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Effects of Sho-saiko-to Extract on Liver Fibrosis in Relation to the Changes in Hydroxyproline and Retinoid Levels of the Liver in Rats

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Abstract

To examine the effects of Sho-saiko-to extract on liver fibrosis, the drug was administered to rats with dimethylnitrosamine-induced liver-injury at various doses. Hydroxyproline and retinoid levels in the liver were measured as indicators of liver function.

In liver-injured rats, the hydroxyproline level in the liver (957 ± 154 nmol g^{-1}) was about 4·16-times that found in normal liver (230 ± 11 nmol g^{-1}), but administration of Sho-saiko-to extract (0·75%, 1·5% or 3%) reduced the hydroxyproline level significantly (554 ± 58, 356 ± 51, 374 ± 66 nmol g^{-1}, $P < 0·01$). Single administration of the active constituents of Sho-saiko-to extract, glycyrrhizin, baicalin or baicalein, decreased the hydroxyproline level significantly compared with the ordinary food group ($P < 0·05$), but the decrease was smaller compared with the Sho-saiko-to extract group. The liver retinoid level was higher in the Sho-saiko-to extract group than the ordinary food group and the value increased dose-dependently. A significant negative correlation, $r = -0·814$ ($P < 0·001$) was detected between the hydroxyproline level and retinoid level in the liver of liver-injured rats. Significant negative correlations, $r = -0·728$ ($P < 0·001$) and $r = -0·873$ ($P < 0·001$), were also detected between the liver hydroxyproline level and the liver concentrations of the active constituents (glycyrretic acid, baicalin and baicalein) in the liver-injured rats.

From these findings, it was considered that the liver concentrations of hydroxyproline and retinoid as well as the active constituents were involved in the improvement of liver fibrosis in the liver-injured rats administered Sho-saiko-to extract. Administration of Sho-saiko-to extract inhibited collagen production while an increase in retinoid level inhibited activation of Ito cells leading to inhibition and prevention of liver fibrosis.

Prevention or suppression of liver fibrosis caused by chronic hepatitis to protect and treat liver cirrhosis and cancer are important. Hepatocirrhosis can be explained as the disturbed control of the proliferation of parenchymal and non-parenchymal cells in the liver (Miyazawa 1993). Therefore, various attempts have been made to prevent or treat hepatocirrhosis by controlling cell proliferation. We reported previously that Sho-saiko-to extract was useful for suppressing Ito cell activation, the cause of liver fibrosis, in rats with liver injury (Miyamura et al 1998). Ito cells are found in the space of Disse in the liver, and function in intact liver lobules as primary storage areas for retinoids, mainly retinol palmitate (Yumoto 1982). In injured liver the Ito cells are activated by cytokines, for instance transforming growth factor β, and release the liver retinoid and produce extracellular matrix components such as collagen (Rojkind et al 1979; Enzan 1992).

In this study we have examined the suppressive effect of Sho-saiko-to extract on Ito cell activation, and the levels of retinoid (the major factor for activation) and hydroxyproline (a collagen-specific

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amino acid) in the liver (McCullough et al 1987; Enzan 1992). The changes in production of retinoid and hydroxyproline after the administration of Sho-saiko-to extract were studied to elucidate the mechanism by which the extract can improve liver fibrosis.

Materials and Methods

Animals
Male Wistar rats, aged seven weeks, 180–200 g, were purchased from NSC Japan. Animals were acclimatized for seven days at $23 \pm 2^{\circ}$C with free access to pellet food (CE-2, Crea Japan) and water. Healthy rats were then selected and seven animals assigned to each group.

Materials
Dimethylnitrosamine was from Nacarai Tesque. Glycyrrhizin, glycyrrhetic acid, baicalin and baicalein for assaying the herbal preparation were from Nacarai Tesque. Sho-saiko-to extract was from Tsumura. Other reagents were extra-pure grade from commercial sources.

Preparation of liver-injured rats
After acclimatization, rats were given an intraperitoneal injection of 35 mg kg^{-1} dimethylnitrosamine.

