Original 和漢医薬学会誌 5, 146－153, 1988

Effect of Syô-saiko-tô on liver regeneration in partially hepatectomized rats

Sakae AMAGAYA[a] Akira MIYAKE[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 23, 1988. Accepted June 27, 1988.)

Abstract

The effect of Syô-saiko-tô, one of Kampô-hôzai (Japanese and Chinese traditional medicines), on liver regeneration was studied in partially hepatectomized rats. Syô-saiko-tô at a dose of 1.2 g/kg body weight facilitated the gain of liver weight, liver RNA content, and mitotic index following partial hepatectomy. The gain of DNA and protein contents in liver also tended to increase by Syô-saiko-tô. Furthermore, the regeneration of hepatocyte collected from the hepatectomized rats pretreated with Syô-saiko-tô increased by comparison with that of hepatocyte collected from rats non-treated with Syô-saiko-tô. However, Syô-saiko-tô did not improve the decrease of mitotic index induced by streptozotocin. These results suggest that the acceleration of Syô-saiko-tô on liver regeneration is due to the increase of pancreatic hormonal secretion. When the action of Syô-saiko-tô on hormonal secretion from pancreas was investigated, glucagon secretion was found to be stimulated and the plasma glucose was also increased with the constant secretion of insulin.

Key words Syô-saiko-tô, liver regeneration, partial hepatectomy, mitotic index, insulin, glucagon, glucose.

Abbreviations DNA, deoxyribonucleic acid ; RNA, ribonucleic acid ; STZ, streptozotocin ; Syô-saiko-tô (Xiao-Chai-Hu-Tang), 小柴胡湯.

Introduction

Recently, Syô-saiko-tô, one of Kampô-hôzai (Japanese and Chinese traditional medicines), has been suggested to be effective for chronic hepatitis by clinical trials.[1,2] As to the pharmacological action of Syô-saiko-tô, there are many reports about saikosaponins,[3-6] the main ingredients of Syô-saiko-tô. On the other hand, we reported that Syô-saiko-tô had both steroidal and non-steroidal anti-inflammatory action as the results of the stimulation of lipocortin-production and the inhibition of cyclooxygenase activity,[7,8] respectively, and increased the anti-inflammatory action of prednisolone[9] with the restoration of prednisolone-induced adrenal atrophy by the stimulation of pituitary-adrenocortical axis function.[10] Furthermore, we reported that Syô-saiko-tô stimulated T cell and macrophage functions.[11] As to the experimental hepatic injury, we reported that Syô-saiko-tô protected the hepatocyte necrosis[12] and inhibited the liver fibrosis formation *in vivo*.[13] This anti-necrosis action is partially explained from the hepatocyte membrane-stabilizing activity of saikosaponins.[14,15] However, few projects were done to examine the influence of Syô-saiko-tô on liver regeneration, an important phenomenon in the therapy of drastic necrosis of hepatocytes or fulminant hepatitis. Many projects have been done to resolve the mechanism of the liver regeneration. A lot of promotors of liver regeneration have been reported[16-20] and many workers have independently showed that the pancreatic hormones, insulin and glucagon, played an important role on the regula-

*〒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**, 146－153, 1998

tion of the liver regeneration,[21-24] although the initiator of liver regeneration remains unclear. The purpose of the present study is to examine the effect of Syô-saiko-tô on liver regeneration following partial hepatectomy in rats and study about the action mechanism of Syô-saiko-tô from the viewpoint of pancreatic hormonal secretion.

Materials and Methods

Reagents : Streptozotocin (STZ) and Tracylol® (aprotinin) were purchased from Sigma Chem. Co. (St. Louis, USA). The kits used for the determination of glucose or pancreatic hormones were as follows : Enzyme kit for glucose (Iatron Labo. Inc., Tokyo, Japan), EIA kit for insulin (Kainos Labo. Inc., Tokyo, Japan), and RIA kit for glucagon (Dainabot Co., Tokyo, Japan). [^{3}H]-Thymidine was purchased from Amersham (Tokyo, Japan). All other reagents were of analytical grade.

