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Laminaria japonica increases plasma exposure of glycyrrhetinic acid following oral administration of *Liquorice* extract in rats

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[ABSTRACT] The present study was designed to investigate the effects of *Laminaria japonica* (Laminaria) on pharmacokinetics of glycyrrhetinic acid (GA) following oral administration of Liquorice extract in rats. Following oral administrations of single-dose and multi-dose Liquorice extract and Liquorice-Laminaria extract, respectively, plasma samples were obtained at various times and the concentrations of GA, liquiritigenin, and isoliquiritigenin were measured by LC-MS. The effects of Laminaria extract on pharmacokinetics of GA were also investigated, following single-dose and multidose of glycyrrhizic acid (GL). The effects of Laminaria extract on intestinal absorption of GA and GL were studied using the *in situ* single-pass intestinal perfusion model. The metabolism of GL to GA in the contents of small and large intestines was also studied. The results showed Liquorice-Laminaria extract markedly increased the plasma concentration of GA, accompanied by a shorter T_{max}. Similar alteration was observed following multidose administration. However, pharmacokinetics of neither liquiritigenin nor isoliquiritigenin was affected by Laminaria. Similarly, Laminaria markedly increased concentration and decreased T_{max} of GA following oral GL were observed. The data from the intestinal perfusion model showed that Laminaria markedly increased GL absorption in duodenum and jejunum, but did not affect the intestinal absorption of GA. It was found that Laminaria enhanced the metabolism of GL to GA in large intestine. In conclusion, Laminaria increased plasma exposures of GA following oral administration of liquorice or GL, which partly resulted from increased intestinal absorption of GL and metabolism of GL to GA in large intestine.

[KEY WORDS] Pharmacokinetics; *Liquorice*; *Laminaria japonica*; Glycyrrhizic acid; Glycyrrhetinic acid; In situ single-pass intestinal perfusion; Intestinal contents

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Introduction

Liquorice (Glycyrrhiza), the root of *Glycyrrhiza uralensis* Fisch, is a traditional herbal medicine with many health benefits such as antiallergic, antiinflammatory, immunomodulating, antiulcerous, antidotal, gastroprotective, antioxidant, and antiviral properties [1]. In China, liquorice is one of the most widely used herbal medicines, as it appears in more than half of Traditional Chinese Medicine (TCM) prescriptions [2]. The main active components in liquorice include liquiritigenin, isoliquiritigenin, liquirtin, isoliquiritin, and glycyrrhizic acid (GL). It is well known that GL, as a prodrug, is metabolized to its active metabolite glycyrrhetinic acid (GA) via intestinal bacterial hydrolysis [3-5]. GL has been widely used in the treatment of patients with chronic hepatitis B and C [5].

In TCM, liquorice is often co-administrated with other herbs to decrease toxic effect or enhance activity of other herbs [6]. However, "Eighteen Incompatible Medicaments" in TCM states that comedication of liquorice and *Sargassum swartzii* may lead severe toxicity. A report has shown that co-administration of *Sargassum* and liquorice showed toxic effects on liver and hematology as well as kidneys in rats [7]. *Laminaria japonica*, another member of brown algae, is the most

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important economic seaweed cultured in the Pacific Ocean [8]. *Laminaria japonica* (Laminaria) has been also used as an herbal medicine in China to treat goiter, scrofula, and dropsy [9-10]. Some reports have shown that the source, chemical composition, medicine property and efficacy of *Laminaria japonica* are similar to that of *Sargassum swartzi*i [11-14], indicating that co-administration of Laminaria and liquorice may lead to increased toxicity. We have previously reported that GA aggravates Clozapin induced hepatotoxicity, which is partly via inhibiting activity and expression of CYP2C11 and CYP2C13 or inducing CYP1A2 [15].

The aims of the present study were to investigate pharmacokinetics of main ingredients of liquorice following oral administration of liquorice alone and co-administration of liquorice and Laminaria to rats and to clarify the possible mechanisms leading to alterations in their pharmacokinetics.

Materials and Methods

Herbal materials and reagents

Glycyrrhiza uralensis Fisch (Liquorice) and *Laminaria japonica* Aresch (Laminaria) were purchased from Meikang Gancao Agriculture Factory (Ningwu, Ningxia, China) and Rongcheng Haidai Agriculture Factory (Weihai, Shandong, China), respectively, which were authenticated by Prof Tang Yu-Ping, Nanjing University of Chinese Medicine, Nanjing, China. Chlorzoxazone (internal standard) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Glycyrrhetinic acid (GA) was purchased from J&K Chemical (Shanghai, China). Glycyrrhizic acid (GL) ammonium salt, liquiritigenin, isoliquiritigenin, liquirtin, isoliquiritin, and laminarin were from Aladdin Industrial Co, Ltd. (Shanghai, China). Ultrapure water was acquired from a Milli-Q system (Millipore, Milford, MA, USA). All other agents were of analytical grade and were commercially available.

