Response of Liver to Glucocorticoid is Altered by Administration of Shosaikoto (Kampo Medicine)

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The effects of Shosaikoto, one of the Kampo medicines used for therapy for chronic hepatitis, on liver functions were studied in mice. Oral administration of Shosaikoto for 5 d enhanced the well-known induction of tyrosine aminotransferase (TAT) activity by dexamethasone. Further, TAT activity in mice treated with Shosaikoto was induced effectively by a smaller dose of dexamethasone, as compared with that in control mice. However, Shosaikoto itself did not induce TAT activity in the liver on oral administration or in cultured hepatocytes by direct addition. Moreover, Shosaikoto did not affect the induction of TAT activity by butyryl-adenosine 3′,5′-cyclic monophosphate. The amplifying effect of Shosaikoto seemed to be specific for induction by dexamethasone. These data suggest that Shosaikoto makes the liver sensitive to glucocorticoid by some unknown mechanism.

Keywords glucocorticoid; tyrosine aminotransferase; Shosaikoto; hepatocyte

Kampo-hozai (traditional herbal medicine in Japan) is a system of drug therapy which has been developed from clinical experience accumulated over some thousands of years in China. Shosaikoto, one of such Kampo prescriptions, is applied clinically as a therapy for chronic hepatitis.[1] In a clinical study, statistically significant improvements were observed in serum glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and γ-guanosine triphosphate (γ-GTP) two or three months later, after the administration of Shosaikoto.[2] In addition, Shosaikoto significantly repressed any increase in serum GOT and GPT levels in the rats treated with CCl_4 or D-galactosamine.[3] Although the mechanism by which serum transaminases are reduced is not understood, one possible explanation is that Shosaikoto stabilizes membranes of the liver and protects the liver from necrosis. In chronic hepatitis and liver cirrhosis, enhancement of the liver function or stimulation of regeneration of liver is necessary to restore the loss of function owing to necrosis, and the enhancement of immune function is necessary to protect the liver from virus infection. We have already shown that Shosaikoto stimulates liver regeneration and immune response.[4-6]

Therefore, in this report, we investigated the effect of Shosaikoto on the liver function in terms of the response to glucocorticoid, which is known to regulate the activities of some enzymes in the liver, and is used for the suppression of inflammation and the induction of seroconversion. Finally, we discuss the usefulness of Shosaikoto as a drug for treating chronic hepatitis.

Materials and Methods

Animals Male ICR mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were used at 6 weeks of age. They were kept in an air-conditioned room (24 °C) and given commercial diet and water *ad libitum*.

Reagents Collagenase was purchased from Nitta Gelatin Co., Ltd., Tokyo, Japan. William's medium E was from Hazleton Research Products, Inc. Dexamethasone, insulin and dibutyryl-adenosine 3′,5′-cyclic monophosphate (dibutyryl-cAMP) were from Sigma Chem. Co. L-Tyrosine and other chemicals were obtained from Nakarai Chemical Co., Kyoto, Japan.

Preparation of Cytosol Fraction for Assay of Tyrosine Aminotransferase (TAT) from Mouse Liver Mice were killed by decapitation between 2:00 to 4:00 in the afternoon, to avoid the influence of the circadian rhythm of TAT activity. The livers were homogenized in 5 volumes of 10 mM Tris–HCl, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM mercaptoethanol and 0.25 M sucrose with a Teflon-glass homogenizer. The homogenate was centrifuged at 105000 × *g* for 1 h at 4 °C to obtain the cytosol fraction used for enzyme assay.

Cell Isolation and Monolayer Culture Parenchymal hepatocytes were isolated from male ICR mice according to the method described by Seglen.[7] After Nembutal anesthesia, the cells were isolated by *in situ* perfusion of the liver with collagenase and were suspended at 5×10^5 cells/ml in William's medium E containing 10% fetal bovine serum (FBS), 10^{-8} M dexamethasone, 10^{-8} M insulin, penicillin 100 U/ml and streptomycin 100 μg/ml. Then they were cultured at a density of 10^5 cells/cm^2 in 6 cm dishes at 37 °C under 5% CO_2 in air. Cell viability was usually over 85%, as measured by the trypan blue exclusion test. After culture for 4 h, the medium was changed to hormone-free William's medium E containing 10% FBS. Following a 16 h culture, dexamethasone or Shosaikoto was added to the dishes at a final concentration from 10^{-9} to 10^{-5} M or of 250 μg/ml, respectively. The cells were incubated for another 6 h and then harvested with a rubber policeman in 1.5 ml of 10 mM Tris–HCl, pH 7.4 containing 1 mM EDTA, 0.1 mM PMSF, and 0.25 M sucrose. The cells were sonicated (2 × 10 s), the homogenate was centrifuged at 30000 × *g* for 30 min, and the resulting supernatant was used as the enzyme preparation for assay of TAT activity.

