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Research Paper

Change in tolbutamide permeability in rat jejunum and Caco-2 cells by Sho-saiko-to (Xiao Chai Hu Tang), a Chinese traditional medicine

Nobuhiro Nishimura[a], Tomichika Uemura[a], Kikuo Iwamoto[b] and Kohji Naora[a]

[a]Department of Pharmacy, Shimane University Hospital, Shimane and [b]Department of Clinical Pharmacokinetics, School of Pharmacy, Aichi Gakuin University, Nagoya, Japan

Abstract

Objectives This study was designed to investigate the effects of Sho-saiko-to (Xiao Chai Hu Tang), a Chinese traditional medicine, on the membrane permeability of tolbutamide in the intestinal tract. We carried out an in-situ loop study with rat jejunum and a transport study with Caco-2 cell monolayers.

Methods In the in-situ loop study, absorption clearance of tolbutamide was estimated from the drug concentrations in the loop and plasma. The apical-to-basolateral and basolateral-to-apical transport of tolbutamide and D-mannitol, a paracellular transport marker, was assessed using Caco-2 cell monolayers cultured on a polycarbonate membrane.

Key findings The absorption clearance of tolbutamide was enhanced by a concomitant dose of Sho-saiko-to over 10 min in the rat in-situ loop. Sho-saiko-to increased the apical-to-basolateral transport of tolbutamide, whereas the basolateral-to-apical transport of this drug was reduced by Sho-saiko-to. On the other hand, in both directions the P_{app} of D-mannitol was reduced by the presence of Sho-saiko-to. Furthermore, the apical-to-basolateral transport of tolbutamide in ATP-depleted Caco-2 cells was diminished by Sho-saiko-to. These findings suggest that Sho-saiko-to can facilitate the epithelial membrane permeability of tolbutamide across the rat jejunum in-situ and Caco-2 cell monolayers. Since Sho-saiko-to suppressed the passive transport of tolbutamide from the apical-to-basolateral side, enhanced permeability may be related to effects of Sho-saiko-to on the energy-dependent transport of tolbutamide in the intestine.

Conclusions Our findings suggest that Sho-saiko-to might facilitate the energy-dependent transport of tolbutamide across the rat jejunum in-situ and Caco-2 cell monolayers.

Keywords Caco-2 cells; permeability; rat jejunal loop; Sho-saiko-to; tolbutamide

Introduction

Recently, Chinese herbal medicines have been used on a grand scale for the treatment of many chronic and acute diseases, such as diabetes mellitus, asthma, hepatitis and rheumatoid arthritis, in Japan, because they appear to boast a mild, wide therapeutic efficacy with relatively low incidence of adverse reactions in comparison with western drugs. In many chronic diseases, some western drugs are commonly administered with Chinese herbal medicines used for supplementary purposes, so that it is important to obtain information about interactions of herbal medicines with co-administered western drugs.[1]

Sho-saiko-to (Xiao Chai Hu Tang) is one of the 'Kampo' formulations, namely traditional Chinese medicines, prescribed most frequently in Japan, and is prepared from seven herbs (Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma). Sho-saiko-to is often used for the treatment of chronic diseases, such as hepatitis, bronchitis and gastroenteropathy. The safety and pharmacokinetics of this herbal medicine administered solely in healthy subjects has been reported.[2] There are several reports on pharmacokinetic interactions of Sho-saiko-to with synthesized drugs.[3–5] In a previous report, it was reported that Sho-saiko-to and other Chinese traditional medicines may be effective against some disorders of lipid

Correspondence: Professor Kohji Naora, Department of Pharmacy, Shimane University Hospital, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan.
E-mail: knaora@med.shimane-u.ac.jp

and mineral metabolism, including diabetes mellitus.[6] Thus, sulfonylurea antidiabetic drugs, such as tolbutamide, are occasionally used with Sho-saiko-to in patients with non-insulin-dependent diabetes mellitus.

