—**Regular Articles**—

Effects of *Sho-saiko-to* (*Xiao Chai Hu Tang*), a Chinese Traditional Medicine, on the Gastric Function and Absorption of Tolbutamide in Rats

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(Received July 31, 2000; Accepted November 7, 2000)

This study was carried out to investigate the effects of *Sho-saiko-to* (*Xiao Chai Hu Tang*), a Chinese traditional medicine, on the gastric function including the gastric emptying rate (GER) and intragastric pH in rats. Additionally, the effects of the GER and intragastric pH on tolbutamide absorption after oral administration were examined. The GER measured at 40 min after dosing was reduced to about 70% by the pretreatment of *Sho-saiko-to* (500 mg/kg). The plasma tolbutamide concentration in the rats treated with a 250 mg/kg dose of *Sho-saiko-to* was significantly lower than that in the control group. Plasma tolbutamide concentrations increased along with the GER in the group co-administered *Sho-saiko-to*, and there were significant correlations between the GERs and plasma levels in both time points at 20 and 40 min after administration. In the study using pylorus-ligated rats, *Sho-saiko-to* significantly elevated the intragastric pH, but induced no change in the concentrations of tolbutamide dissolved in the gastric content. Additionally, *Sho-saiko-to* did not change the area under the plasma concentration-time curve (AUC) of tolbutamide up to 60 min after administration into the stomach loop, and gastric absorption has been considered to minimally contribute to whole absorption of tolbutamide in the gastrointestinal tract. These results indicate that *Sho-saiko-to* has an inhibitory effect on the function of gastric emptying in rats. The reduced gastric emptying could affect gastrointestinal absorption, resulting in the lower plasma concentration of tolbutamide after oral administration. Furthermore, it is suggested that *Sho-saiko-to* can raise the intragastric pH but affect neither the intragastric dissolution nor the gastric absorption of tolbutamide.

Key words——tolbutamide; *Sho-saiko-to*; gastric emptying; gastric pH; gastric absorption; rat

INTRODUCTION

Chinese traditional medicines are being used for an increasing number of patients who are treated with various western drugs. Miller[1] pointed out that clinicians should pay attention to known or potential drug-herb interactions when they prescribe herbal medicinals with western drugs. In fact, many pharmacokinetic and pharmacodynamic interactions between herbal medicinals and western drugs have been reported to date.[2—5] Scientifically based studies on drug-herb interactions are absolutely necessary for clinicians to treat their patients adequately and safely with herbal medicinals and western drugs in concomitant use.

Sho-saiko-to (*Xiao Chai Hu Tang*), a Chinese traditional medicine, is frequently prescribed for the treatment of chronic hepatitis in Japan. Kase *et al.*[6] reported that *Sho-saiko-to* reduced the volume of gastric juice, acid output and pepsin output, causing the rise of intragastric pH in rats. Furthermore, the inhibitory effects of glycyrriza and ginger, crude components included in *Sho-saiko-to*, on gastric acid secretion and gastric emptying have been reported[7—11]. It is likely that such pharmacological effects of the herbal medicinals on the gastric functions give rise to a change in the pharmacokinetics of concomitantly administered drugs.

A prototype of sulphonylurea hypoglycemic agents, tolbutamide, has been widely used in pharmacotherpy for diabetes mellitus. This drug can be absorbed appreciably from the stomach as well as the duodenum in rats,[12] so that the gastric functions such as gastric emptying and gastric acid secretion possibly influence the pharmacokinetics, especially in the absorption process, after oral dosing. It is known that tolbutamide has a fairly low therapeutic index, so pharmacokinetic change of this compound could lead to serious pharmacodynamic reactions.[13] In fact, it has been reported that the bioavailability and hypoglycemic effects of this drug were enhanced by elevation of gastric pH in humans.[14]

Recently, Goto *et al.*[15] reported that *Sho-saiko-to* may be effective against the pathological conditions of diabetes mellitus that involve disorders of lipid and mineral metabolism in rats, so that *Sho-saiko-to* may be prescribed with tolbutamide to treat diabetes mellitus. Therefore, information on the effects of *Sho-saiko-to* on the gastric function and tolbutamide absorption is needed to treat the diabetes patient safely.

