Studies on Medicinal Resources from Livestock. I. Anti-allergic Effects of Pig Bile. (1)[1)]

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Anti-allergic activities of animal biles and commercially available bile acids were evaluated in experimental allergic disease models. Pig bile exhibited marked preventive effects on the models of delayed-type hypersensitivity (type IV allergy), picryl chloride-induced contact dermatitis (PC-CD) and sheep red blood cells (SRBC)-induced footpad swelling in mice. Fel ursi (dried bear gallbladder) also had an inhibitory effect on PC-CD, whereas ox bile, chicken bile and bile acids had no effect on ether of the models.

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

Various animal organs have been widely used in traditional oriental medicine. "Bezoar bovis (bovine gallstone)" and "Fel ursi (bear gallbladder)" are representative examples. According to recent Chinese reports[2,3)] on traditional Chinese medicine, animal biles can be used for therapy of bronchitis, asthma and various hypersensitivities. As regards pharmacological effects of animal biles, Aoki *et al.*[4)] reported that pig bile powder stimulated pancreatic juice secretion and bile flow in rats, promoted the passage of charcoal meal in the small intestine of mice, and produced an increase of liver blood flow in rabbits. The pharmacological effects of animal biles on bronchitis, asthma and hypersensitivities, however, have not yet been studied. Since these diseases are associated with various allergic reactions, we examined anti-allergic effects of animal biles in the present study.

Generally, allergic reactions fall into two categories, acute and delayed types. Furthermore, they can be divided into four or five types on the basis of the mechanisms of allergic diseases. For each type of allergy, experimental disease models have been recently developed. The models used in the present study were picryl chloride-induced contact dermatitis (PC-CD) and sheep red blood cells (SRBC)-induced footpad swelling in mice as delayed type hypersensitivities (type IV).

Experimental

Materials Biles of pigs (triple crosses among Large Yorkshire, Landrace, Duroc and Hampshire strains, and Berkshire strain), oxen (Holstein Friesian strain) and chickens (crosses between female White Plymouth Rock and male White Cornish strains) were collected from gallbladders with sterile plastic syringes, pooled and lyophilized. "Fel ursi" (dried bear gallbladder), a traditional crude drug, and bile acids (hyodeoxycholic acid (HDCA), sodium glycochenodeoxycholate (GCDCA), ursodeoxycholic acid, sodium taurochenodeoxycholate, glycocholic acid, and sodium tauroursodeoxycholate; Sigma Chem. Co.) were obtained commercially.

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

Inhibitory Effect on PC-CD in Mice PC-CD in mice, an experimental model of delayed-type hypersensitivity, was induced according to the method of Asherson and Dtak.[5)] Male ddY mice weighing 18 to 22 g were sensitized by applying 0.1 ml of 7% PC in ethanol to their abdominal skins, which had been shaved on the previous day. After 7 d, contact dermatitis was induced by applying 0.02 ml of 1% PC in olive oil to each ear lobe. After a further 3 d, resensitization was performed in the same manner with 7% PC in ethanol. Seven days later, contact dermatitis was induced again by 1% PC in olive oil (challenge). Each test drug, suspended in distilled water, was orally administered immediately before and 16 h after challenge. Prednisolone (Sigma Chem. Co.,), a steroid drug, was used as a reference standard. Mice in the control group were orally given only distilled water in place of a drug suspension. Ear thickness was measured with a dial thickness gauge (Ozaki Co.,) immediately before (*B*) and 24 h after challenge (*A*). Ear swelling (%) was calculated from the mean value of (*A*) and (*B*) by using the following equation;

$$\text{ear swelling } (\%) = (A/B - 1) \times 100$$

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;

$$\text{inhibition } (\%) = (1 - D/C) \times 100$$

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

Inhibitory Effect on SRBC-Induced Footpad Swelling in Mice Delayed-type hypersensitivity (DTH) was induced by SRBC according to the method of Kettman.[6)] Female ICR mice were sensitized by the s.c. injection of 10^8 SRBC/mouse into their backs. After 4 d, they were challenged by the i.c. injection of 10^8 SRBC/mouse into their right hind footpads. Each test drug, suspended in distilled water, was orally given immediately before and 16 h after challenge. Prednisolone was used as a reference standard. Mice in the control group were orally given only distilled water in place of a drug suspension. Footpad swelling was measured by the use of a dial thickness gauge (Ozaki Co.,) 24 h after challenge. Footpad swelling (%) was calculated by using the following equation;

$$\text{footpad swelling } (\%) = (A/B - 1) \times 100$$

A: Thickness of the right hind footpad 24 h after challenge

B: Thickness of the left hind footpad 24 h after challenge

Calculation of inhibition (%) and measurement of spleen weight were done in the same manner as in the PC-CD test.

