Effect of Syô-saiko-tô and Dai-saiko-tô on experimental hepatic fibrosis in rats

Sakae AMAGAYA[a] Masakane HAYAKAWA[a] Yukio OGIHARA*[a] and Kenji FUJIWARA[b]

[a] *Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nagoya City University*
[b] *First Department of Internal Medicine, Faculty of Medicine, University of Tokyo*

(Received May 10, 1988. Accepted June 16, 1988.)

Abstract

Effect of Syô-saiko-tô and Dai-saiko-tô at a dose of 600 mg/kg body weight on hepatic fibrosis models induced by porcin serum (PS) and dimethylnitrosamine (DMA) were studied in rats. PS and DMA treatment markedly increased hepatic hydroxyproline content and prolonged the prothrombin time, while they showed no significant increase of serum glutamic pyruvic transaminase (sGPT) activity except the 1st month in PS model and the 6th week in DMA model. These data indicate that both fibrosis formations are not originated from the hepatocyte necroinflammation. By the administration of Syô-saiko-tô and Dai-saiko-tô, PS and DMA-induced increase of hydroxyproline content/g liver was inhibited, while the increase of hydroxyproline content/liver was slightly inhibited. This increase of hydroxyproline content/liver, however, was strongly inhibited by the pretreatment of Syô-saiko-tô for 3 months. In addition, both Kampô-hôzai restored the elongation of prothrombin time in both models. These results suggest that Syô-saiko-tô and Dai-saiko-tô directly inhibit hepatic fibrosis formation, and Syô-saiko-tô is more beneficial for the treatment of hepatic fibrosis than Dai-saiko-tô.

Key words Dai-saiko-tô, dimethylnitrosamine, hepatic fibrosis, porcin serum, Syô-saiko-tô.

Abbreviations CCl_4, carbon tetrachloride ; DMA, dimethylnitrosamine, GPT, glutamic pyruvic transaminase ; PS, porcin serum ; Dai-saiko-tô (Da-Chai-Hu-Tang) 大柴胡湯 ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Cirrhosis is the termination of chronic hepatitis and is developed irreversibly. Formation of cirrhosis will be due to the proliferation of connective tissue with inflammation and necrosis of hepatocyte, although its mechanism is not clarified yet. The method of clinical therapy of cirrhosis also was not advanced. In our previous paper, Syô-saiko-tô and Dai-saiko-tô (Kampô-hôzai : Japanese and Chinese traditional medicines) were reported to inhibit the hepatic fibrosis formation induced by the treatment of carbon tetrachloride (CCl_4) for 6 months.[1] Further, we reported that Syô-saiko-tô restored CCl_4-induced necrosis of hepatocytes. This antihepatotoxic action will inhibit the development of CCl_4-induced hepatic fibrosis. Dai-saiko-tô, however, was not effective on necrosis of hepatocytes.[2] These data suggest that Dai-saiko-tô inhibits hepatic fibrosis directly without inhibition of hepatic necroinflammation. Syô-saiko-tô, an inhibitor of hepatic necroinflammation, is also expected to possess similar action to Dai-saiko-tô because of the similar combination of the herbal crude drugs.

In this paper, to examine the direct inhibitory action of Syô-saiko-tô and Dai-saiko-tô on the hepatic fibrosis formation, new hepatic fibrosis

*〒 467 名古屋市瑞穂区田辺通 3-1
名古屋市立大学薬学部生薬学教室 荻原幸夫
3-1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 5, 137－145, 1988

animal models induced by porcin serum (PS)[3-5] and dimethylnitrosamine (DMA),[5-8] which are reported to induce hepatic fibrosis not caused by the hepatocyte necrosis, were applied.

Materials and Methods

Animals : Wistar rats, 5 weeks old (at the initial stage), were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). All animals were maintained on a standard diet and tap water *ad libitum*.

Reagents : Dimethylnitrosamine (DMA) was purchased from Nakarai Chem. Co., Ltd. (Kyoto Japan). Porcin serum was obtained from Nippon Bio-Supp Center (Tokyo, Japan). All other reagents were of analytical grade.

