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Effects of Sho-saiko-to Extract on Fibrosis and Regeneration of the Liver in Rats

MITSUHIKO MIYAMURA, MASAHIDE ONO, SHOJIRO KYOTANI AND YUTAKA NISHIOKA

Department of Pharmacy, Kochi Medical School Hospital, Kohasu, Okoh-cho, Nankoku, Kochi 783, Japan

Abstract

Sho-saiko-to, one of the most widely used Chinese herbal preparations, has long been used for the treatment of chronic liver diseases. We have investigated its effect in retarding the process of liver fibrosis and accelerating liver regeneration, especially its effect on Ito cells that are thought to be deeply involved with liver fibrosis.

Sho-saiko-to extract and its active constituents were orally administered to rats with dimethylnitrosamine-induced liver-injury. After treatment with sho-saiko-to extract hepatic function improved, histopathological results confirmed repair of liver tissue, and retinoid levels increased. On the other hand, when active constituents of sho-saiko-to extract were administered alone, liver retinoid levels remained low, implying that interaction among active constituents of the extract was suppressing Ito cell activation. When sho-saiko-to extract was administered to 70% hepatectomized normal and liver-injured rats, liver weight, the number of S-phase-cells and retinoid levels increased with time. However, these changes were different for normal and liver-injured rats, suggesting that the site of action of sho-saiko-to extract in regenerating liver is different for normal and liver-injured rats.

These results show that sho-saiko-to extract was useful for suppressing the activation of Ito cells.

Recently, the number of cases of liver fibrosis caused by chronic hepatitis, i.e. hepatocirrhosis, has tended to increase year by year and more than 20 000 deaths from this disease occur annually in Japan. Therefore, prevention or suppression of fibrotic changes in the liver, or protection from and treatment of hepatocirrhosis are important. From the standpoint of cell growth, hepatocirrhosis can be explained as disturbed control of 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. Interferon therapy has been used for the treatment of chronic hepatitis to eradicate factors such as viruses, but clinical efficacy is often not sufficient and other therapeutic drugs for hepatopathy have been used concomitantly. Of these, sho-saiko-to extract has been widely used for the treatment of chronic hepatitis.

Sho-saiko-to is a herbal medicine prepared from seven herbs: saiko (*Bupleuri radix*), hange (*Pinelliae tuber*), wogon (*Scutellariae radix*), taiso (*Zizyphi fructus*), ninjin (*Ginseng radix*), kanzo (*Glycyrrhzae radix*) and shokyo (*Zingiberis rhizoma*). The herbs are boiled in water and the decocted liquid is concentrated and evaporated. The effects of sho-saiko-to extract on chronic hepatitis have recently been reported (Amagaya et al 1989; Usami et al 1989; Okita et al 1990). However, most of the reports are concerned with hepatoparenchymal cells, and few studies on non-parenchymal cells have been reported. On the other hand, the deep involvement of Ito cells in fibrosis and regeneration of the liver has recently been demonstrated, and the importance of Ito cells has been indicated (Gohda 1988; Enzan 1992; Enzan et al 1993, 1994, 1995).

Ito cells store fats, mainly retinol palmitate (Yumoto 1982); they are said to be activated by cytokines such as transforming growth factor β producing extracellular matrix such as collagen, as a result enhancing liver fibrosis. Ito cells are activated and retinol palmitate stored in these cells is released in liver-injured rats and, therefore, liver retinoid level is reduced. This level increases in proportion to restoration of liver function (Enzan 1992).

In liver regeneration, hepatocyte growth factor is said to be a factor stimulating DNA synthesis

Correspondence: Y. Nishioka, Department of Pharmacy, Kochi Medical School Hospital, Kohasu, Okoh-cho, Nankoku, Kochi 783, Japan.

(Nakamura et al 1984). Because hepatocyte growth factor is expressed in normal, but not activated, Ito cells (Schirmacher et al 1992) it is necessary to inhibit activation of Ito cells to prevent or suppress liver fibrosis and liver regeneration. However, studies on the effect of sho-saiko-to extract on Ito cells have been not reported.

We therefore examined the effects of sho-saiko-to extract and its active constituents (glycyrrhizin, glycyrrhetic acid, baicalin and baicalein) on fibrosis and regeneration of the liver in rats.

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 ± 2°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, pentobarbital sodium from Dainippon Pharmaceutical, sho-saiko-to extract from Tsumura, bromodeoxyuridine from Wako Pure Chemicals and glycyrrhizin, glycyrrhetic acid, baicalin and baicalein from Nacarai Tesque. Other reagents were extra-pure grade from commercial sources.

Preparation of liver-injured and hepatectomized rats

Dimethylnitrosamine was used for induction of liver injury. After acclimatization, liver-injured rats were prepared by intraperitoneal injection of 35 mg kg^{-1} dimethylnitrosamine. Seven days after treatment the rats, under anaesthesia, underwent resection of approximately two-thirds of the liver according to the method of Higgins & Anderson (1931).

