Effect of Oral Treatment of Perilla frutescens and Its Constituents on Type-I Allergy in Mice

Toshiaki MAKINO,*a Yasuyuki FURUTA,a Hajime FUJI,b Takashi NAKAGAWA,b Hannosuke WAKUSHIMA,a Ken-ichi SAITO,a and Yoshihiro KANOa

Department of Kampo Medicinal Sciences, Hokkaido College of Pharmacy,a Katsuraraoka, Otaru 047–0264, Japan and Research and Development Division, Amino Up Chemical Co., Ltd.,b Sapporo 004–0839, Japan.

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Perilla frutescens BRITTON (perilla, Labiatae) is a medicinal herb prescribed in Saiboku-to (柴朴湯), which is a Kampo formula effective for allergic diseases such as bronchial asthma. The present study was conducted to evaluate the anti-allergic effect of orally administered perilla decoction and to identify the active constituents using mice ear-passive cutaneous anaphylaxis (PCA)-reaction, which is one of the animal models for type I allergy. Perilla decoction significantly suppressed PCA-reaction, and the inhibition % at the dose of 500 mg/kg was 43%. The perilla decoction contains 5.3% of luteolin 7-0-[β-glucuronosyl(2→1)β-glucuronide], 1.6% of apigenin 7-0-[β-glucuronosyl(2→1)β-glucuronide], 0.49% of scutellarin, and 2.5% of rosmarinic acid (weight of compound/dried weight of perilla decoction %), respectively. When these constituents were orally administered to the mice at the dose equivalent to 500 mg/kg of perilla decoction, rosmarinic acid and apigenin 7-0-[β-glucuronosyl(2→1)β-glucuronide] significantly suppressed PCA-reaction, and their inhibition % was 41% (p<0.01) and 32% (p<0.05), respectively. Since the inhibition % or perilla decoction and rosmarinic acid were nearly equal, the anti-allergic effect of perilla decoction depends primarily on rosmarinic acid. The standard Saiboku-to decoction contained 0.013% of rosmarinic acid, which was too low to exhibit anti-allergic activity in a daily dose of Saiboku-to in adults, suggesting that perilla would be prescribed in Saiboku-to to exhibit other pharmacological effects than its anti-allergic activity, such as a sedative.

Key words Perilla frutescens; allergy; rosmarinic acid; apigenin-diglucuronide; Saiboku-to; Labiatae

Industrial advances have brought about chemical pollution in our surroundings, and patients suffering from allergic diseases such as bronchial asthma and atopic dermatitis are heavily affected. Allergies are now the most prevalent and rapidly increasing chronic health problem in industrialized countries. The treatment of allergic diseases is based mainly on allergen avoidance and the use of anti-histamines, steroids, or immunosuppressants. Japanese traditional herbal medicine (Kampo medicine) has recently been advanced for the treatment of allergic diseases, and its effectiveness has received increasing attention.1) One Kampo formula, Saiboku-to (柴朴湯) is clinically used to treat bronchial asthma, and its effectiveness is well documented.2–5) Saiboku-to consists of ten herbs, and the Perilla Herb is believed to be one of the components active in the anti-allergic activity of this formula.6)

The Perilla Herb is dried leaves of Perilla frutescens BRITTON (perilla, Labiatae), and it is reported to have pharmacological activity as a sedative,7) for indigestion,8) or food poisoning.9) Previous in vivo studies showed that intraperitoneal injection of perilla decoction suppressed antigen-specific IgE production in mice,10) histamine-release from peritoneal mast cells in rats11) and induced Th1-type cytokines in mice,12) suggesting that perilla has anti-allergic activities. However, since herbal medicines are usually administered orally, the anti-allergic effects of perilla and its active constituents have not yet been completely demonstrated. In our previous study, we identified 4 phenolic compounds: luteolin 7-0-[β-glucuronosyl(2→1)β-glucuronide], apigenin 7-0-[β-glucuronosyl(2→1)β-glucuronide], scutellarin, and rosmarinic acid, from perilla decoction as the active constituents suppressing the proliferation of cultured murine mesangial cells, and demonstrated that only rosmarinic acid represents the anti-proliferative effect of perilla decoction.13) The present study was conducted to evaluate the anti-allergic effect of orally administered perilla decoction and to identify the contribution of the 4 previously isolated phenolic compounds to the effect of original perilla decoction using passive cutaneous anaphylaxis (PCA)-reaction induced in the ears of mice, which is one of the animal models for type I allergy.14) We also measured the content of an active constituent in Saiboku-to to discuss the contribution of perilla or its constituents to the anti-allergic activity of Saiboku-to.