Preparation of Syô - saiko - tô and Dai - saiko - tô : Powdered extractes of Syô-saiko-tô and Dai-saiko-tô were prepared according to the method mentioned in our previous paper.[2] Human daily dose of the lyophilized extracts of Syô-saiko-tô and Dai-saiko-tô were 7.2 g and 8.0 g. In this experiment, a dose of 600 mg/kg body weight was dissolved in 2 ml distilled water and administered to rats using a stomach tube.

Assay of serum glutamic pyruvic transaminase (sGPT) activity : Blood, 20 μl, was collected from the veniplex of the eyeground of rats using hepatonized capillary tubes for microhematocrits (Terumo Co., Ltd., Tokyo, Japan). SGPT activity was assayed according to the Reitman and Frankel method[9] using transaminase kit (Eiken Chem. Co., Ltd., Tokyo, Japan).

Assay of prothrombin time[10] : Blood, 180 μl, was collected by cardicentesis in a 1-ml syringe containing 20 μl of 3.13% citric acid solution. 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, Meteku, Tokyo, Japan). All procedures were repeated three times and then averaged.

Assay of hepatic hydroxyproline : Hepatic hydroxyproline content was measured by the modified method[11] of Prockop,[12] after hydrolysis of the excised liver tissue in 6 N HCl at 110℃ for 22 hr.

Liver fibrosis model by PS[3-5] : PS, 0.5 ml, was injected twice a week intraperitoneally to rats. Each group consisted of 20−30 rats, maintained for 3 months and exanguinated monthly to get blood and liver. Syô-saiko-tô and Dai-saiko-tô were administered for 3 months and physiological saline was administered in the control group.

Liver fibrosis model by DMA[5-8] : DMA, 30 mg/kg, was injected once intraperitoneally to rats. Each group consisted of 20 − 30 rats. Blood and liver were removed at the 2nd, 4th, and 6th week by the exanguination. Syô-saiko-tô and Dai-saiko-tô were administered to rats for 6 weeks from the day of DMA injection. In the pretreatment experiment of Kampô-hôzai, they were administered from 1 month or 3 month before the DMA injection to the day of exanguination.

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

Results

Hepatic fibrosis induced by PS

Hepatic fibrosis was prepared by the injec-

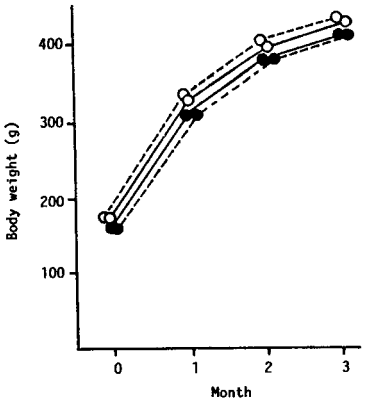

Fig. 1 Effect of Kampô-hôzai on body weight in PS-treated rats.
●···● : normal group, ●—● : PS treated control group, ○—○ : PS and Syô-saiko-tô treated group, ○···○ : PS and Dai-saiko-tô treated group. PS was injected twice a week for 3 months and Kampô-hôzai was administered every day for 3 months. Each point indicates the mean±S.E.M. of 5−9 rats.

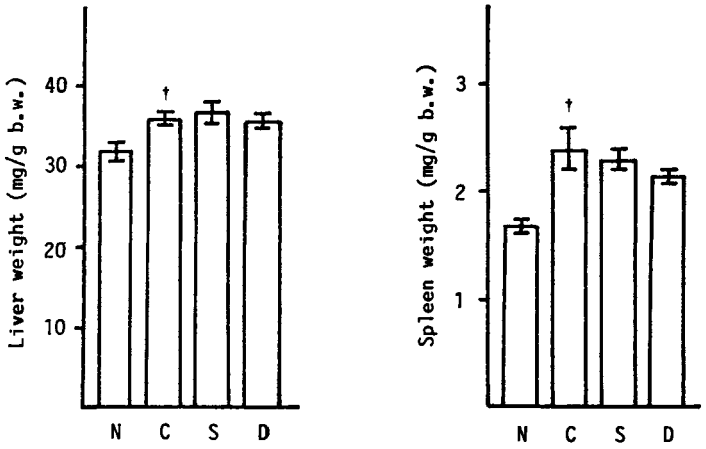

Fig. 2 Effect of Kampô-hôzai on liver and spleen weights in PS-treated rats.
N : normal group, C : PS treated control, S : PS and Syô-saiko-tô treated group, D : PS and Dai-saiko-tô treated group. Each column indicates the mean of 5−9 rats. Vertical bars indicate S.E.M. $^{\dagger}p<0.05$ vs. normal group.