We previously reported that Sho-saiko-to could facilitate the gastrointestinal absorption of tolbutamide, a prototype sulfonylurea hypoglycaemic agent, in rats.[7,8] However, detailed mechanisms for the interaction between them in gastrointestinal absorption processes have not yet been elucidated. Tolbutamide has a fairly low therapeutic index, so that pharmacokinetic changes could lead to serious pharmacodynamic reactions.[9,10] Therefore, to predict and prevent serious reactions induced by drug interaction, it is necessary to characterize the mechanism for increased absorption of tolbutamide after co-administration with Sho-saiko-to. In previous studies, we have shown that Sho-saiko-to did not affect the gastric absorption of tolbutamide in rats[11] and that dissolution of tolbutamide was not changed by Sho-saiko-to.[12] Thus, we focused on the absorption processes of the drug in the small intestine.

We carried out fundamental investigations on the effect of Sho-saiko-to on the intestinal absorption of tolbutamide by using the in-situ loop technique in rats. Moreover, we performed transport studies of tolbutamide in Caco-2 cell monolayers to assess whether Sho-saiko-to could affect tolbutamide transport across the gastrointestinal membrane.

Materials and Methods

Materials

Sho-saiko-to extract granules was purchased from Tsumura & Co. Ltd (Tokyo, Japan). Tolbutamide was obtained from Sigma Chemical Co. (St Louis, USA). [^{14}C]-Tolbutamide and [1, 2-^{3}H]-polyethylene glycol (PEG) 4000 were purchased from American Radiolabeled Chemicals Inc. (St Louis, USA). [1-^{14}C]-D-Mannitol was obtained from Moravek Biochemicals, Co. (Brea, USA). All other chemicals were of highest purity and purchased from Wako Pure Chemicals Industries, Ltd (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan) or Sigma Chemical Co. (St Louis, USA).

In-situ intestinal absorption studies

Male Sprague–Dawley rats, 10–11 weeks old (Nippon SLC, Hamamatsu, Japan), were used. Rats were housed in the laboratory maintained at a 12-h light–dark cycle, controlled room temperature of 23 ± 2°C 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. The experimental protocol and procedures were approved by the Animal Experiment Committee of the Shimane University School of Medicine.

Before the experiment each rat was kept in an individual cage, fasted for approximately 24 h and prevented from taking water for 1–2 h. The drug disappearance and absorption measurements were based on the methods reported previously.[13,14] Under anaethesia with pentobarbital (50 mg/kg, i.p.) the rat's right jugular vein was cannulated with PE 90 tubing[15] for the route of the transfusion. The small intestine was exposed via a midline incision, and a section of lower jejunum, approximately 15 cm long and drained by the corresponding mesenteric vein, was cannulated at the proximal and distal ends with L-shaped plastic cannulas. Two syringes (2.5 ml) were then attached to the cannulas in the jejunum. The mesenteric vein was cannulated with a PE-50 tube while the arterial blood supply was kept intact. The blood lost from the vein was continuously replaced by constant infusion via the jugular vein cannula with an approximately equal volume of heparinized whole blood previously collected from donor rats. The perfusate consisted of Krebs–Henseleit buffer solution containing tolbutamide (500 μg/ml) plus [^{14}C]-tolbutamide as a tracer with or without Sho-saiko-to (75 mg/ml). [1,2-^{3}H]-PEG 4000 was also added to the perfusion solution to act as a non-absorbable marker to help identify any transfer of water into or out of the intestinal lumen and to monitor the structural integrity of the intestinal segment. Samples of the perfusate (50 μl) were collected at zero time and at the midpoint of each blood collection period (0–10, 10–20, 20–30, 30–40, 40–50 and 50–60 min) after the beginning of the perfusion. The absorbed fraction (F_a) of tolbutamide was estimated by correcting minor volume change based on the changes in [1, 2-^{3}H]-PEG 4000 concentrations:

