Effects of Syô-saiko-tô and Dai-saiko-tô on carbon tetrachloride-induced hepatic injury in rats[1)]

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Abstract

Effects of oral administration of Syô-saiko-tô and Dai-saiko-tô on CCl_4-induced hepatic necrosis and functional disorder were studied. Treatment of Syô-saiko-tô restored the CCl_4-induced increase of sGOT and sGPT activities 24 hr after the CCl_4 treatment. Furthermore, Syô-saiko-tô improved the CCl_4-induced elongation of prothrombin time and inhibited the CCl_4-induced increase of cytochrome P-450 activity 24 and 48 hr after the CCl_4 treatment. Dai-saiko-tô, however, showed no effect on the increased sGOT and sGPT activities and the elongation of prothrombin time. These results suggest that Syô-saiko-tô improves the hepatocyte necrosis and functional disorder induced by CCl_4 treatment.

Key words carbon tetrachloride, Dai-saiko-tô, hepatic necrosis, hepatic functional disorder, Syô-saiko-tô.

Abbreviations CCl_4, carbon tetrachloride ; GOT, glutamic oxaloacetic transaminase ; GPT, glutamic pyruvic transaminase ; Dai-saiko-tô (Da-Chai-Hu-Tang), 大柴胡湯 ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Syô-saiko-tô (Xiao-Chai-Hu-Tang), an extract from a mixture of 7 herbs, is one of the traditional Japanese and Chinese medicines prescribed for chronic liver diseases in oral use. Recently, its effectiveness has been suggested by clinical trials for chronic hepatitis.[2,3)] As the pharmacological action of Syô-saiko-tô, steroidal and non-steroidal anti-inflammatory actions,[4-6)] acceleration of pituitary-adrenocortical axis function,[6,7)] and immunopotensiative actions by the stimulation of macrophages and T-lymphocyte functions[8)] were reported. These actions may partially explain the inhibitory effect of Syô-saiko-tô on chronic hepatitis. Furthermore, we reported that Syô-saiko-tô and Dai-saiko-tô whose combined herbal drugs were similar to Syô-saiko-tô inhibited the development of hepatic fibrosis induced by the treatment of carbon tetrachloride (CCl_4) for 6 months,[1,9)] and two inhibitory action mechanisms of Syô-saiko-tô and Dai-saiko-tô on the hepatic fibrosis formation were proposed. One is their anti-necrosis action and the other is the direct anti-fibrosis action. Anti-necrosis action of both Kampô-hôzai (Japanese traditional medicines) was reported by other investigators. Kato *et al.*,[10)] Yamaura *et al.*[11)] and Ota *et al.*[12)] suggested the inhibitory action of Syô-saiko-tô by the intraperitoneal injection on galactosamine-induced liver injury by stabilizing hepatocyte membrane. These actions may be supported from the similar action of saikosaponins involved in *Bupleurum falcatum* root as mentioned by Abe *et al.*[13)] However, the improvement of Syô-saiko-tô by oral administration has not been reported yet. Ota *et al.*[14)] further report-

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ed the anti-hepatotoxic action of Dai-saiko-tô by the inhibition on lipid peroxidation, increase of hepatic ascorbic acid and reduction of glutathione levels. These results suggest that both Kampô-hôzai show anti-necrosis action.

Here, we report the effectiveness of the oral use of Syô-saiko-tô and Dai-saiko-tô for hepatic necrosis and functional disorder in experimental acute liver injury induced by CCl_4.

Materials and Methods

Animals : Wistar rats, 5 weeks old, were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan), housed at 20℃ under normal lighting conditions and maintained on a commercial pellet diet with water *ad libitum* throughout the experiments.

Preparation of Syô-saiko-tô and Dai-saiko-tô : The herbs were mixed as indicated in Table I, suspended in 700 ml of water and extracted at 100℃ for 1 hr. The extracts were then concentrated to 300 ml followed by lyophilization to produce powdered extracts. Yields were 7.2 g in Syô-saiko-tô and 8.0 g in Dai-saiko-tô, respectively. These are the standard human daily doses.

