Studies on Rehmanniae Radix. I. Effect of 50% Ethanolic Extract from Steamed and Dried Rehmanniae Radix on Hemorheology in Arthritic and Thrombosis Rats

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Effects of 50% ethanolic extract (JR-ext) from Chinese Rehmanniae Radix (the steamed and dried root of Rehmannia glutinosa, “Jyuku-Jio” in Japanese) on the hemorheology of inflammatory, thrombosis and intact animals were examined in the in vivo models. JR-ext (200 mg/kg, p.o.) inhibited the reduction of fibrinolytic activity and erythrocyte deformability, the decrease in erythrocyte counts and the increase in connective tissue of the thoracic artery in a chronic inflammatory model, adjuvant-induced arthritis. However, JR-ext was ineffective on the development of edema in the arthritic rats and on acute and chronic inflammation. JR-ext inhibited the reduction of erythrocyte deformability, but not the decrease of coagulative factors in a thrombosis model, endotoxin-induced disseminated intravascular coagulation (DIC). JR-ext also showed a promoting effect on erythrocyte deformability and fibrinolytic activity in intact rats. These results suggest that orally administered JR-ext can prevent an induction of impediment in the peripheral microcirculation of various chronic diseases through the improvement of hemorheology.

Keywords Rehmannia glutinosa; inflammation; adjuvant-induced arthritis; hemorheology; erythrocyte deformability; fibrinolysis

In ancient Chinese herbal literature, it is mentioned that Rehmanniae Radix (the dried root of Rehmannia glutinosa steamed with alcohol, “Jyuku-Jio” in Japanese) is effective for the treatment of Oketsu-syndrome. It is considered that Oketsu-syndrome is closely related to disseminated intravascular coagulation (DIC) disorder, which is an acquired hemorrhagic disorder characterized by the apparent simultaneous activation of blood coagulation, fibrinolysis, and kinin generation, combined with the pathologic consequence of fibrin deposition in the microcirculation.1-3) It is possible that Rehmanniae Radix may be effective against such microcirculation.

The purpose of the present investigation is to study the preventive effects of 50% ethanolic extract from Chinese Rehmanniae Radix on the hemorheology closely related to microcirculation in inflammatory, thrombosis and intact animals.

MATERIALS AND METHODS

Materials Chinese Rehmanniae Radix (the dried root of Rehmannia glutinosa Libosch steamed with alcohol for 18 h, “Jyuku-Jio” in Japanese) was obtained from Beijing Institute for Natural Drug Synthetic Research (Beijing, China) produced in Henan province or Beijing of China. Ten percent (w/v) of Rehmanniae Radix in 50% ethanol was heated for 2 h x 2 times at a temperature of 80 °C. The extract (JR-ext, yield: 59.3%) was obtained through evaporation by mild heating in a vacuum and continuous freeze drying. The following drugs were also used in this study: phenylbutazone (Sigma), dry heat-killed Mycobacterium butyricum (Difco), endotoxin (Escherichia coli 055:B5, Difco), cortisone 21-acetate (cortisone, Nacalai Tesque), pentoxifylline (Sigma), indomethacin (Nacalai Tesque), l-carrageenin (Minsei Rikagaku Co.) and dextran sulphate (Pharmacia Fine Chemicals).

Animals Male KwI: Wistar strain rats (190—220 g or 260—300 g), female Jcl: Sprague-Dawley (SD) strain rats (180—200 g) and male KwI: ddY strain mice (18—22 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 (Clea Japan, Inc.) and water were given freely.

Adjuvant-Induced Arthritis The method was based on that of Nakamura and Shimizu.4) Arthritis was induced by intradermal injection of a 0.05 ml suspension of dry heat-killed Mycobacterium butyricum, 10 mg, in Bayol F, 1 ml, as an adjuvant agent into the tail and right hind paw of SD strain rats. The volume of adjuvant agent injected into the hind paw and the body weight were measured initially and then every other day for 1—11 d thereafter for 27 d, and the volume of edema was determined. The results were expressed as the percentage of hind paw swelling, as the compared with the initial hind paw volume. At 30 d after the injection of the adjuvant agent, whole blood samples were withdrawn from the abdominal vein into plastic syringes while the rats were anesthetized with pentobarbital (44.2 mg/kg, i.p.), and euglobulin lysis time (ELT), erythrocyte deformability, blood platelets, leukocytes, erythrocytes, hemoglobin, hematocrit and reticuloocytes of the blood were measured. And also, the thoracic arteries were removed and the hydroxyproline content was measured by the method of Woessner.5) The test substances suspended in 0.2% carboxymethyl cellulose sodium salt (CMC·Na) solution were administered orally in 30 daily oral doses starting on the day of injection of the adjuvant. Phenylbutazone was used as a standard drug.

