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Bioavailability of glycyrrhizin from Shaoyao-Gancao-Tang in laxative-treated rats

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Abstract

Shaoyao-Gancao-Tang (SGT), a traditional Chinese formulation composed of Shaoyao (Paeoniae Radix) and Gancao (Glycyrrhizae Radix), is frequently used in conjunction with laxatives such as sodium picosulfate in colonoscopy to relieve abdominal pains. We have investigated the alterations of the bioavailability of glycyrrhizin when SGT was co-administered with sodium picosulfate and we tried to identify a regimen that might minimize the alterations. Glycyrrhizin is one of the active glycosides in Gancao and SGT and is hydrolysed into the bioactive metabolite, 18β-glycyrrhetic acid (GA) by intestinal bacteria following oral administration. We found that the maximum plasma concentration (C_{max}) and the area under the mean concentration vs time curve from zero to 24 h ($AUC_{0\text{-}24\,h}$) of GA from a single dose of SGT administered 5 h after a single pretreatment with sodium picosulfate were significantly reduced to 15% and 20% of the control level in rats, respectively. These reductions were still significant four days after sodium picosulfate pretreatment, but were restored by repetitive administration of SGT following sodium picosulfate pretreatment. Similar reductions and recovery were observed for the glycyrrhizin-metabolizing activity of intestinal bacteria in rat faeces. The results warrant clinical studies for co-administration of laxatives such as sodium picosulfate and SGT.

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Introduction

Shaoyao-Gancao-Tang (SGT, Shakuyaku-Kanzo-To in Japanese) is a traditional Chinese formulation composed of Shaoyao (Paeoniae Radix) and Gancao (Glycyrrhizae Radix). Glycyrrhizin, one of the active glycosides in Gancao and SGT, is a pro-drug of the active metabolite 18β-glycyrrhetic acid (GA), which is derived from orally administered glycyrrhizin by intestinal bacteria (Akao et al 1994). Previously we reported that the co-administration of some antibacterial drugs used in peptic ulcer or urinary tract infection therapy with SGT reduced the intestinal metabolism of glycyrrhizin from SGT, such that the maximum plasma concentration (C_{max}) and the area under the mean concentration vs time curve from zero to 24 h ($AUC_{0\text{–}24\,h}$) of GA from SGT were decreased (He et al 2001).

In Japanese clinics, SGT efficiently treats various abdominal pains (Katsura 1995) and muscle cramps accompanying cirrhosis (Kumada et al 1999). SGT is frequently used in colonoscopy to relieve abdominal pains (Arai et al 1994) when laxatives such as sodium picosulfate are generally administered as a pretreatment (Fork et al 1982). To ensure safety and efficacy of drugs in multiple drug therapy, it is important to know about drug–drug interactions. However, to date, no study has reported the interactions between SGT and the co-administered sodium picosulfate. As a part of our ongoing biopharmaceutical analysis of the interactions between traditional Chinese formulations and synthetic drugs (He et al 2003), this study was designed to examine the interactions between SGT and sodium picosulfate, by focusing on the intestinal metabolism of glycyrrhizin and the resulting influences on the bioavailability of GA from SGT.

Materials and Methods

Materials

The freeze-dried extract of SGT (6 g each of Shaoyao and Gancao; yield: 4.02 ± 0.06 g, a common daily dose for adults) used in the animal experiments was prepared as reported by He et al (2001). The voucher specimens of Shaoyao (produced in Japan) and Gancao (imported from China: Dongbei-Gancao in Chinese) were deposited in the Department of Pharmacognosy, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. To assure the homogeneity of the formulations and to prepare batches of stable formulations, HPLC profiles of SGT were analysed (He et al 2001). The major peaks of the profile were clarified to be glycyrrhizin, liquiritin, and liquiritin apioside for Gancao and paeoniflorin for Shaoyao. The content of glycyrrhizin in the freeze-dried extract of SGT was $63.7 \pm 1.0\,mg\,g^{-1}$ ($85.4 \pm 0.3\,mg\,g^{-1}$ in Gancao extract). Glycyrrhizin, GA and sodium picosulfate were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). All of the other chemicals and solvents used were of analytical and/or HPLC grade.

