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Bioavailability Study of Glycyrrhetic Acid after Oral Administration of Glycyrrhizin in Rats; Relevance to the Intestinal Bacterial Hydrolysis

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Abstract

To clarify the metabolic fate of glycyrrhizin when orally ingested, we investigated the bioavailability of glycyrrhetic acid, the aglycone of glycyrrhizin, after intravenous or oral administration of glycyrrhetic acid (5·7 mg kg^{-1}, equimolar to glycyrrhizin) or glycyrrhizin (10 mg kg^{-1}) at a therapeutic dose in rat.

Plasma concentration of glycyrrhetic acid rapidly decreased after its intravenous administration, with AUC of 9200 ± 1050 ng h mL^{-1} and MRT of $1{\cdot}1 \pm 0{\cdot}2$ h. The AUC and MRT values after oral administration were $10\,600 \pm 1090$ ng h mL^{-1} and $9{\cdot}3 \pm 0{\cdot}6$ h, respectively. After oral administration of glycyrrhizin, the parent compound was not detectable in plasma at any time, but glycyrrhetic acid was detected at a considerable concentration with AUC of $11\,700 \pm 1580$ ng h mL^{-1} and MRT of $19{\cdot}9 \pm 1{\cdot}3$ h, while glycyrrhetic acid was not detected in plasma of germ-free rats at 12 h after oral administration of glycyrrhizin. The AUC value of glycyrrhetic acid after oral administration of glycyrrhizin was comparable with those after intravenous and oral administration of glycyrrhetic acid, indicating a complete biotransformation of glycyrrhizin to glycyrrhetic acid by intestinal bacteria and a complete absorption of the resulting glycyrrhetic acid from intestine. Plasma glycyrrhizin rapidly decreased and disappeared in 2 h after intravenous administration. AUC and MRT values were 2410 ± 125 μg min mL^{-1} and $29{\cdot}8 \pm 0{\cdot}5$ min, respectively. Plasma concentration of glycyrrhetic acid showed two peaks, a small peak at 30 min and a large peak at 11·4 h, after intravenous administration of glycyrrhizin, with an AUC of $15\,400 \pm 2620$ ng h L^{-1} and an MRT of $18{\cdot}8 \pm 1{\cdot}0$ h. The plasma concentration profile of the latter large peak was similar to that of glycyrrhetic acid after oral administration of glycyrrhizin, which slowly appeared and declined.

The difference of MRT values (19·9 and 9·3 h) for plasma glycyrrhetic acid after oral administration of glycyrrhizin and glycyrrhetic acid suggests the slow conversion of glycyrrhizin into glycyrrhetic acid in the intestine.

Glycyrrhizin, a main active constituent of licorice root, *Glycyrrhiza glabra* L., is ingested orally as a component of Kampo medicine, and as a sweetening agent. It is hydrolysed to glycyrrhetic acid by human intestinal bacteria (Hattori et al 1983). Glycyrrhetic acid has been reported to show anti-inflammatory action (Finney & Somers 1958). Several studies on the pharmacokinetic disposition of glycyrrhizin and glycyrrhetic acid in human and rat have been reported. However, there are some discrepancies in plasma glycyrrhizin and glycyrrhetic acid levels after oral administration of glycyrrhizin. Glycyrrhizin is not detected in human plasma when given at a therapeutic dose (Nakano et al 1980), which seems to be due to its poor absorption from the intestine (Wang et al 1994). Glycyrrhetic acid is detectable at a significant concentration in plasma of man after oral administration of glycyrrhizin. Our previous study using germ-free and gnotobiote rats has demonstrated that orally administered glycyrrhizin is converted to glycyrrhetic acid by glycyrrhizin-hydrolysing bacteria in intestine and the resulting glycyrrhetic acid is absorbed (Akao et al 1994). Recently, the pharmacokinetic behaviour of orally administered Kampo medicine, Xiao-Chai-Hu-Tang (Sho-saiko-to, TJ-9), containing licorice in healthy subjects has been presented and glycyrrhetic acid detected in plasma at a considerable concentration (Uchida et al 1995). Accordingly, glycyrrhetic acid, not glycyrrhizin, seems to play an important role in the pharmacological action of orally administered glycyrrhizin and Kampo medicine containing licorice. However, there have been few reports on the plasma disposition of glycyrrhetic acid after oral administration of glycyrrhizin at a therapeutic dose.

In this paper we report the bioavailability of glycyrrhetic acid after oral or intravenous administration of glycyrrhizin at a therapeutic dose, or after administration of glycyrrhetic acid, using a highly sensitive determination method in rats.

