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ORIGINAL ARTICLE

Pharmacokinetic study on the effect of ligustrazine–tangeretin co-administration on the pharmacokinetics of ligustrazine and its potential mechanism in rats

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Abstract

Both ligustrazine and tangeretin are usually prescribed in the treatment of cardiovascular diseases, which makes their co-administration possible. The investigation of the interaction between ligustrazine and tangeretin is necessary for the clinical compatibility of their source herbs. This study aimed to investigate the interaction of ligustrazine and tangeretin during their co-administration. The pharmacokinetics of ligustrazine (15 mg/kg) was investigated in the presence of 50, 100, and 150 mg/kg tangeretin in rats with six of each. A single dose of ligustrazine was set as the control. The effect of tangeretin on the in vitro metabolic stability of ligustrazine was also investigated in rat liver microsomes. Tangeretin significantly reduced the system exposure of ligustrazine under all experimental concentrations. Specifically, tangeretin reduced the *AUC* (from 48.86 ± 12.57 to 41.02 ± 4.85 (50 mg/kg tangeretin), 31.47 ± 5.26 (100 mg/kg tangeretin), and 27.55 ± 9.60 (150 mg/kg) μg/mL × h), *MRT* (from 7.05 ± 0.26 to 6.33 ± 0.48, 5.53 ± 0.68, and 5.21 ± 1.31 h), C_{max} (from 7.45 ± 0.44 to 6.03 ± 0.44, 5.24 ± 0.47, and 5.02 ± 0.56 μg/mL), and $t_{1/2}$ (from 5.90 ± 1.27 to 4.84 ± 1.19, 3.48 ± 1.33, 3.09 ± 0.62 h) in rats. In vitro, tangeretin also reduced the metabolic stability of ligustrazine behaved as the decreased half-life and increased intrinsic clearance rate. Co-consumption of ligustrazine with tangeretin induced interactions, which shortens the system exposure of ligustrazine. This study provides theoretical guidance for the clinical prescription of ligustrazine- and tangeretin-containing herbs.

KEYWORDS
CYP3A4, herb–herb interaction, liver microsomes, metabolic stability, pharmacokinetics

Abbreviations: AUC, area under the curve; MRT, mean retention time; Clz/F, clearance rate; Cmax, maximum concentration; $t_{1/2}$, half-life; T_{max}, time reaching the maximum concentration; CYP3A4, cytochromeP 3A4 enzyme; LC-MS/MS, liquid chromatography tandem mass spectrometry.

Dandan Liu and Yunjiao Liu contributed equally to this work.

1 | INTRODUCTION

The clinical application of Chinese traditional medicine has been confirmed in the therapy of cardiovascular diseases, which has achieved a better therapeutic effect. According to patients' disease conditions and pathogens, the prescription usually contains more than two different herbs. The pharmacological activities of ligustrazine have been widely reported, such as anti-inflammation, antioxidation, and antitumor.[1–5] The protective effect of ligustrazine on cardiovascular disorders has also been demonstrated in previous studies. For instance, ligustrazine dramatically attenuated myocardial injury in coronary microembolization rat models via activating the PI3K/Akt signaling pathway.[6] The regulatory effect of ligustrazine on the PI3K/Akt signaling pathway was also revealed to help it exert a neuroprotective effect on cerebral ischemia–reperfusion injury rats.[7] Former research reported that the co-administration of ligustrazine with Radix *Angelicae dahuricae* significantly changed the pharmacokinetic profile of ligustrazine and influenced its therapeutic effect on migraine.[8] Therefore, special attention should be paid to the interaction of ligustrazine with potential combined drugs or compounds providing a reference for the clinical prescription of ligustrazine-containing herbs.

Tangeretin is a common flavone and is rich in the peel of tangerines and citrus species. The medical value of tangerine peel has been widely known, such as antioxidation, anticancer, anti-inflammation, and antibacterial.[9–12] The pharmacological effects of tangeretin were disclosed in cardiovascular-correlated diseases. The antihypertensive effect of tangeretin was found to alleviate the dysfunction and remodeling of the ventricle.[13] The protective effect of tangeretin was illustrated on the brain microvascular endothelial cells from the injury of oxygen–glucose deprivation.[14] Tangeretin could alleviate the myocardial dysfunction induced by sepsis and showed a significant neuroprotective effect in cerebral ischemia–reperfusion injury.[15,16] It can be seen from the previous studies that ligustrazine and tangeretin possessed similar indications and therapeutic effects in cardiovascular diseases, which makes them easily co-exist in one prescription and increase the risk of adverse interaction.