Acetic Acid-Induced Vascular Permeability An acetic acid-induced vascular permeability test was performed by the method of Whittle.6) The ddY strain mice were dosed orally with the test substances suspended with 0.2%
CMC: Na solution 1 h before the intravenous injection of 4% pontamine sky blue (10 ml/kg). Fifteen min after the injection of the dye, 1% acetic acid (10 ml/kg) was injected intraperitoneally. After 20 min, the mice were killed by dislocation of the neck and the cavity was exposed, after a 1 min period, to allow blood to drain away from the abdominal wall. The animal was held by a flap of the abdominal wall and the cavity was irrigated with 10 ml of saline over a Petri dish. The washing was filtered through glass wool and transferred to a test tube. To each tube was added 0.1 ml of 1 N NaOH in order to clear any turbidity due to protein, and the absorbance was read at 590 nm with a Shimadzu model UV-160 spectrophotometer. Vascular permeability was expressed in terms of absorbance value per 20 g weight of mouse which leaked into the intraperitoneal cavity. Indomethacin was used as a standard drug.

**Carrageenin-Induced Edema** The method was based on that of Nakamura et al. The initial hind paw volume of the Wistar strain rats (190–220 g) were determined volumetrically. A 1% solution of λ-carrageenin in saline (0.1 ml/rat) was injected subcutaneously into the right hind paw 1 h after the test substances had been administered orally. Paw volumes were measured 2 and 3 h after the injection of λ-carrageenin solution, and the volume of edema was determined. The results were expressed as the percentage of hind paw swelling, as compared with the initial hind paw volume. Indomethacin was used as a standard drug.

**Cotton Pellet-Induced Granuloma** Cotton pellet-induced granuloma test was carried out by the method of Hicks. Two cotton pellets (50 ± 3 mg, mean ± S.E.) were implanted subcutaneously in Wistar strain rats (190–220 g). The test substances suspended in 0.2% CMC Na were administered in 7 daily oral doses starting on the day of implantation. The rats were killed 7 d after the implantation and the pellets were freed from extraneous tissue. Cortisone was used as a standard drug.

**Endotoxin-Induced DIC** Experimental DIC was induced by a modification of the method of Schoendorf et al. The test substance suspended in water was administered to Wistar strain rats (190–220 g) in 7 daily oral doses. Endotoxin (0.3 mg/kg) was injected intravenously 1 h after the final administration. Blood samples were withdrawn from the abdominal vein into plastic syringes at 4 h after the injection of endotoxin, while the rats were anesthetized with pentobarbitone and platelets, fibrinogen, fibrin degradation products (FDP) and erythrocyte deformability of the blood were measured.

**ELT, Erythrocyte Deformability and Blood Viscosity in Intact Rats** JR-ext suspended in water was administered to Wistar strain rats (190–220 g) for the determination of ELT or same strain rats (260–300 g) for erythrocyte deformability and blood viscosity in 7 daily oral doses, and the whole blood was withdrawn from the abdominal vein 1 h after the final administration. Those hemorheological parameters of the blood were measured. Dextran sulphate or pentoxifylline was used as a standard drug.

**Measurement of Hematological Parameters** The ELT was measured by the method of Kaulf and Schultz. The blood viscosity was measured by the cone-plate rotational viscometer (Biorheolizer, Tokyo Keiki Co., Ltd.). The measurements were carried out at 25°C, a cone angle of 1.56° and at three different shear rates (10, 20 or 50 rpm). Platelets, leucocytes, erythrocytes, hemoglobin, hematocrit and reticulocytes were counted with an automatic blood cell counter (Coulter counter, model S-Plus, Coulter Co., U.S.A.). Fibrinogen was determined by means of the latex agglutination test (FDPL test U, Teikoku Zoki). Erythrocyte deformability was expressed as erythrocyte filterability measured by the method of Reid et al. 11

**Statistical Analysis** The experimental data were tested for statistically significant differences by means of Williams’s Multiple Range test.

**RESULTS**

**Adjuvant-Induced Arthritis**

**Edema of Adjuvant-Induced Arthritis** As shown in Fig. 1, JR-ext (50 or 200 mg/kg) had no inhibitory effect on the development of edema in adjuvant-induced arthritic rats. The standard drug, phenylbutazone (50 mg/kg) showed a strong inhibition on the edema. And also, JR-ext had no effect on the growth of the arthritic rats (data not shown).

**Hematological Parameters** As shown in Table I, the erythrocytes, hemoglobin and hematocrit were decreased and reticulocytes, leukocytes and platelets were increased in the arthritic rats as compared to that of the normal rats. The fibrinolytic activity and the erythrocyte deformability were also reduced. JR-ext (200 mg/kg) was found to significantly inhibit the decrease of erythrocyte count and the reduction of fibrinolytic activity and erythrocyte deformability. A preventive effect of phenylbutazone was clearly recognized on the changes in blood rheology, but not in erythrocyte deformability.

