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Effects of glucose, fructose and 5-hydroxymethyl-2-furaldehyde on the presystemic metabolism and absorption of glycyrrhizin in rabbits

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Abstract

Our previous study reported that co-administration of honey significantly increased the serum levels of glycyrrhetic acid (GA) after oral administration of glycyrrhizin (GZ) in rabbits. The components of honey are sucrose, glucose, fructose and 5-hydroxymethyl-furaldehyde (HMF). To clarify the causative component(s) in honey that altered the metabolic pharmacokinetics of GZ, rabbits were given GZ (150 mg kg^{-1}) with and without glucose (5 g/rabbit), fructose (5 g/rabbit) and HMF (1 mg kg^{-1}), respectively, in crossover designs. An HPLC method was used to determine concentrations of GZ and GA in serum as well as GA and 3-dehydroglycyrrhetic acid (3-dehydroGA) in faeces suspension. A noncompartment model was used to calculate the pharmacokinetic parameters and analysis of variance was used for statistical comparison. Our results indicated that the area under curve (AUC) of GA was significantly increased by 29% when HMF was coadministered, whereas the pharmacokinetics of GZ and GA were not significantly altered by coadministration of glucose or fructose. An in-vitro study, using faeces to incubate GZ and GA individually, indicated that HMF significantly inhibited the oxidation of GA to 3-dehydroGA and this may explain the enhanced GA absorption in-vivo. It was concluded that HMF is the causative component in honey that affects the presystemic metabolism and pharmacokinetics of GZ in-vivo.

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Introduction

Glycyrrhizin (GZ) is a major and active constituent of liquorice. GZ possesses a variety of pharmacological activity, including anti-inflammatory (Baran et al 1974; Okimasu et al 1983), antiviral (Ito et al 1987) and antioxidative (Nagai et al 1992) activity. It has been clinically used as a remedy for chronic hepatitis (Hikino 1985) and immunodeficiency virus infection (Hattori et al 1989). More recently, GZ was reported to inhibit the replication of the severe acute respiratory syndrome-associated viruses (SARS-CV) (Cinati et al 2003). Over-consumption of GZ resulted in the adverse effect of aldosteronism (Shio 1982). Glycyrrhetic acid (GA), an active metabolite of GZ, has pronounced anti-inflammatory activity, even stronger than GZ in-vivo, and is thus responsible for the toxic effect of aldosteronism (Buhler et al 1991).

Our previous study reported that coadministration of honey significantly increased the serum levels of GA after oral administration of GZ in rabbits (Ching et al 2002). Therefore, honey might enhance the in-vivo toxicity of GZ. Sucrose, glucose, fructose and 5-hydroxymethyl-furaldehyde (HMF) are components of honey. Sucrose is hydrolysed into glucose and fructose by sucrase in the intestine. This study attempted to investigate whether glucose, fructose or HMF affected the metabolic pharmacokinetics of GZ. Moreover, the mechanism of the interaction was assessed by incubation of the individual components of honey with GZ and GA individually in rabbit faeces suspension (Kim et al 1998).

Materials and Methods

Materials

GZ (98%), glucose, fructose, HMF and propylparaben were purchased from Sigma Chemical Company (St Louis, MO). GA (99%) was obtained from Yoneyama Yakuhin Kogyo Co. Ltd (Osaka, Japan). 2-Methylanthraquinone (95%) was a product of Aldrich Chemical Company (WI). 3-Dehydroglycyrrhetic acid (3-dehydroGA) was synthesized by a method described in our previous study (Ching et al 2002). All other chemicals and solvents used were of analytical grade or HPLC quality. Milli-Q plus water (Millipore, Bedford, MA) was used for all preparations.

Instrumentation

The HPLC apparatus included one pump (LC-10AS, Shimadzu, Japan) and a UV/VIS detector (SPD-10A; Shimadzu, Japan). The assay employed a LiChrospher 100 RP-18e column (4.0 × 250 mm, 5 μm; Merck). The UV detector was set at 248 nm and the flow rate was 1.0 mL min^{-1}.

Animals

Male New Zealand white rabbits, 2.5~3.2 kg, were used throughout this study. Rabbits were housed in a 12-h light–dark cycle environment maintained at a constant temperature of 25°C and a relative humidity of 50% before study. All rabbits were fasted for one day before drug administration and fasting was continued for 4 h after dosing. Rabbits were allowed free access to water. At least one week was allowed for wash-out between two treatments. The animal study adhered to The Guidebook for the Care And Use of Laboratory Animals (2002), published by the Chinese Society for the Laboratory Animal Science, Taiwan, R.O.C.