Tangeretin was previously demonstrated to induce the activity of CYP3A4, a major isoenzyme of CYP450 and responsible for the metabolism of ligustrazine.[17,18] Therefore, it was speculated that the co-administration of tangeretin with ligustrazine might induce changes in the pharmacokinetics of ligustrazine via inducing CYP3A4, which was investigated in this study.

2 | MATERIALS AND METHODS

2.1 | Experimental design

Adult male Sprague–Dawley rats were obtained from the Harlan and maintained in a cleaning environment on 12 h light/dark cycle at 25 ± 2°C with free access to drinking and food for a week. Before the experiments, the rats fasted overnight and were free to water. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Shanghai Baoshan Luodian Hospital.

The rats were divided into four groups with six of each: (a) treated with a single dose (15 mg/kg body weight) of ligustrazine (>98%, Figure S1, MUST Bio-technology Co. LTD, China); (b) pretreated with 50 mg/kg/d bodyweight tangeretin (>98%, Figure S1, MUST Bio-technology Co. LTD) for a week followed by the administration of 15 mg/kg ligustrazine; (c) pretreated with 100 mg/kg/d bodyweight tangeretin for a week followed by the administration of 15 mg/kg ligustrazine; (d) pretreated with 150 mg/kg/d bodyweight tangeretin for a week followed by the administration of 15 mg/kg ligustrazine. Both the dosage of ligustrazine and tangeretin were selected according to previous reports[8,18,19] and were administered orally by gavage.

2.2 | Pharmacokinetic study

After 5, 15, 30 min, 1, 2, 3, 4, 6, 8, 12, and 24 h of drug administration, 250 μL of blood samples were collected into a heparinized tube. The collected samples were centrifuged at 1445 g for 10 min to isolate the plasma sample. The obtained plasma samples were mixed with methanol and internal standard methanol (1:9, v/v, Fisher Scientific) and vortexed for 1 min. After centrifugation at 16992 g for 10 min, the supernatant was removed into an injection vial and injected for the LC–MS/MS analysis according to the following steps.

2.3 | LC–MS/MS conditions

The Agilent 1290 series liquid chromatography system was employed according to previous studies. The Waters Xbridge C18 column with a diameter of 3.0 μm was used to separate the samples with an isocratic mobile phase (0.1% formic acid, acetonitrile = 65:35, v/v, chromatographic purity, Fisher Scientific) for elution. The column temperature was 25°C and the flowing rate was 0.4 mL/min.

The Agilent 6460 triple-quadrupole mass spectrometer connected to the liquid chromatography system was applied with the MRM mode. The parent/daughter ion pair of ligustrazine was m/z 137.28 → 55.3. The MS/MS conditions were set as: 110 V fragmentor; 3.5 kV capillary voltage; 500 V nozzle voltage; N_2 as the drying gas with a flow rate of 10 L/min and temperature of 350°C. The sheath gas with the flow rate of 11 L/min and the temperature of 400°C. The data were obtained with the Agilent MassHunter B.07 software and analyzed by the Agilent Quantitative analysis software.

2.4 | Metabolic stability evaluation in rat liver microsomes

Rat liver microsomes (20 mg/mL) were obtained from the BD Bioscience and preincubated with tangeretin (0.1 μM) at 37°C for 30 min followed by the addition of ligustrazine (100 μM). There were

triplicate repeats of each treatment. The incubation was conducted for 0, 1, 3, 5, 15, 30, and 60 min, then, 30 μL mixture was collected and prepared as above for LC–MS/MS analysis. The metabolic stability was evaluated by corresponding parameters including half-life ($t_{1/2}$) and intrinsic clearance according to the following equations:

$$t_{1/2} = 0.693 / k;$$

$$V\ (\mu L / mg) = \text{incubation volume} / \text{protein concentration};$$

$$\text{Intrinsic clearance}\ (\mu L / \text{min} / mg) = V \times 0.693 / t_{1/2}.$$

2.5 | Induction assay of CYP3A4

LS180 cells, a human colon adenocarcinoma cell line, was used to evaluate the induction of CYP3A4 by tangeretin according to a previous study.[17,20] Cells (5×10^4) were incubated with 2 and 10 μM tangeretin for 4 days. Then, RNA was extracted from the cultured cells and the expression of CYP3A4 mRNA was evaluated by real-time PCR as previously reported.[17,20] Three independent treatments were set, and the measurement was repeated triplicate.

