Influence of Honey on the Gastrointestinal Metabolism and Disposition of Glycyrrhizin and Glycyrrhetic Acid in Rabbits

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Received May 30, 2001; accepted September 10, 2001

To investigate the effects of honey on the pharmacokinetics of glycyrrhizin and glycyrrhetic acid, administration of glycyrrhizin or glycyrrhetic acid with and without honey was carried out in rabbits in a randomized crossover design. An in vitro study using rabbit fecal flora was employed to elucidate the mechanism of the interaction. HPLC methods were used for the determination of glycyrrhizin, glycyrrhetic acid and 3-dehydroglycyrrhetic acid concentrations in serum and feces. Paired and unpaired Student’s t-tests were used for statistical comparisons for in vivo and in vitro studies, respectively. Our study indicated that the area under the curve (AUC_{\text{in vivo}}) of glycyrrhetic acid was significantly enhanced by 53% when honey was concomitantly given with glycyrrhizin, whereas that of glycyrrhizin was not significantly altered. Nevertheless, lack of effect was observed when honey was concurrently given with glycyrrhetic acid. Fecal study indicated that both the hydrolysis of glycyrrhizin to glycyrrhetic acid and subsequent oxidation of glycyrrhetic acid to 3-dehydroglycyrrhetic acid were significantly affected in the presence of honey to result in more glycyrrhetic acid available for absorption. It could be concluded that honey significantly affected the gastrointestinal metabolism of glycyrrhizin and resulted in the increased glycyrrhetic acid exposure. Therefore, honey might enhance the efficacy and adverse effects of glycyrrhizin.

Key words glycyrrhizin; glycyrrhetic acid; honey; metabolism; interaction

Glycyrrhizin, a glycoside of glycyrrhetic acid, is a major and active constituent of the root of Glycyrriza sp. (licorice) which is the most common herb used in Chinese medicinal prescriptions. In addition to licorice, honey-treated licorice is also often prescribed. The purpose of honey processing of licorice is claimed to be for the enhancement of efficacy. Glycyrrhizin displays a variety of pharmacological actions, including anti-inflammatory, antiviral and antioxidant activities. Overconsumption of glycyrrhizin results in an adverse effect, aldosteronism. Glycyrrhetic acid, the active metabolite of glycyrrhizin and also a minor component of licorice, has pronounced anti-inflammatory activity, even stronger than glycyrrhizin, and is responsible for the toxic effect of aldosteronism after glycyrrhizin intake. Honey is an important additive for traditional Chinese medicines. Our previous study reported that honey significantly decreased the absorption of a flavanone glycoside-naringin from the honey-treated pericarps of Citrus grandis in humans. A subsequent study with rabbits confirmed that honey and its component sugars enhanced the degradation of naringenin by fecal flora and resulted in reduced absorption of naringin. In a continuation of our investigations of the role of honey in Chinese medicine in our laboratory, a screening test had demonstrated that the metabolism of glycyrrhizin by rabbit fecal flora was markedly influenced by honey. Therefore, the aim of the present study was to investigate the effect of honey on the gastrointestinal metabolism and disposition of glycyrrhizin and glycyrrhetic acid in rabbits, moreover the mechanism of interaction was further clarified by using rabbit fecal flora.

MATERIALS AND METHODS

Chemicals Glycyrrhizin and propylparaben were purchased from Sigma Chemical Company (St. Louis, MO, U.S.A.). Glycyrrhetic acid and 2-methylanthraquinone were products of Aldrich Chemical Company Inc. Honey was purchased from a farm in southern Taiwan and collected from Dimocarpus longan Lour. All other chemicals and solvents used were of analytical grade or HPLC quality. Milli-Q plus water (Millipore, Bedford, MA, U.S.A.) was used for all preparations.

Preparation of Honey-Treated Licorice One hundred grams of licorice was macerated with 25 g honey in 50 ml water for 2 h, then baked with stirring on a gas stove until dry.