Administration of sho-saiko-to extract, glycyrrhizin, baicalin and baicalein

Sho-saiko-to extract was administered in pellet food by adding the extract to the food at concentrations of 0·75, 1·5 and 3%. The group administered sho-saiko-to extract was fed with this food from the 7th day after administration of dimethylnitrosamine. The control group were fed with ordinary food. Partially hepatectomized rats were grouped and fed similarly.

For administration of glycyrrhizin, baicalin and baicalein each was added to the ordinary pellet food at 0·75, 1·5 and 3% similarly to sho-saiko-to extract.

Biochemical examination

After administration of sho-saiko-to extract, serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase and total bilirubin in normal and liver-injured rats were measured with a Vision Analyser (Dainabot).

Histopathological examination of the liver

Liver-injured rats were killed periodically after being fed with the ordinary food or sho-saiko-to extract food. The liver was removed, rapidly fixed with formalin, stained with haematoxylin and eosin, and examined histopathologically.

Determination of retinoid concentration in the liver

Retinol palmitate in the liver was measured and expressed as retinoid concentration. Removed rat liver (0·3 g) was weighed and homogenized in chloroform (5 mL) with a cell-homogenizer (Eilard) at 7000 *g* for 20 min in an ice bath. The homogenate was centrifuged (3000 *g*) for 10 min and the bottom layer was collected. The extract was filtered through a 0·5-μm membrane filter (Millipore) and then analysed by HPLC with an Hitachi L-6200 chromatograph fitted with a 4·6 mm × 150 mm i.d. Cosmoseal $_5C_{18}$ column (Nacarai Tesque). The mobile phase was tetrahydrofuran-methanol (1 : 3 v/v); the flow-rate was 1·0 mL min^{-1} and the detection wavelength 280 nm.

Measurement of liver and spleen weight

Remaining liver and spleen were removed 3, 5, 7, 10 and 14 days after partial hepatectomy and weighed (wet weight). Liver regeneration rate was calculated according to the formula:

Liver regeneration rate (%) = (weight of liver remaining/weight of normal rat liver) × 100

The weight of normal rat liver was mean value derived from the expected liver weight for a particular rat body weight.

Counting the number of S-phase cells

Bromodeoxyuridine (40 mg kg^{-1}) was injected intraperitoneally into rats 1, 2, 3, 5, 7, 10 and 14 days after partial hepatectomy. After 1 h the rat was killed under ether anaesthaesia and the liver was removed, quickly fixed with 70% ethanol, dehydrated, embedded in paraffin and sectioned. After removal of the paraffin the section was treated with 4 M hydrochloric acid for monomerization of DNA. The slices were then washed with phosphate buffer (pH 6·8) and S-phase nuclei were stained with 3,3′-diaminobenzidine using anti-bromodeoxyuridine

monoclonal antibody (Becton Dickinson) by the avidin-biotin peroxide complex method. The number of S-phase cells/500 cells was counted under a microscope, and cell-labelling rate was calculated.

Measurement of active constituents of sho-saiko-to extract in the liver and spleen

Removed liver or spleen (ca. 0·3 g) was weighed, and homogenized with a cell-homogenizer (Eilard) in 10 mL 1 : 1 chloroform–methanol at 7000 *g* for 20 min. After centrifugation (3000 *g*, 10 min) the supernatant (8 mL) was collected and dried with an evaporator. Each sample was dissolved in 0·5 mL methanol, filtered through a 0·5-μm membrane filter (Millipore) and analysed by HPLC and LC-MS. An Hitachi M-30B apparatus was used for LC-MS. HPLC and LC-MS conditions were according to the method 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. A P value of $< 0{\cdot}05$ was considered as indicative of statistical significance.

Results

Effects of sho-saiko-to extract on liver fibrosis

Biochemical examination. The results of biochemical examination after administration of sho-saiko-to extract for 14 days are shown in Table 1. Sho-saiko-to extract had no effect on normal rats. In contrast, in the liver-injured rats, the values of the biochemical indices were reduced by administration of the extract.

Pathological examination of liver tissue. Figure 1 shows microphotographs of haematoxylin- and eosin-stained liver tissue from liver-injured rats fed with sho-saiko-to extract food or normal food for 14 days.

Necrosis and defluxion of liver cells were observed for the ordinary food group, and liver fibrosis as a result of increased deposition of collagen fibres was also observed. On the other hand, in the sho-saiko-to extract (1·5%) group, necrosis of liver cells was not observed and injury such as fibrotic change was slight; liver fibrosis had been alleviated by administration of sho-saiko-to extract.

Concentration of retinoid and sho-saiko-to active constituents in the liver. Concentrations of retinoid in the liver after administration of sho-saiko-to extract food or ordinary food for 14 days are shown in Table 2. Liver retinoid concentration is expressed as a percentage of the liver retinoid level in normal rats measured shortly after the end of acclimatization. In normal rats the concentration of liver retinoid increased slightly after administration of sho-saiko-to extract but was not significantly different from the concentration in the rats after administration of ordinary food. In liver-injured rats the concentration of liver retinoid after administration of the ordinary food was low, 21%, whereas that after administration of sho-saiko-to extract food was 47–62%, significantly different from that in the group receiving ordinary food.