In-vivo study

Six rabbits were given GZ orally at a dose of 150 mg kg^{-1} (10 mg mL^{-1} in water) with and without glucose, fructose and HMF, respectively, in a randomized crossover design. The dose for each rabbit was 5 g, 5 g and 1 mg for glucose, fructose and HMF, respectively; doses were individually dissolved in 5 mL of water. Administration of chemicals was carried out via gastric gavage throughout the study. Blood samples (1.2 mL) were withdrawn via a right-ear vein at 0, 1, 2, 4, 6, 8, 10, 12, 24, 36 and 48 h after GZ 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 study

A faeces suspension was prepared by suspending 120 g of fresh rabbit faeces in 360 mL of artificial intestinal fluid (phosphate buffer, pH 7.4). After filtration through gauze, 224 mL of faeces suspension was spiked with 28 mL of GZ solution (1 mg mL^{-1} in methanol–water). The suspension was then divided into four equal volumes, to one of which was added 7 mL of artificial intestinal fluid to afford faeces suspension with a GZ concentration of 100 μg mL^{-1} as control; 7 mL of sugar solution (100 mg mL^{-1} of glucose or fructose or 0.02 mg mL^{-1} of HMF in artificial intestinal fluid) were spiked into the other three samples, respectively. Then the faeces suspension was divided into samples of 700 μL and anaerobically incubated at 37°C on a water bath shaker (Kim et al 1998; Hou et al 2001). Triplicates of samples were withdrawn at 1, 2, 4, 8, 12 and 24 h and frozen immediately at −30 °C until HPLC analysis.

In another study, GA solution (0.5 mg mL^{-1} in methanol) was spiked into faeces suspension with glucose, fructose and HMF, respectively, in the same manner as for GZ, and the procedures followed those described above.

Assay of GZ, GA and 3-dehydroGA in serum and faeces

The methods for determining the quantity of GZ, GA and 3-dehydroGA in serum and faeces, as well as the method validation, were reported previously (Ching et al 2001). For the GZ assay, to 200 μL of serum, 800 μL of methanol containing 0.1 μg mL^{-1} of propylparaben (the internal standard) was added for deproteinization. For the GA assay, to 300 μL of serum, 100 μL of 0.1 M HCl was added, then partitioned with 400 μL of ethyl acetate containing 0.1 μg mL^{-1} of 2-methylanthraquinone (the internal standard). To the faeces sample, 50 μL of 0.1 M HCl was added and then partitioned with 750 μL of ethyl acetate containing 2.5 μg mL^{-1} of 2-methylanthraquinone (the internal standard).

Chromatographic separation was conducted by using a mobile phase consisting of acetonitrile–1% acetic acid (36:64, v/v) for GZ determination and acetonitrile–1% acetic acid (67:33, v/v) for the assay of GA and 3-dehydroGA in serum and faeces.

Data analysis

The peak serum concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained from experimental observation. The pharmacokinetic parameters were analysed by a noncompartmental method with the aid of the program WINNOLIN (version 1.1 SCI software, 1998–1999; Statistical Consulting, Inc., Apex, NC). The area under the serum concentration–time curve ($AUC_{0\text{-}t}$) was calculated using the trapezoidal rule to the last point. Analysis of variance was used to estimate the difference of pharmacokinetic parameters among various treatments. $P < 0.05$ was considered significant.

Results

Effect of glucose and fructose on the oral pharmacokinetics of GZ

Figure 1 depicts the profiles of mean serum concentrations of GZ and GA in six rabbits after oral administration of GZ alone and after coadministration of GZ and glucose or fructose, respectively. The mean T_{max} of GA was much later than