**Tissue Hydroxyproline Content** As shown in Fig. 2, the content of tissue hydroxyproline in the thoracic artery was 7.6 ± 0.8 μg/mg wet tissue in the normal rats. The

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Fig. 1. Effects of 50% Ethanolic Extract from Rehmanniae Radix (JR-ext) and Phenylbutazone on the Paw Edema in Adjuvant-Induced Arthritic Rats

Arthritic was induced by an intradermal injection of a 0.05 ml suspension of dry heat-killed Mycobacterium butyricum, 10 mg, in Bayol F 1 ml as an adjuvant agent into the tail and right hind paw. The paw volumes were measured, and swelling percentage was determined. JR-ext or phenylbutazone was orally administered for 30 d immediately after the injection of adjuvant. The control was orally administered 0.2% CMC Na alone. Each value represents the mean ± S.E. of 8–15 rats. Significantly different from the control group: *p < 0.05, **p < 0.01, ***p < 0.001, normal; ———, control; ○, JR-ext 50 mg/kg; □, JR-ext 200 mg/kg; ▲, phenylbutazone 50 mg/kg.
contents increased to 11.3 ± 0.9 μg/mg in the arthritic rats. When 200 mg/kg of JR-ext was administered to rats, the increase in tissue hydroxyproline content was prevented significantly. Phenylbutazone (50 mg/kg) had a weak effect on the increase of tissue hydroxyproline content.

**Acetic Acid-Induced Vascular Permeability**  When JR-ext (50, 200 mg/kg) was administered to mice, the dye leakage was not reduced, while a standard drug, indomethacin 10 mg/kg significantly reduced the leakage (data not shown).

**Carragenin-Induced Edema**  JR-ext (50, 200 mg/kg) had no inhibitory effect on the edema 2 or 3 h after the injection of λ-carragenin. A standard drug, indomethacin (10 mg/kg) showed potent inhibition (data not shown).

**Cotton Pellet-Induced Granuloma**  When JR-ext (50, 200 mg/kg) was administered daily to rats, the increase in granuloma weight was not inhibited, while a standard drug, cortisone (20 mg/kg) significantly inhibited the increase (data not shown).

**Endotoxin-Induced DIC**  As shown in Fig. 3, the platelet count and fibrinogen level and the erythrocyte deformability were decreased and the FDP level was increased in the DIC rats as compared to that of the normal rats. JR-ext (200 mg/kg) was found to significantly inhibit the reduction of erythrocyte deformability, but not the decrease of platelet count and fibrinogen level. JR-ext caused a further increase in FDP level as compared to that of the DIC rats.

**ELT**  As shown in Fig. 4, JR-ext 500 mg/kg significantly shortened the ELT in intact rats, but doses of 50 or 200 mg/kg were ineffective.

**Erythrocyte Deformability**  As shown in Fig. 5, the erythrocyte deformability was 49.8 ± 3.0 μl/s in normal rats. When 50 or 200 mg/kg of JR-ext was administered one time to rats, the deformability was not increased (data not shown). However, JR-ext (200 mg/kg) administered in 7 daily oral doses significantly increased the deformability. A standard drug, pentoxifylline (25 mg/kg), had an increasing effect on the deformability.

**Blood Viscosity**  JR-ext (50, 200 mg/kg) was ineffective in altering the viscosity of whole blood at three different shear rates (data not shown).

**Blood Cell Counts**  As shown in Fig. 6, the erythrocyte and reticulocyte contents were 6.6 ± 0.1 × 10⁶ cells/μl, 16.9 ± 1.8 × 10⁶ cells/μl in normal rats. When JR-ext (200 mg/kg) was administered daily for 7 d to rats, the counts were significantly increased.

**DISCUSSION**

*Rehmanniae Radix* are used for the treatment of

### Table I. Effects of 50% Ethanolic Extract from Rehmanniae Radix (JR-ext) and Phenylbutazone on Hematological Parameters in Adjuvant-Induced Arthritic Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of rats</th>
<th>Erythrocyte filterability (μl/s)</th>
<th>Erythrocyte (× 10⁸ cells/μl)</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>8</td>
<td>50.1 ± 4.1</td>
<td>7.29 ± 0.10</td>
<td>13.9 ± 0.2</td>
<td>47.7 ± 0.5</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>15</td>
<td>35.1 ± 2.6</td>
<td>6.75 ± 0.09¹</td>
<td>12.0 ± 0.2</td>
<td>43.2 ± 0.6</td>
</tr>
<tr>
<td>JR-ext</td>
<td>200</td>
<td>14</td>
<td>33.9 ± 2.3</td>
<td>6.71 ± 0.07</td>
<td>12.0 ± 0.2</td>
<td>43.2 ± 0.6</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>50</td>
<td>14</td>
<td>44.2 ± 4.5³</td>
<td>6.98 ± 0.06²</td>
<td>12.4 ± 0.1</td>
<td>44.2 ± 0.5</td>
</tr>
</tbody>
</table>