Preparation of Syô-saiko-tô : Powdered extracts of Syô-saiko-tô were prepared according to the method mentioned in our previous paper.[12]

Animals : Male Wistar rats, 5 weeks old, were obtained from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). They were kept in an air conditioned room (24℃) and given a commercial diet and water *ad libitum*.

Partial hepatectomy : Removal of 70% of hepatic parenthyma was achieved according to the method of Higgins and Anderson.[25] The operations were carried out between 10 : 00 a.m. and 11 : 30 a.m. to diminish the influence of diurnal rhythm[26] on the appearance of mitosis.

Experimental procedures : The detailed experimental schedule is shown in Fig. 1. Rats were sacrificed 1, 2, 3, and 5 days after the partial hepatectomy and their livers were removed (Experiment 1). In the case of STZ injection for the inhibition of insulin-secretion, STZ dissolved in physiological saline at a dose of 65 mg/kg was injected intravenously 6 days before the partial hepatectomy. Rats were sacrificed 1 day after the hepatectomy (Experiment 2). Syô-saiko-tô at a dose of 1.2 g/kg body weight was dissolved in 2 ml of distilled water and administered using a stomach tube once a day or twice.

Determination of protein and nucleic acid (DNA and RNA) contents in liver : The protein content of liver homogenate was determined by the method of Lowry *et al*.[27] The DNA and RNA in liver homogenate were fractionated according to the methods of Schmidt and Thannhauser,[28] and Schneider.[29] The DNA content was determined by the modification of the method reported by Burton.[30] The RNA content was determined by the method reported by Mejbaum.[31]

Determination of mitotic activity : Small portions (5×5 mm) of the liver were obtained from the middle part of the lobus. The samples obtained were then fixed in Bouin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin for microscopic observation. The mitotic activity was determined by counting the proportion of parenchymal cell nuclei which were in prophase, metaphase, anaphase, and telophase (mitosis). A minimum of 2000 nucleis were counted in each liver sample, and mitotic index was expressed as the number of mitosis per 100 nucleis.

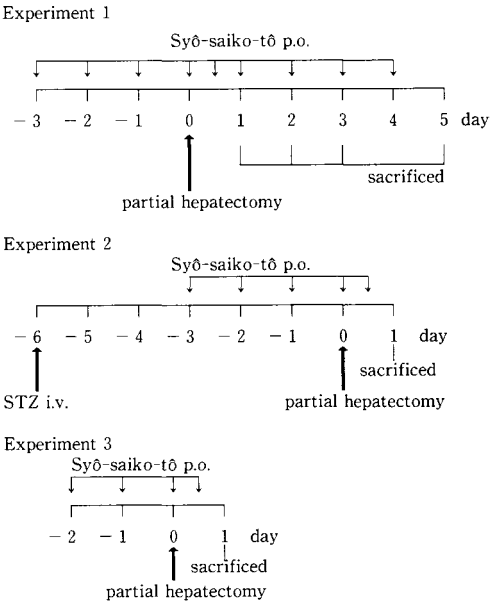

Fig. 1 Experimental schedule.
Each arrow marks the time of indicated treatment.

Primary culture of rat hepatocyte : After the pretreatment of Syô-saiko-tô at a dose of 1.2 g/kg for 3 days using a stomach tube, the rats were partially hepatectomized as shown in Fig. 1 (Experiment 3). At the indicated time after the hepatectomy, parenchymal hepatocytes were isolated from the rats according to the method of the *in situ* two-step collagenase perfusion technique reported by Selgen.[32] Inocula of 10^5 cells were introduced into 1.5 cm-diameter plastic wells (Terumo, Tokyo, Japan). The cells were cultured in 1.0 ml of RPMI 1640 medium supplemented with 5% calf serum under 5% CO_2 in air at 37℃. Two hours after plating, [^{3}H]-thymidine (1.0 μCi) was added to the cultures, and the mixture was incubated for 16 hr. The cell viability at the start of incubation was 80.0±4.0% and that at the end of incubation was 50.0±4.5%. After the incubation, the cells were washed twice with cold saline, and then twice with 10 ml of cold 10% trichloroacetic acid. Radioactivity of the insoluble fraction of 10% trichloroacetic acid was measured by a ALOCA scintillation counter. DNA synthesis was expressed as the incorporation of [^{3}H]-thymidine.