Administration of Sho-saiko-to extract, glycyrrhizin, baicalin and baicalein
Sho-saiko-to extract was added to the pellet food at concentrations of 0·75%, 1·5% or 3·0%. Seven days after dimethylnitrosamine administration, the liver-injured animals were allocated to either the Sho-saiko-to extract group, fed the Sho-saiko-to-treated food, or the normal group, fed ordinary food.

To administer the active constituents of Sho-saiko-to to the animals, glycyrrhizin, baicalin or baicalein was added to the ordinary pellet food at concentrations similar to the Sho-saiko-to extract.

Measurement of hydroxyproline concentration in the liver
Removed liver (about 0·2 g) was weighed and homogenized in 5 mL ethanol by a cell-homogenizer (Eilard, X10/20 model) at 10 000 rev min^{-1} for 5 min in an ice bath. The homogenate was centrifuged (3500 rev min^{-1}, 20 min) and the supernatant was collected. The amount of hydroxyproline in the liver was determined following an HPLC method reported by Sugano et al (1991).

Determination of retinoid concentration in the liver
Retinol palmitate in the liver was measured and expressed as retinoid concentration (Miyamura et al 1998).

Measurement of active constituents in the liver
Although the concentration of glycyrrhizin measured as the active constituent of Sho-saiko-to in the liver was below the detection limit, glycyrrhetic acid (an aglycon) was measured. The liver concentrations of baicalin and baicalein were measured and expressed as combined molarities. This is because Uchida et al (1995) reported their interconversion by in-vivo enzymes.

The amount of each active constituent in the liver was determined by HPLC methods reported previously (Nishioka et al 1992; Miyamura et al 1996).

Statistical analysis
Comparison of results from different groups was performed by one-way analysis of variance. Where there was significant variance, the means were compared by the Bonferroni method. Correlation coefficient was examined by the *t*-test.

Results

Effects of Sho-saiko-to extract on liver concentration of hydroxyproline
The hydroxyproline concentrations in rat liver after administration of Sho-saiko-to-treated or ordinary food for 14 days are shown in Table 1.

Hydroxyproline concentration in the liver of liver-injured rats fed ordinary food was about 4·16-times that in the normal rats. The concentration of

Table 1. Effect of oral administration for 14 days of Sho-saiko-to extract on the hydroxyproline level in the liver of rats.

Treatment	Dose of Sho-saiko-to extract (%)	Hydroxyproline (nmol g^{-1})
Normal rats		
Ordinary food	0·00	230 ± 11*†
Liver-injured rats		
Ordinary food	0·00	957 ± 154†
Sho-saiko-to extract	0·75	554 ± 58*†
	1·50	356 ± 51*
	3·00	374 ± 66*

Each value represents the mean ± s.e. of seven experiments. *$P < 0.05$, compared with the ordinary food groups of liver-injured rats. †$P < 0.05$, compared with the 3·0%-dose liver-injured rats.

Table 2. Effect of oral administration for 14 days of different concentrations of the active constituents of Sho-saiko-to extract on the liver hydroxyproline level in liver-injured rats.

Treatment	Dose of active constituent (%)	Hydroxyproline (nmol g^{-1})
Ordinary food	0·00	957 ± 154
Glycyrrhizin	0·75	636 ± 27*
	1·50	542 ± 44**
	3·00	557 ± 49**
Baicalin	0·75	771 ± 70*
	1·50	668 ± 70**
	3·00	679 ± 59**
Baicalein	0·75	760 ± 47**
	1·50	703 ± 46**
	3·00	696 ± 71**

Each value represents the mean ± s.e. of seven experiments. *$P < 0·05$, **$P < 0·01$, compared with the ordinary food group.