The concentrations of liver retinoid and the levels of active constituents of sho-saiko-to in liver-injured rats after administration of sho-saiko-to extract or its active constituents for 14 days are shown in Table 3. Because glycyrrhizin was always below the detection limit, concentrations of glycyrrhetic acid, its aglycone, are presented as

Table 1. Effect on serum biochemical indices of oral administration of different concentrations of sho-saiko-to extract to rats for 14 days.

Treatment	Dose (%)	Glutamate oxaloacetate transaminase (int. units L^{-1})	Glutamate pyruvate transaminase (int. units L^{-1})	Lactate dehydrogenase (int. units L^{-1})	Total bilirubin (mg dL^{-1})
Normal rats					
Ordinary food		98 ± 35	31 ± 5	218 ± 98	0·25 ± 0·05
Sho-saiko-to extract	0·75	128 ± 75	38 ± 4	368 ± 71	0·25 ± 0·10
	1·50	111 ± 41	37 ± 8	248 ± 58	0·20 ± 0·05
	3·00	137 ± 15	35 ± 10	321 ± 23	0·25 ± 0·10
Liver-injured rats					
Ordinary food		408 ± 78	357 ± 39	323 ± 48	1·50 ± 0·50
Sho-saiko-to extract	0·75	281 ± 57*	178 ± 48*	219 ± 28*	0·80 ± 0·40
	1·50	203 ± 46*	141 ± 38*	198 ± 44*	0·80 ± 0·30*
	3·00	262 ± 54*	201 ± 42	208 ± 33	0·90 ± 0·20*

Each value is the mean ± s.e. of results from seven experiments. *$P < 0{\cdot}05$, significantly different from the result for the ordinary food group.

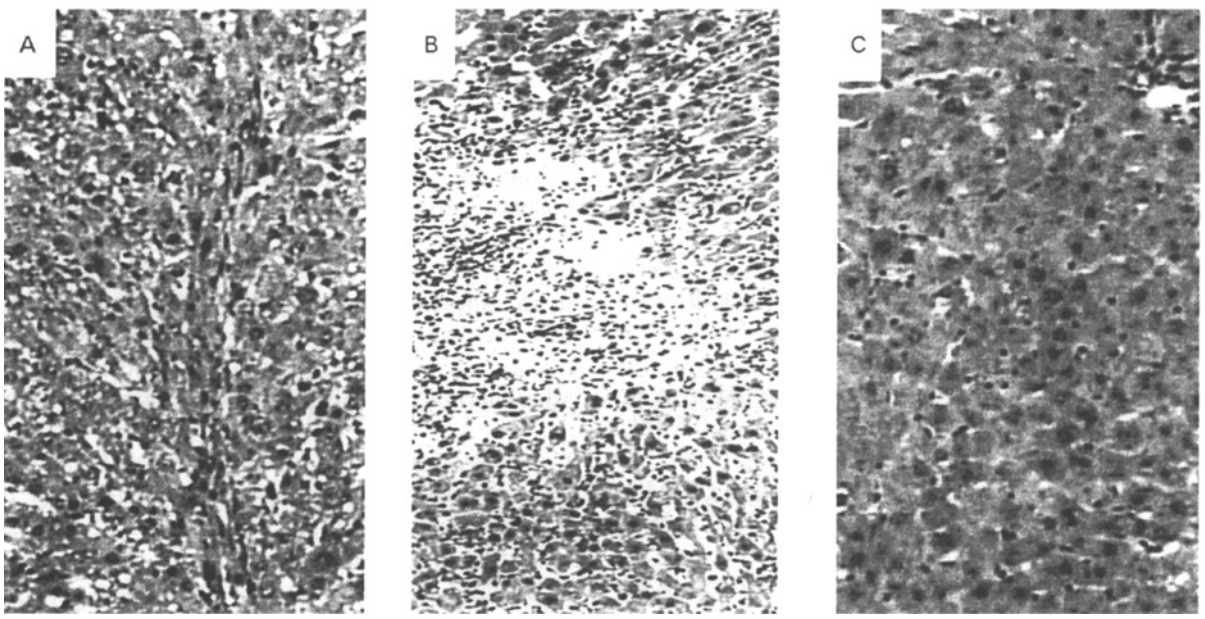

Figure 1. Microphotographs of liver tissue from liver-injured rats (stain haematoxylin and eosin; × 100). A. Dimethylnitrosamine-induced liver injury before experiment; B. Rat-liver-injury after administration of ordinary food; C. Rat-liver-injury after administration of 1·5% sho-saiko-to extract.

Table 2. Effect on retinol palmitate level (%)* of oral administration of different concentrations of sho-saiko-to extract to rats for 14 days.