Determination of plasma insulin, glucagon and glucose : Syô-saiko-tô at a dose of 1.2 g/kg was administered to rats fasted for 20 hr. After 30, 60, 120, and 240 min, 1.0 ml of blood was collected by decapitation from each rat. The blood was mixed with 0.1 ml of physiological saline containing 500 KIU of Tracylol® and 1.2 mg of EDTA-Na_2. After centrifugation at 1600×g, the supernatant was collected and stored at −20℃. After thawing, 1.0 ml of an aliquot was analyzed for insulin, glucagon, and glucose.

Statistics : All values were expressed as mean±S.E.M. The data were statistically analyzed according to Student's t-test.

Results

Hepatic regeneration

Liver weight, protein content, nucleic acid content, and mitotic index were shown as the guidance of the liver regeneration after hepatectomy. As shown in Fig. 2, liver weight steadily increased following partial hepatectomy, and was 67% of that of non-hepatectomized rats 5 days after the hepatectomy. On the other hand, Syô-saiko-tô facilitated the increase of liver weight 3 days after the hepatectomy. Protein (Fig. 3A),

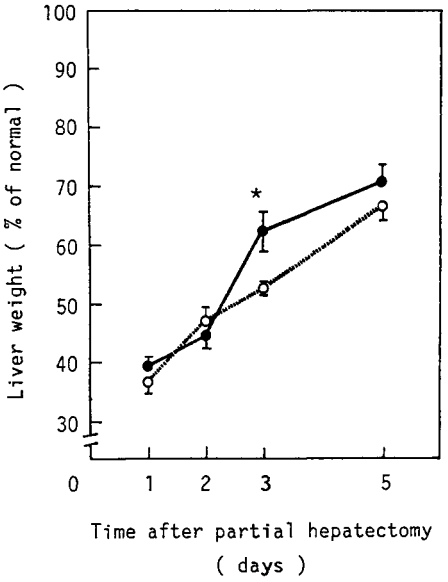

Fig. 2 Liver weight after partial hepatectomy. ○···○ : control, ●—● : Syô-saiko-tô. Each point represents the mean±S.E. of 5 rats. *p<0.05 vs. control group.

RNA (Fig. 3B) and DNA (Fig. 3C) contents were also increased lineally following partial hepatectomy. Especially, RNA contents increased more drastically, and it was 57% of the non-hepatectomized rats at 2nd day and 88% at 5th day. By the treatment of Syô-saiko-tô, the increases of the protein and DNA contents showed the tendency to be stimulated 3 days after the partial hepatectomy (p<0.1). While, the increase of the RNA content was accelerated remarkably at 1st and 3rd day. The mitosis of the hepatocytes as a parameter of the cell proliferation was evaluated and expressed as mitotic index (the number of mitosis/100 nucleis). As shown in Fig. 4, mitotic index was elevated during 3 days after the partial hepatectomy and its maximum peak was achieved on the 1st day. By the treatment of Syô-saiko-tô, the increase of mitosis was promoted on the 1st day in a significant manner.

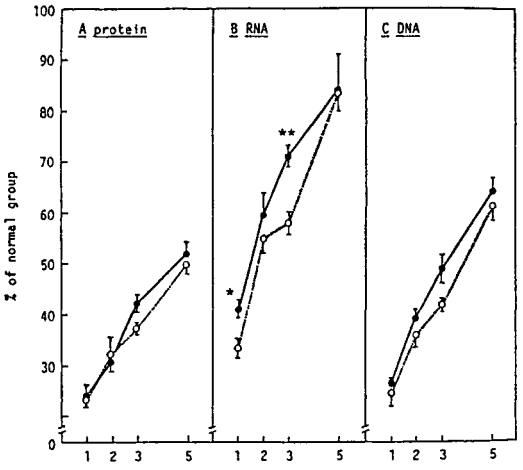

Fig. 3 Protein, RNA and DNA contents in liver after partial hepatectomy.
○---○ : control, ●—● : Syô-saiko-tô. Each point represents the mean±S.E. of 5 rats. $*p<0.05$ and $**p<0.01$ vs. control group.

