLC on a C30 column with a gradient elution of acetonitrile and water both with 0.1 % formic acid. The results showed that the contents of these four iridoids **1**, **3**, **5**, and **6** were obviously different between two botanical origins of Viticis Fructus, *Vitex trifolia* and *V. rotundifolia*. Moreover, preliminary comparison of contents between Viticis Fructus and Plantaginis Semen were carried out. The total content of these four anti-allodynic iridoids was much higher in Viticis Fructus than in Plantaginis Semen.

Conclusion

This study revealed that **1** was one of active compounds responsible for the anti-allodynic activity of Plantaginis Semen. Further confirmatory study on the activity of purified **3** which was isolated from Viticis Fructus supported the involvement of **3**, in addition to **1**, in the anti-allodynic activity of Plantaginis Semen. Moreover, two iridoids **5** and **6** from Viticis Fructus showed anti-allodynic activity. Furthermore, the quantification analysis indicated that the contents of these four iridoids **1**, **3**, **5**, and **6** were obviously different between two species of Viticis Fructus, and the much higher total contents were observed in Viticis Fructus than in Plantaginis Semen, suggesting a higher potential of Viticis Fructus in management of CIPN. This study suggested that four anti-allodynic iridoids **1**, **3**, **5**, and **6** could be promising drug lead compounds, and Viticis Fructus and Plantaginis Semen could be potential crude drug for treatment of CIPN.

Reference

1. Toume K, Hou Z, Yu H, Kato M, Maesaka M, Bai Y, Hanazawa S, Ge Y, Andoh T, Komatsu K. Search of anti-allodynic compounds from Plantaginis Semen, a crude drug ingredient of Kampo formula "Goshajinkigan". J Nat Med 2019; 73: 761-768
2. Yu H, Toume K, Kurokawa Y, Andoh T, Komatsu K. Iridoids isolated from Viticis Fructus inhibit paclitaxel-induced mechanical allodynia in mice. J Nat Med. (in press)