### Table II. Effects of 50% Ethanolic Extract from Rehmanniae Radix (JR-ext) and Phenylbutazone on Hematological Parameters in Adjuvant-Induced Arthritic Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Reticulocyte (× 10⁶ cells/μl)</th>
<th>Leukocyte (× 10⁶ cells/μl)</th>
<th>Platelet (× 10⁶ cells/μl)</th>
<th>ELT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td>18.2 ± 0.8</td>
<td>2.67 ± 0.31</td>
<td>82.1 ± 2.6</td>
<td>182.0 ± 12.1</td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td>29.5 ± 1.7³</td>
<td>5.04 ± 0.40³</td>
<td>133.5 ± 8.1³</td>
<td>404.6 ± 32.1³</td>
</tr>
<tr>
<td>JR-ext</td>
<td>200</td>
<td>30.5 ± 1.6</td>
<td>5.58 ± 0.30</td>
<td>130.6 ± 7.8</td>
<td>390.0 ± 48.8</td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>50</td>
<td>29.7 ± 1.4</td>
<td>4.95 ± 0.69</td>
<td>133.8 ± 8.8</td>
<td>310.9 ± 28.9³</td>
</tr>
</tbody>
</table>

Arthritis was induced by the method in the legend to Fig. 1. JR-ext or phenylbutazone was orally administered for 30 d immediately after the injection of the adjuvant. Blood samples were collected from the abdominal vein, and the hematological parameters were measured. The control was orally administered 0.2% CMC-Na alone. Each value represents the mean ± S.E. Significantly different from the normal group, a) p < 0.05, b) p < 0.01, and from the control group, c) p < 0.05, d) p < 0.01.
Oketsu-syndrome, closely related to the congestive syndrome of chronic inflammation or DIC. Accordingly, in this work, the effect of 50% ethanolic extract (JR-ext) from Chinese Rehmanniae Radix (the steamed and dried root of *Rehmannia glutinosa*, "Jyuku-Jio" in Japanese) against hemorheology in a chronic inflammatory model (adjuvant-induced arthritis) and a thrombocytic model (endotoxin-induced DIC) were examined. It has been considered that adjuvant-induced arthritis is closely related
to either the formation of antibody or the activation of complement and may involve a type III or IV allergic reaction.\(^{12,13}\) In this study, JR-ext did not suppress the development of adjuvant-induced edema in the arthritic rats. Also, JR-ext was inactive in acute inflammatory models. Thus, it seems that JR-ext does not interact with inflammation or type III—IV allergic reactions. However, JR-ext exhibited inhibitory effects on the reduction of fibrinolytic activity and erythrocyte deformability, and on the decrease in erythrocyte counts and the increase in connective tissue in the arthritic model. The changes in these hemorheological parameters causes injury to the microcirculation. So, the inhibitory effect of Rehmanniae Radix on the inducement of impediments to the peripheral microcirculation of various chronic diseases may be expected. Phenylbutazone, an anti-inflammatory drug, strongly inhibited the development of edema, but was inactive in increasing hydroxyproline in the connective tissue.

The effect of JR-ext on the hemorheology was estimated in a thrombotic model, endotoxin-induced DIC. JR-ext exhibited an inhibitory effect on the reduction of erythrocyte deformability, but was inactive in inhibiting the decrease in blood platelets and fibrinogen of experimental DIC. JR-ext caused further increases in the FDP level as compared with that of the control rats. So, it seems that JR-ext promotes fibrinolytic activity.

In intact animals, JR-ext showed a promoting effect on erythrocyte deformability and fibrinolytic activity, and an increasing effect on erythrocyte and reticulocyte counts.

The present study clearly demonstrated that JR-ext has an inhibitory effect on the reduction of erythrocyte deformability in chronic inflammatory and thrombotic models. This is interesting in light of the fact that Rehmanniae Radix has been employed for the treatment of various impedimental diseases in the traditional Chinese system of medicine. It suggests that these findings have potentially important implications for the therapeutic use of Rehmanniae Radix for Oketsu-syndrome. A study is now in progress on the active components and mechanisms of JR-ext on the hemorheology and the effects of fresh root of Rehmannia glutinosa ("Sho-Jio" in Japanese) and the dried root ("Kan-Jio") against the hemorheology of these models.

REFERENCES

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