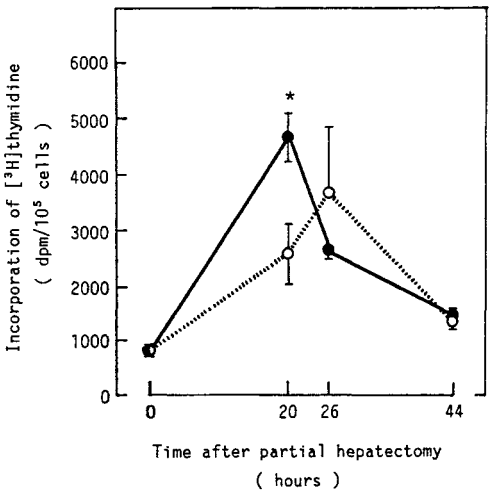

Fig. 5 Incorporation of [^{3}H]-thymidine into hepatocyte isolated from liver after partial hepatectomy.
Syô-saiko-tô was pretreated for 3 days before partial hepatectomy. ○---○ : control, ●—● : Syô - saiko - tô. Each point represents the mean±S.E. of 5－9 rats. $*p<0.05$ vs. control group.

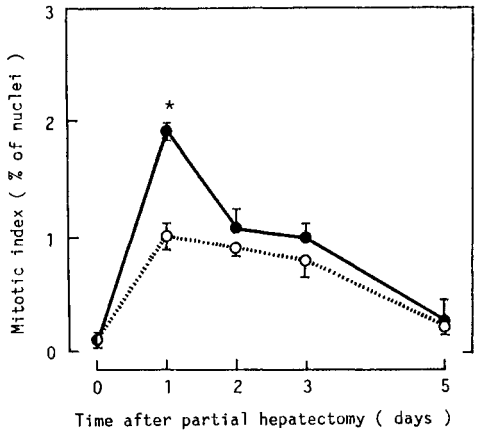

Fig. 4 Mitotic index of liver after partial hepatectomy.
○---○ : control, ●—● : Syô-saiko-tô. Each point represents the mean±S.E. of 5 rats. $*p<0.01$ vs. control group.

Hepatocyte proliferation in vitro

To evaluate the acceleration of Syô-saiko-tô on the hepatic regeneration, *in vitro* method was also used. Fig. 5 shows the incorporation of [^{3}H]-thymidine into hepatocytes isolated from hepatectomized rats. The incorporation of [^{3}H]-thymidine into hepatocytes isolated 20 hr and 26 hr after the partial hepatectomy was higher than that of non-hepatectomized rats. Pre-administration of Syô-saiko-tô for 3 days still increased the incorporation of [^{3}H]-thymidine into hepatocytes isolated 20 hr after the partial hepatectomy, and the incorporated [^{3}H]-thymidine was 2-fold over that of non-hepatectomized rats.

Hepatic regeneration in STZ-injected rats

To know the participation of pancreatic hormones in the liver regeneration-enhancing action of Syô-saiko-tô, STZ-treated rats were hepatectomized. The partial hepatectomy was performed 6 days after the 65 mg of STZ treatment. As shown in Table I, the liver RNA content diminished in a significant manner, and the liver weight and the liver protein content also tended to diminish ($p<0.1$). Furthermore, the mitosis of the hepatocyte was not found. In Syô-saiko-tô treated rats, these diminished parameters by STZ were not restored.

Table I Liver weight, protein content, nucleic acid content, and mitotic index in partially hepatectomized rats pre-treated with STZ.