$$F_a = 1 - [(Ct/Ci)/(Ct'/Ci')] \quad (1)$$

where Ci and Ct are the initial and periodically measured concentration of tolbutamide, respectively, and Ci' and Ct' are those of [1,2-^{3}H]-PEG 4000. Assuming first-order absorption (disappearance), the absorption rate constant (k_a) was estimated as follows:

$$k_a = \ln(1 - F_a)/t \quad (2)$$

where t represents the blood collection period. Moreover, the apparent membrane permeability (disappearance) clearance (CL_{app}) was estimated as follows:

$$CL_{app} = K_a \times V \quad (3)$$

where V is the volume of the intestinal loop (1.5 ml as the administered volume). In addition, the absorption clearance (CL_{abs}) of tolbutamide into the mesenteric blood was estimated as follows:

$$CL_{abs} = (C_p \times V_{blood})/C_{loop} \quad (4)$$

where C_p is the plasma concentration of tolbutamide, C_{loop} is the tolbutamide concentration in the intestinal loop and V_{blood} is the flow rate of mesenteric blood as the mean of the blood collection period.

Caco-2 cell culture

Caco-2 cell line was obtained from American Type Culture Collection (Rockville, USA). Cells of passage 37–43 were used in this study. Caco-2 cells were cultured in Dulbecco's modified Eagle medium supplemented with 1% non-essential amino acids, 10% fetal bovine serum, 1% L-glutamate and 5% antibiotic–antimycotic mixture (10 000 U/ml benzylpenicillin, 10 000 μg/ml streptomycin sulfate and 25 μg/ml

amphotericin B in 0.85% NaCl). All these components were purchased from Gibco-BRL Life Technologies, Inc. (Rockville, USA). Caco-2 cells were grown at 37°C in culture flasks (Nippon Becton Dickinson Co., Ltd, Tokyo, Japan) in humidified air–5% CO_2 atmosphere. The medium was changed every two days. At 80–90% confluence, cells were treated with trypsin EDTA and seeded at a density of 6×10^4 cells/ml into flasks. For the transport studies, cells were seeded on polycarbonate filters (3.0 μm pores, 4.2 cm^2 growth area) inside the 6-well plates (Nippon Becton Dickinson Co., Ltd, Tokyo, Japan) at a density of 4.5×10^5 cells/filter. The culture medium (2.5 ml in the insert and 3.0 ml in the well) was replaced after 72 h and every 48 h thereafter. The transport studies were conducted with the cell monolayers between 16 and 21 days in culture. Sho-saiko-to was added to the apical compartment only. The ATP-depleted Caco-2 cells were prepared by pre-incubation for 30 min with NaN_3 (10 mM) and NaF (10 mM).[16] To evaluate the integrity of the monolayer, we measured the transepithelial electrical resistance (TEER) using EVOM (World Precision Instruments, West Haren, USA) before and after the experiment. All the monolayers used in this study exhibited over 750 ohms cm^2 of TEER values in accordance with a previous paper.[17]

Transport experiments across the Caco-2 cell monolayers

The transport experiments were performed in the transport medium (composition in mM: KCl 5.36, NaCl 137, $NaHPO_4$ $7H_2O$ 0.34, KH_2PO_4 0.44, $NaHCO_3$ 4.17, $CaCl_2$ 1.26, $MgCl_2$ $6H_2O$ 0.49, $MgSO_4$ $7H_2O$ 0.41, glucose 19.45 and Hepes 10.0) using the cell culture compartments. Initially, the Caco-2 cell monolayers were pre-incubated for 30 min at 37°C in the transport medium. Then the new pre-warmed transport medium containing tolbutamide (1 mM) or [^{14}C]-D-mannitol was applied to either the apical (1.5 ml) or basolateral (2.6 ml) side of the Caco-2 cell monolayers and the drug-free transport medium was applied to the opposite side. Sho-saiko-to (50 mg/ml) was invariably applied to the apical compartment in the transport studies of both directions. Samples (0.1 ml) were taken from the apical or basolateral side every 2 min for 10 min and were replaced with equal volumes of the drug-free transport medium. The apical-to-basolateral or basolateral-to-apical permeability coefficient (P_{app}) of each drug was calculated as its flux rate using the following equation:

$$P_{app} = (dQ/dt) \times [1/(A \times C_0)] \quad (5)$$

where A is the surface area of the exposed monolayer (4.2 cm^2), C_0 is the initial concentration of the donor solution and dQ/dt is the rate of concentration change in the receiver solution.

Assay

Tolbutamide concentrations in the medium were determined by an HPLC method in accordance with our previous report.[7] Briefly, sample medium (100 μl) was added to 100 μl of the internal standard solution (25 μg/ml of chlorpropamide) and then shaken for 30 s. After centrifugation at 10 000 rev/min for 5 min, 10 μl of the supernatant was injected into the chromatograph. The radioactivity of the sample, including [^{14}C]-D-mannitol, [^{14}C]-tolbutamide and [1, 2-^{3}H]-PEG 4000, were determined using a liquid scintillation counter (1414 WinSpectral; Wallac, Oy., Turku, Finland).

Statistics

The Mann–Whitney *U*-test was used to estimate a statistical significance of difference between the means of two groups. Multiple comparisons were carried out by using the Kruskal–Wallis test followed by Dunn's test. $P \leq 0.05$ was considered to be statistically significant.

Results

In-situ absorption study

Figure 1a shows the time-course of tolbutamide concentrations in the rat jejunal loop after placing the perfusate containing tolbutamide alone and tolbutamide with Sho-saiko-to. Tolbutamide concentrations declined in accordance with first-order kinetics in both groups. In the group co-administered with Sho-saiko-to, tolbutamide concentrations in the jejunal loop were lower than those of the rats administered tolbutamide alone at 15 min after the beginning of the perfusion ($P < 0.05$). Figure 1b shows the time-course of tolbutamide concentrations in the mesenteric venous plasma during the jejunal loop perfusion. When Sho-saiko-to was added to the perfusate, a significant increase in plasma tolbutamide concentration was observed in the blood collection periods of 0–10 min, 10–20 min and 20–30 min ($P < 0.05$). The mesenteric venous flow rates were 0.322–0.452 and 0.268–0.425 ml/min through 60 min in the rats administered tolbutamide alone and tolbutamide with Sho-saiko-to, respectively. There was no significant change in the blood flow rate between these two groups over the perfusion, so that these blood outflows were replaced by infusion of the donor blood at a flow rate of 0.3 ml/min.

To analyse absorption kinetics in the initial phase, the apparent absorption parameters at 20 min after the beginning of the perfusion were calculated from the amount of tolbutamide remaining in the jejunal loop (Table 1). Significant increase in F_a, k_a and CL_{app} was observed in the rats co-administered Sho-saiko-to ($P < 0.05$). To assess the influence of Sho-saiko-to on tolbutamide absorption in each period, the CL_{abs} of tolbutamide was estimated from the concentration data of tolbutamide in the jejunal loop and the mesenteric venous plasma, which is considered to show actual drug absorption profiles into blood. As shown in Figure 2, in the period of 0–10 min, a 30% increase in CL_{abs} was observed after co-administration with Sho-saiko-to ($P < 0.05$).