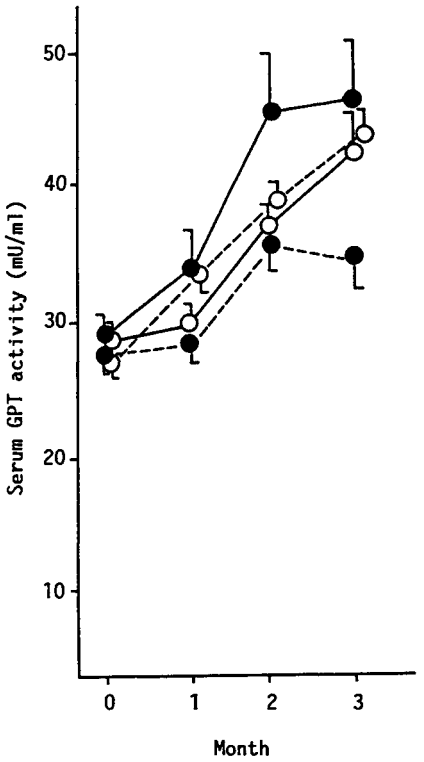

Fig. 3 Effect of Kampô-hôzai on sGPT activity in PS-treated rats.
●···● : normal group, ●—● : PS treated control group, ○—○ : PS and Syô-saiko-tô treated group, ○···○ : PS and Dai-saiko-tô treated group. Each point represents the mean±S.E.M. of 5−9 rats.

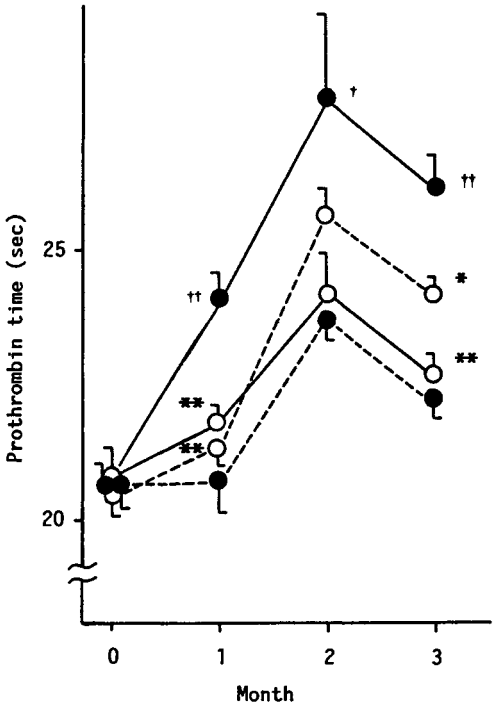

Fig. 4 Effect of Kampô-hôzai on prothrombin time in PS-treated rats.
●···● : normal group, ●—● : PS treated control group, ○—○ : PS and Syô-saiko-tô treated group, ○···○ : PS and Dai-saiko-tô treated group. Each point indicates the mean±S.E.M. of 5−9 rats. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. normal group, $^{*}p<0.05$, $^{**}p<0.01$ vs. control group.

