Protective Effects of Various Chinese Traditional Medicines against Experimental Cholestasis[1)]

Toshinobu SASAKI,[a] Setsuko OHTA,[a] Asahi KAMOGAWA,[b] and Masato SHINODA*,[a]

Faculty of Pharmaceutical Sciences, Hoshi University,[a] 2–4–41 Ebara, Shinagawa-ku, Tokyo 142, Japan and St. Marianna University School of Medicine,[b] 2–16–1, Sugao, Miyamae-ku, Kawasaki 213, Japan. *Received May 11, 1989*

In a previous paper, we reported that methanol extracts obtained from 13 Chinese traditional medicines showed remarkable choleretic effects in normal rats. This paper examines the protective effects against experimental cholestasis induced by carbon tetrachloride (CCl_4) or α-naphthylisothiocyanate (ANIT) in rats. No medicines, including sodium dehydrocholate and 1-phenylpropanol which are used clinically as choleretic drugs, inhibited the decrease of bile flow induced by CCl_4. On the other hand, Intinko-to, Saiko-seikan-to and Bohu-tusyo-san revealed marked improvement of the dysfunction in bile secretion induced by ANIT. These three medicines inhibited the decrease of excretion of bile acid or bilirubin in the bile. They also exerted a protective effect against the alterations of serum components induced by ANIT, *i.e.*, of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase and the concentration of serum bilirubin.

These results indicate that methanol extracts of Intinko-to, Saiko-seikan-to and Bohu-tusyo-san demonstrate not only choleretic effects but also improvement of cholestasis and liver parenchymal injury in rats.

Keywords α-naphthylisothiocyanate; carbon tetrachloride; Chinese traditional medicine; bile flow; cholestasis; liver injury; crude drug

Chinese traditional medicines have recently begun to be used frequently in clinical practice. Effective Chinese traditional medicines such as Intinko-to, Syo-saiko-to and Dai-saiko-to have been identified and utilized especially for liver parenchymal injuries.[2)] Liver injuries can be classified into two types: parenchymal injury and injury (cholestasis) of bile secretion. Experimentally, carbon tetrachloride (CCl_4) is a typical inducer of liver parenchymal injury[3)] that complicates cholestasis.[4)] α-Naphthylisothiocyanate (ANIT) is a typical inducer of experimental cholestasis. The cholestasis induced by CCl_4 is due to dysfunction of bile secretion-independent bile acid excretion. A difference in the experimental cholestasis induced by CCl_4 and ANIT has been reported.[4)]

On the other hand, we previously examined the choleretic effects of 60 methanol extracts of Chinese traditional medicines. Marked increases of bile flow in normal rats were found after administration of 13 methanol extracts obtained, but did not occur with water-soluble fractions obtained from the 13 Chinese traditional medicines.[5)] The choleretic effects of these methanol extracts may stimulate bile secretion-independent bile acid excretion. These extracts are thus assumed to improve the dysfunction of bile secretion induced by CCl_4. There have been few investigations of the protective effect against cholestasis of Chinese traditional medicines. We therefore examined the protective effects of the methanol extracts against the experimental cholestasis induced by CCl_4 or ANIT under the above assumptions.

Experimental

Animals Male Wistar rats (180—220 g) were maintained on an Oriental Yeast MF pellet diet and tap water *ad libitum*. The rats were starved from 24 h before the administration of inducer until the end of the experiment.

Materials ANIT was purchased from Sigma Chemical Co., St. Louis, Mo. CCl_4 was purchased from Wako Pure Chemical Co., Osaka. Sodium dehydrocholate (DHC) and 1-phenylpropanol (1-PP) were purchased from Tokyo Kasei Co., Tokyo. Materials of the Chinese traditional medicines were obtained from Uchida Wakanyaku Co., Tokyo. The materials (100 g), which were prepared according to the recipe,[6,7)] were extracted with 500 ml of methanol three times for 2 h under reflux, and the extract was evaporated to dryness under reduced pressure.

Method of Administration ANIT (50 mg/ml) and CCl_4 (10%) were dissolved in olive oil. At 24 h after the beginning of starvation, ANIT (50 mg/kg) or CCl_4 (0.1 ml/kg) as the inducer of cholestasis was administered intraperitoneally. Extracts of Chinese traditional medicines were dissolved or suspended in pure water. The maximum doses of the extracts were set at 1000 mg/kg, and the maximum volume was 1 ml. DHC was dissolved in pure water at 80 mg/ml and 1-PP was emulsified in 5% arabic gum solution at 80 mg/ml. These samples, the pH of which was regulated at 7—8, were given intraperitoneally 30 min prior to a single dose of the inducer of cholestasis.