Administration of sodium picosulfate and SGT extract to rats

Male Wistar rats (8-weeks-old, approximately 250 g) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The animals were maintained on a 12-h light/dark cycle at 21–24°C and given free access to water and standard laboratory chow throughout the study. All animal experiments were carried out in accordance with the Guidelines of the Animal Care and Use Committee of Toyama Medical and Pharmaceutical University approved by the Japanese Association of Laboratory Animal Care.

Sodium picosulfate was administered orally to rats ($n = 5$ for each group) as a single dose ($50\,mg\,kg^{-1}$, 10-fold the common daily dose for man) at 0900 h on day 0. SGT extract was administered orally twice daily at a total daily dose ($645\,mg\,kg^{-1}$, equivalent to $41\,mg\,kg^{-1}$ glycyrrhizin) that was 10-fold the common daily dose for man, as shown in Figures 1 and 2. The reason for dosing rats at 10-fold the human dose was because smaller animals eliminate drugs more rapidly than man. To achieve the same pharmacokinetic values as in man the rat needs to be administered with relatively high doses (Boxenbaum 1982). Previous studies indicated that when rats were administered orally with SGT in a single dose that was 10-fold the human dose, the values of C_{max} and AUC of GA (He et al 2001) were similar to those for man (Bando et al 2000). There have been similar cases for rats (Sakamoto et al 1985) and man (Welling et al 1977) when synthetic drugs, such as the antibiotic amoxicillin, have been administered.

Determination of water content and glycyrrhizin-metabolizing activity in rat faeces

Faecal samples (approximately 0.5 g each) were collected at 0900 h (just before sodium picosulfate administration,

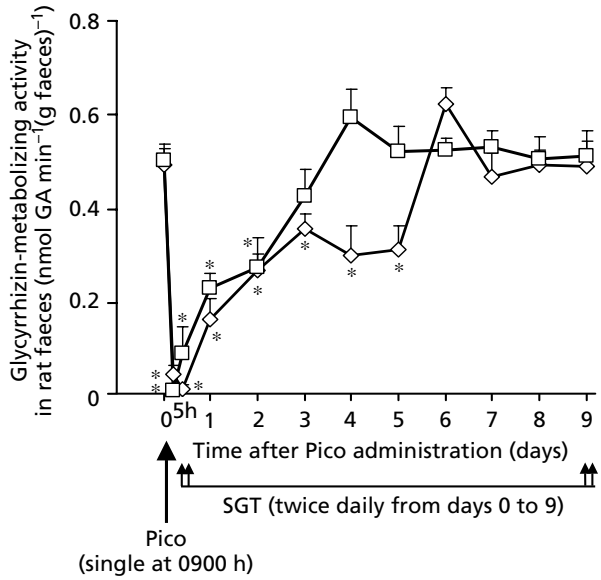

Figure 1 Alteration of glycyrrhizin-metabolizing activity in rat faeces after a single administration of sodium picosulfate (Pico) with or without repetitive SGT. SGT extract was administered orally twice daily (at 1400 and 1900 h on day 0; at 0900 and 1900 h from days 1 to 9) at a total daily dose 10-fold the common daily dose for man. ◇, Sodium picosulfate alone; □, sodium picosulfate with repetitive SGT. Each point represents the mean ± s.e. ($n = 5$). *$P < 0.01$, significantly decreased vs initial value (mean 0.50 ± 0.03).