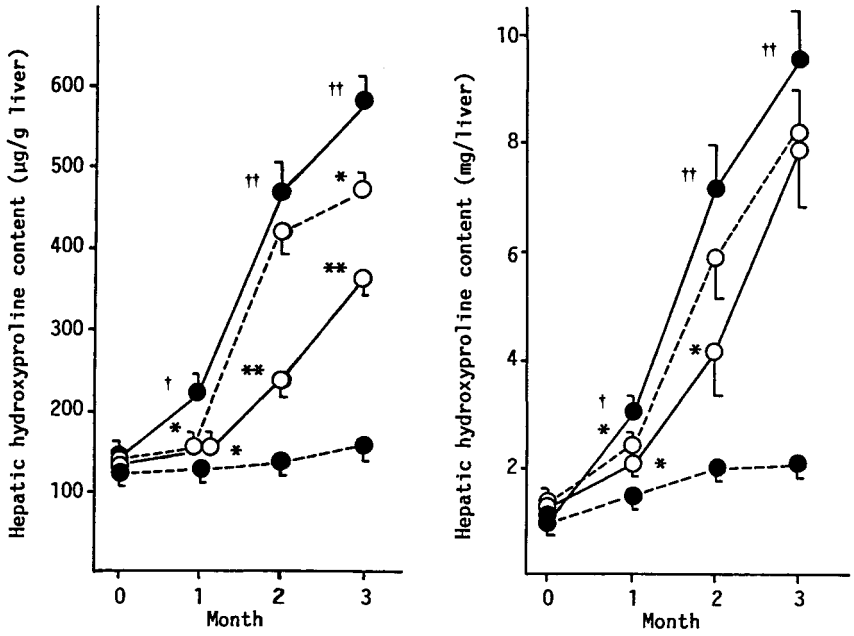

Fig. 5 Effect of Kampô-hôzai on hepatic hydroxyproline content in PS-treated rats.
●···● : normal group, ●—● : PS treated control group, ○—○ : PS and Syô-saiko-tô treated group, ○···○ : PS and Dai-saiko-tô treated group. Each point indicates the mean±S.E.M. of 5−9 rats. †$p<0.05$, ††$p<0.01$ vs. normal group, *$p<0.05$, **$p<0.01$ vs. control group.

tion of PS. As shown in Fig. 1, the increase of body weight was not changed by the injection of PS, and Kampô-hôzai showed no effect on body weight. Fig. 2 shows the liver and spleen weights. Although the liver and spleen weights/g body weight were increased in control rats, Kampô-hôzai had no effect on the increased liver and spleen weights. As shown in Fig. 3, sGPT activity showed the tendency to increase at the 3rd month by the treatment of PS, but both Kampô-hôzai never showed significant effect on sGPT activity. Prothrombin time was remarkably prolonged by PS throughout the experimental period as shown in Fig. 4. Syô-saiko-tô and Dai-saiko-tô inhibited the elongation of prothrombin time at the 1st and 3rd month and showed the tendency to inhibit it at the 2nd month. Fig. 5 shows the hepatic hydroxyproline content. Hydroxyproline content increased remarkably by the treatment of PS. When Syô-saiko-tô was treated, the increase of hydroxyproline content/g liver was inhibited throughout the

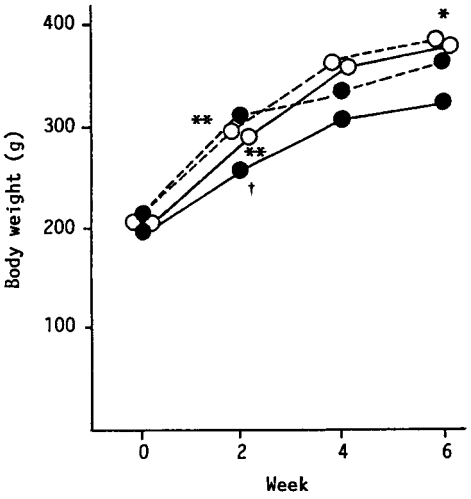

Fig. 6 Effect of Kampô-hôzai on body weight in DMA-treated rats.
●···● : normal group, ●—● : DMA treated control group, ○—○ : DMA and Syô-saiko-tô treated group, ○···○ : DMA and Dai-saiko-tô treated group. DMA was injected at day 0 and Kampô-hôzai was administered every day for 6 weeks. Each point indicates the mean± S.E.M. of 6−8 rats. †$p<0.01$ vs. normal group, *$p<0.05$, **$p<0.01$ vs. control group.