Method of Bile Collection At 24 h after administration of ANIT or CCl_4, rats were anesthetized with urethane (Wako Pure Chemical, 1.0 mg/kg) and laparotomized. A polyethylene cannula (0.9 × 150 mm, Igarashi Ika Kogyo) was inserted into the common bile duct and 0.9% NaCl (1 ml) was administered intraperitoneally to prevent dehydration. Bile collection was begun at 30 min after this operation and was continued for 120 min.

Assay Method of the Concentration of Various Components in Bile The bile volume was measured using a microsyringe. The biliary concentration of bile acid was estimated by the enzymic method[8)] (Enzabile, Daiichi Kagaku Yakuhin), and the bilirubin concentration was measured by the Jendrassik method[9)] (Denka Seiken) employing an Auto Biochemical Analyzer (TBA-380, Toshiba Medical). The biliary sodium and potassium concentrations were determined by the flame photometric method (170-50, Hitachi Industry). The excretion of these components for a fixed time was estimated from their concentration and the bile flow.

The protective potencies were calculated using the following equation:

$$\text{protective potency against experimental cholestasis (\%)} = \frac{\text{(sample + inducer) treated group} - \text{inducer treated group}}{\text{normal group} - \text{inducer treated group}} \times 100$$

Assay Method of the Protective Effects against Liver Injury Induced by ANIT The experimental designs used were the same until the administration of ANIT. Rats were laparotomized under anesthesia with diethyl ether at 48 h after administration of the sample or ANIT. Venous blood was collected from the vena cave inferior by puncture, and the serum was obtained by centrifugation. The activities of serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured by the Karmen method[10)] (Denka Seiken). The alkaline phosphatase (ALP) in the serum was estimated by the GSCC method[11)] (Denka Seiken). The concentration of serum bilirubin was measured by the Jendrassik method[9)] (Denka Seiken) employing an Auto Biochemical Analyzer (TBA-380, Toshiba Medical).

The protective potencies were calculated from each value using the following equation:

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$$\text{protective potency against ANIT induced liver injury (\%)} = \frac{\text{ANIT treated group} - (\text{sample} + \text{ANIT}) \text{ treated group}}{\text{ANIT treated group} - \text{normal group}} \times 100$$

Statistical Analysis Statistically significant differences between two values were estimated by Student's t test with a p value of less than 0.05 being considered as representing a significant protective potency.

Results

Protective Effects of Methanol Extracts of Chinese Traditional Medicines against Cholestasis Induced by CCl_4 The protective effects of samples given 30 min prior to a single dose of CCl_4 (0.1 ml/kg, i.p.) were investigated (Fig. 1).

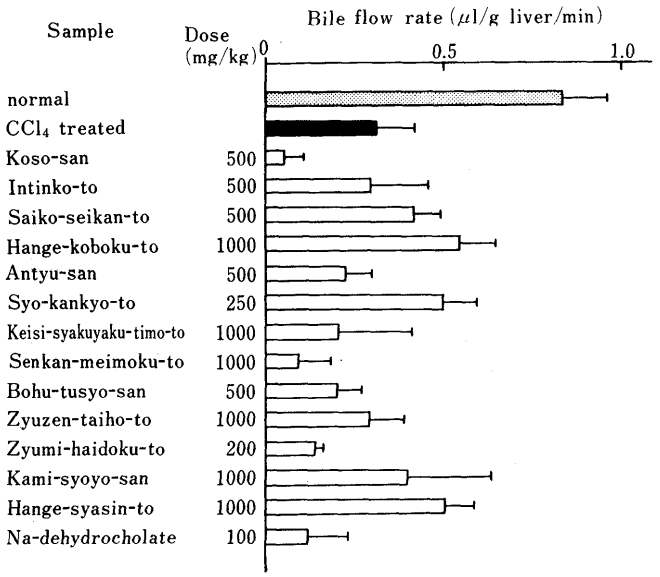

Fig. 1. Effects of Methanol Extracts of Chinese Traditional Medicines on Alterations of Bile Flow Induced by CCl_4

Values are the means ± S.E. ($n=4$).

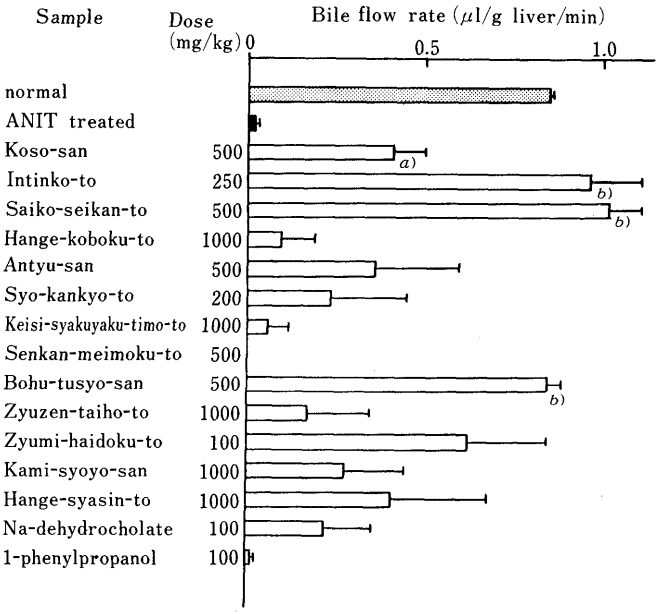

Fig. 2. Effects of Methanol Extracts of Chinese Traditional Medicines on Alterations of Bile Flow Induced by ANIT