Animals Male New Zealand white rabbits, weighing 2—3 kg, were used throughout this study. Animals were housed in a 12-h light—dark, constant temperature environment prior to study. All rabbits were fasted for 1 d before and continued for 4 h after drug administration. Water was supplied ad libitum. At least one week was allowed for wash-out between two treatments. Drug administration was carried out via gastric gavage throughout the study. The animal study adhered to the “Principles of Laboratory Animal Care” (NIH publication #85—23, revised 1985).

In Vivo Studies (a) Eight rabbits were orally given glycyrrhizin with and without honey in a randomized crossover design study. For the treatment with glycyrrhizin alone, rabbits were given 5 ml of water immediately before glycyrrhizin, which was administered as an aqueous solution (10 mg/ml) at a dose of 150 mg/kg. For combined treatment, honey aqueous solution (5 g in 5 ml water) was given immediately before glycyrrhizin aqueous solution. Blood samples (1.2 ml) were withdrawn via the right ear vein at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36 and 48 h after glycyrrhizin administration.
All blood samples were centrifuged at 9860 g for 15 min and the serum obtained was stored at −30 °C until analysis.

(b) Six rabbits were orally given glycyrrhetic acid with and without honey in a randomized crossover design study. For the treatment with glycyrrhetic acid alone, rabbits were given 15 ml water immediately before glycyrrhetic acid which was administered at a dose of 84 mg/kg (42 mg/ml in glycofurol). For the combined treatment, honey aqueous solution (5 g in 15 ml water) was given prior to glycyrrhetic acid administration. Blood samples (1.2 ml) were withdrawn via the right ear vein at 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, and 8 h after glycyrrhetic acid administration. All blood samples were centrifuged at 9860 g for 15 min and the serum obtained was stored at −30 °C until analysis.

**In Vitro Studies** Fecal suspension was prepared by suspending 60 g fresh rabbit feces in 180 ml artificial intestinal fluid (pH=7.4).

(a) After filtration through gauze, 112 ml feces suspension was spiked with 14 ml glycyrrhizin solution (1 mg/ml in methanol/water), then it was equally divided into two aliquots of 63 ml each. To an aliquot of 63 ml fecal suspension, 7 ml artificial intestinal fluid was added as control, whereas 7 ml honey solution (100 mg/ml in artificial intestinal fluid) was spiked into the other aliquot. Then the feces suspensions were divided into aliquots of 700 µl each, and anaerobically incubated at 37 °C in a water bath shaker. Samples in triplicate were withdrawn at 1, 2, 4, 8, 12 and 24 h and frozen immediately at −30 °C until analysis.

(b) In another series of experiments, 112 ml feces suspension was spiked with 14 µl glycyrrhetic acid solution (0.5 mg/ml in methanol), then it was equally divided into two aliquots of 63 ml each. The subsequent procedures followed those described in (a).

**Synthesis of 3-Dehydroglycyrrhetic Acid** Our method was modified from a previous report. Glycyrrhetic acid (1 g) was dissolved in 200 ml ether, to which 1.7 ml of Jones reagent was added portionwise with stirring at room temperature. The reaction was continued for 24 h and filtered, then dehydrated with anhydrous sodium sulfate. The solution was evaporated to dryness in vacuo and the residue was recrystallized from ethyl acetate to afford 3-dehydroglycyrrhetic acid, which was subjected to electron impact (EI)-MS, 1H- and 13C-NMR spectral analysis.

**Preparation of Calibration Curves of Glycyrrhetic Acid and 3-Dehydroglycyrrhetic Acid in Feces** Various concentrations of 70 µl glycyrrhetic acid and 70 µl 3-dehydroglycyrrhetic acid were spiked into 560 µl feces suspension to afford a series of standards consisting of 200.0, 100.0, 50.0, 25.0, 12.5 and 6.2 µg/ml glycyrrhetic acid and 100.0, 50.0, 25.0, 12.5, 6.2 and 3.1 µg/ml 3-dehydroglycyrrhetic acid. To each 700 µl feces standard, 50 µl 0.1 N HCl were added, followed by partition with 750 µl EtOAc containing 2.5 µg/ml 2-methylanthraquinone as the internal standard. The mixture was vortexed for 20 s and then centrifuged at 9860 g for 15 min, the EtOAc layer was removed and evaporated to dryness under N-EVAP™ 112 nitrogen evaporator (Organamation Associates, Inc., U.S.A.), then the residue was reconstituted with 200 µl CH3CN, of which 20 µl was subjected to HPLC analysis.