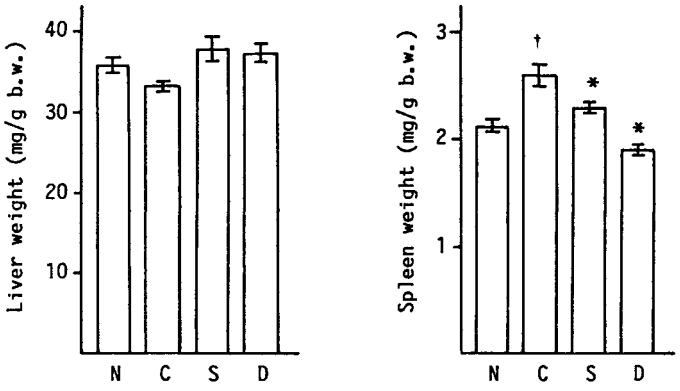

Fig. 7 Effect of Kampô-hôzai on liver and spleen weights in DMA-treated rats.
N : normal group, C : DMA treated control, S : DMA and Syô-saiko-tô treated group, D : DMA and Dai-saiko-tô treated group. Each column indicates the mean of 6−8 rats. Vertical bars indicate S.E.M. †$p<0.05$ vs. normal group, *$p<0.05$ vs. control group.

experimental period and that of hydroxyproline content/liver was also inhibited at the 1st and 2nd month. The inhibitory action of Dai-saiko-tô on the increase of hydroxyproline was milder than that of Syô-saiko-tô.

Hepatic fibrosis induced by DMA

Another liver fibrosis model was prepared by the single injection of DMA. As shown in Fig. 6, the increase of body weight showed the tendency to decrease by the treatment of DMA and both Kampô-hôzai restored the DMA-induced decrease of body weight at the 2nd week. Furthermore, they showed the tendency to restore the decrease of body weight at the 4th and 6th week. Fig. 7 shows the liver and spleen weights. DMA showed no effect on liver weight/g body weight, while it increased spleen weight/g body weight. Syô-saiko-tô and Dai-saiko-tô showed no effect on the liver weight, but they inhibited the increase of spleen weight/g body weight. As shown in Fig. 8, sGPT activity was enhanced by the treatment of DMA only at the 2nd week and it was decreased to the normal level at the 4th and 6th week. In Syô-saiko-tô treated group, the increase of sGPT activity at the 2nd week was inhibited. Dai-saiko-tô, however, showed no inhibitory action at the 2nd week. They showed no effect on the sGPT activities at the 4th and

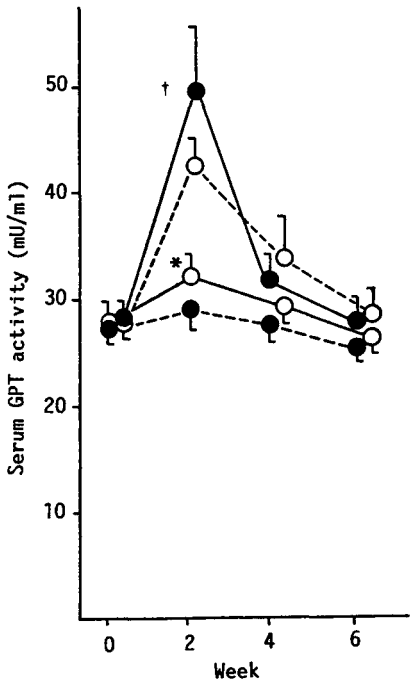

Fig. 8 Effect of Kampô-hôzai on sGPT activity in DMA-treated rats.
●···● : normal group, ●—● : DMA treated control group, ○—○ : DMA and Syô-saiko-tô treated group, ○···○ : DMA and Dai-saiko-tô treated group. Each point indicates the mean±S.E.M. of 6−8 rats. †$p<0.05$ vs. normal group, *$p<0.05$ vs. control group.