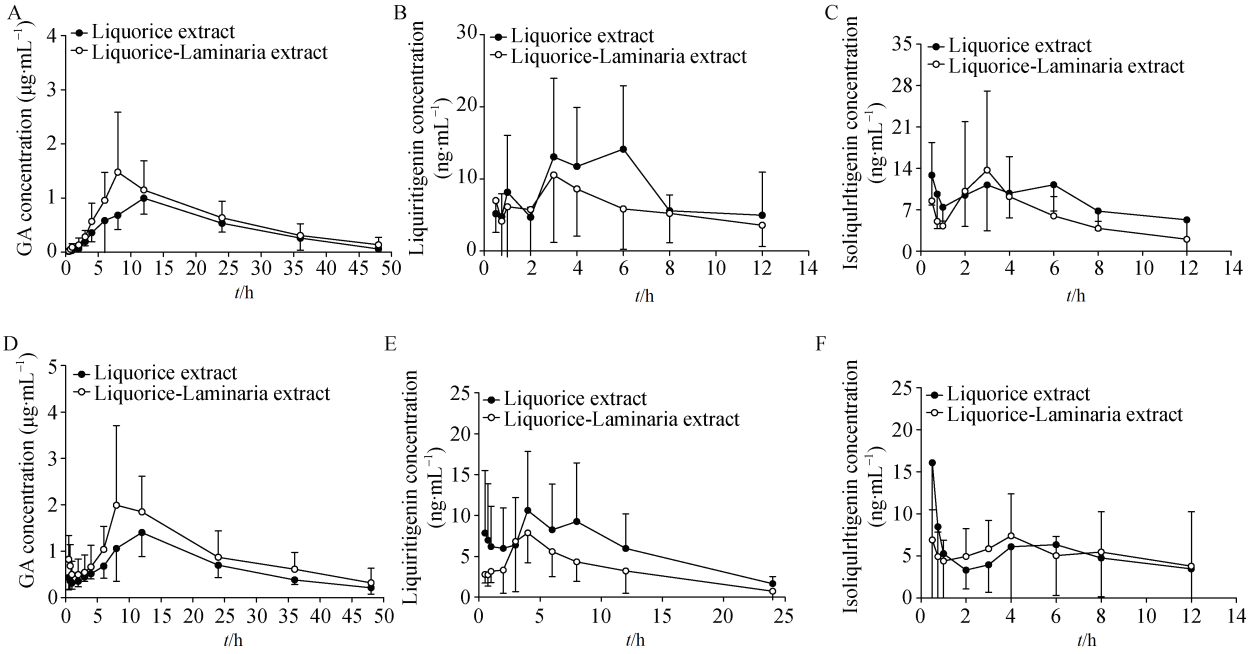

Fig. 1 Plasma concentrations of GA (A, D), liquiritigenin (B, E) and isoliquiritigenin (C, F) following oral administration of Liquorice extract (0.64 g·kg^{-1}) or Liquorice-Laminaria extract (1.2 g·kg^{-1}) to rats. Single dose: A, B, and C; Multidose: D, E, and F. The data are expressed as mean ± SD (n = 8)

Preparation of Liquorice, Laminaria, and their combined extracts

The three preparations were made in similar procedure. To prepare the Liquorice extract, 3 kg liquorice was extracted twice with boiling water (1 : 10) for 1 h. The decoction was concentrated to dryness on a rotary vacuum evaporator, affording 193 g powder (yield: 6.43%). For preparation of Liquorice-Laminaria extract, Liquorice and Laminaria, in a ratio of 1 : 1, were mixed to reach a final weight of 2 kg. 121 g powder (yield: 6.05%) was finally achieved. For Laminaria extract, 3.7 kg condensed extract (yield: 37%) was obtained from 10 kg Laminaria. All extracts were stored at 4 ºC and suspended in 0.25% carboxymethyl cellulose sodium salt (CMC-Na) before oral administration.

The contents of five active ingredients in Liquorice extract and Liquorice-Laminaria extract were quantitatively analyzed by HPLC-UV as described previously [16]. The contents (*W*/*W*) of GL, liquirtin, liquiritigenin, isoliquiritigenin and isoliquiritin in Liquorice extract were measured to be 12.08%, 2.23%, 1.12%, 0.32%, and 0.11%, respectively. The contents (*W*/*W*) of GL, liquirtin, liquiritigenin, isoliquiritigenin and isoliquiritin in Liquorice-Laminaria extract were

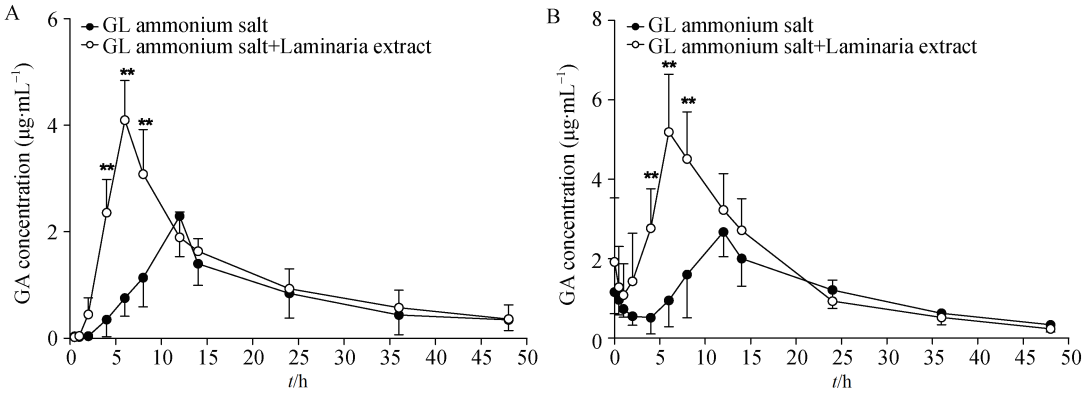

Fig. 2 Plasma concentrations of GA following oral administration of GL ammonium salt (77 mg·kg^{-1}) alone or co-administrated with Laminaria extract (3.7 g·kg^{-1}) for single dose (A) and multidose (B). The data were expressed as mean ± SD (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* GL ammonium salt alone

measured to be 6.88%, 0.81%, 0.4%, 0.12%, and 0.05%, respectively. No GA was detected in the two preparations.