**Analytical Methods for Glycyrrhizin and Glycyrrhetic Acid in Serum and Feces** The quantitation of glycyrrhizin and glycyrrhetic acid in serum followed the previous methods. For glycyrrhizin assay, 200 µl serum was deproteinized with 800 µl methanol containing 0.1 µg/ml propylparaben as the internal standard. For glycyrrhetic acid assay, 300 µl serum was acidified with 100 µl 0.1 N HCl, then partitioned with 400 µl ethyl acetate containing 0.1 µg/ml of 2-methylanthraquinone.

For the determination of glycyrrhetic acid and 3-dehydroglycyrrhetic acid in feces, 50 µl 0.1 N HCl was added to 700 µl feces suspension, and then partitioned with 750 µl ethyl acetate containing 2.5 µg/ml 2-methylanthraquinone as the internal standard. The subsequent procedures were identical to those for feces calibrators.

The HPLC apparatus included one pump (LC-10AS, Shimadzu, Japan) and an UV/VIS detector (SPD-10A, Shimadzu, Japan). A LiChrospher 100 RP-18e column (4.0×250 mm, 5 µm, Merck) was used. The UV detector was set at 248 nm and the flow rate was 1.0 ml/min. Mobile phase consisted of acetonitrile and 1% acetic acid (36 : 64, v/v) for glycyrrhizin determination. For the assay of glycyrrhetic acid and 3-dehydroglycyrrhetic acid in serum and feces, a mobile phase consisting of acetonitrile and 1% acetic acid (67 : 33, v/v) was used.

**Validation of Assay Method of Glycyrrhetic Acid and 3-Dehydroglycyrrhetic Acid in Feces** Good linearity was obtained from linear regression of the calibrators in the concentration range of 6.2—200.0 µg/ml and 3.1—100.0 µg/ml for glycyrrhetic acid and 3-dehydroglycyrrhetic acid, respectively. The precision and accuracy of this method was evaluated by the intra-day and inter-day analysis of triplicate feces standards for three consecutive days.

**Data Analysis** The pharmacokinetic parameters of glycyrrhizin and glycyrrhetic acid were calculated using a non-compartment model with the aid of WINNONLIN (version 1.1, S.CI software, Statistical Consulting, Inc., Apex, NC). Paired and unpaired Student’s t-tests were used for statistical comparisons for *in vivo* and *in vitro* studies, respectively. A *p* value of less than 0.05 was considered significant.
RESULTS

Analytical Methods for Glycyrrhizin, Glycyrrhetic Acid and 3-Dehydroglycyrrhetic Acid in Serum and Feces

The analytical method and validation of glycyrrhizin and glycyrrhetic acid analysis in serum has been reported.11) The regression lines for glycyrrhizin and glycyrrhetic acid were $Y = 0.194X - 0.025$ ($r = 0.999$) and $Y = 1.016X + 0.008$ ($r = 0.999$), respectively. In this study, the assay method of glycyrrhetic acid and 3-dehydroglycyrrhetic acid in feces was established.

The regression lines for glycyrrhetic acid and 3-dehydroglycyrrhetic acid were $Y = 0.041X^2 + 0.051$ ($r = 0.999$) and $Y = 0.041X + 0.008$ ($r = 0.999$), respectively. Validation of the method showed that the intra-day and inter-day coefficient of variations (CVs) were below 11.2% and 7.7%, respectively, and the intra-day and inter-day relative errors were below 17.3% and 7.7%, respectively.

Effect of Honey on the Oral Pharmacokinetics of Glycyrrhizin

Figure 1 depicts the profiles of mean serum concentrations of glycyrrhizin and glycyrrhetic acid in eight rabbits, respectively, after the administrations of glycyrrhizin with and without honey. Table 1 lists the pharmacokinetic parameters of glycyrrhizin and glycyrrhetic acid after the two treatments. Statistical comparison indicated that the concomitant intake of honey significantly enhanced the area under the blood concentration–time curve ($AUC_{0\rightarrow t}$) of glycyrrhetic acid by 53%, whereas the $AUC_{0\rightarrow t}$ and $C_{\text{max}}$ of glycyrrhizin were not significantly affected.