STZ treatment	Syô-saiko-tô treatment	Liver weight (g)	Protein (g/liver)	RNA (mg/liver)	DNA (mg/liver)	Mitotic index (% of nuclei)
−	−	2.7±0.2	0.58±0.04	33.2±2.5	7.0±0.8	0.40±0.17
+	−	2.2±0.2	0.48±0.04	25.2±1.6*	6.8±0.5	0
+	+	2.2±0.1	0.47±0.02	27.4±0.7*	6.9±0.6	0

*$p<0.05$ vs. normal group non-treated with STZ and Syô-saiko-tô. Each value indicates the mean± S.E. of 6 rats.

Plasma insulin, glucose and glucagon levels

In order to explain the participation of pancreatic hormones in the stimulative action of Syô-saiko-tô on the hepatocyte regeneration, plasma insulin, glucose and glucagon levels after the Syô-saiko-tô treatment was investigated. As shown in Fig. 6A, plasma insulin level was not changed after the Syô-saiko-tô treatment up to 240 min. Syô-saiko-tô, however, elevated the plasma glucose level throughout the experimental period as shown in Fig. 6B. Moreover, Syô-saiko-tô elevated the plasma glucagon level 30 min after its administration as shown in Fig. 6C.

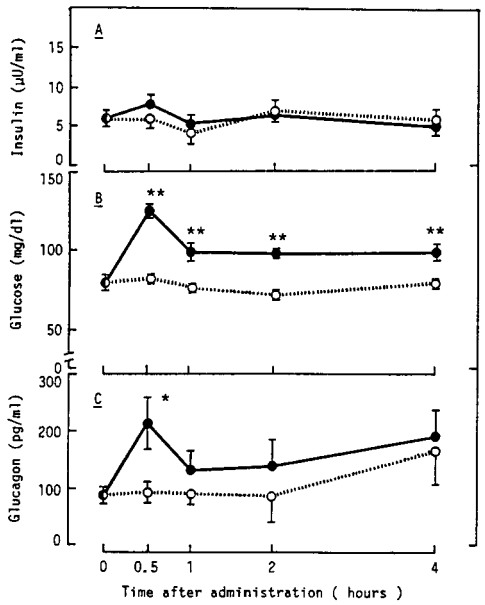

Fig. 6 Plasma insulin, glucose and glucagon levels after Syô-saiko-tô treatment.
A : plasma insulin, B : plasma glucose, C : plasma glucagon. ○---○ : control, ●—● : Syô-saiko-tô. Each point represents the mean±S.E. of 4−6 rats. *$p<0.05$ and **$p<0.01$ vs. control group.

Discussion

The enhancement of liver regeneration by Syô-saiko-tô was well-reflected by the changes of liver weight, protein content and nucleic acid content (Figs. 2 and 3). These results suggest that Syô-saiko-tô accelerates the proliferation of hepatocyte. This stimulative action of Syô-saiko-tô was further explained from both the increase in the number of mitosis in hepatocytes *in vivo* (Fig. 4) and the enhancement of the hepatocyte proliferation *in vitro* (Fig. 5). The peak of the incorporation of thymidine in Syô-saiko-tô treated rats was 20 hr after the partial hepatectomy, although it was 26 hr in Syô-saiko-tô non-treated rats. This advancement of the peak of the incorporation of thymidine in Syô-saiko-tô treated rats suggests that Syô-saiko-tô stimulates the proliferation cell-cycle of hepatocyte isolated from partially hepatectomized rats. The increases of liver weight, protein content and nucleic acid content 1 or 3 days after the partial hepatectomy also indicate the stimulation of proliferation cell-cycle in Syô-saiko-tô treated rats. On the other hand, STZ damages pancreatic β-cells, resulting in the decrease of the secretion of insulin[33] which is shown to be one of the promotors in liver regeneration,[21-24] and the inhibitory effect of STZ on liver regeneration is dependent on the decrease of insulin secretion. Since Syô-saiko-tô did not improve the STZ-induced suppression on liver regeneration in partially hepatectomized rats, the liver regeneration-enhancing activity of Syô-saiko-tô is closely related to the insulin secretion. Nevertheless, plasma insu-

