Interaction of Licorice on Glycyrrhizin Pharmacokinetics

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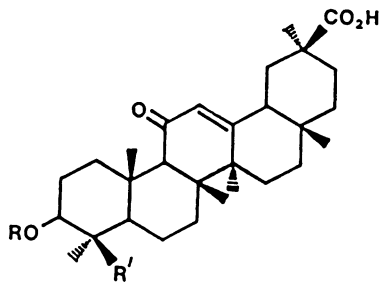
The effects of components of aqueous licorice root extract (LE) on the pharmacokinetics of glycyrrhizin (G) and glycyrrhetic acid (GA) were investigated in rats and humans. The aim of this work was to define the role of pharmacokinetics in G toxicity. In the procedure, G and GA were detected in biological fluids by means of recently improved HPLC methods. Significantly lower G and GA plasma levels were found in rats and humans treated with LE compared to the levels obtained with those in which G alone was administered. The pharmacokinetic curves showed significant differences in the areas under the plasma-time curve (AUC), C_{max}, and T_{max} parameters. The data obtained from urine samples are in agreement with the above results and confirm a reduced bioavailability of G present in LE compared to pure G. This should be attributed to the interaction during intestinal absorption between the G constituent and the several components in LE. The modified bioavailability could explain the various clinical adverse effects resulting from the chronic oral administration of G alone as opposed to LE. —Environ Health Perspect 102(Suppl 9):65–68 (1994)

Key words: chemical interactions, bioavailability, licorice, glycyrrhizin, pharmacokinetics

Introduction

Aqueous liquorice root extract (LE) is prepared from *Glycyrrhiza glabra L.* varieties and is a black mass containing several constituents and having a characteristic sweet taste. In particular, LE contains glycyrrhizin (G) (or glycyrrhizinic acid), a saponinlike glycoside that is 50 times sweeter than sugar. Upon hydrolysis, the glycoside loses its sweet taste and is converted to aglycone glycyrrhetic acid, a pentacyclic triterpene derivative of the β-amyrin type (Figure 1), plus two molecules of glucuronic acid. Other components of LE include flavonoid glycosides (liquiritin, isoliquiritin, liquiritoside, isoliquiritoside, rhamnoliquiritin, and rhamnoisoliquiritin), coumarin derivatives (herniarin and umbelliferone), asparagine, 22,23-dihydrostigmasterol, glucose, mannitol and about 20% starch (*1*).

LE is used for some therapeutic purposes. It was the Dutch physician Revers who discovered that LE contains a substance with spasmolytic power and with a beneficial influence on the healing process of gastric ulcers (*2*). In addition, glycyrrhetic acid has also been shown to possess antiinflammatory action which has led to its use in ointments for dermatological disorders (*3*). Other possible therapeutic properties of G have been studied such as antiallergic (*4*), antiarthritic (*5*), anticholinergic (*6*), antiestrogenic (*7*), antihepatotoxic (*8*) antileukaemogenic (*9*), and antiviral (*10*). Furthermore, G is used as a sweetening agent in many food and luxury products such as licorice, chocolates, beer, liquors and chewing gum. However, serious side effects related to glycyrrhizin ingestion, including headaches, edema, body weight increase, and disturbances in body-electrolyte balance were observed either after daily high LE personal consumption or in clinical use (*11*).



I R = gluA (1→2) gluA (1→), R' = CH_3

II R = H, R' = CH_3

Figure 1. Chemical structure of glycyrrhizin (I) and glycyrrhetic acid (II).

In the present work, which is part of an extensive study on the behavior and toxicity of G, an attempt was made to compare the bioavailabiliy of G after oral administration of G alone or G in LE in rats and humans to evaluate the interactive effects between G and several LE components during intestinal absorption.