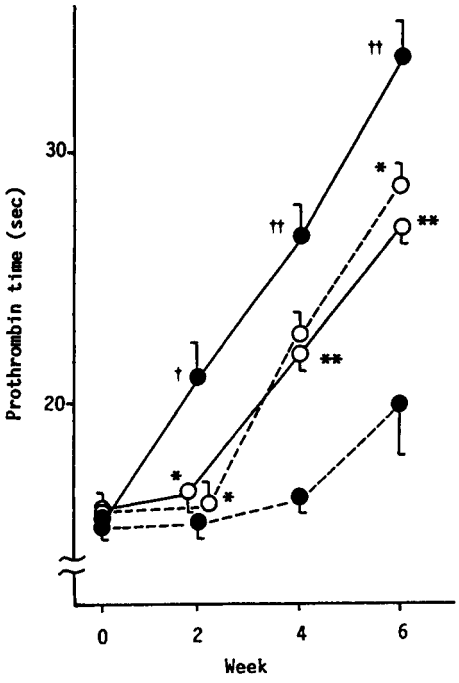

Fig. 9 Effect of Kampô-hôzai on prothrombin time in DMA-treated rats.
●···● : normal group, ●—● : DMA treated control group, ○—○ : DMA and Syô-saiko-tô treated group, ○···○ : DMA and Dai-saiko-tô treated group. Each point indicates the mean±S.E.M. of 6−8 rats. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ vs. normal group, $^{*}p<0.05$, $^{**}p<0.01$ vs. control group.

6th week. Prothrombin time was prolonged by DMA throughout the experimental period and both Kampô-hôzai inhibited it as shown in Fig. 9. The results of hepatic hydroxyproline content are shown in Fig. 10. By the treatment of DMA, hepatic hydroxyproline content increased and reached the maximum level at the 6th week. When Syô-saiko-tô and Dai-saiko-tô were administered, the increase of hydroxyproline content/g liver was inhibited throughout the experimental period. However, both Kampô-hôzai were inactive on the increase of hydroxyproline content/liver except at the 4th week. Therefore, the effect of the pretreatment of Syô-saiko-tô and Dai-saiko-tô on the fibrosis formation induced by DMA was investigated. As shown in Fig. 11, the pretreatment of Syô-saiko-tô for 1 or 3 months inhibited the increase of both hydroxyproline content/g liver and hydroxyproline content/ liver ($p < 0.1$ by the pretreatment for 1 month), while Dai-saiko-tô inhibited only the increase of hydroxyproline content/g liver by its pretreatment for 3 months. Further, pretreatment of Syô-saiko-tô and Dai-saiko-tô inhibited the elongation of prothrombin time in a significant manner.

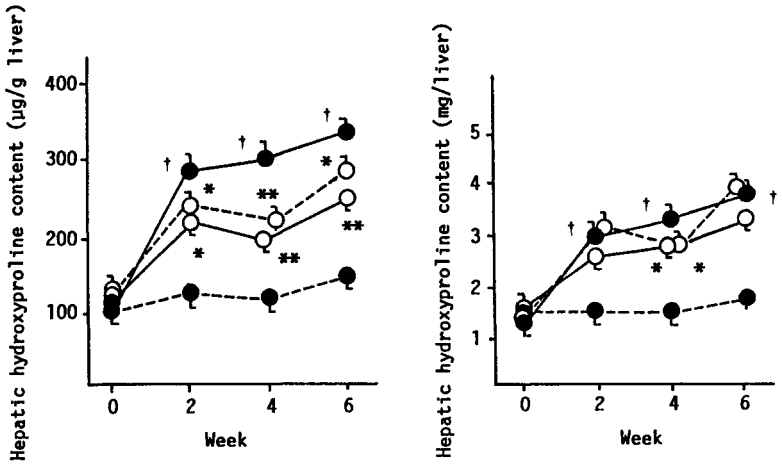

Fig. 10 Effect of Kampô-hôzai on hepatic hydroxyproline content in DMA-treated rats.
●···● : normal group, ●—● : DMA treated control group, ○—○ : DMA and Syô-saiko-tô treated group, ○···○ : DMA and Dai-saiko-tô treated group. Each point indicates the mean±S.E.M. of 6−8 rats. $^{\dagger}p<0.01$ vs. normal group, $^{*}p<0.05$, $^{**}p<0.01$ vs. control group.