a, b) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. Values are the means ± S.E. ($n=4$).

The bile flow of the normal group was approximately 0.8 μl/g liver/min. The bile flow of the CCl_4-treated group was reduced to less than half of that of the normal group, so that marked cholestasis was found in this group. No medicine inhibited the decrease of bile flow induced by CCl_4. In the case of DHC administration, a decrease in bile secretion was also found. Thus, none of the drugs tested exerted a protective effect against the cholestasis induced by CCl_4.

Protective Effects of Methanol Extracts of Chinese Traditional Medicines against Cholestasis Induced by ANIT As in the case of CCl_4, the effects of samples given 30 min prior to a single dose of ANIT (50 mg/kg i.p.) were investigated (Fig. 2).

The bile flow of the normal group was 0.8 μl/g liver/min. The bile flow of the ANIT-treated group was minimal, so that bile secretion almost stopped. In the case of administration of methanol extracts of Koso-san, Intinko-to, Saiko-seikan-to and Bohu-tusyo-san, the decrease in bile flow was inhibited, with a significant different from the ANIT-treated group. In particular, with Intinko-to, Saiko-seikan-to and Bohu-tusyo-san, the bile flow was maintained at a normal level (0.8—1.0 μl/g liver/min). These medicines thus exert a strong protective effect against this type of cholestasis. There were no effects in the case of DHC and 1-PP.

Effects of Medicines with Protective Effects on Cholestasis, on Alterations of Biliary Components Induced by ANIT The excretion of various biliary components in cholestasis induced by ANIT was kept at a normal level by the administration of Intinko-to, Saiko-seikan-to and Bohu-tusyo-san. The protective potencies of these com-

Table I. Protective Effects of Methanol Extracts of Chinese Traditional Medicines against Cholestasis Induced by ANIT

Sample	Dose (mg/ml)	Protective potency (%)				
		B.F.	B.A.	T-BIL	Na	K
Intinko-to	250	113.9$^{a)}$	52.5$^{a)}$	114.3$^{a)}$	98.2$^{a)}$	95.8$^{a)}$
Saiko-seikan-to	500	120.2$^{b)}$	93.2$^{a)}$	200.8$^{a)}$	111.6$^{b)}$	102.9$^{b)}$
Bohu-tusyo-san	500	99.5$^{b)}$	86.0$^{a)}$	129.7$^{a)}$	98.9$^{b)}$	101.2$^{b)}$

B.F.: bile flow. B.A.: bile acid.

$$\text{protective potency (\%)} = \frac{(\text{ANIT} + \text{sample}) \text{ treated group} - \text{ANIT treated group}}{\text{normal group} - \text{ANIT treated group}} \times 100$$

a, b) Significantly different from the ANIT treated group at $p<0.05$ and $p<0.01$, respectively ($n=4$).

Table II. Protective Effects of Methanol Extracts of Chinese Traditional Medicines against Alterations of Serum Components Induced by ANIT

Sample	Dose (mg/kg)	Protective potency (%)				
		S-GOT	S-GPT	S-ALP	S-T-BIL	S-D-BIL
Intinko-to	250	98.4$^{b)}$	98.5$^{b)}$	123.2$^{b)}$	99.3$^{b)}$	99.5$^{b)}$
Saiko-seikan-to	500	93.2$^{b)}$	98.0$^{b)}$	119.2$^{b)}$	99.5$^{b)}$	99.8$^{b)}$
Bohu-tusyo-san	500	19.1	45.4	70.6$^{a)}$	73.7$^{b)}$	74.7$^{b)}$

$$\text{protective potency (\%)} = \frac{\text{ANIT treated group} - (\text{ANIT} + \text{sample}) \text{ treated group}}{\text{ANIT treated group} - \text{normal group}} \times 100$$

a, b) Significantly different from the ANIT treated group at $p<0.05$ and $p<0.01$, respectively ($n=4$).

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ponents were calculated (see Table I).