Animals

Adult female and male Sprague-Dawley (SD) rats, weighing 180–220 g, from Sino-British Sippr/BK Laboratory Animal Ltd. (Shanghai, China) were housed under controlled environmental conditions with a temperature of (23 ± 1) ºC, a humidity level of 55% ± 10%, and a 12-h/12-h light/dark cycle. The rats were allowed free access to food and water. The animals were acclimatized to the facilities for five days, and fasted for 12 h before all experiments. All the experiments were carried out in accordance with guidelines on the Care and Use of Animals developed by the National Advisory Committee for Laboratory Animal Research. The number of rats was kept as low as possible and the suffering of animals was minimized. All animals received humane care. And all animal use and care and experimental protocols were reviewed and approved by the Animal Ethics Committee of China Pharmaceutical University, Shenyang, China (No. CPU-PCPK- S1110332).

Pharmacokinetics of GA, liquiritigenin, and isoliquiritigenin following oral administration of Liquorice extract and Liquorice-Laminaria extract in rats

Sixteen SD rats were randomly divided into two groups of four male and four female in each, and orally administered Liquorice extract (0.64 g·kg^{-1}) or Liquorice-Laminaria extract (1.2 g·kg^{-1}) on Day 1 for the single-dose study. Blood samples (0.25 mL) were collected under light ether anesthesia via the oculi chorioideae vein at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h post- dosing. Plasma samples were obtained by centrifugation of blood samples at 8 000 rpm for 5 min and stored at −80 ºC until analysis. On Day 3, the same rats were continuously administered their original daily dosages for another 7 days. Blood samples were taken on Day 9 as described above for single-dose study.

Effects of Laminaria extract on pharmacokinetics of GA following oral administration of GL in rats

Twelve male SD rats were randomly divided into two groups of six in each, and orally administered GL ammonium salt (77 mg·kg^{-1}) alone and in combination with Laminaria extract (3.7 g·kg^{-1}) on Day 1. Blood samples (0.25 mL) were collected under light ether anesthesia via the oculi chorioideae vein at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h post dosing. Plasma samples were prepared as above and stored at −80 ºC until analysis. On Day 3, the same rats were continuously administered their original daily dosages for another 7 days. Blood samples were obtained on Day 9 as described above.

Intestinal absorption of GL and GA

In situ single-pass intestinal perfusion model was used to investigate the intestinal absorption of GA and GL according to the methods described previously [17]. Briefly, male SD rats, fasted overnight, were anesthetized by intraperitoneal injection of 45 mg·kg^{-1} of pentobarbital sodium salt (dissolved in 0.9% saline solution). The abdomen was opened through a middle incision. Duodenum and jejunum were isolated between two cannulas by 10 cm, which were fixed by ligation. The manipulation was practiced carefully to minimize any intestinal blood supply disturbances. The isolated intestinal segments were pre-perfused with 0.9% saline at a rate of 0.2 mL·min^{-1} (37 ºC) for 10 min to reach a steady state for water and solute absorption, and then Krebs-Henseleit (K-H) buffer containing the experimental agents (GA, GA + Laminaria extract, GL and GL + Laminaria extract) was replaced. Inlet concentrations (C_{in}) of GA and GL ammonium salt perfusion buffer were kept to be 20 μg·mL^{-1} and concentration of Laminaria extract was set to be 1 mg·mL^{-1}. After reaching the steady-state (10 min), consecutive effluent (at 15 min intervals) was collected via the distal cannula for 120 min. At the end of the experiments, the animals were sacrificed and the areas of perfused intestinal segments (area) were measured. The cumulative fraction of absorption was estimated and the effective permeability across intestine (P_{eff}) was calculated using the following equation:

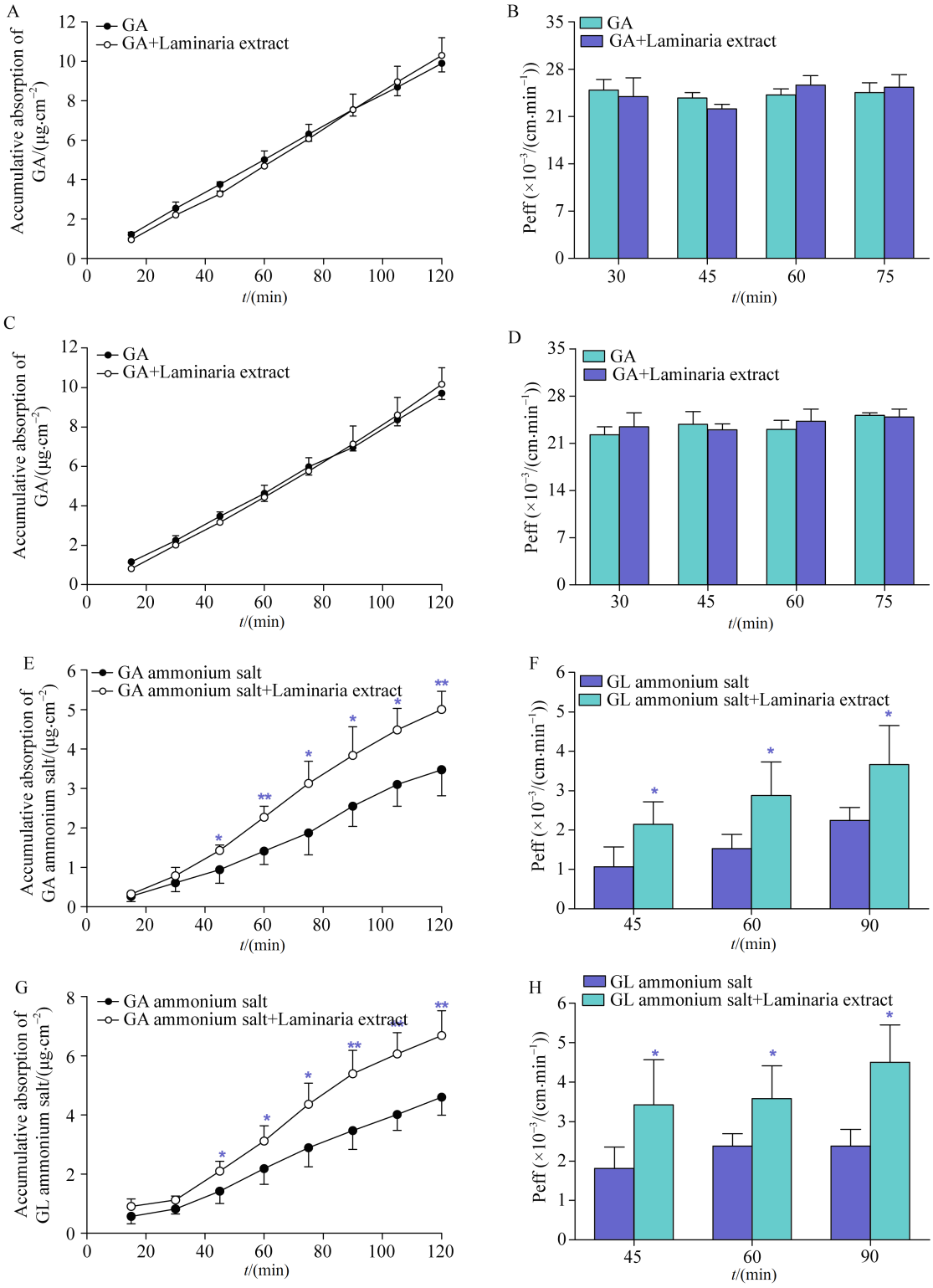

Fig. 3 Effects of Laminaria extract on absorption of GA (A-D) and GL ammonium salt (E-H) in duodenum and jejunum. Inlet concentrations of GA and GL ammonium salt in perfusion buffer were kept to be 20 μg·mL^{-1}, and concentration of Laminaria extract was set to be 1 mg·mL^{-1}. Accumulative absorption of duodenum (A, E), jejunum (C, G) and P_{eff} values of duodenum (B, F), jejunum (D, H) of indicated compounds. The data are expressed as mean ± SD (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* GL ammonium salt alone

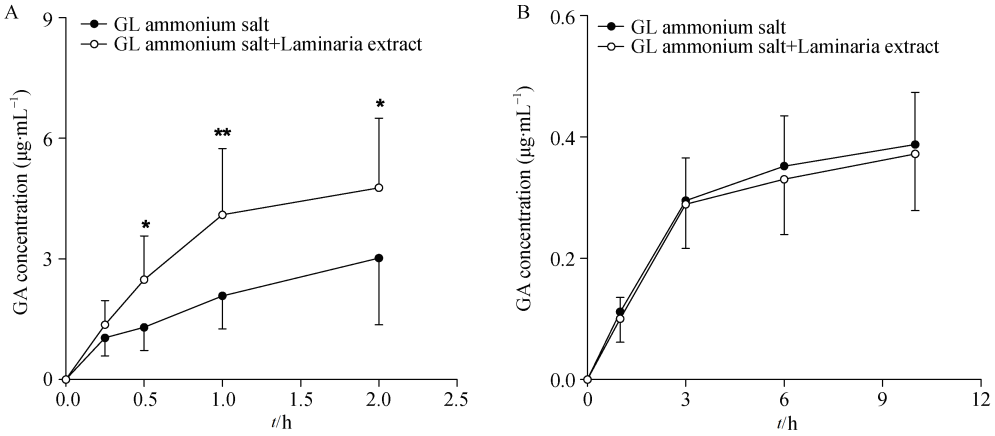

Fig. 4 Effects of Laminaria extract on GL metabolism into GA in large intestine content (A) and small intestine content (B). Initial concentration of GL ammonium salt and Laminaria extract were set to be 20 μg·mL^{-1} and 1 mg·mL^{-1}, respectively. The data are expressed as mean ± SD (n = 4). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* GL ammonium salt alone

$$P_{eff} = -Q\ln(C_{out}/C_{in})/area$$

Where C_{in} and C_{out} are the inlet concentration and the outlet concentration of compounds, respectively, which are corrected by phenol red concentration, and Q is the flow rate (0.2 mL·min^{-1}).

Metabolism of GL in intestine contents

Male rats were sacrificed by decapitation under ether anesthesia, and the gastrointestinal tract including small and large intestines was removed. The contents of large intestine were collected and then homogenized in 6-fold ice-cold phosphate buffered saline (PBS, pH 7.0) while the small intestine was homogenized in 3-fold PBS according to our previous studies. The homogenates were centrifuged at 3 000 rpm for 10 min at 4 ºC. The supernatants were separated to obtain fresh cultural solution of the content of large and small intestine. The contents of the large and small intestine were processed under an anaerobic environment [18-21]. A 1.9-mL aliquot of pre-incubated fresh cultural solution of the content of large and small intestine mixed with 0.1 mL of buffer solution containing tested agents was incubated in a shaking water-bath at 37 ºC. Final concentrations of GL ammonium salt and Laminaria extract were set to be 20 μg·mL^{-1} and 1 mg·mL^{-1}, respectively. For the large intestine, a 200-μL aliquot of the mixture was removed at 0, 0.25, 0.5, 1, and 2 h after incubation, while the sampling times were 0, 1, 3, 6, and 10 h for the small intestine.