Tolbutamide transport across Caco-2 cell monolayers

The time-course of tolbutamide transported from the apical compartment to the basolateral compartment in Caco-2 cell monolayers was linear in both the groups administered tolbutamide alone (control) and tolbutamide with Sho-saiko-to (Figure 3). The permeated amounts of tolbutamide were

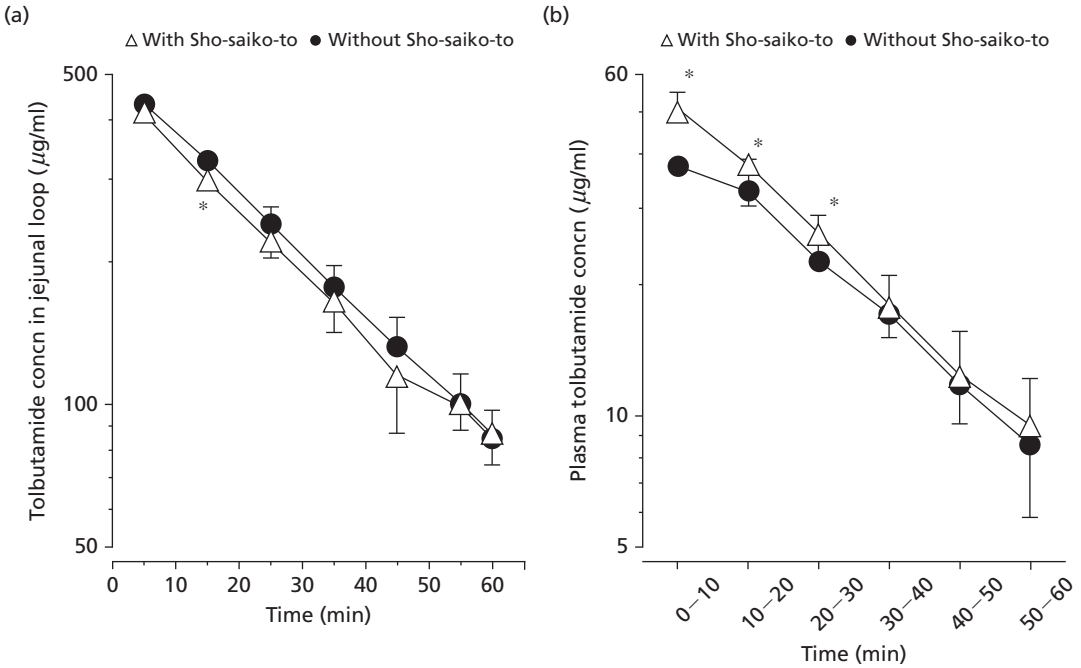

Figure 1 Tolbutamide concentration–time profiles in rat jejunal loop (a) and mesenteric venous blood (b) after the beginning of the perfusion. Tolbutamide (500 μg/ml) was added in the perfusate with or without (control) Sho-saiko-to (75 mg/ml). Each point represents the mean ± SD of three or four rats. $^{*}P < 0.05$, compared with the control (Mann–Whitney *U*-test)

Table 1 Effect of Sho-saiko-to on apparent absorption parameters of tolbutamide in rat jejunal loop

Group	F_a (%)	k_a (min^{-1})	CL_{app} (μl/min)
Control	34.3 ± 2.8	0.0280 ± 0.0028	42.02 ± 4.28
With Sho-saiko-to	40.6 ± 2.4*	0.0348 ± 0.0027*	52.13 ± 4.00*

Each kinetic parameter was calculated at 20 min after the beginning of the jejunal loop perfusion. Each value represents the mean ± SD of three or four rats. $^{*}P < 0.05$, compared with the control (Mann–Whitney *U*-test).

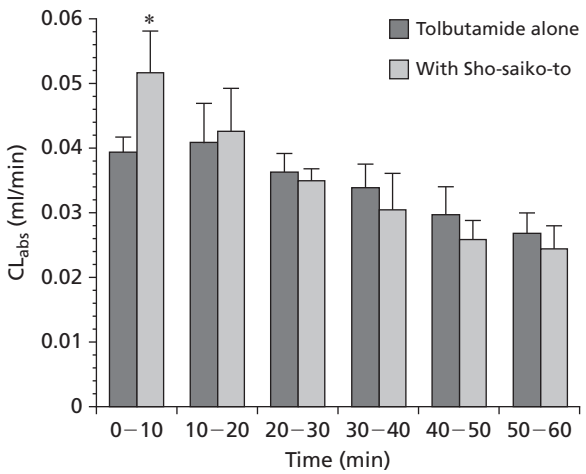

Figure 2 Absorption clearance (CL_{abs}) of tolbutamide during rat jejunal loop perfusion with or without Sho-saiko-to. Each column represents the mean and SD of three or four rats. $^{*}P < 0.05$, compared with the control (Mann–Whitney *U*-test)