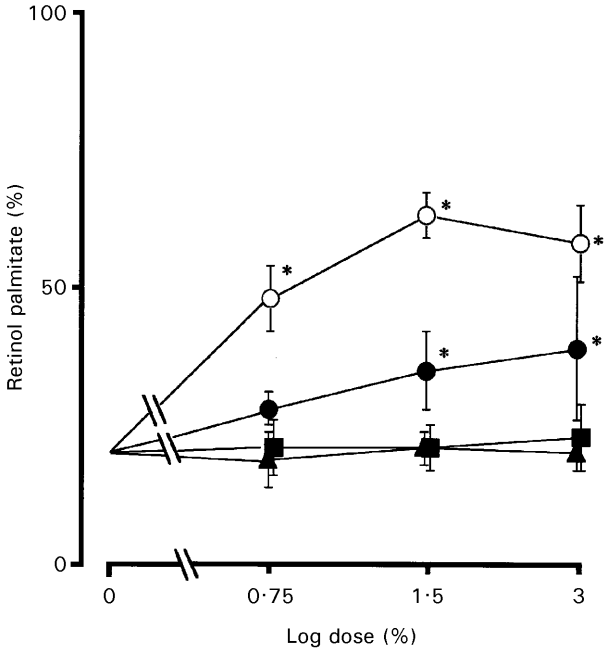

Figure 1. Effect of oral administration of different doses of Sho-saiko-to extract or its active constituents on liver retinol palmitate level (%) in liver-injured rats for 14 days. ○ Sho-saiko-to extract, ● glycyrrhizin, ▲ baicalin, ■ baicalein. Each value represents the mean ± s.e. of seven experiments. * $P < 0·05$, compared with the result for 0% dose (ordinary food group).

liver hydroxyproline decreased in the liver-injured rats administered Sho-saiko-to extract. The rate of decrease was dependent on the dose of Sho-saiko-to extract. However, no significant difference was detected between the 1·5% and 3% doses ($P < 0·05$).

Effects of the active constituents on liver concentration of hydroxyproline

The hydroxyproline concentrations in rat liver after administration of the active constituents of Sho-saiko-to extract or ordinary food for 14 days are shown in Table 2.

Liver hydroxyproline concentrations of the active constituent group were lower than the ordinary food group. In the glycyrrhizin food group the liver hydroxyproline level was lower than that in the groups given the other active constituents. The liver hydroxyproline concentration was higher in the groups given active constituents than the groups administered the same dose of Sho-saiko-to extract (Table 1).

Effects of Sho-saiko-to extract or active constituents on retinoid concentration in the liver

The retinoid concentrations in rat liver after administration of Sho-saiko-to extract, active constituents or ordinary food for 14 days are shown in Figure 1.

Liver retinoid concentration was expressed as a percentage of the liver retinoid level in normal rats measured shortly after the end of acclimatization, set as 100%.

In liver-injured rats, the concentration of liver retinoid in the ordinary group (0% dose) was very low (21%). In the Sho-saiko-to extract food group, the concentration of liver retinoid increased 2·23–2·95-times compared with the ordinary food group.

Liver retinoid concentrations increased 1·29–1·81-times in the group receiving glycyrrhizin compared with the ordinary food group. However, the value was lower than in the groups administered Sho-saiko-to extract. In the groups receiving baicalin or baicalein, there was no difference from the group receiving the ordinary food.

Concentrations of active constituents in the liver

The liver concentrations of glycyrrhetic acid, baicalin and baicalein after administration of Sho-saiko-to extract or active constituents for 14 days are shown in Figure 2.

The liver concentration of glycyrrhetic acid was higher in the group receiving Sho-saiko-to extract than in the group receiving glycyrrhizin ($P < 0·01$). Groups receiving Sho-saiko-to extract or glycyrrhizin tended to show a dose-dependent increase in the liver concentrations of glycyrrhetic acid (143–218 nmol g^{-1}, Sho-saiko-to group; 47–98 nmol g^{-1}, glycyrrhizin group). However, no