MATERIALS AND METHODS

Materials Herbal medicines used in this study all conformed to Japanese Pharmacopoeia XIII and were purchased from Tochimoto Tenkaido (Osaka, Japan). Ovalbumin (OVA) was purchased from Seikagaku Corporation (Tokyo, Japan). Tranilast was generously supplied by Kissei Pharmaceutical Co., Ltd. (Tokyo). Rosmarinic acid was bought from Extrasythése (Genay, France).

Preparation of the Decoctions Perilla decoction was prepared by boiling 200 g of Perilla Herb in 4 l of distilled water for 1 h (dried weight, 40 g). A standard decoction of Saiboku-to was prepared by boiling 10 herbal medicine mixtures (3.0 g of Magnolia Bark, 2.0 g of Perilla Herb, 7.0 g of Bupleum Root, 5.0 g of Pinellia Tubers, 1.0 g of Ginger, 3.0 g of Scutellaria Root, 3.0 g of Ginseng, 3.0 g of Jujube, 2.0 g of Glycyrhriza and 5.0 g of Poria Sclerotium) in 680 ml distilled water for 1 h (dried weight, 7.0 g; extract ratio: 21%).

Isolation of Phenolic Constituents Leaves of P. frutescens forma viridis were harvested in the experimental garden of Amino-Up Chemical Co., Ltd. (Sapporo, Japan) in August, 2000, and 300 g of the dried leaves were boiled in 6 l
of water for 1 h to yield perilla extract (dried weight, 96 g). This extract was applied to a Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) column (4.0 × 30 cm) and eluted successively by H₂O and MeOH. The H₂O-eluate was further applied to a Sephadex LH-20 (Pharmacia, Buckinghamshire, U.K.) column eluted with the solvent of decreasing polarity from H₂O to MeOH. The H₂O/MeOH 70 : 30 eluate was further applied to MCI gel CHP-20 with the same solvent, and then to Capsel-gel-ODS (2.5 × 20 cm, Shiseido) eluted with H₂O/acetonirole 95 : 5 to give lutelenin 7-Ο-β-D-glucuronosyl(2→1)β-D-glucuronic acid (1) and apigenin 7-Ο-β-D-glucuronosyl(2→1)β-D-glucuronic acid (2). The H₂O/MeOH 50 : 50-eluate of the Diaion HP-20 column was applied to Sephadex LH-20 eluted with H₂O/ MeOH 70 : 30, then to MCI gel CHP-20 with the same solvent to yield scutellarin (3). The Rf values of TLC (Merck Kiesel gel F₂₅₄, n-BuOH/MeOH/H₂O 7 : 1 : 2) of 1—3 were 0.1, 0.15, and 0.3, respectively. Compounds 1—3 were identified based on their 1H-, 13C-NMR and FAB-MS data.13 and were collected and subjected to the experiment with repeated chromatography. 