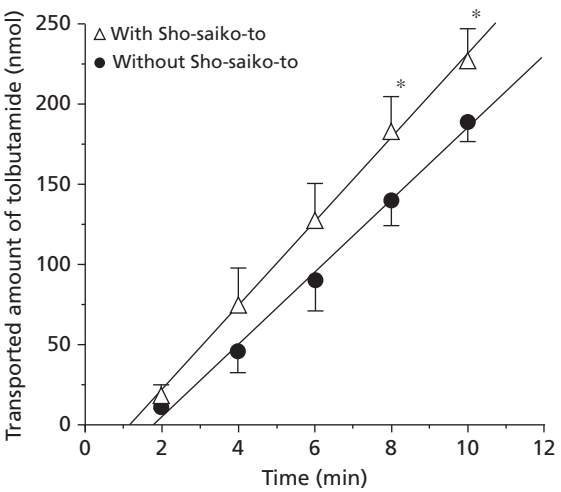

Figure 3 Effect of Sho-saiko-to on the apical-to-basolateral transport of tolbutamide across Caco-2 monolayers. Tolbutamide (1 mM) was added to the apical side with or without (control) Sho-saiko-to (50 mg/ml). Each point represents the mean ± SD of four or five experiments. $^{*}P < 0.05$, compared with the control (Mann–Whitney *U*-test)

significantly increased by the presence of Sho-saiko-to in the apical compartment. Table 2 demonstrates the effects of Sho-saiko-to on the P_{app} of tolbutamide as well as D-mannitol, a paracellular marker of membrane transport across the Caco-2 cells. In the apical-to-basolateral direction, the P_{app} of tolbutamide showed a 20% increase upon addition of Sho-saiko-to ($P < 0.05$). In contrast, the P_{app} of D-mannitol with Sho-saiko-to was significantly reduced to 62% of the

Table 2 Effect of Sho-saiko-to on the permeability coefficients of tolbutamide and D-mannitol across Caco-2 cell monolayers

Direction	Control	With Sho-saiko-to
Apical-to-basolateral		
Tolbutamide	86.3 ± 8.1	104.0 ± 6.5*
D-Mannitol	0.681 ± 0.054	0.419 ± 0.032*
Basolateral-to-apical		
Tolbutamide	13.82 ± 0.43	9.56 ± 0.46*
D-Mannitol	0.738 ± 0.073	0.509 ± 0.022*

Tolbutamide (1 mM) or [^{14}C]-mannitol (50 μM) was added to the apical or basolateral compartment. Sho-saiko-to (50 mg/ml) was added to the apical compartment. Each value represents the mean ± SD of four or five experiments. *$P < 0.05$, compared with the control (Mann–Whitney *U*-test).