lin level after the administration of Syô-saiko-tô was not different from those of Syô-saiko-tô non-treated rats (Fig. 6A). In contrast, Syô-saiko-tô elevated the plasma glucose and glucagon levels (Figs. 6B and 6C). Glucagon is also one of the promotors of liver regeneration as well as insulin.[21-24] Leffert,[23] and Bucher and Swaffield[22] suggest that insulin and glucagon interact in a synergistic fashion to facilitate liver regeneration. In fact, this synergistic effect of insulin and glucagon, which is called G-I therapy, is clinically applied in acute or fulminant hepatitis. Therefore, the liver regeneration-enhancing action of Syô-saiko-tô may be considered to attribute to the synergistic effect of glucagon increased by Syô-saiko-tô and insulin unchanged. Furthermore, it is reported that the synergistic action of glucagon and insulin protects the progress of hepatitis.[34] These suggestions could well support that Syô-saiko-tô is effective against the necrosis of hepatocytes, hepatitis, and cirrhosis. Plasma glucose elevation also prove the increase of glucagon secretion without the increase of insulin secretion. On the other hand, Syô-saiko-tô itself contains much sugars and oligosaccharides which are mainly involved in *Zizyphus vulgaris* LAM (Taisô) and *Pinelliae ternata* BREITENBACH (Hange), and the increased plasma glucose is expected to depend on the sugars absorbed from the intestine when Syô-saiko-tô is administered. But, in our preliminary experiment Syô-saiko-tô never increased the plasma glucose level in STZ-treated rats. These results suggest that the elevation of plasma glucose is not due to the sugars involved in Syô-saiko-tô, but is due to the secretion of glucagon. The secretion of glucagon is controlled by adrenalin, a stimulator of glucagon and an inhibitor of insulin, and adrenalin facilitates the ACTH-releasing, resulting in the induction of glucocorticoid secretion from the adrenal gland to promote the glycogenolysis in liver. Since, Syô-saiko-tô is reported to induce the glucocorticoid secretion,[9] Syô-saiko-tô may control adrenalin secretion from the adrenal gland. Another possibility of the action mechanism of Syô-saiko-tô is the increase of the sensitivity or the number of glucagon receptors. Furthermore, the increased glucose by Syô-saiko-tô may be also related to the stimulation of liver regeneration, since the increase of glucose causes the cellular increase of Ca^{2+} concentration which is neccessary for the cell proliferation.[35,36] Moreover, we proved that Syô-saiko-tô stimulated the plasma PGI_2 formation by the stimulation of endothelial cell function (data are not shown), although it inhibits the cyclooxygenase activity.[8] Increased plasma PGI_2 may stimulate the utility of glucose in liver by the enhancement of blood stream. In our studies, the plural mechanisms of stimulative action of Syô-saiko-tô on hepatic regeneration were forecasted. To make clear the enhancing action of Syô-saiko-tô on liver regeneration, further study is needed in future.

和文抄録

小柴胡湯の肝再生に及ぼす影響を部分肝切除ラットを用いて検討した。小柴胡湯 1.2 g/kg を経口投与すると，部分切除された肝臓重量及び肝 RNA 含量の回復が有意に促進され，また，mitotic index（肝細胞100個当りの mitosis の数）が有意に増加した。さらに，肝 DNA 及び肝蛋白含量の回復も促進される傾向を示した。次に，部分肝切除後の肝細胞への [^{3}H]-thymidine の取り込みを *in vitro* の系で検討してみると，小柴胡湯を前投与したラットより単離した肝細胞では，コントロールとして水を投与したラットの肝細胞に比較して [^{3}H]-thymidine の取り込みが有意が促進された。そこで，小柴胡湯の肝再生促進作用における膵臓ホルモンの影響を検討するために，β細胞障害性を有する streptozotocin を投与したラットを用いて同様の実験を行ってみると，小柴胡湯の肝再生促進効果が消失した。次に，血漿 insulin 濃度を測定してみると，小柴胡湯投与により全く変化が認められなかった。しかし，小柴胡湯は，血漿 glucose 及び血漿 glucagon 濃度を有意に上昇させた。これらの結果は，小柴胡湯の肝再生促進作用に，glucagon の上昇が glucagon-insulin の共同作用を介して関与している可能性を示唆している。

References

1) Oka, H.: Xiao-Chai-Hu-Tang and Gui-Zhi-Fu-Ling-

Wan for treatment of chronic hepatitis. In "Proceedings of Symposium 9 and Satelite Symposium 8 of the 17th International Congress of Internal Medicine, Kyoto, October 1984" International Congress Series No. 693, *Excepta Medica*, Tokyo, Japan, 1985, p. 232.