Assay of serum glutamic oxaloacetic transaminase (sGOT) and glutamic pyruvic transaminase (sGPT) activities[15] : Blood, 20 μl, was collected from the veniplex of the eyeground of rats using capillary tubes for microhematocrits (Terumo Co., Ltd., Tokyo, Japan) 24 and 48 hr after the CCl_4 treatment. sGOT and sGPT activities were assayed using transaminase kits (Eiken Chem. Co., Ltd., Tokyo, Japan).

Assay of prothrombin time[16] : Blood, 180 μl, was collected by cardicentesis in a 1-ml syringe containing 20 μl of 3.13% citric acid solution 24 and 48 hr after the CCl_4 treatment. The plasma obtained by the centrifugation at $3000 \times g$ was added to the 0.1 ml of thromboplastin solution (Japan Travenol, Tokyo, Japan) under 37℃. Finally, 0.1 ml of 0.02 M $CaCl_2$ was added to the mixed solution and the prothrombin time was measured by fibrometer (Clotek-II TS, Metek, Tokyo, Japan). All procedures were repeated three times and averaged.

Assay of cytochrome P-450 activity : Rats were decapitated 24 and 48 hr after the CCl_4

Table I Combination of herbal drugs in Kampô-hôzai.

	Syô-saiko-tô	Dai-saiko-tô
Bupleuri Radix (*Bupleurum chinense* DC., China)	7	6
Pineliae Tuber (*Pinellia ternata* BREITENBACH, China)	5	4
Zingiberis Rhizoma (*Zingiber officinale* ROSCOE, Japan)	4	4
Scutellariae Radix (*Scutellaria baicalensis* GEORGI, China)	3	3
Zizyphi Fructus (*Zizyphus vulgaris* LAM., China)	3	3
Ginseng Radix (*Panax ginseng* C.A.MEYER, Korea)	3	
Glycyrrhizae Radix (*Glycyrrhiza glabra* L., China)	2	
Paeoniae Radix (*Paeonia lactiflora* PALLAS, Japan)		3
Auranti Fructus Immaturus (*Citrus aurantium* L., Japan)		2
Rhei Rhizoma (*Rheum tanguticum* MAXIM, China)		2

Each number indicated in the Table shows the grams of composed herbal drug per daily dose of a human.

treatment. Rat liver perfused with tyrode solution were homogenized in 0.035 M tris-HCl buffer (pH 7.5) containing 0.155 M KOH using potter homogenizer with a teflon pistol and centrifuged at 15000×*g* for 15 min by Hitachi SCR 20 BA centrifuge. The supernatant obtained was centrifuged at 105000×*g* for 60 min by Beckman C8-80 centrifuge. After the removal of supernatant, the residue was suspended in 25 ml of tris-HCl buffer and centrifugation at 105000×*g* was repeated for 45 min. The residue obtained was suspended in 10 ml of 0.02 M potasium phosphate buffer (KPB : pH 7.5). The protein contents in the suspended fraction was determined by the Lowry method.[17] The absorbance of the microsomal suspension (2 mg protein/ml) was measured after the bubbling of CO gas and the addition of 10 mg of $Na_2S_2O_4$. Cytochrome P-450 activity was calculated according to the method reported by Omura and Sato.[18,19]

Experimental procedure : Rats were divided into 4 groups. Each group consisted of 6—10 rats. Subcutaneous injection of 0.8 ml of 50% CCl_4 in olive oil were received by 3 groups of rats. Syô-saiko-tô and Dai-saiko-tô at a dose of 600 mg/kg body weight, dissolved in 2 ml of distilled water, were administered to 2 groups of rats treated with CCl_4, respectively, for 6 days from 4 days before to 1 day after the CCl_4 injection using a stomach tube. In the normal group, physiological saline was administered.

Presentation of data : Significant differences were calculated by Student's *t*-test.

Results

Effects on serum transaminase activities

The subcutaneous treatment of CCl_4 increased sGOT and sGPT activities which are the indices of hepatic necrosis as twice those of the normal group at 24 hr as shown in Fig. 1 and Fig. 2. These levels were decreased to the normal level 48 hr after the CCl_4 treatment. Treatment of Syô-saiko-tô inhibited the increase of sGOT and sGPT activities at 24 hr. At that time, sGPT activity of Syô-saiko-tô treated group reached that of the normal group. Dai-saiko-tô, however, showed no effect on the increased sGOT and sGPT activities 24 hr after the CCl_4 treatment.