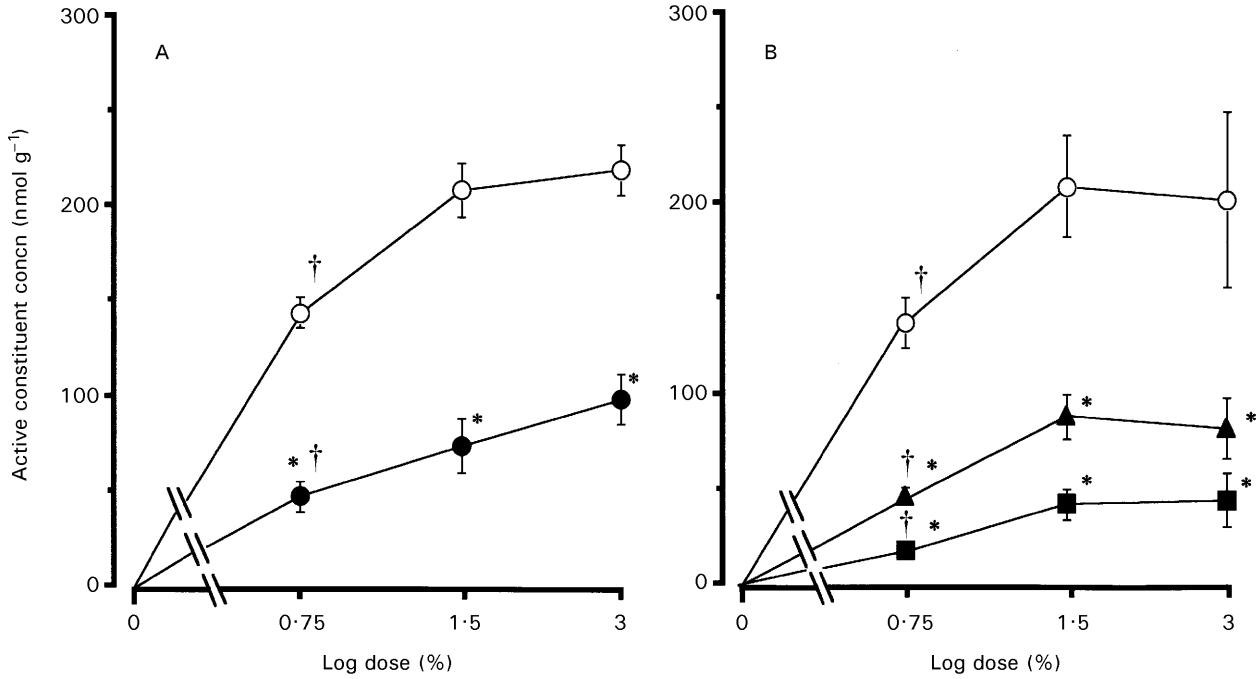

Figure 2. Effect of oral administration of different doses of Sho-saiko-to extract or its active constituents on concentrations of active constituents of the liver in liver-injured rats for 14 days. A. Glycyrrhetic acid, B. baicalin and baicalein. ○ Sho-saiko-to extract, ● glycyrrhizin, ▲ baicalin, ■ baicalein. Each value represents the mean ± s.e. of seven experiments. *$P < 0.01$, compared with the result for Sho-saiko-to extract. †$P < 0.01$, compared with the result for 3% dose.

significant difference was detected between the groups receiving doses of 1·5% and 3% ($P < 0.01$).

Liver concentrations of baicalin and baicalein were higher in the group receiving Sho-saiko-to extract than in the groups receiving baicalin or baicalein ($P < 0.01$).

The groups given Sho-saiko-to extract, baicalin or baicalein tended to show a dose-dependent increase in liver glycyrrhetic acid concentration (137–209 nmol g^{-1}, Sho-saiko-to group; 46–88 nmol g^{-1}, baicalin group; 18–44 nmol g^{-1}, baicalein group). However, no significant difference was detected between the groups receiving doses of 1·5% and 3% ($P < 0.01$).

Relationship between liver concentrations of hydroxyproline and retinoid

Figure 3 shows the relationship between liver concentrations of hydroxyproline and retinoid after administration of Sho-saiko-to extract and the respective active constituents for 14 days to liver-injured rats.. A significant negative correlation of $r = -0.814$ ($P < 0.001$) was shown.