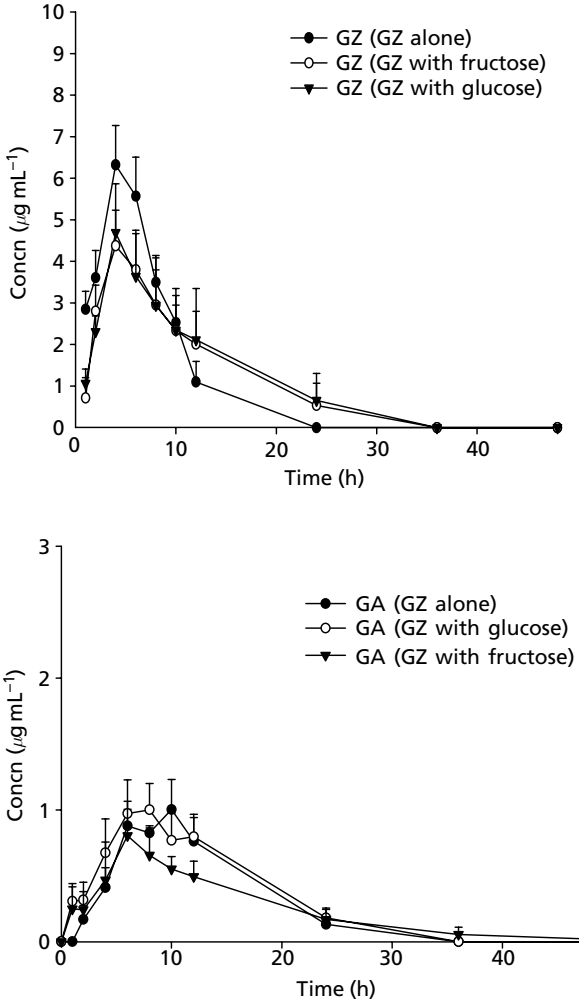

Figure 1 Mean (± s.e.) serum concentration–time profiles of glycyrrhizin (GZ) and glycyrrhetic acid (GA) after oral administration of GZ ($150\,mg\,kg^{-1}$) alone or after coadministration with fructose (5 g) or glucose (5 g) to 6 rabbits.

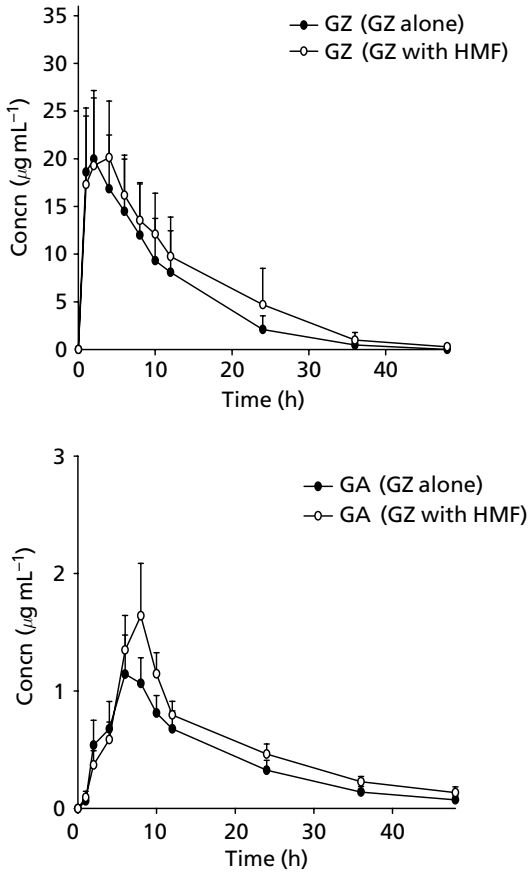

Figure 2 Mean (± s.e.) serum concentration–time profiles of glycyrrhizin (GZ) and glycyrrhetic acid (GA) after oral administration of GZ ($150\,mg\,kg^{-1}$) alone or after coadministration with 5-hydroxymethyl-furaldehyde (HMF, 1 mg/rabbit) to 10 rabbits.

that of GZ and the C_{max} of GA was much lower than that of GZ for the three treatments. The C_{max} values of GZ were 6.7 ± 0.9, 5.0 ± 1.2 and $4.9 \pm 0.9\,\mu g\,mL^{-1}$, and the $AUC_{0\text{-}t}$ values were 44.8 ± 6.5, 45.5 ± 20.3 and $39.8 \pm 10.6\,\mu g\,h\,mL^{-1}$ after administration of GZ alone, coadministration with glucose and coadministration with fructose, respectively. The C_{max} values of GA were 1.2 ± 0.2, 1.1 ± 0.2 and $1.0 \pm 0.2\,\mu g\,mL^{-1}$, and the $AUC_{0\text{-}t}$ values were 11.3 ± 2.5, 13.6 ± 3.2 and $10.6 \pm 2.5\,\mu g\,h\,mL^{-1}$ after administration of GZ alone, coadministration with glucose and coadministration with fructose, respectively. Statistical comparison indicated that the concomitant intake of glucose or fructose did not significantly affect the pharmacokinetics of GZ and GA.