The protective potencies for the excretion of sodium and potassium ions in the three groups were almost 100%, representing the condition of normal excretion of both components approximately. However, the protective potency for the excretion of bile acid with Intinko-to was only approximately 50%. The protective potency for the excretion of bilirubin with Saiko-seikan-to was nearly 200%, while in the case of the other two medicines, the protective potencies were nearly 100% (*i.e.*, normal excretion was maintained).

Effects of Medicines on Liver Injury Induced by ANIT In the normal group, the cholestasis group induced by ANIT and the Intinko-to, Saiko-seikan-to and Bohu-tusyo-san treated groups, the activities of serum GOT, GPT, ALP and the concentrations of total and direct bilirubin (T-BIL and D-BIL) were measured, and the protective potencies were calculated (see Table II).

Increases of these components were inhibited by the administration of Intinko-to and Saiko-seikan-to; the protective potencies were almost 100%. In the case of Bohu-tusyo-san, the increases of ALP and bilirubin were inhibited; the protective potencies exceeded 70%.

Discussion

The protective potencies against experimental cholestasis were examined in 13 Chinese traditional medicines recognized to have choleretic effects.[5] Bile has been divided into two types according to its mechanism of formation[12]: one is the bile acid-dependent fraction and the other is the bile acid-independent fraction. Formation of the former is related to the permeability of bile acid, and the latter to sodium, potassium,[13] bicarbonate,[14] *etc.* However, the mechanism of the latter has not been fully elucidated, and many hypothesis have been proposed for it. The cholestasis induced by CCl_4 is due to dysfunction of bile secretion-independent bile acid excretion, but ANIT injures the bile acid excretion and stops the bile flow.[4] In a previous study, we found that 13 kinds of methanol extracts obtained from Chinese traditional medicines stimulated the bile secretion-independent bile acid excretion and so increased the bile flow of normal rats. These extracts were therefore assumed to be able to improve the cholestasis induced by CCl_4. The 13 kinds of methanol extracts in fact had no protective effect on the cholestasis induced by CCl_4, but remarkable effects were exerted by Intinko-to, Saiko-seikan-to and Bohu-tusyo-san on the cholestasis induced by ANIT. Intinko-to protects against the experimental liver injury induced by ANIT,[15] and improves the cholestasis induced by cholestatic factor.[16] This medicine is employed for the treatment of jaundice in clinical practice. No previous investigations have examined the protective potencies of Saiko-seikan-to and Bohu-tusyo-san in clinical or experimental cholestasis. In an earlier report, marked effects on the cholestasis induced by both CCl_4 and ANIT were noted for cysteamine (MEA).[4] These findings suggest that the mechanism of protection of the three extracts may differ from that of MEA.

In the present study, no medicine including DHC and 1-PP, which are employed clinically as choleretic drugs, exerted protective effects against CCl_4. Thus, protective effects against cholestasis can not be predicted from results for choleretic effects.

Intinko-to, Saiko-seikan-to and Bohu-tusyo-san improved not only the dysfunction of bile secretion but also the excretion of various components in the bile. An examination was made of whether these three medicines have protective effects on liver injury, whose index is alterations of serum components and which was induced by ANIT. The activities of serum GOT and GPT that exist in hepatocytes can be utilized as indices of liver parenchymal injury. The activity of serum ALP present in the bile canaliculus membrane and the concentration of serum bilirubin represent indices of bile ductular injury.[17] Although the ANIT-induced injuries were mainly bile ductular injuries, it is known that hepatocytes of the portal area are also damaged by mass administration.[18] In the present study, the protective potencies were almost 100% for all items with Intinko-to and Saiko-seikan-to. Bohu-tusyo-san revealed high protective potencies, of more than 70%, for ALP and bilirubin. Remarkable protective effects against the experimental liver injury induced by ANIT, as reflected by alterations of the serum components, were also found. The degrees of protective potency in the three medicines differed from each other for the various parameters in the bile and serum at cholestasis or liver injury. These findings suggest differences of protective mechanism among the three medicines.

Gardenia Fructus is a common component of Intinko-to, Saiko-seikan-to and Bohu-tusyo-san with remarkable protective effects against the cholestasis induced by ANIT. Its component ratio in the three Chinese traditional medicines was 35.7%, 6.5% and 4.6%, respectively. Methanol extracts obtained from Gardenia Fructus increased the bile flow of normal rats,[19] but have not been examined for their protective effects on cholestasis. In the present materials, Gardenia Fructus could also be found in Kami-syoyo-san and Senkan-meimoku-to at component ratios of 5.9% and 8.7%, respectively, which were more than that in Bohu-tusyo-san. However these two medicines did not show improvement of the cholestasis induced by ANIT. It is difficult to conclude therefore that the protective effects against cholestasis could be due only to Gardenia Fructus. There may be synergism between Gardenia Fructus and other crude drugs for the improvement of the experimental cholestasis induced by ANIT. Elucidation of the precise details of the protective effects must await further research.

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