Treatment	Dose (%)	Normal rats	Liver-injured rats
Ordinary food	–	103 ± 15	21 ± 3
Sho-saiko-to extract	0·75	105 ± 16	47 ± 6†
	1·50	118 ± 5	62 ± 4†
	3·00	129 ± 10	57 ± 3†

*Percentage of the liver retinoid level of normal rats, measured shortly after the end of acclimatization. Each value is the mean ± s.e. of results from seven experiments. †$P < 0.05$, significantly different from the result from the ordinary food group.

molarities. Because baicalin and baicalein are a glucuronide and its aglycone, and their enzyme-catalyzed in-vivo interconversion has been reported (Uchida et al 1995), the combined molarities of these two compounds are given.

The liver retinoid level in the glycyrrhizin group was slightly higher than that in the ordinary group, but the value was lower than that administered in the same dose of sho-saiko-to extract. The retinoid levels in the baicalin or baicalein groups were not significantly different from those in the ordinary group.

Glycyrrhetic acid, baicalin and baicalein levels in the livers of the sho-saiko-to extract group were higher than in those of the active constituent groups. The values found for the group administered 1·5% were higher than for those given 0·75%, but there was no significant differences in the levels found in those animals given 1·5 and 3%.

Effects of sho-saiko-to extract on liver regeneration
Changes in liver regeneration rate and liver retinoid level. Changes in liver regeneration rate and liver retinoid levels in partially hepatectomized rats are shown in Figure 2. In normal rats the rate of liver regeneration increased rapidly until five days after the operation, irrespective of whether the diet contained sho-saiko-to extract, and became steady thereafter. On the other hand, the rate of liver regeneration in the liver-injured rats was affected by sho-saiko-to extract, the rate for the ordinary food group being lower than that for the sho-saiko-to extract group. In the sho-saiko-to extract group there was no significant difference between the rates of liver regeneration after doses of 1·5 or 3%.

In normal rats the liver retinoid level decreased to 50–65% three days after hepatectomy irrespective of whether sho-saiko-to extract was administered, but rapidly rose thereafter. From five days after hepatectomy the level in the rats in the sho-saiko-to extract group was higher than that in the ordinary group. No dose-dependent difference was observed. In liver-injured rats higher liver retinoid levels were found after administration of sho-saiko-to extract. In the sho-saiko-to group, the liver retinoid level tended to be higher at larger doses, although there were no differences in the levels measured after doses of 1·5 and 3%.

Table 3. Concentrations of liver retinoid and sho-saiko-to active constituents in liver-injured rats after administration of sho-saiko-to extract or its active constituents for 14 days.

Treatment	Dose (%)	Retinol palmitate (%)*	Glycyrrhetic acid (nmol g^{-1})	Baicalin and baicalein (nmol g^{-1})
Ordinary food		21 ± 3		
Glycyrrhizin	0·75	27 ± 3†	47 ± 8	
	1·50	34 ± 7†	73 ± 14	
	3·00	38 ± 13†	98 ± 13	
Baicalin	0·75	18 ± 5		46 ± 5
	1·50	20 ± 3		88 ± 12
	3·00	19 ± 3		82 ± 16
Baicalein	0·75	20 ± 5		18 ± 5
	1·50	20 ± 4		42 ± 8
	3·00	22 ± 6		44 ± 14
Sho-saiko-to extract	0·75	47 ± 6†§	143 ± 8‡§	137 ± 13‡§
	1·50	62 ± 4†	208 ± 16‡	209 ± 27‡
	3·00	57 ± 7†	218 ± 21‡	202 ± 46‡

Each value is the mean ± s.e. of results from seven experiments. *Percentage of the liver retinoid level of normal rats, measured shortly after the end of acclimatization. †$P < 0·05$, significantly different from retinoid values of ordinary food group. ‡$P < 0·05$, significantly different from value after administration of same-dose of active constituent. §$P < 0·05$, significantly different from result for 3% dose.

Changes in the number of S-phase cells. Bromodeoxyuridine-stained liver tissue of liver-injured rats after hepatectomy is shown in Figure 3. More S-phase nuclei that were synthesizing DNA were found in the group receiving 1·5% sho-saiko-to extract than in the ordinary group. Changes in the labelling index of S-phase cells after hepatectomy are shown in Figure 4.

In normal rats, the number of S-phase cells reached a peak on day 1 after hepatectomy irrespective of the administration of sho-saiko-to extract, and decreased rapidly after two days. In contrast, in liver-injured rats the number of S-phase cells was affected by sho-saiko-to extract, the rate of increase in the sho-saiko-to extract group being higher than in the ordinary group. The peak of S-phase cells occurred three to seven days after administration. The rate of increase of the number of S-phase cells on the third day of administration was elevated as the dose was increased. The rate of increase in the number of S-phase cells tended to decrease after three days.

Changes in spleen weight. Changes in spleen weight after partial hepatectomy are shown in Figure 5. In the normal rats, the weight peaked after administration of sho-saiko-to extract for seven days, and decreased thereafter. In the liver-injured rats, the rate of increase in spleen weight was greater than in the normal rats, and the rate increased with increasing doses of sho-saiko-to extract.

