

The Purgative Action of Rhei Rhizoma Is Increased by Raphani Semen

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Abstract – Herbal medicinal interaction on the purgative action of Chungpesagan-tang, which is one of the traditional Korean medical prescriptions that has been most frequently used for stroke was investigated. Chungpesagan-tang had more potent purgative activity than Rhei Rhizoma alone. When each Chungpesagan-tang composing herbal medicine with Rhei Rhizoma was extracted with water, water extract of Raphani Semen with Rhei Rhizoma had the most potent purgative activity. However, Raphani Semen had not purgative activity. When Rhei Rhizoma with Raphani Semen was extracted, sennoside A content in the water extract was increased. The transformation of sennoside A of Rhei Rhizoma to rheinanthrone by human intestinal microflora was weakly induced by Raphani Semen. These results suggest that the purgative action of Chungpesagan-tang could be controlled by the addition and subtraction of Chungpesagan-tang-composing herbal medicines.

Key words – Purgative action, Chungpesagan-tang, Rhei Rhizoma, Raphani Semen, sennoside A.

Introduction

The herbal medicinal polyprescription is composed of several herbal medicines. This herbal medicinal polyprescription has been formulated according to the four regular components theory of Oriental medicines, which consisted of Monarch, Minister, Assistant and Laborer. However, this theory has not been elucidated by the scientific research method until now. If this theory could be understood through the experiment, biologically active polyprescription could be developed. To scientifically understand the fundamental formulation theory of traditional herbal medicinal polyprescription, we tried to investigate herbal medicinal interaction on the purgative action of Chungpesagan-tang, which have been frequently used for patients who suffer from stroke with constipation (Lee, 1996). Because this Chungpesagan-tang have been thought to have better purgative activity than Rhei Rhizoma or Yuldahanso-tang. This hypothesis was supported by our previous report that the purgative activity of Chungpesagan-tang was more potent than that of Rhei Rhizoma (Jeon *et al.*, 1999). The main purgative component in Rhei Rhizoma is a sennoside (Nonaka *et al.*, 1977; Oshino *et al.*, 1972; Oshino *et al.*, 1974; Oshino *et al.*, 1978).

This sennoside is a prodrug which is transformed into the active component, rheinanthrone, by intestinal bacteria (Kobashi *et al.*, 1980). Therefore, we investigated the synergistic effect of Chungpesagan-tang-composing herbal medicines, particularly Raphani Semen, on the purgative action of Rhei Rhizoma and on the metabolism of sennoside A of Rhei Rhizoma by human intestinal bacteria.

Materials and Methods

Materials – Rhei Rhizoma, Puerariae Radix, Scutellariae Radix, Angelicae Tenuissimae Radix, Platycodi Radix, Raphani Semen, Cimicifugae Rhizoma and Angelicae Dahuricae Radix were purchased from Heungin-Yakup Co., (Seoul, Korea). The botanical identity of these herbal medicines was verified and vouched at College of Pharmacy, Kyung Hee University (Korea). Culture media for human intestinal bacteria were purchased from Difco, Co. (U.S.A.). The other chemicals were of analytical reagent grade.

Extraction and sennoside A determination of polyprescriptions – Fifty grams of each Chungpesagan-tang-composing herbal medicine, were extracted with 450 ml of water in a boiling water bath, filtrated, concentrated and dried with a freezing dryer. The combination of each herbal medicine composing Chungpesagan-tang with Rhei Rhizoma were also

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extracted with water. Chungpesagan-tang (which is consisted of 16 g *Puerariae Radix*, 8 g *Scutellariae Radix*, 8 g *Angelicae Tenuissimae Radix*, 4 g *Platycodi Radix*, 4 g *Raphani Semen*, 4 g *Cimicifugae Rhizoma*, 4 g *Angelicae Dahuricae Radix* and 4 g *Rhei Rhizoma* per a package) was also extracted. All extracts were dried with a freezing dryer and used if necessary. One hundred milligrams of each dried extract of all polyprescriptions was dissolved and made to 10 ml with methanol. The quantity of sennoside A was assayed with HPLC. HPLC (Younglin system, Korea) was carried out as follows: column, μ -Bondapak C₁₈ 7.8×300 mm; solvent, 0.2 M phosphate buffer (pH 8.0)-acetonitrile (84:16); wavelength of detector, 280 nm.

Metabolism of sennoside A of each polyprescription by human intestinal bacteria – Human intestinal bacteria (1 g of fresh human feces) were suspended with 10 ml of the anaerobic dilution medium and centrifuged for 5 min at 500 rpm. The supernatant (1 ml) were mixed with the above polyprescription extracts (mg as sennoside A) and then incubated for 3 h at 37°C. Into 5 ml of the reaction mixture, 5 ml of methanol was added and the quantity of sennoside A was assayed by TLC scanner (Shimadzu CS-920, Japan).