The purpose of this study is to clarify whether *Sho-saiko-to* could affect the gastric function, including the gastric emptying rate (GER) and intragastric pH, in rats. Additionally, the effects of the GER and intragastric pH on the absorption of tolbutamide after oral administration were examined.

MATERIALS AND METHODS

1. Materials Tolbutamide was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). *Sho-saiko-to* extract granules, an oral dosage form for therapeutic use, were purchased from Tsumura & Co. Ltd. (Lot No. 13053772, Tokyo, Japan). *Sho-saiko-to* was manufactured from an extract of bupleurum root (29.2%), pinellia tuber (20.8%), scutellaria root (12.5%), jujube fruit (12.5%), ginseng root (12.5%), glycyrrhiza root (8.3%) and ginger rhizome (4.2%), and the extract granules consisted of a 6 : 4 (w/w) mixture of the extract and additive. All other chemicals were of analytical grade and purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan) or Nacalai Tesque, Inc. (Kyoto, Japan).

2. Animals Male Sprague-Dawley rats (Japan SLC Inc., Hamamatsu, Japan) were used, weighing from 215 to 347 g, and 8–10 weeks of age. Each rat was fasted for approximately 24 h and given no water for 1–2 h before the experiment in individual cages. All rats used in this study were housed in a laboratory maintained at a 12 h light-dark cycle, with a controlled room temperature of 23 ± 2℃ and relative humidity of 50 ± 10%. Animal experiments were all carried out in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science.

3. Gastric Emptying Rate (GER) Study Gastric emptying was evaluated according to the methods reported by Scarpignato *et al.*[16] and Ohba *et al.*[17] with a few modifications. Unanesthetized rats were given 1%(w/v) of arabic gum suspension (vehicle, 2 ml/kg), with or without *Sho-saiko-to* (250-750 mg/kg), 1 h before oral administration of a prewarmed test meal (37℃, 4 ml/kg) containing 0.05% (w/v) phenol red, 2%(w/v) aqueous methylcellulose (400 cps) and tolbutamide (12.5 mg/ml) administered with a gastric catheter. Since the preliminary study indicated the test meal mixed with *Sho-saiko-to* in arabic gum suspension was too viscous to administer by gastric incubation, *Sho-saiko-to* was given in the pretreatment according to the previous method of GER measurement[17]. The rats were killed by cervical dislocation at 0 min (standard stomach), 20 or 40 min (test stomach) after administration of the test meal. The stomach was exposed by laparotomy, quickly ligated at the pylorus and cardia and removed with its contents. At the same time as the sacrifice, the blood was withdrawn from the abdominal aorta to collect the plasma by centrifugation at 1200 rpm for 5 min. Plasma was frozen and kept until determination of tolbutamide concentrations were complete. The isolated stomach was homogenized with a Polytron homogenizer (model PY10TSKR: Kinematica AG, Littau-Luzern, Switzerland) in 100 ml of 0.1 N NaOH. Five milliliters of the homogenate was added to 0.5 ml of a 20%(w/v) trichloroacetic acid solution, followed by vortexing for 1 min and centrifugation at 2500 g for 20 min. The supernatant (5 ml) was added to 4 ml of 0.5 N NaOH, and then vortexed for 1 min. The absorption of the solution was measured at 560 nm with a spectrophotometer (UV-160A: Shimadzu, Kyoto, Japan). The GER for each rat was calculated according to the following equation:

$$\mathrm{GER}(\%) = \left(1 - \frac{\begin{array}{c}\text{Amount of phenol red recovered}\\ \text{from the test stomach/}\\ \text{body weight of the rat}\end{array}}{\begin{array}{c}\text{Average of (phenol red amount}\\ \text{recovered from the standard}\\ \text{stomach/body weight of the rat)}\end{array}}\right) \times 100$$