Changes in the levels of sho-saiko-to active constituents in the liver and spleen. Changes in the levels of sho-saiko-to active constituents in the livers and spleens of partially hepatectomized liver-injured rats are shown in Table 4. Both in normal and in liver-injured rats, hepatic levels of glycyrrhetic acid, baicalin and baicalein increased three days after administration of sho-saiko-to extract. The levels of these compounds were higher in the liver-injured rats. The levels of the active constituents in the spleens of normal rats were below the detection limits. In the liver-injured rats, levels of the active constituents increased from three days after the administration of sho-saiko-to extract. In the liver and spleen the levels of glycyrrhetic acid, baicalin and baicalein rose as doses of sho-saiko-to extract were increased. However, at the 7th and 14th days there were no significant differences between the levels found in rats administered doses of 1·5 and 3%.

Table 5 lists the apparent absorption parameters calculated from the liver levels of sho-saiko-to active constituents after administration of sho-saiko-to extract in partial hepatectomy rats. The values of these parameters were higher for liver-injured rats than for normal rats. Of these, the three to seven day values were higher than the others. In particular the levels were highest in the liver-injured rats administered 1·5% sho-saiko-to extract; the absorption parameter for glycyrrhetic acid was 68·5 nmol g^{-1} per day and that for baicalin and baicalein was 82·1 nmol g^{-1} per day.

Discussion

The effects of sho-saiko-to extract on activated Ito cells were examined by administering the extract to

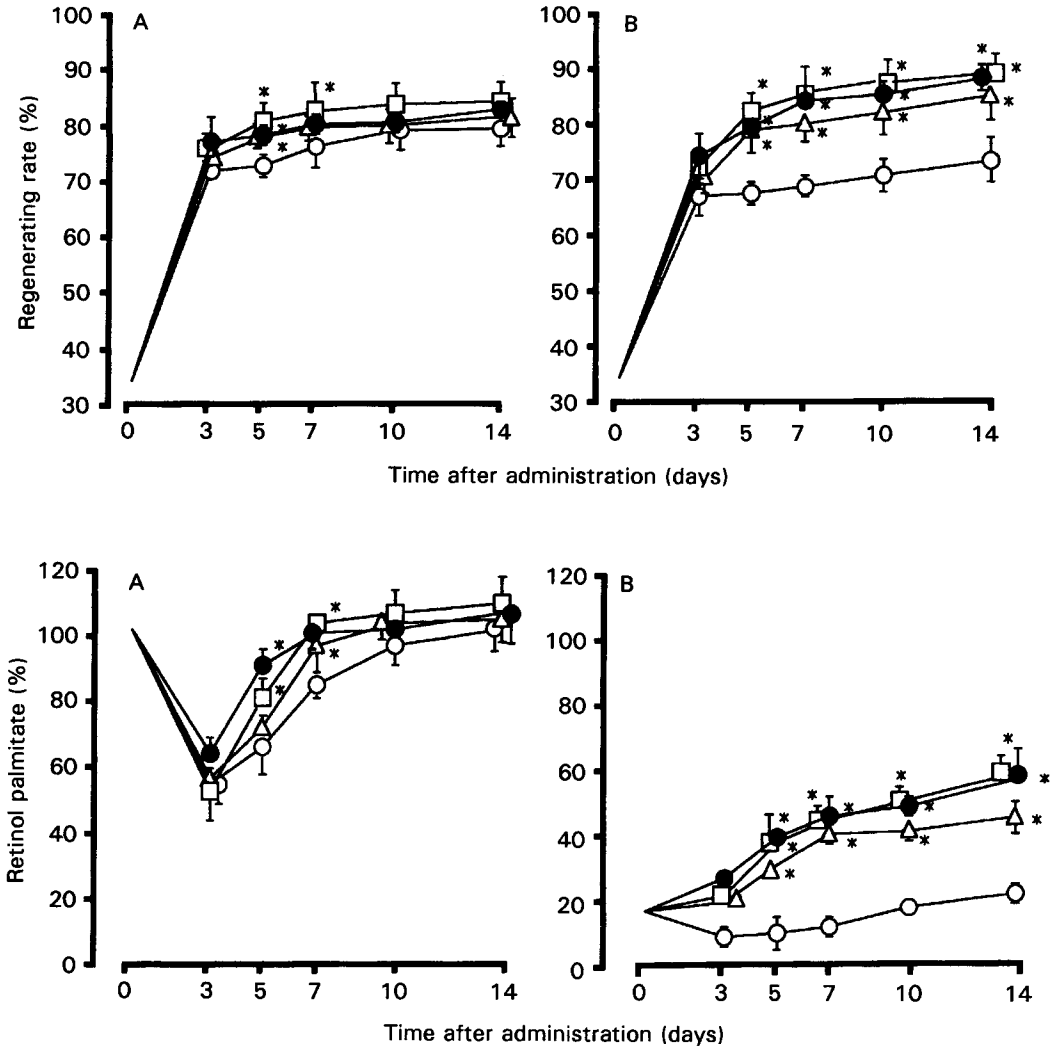

Figure 2. Effect of different concentrations of sho-saiko-to extract (oral administration for 14 days) on the rate of regeneration of rat liver and on liver levels of retinol palmitate after hepatectomy: A, normal rats; B, liver-injured rats; ○, ordinary food; △, concentration 0·75%; □, concentration 1·5%; ●, concentration 3%. Each point shows the mean ± s.e. of results from seven experiments. *$P < 0.05$, significantly different from the result for the ordinary food group.