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Materials and Methods

Materials

Glycyrrhizin was purchased from Wako Pure Chemical Industry (Osaka, Japan). Glycyrrhetic acid was purchased from Tokyo Kasei Co. (Tokyo, Japan) and purified by column chromatography and repeated recrystallization. β-Galactosidase was purchased from Boehringer Mannheim GmbH.

Bovine serum albumin and 7-β-D-galactopyranosyloxy-4-methyl coumarin were purchased from Sigma, physiological saline from Fusou Pharmaceutical Industry (Osaka, Japan), Freund's complete adjuvant from Difco and goat anti-rabbit IgG (Marcella 10) for enzyme-immunoassay from Dainippon Pharmaceutical Industry (Osaka, Japan). All other reagents were of special or HPLC analytical grade obtained from Wako Pure Chemical Industry (Osaka, Japan).

Animals, treatment and sampling

Male Sprague Dawley rats, 220–290 g, were purchased from Charles River (Atsugi, Japan). Male Wistar rats, 188-200 g, and germ-free rats, 149–187 g, were purchased from Clea Japan, Inc. (Tokyo, Japan). Animals were fed standard laboratory chow with water freely available. Germ-free rats were maintained in an isolator cage under sterilized conditions, and autoclaved water and germ-free laboratory chow were freely available. Animals were fasted overnight before the experiment after accommodation for one week.

Glycyrrhizin was dissolved in 1% sodium carbonate solution, and orally or intravenously administered at a dose of 10 mg kg^{-1} to Sprague Dawley rats (n = 5). Glycyrrhetic acid was suspended in distilled water or dissolved in distilled water containing Tween 20 (2% at final concentration), and orally or intravenously administered, respectively, at a dose of 5·7 mg kg^{-1}, equimolar to glycyrrhizin of 10 mg kg^{-1}, to Sprague Dawley rats (n = 6). The dose of 2 mg glycyrrhizin/rat (ca 10 mg kg^{-1}) after sterilizing with membrane filter (Millipore) was given to Wistar germ-free rats (n = 4) and conventional rats (n = 5). Each solution was administered orally or intravenously through the caudal vein. Blood samples were taken at appropriate time intervals from the jugular or femoral vein after being cannulated with polyethylene tubing (PE-10), which was filled with sodium heparin at a concentration of 100 int. units mL^{-1}, under ether anaesthesia. For the germ-free rats and conventional rats, blood samples were taken from the vena cava after anaesthetization with ether. The plasma was collected by centrifugation and stored at −20°C until analysis.

Determination of glycyrrhizin and glycyrrhetic acid

The concentration of glycyrrhizin in plasma was determined by HPLC (Akao et al 1994) with minor modifications. The limit of quantitation was 500 ng mL^{-1} of plasma. EIA of glycyrrhetic acid was carried out using plasma without extraction (Kanaoka et al 1983). The limit of quantitation was 5 ng mL^{-1} of plasma.

Data analysis

Pharmacokinetic evaluation was performed by non-compartmental analysis of the plasma concentration-time data based on the statistical moment. The area under the plasma concentration-time curves ($AUC_{0-\infty}$) and the mean residence time (MRT_{0-lim}) were calculated by the trapezoidal rule with a monoexponential extrapolation of the terminal phase. The bioavailability of glycyrrhetic acid was assessed by the comparison of AUCs after intravenous and oral administration of glycyrrhetic acid or oral administration of glycyrrhizin. Data were expressed as mean ± s.e.m. of 5 to 6 rats. Statistical analysis was carried out by analysis of variance.

Results

Plasma concentration of glycyrrhetic acid after intravenous or oral glycyrrhetic acid administration

Plasma concentration of glycyrrhetic acid decreased bi-exponentially after its intravenous administration in rats (Fig. 1). The AUC and MRT values (Table 1) were compatible with previous results (Ishida et al 1989; Yamamura et al 1991).

There was no significant difference between the AUC values after the oral and intravenous administrations, indicating a complete absorption of orally administered glycyrrhetic acid.

Plasma concentrations of glycyrrhizin and glycyrrhetic acid after oral glycyrrhizin administration

Glycyrrhizin was not detectable in rat plasma at any sampling time after oral administration of glycyrrhizin at a dose of 10 mg kg^{-1}, but its major metabolite, glycyrrhetic acid, was detected at a considerable concentration, as observed in man (Nakano et al 1980). The pharmacokinetic parameters are listed in Table 1. The AUC value was comparable with AUC values after intravenous and oral administration of glycyrrhetic acid. This suggests a complete biotransformation of glycyrrhizin into glycyrrhetic acid by intestinal bacteria followed by complete absorption. Glycyrrhetic acid was not detected in plasma of germ-free rats 12 h (about the expected T_{max}) after oral administration of glycyrrhizin, while it was detected at significant concentration of 470·2 ± 86·2 ng mL^{-1} in conventional rats. The T_{max}, one of pharmacokinetic parameters indicating the absorption rate, was markedly prolonged when compared with that after oral administration of glycyrrhetic acid.