Enzyme Assay The activity of TAT in the cytosol was measured by the method of Diamondstone.[8] Protein was determined by the method of Lowry *et al.*,[9] using bovine serum albumin (BSA) as the standard.

Preparation of Shosaikoto Shosaikoto (dose per person per day) was prepared as follows. Bupleuri Radix (7 g), Pinelliae Tuber (5 g), Scutellarae Radix (3 g), Ginseng Radix (3 g), Zingbers Rhizoma (4 g), Zizyphi Fructus (3 g) and Glycyrrhizar Radix (2 g) were added to 700 ml of water, boiled for 1 h and concentrated to 300 ml. This decoction was lyophilized to give 7.2 g of powdered extract.

Results

We assessed the action of Shosaikoto on the response of liver to glucocorticoid by measuring the activity of TAT, which is known to be induced by glucocorticoid. There are several reports that Shosaikoto stimulates corticosterone secretion in 1 h after its administration and that Shosaikoto contains glucocorticoid-like components.[10] First, we ascertained whether the corticosterone secreted or Shosaikoto administered orally could induce TAT activity in liver. Following the oral administration of Shosaikoto (1.2 g/kg of body weight) at 8 a.m., TAT activity in liver was measured at the time indicated. As shown in Fig. 1, TAT activities of both groups showed an similar pattern of circadian rhythm. However, oral administration of Shosaikoto did not influence TAT activity in the liver until 12 h after administration. Taking account of circadian rhythm, the following experiments of TAT induction were performed from 10 a.m. to 4 p.m., unless otherwise indicated. In order to further examine the presence of

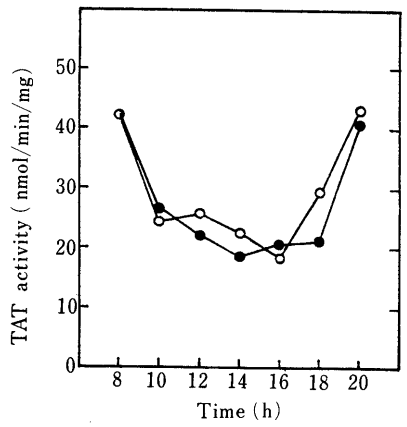

Fig. 1. Effect of Shosaikoto Orally Administered on TAT Activity in Liver

Shosaikoto was administered at 8 a.m. Open circles, H_2O *p.o.*; closed circles, Shosaikoto *p.o.* Values are the averages of two experiments carried out in duplicate.

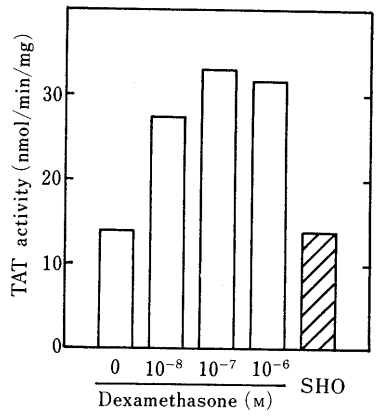

Fig. 2. TAT Induction by Dexamethasone and Shosaikoto in Isolated Hepatocytes

Shosaikoto was added at 250 μg/ml final concentration. Data are the averages of two experiments. SHO = Shosaikoto.

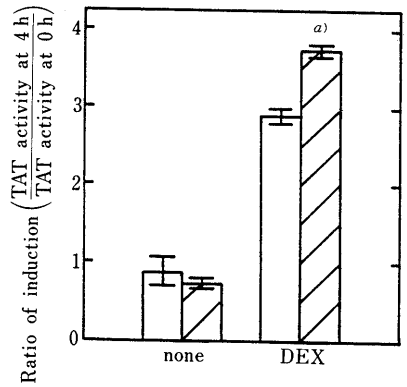

Fig. 3. TAT Induction by Dexamethasone in Liver

Dexamethasone (100 μg/kg of mouse) was injected s.c. at 10 a.m. TAT activity was measured at 4 h after injection, when the induction reached a maximum. Open columns, control mice; hatched columns, mice treated with Shosaikoto (1.2 g/kg of body weight) once a day for 5 consecutive days. Data represent the ratio of TAT activity at 4 h after injection to TAT activity at 0 h. Values are shown as means ± S.E. of four separate experiments. *a*) $p<0.05$ *vs.* control group. DEX = dexamethasone.

glucocorticoid-like components in Shosaikoto, we used cultured hepatocytes isolated from mice according to the method of Seglen.[7] It was found that 10^{-8} M dexamethasone significantly induced TAT activity in cultured