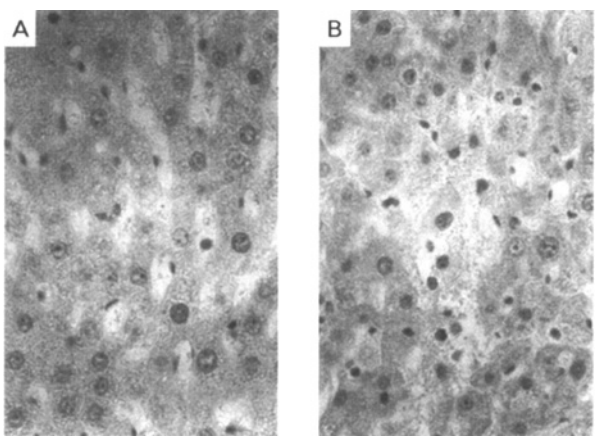

Figure 3. Microphotographs of liver tissue from liver-injured rats after hepatectomy (stain bromodeoxyuridine; ×400). A. Rat-liver-injury after administration of ordinary food. B. Rat-liver-injury after administration of 1·5% sho-saiko-to extract.

liver-injured rats and measuring liver retinoid levels and the levels of active constituents of the extract. The sho-saiko-to extract had no effects on results from biochemical examination of normal rats whereas serum levels of glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, lactate dehydrogenase and total bilirubin in liver-injured rats increased after treatment with this extract, suggesting its usefulness for improving liver function. Histopathological examination showed that fibrotic changes in the liver were suppressed by administration of this extract. In normal rats the liver retinoid level was constant, irrespective of treatment with sho-saiko-to extract, whereas that in liver-injured rats was greatly increased by administration of the extract. This increase seems to indicate the large effect of sho-

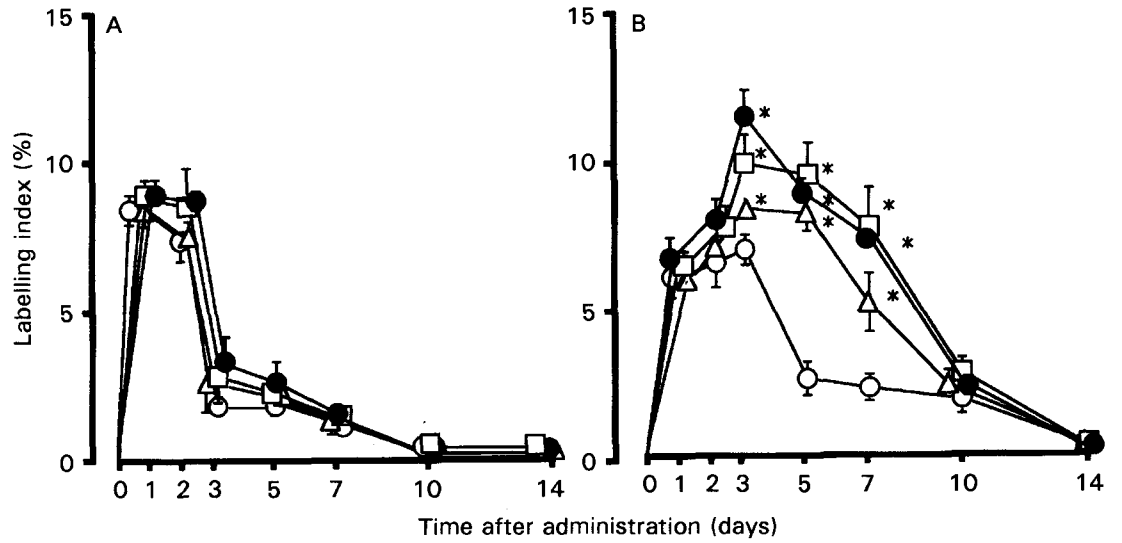

Figure 4. Effect of different concentrations of sho-saiko-to extract on the bromodeoxyuridine labelling index in rats after hepatectomy: A, normal rats; B, liver-injured rats; ○, ordinary food; △, concentration 0·75%; □, concentration 1·5%; ●, concentration 3%. Each point shows the mean ± s.e. of results from seven experiments. *$P < 0·05$, significantly different from the result for the ordinary food group.