Effect of Honey on the Oral Pharmacokinetics of Glycyrrhetic Acid

Figure 2 depicts the mean serum concentration–time profiles of six rabbits, after the administration of glycyrrhetic acid with and without honey, respectively. Table 2 lists the pharmacokinetic parameters of glycyrrhetic acid after the two treatments. No significant difference between the two treatments was observed.

Effect of Honey on the Metabolism of Glycyrrhizin by Fecal Flora

Figure 3 depicts the metabolic time courses of glycyrrhizin to form glycyrrhetic acid and 3-dehydroglycyrrhetic acid when glycyrrhizin was incubated with feces in the presence or absence of honey. At 1 h after incubation,

Table 1. Mean (±S.E.) Pharmacokinetic Parameters of Glycyrrhizin and Glycyrrhetic Acid in Eight Rabbits after Giving Glycyrrhizin (150 mg/kg) Alone and Coadministration with Honey (5 g/Rabbit)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glycyrrhizin alone</th>
<th>Glycyrrhetic acid</th>
<th>Glycyrrhizin + honey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)$^a$</td>
<td>5.4±1.1</td>
<td>9.0±0.7</td>
<td>5.0±0.7</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)$^b$</td>
<td>20.1±4.0</td>
<td>0.9±0.2</td>
<td>19.0±4.3</td>
</tr>
<tr>
<td>$AUC$ (µg/ml·h)$^c$</td>
<td>208.4±44.1</td>
<td>10.9±2.1</td>
<td>178.2±45.2</td>
</tr>
<tr>
<td>$MRT$ (h)$^d$</td>
<td>8.2±0.6</td>
<td>13.4±1.3</td>
<td>8.1±0.7</td>
</tr>
</tbody>
</table>

$a$ Time of peak plasma level. $b$ Concentration of peak plasma level. $c$ Area under plasma concentration–time curve to the last point. $d$ Mean residence time. $^*$ p<0.05 compared with glycyrrhizin alone.
Table 2. Mean (±S.E.) Pharmacokinetic Parameters of Glycyrrhetic Acid in Six Rabbits after Giving Glycyrrhetic Acid (84 mg/kg) Alone and Co-administration with Honey (5 g/rabbit)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glycyrrhetic acid alone</th>
<th>Glycyrrhetic acid + honey</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (h)$^a$</td>
<td>1.7±0.7</td>
<td>1.3±0.4</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/ml)$^b$</td>
<td>1.3±0.3</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>AUC (µg/ml·h)$^c$</td>
<td>3.0±0.5</td>
<td>2.7±0.7</td>
</tr>
<tr>
<td>MRT (h)$^d$</td>
<td>2.8±0.3</td>
<td>2.5±0.5</td>
</tr>
</tbody>
</table>

$^a$—$^d$ as in Table 1.

**DISCUSSION**

The metabolism and disposition of glycyrrhizin in humans and in rats were described in several studies. The results suggested that glycyrrhizin could be absorbed per se from small intestine and its active metabolite glycyrrhetic acid was then absorbed from the large intestine. However, the time profiles of glycyrrhizin and glycyrrhetic acid after oral dosing of glycyrrhizin were not in good agreement among different laboratories.

In contrast to previous studies, the present investigation used rabbits for the pharmacokinetic studies of glycyrrhizin and glycyrrhetic acid. Our results indicated that after oral dosing of glycyrrhizin, glycyrrhizin was absorbed faster and to a greater extent than glycyrrhetic acid. The $T_{\text{max}}$ and mean residence time (MRT) of glycyrrhetic acid were much more prolonged than those of glycyrrhizin, which corresponds to data reported for rats.