Effect of HMF on the oral pharmacokinetics of GZ

Figure 2 depicts the profiles of mean serum concentrations of GZ and GA in ten rabbits after administration of GZ alone and after coadministration of GZ with HMF, respectively. Table 1 lists the pharmacokinetic parameters of GZ and GA after administration of GZ alone and after coadministration of GZ with HMF. Statistical comparison indicated that the concomitant intake of HMF significantly elevated the $AUC_{0\text{-}t}$ of GA by 29%, whereas that of GZ was not significantly altered.

Table 1 Pharmacokinetic parameters of glycyrrhizin (GZ) and glycyrrhetic acid (GA) after giving GZ ($150\,mg\,kg^{-1}$) alone and after coadministration with 5-hydroxymethyl-furaldehyde (HMF, 1 mg) to 10 rabbits

Parameters	GZ Alone		GZ with HMF	
	GZ	GA	GZ	GA
T_{max} (h)	2.2 ± 0.3	8.0 ± 1.9	3.9 ± 0.8	7.2 ± 0.6
C_{max} ($\mu g\,mL^{-1}$)	20.7 ± 7.1	1.5 ± 0.3	22.9 ± 7.1	2.0 ± 0.4
AUC ($\mu g\,h\,mL^{-1}$)	229.6 ± 106.9	18.7 ± 3.2	296.7 ± 122.0	24.1 ± 3.2*
MRT (h)	5.7 ± 0.9	13.4 ± 1.9	7.4 ± 1.3	15.6 ± 1.6

Data expressed as mean ± s.e. *$P < 0.05$ compared with GZ alone.

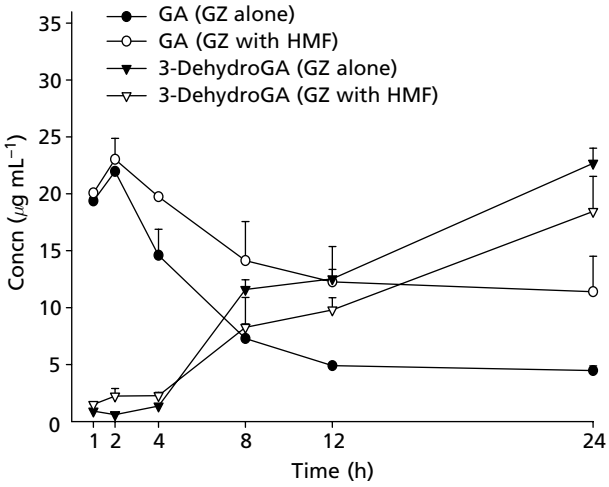

Figure 3 Mean (± s.e.) concentration–time profiles of glycyrrhetic acid (GA) and 3-dehydroGA after incubation of glycyrrhizin (GZ, 100 μg mL^{-1}) alone or with 5-hydroxymethyl-furaldehyde (HMF, 2 μg mL^{-1}) in rabbit faeces suspension (n = 3).

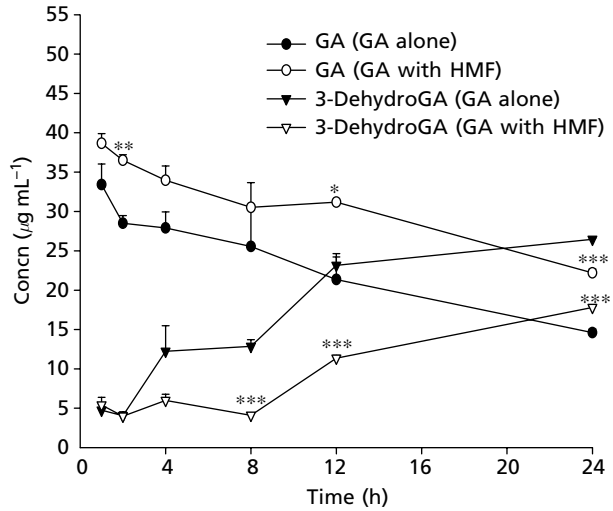

Figure 4 Mean (± s.e.) concentration–time profiles of glycyrrhetic acid (GA) and 3-dehydroGA after incubation of GA (50 μg mL^{-1}) alone or with 5-hydroxymethyl-furaldehyde (HMF, 2 μg mL^{-1}) in rabbit faeces suspension (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with GA alone.