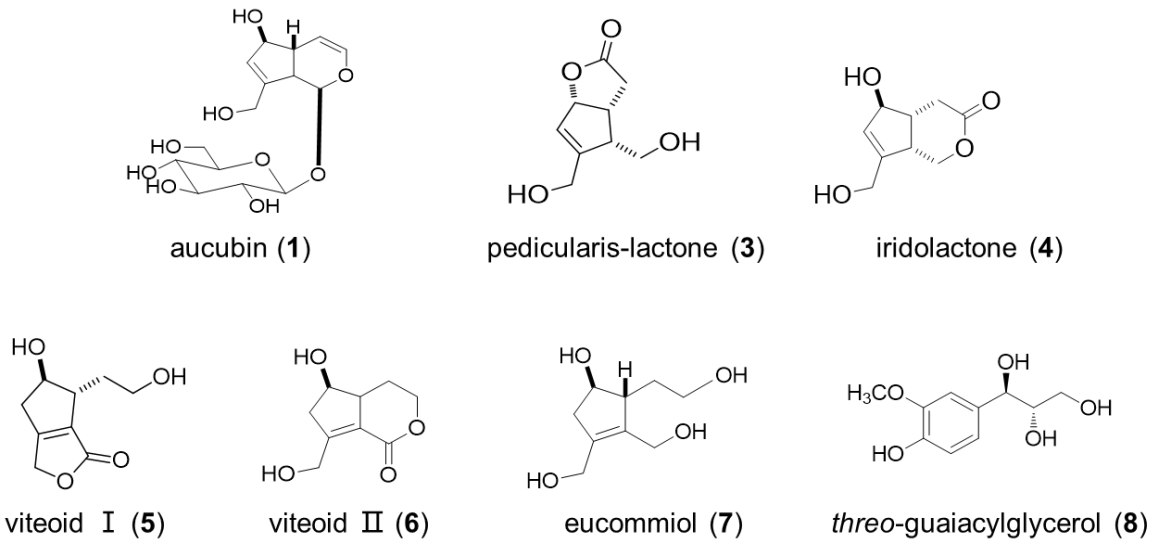

Figure 1 Chemical structures of isolated compounds

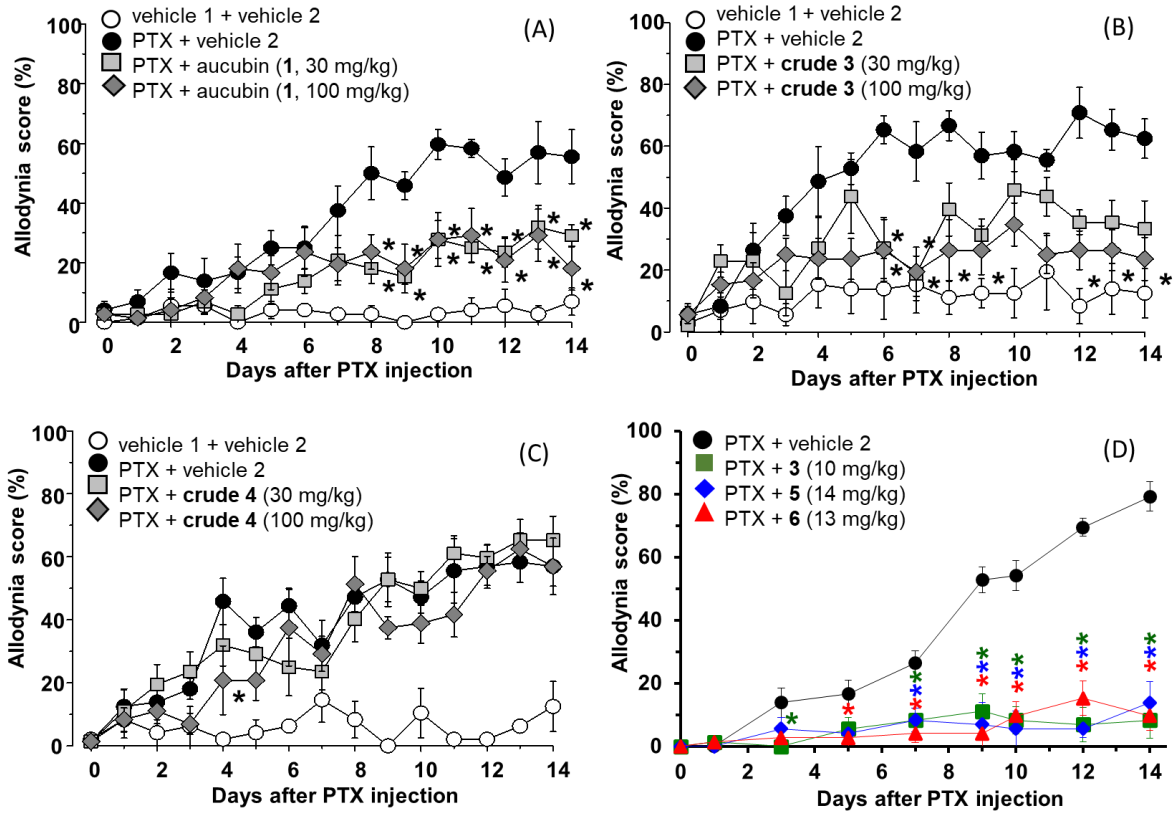

Figure 2 Effects of repeated oral administration of **1** (A), **crude 3** (B), **crude 4** (C) and purified **3**, **5** and **6** (D) or vehicle 2 on PTX-induced mechanical allodynia. PTX (5 mg/kg) or vehicle 1 (physiological saline containing 10% Cremophor EL® (Sigma) and 10% ethanol) was injected intraperitoneally in mice. Samples or vehicle 2 (5% gum arabic) were administered orally once daily starting the day after PTX injection. Data are presented as mean ± standard error of the mean (N = 4-6). *p< 0.05 vs. PTX + vehicle 2 (Bonferroni multiple comparisons)

