Studies on Medicinal Resources from Livestock. II. Anti-allergic Effects of Pig Bile. 1,2) (2)
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Anti-allergic activities of lyophilized pig bile [PB] were examined in mice with picroly chloride-induced contact dermatitis (PC-CD), an experimental model of delayed-type hypersensitivity (DTH; type-IV allergy). PC-CD was markedly inhibited by an oral administration of [PB] within 4h after but not during 8 to 16h after challenge with picroly chloride.

Anti-inflammatory activities of [PB] were also examined in acetic acid-induced mouse increased vascular permeability, hypotonic-hyperthermic lysis of rat erythrocytes and carrageenin-induced rat hind paw edema. [PB] had no effect on these models. The present study suggests that [PB] inhibits PC-CD through its immuno-modulation in the inductive phase of DTH rather than by an anti-inflammatory action.

Keywords anti-allergic effect; pig bile; delayed type hypersensitivity; picroly chloride induced contact dermatitis; anti-inflammatory effect

Recent Chinese reports 2,4) on traditional Chinese medicine show that animal biles can be used for therapy of bronchitis, asthma and hypersensitivities. Since these diseases are associated with various allergic reactions, the anti-allergic effects of animal biles were examined through some experimental allergic disease models in the previous study. 11 It was found that pig bile [PB] markedly prevented picroly chloride-induced contact dermatitis (PC-CD) in mice and sheep red blood cells (SRBC)-induced footpad swelling in mice, both of which are experimental models of delayed-type hypersensitivity (DTH; type-IV allergy). In order to study the mode of action of [PB], the effects of various treatment regimens with [PB] on PC-CD inhibition and anti-inflammatory effects in some experimental models were examined in the present study.

Experimental
Materials Biles of pigs (triple crosses among Large Yorkshire, Landrace, Duroc and Hampshire strains) were collected from gallbladders with a sterile plastic syringe, pooled and lyophilized (PB). The result of chemical analysis of [PB] was reported in the previous paper. 11

Animals Male Wistar rats and male ddY mice were used. They were housed in an air-conditioned room with a commercial chow (Orientai Yeast Co., Ltd.) and tap water ad libitum.

Methods 1) Effects of Various Treatment Regimens with [PB] on PC-CD Inhibition PC-CD was induced according to the method of Asherson and Dtk. 21 Male ddY mice weighing 18 to 22 g were sensitized by applying 0.1 ml of 7% picroly chloride (PC) in ethanol to their abdominal skins which had been shaved on the previous day. After 7d, contact dermatitis was induced by applying 0.02 ml of 1% PC in olive oil to both ear lobes of the mice. After a further 3d, the mice were re-sensitized with 7% PC in ethanol. Seven days after re-sensitization, the mice were challenged with 1% PC in olive oil to induce contact dermatitis again (challenge). Each test drug, suspended in distilled water, was orally administered before and/or after challenge with 1% PC. Prednisolone (Sigma Chem. Co.) was used as a reference standard. Mice in the control group were orally given distilled water in place of a drug suspension. Ear thickness was measured with a dial thickness gauge (Ozkaki Co.) immediately before (B) and 24h after challenge (A). Ear swelling (%) was calculated from the mean value of (A) and (B) by using the following equation:

ear swelling (%) = (A - B) / (1 - X 100

Inhibition rate (%) was calculated from ear swelling (%) of the control group (C) and that of each test drug group (D) by using the following equation:

inhibition (%) = (1 - D / C) X 100

Immediately after the final measurement of ear swelling, their spleens of mice were removed to measure their wet weights.

a) Oral Administrations Immediately before and/or 16h after Challenge: Each drug suspension was orally administered immediately before, or 16h after, or both immediately before and 16h after challenge.

b) Oral Administrations during 16h after Challenge: Each drug suspension was orally given 0, 4, 8, 12 or 16h after challenge.

2) Anti-inflammatory Effects a) Effect of Increased Vascular Permeability Induced by Acetic Acid in Mice: Male ddY mice weighing around 20g were fasted overnight. Indomethacin (Sigma Chem. Co.) was used as a reference standard [PB] and indomethacin, suspended in distilled water, were orally administered 30min before the i.v. injection of 4% pottamine sky blue (PSB) solution in saline (0.1ml/10g body weight). With mice in the control group, distilled water was orally administered in place of a drug suspension. Five min after the injection of PSB, 0.6% acetic acid solution in saline was injected intraperitoneally (0.1ml/10g body weight) to induce increased vascular permeability according to the method of Kostar et al. 22) After 20min, the mice were killed by decapitation. Distilled water was injected intraperitoneally (10ml/animal) and fluid in the peritoneal cavity was collected after a gentle 30-s massage of the abdomen. The fluid was centrifuged at 3000 rpm for 10min with 0.1ml of 0.1N NaOH to remove protein. The absorbance of the supernatant was measured at 540nm to calculate inhibition (%) to the control group.

b) Effect of Hypotonic-Hyperthermic Lysis of Rat Erythrocytes: The following experiments were carried out according to the method of Glenn and Bowman. 24)

1. Hypotonic-Hyperthermic Lysis: A 10% rat erythrocyte suspension was prepared by centrifuging freshly obtained heparinized blood of male Wistar rats and adding 0.15m phosphate buffer (pH 7.4). A 3.0ml portion of 0.015m phosphate buffer containing each drug at the designated concentration was added to 3.0ml of the erythrocyte suspension. In order to produce 100% hemolysis, 0.1%, Na2CO3 solution in distilled water was added to the erythrocyte suspension in place of 0.015m phosphate buffer. The mixture was heated at 53°C for 20min in a water bath, chilled for 5min in an ice bath and centrifuged. The absorbance of the supernatant was measured at 540nm to determine hemolysis (%). Sodium citrate was used as a reference standard. Each assay was carried out in triplicate.