Materials and Methods

Chemicals

Glycyrrhizin (glycyrrhizic acid ammonium salt), glycyrrhetic acid, and propyl-4-hydroxybenzoate or Paraben (PPB) were purchased from Fluka (Buchs, Switzerland). Aqueous licorice root extract with a G content of 7.64% was supplied by SAILA S.p.A. (Silvi Marina (TE), Italy). Methanol, ethanol, acetonitrile, acetic acid, and all other reagents were obtained from Merck (Darmstadt Germany). Analytical grade ultrapure water from Millipore milli-Q (Millipore) was used.

The stock solutions of G and GA were prepared by dissolving 50 mg of G or GA in 50 ml of ethanol. The stock solution of PPB (2×10^{-2}M) was obtained by dissolving 72 mg of PPB in 20 ml of methanol. The standard solutions were all prepared by diluting the stock solution with methanol (*11*).

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Apparatus and Chromatographic Conditions

The HPLC system consisted of a Varian 9001 chromatographic pump and Water (Millipore) 481 detector. Separation was achieved on a reverse-phase column (250 mm × 4 mm I.I., 5 μm, RP-18, Merck) fitted with a column inlet filter.

The mobile phase consisted of acetonitrile–water–acetic acid (36:64:0.5, v/v) for G and methanol–water–acetic acid (83:17:0.5, v/v) for GA. The mobile phase was used at a flow rate of 1.0 ml/min for G and of 0.85 ml/min for GA. These experimental conditions are based on those of Zhang et al. (*12*) with some modifications. The column was maintained at room temperature and the chromatograms were monitored at a wavelength of 251 nm throughout the experiments. Data processing was handled by a Waters (Millipore) 745 integrator.

Experimental Procedure in Rats

Sprague-Dawley rats (300 ± 30 g, bw) were divided into groups (four male and four female) with fasting for about 16 hr prior to experiments but with water allowed *ad libitum*. G and LE in water solution were administered by gavage at proportional doses with G ranging from 40 to 480 mg/kg, body weight. At 1, 2, 4, 6, 8, 10, 12, 16, 24, 36 hr after the treatments, the animals were anesthetized with ethyl urethane (15% solution, ip). Blood was collected in EDTA tubes by cardiac puncture and centrifuged at 3000 rpm for 30 min. Subsequently, the plasma was separated and stored at −20°C until analysis.

The urinary excretion of G and GA was studied over 12-, 24- and 36-hr intervals after oral administraton of the maximum dosage (G: 480 mg/kg bw; LE: 6276 mg/kg bw). Each treated rat was housed separately in a clean metabolic cage for urine collection with only drinking water available. The exact volume of urine collected from each animal was measured and each urine sample centrifuged at 1500 rpm for 10 min and stored in freezer (at −20°C) until analysis.

Experimental Procedure in Humans

Eight healthy volunteers of both sexes were used in different combinations to detect G and GA concentration levels in their plasma samples. G and LE in water solution were orally administered in doses of either 800 or 1600 mg as G. Venous blood samples were collected in EDTA tubes at 1-, 2-, 4-, 6-, 8-, 12-, 24- and 36-hr intervals after treatment, centrifuged and the plasma separated and stored at −20°C until analysis.

The urine of each volunteer was collected over 36 hr after G or LE administration at 0-, 4-, 8-, 12-, 24- and 36-hr intervals.

Analytical Procedure

Samples of 1 ml for plasma and urine were used for G and GA determination. After vigorous shaking with 5 ml of methanol for 20 min, the samples were centrifuged for 30 min at 2000 rpm (*13*). For the G analysis, to the methanol used was added an internal standard PPB (2.5×10^{-7} moles/l). Then, the supernatant liquid was vacuum dried. The residue was dissolved in 400 μl of methanol and filtered through a Millipore membrane filter (0.45 μm). A 50-μl aliquot of the solution was injected for each time course into the HPLC.

The G concentration was calculated by the peak area method using an internal standard (PPB), and GA by the peak area method with external standard because there is no appropriate internal standard for GA.