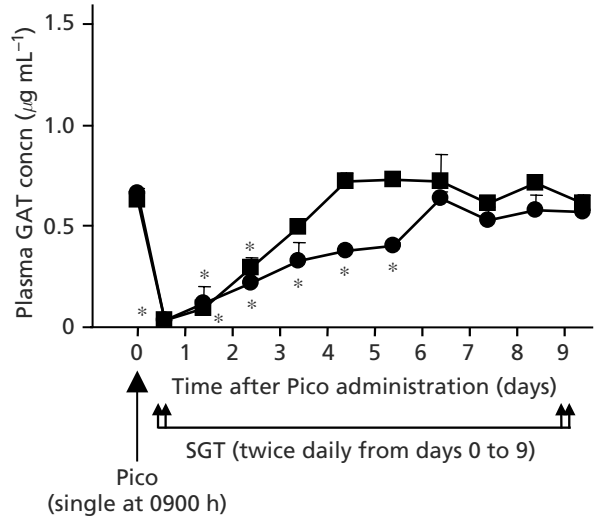

Figure 2 Alteration of daily plasma GA concentration after a single administration of sodium picosulfate (Pico) with or without repetitive SGT. In the group with repetitive SGT, SGT extract was administered orally twice daily (at 1400 and 1900 h on day 0; at 0900 and 1900 h from days 1 to 9) at a total daily dose 10-fold the common daily dose for man. In the group without SGT, SGT extract was administered at a single dose at 1400 h on day 0. Blood samples were collected 9 h after SGT administration at 1400 h on day 0 and at 0900 h from days 1 to 9 as reported previously (He et al 2003). The 9 h time point after SGT administration corresponded to t_{max} of GA (He et al 2001). ●, Sodium picosulfate with a single SGT; ■, sodium picosulfate with repetitive SGT. Each point represents the mean ± s.e. ($n = 5$). *$P < 0.01$, significantly decreased vs initial value (mean 0.65 ± 0.03).

initial time point), 1400 and 1900 h on day 0 and at 0900 h from days 1 to 9. The water content in faeces was determined by calculating the weight reduction of faecal samples before and after drying at 120°C for 4 h. The condition was defined as diarrhoea when the water percentage in the faeces was 80% or more.

The glycyrrhizin-metabolizing activity (the rate of metabolism of glycyrrhizin into GA) in fresh rat faeces was estimated by determining the amount of GA formed in a reaction mixture containing rat faecal suspension and 0.5 mM glycyrrhizin, using HPLC with a YMC-Pack ODS-A-303 column and spectrophotometric detection at 251 nm (He et al 2001). The recovery time (expressed in days) was defined as the average number of days from the time when the activity was reduced to 70% or less of the initial level to when it recovered to 90% or more (Abe et al 2001).

Determination of plasma GA concentration

Blood samples were collected according to the specific schedule described in the legends to Figures 2 and 3. The collected blood samples were immediately centrifuged at 1100 g for 10 min and the obtained plasma was stored at −20°C until analysis. Plasma GA concentration was measured using the same HPLC conditions as described in the determination of glycyrrhizin-metabolizing activity. The C_{max} and the time to reach C_{max} (t_{max}) were determined directly from the actual drug levels in the plasma. The $AUC_{0-24\,h}$ was calculated by using the trapezoidal rule.

Statistical analysis

Differences between values before and after treatment were statistically analysed using a paired two-tailed Student's *t*-test. Comparisons between two groups and among more than two groups were performed using the unpaired two-tailed Student's *t*-test and one-way analysis of variance followed by Tukey's post-hoc test, respectively. Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Duration of diarrhoea caused by sodium picosulfate

Diarrhoea occurred 5 h after a single dose of sodium picosulfate and continued for approximately two days (2.0 ± 0.5 days). Diarrhoea was not improved by repetitive administration of SGT twice a day for two days following the single pretreatment with sodium picosulfate on day 0. The duration of diarrhoea in the presence of SGT administration (2.2 ± 0.7 days) was as long as that in the absence of SGT administration.

These results indicated that SGT had no effect on improving diarrhoea and the laxative effect of sodium picosulfate was not affected by co-administering SGT. It was reported that the laxative effect of orally administered sodium picosulfate was attributable to the sodium picosulfate hydrolysis product in the gut (Jauch et al 1975), which was formed from sodium picosulfate biotransformation by intestinal bacteria (Kim et al 1992). Although this study did not directly examine the alteration of sodium picosulfate biotransformation by co-administration of SGT, the results suggested that sodium picosulfate biotransformation was not influenced in this regard.