2. Hypotonic Lysis: The mixture was heated at 37°C for 1h (instead of 53°C for 20min) in the experiment described in 1.

3. Hyperthermic Lysis: A 3.0ml portion of 0.15m phosphate buffer (instead of 0.015m phosphate buffer) containing each drug was added to 3.0ml of the erythrocyte suspension in the experiment described in 1.

c) Effect, on Carrageenin-Induced Hind Paw Edema in Rats: The experiment was carried out by the method of Winter et al. 25) Male Wistar rats weighing 120 to 140g were fasted overnight. Indomethacin (Sigma Chem. Co.) was used as a reference standard. [PB] and indomethacin, suspended in distilled water, were orally administered. With rats in the control group, distilled water was orally given in place of a drug suspension. At 1h after the oral administration of the test drug, 0.1ml of 1% carrageenin solution in sterile saline was injected subcutaneously into the plantar surface of the right hind paw of each rat. Hind paw volumes were measured by the displacement method in a water bath at every 1h from 0 to 5h after the injection of carrageenin. Increase (%) in hind paw volume was calculated by using the following equation:

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increase \( (\%) = (A - B - 1) \times 100 \)

A: Hind paw volume at every 1 h after the injection.
B: Hind paw volume at 0 h (immediately) after the injection.

3) Statistical Analysis All data are mean values ± S.E. Statistical analysis was performed by Yamazaki’s ASSFT method \(^{10}\) (based on Dunnet’s \(^{11}\) and Schefﬂe’s method \(^{12}\)).

Results

1) Effects of Various Treatment Regimens with [PB] on PC-CD Inhibition

a) Oral Administrations Immediately before and/or in 16 h after Challenge

Figure 1 shows the results of one or two treatments with [PB]. An effective PC-CD inhibition was observed when [PB] was orally administered immediately before or immediately before and 16 h after challenge with PC. However, no PC-CD inhibition was observed when it was administered 16 h after challenge. Prednisolone markedly inhibited PC-CD whenever it was administered. There was no significant difference in spleen weight between the control and the treated groups.

b) Oral Administrations during 16 h after Challenge

Figure 2 indicates the results of treatments with [PB] during 16 h after challenge with PC. [PB] inhibited PC-CD effectively when orally administered within 4 h after, but not 8 to 16 h after challenge. Prednisolone markedly inhibited PC-CD whenever orally administered.

2) Anti-inﬂammatory Activities of [PB]

Increased vas-

### Table I. Effect of [PB] on Hypotonic-Hypothermic Lysis of Rat Erythrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg/ml)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>97.0 ± 0.8</td>
</tr>
<tr>
<td>[PB]</td>
<td>100</td>
<td>99.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>99.8 ± 0.8</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1000</td>
<td>95.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>49.0 ± 0.2</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 6 tubes.

### Table II. Effect of [PB] on Hypotonic Lysis of Rat Erythrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg/ml)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>80.4 ± 0.6</td>
</tr>
<tr>
<td>[PB]</td>
<td>100</td>
<td>73.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>78.9 ± 0.2</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1000</td>
<td>45.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 6 tubes.

### Table III. Effect of [PB] on Hyperthermic Lysis of Rat Erythrocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μg/ml)</th>
<th>Hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>60.9 ± 1.4</td>
</tr>
<tr>
<td>[PB]</td>
<td>100</td>
<td>40.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>96.5 ± 0.2</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>1000</td>
<td>50.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>38.7 ± 0.6</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. of 6 tubes.
cular permeability induced by acetic acid was inhibited significantly by oral administration of indomethacin to mice at a dose of 10 mg/kg, but not by [PB] at the doses tested (Fig. 3).

The inhibitory effect of [PB] on hypotonic-hyperthermic lysis of rat erythrocytes was examined at concentrations of 100 and 500 µg/ml, while [PB] at concentrations of more than 500 µg/ml interfered with the measurement of hemolysis due to its bile pigment. Table I indicates that [PB] did not inhibit hypotonic-hyperthermic lysis of rat erythrocytes at the given concentrations. Effects of [PB] on hypotonic and hyperthermic lysis are shown in Tables II and III, respectively. Lysis was slightly inhibited in both models, but was not dependent on the concentration of [PB]. Sodium citrate inhibited lysis at concentrations of 1000 and 5000 µg/ml.

The inhibitory effect of [PB] on rat hind paw edema induced by carrageenin was examined (Fig. 4). Indomethacin inhibited the edema at a dose of 10 mg/kg (p.o.) 3, 4 and 5 h after the injection of carrageenin, but [PB] did not even at doses of 200 and 500 mg/kg (p.o.).

Discussion

In order to find medicinal resources from livestock, the authors first examined the anti-allergic activities of animal bile and reported that [PB] markedly inhibited experimental models of DTH.11 In the present study, the anti-allergic and anti-inflammatory activities of [PB] were further examined to elucidate the mode of action of [PB].

To examine the effects of various treatment regimens with [PB] on PC-CD inhibition, [PB] was orally adminis-

References and Notes

2) Part of this paper was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April, 1986.
5) G. L. Asherson and W. Duk, Immunology, 15, 405 (1968).
12) H. Scheffe, Biometrika, 40, 87 (1953).