Cellular uptake and metabolism of GL in caco-2 cells

Caco-2 cells obtained from Chinese Academy of Medical Sciences were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NEW YORK, USA) with 10% fetal bovine serum (Gibco) and 1% nonessential amino acids (Gibco) in a humidified incubator with 5% CO_2 and 95% air atmosphere at 37 ºC. Caco-2 cells were seeded into 24-well plates at a density of 3×10^4 cells/cm^2. The experiments were conducted to examine the concentration- and time-dependent elements of GL. The concentration-dependent study was conducted with a 90-min incubation period in presence of different concentrations of GL (5, 10, 25, 50, 100, and 200 μmol·L^{-1}). The time-dependent experiment was performed with 200 μmol·L^{-1} of GL for various incubation times (2, 5, 10, 30, 60, and 90 min). In brief, afer 7 days of culture, the culture medium was removed, and 0.5 mL of Hank's Balanced Salt Solution (HBSS) containing GL was added to each well. At the end of designated incubation period, the solution was removed to analysis the conversion of GL to GA. 1 mL of ice-cold HBSS was added to terminate the assay, and the cells were then washed 3 times with 1 mL of ice cold HBSS. Then 0.4 mL of purified water was added into each well, and the cells were frozen and thawed three times. The uptake of GL by the Caco-2 cells was measured using the HPLC-UV method, and GA was measured by LC-MS. Protein concentrations were measured using a BCA protein assay kit (Beyotime Institute of biotechnology, Jiangsu, China). All experiments were conducted in triplicate. Effects of laminarin, a main active compound in Laminaria, at different levels (10, 100, and 400 μmol·L^{-1}) on the uptake of GL (200 μmol·L^{-1}) by Caco-2 cells and GL metabolism were also investigated in incubation periods of 2 and 60 min.

Drug analysis

The oncentrations of GA, liquiritigenin, and isoliquiritigenin were determined using a Shimadzu LCMS-2020 system (Shimadzu, Japan). Chlorzoxazone in a volume of 10 μL (1 μg·mL^{-1}, internal standard) and 1 mL of ethyl acetate were added to each of the 100 μL of plasma and cell samples or 200 μL intestine content samples. The mixture was shaken for 10 min and centrifuged at 10 000 *g* for 5 min. The organic layer was transferred and evaporated to dryness in a vacuum evaporator (Thermo, Waltham, MA, USA). The residue was reconstituted in 100 μL of methanol. An aliquot of 5 μL was injected into the LC-MS system after centrifugation at 14 000 *g* for 10 min. The eparation was performed at 40 ºC on a Symmetry C_{18} column (5.0 μm, 2.1 mm × 150 mm, Waters, Milford, Massachusetts, USA). The mobile phase was composed of methanol-1mmol·L^{-1}