Effects on prothrombin time and cytochrome P-450 activity

To know the further restorative effect of

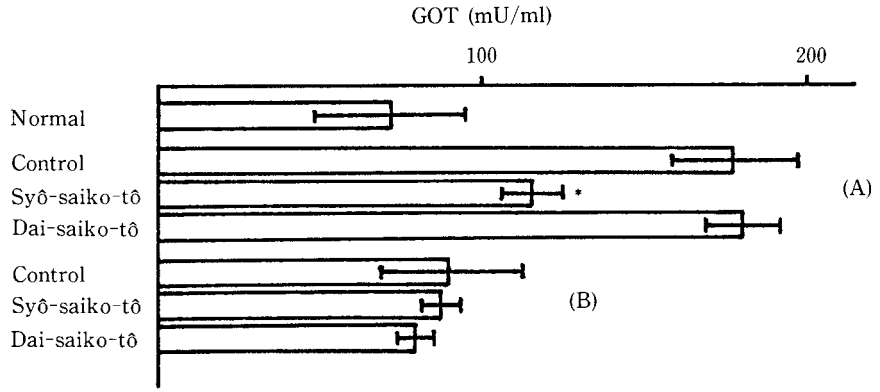

Fig. 1 Effects of Syô-saiko-tô and Dai-saiko-tô on serum GOT activity in rats treated with CCl_4.

Syô-saiko-tô and Dai-saiko-tô at a dose of 600 mg/kg body weight were administered using a stomach tube for 6 consecutive days from 4 days before to 1 day after the CCl_4 injection. (A) : 24 hr after the CCl_4 injection, (B) : 48 hr after the CCl_4 injection. Each column indicates the mean±S.E.M. of 5—8 rats. $^*p<0.01$ vs. control.

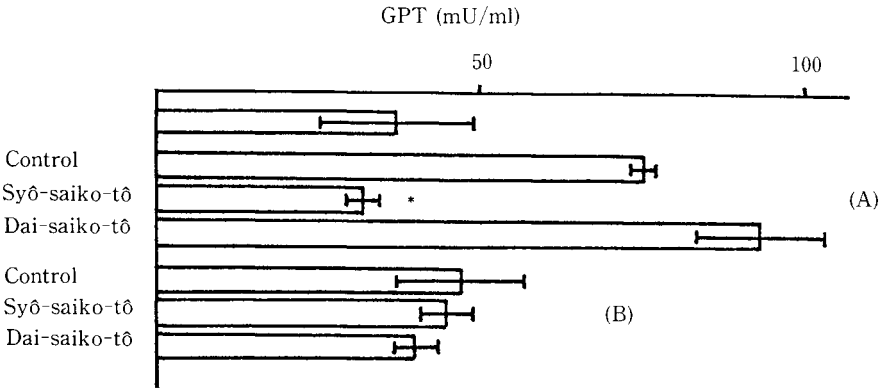

Fig. 2 Effects of Syô-saiko-tô and Dai-saiko-tô on serum GPT activity in rats treated with CCl_4.
Details are the same as in the legend to Fig. 1. (A) : 24 hr after the CCl_4 injection, (B) : 48 hr after the CCl_4 injection. Each column indicates the mean±S.E.M. of 5—8 rats. $*p<0.01$ vs. control.

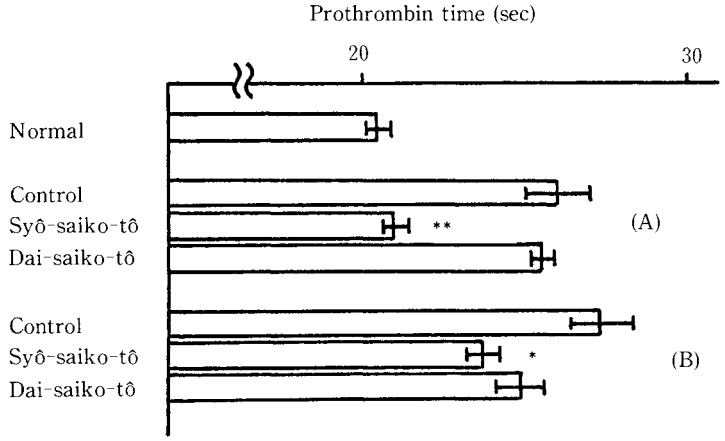

Fig. 3 Effects of Syô-saiko-tô and Dai-saiko-tô on prothrombin time in rats treated with CCl_4.
Details are the same as in the legend to Fig. 1. (A) : 24 hr after the CCl_4 injection, (B) : 48 hr after the CCl_4 injection. $*p<0.05$, $**p<0.01$ vs. control.