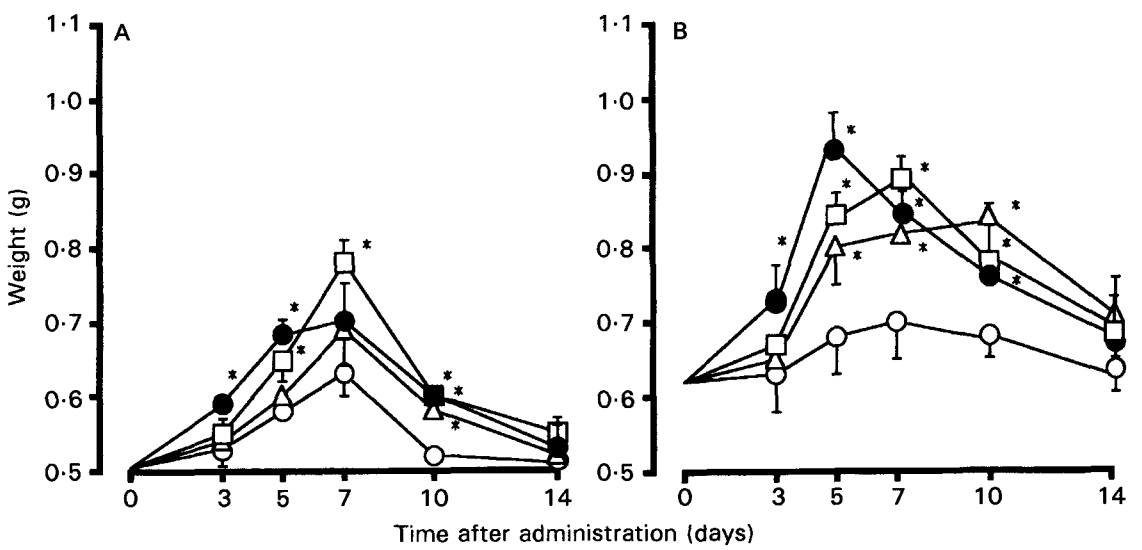

Figure 5. Effect of different concentrations of sho-saiko-to extract on rat spleen weight after hepatectomy: A, normal rats; B, liver-injured rats; ○, ordinary food; △, concentration 0·75%; □, concentration 1·5%; ●, concentration 3%. Each point shows the mean ± s.e. of results from seven experiments. *$P < 0·05$, significantly different from the result for the ordinary food group.

saiko-to extract on the storage and release of retinoid by Ito cells.

When glycyrrhizin, baicalin and baicalein, the active constituents of sho-saiko-to extract, were administered only glycyrrhizin led to an increase in liver retinoid level, and this increase was much less than that observed after administration of sho-saiko-to extract.

Liver retinoid level was not affected by administration of baicalin and baicalein. These results seem to show that glycyrrhizin participates in increasing the liver retinoid level, but the effect of glycyrrhizin alone is small and the action is somehow affected by other active constituents such as baicalin and baicalein. The concentration of glycyrrhetic acid in the liver increased markedly in liver-injured rats treated with sho-saiko-to extract. However, treatment with glycyrrhizin alone did not result in an increase in the glycyrrhetic acid level greater than that resulting from the extract, suggesting that other active constituents in the extract have a marked effect on the absorption of glycyrrhizin. As reported in our previous studies (Nishioka et al 1992; Miyamura et al 1996), the partition

Table 4. Changes in the levels of sho-saiko-to active constituents in the liver and spleen after administration of sho-saiko-to extract to rats after partial hepatectomy.

Sho-saiko-to dose (%)	Level of glycyrrhetic acid (nmol g^{-1})			Level of baicalin + baicalein (nmol g^{-1})		
	3 days	7 days	14 days	3 days	7 days	14 days
Liver: normal rats						
0·75	7·8 ± 2·0*	23·5 ± 3·7*	48·5 ± 5·7*	5·1 ± 1·3*	33·1 ± 7·1*	65·8 ± 15·2*
1·50	8·1 ± 3·5*	40·7 ± 4·8	76·3 ± 8·8	5·6 ± 1·8*	52·0 ± 13·0	103·3 ± 25·0
3·00	16·3 ± 2·6	45·1 ± 2·5	97·2 ± 7·5	7·3 ± 2·9	48·0 ± 8·5	92·9 ± 10·8
Liver: liver-injured rats						
0·75	10·1 ± 3·1*	189·3 ± 25·2*	211·1 ± 21·2*	40·9 ± 2·3*	217·7 ± 23·9*	236·6 ± 18·7*
1·50	21·2 ± 3·5*	295·3 ± 10·4	325·0 ± 23·1	72·6 ± 6·2*	401·0 ± 14·3	477·2 ± 15·9
3·00	98·0 ± 14·0	289·3 ± 15·1	295·0 ± 15·6	135·0 ± 15·0	385·0 ± 33·0	447·0 ± 49·1
Spleen: liver-injured rats						
0·75	7·8 ± 3·2*	43·0 ± 5·6*	75·0 ± 7·0*	20·8 ± 3·1*	63·0 ± 8·9*	75·0 ± 5·8*
1·50	18·2 ± 5·1*	89·1 ± 4·5	91·8 ± 14·1	23·5 ± 5·4*	135·0 ± 18·2	145·0 ± 15·5
3·00	32·0 ± 3·5	75·0 ± 13·8	86·0 ± 15·3	43·0 ± 4·0	120·5 ± 15·6	140·5 ± 20·0

Each value is the mean ± s.e. of results from seven experiments. *$P < 0·05$, significantly different from result for 3% dose.