Effect of HMF on the generation of GA and 3-dehydroGA from GZ in faeces

Figure 3 shows the metabolic profiles of GA and 3-dehydroGA when GZ was incubated with faeces in the presence and absence of HMF. The concentration of GA was increased by the presence of HMF, whereas the formation of 3-dehydroGA was inhibited.

Effect of HMF on 3-dehydroGA generation from GA in faeces

For direct observation of the influence of HMF on the presystemic metabolism of GA, GA was incubated with faeces in the presence and absence of HMF. Figure 4 shows the metabolic profiles of GA and 3-dehydroGA when GA was incubated with and without HMF. The generation of 3-dehydroGA was significantly inhibited by the presence of HMF and resulted in an increase in GA concentration.

Discussion

GZ can be absorbed in both intact and metabolite form (e.g. GA, which is formed after hydrolysis of GZ and thus absorbed much later than GZ). Because two groups of rabbits were used for GZ pharmacokinetic studies, some differences in pharmacokinetic behaviour of GZ, as shown in Figures 1 and 2, were attributable to great variation among individuals. There were 4 rabbits in the HMF study (Figure 2) that exhibited extraordinarily high GZ serum levels. Because a crossover design was used in this study, those outliers were not excluded and thus resulted in a much higher mean GZ level than that in Figure 1. However, not much difference in GA pharmacokinetics was observed between the two groups.

Major components of honey include sucrose, glucose, fructose and HMF, etc. Sucrose is hydrolysed into glucose and fructose by sucrase in the intestine, so the effect of sucrose on the pharmacokinetics of GZ was not investigated in this study. According to a previous study of honey (Wen et al 1995), the glucose and fructose content is 17–36% and 21–47%, respectively, whereas the content of HMF is as low as 92 ppm. After being assayed in this study, therefore, their doses coadministered with GZ to rabbits were designed as 5 g, 5 g and 1 mg, respectively. Our results indicated that coadministration of glucose and fructose with GZ did not significantly influence the pharmacokinetics of GZ and GA. This might be explainable by the fact that glucose and fructose are readily absorbed in the small intestine via a transporter (Thomson et al 1996). On the other hand, the minor constituent HMF significantly enhanced the $AUC_{0\text{-}t}$ of GA by 29%. The two profiles of GA began to diversify at 6 h after dosing, indicating that the interaction between HMF and GZ became prominent when GA was generated from GZ to a great extent. The in-vivo anti-inflammatory activity of GA was 10–50 times that of GZ (Kumagai et al 1981) and GA was responsible for the adverse effect of aldosteronism, therefore the enhanced GA serum level caused by HMF might result in increased toxicity of GZ. HMF is a minor constituent in honey and its concentration increases with storage time (Vinas et al 1992). Therefore, aging honey would exert a more pronounced effect on GZ pharmacokinetics.

GZ is reported to be absorbed per se in the small intestine and the remaining GZ is then hydrolysed into GA in the colon; moreover, GA is further oxidized into 3-dehydroGA by enteral microflora (Wang et al 1994). Our previous study had explored the mechanism of effects of

honey and sugars on the presystemic metabolism of naringin by using rabbit faeces (Hou et al 2001). The mechanism of HMF effect on GZ pharmacokinetics was investigated by the same methodology. As shown in Figure 3, GA was found to increase and then decline gradually as incubation progressed. On the contrary, the oxidation metabolite of GA, 3-dehydroGA, climbed upward gradually. These results indicated that HMF significantly inhibited the oxidation of GA to 3-dehydroGA and resulted in more GA being available for absorption. Furthermore, when GA was incubated with faeces in the absence and presence of HMF, the results were in good agreement with those of GZ incubated without and with HMF, confirming that oxidation of GA was significantly inhibited by HMF. These in-vitro results can explain the mechanism of the in-vivo interaction between HMF and GZ. However, 3-dehydroGA was not detected in any serum samples of rabbits after GZ administration. This might be due to the large volume of distribution of 3-dehydroGA.