Plasma concentrations of glycyrrhizin and glycyrrhetic acid after intravenous glycyrrhizin administration

Plasma concentration of glycyrrhizin rapidly decreased after its intravenous administration at a dose of 10 mg kg^{-1} in rats, and 94% of glycyrrhizin, calculated from AUC, disappeared within 2 h (Fig. 2). The pharmacokinetic parameters are listed in

Table 1. Pharmacokinetic parameters of glycyrrhetic acid and glycyrrhizin after oral and intravenous administrations.

Drug	Dose (mg kg^{-1})	Measurement article	T_{max} (h)	C_{max} (ng mL^{-1})	$AUC_{0-\infty}$ (ng h mL^{-1})	MRT_{0-lim} (h)
Glycyrrhetic acid (i.v.)	5·7	glycyrrhetic acid			9200 ± 1050	1·1 ± 0·2
Glycyrrhetic acid (p.o.)	5·7	glycyrrhetic acid	2·5 ± 0·7	1400 ± 300	10600 ± 1090	9·3 ± 0·6
Glycyrrhizin (p.o.)	10	glycyrrhetic acid	15·6 ± 1·5	606 ± 80·1	11700 ± 1580	19·9 ± 1·3
Glycyrrhizin (i.v.)	10	glycyrrhizin			2410 ± 125[a]	29·8 ± 0·5[b]
Glycyrrhizin (i.v.)	10	glycyrrhetic acid	11·4 ± 2·0	977 ± 329	15400 ± 2620	18·8 ± 1·0

Each value represents the mean ± s.e.m. [a]μg min mL^{-1} ; [b]min.

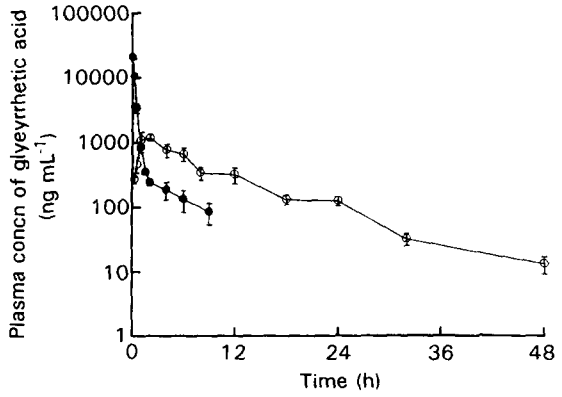

FIG. 1. Plasma concentration profile of glycyrrhetic acid after its intravenous (●) or oral (○) administration at a dose of 5.7 mg kg^{-1}, equimolar to 10 mg kg^{-1} of glycyrrhizin, in rats. Each value represents mean ± s.e.m. of 6 rats.

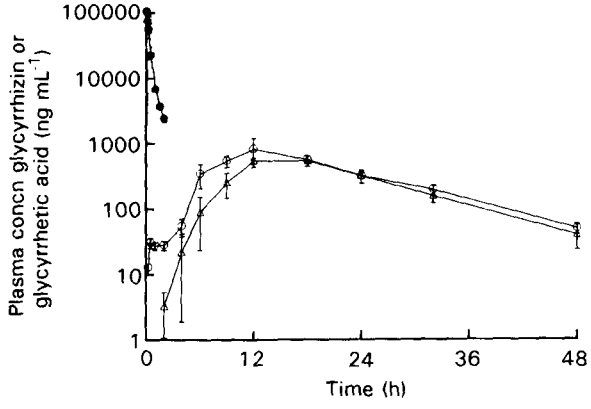

FIG. 2. Plasma concentration profile of glycyrrhizin or glycyrrhetic acid after intravenous or oral administration of glycyrrhizin at a dose of 10 mg kg^{-1} in rats. ● Glycyrrhizin after intravenous administration of glycyrrhizin; ○ glycyrrhetic acid after intravenous administration of glycyrrhizin; △ glycyrrhetic acid after oral administration of glycyrrhizin. Each value represents mean ± s.e.m. of 5 rats.

Table 1. Glycyrrhetic acid was also detected in plasma after intravenous administration of glycyrrhizin as two broad peaks, a small peak at 30 min and a large peak at 11·4 h (Fig. 2); the pharmacokinetic parameters obtained were close to those after oral administration of glycyrrhizin (Table 1), and no significant difference was observed between two AUC values.