Syô-saiko-tô and Dai-saiko-tô on the hepatocellular functional disorder induced by CCl_4, prothrombin time in plasma and cytochrome P-450 activity in liver were studied. Elongation of prothrombin time was induced 24 and 48 hr after the CCl_4 treatment as shown in Fig. 3. This prolonged prothrombin time was inhibited by Syô-saiko-tô at 24 and 48 hr. The increased prothrombin time, however, was not changed by the treatment of Dai-saiko-tô. On the other hand, CCl_4 treatment decreased the liver cytochrome P-450 activity as shown in Fig. 4. Treatment of Syô-saiko-tô restored the decreased cytochrome P-450 activity 24 and 48 hr after the CCl_4 injection. In this experiment, Dai-saiko-tô also inhibited the decrease in cytochrome P-450 activity at 48 hr.

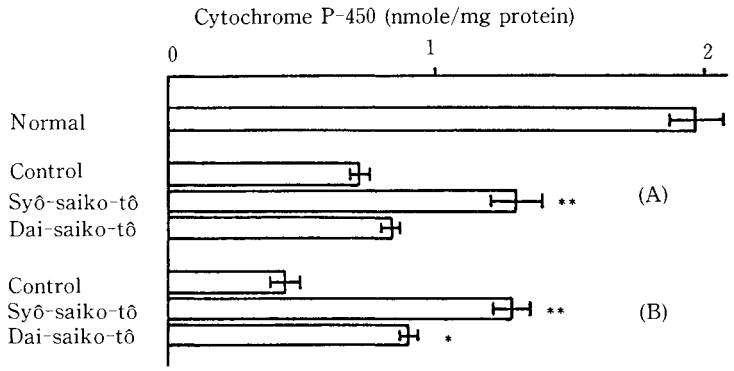

Fig. 4 Effects of Syô-saiko-tô and Dai-saiko-tô on cytochrome P-450 activity in rats treated with CCl_4.
Details are the same as in the legend to Fig. 1. (A) : 24 hr after the CCl_4 injection, (B) : 48 hr after the CCl_4 injection. $^*p<0.05$, $^{**}p<0.01$ vs. control.

Discussion

Hepatic diseases are diagnosed by some serum indices, and serum transaminase activities are most popular among the indices. Transaminase activities well represent the necrosis of hepatocyte, while they are not adequit to diagnose the subsequent hepatocyte functional disorder, active chronic hepatitis and cirrhosis. After the improvement of drastical hepatocellular necrosis, the transaminase activities are quickly lowered. Recently, these chronic stages of hepatic diseases are clinically diagnosed by measuring prothrombin time. In this experiment prothrombin time and cytochrome P-450 activity which also show the activity of hepatic function were determined to know the hepatocyte functional disorder in a CCl_4-induced acute hepatic injury in addition to measure serum transaminase activities to know the hepatocyte necrosis.