In summary, HMF enhanced the absorption of the more potent metabolite, GA, and it might increase the pharmacological effects and possibly the toxicity of GZ.

References

Baran, J. S., Langford, D. D., Liang, C., Pitzele, B. S. (1974) Synthesis and biological activities of substituted glycyrrhetic acids. *J. Med. Chem.* **17**: 184–191

Buhler, H., Perschel, F. H., Hierholzer, K. (1991) Inhibition of rat renal 11 beta-hydroxysteroid dehydrogenase by steroidal compounds and triterpenoids; structure/function relationship. *Biochim. Biophys. Acta* **1075**: 206–212

Ching, H., Hsiu S. L., Hou, Y. C., Chen, C. C., Chao, P. D. L. (2001) Comparison of pharmacokinetics between glycyrrhizin and glycyrrhetic acid in rabbits. *J. Food Drug Anal.* **9**: 67–71

Ching, H., Hou, Y. C., Hsiu, S. L., Tsai, S. Y., Chao, P. D. L. (2002) Influence of honey on the gastrointestinal metabolism and disposition of glycyrrhizin and glycyrrhetic acid in rabbits. *Biol. Pharm. Bull.* **25**: 87–91

Cinati, J., Morgenstern, B., Bauer, G., Chandra, P., Rabenau, H., Doerr, H. W. (2003) Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. *Lancet* **361**: 2045–2046

Hattori, T., Ikematsu, S., Koito, A., Matsushita, S., Maeda, Y., Hada, M., Fujimaki, M., Takatsuki, K. (1989) Preliminary evidence for inhibitory effect of glycyrrhizin on HIV replication in patients with AIDS. *Antiviral Res.* **11**: 255–261

Hikino, H. (1985) Antihepatotoxic activity of crude drugs. *J.Pharm. Soc. Jpn* **105**: 109–118

Hou, Y. C., Hsiu, S. L., Huang, T. Y., Yang, C. Y., Tsai, S. Y., Chao, P. D. L. (2001) Effect of honey and sugars on the metabolism and disposition of naringin in rabbits. *Planta Med.* **67**: 538–541

Ito, M., Nakashima, H., Baba, M., Pauwels, R., De Clercq, E., Shigeta, S., Yamamoto, N. (1987) Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodeficiency virus [HIV (HTLV-III/LAV)]. *Antiviral Res.* **7**: 127–137

Kim, D. H., Jung, E. A., Sohng, I. S., Han, J. A., Kim, T. H., Han, M. J. (1998) Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Arch. Pharm. Res.* **21**: 17–23

Kumagai, A., Tamura, Y., Chang, Y. I. (1981) Biochemistry of glycyrrhizae radix. The mode of actions of glycyrrhizin and gllycyrrhetinic acid. *Gendai Toyo Igaku*. **2**: 38–45

Nagai, T., Egashira, T., Kudo, Y., Yamanaka, Y., Shimada, T. (1992) Attenuation of dysfunction in the ischemia-reperfused liver by glycyrrhizin. *Jpn. J. Pharmacol.* **58**: 209–218

Okimasu, E., Moromizato, Y., Watanabe, S., Sasaki, J., Shiraishi, N., Morimoto, Y. M., Miyahara, M., Utsumi, K. (1983) Inhibition of phospholipase A2 and platelet aggregation by glycyrrhizin, an antiinflammation drug. *Acta Med. Okayama* **37**: 385–391

Shio, H. (1982) A case of pseudoaldosteronism induced by glycyrrhizin and study of all cases in Japan. *Clin. Endocrinol.* **30**: 57–62

Thomson, A. B., Keelan, M., Wild, G. E. (1996) Nutrients and intestinal adaptation. *Clin. Invest. Med.* **19**: 331–345

Vinas, P., Campillo, N., Hernandez Cordoba, M. (1992) Simultaneous liquid chromatographic analysis of 5-(hydroxymethyl)-2-furaldehyde and methyl anthranilate in honey. *Food Chem.* **44**: 67–72

Wang, Z., Kurosaki, Y., Nakayama, T., Kimura, T. (1994) Mechanism of gastrointestinal absorption of glycyrrhizin in rats. *Biol. Pharm. Bull.* **17**: 1399–1403

Wen, H. M., Chern, J. C., Chen, S. H. (1995) Quality survey of commercial honey products. *J. Food Drug Anal.* **3**: 295–306