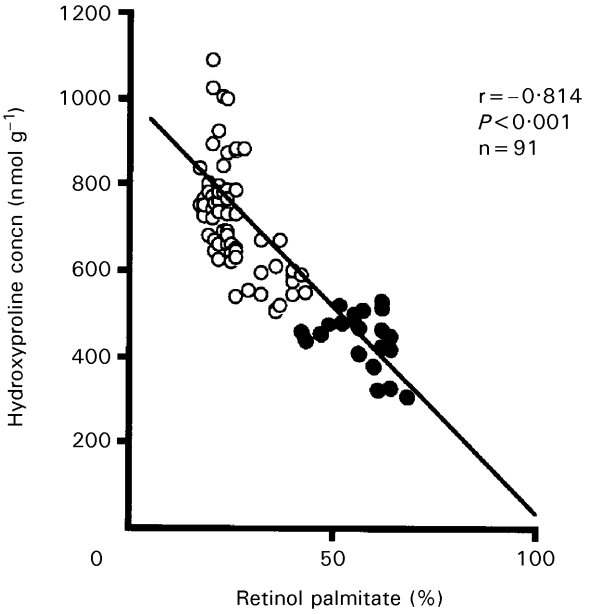

Figure 3. Correlation of liver hydroxyproline and liver retinol palmitate after oral administration of foods for 14 days in liver-injured rats. ○ Ordinary food and active constituent groups, ● Sho-saiko-to extract groups.

Relationship between liver concentrations of hydroxyproline and active constituents

Figure 4 shows the relationship between liver concentrations of hydroxyproline and active constituents of Sho-saiko-to extract after administration of the extract and the respective active constituents for 14 days. Significant negative correlations of $r = -0.728$ ($P < 0.001$) and $r = -0.873$ ($P < 0.001$) were shown between the liver con-

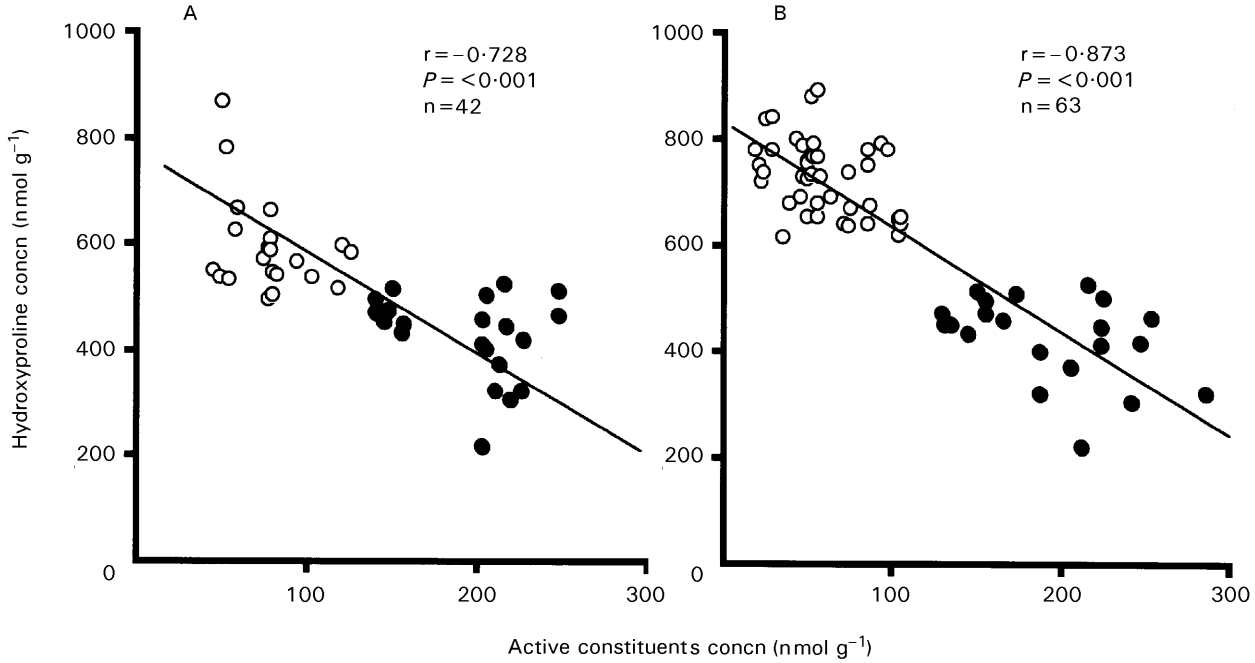

Figure 4. Correlation of liver hydroxyproline and liver active constituent level after oral administration of foods for 14 days in liver-injured rats. A. Glycyrrhetic acid, B. baicalin and baicalein. ○ Active constituent groups, ● Sho-saiko-to extract groups.

centration of hydroxyproline and that of glycyrrhetic acid and that of baicalin and baicalein, respectively.