2) Fujiwara, K. : Treatment trial of traditional oriental medicine in chronic hepatitis. In "New Trends in Peptic Ulcer and Chronic Hepatitis" Part II Chronic hepatitis. *Excepta Medica*, Tokyo, Japan, 1987, p. 141.

3) Yokoyama, H., Hiai, S. and Oura, H. : Chemical structures and corticosterone secretion-inducing activity of saikosaponins. *Chem. Pharm. Bull.* **29**, 500-504, 1981.

4) Hiai, S., Yokoyama, H., Nagasawa, T. and Oura, H. : Stimulation of the pituitary-adrenocortical axis by saikosaponin of Bupleuri Radix. *Chem. Pharm. Bull.* **29**, 495-498, 1981.

5) Yamamoto, M., Kumagai, A. and Yokoyama, Y. : Structure and actions of saikosaponins isolated from *Bupleurum falcatum* L. *Arzneim.-Forsch.* **25**, 1021-1040, 1975.

6) Takagi, T. and Shibata, M. : Pharmacological studies on *Bupleurum falcatum* L. II. Anti-inflammatory and other pharmacological actions of crude saikosides. *Yakugaku-Zasshi* **89**, 1367-1378, 1969.

7) Amagaya, S., Higuchi, H. and Ogihara, Y. : Blockade by anti-glucocorticoids, actinomycin D and cycloheximide of the anti-inflammatory action of some kampohozai (Chinese traditional medicines) against serotonin. *J. Pharmacobio-Dyn.* **7**, 707-717, 1984.

8) Amagaya, S., Umeda, M. and Ogihara, Y. : Inhibitory action of Shosaikoto and Daisaikoto (traditional Chinese medicine) on collagen-induced platelet aggregation and prostaglandin biocynthesis. *Planta Medica* **52**, 345-349, 1986.

9) Shimizu, K., Amagaya, S. and Ogihara, Y. : Combined effects of Shosaikoto (Chinese traditional medicine) on the anti-inflammatory action of steroid. *J. Pharmacobio-Dyn.* **7**, 891-899, 1984.

10) Iwama, H., Amagaya, S. and Ogihara, Y. : Studies of the combined use of steroid and Shosaikoto, one of the Kampohozai (Chinese traditional medicine), on pituitary adrenocortical axis function and immune responses. *J. Pharmacobio-Dyn.* **9**, 189-196, 1986.

11) Iwama, H., Amagaya, S. and Ogihara, Y. : Effects of Kampohozai (traditional Chinese medicine) on immune responses. *In vivo* studies of Syô-saiko-tô and Dai-saiko-tô on antibody responses to sheep red blood cell and lipopolysaccharide. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 8-19, 1987.

12) Amagaya, S., Hayakawa, M., Ogihara, Y. and Fujiwara, K. : Effects of Syô-saiko-tô and Dai-saiko-tô on carbon tetrachloride-induced hepatic injury in rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 129-136, 1988.

13) Amagaya, S., Hayakawa, M., Ogihara, Y. and Fujiwara, K. : Effect of Syô-saiko-tô and Dai-saiko-tô on experimental hepatic fibrosis in rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **5**, 137-145, 1988.

14) Abe, H., Sakaguchi, M., Konishi, H., Tani, T. and Arichi, S. : The effect of saikosaponins on biological membranes. I. The relationship between the structures of saikosaponins and hemolytic activity. *Planta Medica* **34**, 160-166, 1987.