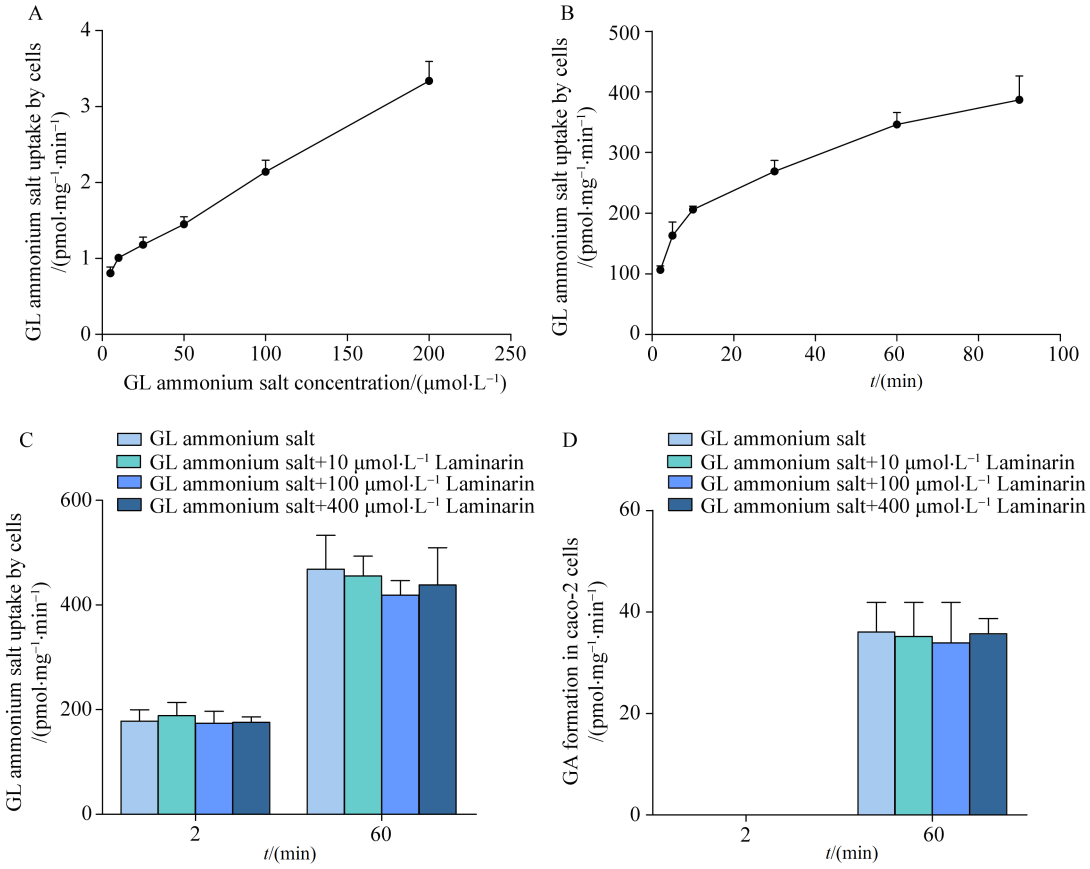

Fig. 5 Concentration-dependent uptake of GL by Caco-2 cells (A). The cells were incubated in HBSS containing different concentrations of GL ammonium salt at 37 ºC for 90 min. Time-dependent GL uptake by Caco-2 cells (B). The cells were incubated in HBSS containing 200 μmol·L^{-1} GL ammonium salt at 37 ºC for different times. Effects of Laminarin on GL uptake (C) and GL metabolism (D) in Caco-2 cells. The cells were incubated with GL ammonium salt (200 μmol·L^{-1}) and Laminarin (10, 100 and 400 μmol·L^{-1}) for designated times. The data are expressed as mean ± SD (n = 3)

of ammonium formate at a flow rate of 0.2 mL·min^{-1}. The gradient cycle was consisted of an initial 40% methanol, increased to 60% within 1 min, and maintained from 1 to 4.5 min, then increased to 85% within 2 min，and maintained from 6.5 to 8.5 min, then returned to 40% at 13 min, and maintained up to 14 min for column equilibration. The mass spectrometer was operated in the negative electrospray ionization mode using selective ion monitoring data acquisition: GA [M – H]$^-$ *m/z* 469.25, Chlorzoxazone [M – H]$^-$ *m/z* 167.9, liquiritigenin [M – H]$^-$ *m/z* 254.95, and isoliquiritigenin [M – H]$^-$ *m/z* 254.95. Mass spectrometric conditions were optimized as follows: heat block: 350 ºC; DL temperature: 270 ºC; drying gas: 10.0 L·min^{-1}; nebulizing: 1.5 L·min^{-1}; and interface voltage: −4.5 kV. The recoveries were greater than 70%. The relative standard deviations of intra-day and inter-day assays were less than 15%. The linear range of GA, liquiritigenin, and isoliquiritigenin were 19.5–5 000 ng·mL^{-1}, 1.17–300 ng·mL^{-1} and 0.625–160 ng·mL^{-1}, respectively. The lower limits of quantification (LLOQ) of GA, liquiritigenin, and isoliquiritigenin were 19.5, 1.17 and 0.625 ng·mL^{-1}, respectively the results indicated that no obvious matrix effect was observed. The representative LC-MS chromatograms are shown in Fig. 6.

The concentrations of GL and GA in perfusate were determined using a Shimadzu HPLC system consisting of an LC-10AD pump (Shimadzu Ltd., Kyoto, Japan), a model SPD-10A UV absorbance detector (Shimadzu) set at 254 nm, and a Waters Symmetry C_{18} column (5.0 mm, 150 mm × 4.6 mm, Waters, USA). The mobile phase was consisted of acetonitrile-10m mol·L^{-1} ammonium acetate and 0.5% acetic acid (38/62 *V/V* in GL ammonium salt and 80/20 *V/V* in GA) at a flow rate of 1 mL·min^{-1}. 20 μL of the perfusate samples was injected onto the HPLC system after centrifugation at 14 000 *g* for 10 min twice as described previously [22].

400 μL of methanol were added to each 200 μL of cell lysate samples for the analysis of GL ammonium salt. The mixture was shaken for 10 min and centrifuged at 10 000 *g* for 5 min. The organic layer was transferred and evaporated to dryness in a vacuum evaporator. The residue was reconstituted in 100 μL of mobile phase and 20 μL was injected onto the HPLC system (same as perfusate samples) after centrifugation at 14 000 *g* for 10 min. The linear range of GL ammonium salt was 0.156–2.5 μg·mL^{-1}.

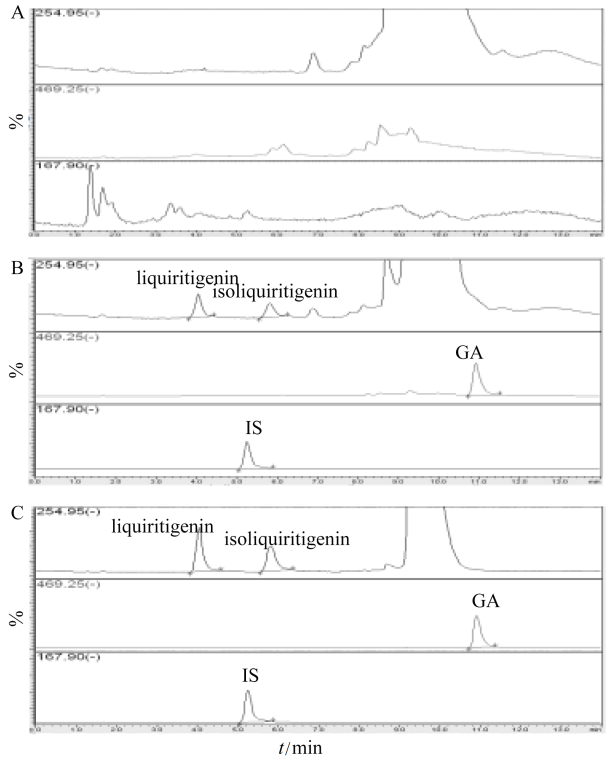

Fig. 6 LC-MS chromatograms of various samples : (A) blank rat plasma; (B) rat plasma spiked with standards of GA, liquiritigenin, and isoliquiritigenin; (C) rat plasma sample after oral administration of Liquorice extract. IS: internal standard