Discussion

About 80% of liver cells are parenchymal cells and the remaining 20% are occupied by connective tissue cells such as Glisson's sheath cells and sinusoid wall cells. However, these connective tissue components in the remaining 20%, in particular the extracellular matrix component, are known to participate in the formation of hepatocytes, the control of their proliferation and their expression of function. It is also known that nonparenchymal Ito cells accelerate production of this extracellular matrix at the time of liver injury (Takahara et al 1988). Thus, control and prevention of liver fibrosis depends greatly on controlling activation of Ito cells.

In this study, we have examined liver concentration of retinoid as an indicator of the activation of Ito cells and that of hydroxyproline in collagen as an indicator of the extracellular matrix. The relationship between the suppressive effect of Sho-saiko-to extract on Ito cell activation and suppression of extracellular matrix production has been elucidated.

Hydroxyproline liver concentration in liver-injured rats decreased significantly in the group receiving Sho-saiko-to extract compared with the ordinary food group. The effect was dose-dependent. This result demonstrated that the administration of Sho-saiko-to extract would inhibit collagen production and, thus, would be useful for improving liver fibrosis. However, there was no significant difference between the groups receiving Sho-saiko-to extract at doses of 1·5 and 3%, indicating the necessity for further study of the dosage. In the groups receiving active constituents of the extract, the liver concentration of hydroxyproline was decreased compared with the group receiving ordinary food, although the rate of decrease was smaller than that shown in the Sho-saiko-to extract group. This indicated participation of the active constituents in lowering hydroxyproline level in the liver and, hence, lowering the collagen production. Of the groups receiving active constituents, the groups receiving glycyrrhizin showed the greatest decrease in the liver hydroxyproline level but the degree was still smaller than that shown in the Sho-saiko-to extract group. Thus, it was considered that the effect on the liver hydroxyproline level was not derived from glycyrrhizin alone but attributable to mutual actions of the active constituents.

Liver concentration of retinoid in the liver-injured rats increased significantly in the group

receiving Sho-saiko-to extract compared with the ordinary food group and the effect was dose-dependent. Among the groups receiving active constituents of the extract, the group receiving glycyrrhizin showed an increase in liver retinoid level but the degree of increase was smaller than that in the Sho-saiko-to extract group. Thus, administration of Sho-saiko-to extract resulted in an increase in liver retinoid and a decrease in liver hydroxyproline indicating some relationship between the two phenomena. When the relationship between the two substances was examined, a significant negative correlation was shown. This result was considered to mean that Sho-saiko-to improved liver fibrosis by promoting the increase in liver retinoid levels and, as a result, decreased the hydroxyproline levels, or collagen production. It was also assumed that advancement of liver fibrosis was predictable by measuring these two compounds.

The liver concentrations of the active constituents of Sho-saiko-to extract in the liver-injured rats increased significantly in the group receiving Sho-saiko-to extract compared with the groups receiving the respective constituents and the increase was dose-dependent. As a negative correlation was detected between the liver hydroxyproline level and the concentrations of the active constituents in the liver, it was suggested that the decrease in collagen production was influenced by the increased concentrations of the active constituents in the liver.

These results suggested that the levels of liver retinoid and hydroxyproline were associated with the improvement of liver fibrosis in the liver-injured rats receiving Sho-saiko-to extract. Sho-saiko-to extract suppressed the activation of Ito cells in liver-injured rats. To increase the liver retinoid level and decrease the hydroxyproline level, or lower collagen production, in liver-injured rats, it was shown that it was more useful to administer Sho-saiko-to extract than to administer its active constituents individually.

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