Results and Discussion

Animal Study

In the rat, plasma levels of G after oral administration of G at doses of 40, 80, and 120 mg/kg bw or at the proportional dosages of LE (523, 1046, 1569 mg/kg/bw) were lower than the limit of sensivity of HPLC (0.2 μg/ml). However, as can be seen from Figure 2*a*, it was possible to determine G concentration in the plasma of the rats treated with 160 mg/kg/bw, of G while, on the contrary, G concentrations were below or near to the limit of sensitivity (0.2 μg/ml) in the samples treated with a corresponding LE dose, that contains the same amount of G as in the pure compound, of 2092 mg/kg/bw. To detect a more appreciable amount of the test compound in the plasma, both for G and LE, it was necessary to administer higher quantities of both G (480 mg/kg/bw) and LE (6276 mg/kg/bw). This is supported by earlier studies in the available literature (*12,14,15*). From these higher doses, Figure 2*b* shows that the plasma levels of G in rats treated with LE were considerably and significantly lower for each sample-time than those obtained after administration of G. This supports findings at the lower dose, Figure 2*a* although results for LE were largely undetectable. To define the different pharmacokinetics of G, Table 1 gives some parameters of the above preparation. In fact, the area under the plasma time-curve (AUC) values for G treatment were more than two times higher than the AUC LE. On the other hand, the C_{max} of G is increased 3-fold in comparison with the C_{max} obtained from animals treated with LE. The time required for a maximum concentration (T_{max}) of G was 12 hr after administration of LE, while G reaches T_{max} at 10 hr after administration of G alone. Similar results are reported in the literature from experiments in rat (*14*) and rabbit (*12*).

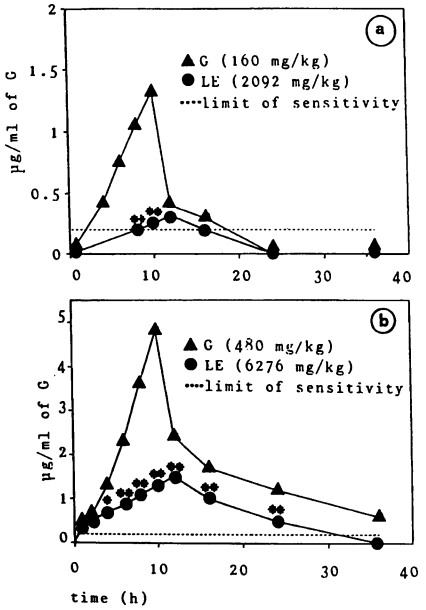

Figure 2. Plasma levels in rat of glycyrrhizin (G) after oral aministration of glycyrrhizin (G) or aqueous licorice root extract (LE). Each point represents the mean value from six to eight animals. The statistical analysis was carried out by Student's *t*-test. ** Significant level at $p < 0.01$; * Significant level at $p < 0.05$.

In urine experiments the results obtained for G and GA contents at 12-, 24-, and 36-hr intervals after oral treatment are reported in Figures 3*a* and 3*b* respectively. If compared with administered dosage, the G and GA excreted is very low reflecting the low plasma concentration. However, at all these periods of time it is clearly evident that both G and GA excretions are significantly higher in animals treated with G alone than in those treated with LE. Thus, the above data and preliminary results

Table 1. Pharmacokinetic parameters of g in plasma after oral administration of aqueous solution of LE or G in rats.[a]

Compounds	AUC36h, μg x hr/ml	C_{max}, μg/ml	T_{max}, hr
Glycyrrhizin (480 mg/kg)	53.4 ± 6.3	4.8 ± 0.5	10
Licorice (6276 mg/kg)	20.9 ± 3.2[b]	1.5 ± 0.2[b]	12

[a] Data are the mean ± SE of six to eight rats; [b] $p < 0.01$.

