Study on Anti-Oketsu Activity of Rhabarb II. Anti-allergic Effects of Stilbene Components from Rhei undulati Rhizoma (Dried Rhizome of Rheum undulatum Cultivated in Korea)

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Methanol extract (RM-ext) obtained from the dried rhizome of Rheum undulatum was screened for activity in experimental models of type I allergy. RM-ext exhibited the inhibition on 48-h homologous passive cutaneous anaphylaxis (PCA) in rats and an antigen-induced histamine release from rat peritoneal mast cells. Among nine stilbenes isolated from RM-ext, seven inhibited the histamine release. Rhapontigenin (compound 1), piceatannol (2) and piceatannol 3'-β-D-glycoside (6) with oral administration showed the inhibition on PCA. Compounds 1 and 2 exhibited the inhibitory effect on sheep red blood cell-induced delayed-type hyper sensitivity (SRBC-DTH) of type IV allergic model. These results indicated that the rhizome of Rheum undulatum inhibits the allergic reactions and that these inhibitory effects may be partially attributable to the stilbenes mentioned above.

Key words Rheum undulatum; anti-allergic activity; stilbene component; rhaponticin

Rhubarb varieties have been very commonly used as a purgative since the early days of Western medicine. According to ancient Chinese herbal literature, rhubarb is stated to have been used as an oral haemostatic agent for the treatment of hematemesis, melena and metrorrhagia, and also for pains of the legs and waist, which are believed to be caused by Oketsu symptoms closely related to disseminated intravascular coagulation (DIC), chronic inflammation and allergic disease. In present Chinese medicine, it is largely employed as a purgative and is also applied as a haemostatic agent. Pharmacological studies have reported its cathartic effects,2,3) with a decreasing effect on the urea and nitrogen levels in blood,4,5) as well as an anti-herpes effect.6) However, the effect of Japanese Pharmacopoeia (JP) rhubarbs originated in Rheum officinale, R. palmatum and R. coreanum against Oketsu symptoms have not been investigated.7) A Kampo doctor in the Edo era used rhubarb originated from R. undulatum to contract Oketsu symptoms.8) In a previous paper, we found that the extract from Rhei undulati Rhizoma (rhizome of R. undulatum L. cultivated in Korea) has been screened for the activities against type I and IV allergic and inflammatory reactions.9) This study was designed to identify the anti-allergic components in methanol extract from the rhizome.

MATERIALS AND METHODS

Plant Material  Rhei undulati Rhizoma originated from the rhizome of R. undulatum L. cultivated in Korea was used in this study. The plant materials were identified by Dr. Ilhyuck Kim of Chuang Ang University, Korea. Voucher specimens were deposited in Kinki University, Osaka, Japan.

Extraction of the Rhizome  The dried rhizome of R. undulatum (5.8 kg) was extracted with methanol at room temperature (24 h, ×3). The methanol extract (RM-ext) was evaporated to dryness (yield; 33.8%).

Isolation of Stilbene Components from the Rhizome  RM-ext was subjected to Diaion HP-20 column chromatography (H2O→MeOH→acetone) to give the H2O-eluted fraction (13.3%), MeOH-eluted fraction (18.7%), and acetone-eluted fraction (1.8%). The MeOH-eluted fraction was subjected to silica gel column chromatography [CHCl3 : MeOH (10:1, v/v)→(4:1)→CHCl3 ; MeOH : H2O (10:3:1, lower layer)→MeOH] to give five fractions (frs. 1—5). Each fraction was subjected to ODS column chromatography (MeOH–H2O) and finally to HPLC [YMC-pack R&D ODS-5-A, 250×20 mm i.d., MeOH–H2O or CH3CN–H2O] to give rhapontigenin (compound 1, 0.58%), piceatannol (2, 0.073%), desoxyrhapontigenin (3, 0.015%), resveratrol (4, 0.048%), rhaponticin (5, 3.3%), piceatannol 3'-O-β-D-glycoside (6, 2.0%), desoxyrhaponticin (7, 0.048%), rhaponticin 2'-O-gallate (8, 0.21%), and rhaponticin 6'-O-gallate (9, 0.087%).

Animals  Slc: Male Wistar strain rats (180—200 g) and female ICR strain mice (25—28 g) were used. They were maintained in an air-conditioned room with light from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. A laboratory pellet chow (Labo MR Stock, Nihon Nosan Kogyo) and water were given freely.