Chemical Analysis The total bile acid content was determined by the enzyme method.[7)] The cholesterol content was determined by the *o*-phthalaldehyde method.[8)] The fatty acid content was determined by the bathocupuroine method.[9)] The phospholipid content was determined by the molybdenum blue method.[10)] The total neutral sugar content was determined by the phenol–sulfate method.[11)] The protein content was determined by the Lowry method[12)] with bovine serum albumin as a standard protein.

Statistical Analysis All data are means ± S.E. Statistical analysis was performed by using Student's *t* test.

Results and Discussion

The inhibitory effects of the various animal biles on the models of DTH in mice were examined. Figure 1 indicates that pig bile remarkably inhibited the PC-induced ear swelling in mice at a dose of 100 mg/kg. Bear bile also showed an inhibitory effect, whereas ox and chicken biles had no effect. Pig bile prepared from Berkshire strain was also effective, showing 34.0% inhibition at a dose of 100 mg/kg (data not shown). The effect on SRBC-induced footpad swelling in mice is shown in Fig. 2. Pig bile

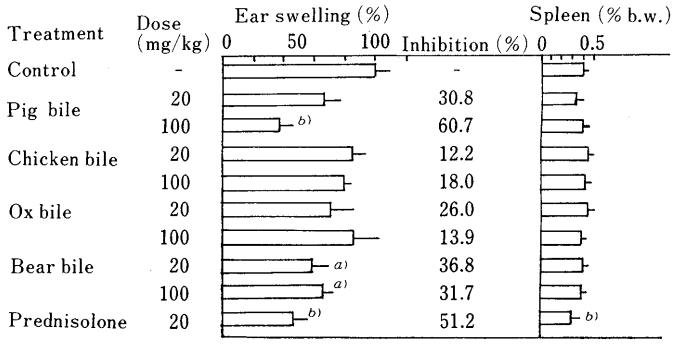

Fig. 1. Effects of Biles Prepared from Various Species of Animals on PC-CD in Mice

Each group includes 10 animals. Values are means±S.E. Significantly different from the control group at *a*) $p<0.05$, *b*) $p<0.01$.

exhibited an inhibitory effect at a dose of 500 mg/kg, whereas ox and chicken biles had no effect, as in the case of PC-CD. Pig bile from Berkshire strain had no significant effect in this experiment (data not shown).

Animal biles contain bile acids, bile pigment, lipids and glycoproteins as major solid components. The result of chemical analysis of the lyophilized pig bile is shown in Table I. Namba *et al.*[13] reported that glycohyodeoxycholic acid and glycochenodeoxycholic acid made up approximately 90% of bile acids in pig bile. Aoki *et al.*[14] also reported that HDCA and chenodeoxycholic acid were main components of pig bile powder. In this study, six kinds of commercially available bile acids including HDCA and GCDCA were examined for inhibitory effects on the models of DTH to find the active components of pig bile.

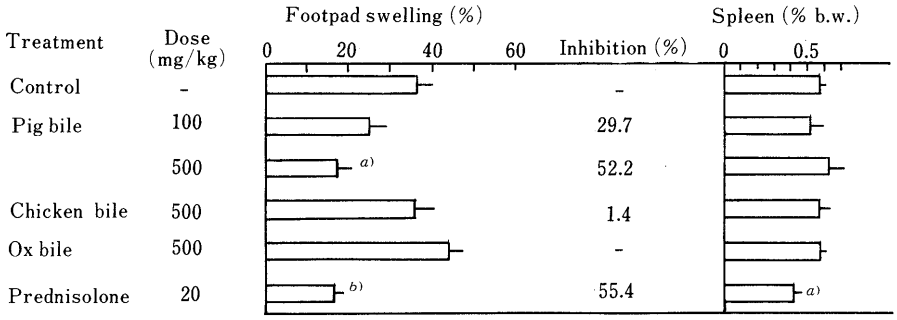

Fig. 2. Effects of Biles Prepared from Various Species of Animals on SRBC-DTH in Mice

Each group includes 10 animals. Values are means±S.E. Significantly different from the control group at *a*) $p<0.05$, *b*) $p<0.01$.