Statistical analyses

All results were expressed as the mean ± standard deviation (SD). Noncompartmental analysis was employed to estimate the pharmacokinetic parameters with Phenix WinNonlin 6.3 (Pharsight, St. Louis, MO, USA). An independent-sample t-test (SPSS 13.0) was used to compare the results under two conditions. $P < 0.05$ was regarded statistically significant.

Results

Pharmacokinetics of GA, liquiritigenin, and isoliquiritigenin after oral administration of Liquorice extract and Liquorice-Laminaria extract

The plasma concentrations of GA, liquiritigenin and isoliquiritigenin were simultaneously measured (Fig. 1) by HPLC-MS following single and multidose administrations of Liquorice extract and Liquorice-Laminaria extract in rats. The corresponding pharmacokinetic parameters were estimated (Table 1). The results showed that Liquorice-Laminaria extract markedly increased the plasma concentrations of GA (Fig. 1), leading to significant increases in AUC and C_{max} by 41% and 61% compared to that of liquorice extract administration alone. Significant increase in plasma exposures of GA were observed in rats treated with Liquorice-Laminaria extract following multidose administration, whose extents of increase in AUC_{0-48h} (by 1.52-fold) and C_{max} (by 2.05-fold) of GA were significantly greater than that following the single dose regimen. Co-administration of Laminaria also shortened the time to peak concentration of GA (T_{max}), although no statistical significance was observed.

Table 1 Pharmacokinetic parameters of GA, liquiritigenin, and isoliquiritigenin after oral administration of Liquorice extract (0.64 g·kg^{-1}) and Liquorice-Laminaria extract (1.2 g·kg^{-1}) with single dose and multidose regimens (mean ± SD, n = 8)

	PK Parameters	Liquorice extract	Liquorice-Laminaria extract
Single dose			
GA	$T_{1/2}$ /h	8.72 ± 2.42	11.09 ± 6.64
	T_{max} /h	10.75 ± 2.38	8.50 ± 2.33
	C_{max} /(μg·mL^{-1})	1.13 ± 0.38	1.82 ± 0.84*
	AUC_{0-48h} /(μg·h·mL^{-1})	21.17 ± 6.27	29.93 ± 10.14*
liquiritigenin	T_{max} /h	3.0 ± 2.1	2.9 ± 1.0
	C_{max} /(ng·mL^{-1})	17.0 ± 9.6	11.8 ± 8.5
	AUC_{0-48h} /(ng·h·mL^{-1})	128.5 ± 81.6	78.2 ± 67. 8
isoliquiritigenin	T_{max} /h	1.3 ± 1.4	2.9 ± 2.2
	C_{max} /(ng·mL^{-1})	18.4 ± 6.7	15.4 ± 16.3
	AUC_{0-48h} /(ng·h·mL^{-1})	132.8 ± 61.8	84.9 ± 57.4
Multidose			
GA	$T_{1/2}$ /h	15.04 ± 5.57	18.59 ± 14.12
	T_{max} /h	10.50 ± 2.07	8.75 ± 2.12
	C_{max} /(μg·mL^{-1})	1.30 ± 0.50	2.66 ± 1.23**
	AUC_{0-48h} /(μg·h·mL^{-1})	28.97 ± 7.97	44.00 ± 16.64*
liquiritigenin	T_{max} /h	3.5 ± 4.0	2.9 ± 1.4
	C_{max} /(ng·mL^{-1})	18.8 ± 9.7	11.8 ± 7.0
	AUC_{0-48h} /(ng·h·mL^{-1})	151.2 ± 106.6	66.27 ± 39.7
isoliquiritigenin	T_{max} /h	1.6 ± 1.7	2.5 ± 4.1
	C_{max} /(ng·mL^{-1})	17.7 ± 35.8	12.8 ± 5.3
	AUC_{0-48h} /(ng·h·mL^{-1})	83.5 ± 98.8	86.0 ± 72.7

*$P < 0.05$, **$P < 0.01$ *vs* Liquorice extract alone

The results showed that the concentrations of liquiritigenin and isoliquiritigenin were very low, accompanied by large individual differences. It was also found that Laminaria extract affected to less extent on pharmacokinetics of liquiritigenin and isoliquiritigenin.

Effects of Laminaria extract on the pharmacokinetics of GA following oral administration of GL ammonium salt

The plasma concentrations of GA following single dose and multidose administrations of GL ammonium salt alone or in combination with Laminaria extract were measured (Fig. 2) and the corresponding pharmacokinetic parameters were estimated (Table 2). It was consistent with the findings in rats treated with Liquorice-Laminaria extract, suggesting that co-administration of Laminaria extract markedly increased plasma exposure of GA, increasing $AUC_{0–48h}$ and C_{max} by 48% and 55% of GL alone, respectively. It was also found that T_{max} of GA was significantly shortened. Similar alteration was also found following the multidose regimen.