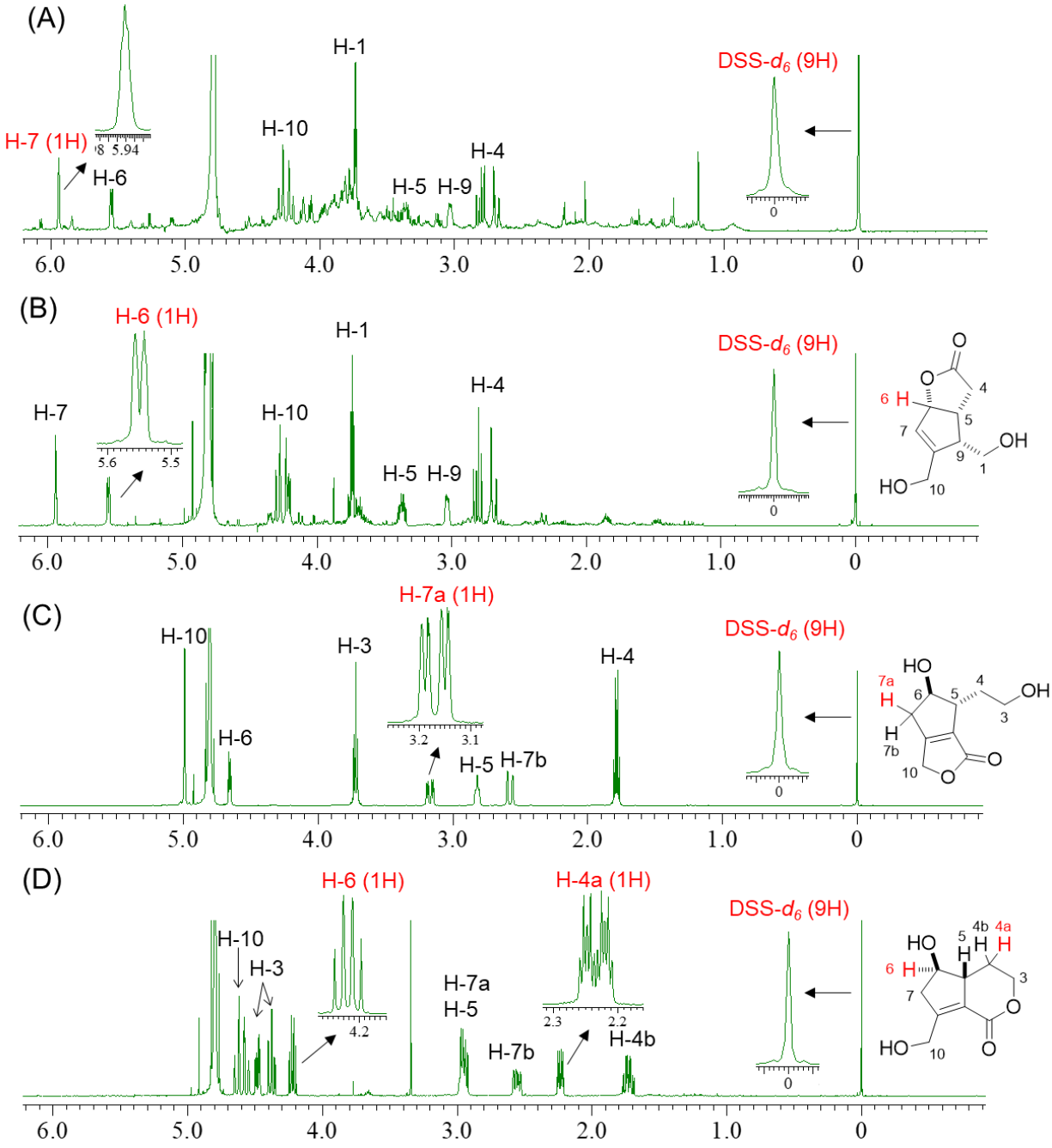

Figure 3 ^{1}H-NMR spectrum of **crude 3** (A), pedicularis-lactone (**3**, B), viteoid I (**5**, C), and viteoid II (**6**, D) in D_2O containing DSS-d_6. The red colored signals were chosen for the quantitative determination.