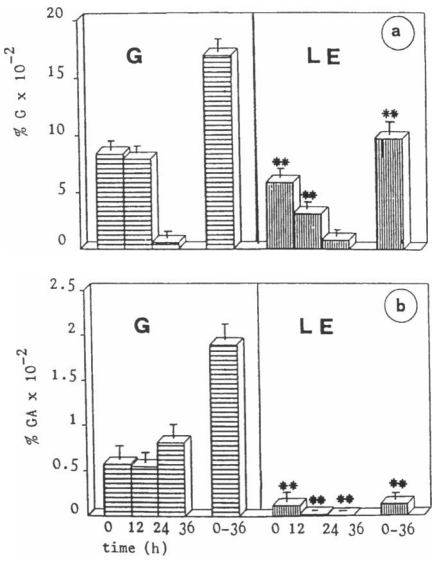

Figure 3. Urinary excretion in rat of glycyrrhizin (G) (*a*) or glycyrrhetic acid (GA) (*b*) after oral administration of glycyrrhizin (G) or aqueous licorice root extract (LE). Each bar represents the mean ± SD of the percentage of G or GA excreted at each interval period and cumulatively (0–36 hr) by six to eight animals.The statistical analysis was carried out by the Student's *t*-test. ** Significant level at $p < 0.01$;* Significant at $p < 0.05$.

from a bile excretion study (0–16 hr) in progress confirm the plasma level values which indicate that when G is a component of LE, G has a significantly reduced intestinal absorption rate than if it is administered alone.

Human Study

In experiments with human volunteers, it was not possible to detect G content in plasma samples at all considered interval times within 36 hr after single G and LE oral administration (G, 0.8 g, LE, 10.5 g). Only from analysis of one volunteer, who had been given double quantities of G (1.6 g), did we find, and then only during some of the test periods, detectable plasma levels of G; however, a week later and in the same volunteer, after LE administration (21 g) no detectable G level was found as shown in Figure 4.

After this finding, a set of experiments were performed in another volunteer group to establish their plasma levels of GA, which is the principal G metabolite derived from hydrolysis of the sugar chain. For example, Figure 5 shows the plasma pharmacokinetics of GA in two individual volunteers, one male (Figure 5*a*) and one female (Figure 5*b*), after a single oral administration of G alone (1.6 g) and then an LE dosage 1 week later (21 g). The G plasma levels in both cases are lower at all intervals (0–36 hr) in

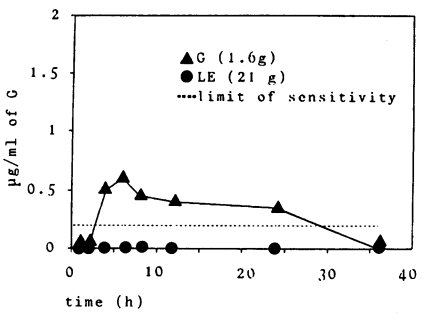

Figure 4. Plasma levels of glycyrrhizin (G) after oral administration of glycyrrhizin (G) or aqueous licorice root extract (LE) in one male volunteer.

LE administration when compared with single component (G) administration. However, the spread between male and female results, particularly up to 12 hr, is considerable in the volunteers.

Similar to the results obtained in rat experiments, the amounts of G or GA excreted were also very low in urine collected from the two above-mentioned volunteers at 4, 8, 12, 24, and 36 hr after either G or LE administration at the highest dosage. However, at all the time intervals considered, Figure 6*a* shows that the G excretion averaged a lower value with LE than with G administration. The results having been based on only two volunteers are valuable only as a trend indication, but