4. Stomach Loop Study Under ether anesthesia, the rat stomach loop was prepared by the pylorus ligation. The abdomen was then closed. Thirty minutes later, tolbutamide (50 mg/kg) suspended in 1% potato-starch/0.9% NaCl solution (potato starch saline), with or without *Sho-saiko-to* (500 mg/kg), was placed into the stomach loop of the conscious rats. At 20, 40 or 60 min after placing the drug, the rats were killed by cervical dislocation and the stomach was removed. Immediately, blood samples were withdrawn from the abdominal aorta, and the serum was separated from the blood by centrifugation with the serum separator (Fibritin: Takazono Sangyo Co., Ltd., Osaka, Japan). A portion of the serum was ultrafiltrated with a micropartition device (Centrifree: Millipore, Co.; Bedford, MA, U.S.A.) at 4000 rpm for 20 min at room temperature. The intragastric pH was measured using an electric pH meter (F-8 AT: Horiba Seisakusho, Ltd., Kyoto, Japan) with a fine-tip combination electrode (6029-10T: Horiba Seisakusho, Ltd.) directly inserted into the stomach from the pylorus as soon as the stomach was isolated from the rats. After the measurement of the pH, the supernatant of the gastric content was collected by centrifugation at 12000 rpm for 5 min to determine the concentrations of tolbutamide

dissolved in the stomach content. Every sample was frozen and kept until the determination of tolbutamide concentrations were complete.

5. Analytical Procedure Tolbutamide concentrations in each biological sample were determined by a high performance reversed phase liquid chromatographic method developed previously for the assay of plasma drug levels.[18] No interfering peak was observed in chromatograms for the sample of the gastric contents.

6. Statistics These results were expressed as the mean ±SD for the indicated numbers of experiments. The Student's t-test was utilized to estimate the statistical significance of difference between the means of two groups. One way analysis of variance was used in the dose-dependence experiment in the GER study. A p value of 0.05 or less was considered to be statistically significant.

RESULTS

1. GER Study In both the control group and the *Sho-saiko-to* pretreatment group, the GER measured at 20 min after administration of the test meal was about 35%. At 40 min after dosing, the control group showed a GER of 80.9%, whereas a significantly lower GER of 58.2% was observed in the rats treated with 500 mg/kg of *Sho-saiko-to* (Fig. 1). Effects of the pretreatment dose of *Sho-saiko-to* on the GER and plasma tolbutamide concentration, which were determined at 40 min, are summarized in Table 1. In results similar to those obtained from the dose of 500 mg/kg, pretreatment of *Sho-saiko-to* in doses of 250 and 750 mg/kg tended to reduce the GERs to about 70% of the control group. The plasma tolbutamide concentration in the rats treated with the 250 mg/kg dose of *Sho-saiko-to* was significantly lower than that in the control group. However, dose-dependent effects were not detected in either the GER or the plasma tolbutamide concentration. Individual plasma data of the tolbutamide concentration obtained in the group given *Sho-saiko-to* (500 mg/kg) were plotted against the corrsponding GER data. The results are shown in Fig. 2. Significant correlations ($p < 0.05$) between the GER and plasma level were observed in both time points (Fig. 2, Panel A; 20 min: Panel B; 40 min).

2. Stomach Loop Study The intragastric pH and tolbutamide concentrations dissolved in the gastric content after placing the tolbutamide suspension with or without *Sho-saiko-to* are summarized in Table 2. In the preliminary study using rats without the stomach loop, the pH values of the stomach contents measured at 30 min after administration of potato starch saline were 1.81±0.03 and 2.60±0.50 for the control (without *Sho-saiko-to*) and *Sho-saiko-to* treated rats, respectively. Since the results shown in Table 2 coincided with those pH values in the preliminary experiments, it is considered that the preparation of the stomach loop could not influence the intragastric pH during the experiments. The group given tol-

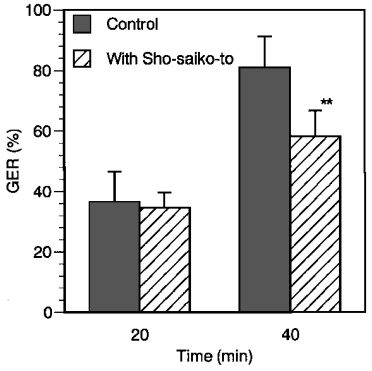

Fig. 1. Effects of Pre-administration of Sho-saiko-to Extract Granules (500 mg/kg) on the GER (%) at 20 or 40 min after Administration of the Test Meal in Rats

The vehicle alone (control) and the vehicle with Sho-saiko-to was administered 1h before the test meal administration. Each column and vertical bar represents the mean and SD of 3 or 5 rats. There is a significant difference from the control (Student's t-test: $^{**}p < 0.01$).