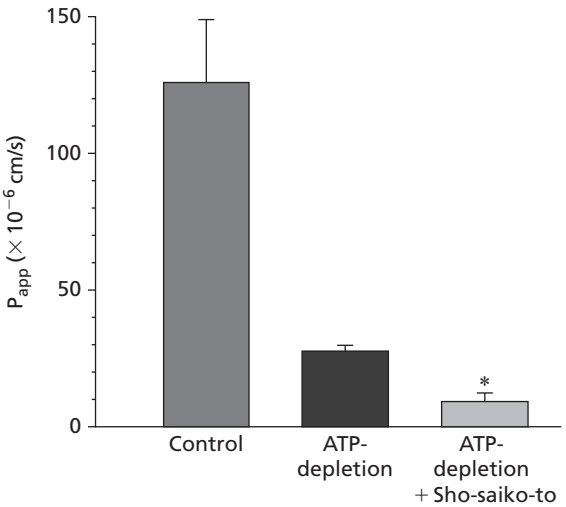

Figure 4 Effect of Sho-saiko-to on the apical-to-basolateral transport of tolbutamide across ATP-depleted Caco-2 cell monolayers. Caco-2 cells were pre-incubated in the presence or absence (control) of 10 mM NaN_3 and 10 mM NaF for 20 min. Tolbutamide (1 mM) was added to the apical side with or without Sho-saiko-to (50 mg/ml). Each column represents the mean and SD of four experiments. *$P < 0.05$, compared with the control (Kruskal–Wallis test followed by Dunn's multiple comparison test)

group receiving D-mannitol alone ($P < 0.05$). In the basolateral-to-apical direction, the P_{app} of tolbutamide and D-mannitol were decreased by about 30% in the presence of Sho-saiko-to.

To investigate the effect of Sho-saiko-to on ATP-dependent specific transport systems for tolbutamide reported by Nishimura *et al.*,[18] we performed a transport experiment for tolbutamide in the apical-to-basolateral direction by using ATP-depleted cells. In the ATP-depleted cells, the P_{app} of tolbutamide decreased to 22.5% of that of the intact cells (control) (Figure 4). Furthermore, 65% reduction in the apical-to-basolateral transport of tolbutamide was observed in the ATP-depleted cells when Sho-saiko-to was added in the apical compartment.

Discussion

In a previous study, the mechanism and interaction of intestinal absorption of various compounds, including herbal constituents, were investigated by using in-vitro and in-situ methods.[19–21] In the first part of this study, we applied the in-situ approach to collect the mesenteric venous plasma and luminal drug solution periodically after placing the drug solution into the jejunal segment in rats. The time profiles of periodical jejunal concentrations of tolbutamide indicate that in-situ jejunal absorption of this drug took place according to apparent first-order kinetics (Figure 1a). Matsuda *et al.*[22] reported that the permeability of L-carnitine across the rat intestine in-situ was reflected in the in-vivo absorption rate after oral administration. So we estimated the apparent permeability parameters of tolbutamide in rat intestinal lumen to assume in-vivo absorption. Since the concomitant administration of Sho-saiko-to significantly decreased and increased tolbutamide concentrations in the lumen and plasma, respectively, during the early phase (Figure 1a), the apparent absorption parameters of tolbutamide were estimated by the drug concentration at 20 min after placing the drug solution in the loop. Significantly increased apparent absorption parameters, F_a, k_a and CL_{app}, of tolbutamide were observed when co-administered with Sho-saiko-to (Table 1). These results suggest that Sho-saiko-to could facilitate the disappearance of tolbutamide from the jejunal loop in rats. Otherwise, it should be considered that degradation, metabolism and accumulation of the drug in the jejunal lumen affect drug absorption in the intestine, so we evaluated the intestinal membrane permeability of tolbutamide in-situ by estimating the actual absorption parameter, CL_{abs}. The CL_{abs} of tolbutamide was increased by co-administration of Sho-saiko-to within 10 min of drug placement. Since it was observed that Sho-saiko-to did not affect the blood flow rate in this study, the change in the CL_{abs} of tolbutamide by Sho-saiko-to was not related to the flow rate in the mesenteric vein. These results indicate that Sho-saiko-to could augment the epithelial permeability of tolbutamide in rat jejunum to cause the elevation of plasma tolbutamide concentrations in the early phase. There is a previous report that the rise in initial absorption rate of sulphonylureas, including tolbutamide, could lead to hypoglycaemia in humans.[23] Therefore, it is necessary that careful attention is paid to the concomitant dose of tolbutamide with Sho-saiko-to in the clinical setting.