It is clear that oral administration of Syô-saiko-tô was effective in reducing hepatocellular necrosis induced in rats by the single injection of CCl_4. The improvement of Syô-saiko-tô on the hepatic fibrosis induced in mice by repeated CCl_4 injection for 6 months as reported in our previous paper[1,9] is suggested to be due to its inhibitory action on hepatocellular necrosis. It is possible that Syô-saiko-tô inhibits the necroinflamation.[20] Since hepatotoxicity of CCl_4 is due to the action of its radical generation in the liver,[21] the possibility of reduced CCl_4 metabolism by Syô-saiko-tô is expected. In an experiment using mice,[1,9] Syô-saiko-tô, however, inhibited the hepatic necrosis induced by galactosamine whose hepatotoxic action is different from that of CCl_4.[22] By the intraperitoneal injection of Syô-saiko-tô, Kato *et al*,[10] Yamaura *et al*,[11] and Ota *et al*.[12] also reported its anti-hepatotoxic action using a galactosamine-induced hepatic injury model. These results suggest that Syô-saiko-tô has cytoprotective action. On the other hand, our experimental data show that Dai-saiko-tô is not effective on the hepatocyte necrosis. Ota *et al*.[14] reported that Dai-saiko-tô is not effective on the galactosamine-induced hepatic injury, but it is effective on the CCl_4-induced hepatic injury. They concluded the action of Dai-saiko-tô is due to the inhibition of CCl_4-dependent lipid peroxidation, the increase in hepatic ascorbic acid and the reduction of glutathione level. Disagreement of the results may be explained by the different extent of necrosis induced by CCl_4. sGOT and sGPT levels obtained by the intraperitoneal CCl_4 injection in their control group were higher than those obtained by the subcutaneous CCl_4 injection in our control group. In our experiment, sGOT and sGPT levels, 180 and 75 mU/ml serum respectively, may be too low to exhibit the inhibitory action for Dai-saiko-tô. One more reason expected is the difference of the doses of Dai-saiko-tô. The dose of Dai-saiko-tô in our experiment is 600 mg/kg which is almost equivalent to

a 5 times dose for a human per day, while their applied dose is 1 g/kg which is about twice as high as that which we used. Anyway, the anti-necrosis action of Dai-saiko-tô is markedly weaker than that of Syô-saiko-tô. Functional disorder of hepatocyte also increased by the treatment of CCl_4. The peak of sGOT and sGPT were shown 24 hr after the CCl_4 injection and lowered to normal level at 48 hr, while the elongation of prothrombin time and decrease of cytochrome P-450 activity lasted even at 48 hr, and their maximum and minimum level, respectively, were expected to appear after 48 hr. These data suggest the existence of a time lag between the development of hepatic necrosis and functional disorder. And, Syô-saiko-tô protected the hepatocyte functional disorder as well as hepatocyte necrosis judging from the indices used in our experiment. The protective action of Dai-saiko-tô on the hepatocyte functional disorder is very weak. It restored only the decreased cytochrome P-450 activity 48 hr after the CCl_4 injection.

Among components involved in Syô-saiko-tô, several saponins and their metabolites are known[23-36] and some of them are reported to possess membrane stabilizing action.[13] Most of those saponins are involved in *Bupleurum falcatum* root, *Panax ginseng* root, and *Glycyrrhiza glabra* root. *Bupleurum falcatum* root is a common composed drug of Syô-saiko-tô and Dai-saiko-tô. *Panax ginseng* root and *Glycyrrhiza glabra* root, however, are involved in Syô-saiko-tô only. Since Dai-saiko-tô showed no improvement on hepatic necrosis and functional disorder, saponins involved in *Panax ginseng* root and *Glycyrrhiza glabra* root are pharmacologically interested.

In conclusion, oral administration of Syô-saiko-tô may be beneficial for the treatment of necrosis and functional disorder of hepatocyte. Its inhibitory action on the hepatic fibrosis also may be due to the protecting activity against necrosis and functional disorder of hepatocyte. The fact that Dai-saiko-tô, which possesses a similar combination of composed herbal drugs to Syô-saiko-tô, showed no hepatocyte protecting action in this experiment and inhibited the fibrosis formation[1,9] suggest the existence of other inhibitory action mechanisms of both Kampô-hôzai on the development of hepatic fibrosis. Further study using hepatic fibrosis model which lacks hepatic necrosis in its developing stage is needed to explain the anti-fibrosis action mechanism of Syô-saiko-tô and Dai-saiko-tô.

和文抄録

小柴胡湯，大柴胡湯の四塩化炭素による肝細胞壊死，肝機能障害に対する効果を経口投与により検討した。小柴胡湯は，四塩化炭素投与24時間後の血清GOT，血清GPTの上昇を抑制し，さらに，肝機能障害の指標に選んだプロトロンビンタイムの延長，肝チトクロームP-450活性の低下を，四塩化炭素投与24時間及び48時間後に有意に改善した。しかし，大柴胡湯は，血清GOT，血清GPTの上昇，プロトロンビンタイムの延長を抑制しなかった。以上の結果は，小柴胡湯が，四塩化炭素による肝細胞壊死，肝機能障害を改善させることを示唆している。

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