Table 5. Apparent absorption parameters for active constituents of sho-saiko-to in liver after administration of sho-saiko-to extract to partial hepatectomy rats.

Dose of Sho-saiko-to (%)	Subject	Glycyrrhetic acid (nmol g^{-1} day^{-1})			Baicalin + baicalein (n mol g^{-1} day^{-1})		
		0–3 days	3–7 days	7–14 days	0–3 days	3–7 days	7–14 days
0·75	Normal rats	2·6	3·9	3·6	1·7	7·0	4·7
	Liver-injured rats	3·7	44·8	3·1	13·6	44·2	2·7
1·50	Normal rats	2·7	8·2	5·1	1·9	11·6	17·1
	Liver-injured rats	7·1	68·5	4·2	24·2	82·1	10·9
3·00	Normal rats	5·4	7·2	7·5	2·4	10·2	6·4
	Liver-injured rats	32·7	47·8	0·8	45·0	62·5	9·9

coefficient of glycyrrhizin in-vitro is elevated by addition of baicalin or baicalein. Therefore, absorption of glycyrrhizin seems to be enhanced by interaction with other active constituents such as baicalin and baicalein.

The effects of sho-saiko-to extract on the regeneration of the liver and the repair of liver injury was examined by administering the extract to liver-injured rats after hepatectomy. The rate of liver regeneration in liver-injured rats was increased by administration of the sho-saiko-to extract, suggesting that the extract repairs liver injury and simultaneously enhances liver regeneration. Histopathological examination of liver regeneration revealed that, irrespective of treatment with sho-saiko-to extract, in normal rats the number of S-phase cells reached a peak 1 day after partial hepatectomy and decreased rapidly thereafter. However, in liver-injured rats the number of S-phase cells increased greatly after treatment with sho-saiko-to extract with a peak occurring three to seven days after hepatectomy. These results again indicate that sho-saiko-to extract aids liver regeneration. The difference between the peaks in the number of S-phase cells in normal and liver-injured rats, and the report by Miyazawa (1993) that regeneration of liver parenchymal cells was complete one day after partial hepatectomy whereas that of non-parenchymal cells continued until two to three days after hepatectomy, implies that the effect of sho-saiko-to extract during liver regeneration appears to be mainly on non-parenchymal liver cells. No difference in the number of S-phase cells resulted from doses of 1·5% and 3%. According to Okita et al (1990) administration of a high concentration of glycyrrhizin resulted in accumulation of liver cells in G_1/G_0-phase and a decrease in the number of S-phase cells. Therefore, there seems to be an optimum dose of sho-saiko-to and exceeding this might suppress, rather than enhance, liver regeneration.

The liver retinoid level in partially hepatectomized rats decreased for three days after hepatectomy and then increased rapidly in normal rats irrespective of the administration of sho-saiko-to extract. In liver-injured rats the retinoid level was

greatly elevated as a result of administration of sho-saiko-to extract. Because the change in liver retinoid level was similar to that in the rate of liver regeneration, participation of non-parenchymal cells, Ito cells in particular, in liver regeneration is likely.

Spleen weight for liver-injured rats was larger than for normal rats, and the difference was affected by administration of sho-saiko-to extract. Concentrations of sho-saiko-to active constituents in the spleen of liver-injured rats increased with increasing dose of sho-saiko-to extract. In contrast, none of these constituents was detected in normal rats. This suggests participation of both the spleen and the liver in liver regeneration. It has recently been reported that hepatocyte growth factor produced in the liver and the spleen is an important factor in liver regeneration (Nakamura et al 1984; Nakatukasa et al 1991; Tamura et al 1993; Okazaki 1994). We plan to study in the effect of sho-saiko-to extract on hepatocyte growth factor.

After administration of sho-saiko-to extract to partial hepatectomy rats, the size of the three to seven day apparent absorption parameters of the active constituents mirrored those of S-phase cells. Levels after a dose of 1·5% were higher than after a dose of 0·75% but there were no differences between the results obtained after doses of 1·5 and 3%. These results suggest that the increase in the number of S-phase cells depends on the apparent absorption parameters, and that there seems to be an optimum dose of sho-saiko-to extract.

In conclusion, sho-saiko-to extract seems to be useful for repair of liver injury, improvement of fibrotic changes of the liver and promoting liver regeneration. This improvement seems to depend greatly on the suppression of activation of Ito cells, non-parenchymal cells in the liver, and the improvement of retinoid storage in the cells.

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