Table 1. Effects of Sho-saiko-to on the GER and Plasma Concentration of Tolbutamide at 40 min after Administration of the Test Meal with the Pretreatment of Sho-saiko-to

Dose of Sho-saiko-to(mg/kg)	GER (%)[a]	Tolbutamide conc. (μg/ml)
None (control)	80.94±10.27	268.2±19.0
250	58.17±18.37	203.1±36.0**
500[b]	58.18± 8.55	251.1±24.6
750	56.41±19.37	234.0±10.5

Data are expressed as the mean±SD of 4-6 rats. *a*) The values were estimated from the eqation:

$$\text{GER (\%)} = \left(1 - \frac{\text{Amount of phenol red recovered from the test stomach/body weight of the rat}}{\text{Average of (phenol red amount recovered from the standard stomach/body weight of the rat)}}\right) \times 100$$

b) Data are adapted from Fig. 1. There is a significant difference from the control (One way analysis of variance: ** $p < 0.01$).

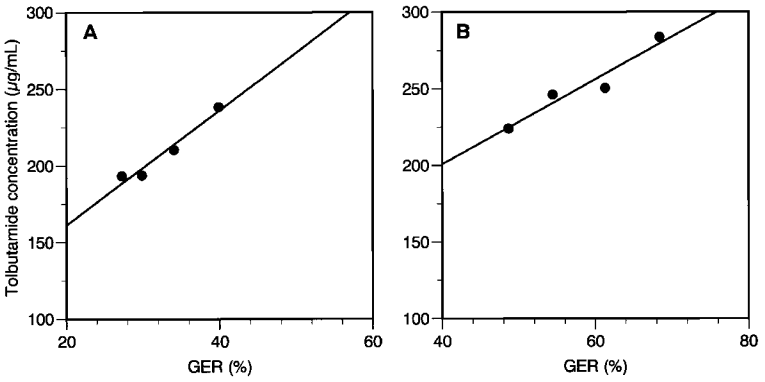

Fig. 2. Relationship between the GER and Plasma Tolbutamide Concentration after Test Meal Administration with Pretreatment of the Sho-saiko-to Extract Granules (500 mg/kg) in Two Time Points (Panel A: 20 min, Panel B: 40 min)
The solid lines were obtained by the least-squares method.

Table 2. Effects of Sho-saiko-to (500 mg/kg) on the Intragastric pH and Concentration of Tolbutamide Dissolved in the Gastric Content after Administration of Tolbutamide with or without Sho-saiko-to in the Stomach Loop

Time (min)	Intragastric pH		Tolbutamide conc. (μg/ml)	
	Tolbutamide alone	With Sho-saiko-to	Tolbutamide alone	With Sho-saiko-to
20	1.79±0.53	3.48±0.65**	50.0±2.5	57.5±8.1
40	1.86±0.43	3.04±0.59*	53.5±8.1	55.7±6.6
60	1.85±0.64	2.53±0.29	48.0±3.5	50.7±3.1

Each drug suspension was adjusted to a pH of 5.1 before administration. Each value is expressed as the mean±SD of 3-5 rats. There is a significant difference from the group of tolbutamide alone (Student's t-test: * $p<0.05$, ** $p<0.01$).

butamide alone kept the intragastric pH of about 1.8 over 60 min, while concomitant administration with *Sho-saiko-to* significantly raised the intragastric pH to more than 2.5. However, the concentrations of tolbutamide dissolved in the gastric content were not changed by the concomitant dose of *Sho-saiko-to* throughout the experiment.