Animals, treatment and sampling – Male mice (ICR 20–25 g) were purchased from Daehan Animal Co. (Korea), and maintained for two weeks before use and then kept in metabolic cages for the experiments: Pellet foods (Samyang Co., Korea) and water were freely available. All animal experiments carried out on 20–22°C and 50±10% humidity. *Rhei Rhizoma* (25 mg/25 g mouse) or its containing polyprescriptions (25 mg/25 g mouse as *Rhei Rhizoma*) were orally administered to 10 mice, and saline to 10 mice as the control. Fresh feces were compulsively obtained just before and at 2, 3.5, 4.5 and 6 h after the administration, and their moisture content (%) was determined according to the following formula.

Moisture content (%) = [(fresh feces weight-dry feces weight) / fresh feces weight] × 100

Results and Discussion

Effect of herbal medicines on the purgative action of *Rhei Rhizoma* – The water extracts of Chungpesagan-tang or Yuldahanso-tang were administered into mice and their purgative activities

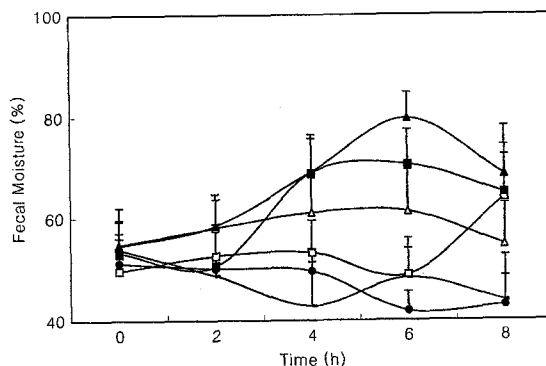


Fig. 1. The purgative action of *Rhei Rhizoma* and Chungpesagan-tang. —, treated with saline; ●, 150 mg Yuldahanso-tang; □, 12.5 mg *Rhei Rhizoma*; ■, 25 mg *Rhei Rhizoma*; △, 12.5 mg Chungpesagan-tang as *Rhei Rhizoma*; ▲, 25 mg Chungpesagan-tang as *Rhei Rhizoma*.

were measured (Fig. 1). The purgative action of Chungpesagan-tang was more potent than that of *Rhei Rhizoma*. However, Yuldahanso-tang did not have the purgative action. These results suggest that the purgative action of *Rhei Rhizoma* could be increased by Yuldahanso-tang, because Chungpesagan-tang is consisted of Yuldahanso-tang and *Rhei Rhizoma*.

The water extract of each herbal medicine, which is the constituent herbal medicine of Chungpesagan-tang, combined with *Rhei Rhizoma* was administered into mice and the purgative action was measured (Table 1). Fecal moisture of normal control group

Table 1. Effect of some herbal medicines on the purgative action of *Rhei Rhizoma*

Prescription ^{a)}	Moisture (%)
Control	56.0±2.6
Rh	67.3±6.7 ^b
Rh : Pu (1:1)	66.0±4.0 ^b
Rh : Sc (1:1)	68.0±7.0 ^b
Rh : At (1:1)	74.7±2.5 ^{b,c}
Rh : Ra (1:1)	75.7±1.2 ^{b,c}
Rh : Ci (1:1)	70.7±4.2 ^b
Rh : Pl (1:1)	66.7±4.2 ^b
Rh : Ad (1:1)	63.01±5.3 ^b
Chungpesagan-tang	71.0±6.6 ^b
Yuldahanso-tang	54.6±4.2

^{a)}Rh, *Rhei Rhizoma*; Pu, *Puerariae Radix*; Sc, *Scutellariae Radix*; At, *Angelicae Tenuissimae Radix*; Ra, *Raphani Semen*; Pl, *Platycodi Rhizoma*; Ci, *Cimicifugae Radix*; Ad, *Angelicae Dahuricae Rhizoma*; Chung, Chungpesagan-tang.

^{b)}Significantly different from that of control group (n = 10).

^{c)}Significantly different from that of Rh group (n = 10).

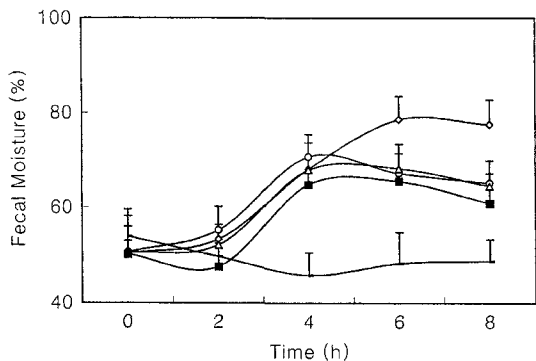


Fig. 2. Effect of Rhapsani Semen on the purgative action of Rhei Rhizoma. —, treated with saline; ■, 25 mg Rhei Rhizoma; ○, water extract of 25 mg Rhei Rhizoma combined with 12.5 mg Raphani Rhizoma; ◇, water extract of 25 mg Rhei Rhizoma combined with 25 mg Raphani Rhizoma; △, water extract of 25 mg Rhei Rhizoma combined with 50 mg Raphani Rhizoma.

was $56.0 \pm 2.6\%$. When water extract of Rhei Rhizoma alone was administered on mice, the fecal moisture was $67.3 \pm 6.7\%$ at 3.5-6 h after its administration. Water extract of Raphani Semen, *Angelicae Tenuissimae Radix* or *Cimicifugae Rhizoma* with Rhei Rhizoma increased the fecal moisture. Particularly, Raphani Semen potently increased the purgative action of Rhei Rhizoma, although Raphani Semen alone displayed no purgative action. This synergism was dependent on the dose of Raphani Semen (Fig. 2). The purgative action was best, when Rhei Rhizoma was extracted with the same ratio of Raphani Semen. However, *Angelicae Dahuricae Radix* and *Puerariae Radix* inhibited the purgative action of Rhei Rhizoma. The other herbal medicines, *Platycodi Radix* and *Scutellariae Radix* did not affect the purgative action of Rhei Rhizoma.