Because honey is a popular worldwide daily diet constituent, and also a common additive for traditional Chinese medicines, the effect of honey on the oral pharmacokinetics of glycyrrhizin was investigated in this study. The results indicated that concomitant ingestion of honey with glycyrrhizin significantly enhanced the systemic exposure of the active metabolite glycyrrhetic acid by 53%, but did not alter that of the parent compound. The in vivo anti-inflammatory activity of glycyrrhetic acid was shown to be 10 to 50 times higher than that of glycyrrhizin and glycyrrhetic acid was responsible for the adverse effect of aldosteronism, therefore, enhanced glycyrrhetic acid exposure might result in important clinical consequences. Honey might enhance the efficacy of glycyrrhizin and possibly increase the risk of aldosteronism.

The mechanism of the honey effect on glycyrrhizin pharmacokinetics was investigated in vitro by using fresh rabbit feces. It was reported that Ruminococcus sp. PO1-3, an intestinal bacterium isolated from human feces, metabolized glycyrrhizin to glycyrrhetic acid and 3-dehydroglycyrrhetic acid. As the incubation started, glycyrrhetic acid and 3-dehydroglycyrrhetic acid were found to emerge from glycyrrhizin. Glycyrrhetic acid increased at the early phase, but declined gradually thereafter, whereas 3-dehydroglycyrrhetic acid kept increasing during the whole incubation period. Our results, as shown in Fig. 3, indicated that honey enhanced the bacterial hydrolysis of glycyrrhizin to glycyrrhetic acid and moreover, inhibited the oxidation of glycyrrhetic acid to 3-dehydroglycyrrhetic acid. Therefore honey resulted in significantly more glycyrrhetic acid for absorption.

In order to measure directly the honey effect on the oxidation of glycyrrhetic acid, fecal suspension was incubated with glycyrrhetic acid in the presence or absence of honey. The metabolic time courses of glycyrrhetic acid when incubated with fecal flora with and without honey revealed that it markedly inhibited the oxidation of glycyrrhetic acid to form 3-dehydroglycyrrhetic acid and left more glycyrrhetic acid for absorption. Unfortunately, 3-dehydroglycyrrhetic acid has significantly more glycyrrhetic acid was generated in the presence of honey when compared to that without honey. Moreover, at more than 4 h of incubation, the generation of 3-dehydroglycyrrhetic acid, an oxidation metabolite of glycyrrhetic acid, was significantly inhibited in the presence of honey.

**Effect of Honey on the Metabolism of Glycyrrhetic Acid by Fecal Flora** Figure 4 depicts the metabolic time courses of glycyrrhetic acid to form 3-dehydroglycyrrhetic acid when glycyrrhetic acid was incubated with feces in the presence or absence of honey. At 1 h after incubation, significantly reduced amount of 3-dehydroglycyrrhetic acid were generated from glycyrrhetic acid in the presence of honey.

![Image 1](image1.png)

![Image 2](image2.png)

![Image 3](image3.png)
not been detected in any serum samples of rabbits after glycyrrhizin and glycyrrhetic acid administrations with or without honey in this study. A literature search indicated that no blood data for 3-dehydroglycyrrhetic acid has been reported for any animal species. This might be because of its poor bioavailability or due to a large volume of distribution.

The effect of honey on the oral pharmacokinetics of glycyrrhetic acid in rabbits was also investigated in this study. Our result indicated that lack of effect on the oral pharmacokinetics of glycyrrhetic acid was found with the concomitant intake of honey. Our previous study comparing the oral pharmacokinetics of equal molar glycyrrhizin and glycyrrhetic acid indicated that the absorption of glycyrrhetic acid was much faster and MRT was much shorter than that hydrolyzed from glycyrrhizin.11) These results suggested that being a relatively nonpolar aglycone, glycyrrhetic acid was predominantly absorbed in the small intestine and thus the influence of honey on the fate of glycyrrhetic acid transformed from glycyrrhizin in the large intestine did not occur to the oral dose of glycyrrhetic acid.

In summary, concomitant intake of honey with glycyrrhizin significantly increased the absorption of the even stronger active metabolite–glycyrrhetic acid, therefore honey might enhance the efficacy and increase the risk of adverse reactions of glycyrrhizin.

Acknowledgements This work was financially supported by National Science Council, R.O.C. (90-2320-B-039-016) and China Medical College Hospital (DMR-90-139).

REFERENCES