Quantitation of Four Phenolic Compounds in Perilla Decoction 
Perilla decoction (dried weight, 12.5 μg) was applied to HPLC (YMC J’sphere ODS-H80, 150×4.6 mm I.D. YMC, Tokyo; mobile phase, H₂O/MeOH/AcOH 65 : 35 : 0.1, 0—30 min linear gradient; flow rate, 1 ml/min; temperature, 40 °C; detection, UV absorption at 328 nm) to give peaks of 1 (tₜ 19.3 min), 2 (tₜ 11.0 min), 3 (tₜ 15.9 min), and rosmarinic acid (tₜ 25.3 min). Each compound was applied to the column, and the calibration lines were made from their peak areas. Quantifiable ranges of these compounds were 0.2—2 μg of 1, 0.1—1 μg of 2, 0.04—0.4 μg of 3, 0.1—1 μg of rosmarinic acid. Data were expressed with mean percentages (weight of the compounds/dried weight of perilla decoction %) in two different quantitative analyses.

Quantitation of Rosmarinic Acid in Saiboku-to 
Standard decoction of Saiboku-to (dried weight, 500 μg) and marketing extract-granules of Saiboku-to (Matsusuka Industries, Nagoya, Japan) (500 μg) were applied to HPLC (YMC J’sphere ODS-H80, 150×4.6 mm I.D.): mobile phase, H₂O/MeOH/AcOH 60 : 30 : 0.1→45 : 55 : 0.1, 0—30 min linear gradient; flow rate, 1 ml/min; temperature, 40 °C; detection, UV absorption at 328 nm) to give a peak of rosmarinic acid (tₜ 22.8 min). Rosmarinic acid was applied to the column, and the calibration line (25—250 ng) was made from its peak area. Data was expressed with mean percentages (weight of rosmarinic acid/dried weight of Saiboku-to decoction %) from two different quantitative analyses.

Preparation for Anti-OVA Serum 
The procedure employed was a slight modification of the method of Inagaki et al.5) Ovalbumin was suspended in 20 mg/ml Al(OH)₃ solution (final concentration of OVA, 10 μg/ml), and 0.2 ml of this suspension (OVA, 2 μg) was intraperitoneally injected into 4-week-old female Balb/c mice (Japan SLC, Hamamatsu, Japan) 5 times at 2 week intervals. Ten days after the 5th injection of OVA, whole blood was collected to obtain anti-OVA serum. After 48 h, OVA (10 mg/kg body weight) in saline containing 0.5% Evansblue (Merck, Dermstadt, Germany) was intravenously injected to induce the PCA-reaction. Perilla decoction and its constituents were orally administered to mice 30 min before antigen-challenge. As a positive control, tranilast was used at a dose of 500 mg/kg. Thirty minutes after antigen-challenge, mice were sacrificed and their ears were collected. The ears were dissolved in 300 μl of 1 N KOH and Evansblue was extracted with 700 μl of acetone/10 N phosphoric acid 67 : 3. The concentration of Evansblue was measured colorimetrically at 620 nm. Data were expressed as amount of Evansblue per ears and as inhibition %, which was calculated using the following equation:

\[
\text{inhibition\%} = \left( \frac{\text{dye amount of sample – treated group} - \text{dye amount of background}}{\text{dye amount of control} - \text{dye amount of background}} \right) \times 100
\]

Statistical Analysis  Data were expressed as mean±S.E. One-way analysis of variance (ANOVA) followed by a multiple comparison procedure according to Fisher’s protected least significant difference (PLSD) was employed to evaluate the effect of perilla decoction. Student’s t-test was employed for comparison between the control and compound-treated groups. A difference of p<0.05 was considered significant.

RESULTS AND DISCUSSION

Perilla Herb (200 g) was extracted in boiling water according to the standard formation process of Kampo decoctions to yield perilla decoction (dried weight, 40 g; extract ratio, 20%), and then the suppressive effect of the decoction on mice ear-PCA-reaction was evaluated. Compared with the background group, cutaneous injection of anti-OVA serum induced prominent enhancement of vascular permeability as exhibited by dye elusion into the ears in the control mice; this was significantly, but not dose-dependently, suppressed by oral treatment of perilla decoction, and the maximum suppressive effect was observed at the dose of 500 mg/kg (inhibition %, 43%, p<0.01, Fig. 1). Kampo medicine sometimes exhibits anti-dose-dependent pharmacological effects, since it contains several constituents and its entire pharmacological effect is explained as the sum of each constituent. In the fol-
were represented as mean ± SE (n = 7). *p < 0.05, **p < 0.01.

following experiment, the doses of perilla extract and its constituents were determined to be equivalent to 500 mg/kg of perilla extract, and the isolation of the active constituents in perilla decoction was attempted.