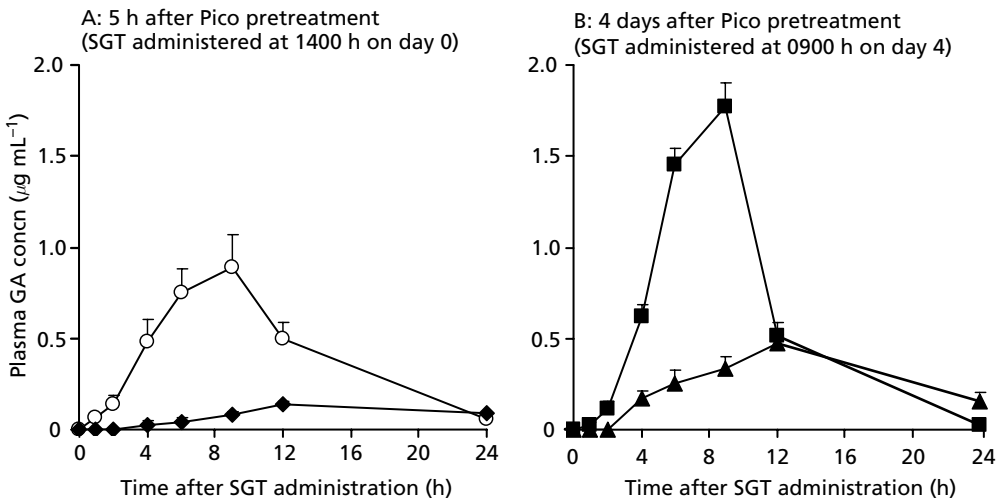

Figure 3 Plasma GA concentration from SGT administered 5 h (A) or four days (B) after a single sodium picosulfate (Pico) pretreatment in rats. Blood samples (approximately 0.24 mL each) were collected from the tail vein using heparinized microcapillary tubes at 0, 1, 2, 4, 6, 9, 12 and 24 h after SGT (323 mg kg^{-1}) administration at 1400 h on day 0 (A) or 0900 h on day 4 (B). A: ◆, Single dose of SGT administered 5 h after sodium picosulfate pretreatment; ○, single dose of SGT administered alone (control). B: ▲, Single dose of SGT administered four days after sodium picosulfate pretreatment without repetitive SGT; ■, Single dose of SGT administered four days after sodium picosulfate pretreatment with repetitive SGT. Each point represents the mean ± s.e. (n = 5) determined by HPLC (He et al 2001).

Glycyrrhizin-metabolizing activity of intestinal bacteria in rat faeces

Figure 1 shows that glycyrrhizin-metabolizing activity of intestinal bacteria in rat faeces was reduced to approximately 10% of the initial level 5 h after pretreatment with sodium picosulfate. This reduction was significant but not as serious as that caused by antibacterial drugs (amoxicillin and metronidazole), which reduced glycyrrhizin-metabolizing activity to an undetectable level lasting for three days (He et al 2003).

The reduced glycyrrhizin-metabolizing activity did not recover until day 6 (6.2 ± 0.5 days). However, the recovery period was significantly shortened to approximately three days (3.6 ± 0.2 days) by repetitively administrating SGT for four days following the single sodium picosulfate pretreatment on day 0. This restorative effect of repetitive SGT on bacterial glycyrrhizin metabolism reduced by laxative was comparable with that on bacteria glycyrrhizin metabolism reduced by antibacterial drugs (He et al 2003). Mechanisms for the restorative effect are suggested to be attributable to glycyrrhizin in SGT, which is believed to induce the glycyrrhizin β-D-glucuronidase, which in turn allows the enzyme-producing bacteria to proliferate (Akao et al 1988). The enhancing effect of crude drugs on bacterial metabolizing activity was also proved by other researchers, who reported that consecutive intakes of ginseng stimulated ginsenoside-hydrolysing bacteria and thus enhanced the ginsenoside-hydrolysing potential of the intestinal flora (Hasegawa & Uchiyama 1998).

However, after recovering from the reduction phase, the glycyrrhizin-metabolizing activity did not gradually increase to a high level greater than the initial value, as was observed in a previous study using antibacterial drugs (He et al 2003). Although the reason for this remains unclear, it might have been due to the difference in potency and patterns between laxative drugs and antibacterial drugs, as these drugs interfere with the intestinal flora.

Plasma GA concentration

In the pharmacokinetic experiments of sodium picosulfate pretreatment with or without repetitive use of SGT, daily plasma GA concentration (equivalent to C_{max}) was measured 9 h after SGT administration. As shown in Figure 2, in groups either with or without repetitive SGT, plasma GA concentration on day 0 was significantly reduced by sodium picosulfate pretreatment to 4.5% of the initial level. In the group without repetitive SGT, the GA level was still significantly low (50% of initial level) on day 3, and did not recover to the initial level until day 6. However, in the group with repetitive SGT, the GA level returned to the initial level on day 3 (78% of initial level). The recovering profiles of plasma GA concentration were similar to that of the glycyrrhizin-metabolizing activity. These results agreed very well with the previous finding that glycyrrhizin-metabolizing activity of intestinal bacteria showed a good positive correlation with plasma GA concentration (He et al 2001).