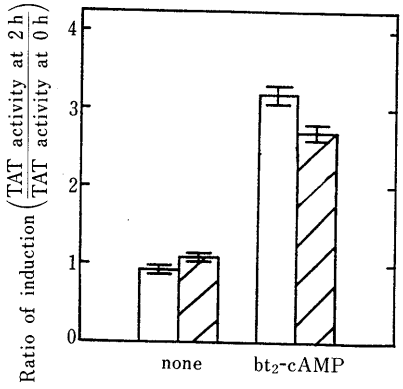

Fig. 4. TAT Induction by Dibutyryl-cAMP in Liver

Dibutyryl-cAMP (100 mg/kg of mouse) was injected s.c. at 10 a.m. TAT activity was measured at 2 h after injection, when the induction reached a maximum. Open columns, control mice; hatched columns, mice treated with Shosaikoto (1.2 g/kg of body weight) once a day for 5 consecutive days. Data represent the ratio of TAT activity at 2 h after injection to TAT activity at 0 h. Values are shown as means ± S.E. of three separate experiments. bt_2-cAMP = dibutyryl-cAMP.

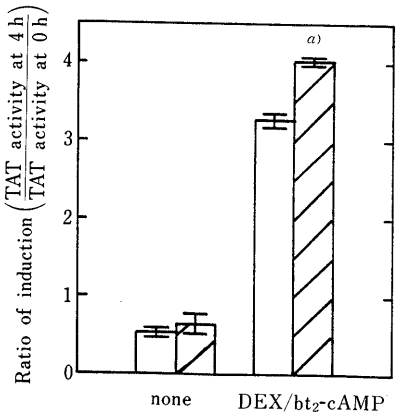

Fig. 5. TAT Induction by Dexamethasone and Dibutyryl-cAMP in Liver

Dexamethasone (100 μg/kg of mouse) and dibutyryl-cAMP(100 mg/kg of mouse) were injected s.c. at 10 a.m. TAT activity was measured at 4 h after injection, when the induction reached a maximum. Open columns, control mice; hatched columns, mice treated with Shosaikoto (1.2 g/kg of body weight) once a day for 5 consecutive days. Data represent the ratio of TAT activity at 4 h after injection to TAT activity at 0 h. Values are shown as means ± S.E. of three separate experiments. *a*) $p<0.05$ *vs.* control group.

hepatocytes and the induction reached a plateau at 10^{-7} M dexamethasone (Fig. 2). On the other hand, Shosaikoto did not induce TAT activity even at a dose of 250 μg/ml, the maximum concentration that did not injure cells. Further, when Shosaikoto was added to cultured hepatocytes simultaneously with dexamethasone (10^{-9} or 10^{-8} M), Shosaikoto did not influence the induction of TAT by dexamethasone (data not shown). We next investigated the effect of Shosaikoto administered for a long period on liver function. We orally administered Shosaikoto (1.2 g/kg of body weight) to mice once a day for 5 d and then injected dexamethasone (100 μg/kg of body weight) subcutaneously on the 6th day. The induction of TAT activity reached a maximum at 4 h after the injection of dexamethasone. As shown in Fig. 3, the induction of TAT activity in mice treated with Shosaikoto was significantly greater than that in control mice. When dibutyryl-cAMP was injected instead of dexamethasone, the induction of TAT was maximum at 2 h after the injection. However, the treatment with Shosaikoto repressed the induction slightly in contrast with

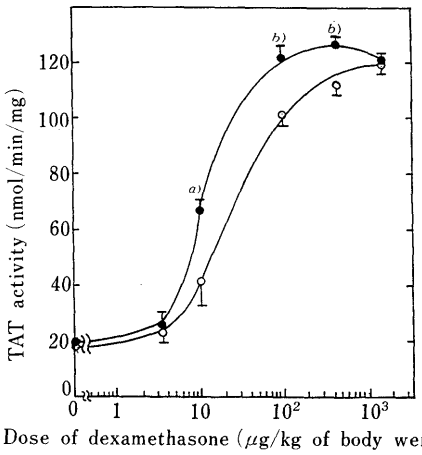

Fig. 6. Dependence of TAT Induction in Liver on the Concentration of Dexamethasone

Open circles, control mice; closed circles, mice treated with Shosaikoto (1.2 g/kg of body weight) once a day for 5 consecutive days. Data are shown as means ± S.E. of three separate experiments. *a*) $p<0.05$. *b*) $p<0.01$ *vs.* respective control.