Discussion

Several studies on the pharmacokinetic disposition of glycyrrhizin and glycyrrhetic acid have been carried out in human and rat. However, that of glycyrrhetic acid after its oral administration and after oral and intravenous administration of glycyrrhizin at therapeutic doses has not yet been elucidated. In this study, the AUC values of glycyrrhetic acid after its oral and intravenous administration to rats were very similar, indicating a complete absorption of orally administered glycyrrhetic acid from the gastrointestinal tract. Moreover, it was clarified that orally administered glycyrrhizin was completely transformed to glycyrrhetic acid by intestinal bacteria followed by a complete absorption of the resulting glycyrrhetic acid, based on the AUC value and no plasma glycyrrhetic acid in germ-free rats (Akao et al 1994).

Although Wang et al (1994) reported that the bioavailability of glycyrrhetic acid after oral administration of glycyrrhizin was 14·2%, a much larger dose (200 mg kg^{-1}) was used in their experiment.

Plasma concentration profiles of glycyrrhetic acid after oral administration of glycyrrhizin and Kampo medicine containing licorice in human beings (Nakano et al 1980; Yamamura et al 1992; Uchida et al 1995) are similar to our present profile at the therapeutic dose in rats. In these human and rat plasma, glycyrrhizin has not been detected at any time. These results suggest that, when glycyrrhizin is orally ingested as therapy and as a sweetener, glycyrrhetic acid, a major metabolite, plays an important role in its pharmacological action and in its side-effects.

Glycyrrhetic acid is detected in plasma of human and rat when glycyrrhizin is given intravenously. The biphasic profile of glycyrrhetic acid has been found both at the large dose (Ishida et al 1989) and at the therapeutic dose (Fig. 1) of glycyrrhizin in normal rats, but only one early peak in bile-fistulated rats (Ishida et al 1989). Accordingly, the early small peak (30 min) in Fig.1 that we detected seems to be caused by biotransformation of glycyrrhizin in the rat body and the second broad peak is caused by bacterial hydrolysis of glycyrrhizin after secretion into the intestine via the bile, as suggested by Ishida et al (1989). In fact, more than 80 % of intravenously given glycyrrhizin is recovered in the unchanged form in bile (Ichikawa et al 1984). However, no glycyrrhetic acid has been detected in human plasma up to 5 h after intravenous administration of glycyrrhizin, but the broad peak detected around 24 h (Nakano et al 1980), suggests no transformation of glycyrrhizin to glycyrrhetic acid in man. This finding is compatible with the results that rat liver β-glucuronidase hydrolyses glycyrrhizin to glycyrrhetic acid via glycyrrhetic acid monoglucuronide, but human liver enzyme does not hydrolyse glycyrrhizin to glycyrrhetic acid (Akao et al 1991). The presumed metabolic fate of glycyrrhetic acid after oral or intravenous administration of glycyrrhizin is shown in Fig. 3.

The T_{max}, one of the pharmacokinetic parameters indicating the absorption rate, of glycyrrhetic acid after oral administration of glycyrrhizin is markedly prolonged, when compared with that after oral administration of glycyrrhetic acid, sug-

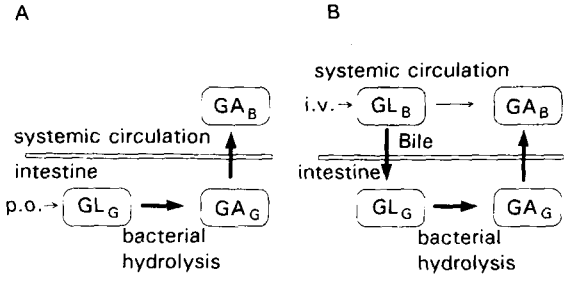

FIG. 3. Schematic representation of metabolic fate after oral (A) or intravenous (B) administration of glycyrrhizin. GL_G = intestinal compartment of glycyrrhizin, GL_B = blood compartment of glycyrrhizin, GA_G = intestinal compartment of glycyrrhetic acid, GA_B = blood compartment of glycyrrhetic acid.

gesting slow transformation of glycyrrhizin to glycyrrhetic acid by intestinal bacteria. MRT values for plasma glycyrrhetic acid after oral administrations of glycyrrhizin and glycyrrhetic acid were 19·9 h and 9·3 h, respectively. Accordingly, the mean transit time (MTT) required for the conversion in the intestine was estimated to be 10·6 h. The plasma concentration profile of glycyrrhetic acid, which appeared slowly and remained in the concentration range of 0·2–0·8 $\mu g\ mL^{-1}$ for 6 to 40 h (Fig. 1), after oral administration of glycyrrhizin suggests a natural prodrug; intestinal bacteria take part in activation by hydrolysis of natural prodrugs such as glycyrrhizin (Kobashi et al 1992).

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