Drugs  The following drugs were used in this study: aluminum hydroxide gel (Maruishi), egg albumin (EWA,
Sheep Red Blood Cell-Induced Delayed-type Hypersensitivity (SRBC-DTH, Type IV Allergic Model)  The test was carried out using peritoneal mast cells which were obtained from the peritoneal cavity according to the modified method of Kettman. Fourteen days later, they were anesthetized with pentobarbital (44.2 mg/kg, i.p.), blood was withdrawn from the carotid arteries and rat anti-EWA serum was obtained. Serum was stored at -80 °C until use. The anti-EWA IgE antibody (1:32) was determined by PCA in Wistar rats. The PCA titer was expressed as the highest dilution causing a lesion more than 5 mm in diameter. The antisera diluted 8-fold with saline was injected into 2 sites on the shaved dorsal skin of male Wistar strain rats intradermally in a 0.05 ml dose. Forty-eight hours after sensitization, the rats were challenged with 0.5 ml of saline containing 2 mg of EWA and 5 mg of Evans blue via the tail vein. Thirty min later, they were sacrificed, the dorsal skin was removed for measurement of the blue area, the amount of leaked dye was then determined colorimetrically after incubation with 1.0N KOH, followed by extraction with a mixture of acetone and phosphoric acid. The dye concentration was measured spectrophotometrically at 620 nm. The dye leakage was calculated from the dye concentration and the area. The results were determined as a percentage of dye leakage induced by PCA, but compound 5 showed a weak inhibition (86.6, 40.0 and 30.8%, previously), but frs. 1, 2, and 4 did not inhibit. Compounds 2, 4, and 5 at a concentration of 100 µg/ml showed 16.4±0.4% inhibition against the inhibition on histamine release from mast cells. The bioassay-guided fractionation for RM-ext was examined against the inhibition on histamine release from mast cells. Fractions 2, 4, and 5 at a concentration of 100 µg/ml showed the inhibition (86.6, 40.0 and 30.8%, previously), but frs. 1 and 3 did not inhibit. Compounds 1, 2, 4, 5, 6, 8, and 9 isolated from frs. 2 or 4 exhibited the activity. The bioassay-guided fractionation for RM-ext was examined against the inhibition on histamine release from mast cells. Fractions 2, 4, and 5 at a concentration of 100 µg/ml showed the inhibition (86.6, 40.0 and 30.8%, previously), but frs. 1 and 3 did not inhibit. Compounds 1, 2, 4, 5, 6, 8, and 9 isolated from frs. 2 or 4 exhibited the activity.

RESULTS

Effect of RM-ext on 48-h Homologous PCA  As shown in Fig. 2, the total dye amount which leaked into the skin was 16.7±3.3 µg/site in the control group. When RM-ext (500 mg/kg, p.o.) was administered to rats, the dye leakage was significantly inhibited. A control agent, DSCG (2 mg/kg, i.v.), also inhibited this leakage.

Effects of RM-ext and Stilbenes on Histamine Release from Mast Cells  Total histamine contents in mast cells were 16.4±0.4 ng/ml. As shown in Table 1, the control value for antigen-induced histamine release was 42.0±0.8% and spontaneous release was 4.1±1.3%. Treatment with RM-ext (10, 50 or 200 µg/ml) and the positive control agent, DSCG (200 µg/ml), significantly suppressed the histamine release. As shown in Table 1, the control value for antigen-induced histamine release was 42.0±0.8% and spontaneous release was 4.1±1.3%. Treatment with RM-ext (10, 50 or 200 µg/ml) and the positive control agent, DSCG (200 µg/ml), significantly suppressed the histamine release.

Fig. 2. Effects of Methanol Extract from Rhei undulati Rhizoma (RM-ext) and Disodium Cromoglycate (DSCG) on 48-h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats

RM-ext suspended with 0.2% CMC·Na was orally administered to rats mediated by the rat anti-EWA serum 1 h before the challenge with antigen. DSCG dissolved in saline was intravenously administered 1 min before the challenge. Control was orally administered 0.2% CMC·Na or intravenously administered saline alone. Each value represents the mean±S.E. of 7—8 rats. Significantly different from the control group, *p<0.05; **p<0.01.

Statistical Analysis  The experimental data were tested for statistically significant differences by the Bonferroni/Dunn test.

Effect of Four Stilbenes on 48-h Homologous PCA  As shown in Fig. 3, compounds 1, 2, and 6 inhibited the dye leakage induced by PCA, but compound 5 was weak.

Effects of Four Stilbenes on SRBC-DTH  Effects of four stilbenes on the effector phase of SRBC-DTH are shown in Fig. 4. Compounds 1 and 2 (100 mg/kg, p.o., respectively) had significant inhibition on the footpad swelling 24 h after the challenge, but compounds 5 and 6 were ineffective.
caused by chemical mediators such as histamine and leukotrienes released from mast cells and bathophils by an IgE-related mechanism, the effect of RM-ext was examined on histamine release from mast cells induced by IgE antibody. As predicted, RM-ext inhibited histamine release from mast cells. Pursuit of the active components was done by monitoring the inhibitory effect of the histamine release. The inhibitory effects were observed on the fraction containing nine kinds of stilbene compounds, among those isolated, compounds 1, 2, 4, 5, 6, 8, and 9 showed the inhibitory effects. Inamori et al. reported that compound 2 isolated from Cassia garrettia inhibited histamine release from human bathophils and mast cells of the rat peritoneal cavity.16,17)

Compounds 1, 5, 6, and 2 (aglycone of compound 6) are the main components in the fraction. Compounds 1, 6, and 2, when given per os to rats at a dosage of 20—100 mg/kg, inhibited 48-h PCA reaction, but the effect of compound 5 was weak despite the strong inhibitory effect in \textit{in vitro} models. It is thought that compound 5 is hard for the digestive tract into the interior of the body. Compounds 1 and 2 exhibited the inhibitory effect on paw edema induced by SRBC-DTH reaction in mice which are classified as type IV allergic reaction.