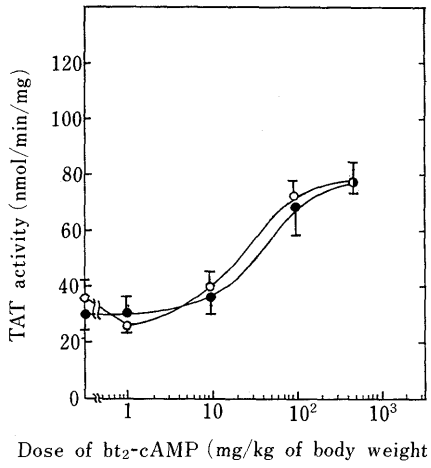

Fig. 7. Dependence of TAT Induction in Liver on the Concentration of Dibutyryl-cAMP

Open circles, control mice; closed circles, mice treated with Shosaikoto (1.2 g/kg of body weight) once a day for 5 consecutive days. Values are shown as means ± S.E. of three separate experiments.

dexamethasone (Fig. 4). In the experiment shown in Fig. 5, dexamethasone and dibutyryl-cAMP were injected subcutaneously at the same time. As in the case of the injection of dexamethasone, TAT activity was induced maximally at 4 h after the injection and was much greater in mice treated with Shosaikoto than in control mice. Figure 6 shows the dose-response relation for induction of TAT activity by dexamethasone. Dexamethasone (10 μg/kg) significantly induced TAT activity in mice treated with Shosaikoto, whereas 10 μg/kg of dexamethasone was not enough to induce TAT activity in control mice. However, at a dose of 1.67 mg of dexamethasone per kg of body weight, TAT activities were induced to the same extent in both groups. On the other hand, as shown in Fig. 7, Shosaikoto treatment did not cause an increase of TAT activity induced by dibutyryl-cAMP at any dose. These results indicated that long-term treatment with Shosaikoto affected the pathway of TAT induction by dexamethasone and altered the response of the liver to dexamethasone. Therefore, we next measured glucocorticoid receptors in the liver using ^{3}H-dexamethasone. Although the data are not shown, no difference was seen in the K_d values.

Discussion

Most biochemical experiments concerning Kamp-hozai reported previously were done *in vitro* or by i.p. administration, which is different from the route by which Kampohozai is clinically administered. Therefore, in this paper we orally administered Shosaikoto to mice and investigated the effect biochemically. Based on the *in vitro* experiments reported to date, Shosaikoto contains many physiologically active components, for instance, saikosaponin, glycyrrhizin, *etc.* However, their actions have not been fully confirmed *in vivo* and their actions *in vitro* were different from those *in vivo* in some cases. Therefore, to investigate the effect of Shosaikoto on liver function, we first examined whether corticosterone, which was released upon Shosaikoto administration either orally or intraperitoneally, showed extensive action on the liver and whether glucocorticoid-like components in Shosaikoto actually showed glucocorticoid-like action on the liver. For this purpose, we measured TAT activity, since it is known to be induced by glucocorticoid. As TAT shows circadian rhythm, the induction of TAT activity was measured from 10 a.m. to 4 p.m. when TAT activity was at a nadir. In contrast to the prediction based on previous data, Shosaikoto did not induce TAT activity when administered orally or added to cultured hepatocytes. These data suggested that corticosterone secreted after Shosaikoto administration and glucocorticoid-like components in Shosaikoto were insufficient to induce TAT in the liver or that glucocorticoid-like components did not exist in Shosaikoto. Many components of Shosaikoto were converted into other components in the stomach-intestinal tract by acid or flora. Thus, the effective concentration of such components in blood was thought to be much lower than had been expected. However, when Shosaikoto was administered for 5 d, the induction of TAT activity by dexamethasone but not dibutyryl-cAMP was enhanced in mice treated with Shosaikoto as compared with control mice. These results indicated that Shosaikoto modified the pathway of TAT induction by dexamethasone and made the liver responsive to low concentrations of dexamethasone. Hence, we measured glucocorticoid receptor in the liver using ^{3}H-dexamethasone. The data obtained did not explain the enhancement of TAT induction by dexamethasone after the treatment with Shosaikoto, in terms of K_d and B_m values. Shosaikoto, indeed, facilitates the secretion of corticosterone, but since Shosaikoto does not induce TAT activity in the liver, the induction may require a higher concentration of corticosterone than that made available by Shosaikoto. Consequently, the enhancement of TAT induction by dexamethasone after Shosaikoto treatment might be due to components other than glucocorticoid, that influence the pathway of TAT induction by dexamethasone. Kido *et al.* reported that diacylglycerol and phorbol ester derivatives (stimulators of protein kinase C) enhanced the induction of TAT by various glucocorticoids but had no effect on the induction of TAT by glucagon or insulin.[11,12] Hence, it is conceivable that Shosaikoto contains such components as diacylglycerol or phorbol ester that stimulate protein kinase C. However, we can not exclude

the possibility that the permeability to dexamethasone in the tissue into which dexamethasone was injected was altered by Shosaikoto treatment, resulting in more efficient transport of dexamethasone to the blood, and hence to the liver, where TAT is induced. Further experiments are required to clarify the action of Shosaikoto.

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