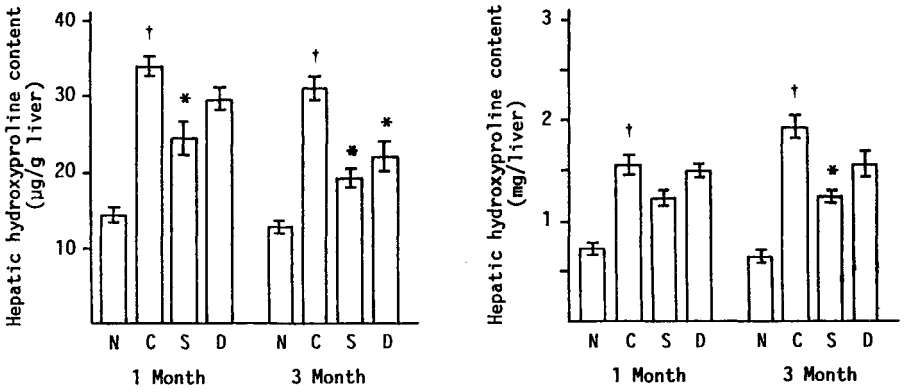

Fig. 11 Effect of pretreatment of Kampô-hôzai on the heptic hydroxyproline content in DMA-treated rats.

Kampô-hôzai was treated from 1 month or 3 month before the DMA-injection and hydroxyproline content was determined at the 4th week after the DMA-injection. N : normal group, C : DMA treated control group, S : DMA and Syô-saiko-tô treated group, D : DMA and Dai-saiko-tô treated group. Each column indicates the mean of 6－8 rats. Vertical bars indicate S.E.M. $^{\dagger}p<0.01$ vs. normal group, $^{*}p<0.01$ vs. control group.

Discussion

Although the mechanism of the occurrence of cirrhosis is unknown, the fibrosis formation is one of the important phenomenon in the process of the development of cirrhosis and it is proportional to the development of cirrhosis. These show the possibility that the inhibition of hepatic fibrosis is expected partially to contribute to the restoration of cirrhosis. On the other hand, Syô-saiko-tô and some other Kampô-hôzai are recently reported to cure the chronic hepatitis and cirrhosis,[13,14] and have begun to be applied widely for the treatment of hepatitis in Japan. But, their action mechanisms are not clarified completely. Therefore, we investigated the effect of Syô-saiko-tô and Dai-saiko-tô on the experimental fibrosis models. In our previous paper,[1] Syô-saiko-tô and Dai-saiko-tô inhibited the hepatic fibrosis formation induced by the long term (6 months) treatment of CCl_4 and two mechanisms of their inhibitory actions were expected. One is the inhibition of necrosis of hepatocyte, whose chronic development induces hepatic fibrosis. The other is the direct inhibition on fibrosis formation. We reported that Syô-saiko-tô at a dose of 600 mg/kg strongly inhibited CCl_4-induced necrosis of hepatocyte, and the same dose of Dai-saiko-tô showed no effect on CCl_4-induced necrosis of hepatocyte.[2] These results suggest that the inhibitory effect of Syô-saiko-tô on the fibrosis formation is partially due to its inhibition on hepatic necroinflammation, and that of Dai-saiko-tô is expected to inhibit the hepatic fibrosis directly. Syô-saiko-tô is also expected to possess direct anti-fibrosis action because of its similar combination of crude drugs to Dai-saiko-tô. In this paper, to explain the above questions the effect of Syô-saiko-tô and Dai-saiko-tô on the fibrosis models induced by DMA and PS were studied. DMA and PS-induced fibrosis were reported to form by the inhibition of collagenase activity and stimulation of prolylhydroxylase activity, respectively,[5] and they are not concerned in the hepatocyte necroinflammation.[3-8] By the injection of DMA, sGPT activity was not changed at the 4th and 6th week, while it increased at the 2nd week. Hepatic hydroxyproline content, however, increased straightly from the 2nd to 6th week. These results suggest that only in an early stage of liver fibrosis formation DMA induces the necrosis of hepatocyte and the fibrosis formatiom observed in latter stage never need the necroin-