In the next part of this study, we examined the epithelial membrane transport of tolbutamide by using Caco-2 cell monolayers to elucidate the mechanisms involved in the enhancement of gastrointestinal absorption by Sho-saiko-to. Caco-2 cells are known to form confluent monolayers of well-differentiated enterocyte-like cells with the functional properties of transporting epithelia and have been used to study the transport of drugs.[17,24] Caco-2 cells have also been used for investigating the transport of natural products.[19] The addition of Sho-saiko-to into the apical compartment significantly increased tolbutamide transport from the apical compartment to the basolateral one 10 min after beginning incubation (Figure 3). Also, Sho-saiko-to induced a 20% increase in the P_{app} of tolbutamide in the

absorption direction (Table 2). Imai *et al.*[25] reported that glycyrrhizin and glycyrrhetinic acid, the constituents of Sho-saiko-to, could enhance paracellular transport in Caco-2 cells. Accordingly, we investigated the effect of Sho-saiko-to on the paracellular transport of tolbutamide. As the results showed, the P_{app} of D-mannitol, the paracellular marker of membrane transport across Caco-2 cells,[26] was significantly reduced by Sho-saiko-to. This phenomenon implies that Sho-saiko-to could suppress the paracellular passive transport in the absorption direction across the Caco-2 cell monolayers. This inhibitory effect may be ascribed to crude component(s) other than glycyrrhizin in Sho-saiko-to.

We reported that tolbutamide was translocated in the apical-to-basolateral direction across the Caco-2 cells by some carrier-mediated transport system with a pH and energy dependency.[18] Thus, we carried out the transport studies by using ATP-depleted cells, which allows us to reveal the effects of Sho-saiko-to on the passive transport of tolbutamide. The P_{app} of tolbutamide was reduced by ATP depletion (Figure 4). Moreover, Sho-saiko-to significantly diminished the tolbutamide transport in the ATP-depleted Caco-2 cells, which shows that passive transport of tolbutamide is not enhanced, but rather inhibited, by Sho-saiko-to. Although this inhibitory effect of Sho-saiko-to on passive transport is supported by the results with D-mannitol described above, this effect cannot explain the enhancement in gastrointestinal absorption and membrane permeability of tolbutamide by Sho-saiko-to. As the results of ATP-depleted experiments indicate that some energy-dependent carrier-mediated transport system(s) may function in the Caco-2 cells, Sho-saiko-to could activate the ATP-dependent specific transport system of tolbutamide in Caco-2 cells.

There are a number of papers that have shown crude components in Sho-saiko-to to enhance absorption of co-administered drugs. For example, Zingiberaceae extract increased intestinal absorption of sulfaguanidine in rats,[27] and rectal absorption of amphotericin B was facilitated by glycyrrhizinate in rabbits.[28] So it is well considered that such crude drugs contained in Sho-saiko-to may affect the gastrointestinal absorption of the co-administered drug tolbutamide. Alternatively, Ohtake *et al.*[29] separated and isolated the active principles of Sho-saiko-to, and this Chinese traditional medicine had a huge number of biologically active compounds. In our study, we did not clarify an active compound causing the acceleration of tolbutamide absorption. Chinese traditional medicines are considered to exert their activity by the additive or synergistic effects of the several biological active compounds contained in the multiple herbal medicines. Therefore, it is considered that Sho-saiko-to may exhibit facilitated absorption as a complex preparation.

Conclusions

This study suggests that Sho-saiko-to can enhance the epithelial membrane permeability of tolbutamide across the rat jejunum in-situ and in Caco-2 cell monolayers. It also suggests that the enhancing effects are not due to facilitation of the passive transport but presumably activation of the energy-dependent transport of tolbutamide. This result may be relevant to the accelerated in-vivo absorption rate of tolbutamide by concomitant dosing with Sho-saiko-to in rats.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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