Sennoside A content in water extracts of Rhei Rhizoma combined with Raphani Semen and its metabolism by human intestinal bacteria – To understand why the purgative action of Rhei Rhizoma was induced by herbal medicines such as Raphani semen, sennoside A content of water extract of each polyprescription were determined (Table 2). Sennoside A content in water extract of Rhei Rhizoma was 3.0 ± 0.22 mg per a gram of dried Rhei Rhizoma. However, its content in water extract of Chungpesagan-tang was 0.41 ± 0.28 mg per a gram of dried Rhei Rhizoma. Sennoside A content in water extract of Raphani Semen combined with Rhei Rhizoma was increased according to the increment of Raphani

Table 2. Sennoside A content of water extract of Rhei Rhizoma and Rhei Rhizoma-containing polyprescriptions

Polyprescription	Content (%)
Rh ^{a)}	0.30 ± 0.04
Chungpesagan-tang	0.41 ± 0.03^b
Yuldahanso-tang	0
Rh:Ra (1:0.5)	0.43 ± 0.04^b
Rh:Ra (1:1)	0.50 ± 0.05^b
Rh:Ra (1:2)	0.35 ± 0.05^b

^{a)}Rh, Rhei Rhizoma; Ra, Raphani Semen.

^{b)}Significantly different from that of Rh.

Semen. Sennoside A is a genuine purgative compound in Rhei Rhizoma. Therefore, these results suggest that Raphani Semen could induce the purgative action, because Raphani Semen increased the extract level of sennoside A from Rhei Rhizoma. Furthermore, this result suggested that the purgative action of Chungpesagan-tang was more potent than that of Rhei Rhizoma alone. This synergism of sennoside A extraction level from Rhei Rhizoma by Raphani Semen could affect the purgative action of Chungpesagan-tang.

Sennoside A is a prodrug which can be activated to rheinanthrone, a genuine purgative component, by human intestinal bacteria. Therefore, to evaluate herbal drug interaction and the purgative action of Rhei Rhizoma in Chungpesagan-tang, the activity

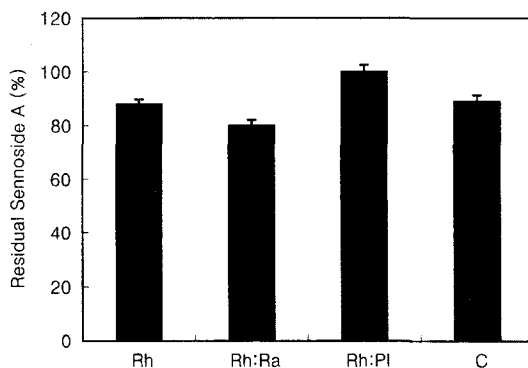


Fig. 3. The activity transforming sennoside A of water extract of Rhei Rhizoma combined with and Chungpesagan-tang to rheinanthrone by human intestinal bacteria. Water extracts of Rhei Rhizoma and its containing polyprescriptions, which contained 5 mg sennoside A, were incubated with human intestinal bacteria for 3 h at 37°C and determined sennoside A. Rh, water extract of Rhei Rhizoma; Rh:Ra, water extract of 5 g Rhei Rhizoma combined with 5 g Raphani Rhizoma; Rh:Pl, water extract of 5 g Rhei Rhizoma combined with 5 g *Platycodi Radix*; C, Chungpesagan-tang.

transforming sennoside A in each polyprescription to rheinanthrone was measured (Fig. 3). When Rhei Rhizoma was incubated with human intestinal bacteria, 11% sennoside A in Rhei Rhizoma was transformed to rheinanthrone within 3 h. This activity transforming sennoside A to rheinanthrone was induced two-fold by Raphani Semen. The transforming activity of sennoside A in Chungpesagan-tang to rheinanthrone was similar to that of Rhei Rhizoma alone. These results suggested that Raphani Semen could induce the purgative action of Rhei Rhizoma, because Raphani Semen increased the transforming activity of sennoside A of Rhei Rhizoma to rheinanthrone as well as the extraction level of sennoside A from Rhei Rhizoma. Furthermore, the transforming activity and extraction efficacy of sennoside A from Rhei Rhizoma could be affected by the other herbal medicines. These results suggest that, by the addition and subtraction of Chungpesagan-tang-composing herbal medicines, the sennoside A extract level could not only be controlled, but the potency of the purgative action could be also controlled.

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