It is anticipated that certain anti-allergic effects of this rhubarb consisting of dried rhizome of \textit{Rheum undulatum} are due to stilbene compounds.

Rhubarb has been commonly used worldwide as a purgative medicine from ancient times while in the traditional Chinese system, it has also been used as a therapeutic medicine of Oketsu (\textit{おくせつ} syndrome). Pharmacological studies of rhubarb on Oketsu syndrome are seldom found, although its purgative activities have been rather extensively reported.

JP Rhubarbs originated in \textit{R. officinale}, \textit{R. palmatum} and \textit{R. coreanum} are wellknown to contain a purgative component, sennosides in quantity and to possess a clear purgative effect. These JP rhubarbs are also obvious to be free from stilbene compounds and ineffective on an anti-allergic activities, and had no the inhibitory effects on endotoxin-induced DIC and on the inhibition of blood platelet aggregation.8)

It was proved that in rhubarb consisting of dried rhizome of \textit{R. undulatum} has anti-allergic activity and its effects are

## Table 1. Inhibitory Effects of Methanol Extract from \textit{Rheum undulatum} Rhizoma (RM-ext), Stilbene Compounds from RM-ext and Disodium Cromoglycate (DSCG) on Histamine Release from Mast Cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>Release (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>42.0±0.8</td>
<td>—</td>
</tr>
<tr>
<td>RM-ext</td>
<td>10</td>
<td>34.9±1.9**</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>26.5±1.4**</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>22.1±3.2**</td>
<td>47.4</td>
</tr>
<tr>
<td>Compound 1</td>
<td>20</td>
<td>5.6±2.6**</td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4.9±0.5**</td>
<td>90.3</td>
</tr>
<tr>
<td>Compound 2</td>
<td>20</td>
<td>7.1±0.5**</td>
<td>86.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.0±1.0**</td>
<td>94.0</td>
</tr>
<tr>
<td>Compound 3</td>
<td>100</td>
<td>56.4±3.0</td>
<td>—</td>
</tr>
<tr>
<td>Compound 4</td>
<td>20</td>
<td>5.3±2.0**</td>
<td>88.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.7±0.2**</td>
<td>98.5</td>
</tr>
<tr>
<td>Compound 5</td>
<td>20</td>
<td>26.4±7.8**</td>
<td>48.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.2±0.7**</td>
<td>60.5</td>
</tr>
<tr>
<td>Compound 6</td>
<td>20</td>
<td>18.4±4.9**</td>
<td>64.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15.8±2.0**</td>
<td>69.1</td>
</tr>
<tr>
<td>Compound 7</td>
<td>100</td>
<td>61.2±8.5</td>
<td>—</td>
</tr>
<tr>
<td>Compound 8</td>
<td>20</td>
<td>25.7±2.7**</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.4±1.4**</td>
<td>94.6</td>
</tr>
<tr>
<td>Compound 9</td>
<td>20</td>
<td>42.8±1.7*</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>20.6±5.4**</td>
<td>53.3</td>
</tr>
<tr>
<td>DSCG</td>
<td>200</td>
<td>6.7±1.7**</td>
<td>84.0</td>
</tr>
</tbody>
</table>

Mast cells prepared from the peritoneal cavity fluid of rats were suspended in Tyrode solution at 2.9×10^7 cells/ml. Suspensions of these cells treated with samples for 5 min were stimulated by EWA (2 mg/ml) for 10 min at 37°C, and histamine release was measured. Each value represents the mean±S.E. of 3 experiments. Significantly different from the control group, *, p<0.05; **, p<0.01.

DISCUSSION

We previously reported\textsuperscript{9)} that an aqueous extract of rhubarb of Korea growth (dried rhizome of \textit{R. undulatum} L.) exhibited in the inhibitory effects on experimental models of Type I—IV allergy.

In this work, the active compounds which possess anti-type I allergy in this rhubarb were explored. Methanol extract (RM-ext) of the rhubarb inhibited the leakage of dye induced by 48-h homologous PCA. As type I allergy is known to be

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\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Effects of Four Stilbene Compounds and Disodium Cromoglycate (DSCG) on 48-h Homologous Passive Cutaneous Anaphylaxis (PCA) in Rats}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Effects of Four Stilbene Compounds and Prednisolone (Pred.) on Sheep Blood Cell-Induced Delayed-type Hypersensitivity (SRBC-DTH) in Mice}
\end{figure}
attributable mainly to stilbene compounds such as compound 6 (piceatannol 3'-O-β-D-glycopyranoside).

Ko found inhibitory effects on blood platelet aggregation in stilbene compounds isolated from this rhubarb. In JP, this rhubarb is classified as a non-medicinal rhubarb to distinguish it from JP rhubarbs.

In contemporary Japanese and Chinese systems of medicine, the principle virtues of rhubarb are divided broadly into a purgative effect and a therapeutical effect on Oketsu syndrome, so that considered necessary to use practically this rhubarb showed be used for treatment of Oketsu syndrome.

REFERENCES