flamation of hepatocyte. By the injection of PS, sGPT activity was slightly increased in the latter stage of the experimental period. These suggest that the necrosis of the hepatocyte may be partially participated in the PS-induced hepatic fibrosis, although the increased sGPT levels were remarkably lower than those induced by CCl_4 injection.[2] In the experimental model induced by DMA, Syô-saiko-tô inhibited the fibrosis formation even at the 4th and 6th week when the transaminase activity did not increase. This shows that Syô-saiko-tô inhibits the fibrosis formation directly. Inhibitory effect of Syô-saiko-tô on the PS-induced hepatic fibrosis also supports this direct antifibrosis action. Moreover, the stronger inhibition of Syô-saiko-tô by its pretreatment for 1 or 3 months on the hydroxyproline content/liver suggests that Syô-saiko-tô increases the energy of resistance to the toxins in the body to keep the homaeostasis normal. Furthermore, Syô-saiko-tô inhibited the elongation of prothrombin time remarkably in both DMA and PS-treated fibrosis models. Since prothrombin time indicates the degree of hepatic functional disorder which may be induced from the development of hepatocyte fibrosis, the improvement of prothrombin time by Syô-saiko-tô also indicates the anti-fibrosis action of Syô-saiko-tô. The inhibitory action of Syô-saiko-tô on hepatocyte necrosis, which was reported in our previous paper,[2] suggest that Syô-saiko-tô stabilizes hepatocyte membrane. This membrane stabilization effect may be derived from the actions of saponins,[15] ingredients of Syô-saiko-tô. From these results, the inhibition of Syô-saiko-tô on the fibrosis formation could be explained by its plural actions which were the protection of the hepatocyte functions and the inhibitions on both necrosis of hepatocyte and fibrosis formation. On the other hand, the inhibitory action of Dai-saiko-tô on the DMA and PS-induced hepatic fibrosis suggests that Dai-saiko-tô also possess direct antifibrosis action, although it never inhibited the hepatic necroinflammation. The milder antifibrosis action of Dai-saiko-tô than that of Syô-saiko-tô may be explained by the lack of antinecrosis action in Dai-saiko-tô. In an hepatic necrosis model Dai-saiko-tô never improved the elongation of prothrombin time,[2] while it improved the elongation of prothrombin time in a chronic fibrosis model. This phenomenon suggests the difference between the mechanism in developing the hepatic disorder induced by necrosis and that induced by fibrosis. The direct inhibitory mechanisms of Syô-saiko-tô and Dai-saiko-tô on the formation of fibrosis are not known and further study will be needed in future.

和文抄録

小柴胡湯及び大柴胡湯 600 mg/kg を経口投与し，porcin serum (PS) 及び dimethylnitrosamine (DMA) により作製したラット肝線維化に対する効果を検討した。PS 及び DMA 処理により，肝 collagen の指標である hydroxyproline 含量の上昇及び肝機能の指標である prothrombin time の延長が認められたが，肝細胞壊死の指標となる血清 GPT 活性は，PS モデルの 1 カ月目，DMA モデルの 6 週間目を除いて上昇しなかった。これらの事実は，今回検討した肝線維化モデルが肝細胞壊死に由来しない発症機序を有していることを示唆している。そこで，両線維肝モデルに対する小柴胡湯，大柴胡湯の効果を検討してみると，両方剤とも肝臓 1 g 当りの hydroxyproline 含量増加を有意に抑制したが，肝臓当りの hydroxyproline 含量増加に対しては強い抑制を示さなかった。そこで，両漢方方剤の 1 カ月及び 3 カ月間前投与による効果を検討してみると，小柴胡湯に肝臓当りの hydroxyproline 含量増加抑制が認められた。また，両線維肝モデルにおける prothrombin time の延長は，両方剤により有意に抑制された。これらの結果より，小柴胡湯，大柴胡湯に肝細胞壊死抑制を介さない肝線維化抑制作用を有することが示唆され，特に小柴胡湯により強い抑制作用が確認された。

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