Figure 3 is time-course profiles of serum tolbutamide levels after administration to the stomach loop. Co-administration of *Sho-saiko-to* induced about a 40% increase in the serum tolbutamide concentrations at 60 min after administration, although some decrease was found at 20 min. Fractions of tolbutamide bound to the serum protein determined at the three sampling time-points were 93.2±2.1% and 91.3±3.3% for the rats treated with tolbutamide alone and tolbutamide with *Sho-saiko-to*, respectively. No change by the presence of *Sho-saiko-to* was observed in the bound fractions of tolbutamide. The AUCs of serum tolbutamide concentrations up to 60 min in the stomach loop study were calculated as a gastric AUC by the trapezoidal method using a program WinNonlin (Scientific Consulting Inc.; Cary, USA). The respective AUCs for tolbutamide alone and for the *Sho-saiko-to* co-administered group were 18.9 μg h/ml and 20.9 μg h/ml.

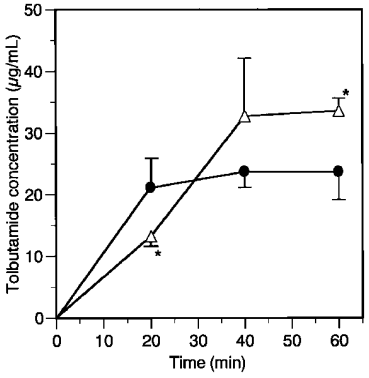

Fig. 3. Effects of Sho-saiko-to on Serum Tolbutamide Concentrations after Placing Tolbutamide (50 mg/kg) with (△) or without (●, control) Sho-saiko-to (500 mg/kg) in the Rat Stomach
Each point and vertical bar represents the mean and SD of results from 3 or 6 rats. There is a significant difference from the control (Student's t-test: * $p<0.05$).

DISCUSSION

In the GER study, the pretreatment of *Sho-sai-*

ko-to did not reduce the GER at 20 min after administration of the test meal, but the significantly reduced GER was obtained at 40 min (Fig. 1). Additionally, the inhibitory effect on the GER at 40 min was also obtained in the other two doses (250 and 750 mg/kg, Table 1). Intragastric pH is known to be a factor affecting the gastric emptying fuction.[19,20] In the present study, however, *Sho-saiko-to* altered the GER only at 40 min after administration, while the rise of intragastric pH was observed at both 20 and 40 min. These results suggest that the reduced GER by *Sho-saiko-to* is caused by factors other than gastric pH. Several previous studies using the components of *Sho-saiko-to* described the effects on the gastric emptying function. Wang *et al.*[11] reported that the extract of glycyrrhiza (1354 mg/kg), a major constituent of *Sho-saiko-to*, reduced the GER to 50—70 % of the control in rats. In our study, a similar reduction of the GER was observed in spite of the smaller dose of glycyrrhiza, about 13—40 mg/kg. Additionally, Aburada *et al.*[21] reported that i.v. administration of [6]-gingerol suppressed the gastric contraction by reflection of the action on the central nervous system. [6]-Gingerol is a component of the ginger rhizome, which is one of the constituents of *Sho-saiko-to*. This component is known to be absorbed, to a considerable extent, from the intestines in rats.[22,23] From these aspects, it is suggested that the suppression of gastric emptying in the rats treated with *Sho-saiko-to* may be induced by the combined effects of glycyrrhiza with other active components included in *Sho-saiko-to*. The reasons why *Sho-saiko-to* did not alter the GER at 20 min is unclear. It is supposed that the active components in *Sho-saiko-to* had not yet been sufficiently absorbed from the gastrointestinal tract to show the pharmacological effects in this relatively short period of time.

It is well known that the GER could influence the absorption rate of many kinds of drugs.[17,24—27] In order to elucidate whether the GER reduced by *Sho-saiko-to* could change tolbutamide absorption from the gastrointestinal tract, tolbutamide concentrations in plasma were simultaneously determined in the GER study and the correlation between plasma tolbutamide concentrations and GERs was examined. As the results in Table 1 show, plasma tolbutamide concentrations in the group given 250 mg/kg doses of *Sho-saiko-to* were significantly lower than those in the control group. In addition, the proportional increase of plasma tolbutamide levels with the GER was found in both time-points (Fig. 2), indicating that the GER is an important factor determining the plasma tolbutamide concentration after oral administration. Thus, it is clarified that *Sho-saiko-to* has an inhibitory effect on the gastric emptying function to delay gastrointestinal absorption of tolbutamide, leading to a decrease in the plasma concentration of this drug after oral administration. We have reported that *Sho-saiko-to* elevated plasma concentrations of tolbutamide up to 1 h after oral co-administration.[18,28] In the present studies, plasma tolbutamide concentration tended to be decreased by pre-treatment of *Sho-saiko-to* up to 40 min after dosing. Thus, the present results were not consistent with our previous findings. In the GER study, *Sho-saiko-to* was administered 1 h before the tolbutamide dosing, while *Sho-saiko-to* was given simultaneously with tolbutamide in the previous studies. Thus, the difference of the dosing methods may be related to this inconsistency.