In our previous study, we identified 4 phenolic compounds; luteolin 7-O-β-glucuronosyl(2→1)β-glucuronide (1), apigenin 7-O-β-glucuronosyl(2→1)β-glucuronide (2), scutellaran (3), and rosmarinic acid, from perilla decoction as the active constituents suppressing the proliferation of cultured murine mesangial cells (Fig. 2).13) The quantitative analysis of these phenolic compounds in the perilla decoction revealed that their contents were 5.3% of luteolin, 1.6% of apigenin, 0.49% of scutellaran, and 2.5% of rosmarinic acid, respectively. Then, the contribution of these compounds to the anti-allergic effect of perilla decoction was evaluated. Figure 3 shows the effect of oral treatment of perilla decoction (500 mg/kg) and the relative amount of each constituent (27 mg/kg of 1, 8.0 mg/kg of 2, 2.7 mg/kg of 3, and 13 mg/kg of rosmarinic acid) on PCA reaction induced in the ears of mice. Compound 2 and rosmarinic acid significantly suppressed PCA-reaction, and the inhibition % was 32% (p<0.05) and 41% (p<0.01), respectively. Since the inhibition % of perilla decoction and rosmarinic acid were nearly equal, it is proposed that rosmarinic acid was the representative anti-allergic constituent in the decoction, and compound 2 partly contributed to the decoc-

tion's effect. The combination of these compounds may explain the anti-dose-dependent effect of perilla decoction. Inhibition % of rosmarinic acid at the dose of 13 mg/kg (41%) was comparable to that of tranilast at the dose of 500 mg/kg (50%), indicating that the anti-allergic titer of rosmarinic acid was much higher than tranilast. As the anti-allergic constituent of perilla decoction, glycoprotein (ca. 6.0 kDa), which suppressed histamine-release from cultured mast cells, was isolated from perilla aqueous extract.16) However, when perilla decoction was administered orally, such macromolecular compounds would not contribute to the anti-allergic effect of perilla decoction, since they were hardly absorbed from the gastrointestinal tract into the circulation.

Saiboku-to is a well-known Kampo formula used for allergic bronchial asthma, and Perilla Herb is believed to be one of the active herbs in the formula.9) We therefore sought to evaluate whether rosmarinic acid could be identified as the anti-allergic constituent of Saiboku-to. Figure 4 depicts a HPLC-profile of the Saiboku-to standard decoction prepared in our laboratory. Quantitative analysis revealed that the Saiboku-to usually have restlessness, fear, and a tendency toward depression,12) Perilla Herb may be prescribed as part of Saiboku-to in order to exhibit its sedative activity.8) In conclusion, oral treatment of perilla decoction suppresses allergic reaction in mice, and the active constituents are rosmarinic acid and apigenin-diglucuronide. The anti-allergic titer of rosmarinic acid is higher than tranilast, and rosmarinic acid is a promising compound to prevent or treat al-

Fig. 3. Suppressive Effect of Perilla Decoction and Its Constituents on PCA-Reaction Induced in the Ears of Mice

Doses were equivalent to 500 mg/kg of perilla extract: luteolin 7-O-β-glucuronosyl(2→1)β-glucuronide (1), 27 mg/kg; apigenin 7-O-β-glucuronosyl(2→1)β-glucuronide (2), 8.0 mg/kg; scutellaran (3), 2.7 mg/kg; rosmarinic acid (4), 13 mg/kg. Data were represented as mean ± S.E. (n = 7). *p < 0.05, **p < 0.01.
Allergic diseases.

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