Table 2 Pharmacokinetic parameters of GA after oral administration of GL ammonium salt alone (77 mg·kg^{-1}) or co-administrated in combination with Laminaria extract (3.7 g·kg^{-1}) with single dose and multidose regimens (mean ± SD, n = 6)

	PK Parameters	GL alone	GL+ Laminaria extract
Single dose	$T_{1/2}$ /h	16.46 ± 8.42	19.18 ± 15.03
	T_{max} /h	12.33 ± 0.82	7.33 ± 2.42**
	C_{max} /(μg·mL^{-1})	2.64 ± 0.76	4.1 ± 1.08*
	AUC_{0-48h} /(μg·h·mL^{-1})	37.86 ± 13.14	56.16 ± 12.35*
Multidose	$T_{1/2}$ /h	12.66 ± 4.68	10.65 ± 2.58
	T_{max} /h	12 ± 2.19	7.33 ± 2.42**
	C_{max} /(μg·mL^{-1})	2.97 ± 0.78	5.34 ± 1.84*
	AUC_{0-48h} /(μg·h·mL^{-1})	52.81 ± 15.14	75.77 ± 15.67*

*P < 0.05, **P < 0.01 *vs* GL alone

Effects of Laminaria extract on intestinal absorption GA and GL

In situ single-pass intestinal perfusion model was used to investigate absorption of GA and GL in duodenum and jejunum (Fig. 3). The results showed that both GA and GL ammonium salt were absorbed via duodenum and jejunum. The absorption of GA was greater than that of GL ammonium salt, as evidenced by higher P_{eff} values. Addition of Laminaria extract had little effect on the intestinal absorption of GA, but significantly increased the absorption of GL in both duodenum and jejunum.

Metabolism of GL into GA in intestinal contents

It is generally accepted that GL is converted to GA by intestinal bacteria [23]. Effects of Laminaria extract on GL metabolism into GA was studied in both small intestine and large intestine contents. The results showed that GL could be converted to GA both in the content of small and large intestine (Fig. 4) and a higher formation of GA was observed in large intestine, indicating that the conversion rate in large intestine was markedly higher than that in small intestine. Addition of Laminaria extract markedly enhanced the GL metabolism in the large intestine, but had no effect on that in small intestine.

Effects of laminarin on uptake and metabolism of GL in Caco-2 cells

The uptake of GL ammonium salt by Caco-2 cells over time at a concentration of 200 μmol·L^{-1} at 37 ºC was measured (Fig. 5). It was found that the accumulation of GLwas time-dependent. The uptake at 2 and 60 min was used for evaluating the GL transport and effects of laminarin on the transport of GL. The result demonstrated the uptake of GL by Caco-2 cells was in a concentration-dependent manner. GA was also detected in incubation solution, verifying GL metabolism in Caco-2 cells. Effects of laminarin, a polysaccharide in Laminaria, on both GL uptake and metabolism in Caco-2 cells were further investigated. Surprisingly, laminarin did not affect GL uptake or GL metabolism in Caco-2 cells.

Discussion

Laminaria, a widely used TCM herb, is also a popular dietary supplement and traditional marine foodstuff in Korea, Japan and China. Recent reports have shown that Laminaria exhibits various properties beneficial to health, including antimicrobial, antioxidant, antidiabetic, and hypolipidemic effects [24-26]. Liquorice is also a widely used herb, and liquorice and Laminaria are co-administrated in Haizao Yuhu Decoction for treating thyroid tumors and breast hyperplasia [27]. “Eighteen Incompatible Medicaments” in TCM warns that liquorice should not be co-administrated with *Sargassum swartzii*, otherwise severe toxicity occurs. Laminaria, in its source, chemical composition, medicine property and efficacy, is similar to *Sargassum swartzii*, inferring that co-administration of Laminaria and liquorice may induce toxicity. The aim of the present study was to investigate the effects of Laminaria on pharmacokinetics of main ingredients in liquorice. The main findings were that Liquorice-Laminaria extract markedly increased plasma exposure of GA following oral administration of liquorice extract. The dosage of GL was corrected by contents of GL in the two preparations,

indicating that the increase was not due to differences in GL dose tested. This finding was further verified by the fact that Laminaria extract markedly increased plasma exposure of GA following oral administration of GL ammonium salt.

GL is considered to be the main ingredient in liquorice [28] and is metabolized to GA via intestinal microflora [29]. In the present study, we found that the absorption of GL in both duodenum and jejunum was markedly enhanced by Laminaria extract, which was different from the results with GA. GL was metabolized to GA both in small intestine and large intestine, but the conversion rate in large intestine was significantly higher than that in the small intestine. Additionally, the GL metabolism to GA in large intestine was enhanced by the Laminaria extract. According to the intestinal enzyme preparation [30-32], it was mainly the enzymes in the supernatants leading to the GA generation, indicating that *Laminaria japonica* increased GL metabolism to GA in large intestine via affecting enzyme activities. However, the effects of microbiota were excluded. Further studies are needed to clarify the underlying mechanism(s).

The results that GL was transformed to GA in Caco-2 cells and Laminaria enhanced the intestinal absorption of GL could partly elucidate the mechanisms for the decreased *Tmax* of GA. In the present study, laminarin, a main polysaccharide in *Laminaria japonica* [26], was used to identify ingredient of Laminaria affecting GL absorption and metabolism. Our result was negative，indicating that laminarin was not the ingredient of *Laminaria japonica* affecting the absorption of GL. Further studies are needed to clarify which ingredients in Laminaria lead to the increase in plasma exposure of GA.

In conclusion, Laminaria extract increased plasma exposure of GA following oral administration of Liquorice extract or GL via enhancing GL absorption and GL metabolism to GA.

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