Tolbutamide is a weak acid with low aqueous solubility at acidic pH, so that the variation of the intragastric pH may change the absorption of this drug. In the second part of this study, focusing on the effect of *Sho-saiko-to* on the intragastric pH, a stomach loop technique was utilized to assess intragastric dissolution and gastric absorption of tolbutamide. As is obvious in Table 2, the intragastric pH was significantly elevated in the presence of *Sho-saiko-to*. Recently, it has been reported that *Sho-saiko-to* can inhibit the gastric acid secretion.[6,29] Additionally, similar inhibitory effects by some crude drugs included in *Sho-saiko-to* were reported in laboratory animals.[7,10] The results of the present study are consistent with these aspects reported previously. However, the concentrations of tolbutamide dissolved in the gastric content were not changed by concomitant administration of *Sho-saiko-to* (Table 2). In general, the solubility of a drug is substantially affected by a small change in the pH around the pK_a. In the case of this study, the pK_a of tolbutamide, 5.4, diverged considerably from the intragastric pH range produced by *Sho-saiko-to*. Therefore, it is understandable that the increase in the intragastric pH from 1.8 to 3.5 induced by *Sho-saiko-to* could not affect the concentration of tolbutamide dissolved in the stomach. It was reported that the absorption of albendazole, a basic drug with a pK_a of 2.68 and 11.83, is greatly altered in concurrence with the intragastric pH, resulting from an increase in the solubility of the drug.[30] It is possible that the increase in intragastric pH obtained in the present study can induce an 85% decrease in albendazole solubility, leading to reduction of the bioavailability of this drug. Thus, the rise of intragastric pH by *Sho-saiko-to* may affect absorp-

tion of concomitantly administered drugs which have the pK_a around the gastric pH.

Co-administration of *Sho-saiko-to* decreased the serum tolbutamide concentration at 20 min after administration, while at 60 min, serum tolbutamide levels in the rats given *Sho-saiko-to* was significantly higher than that of the control group (Fig. 3). Although some changes by *Sho-saiko-to* were observed in the time-profile of serum tolbutamide levels after administration into the stomach, the gastric AUC of tolbutamide was not altered by *Sho-saiko-to*. These findings indicate that co-administration of *Sho-saiko-to* has no effect on the gastric absorption of tolbutamide.

Gastric AUCs in both groups were at most 10% of the oral AUCs (158 ± 52 μg h/ml for tolbutamide alone, 183 ± 30 μg h/ml for the *Sho-saiko-to* co-administered group) obtained from the *in-vivo* oral administration study reported previously.[28] This comparison means that gastric absorption minimally contributes to whole absorption of tolbutamide in the gastrointestinal tract. Consequently, it is considered that a large parcentage of tolbutamide, after oral administration, could be absorbed from the intestinal tract. Since it has been recently reported that glycyrrhizin and its salts can enhance intestinal absorption or cellular transport of the poorly absorbed drugs,[31—34] the detailed investigation on the effects of *Sho-saiko-to* at the small intestine may be needed to clarify the effects of co-administration with *Sho-saiko-to* on the tolbutamide absorption after oral dosing.

In conclusion, this study indicates that *Sho-saiko-to* has an inhibitory effect on the function of the gastric emptying in rats. The reduced gastric emptying could affect the gastrointestinal absorption, resulting in the lower plasma concentration of tolbutamide after oral administration. Furthermore, it is suggested that *Sho-saiko-to* can raise the intragastric pH but affect neither the intragastric dissolution nor the gastric absorption of tolbutamide.

Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research (No. 10922085) provided by the Ministry of Education, Science and Culture of Japan.

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