Figure 3A compares the plasma GA concentration in a full time course at 5 h on day 0 between groups with or without (control) sodium picosulfate pretreatment. The plasma GA concentration in the group with sodium picosulfate pretreatment was significantly decreased. Figure 3B compares the plasma GA concentration in a full time course on day 4 between groups with or without repetitive administration of SGT following sodium picosulfate pretreatment. The plasma GA concentration in the group repetitively treated with SGT was significantly greater compared with the group which did not receive the same regimen.

Table 1 summarizes the pharmacokinetic parameters for the four groups. The C_{max} and $AUC_{0-24\,h}$ of GA from the single dose of SGT administered 5 h after pretreatment with sodium picosulfate was significantly reduced to approximately 15% and 20% of the control levels, respectively. The values were still significantly small ($P < 0.05$, 52% and 66% of the control levels, respectively) even

Table 1 Pharmacokinetic parameters of 18β-glycyrrhetic acid (GA) from Shaoyao-Gancao-Tang (SGT) administered 5 h or four days after pretreatment with sodium picosulfate (Pico) on day 0 in rats

Parameters	5 h after Pico pretreatment		4 days after Pico pretreatment	
	SGT alone control[a]	SGT with Pico pretreatment 1[b]	SGT with Pico pretreatment 2[c]	SGT with Pico pretreatment 3[d]
t_{max} (h)	8.40 ± 0.60	12.00 ± 0.00**	11.40 ± 0.60**	$9.00 \pm 0.00^{\dagger,\ddagger}$
C_{max} (μg mL^{-1})	0.92 ± 0.18	0.14 ± 0.02**	0.48 ± 0.05*,†	1.77 ± 0.13**,†,‡
(C_{max}%)[e]	100%	15.3 ± 1.9	51.6 ± 5.1	192.9 ± 14.2
$AUC_{0-24\,h}$ (μg h mL^{-1})	9.84 ± 1.44	1.98 ± 0.36**	6.47 ± 0.24*,†	14.39 ± 1.25*,†,‡
($AUC_{0-24\,h}$ %)[e]	100%	20.1 ± 3.6	65.7 ± 6.7	146.3 ± 12.7

Each value represents the mean ± s.e. (n = 5). Plasma GA concentration was determined using HPLC (He et al 2001). [a]SGT was administered alone at a single dose of 322.5 mg kg^{-1}. [b]SGT was administered at a single dose 5 h after pretreatment with sodium picosulfate. [c]SGT was administered at a single dose four days after pretreatment with sodium picosulfate. [d]SGT was administered repetitively (twice a day with a total daily dose of 645 mg kg^{-1} from days 0 to 3 and at a single dose of 322.5 mg kg^{-1} on day 4). [e]Percentage relative to the value of control group. *$P < 0.05$ and **$P < 0.01$ vs the control value; $^{\dagger}P < 0.01$ vs SGT with sodium picosulfate pretreatment 1; $^{\ddagger}P < 0.01$ vs SGT with sodium picosulfate pretreatment 2.

when the SGT was administered four days after sodium picosulfate pretreatment, although they were significantly ($P < 0.01$) increased compared with those obtained at 5 h. However, in animals treated with repetitive SGT following sodium picosulfate pretreatment, the C_{max} and $AUC_{0-24\,h}$ on day 4 recovered from the early reductions and increased to greater values as high as 1.9- and 1.5-fold the control values, respectively. These enhancing effects of repetitive SGT on GA pharmacokinetics reduced by the laxative were consistent with those on GA pharmacokinetics reduced by antibacterial drugs (He et al 2003).

Since SGT was administered at the time when the diarrhoea occurred, the mechanism of the effect of sodium picosulfate on GA availability is suggested to be attributable to its laxative effect flushing out bacteria faster than they could recolonize, such that the bacterial content in faeces was decreased.

Conclusions

This study examined the alterations of the intestinal bacterial metabolism and bioavailability of glycyrrhizin from SGT in rats upon pretreatment with sodium picosulfate. Sodium picosulfate administration caused serious diarrhoea and reduced glycyrrhizin-metabolizing activity in rat faeces. The recovery of the reduced glycyrrhizin-metabolizing activity was accelerated by repetitive administration of SGT following sodium picosulfate pretreatment. Similar results were observed for GA pharmacokinetics from SGT. The results warrant clinical studies of the concurrent use of laxatives such as sodium picosulfate and traditional Chinese formulations containing Gancao such as SGT, to ensure bioavailability of glycyrrhizin.

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