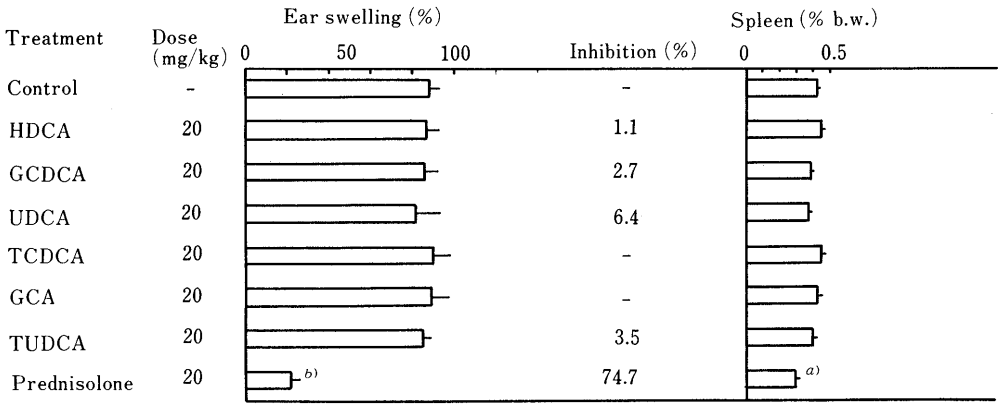

Fig. 3. Effects of Some Bile Acids on PC-CD in Mice

Each group includes 10 animals. Values are means±S.E. Significantly different from the control group at *a*) $p<0.05$, *b*) $p<0.01$.
HDCA, hyodeoxycholic acid; GCDCA, sodium glycochenodeoxycholate; UDCA, ursodeoxycholic acid; TCDCA, sodium taurochenodeoxycholate; GCA, glycocholic acid; TUDCA, sodium tauroursodeoxycholate.

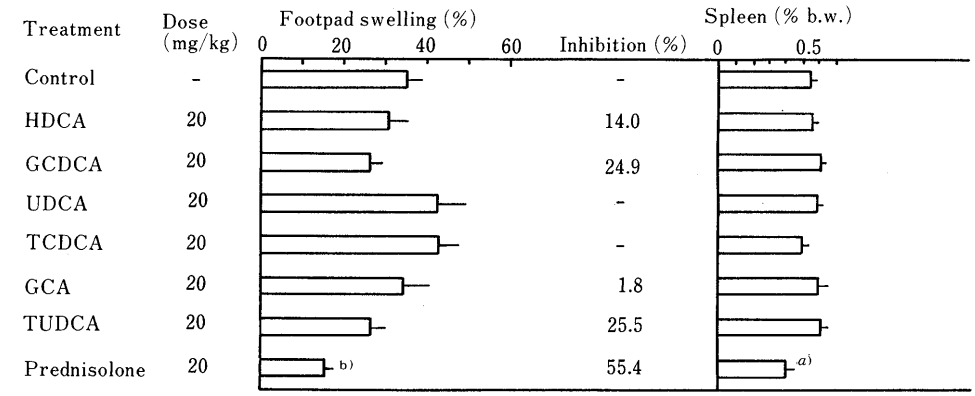

Fig. 4. Effects of Some Bile Acids on SRBC-DTH in Mice

Each group includes 10 animals. Values are means±S.E. Significantly different from the control group at *a*) $p<0.05$, *b*) $p<0.01$.

TABLE I. Analysis of Lyophilized Pig Bile

	mg/g
Total bile acid[a]	572
Cholesterol[b]	25
Fatty acid[c]	80
Phospholipid[d]	6
Total sugar[e]	52
Protein[f]	48

a) Enzyme method. *b*) *o*-Phthalaldehyde method. *c*) Bathocupuroine method. *d*) Molybdenum blue method. *e*) Phenol–sulfate method. *f*) Lowry method.

However, those bile acids had no effect on either of the models at a dose of 20 mg/kg (Figs. 3 and 4). The dose of 20 mg each bile acid/kg is equivalent to that of 100—500 mg or more of pig bile powder/kg on the basis of each bile acid content in pig bile powder according to the study of Aoki *et al.*[14] The above findings suggest that the active components of pig bile are not bile acids but some other species-specific substances (*e.g.* glycoproteins).

Prednisolone, a reference standard, exhibited a strong preventive effect on PC-CD and SRBC-induced footpad swelling at a dose of 20 mg/kg. It was also shown that treatment with prednisolone significantly reduced the spleen weight of mice (Figs. 1, 2, 3 and 4).

Aoki *et al.*[15] reported that acute and chronic toxicities of pig bile powder were very low. In the present study, pig bile showed no serious side effect such as the splenic atrophy caused by steroid drugs (*e.g.* prednisolone). It is remarkable that pig bile produces a potent inhibitory effect on DTH without apparent adverse effect.

Further studies are in progress on the active components in pig bile and on the mode of action.

References and Notes

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