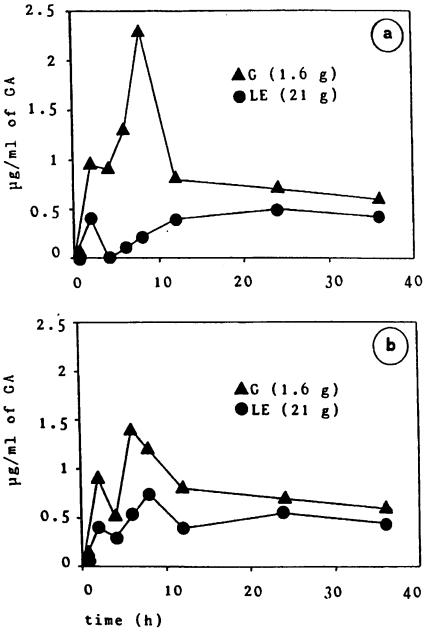

Figure 5. Plasma levels of glycyrrhetic acid (GA) after oral administration of glycyrrhizin (G) or aqueous licorice root extract (LE) in male (*a*) and female (*b*) volunteers.

do, however, confirm the results obtained on the rat experiments. In comparing G and LE treatments, GA excretion in humans showed no differences, and GA was detected at essentially similar quantities in both treatments only in urine excretion between 12 and 24 hr (Figure 6*b*). Thus, the human results are in total agreement with those obtained in the animal study. Therefore, it is possible to postulate that there is a significant reduced intestinal absorption rate of G when it is a component of LE as opposed to administration alone.

Conclusions

The excellent sensitivity of the HPLC procedure, modified especially for this experiment (*16*), assures the accurate reproducible results obtained throughout this study. The findings both in animal and human models clearly confirm a lower bioavailability of G when orally consumed as a component of LE. A reduced rate of intestinal absorption explains the lower plasma and urine levels of G during the 36 hr observation period after LE administration as compared with that obtained after G administration alone at a corresponding quantity ratio.

One can, therefore, assume that there is positive interaction between G and one or more of the several substances that are components of aqueous licorice root extract (LE).

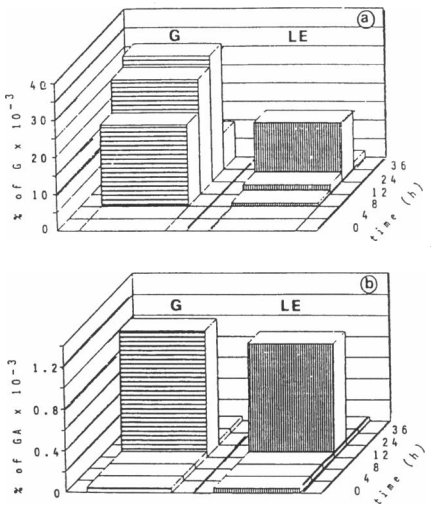

Figure 6. Urinary excretion in humans of glycyrrhizin (G) (*a*) or glycyrrhetic acid (GA) (*b*) after oral administration of glycyrrhizin (G) or aqueous licorice root extract (LE). Each bar represents the mean of the percentage of G and GA excreted, respectively, at each interval period by the same two volunteers of Figures 4,5.

It is well known that several components in food constituents can interact with drugs and produce modified intestinal absorption (*17*). In general, an altered bioavailability of a drug can result in reduced therapeutic action or can increase undesirable side effects (*17*).

The clinical implications of this phenomenon can prove highly important when the drug is consumed orally and for long periods of time. In the present situation, the interaction between G and the LE components represents an advantage since the lower intestinal absorption of G means a reduced incidence of side effects. In fact, a recently completed parallel clinical study on the chronic oral consumption of aqueous liquorice root extract (LE) in high doses for 4 week confirms our findings (*18,19*).

In conclusion, it is important to restate that this study clearly indicates that the continuous consumption of licorice root extract in daily use as food or for therapeutic purposes is safer than the use of glycyrrhizin alone or when the latter is added to man-made products (sweets, chewing gum, drinks, drugs, etc.). From the results of this paper, it is well demonstrated that LE reduces the toxicity of G probably due to component interaction before and/or during intestinal absorption, providing an increasing measure of safety.

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