15) Abe, H., Sakaguchi, M., Odashima, S. and Arichi, S. : Protective effect of saikosaponin-d isolated from *Bupleurum falcatum* L. on CCl_4-induced liver injury in the rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **320**, 266-271, 1982.

16) Fisher, B., Azuch, P., Levine, M. and Fisher, E.R. : A ported blood factor as the humoral agent in liver regeneration. *Science* **171**, 575, 1971.

17) Miura, Y. and Fukui, N. : Prostaglandins as possible trigger for liver regeneration after partial hepatectomy. *Cell. Mol. Biol.* **25**, 179-184, 1979.

18) Yoshikawa, T. and Terayama, H. : Tissue producing the serum factor stimulating the release of cathepsin D from lysozomes *in vitro*. *J. Cong. Biochem. Physiol.* **77A**, 39-44, 1984.

19) Hayashi, S. and Kameji, T. : Ornithine decarboxylase (rat liver). *Methods in Enzymology* **94**, 154-158, 1983.

20) Ebina, Y., Iwai, H., Fukui, N., Ohtsuka, H. and Miura, Y. : Prereplicative enzyme changes in regenerating rat liver. *J. Biochem.* **77**, 641-645, 1975.

21) Busher, N.L.R. and Swaffield, M.V. : Reguration of hepatic regeneration in rats by sinergistic action of insulin and glucagon. *Proc. Natl. Acad. Sci. USA* **72**, 1157-1160, 1975.

22) Bucher, N.L.R. and Swaffield, N.M. : Synergistic action of glucagon and insulin in regulation of hepatic regeneration. *Adv. Enz. Regul.* **13**, 281-293, 1975.

23) Leffert, H.L., Koch, K.S., Moran, T. and Rubalcava, B. : Hormonal control of rat liver regeneration. *Gastroenterologya* **76**, 1470-1482, 1979.

24) Okita, K. and Matsuda, S. : Clinical use of glucagon and insulin in the therapy of fulminant hepatic failure. *Gastroenterologia Jap.* **14**, 453-457, 1979.

25) Higgins, G.M. and Anderson, R.M. : Experimental pathology of liver : Restoration of liver of white rat following partial surgical removal. *AMA. Arch. Pathol.* **12**, 186-202, 1931.

26) Heine, W.D. and Klinge, O. : Workshop on experimental liver injury, Freiburg, October, pp. 320-329, 1973.

27) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.J. : Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.

28) Schmidt, G., Thannhauser, S.J. : A method for the detection of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. *J. Biol. Chem.* **161**, 83-89, 1945.

29) Schneider, W.C. : Phosphorus compounds in animal tissues III. Comparison of methods for the estimation of nucleic acids. *J. Biol. Chem.* **164**, 747-751, 1946.

30) Burton, K. : A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of dexyribonucleic acid. *Biochem. J.* **62**, 315-323, 1956.
31) Mejbaum, W. : Estimation of small amounts of pentose especially in derivatives of adenylic acid. *Z. Physiol. Chem.* **258**, 117-120, 1939.
32) Seglen, P.O. : Preparation of isolated rat liver cells. *Methods. Cell. Biol.* **13**, 29-83, 1976.
33) Alain, J., Andre, E.L., Werner, S. and Albert, E.R. : Diabetogenic action of streptozotocin : Relationship of dose to metabolic response. *J. Clin. Invest.* **48**, 2129-2139, 1969.
34) Nakasone, K. : Effect of pancreatic hormones on liver regenerations. Morphometric analysis of mitochondria of rat hepatocyte. *Igaku-kenkyu* **55**, 77-96, 1985.
35) Balk, S.D., Whitfield, J.F., Toudale, T. and Braun, A. C. : Roles of calcium, serum, plasma and folic acid in the control of proliferation of normal and rous sarcoma virus-injected chicken fibroblasts. *Proc. Natl. Acad. Sci. USA* **70**, 675-679, 1973.
36) Rixon, R.H. and Whitfield, J.F. : The control of liver regeneration by parathyroid hormone and calcium. *J. Cell. Physiol.* **87**, 147-155, 1976.