2.6 | Statistical analysis

The pharmacokinetic data were analyzed with DAS 3.0 pharmacokinetic software to obtain correlated parameters, including area under the curve (*AUC*), half-life ($t_{1/2}$), time reaching the maximum concentration (T_{max}), maximum concentration (C_{max}), and clearance rate (*Clz/F*). The difference between groups was assessed by student's *t*-test (between two groups) or one-way ANOVA followed by the Turkey post-hoc test (among multiple groups). The statistical significance was indicated by $p < .05$.

3 | RESULTS

3.1 | Effect of tangeretin on the pharmacokinetics of ligustrazine in rats

The co-administration of tangeretin dramatically influenced the pharmacokinetic profile of ligustrazine in rats (Figure 1). In the presence of 50 mg/kg tangeretin, the $AUC_{(0\text{-}t)}$ of ligustrazine decreased 16.05%, while $AUC_{(0\text{-}\infty)}$ reduced 14.49%. The $t_{1/2}$ was also found to be shortened from 5.90 ± 1.27 to 4.84 ± 1.19 h. The clearance rate was enhanced 1.45-fold by 50 mg/kg tangeretin (0.45 ± 0.036 vs. 0.31 ± 0.079 L/h/kg). A significant decrease of 19.17% was also observed in the C_{max} and *MRT* reduced 10.22% (from 7.05 ± 0.26 to 6.33 ± 0.48 h) ($p < .05$, Table 1).

With the increase of tangeretin concentration, the promotion of ligustrazine pharmacokinetics was enhanced. The co-administration of 100 mg/kg tangeretin reduced 35.60% of $AUC_{(0\text{-}t)}$ and 36.43% of $AUC_{(0\text{-}\infty)}$ of ligustrazine. The *MRT* decreased 21.57%, and the $t_{1/2}$ was also suppressed 41.02%. The clearance rate increased 2.13-fold to 0.66 ± 0.48 L/h/kg (Table 1). The combination with 150 mg/kg tangeretin reduced 43.62% of $AUC_{(0\text{-}t)}$ and 45.93% of $AUC_{(0\text{-}\infty)}$ to 27.55 ± 9.60 and 27.76 ± 9.77 μg/mL × h, respectively with a 47.63%-decreasing $t_{1/2}$. The clearance rate was accelerated to 0.72 ± 0.28 L/h/kg ($p < .05$, Table 1).

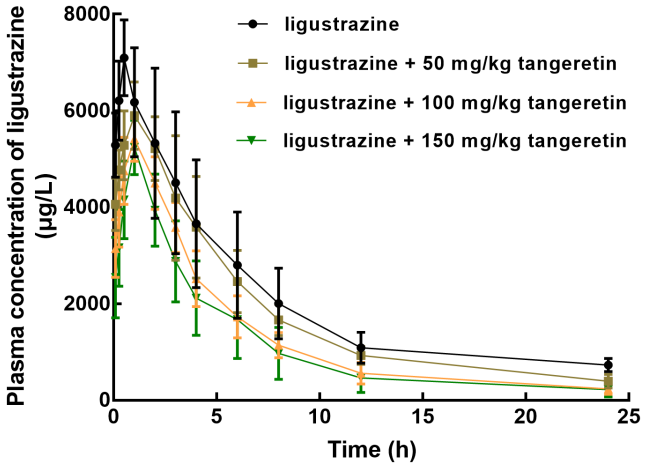

FIGURE 1 The plasma concentration-time curve of ligustrazine in the presence of 50, 100, and 150 mg/kg tangeretin.

3.2 | Effect of tangeretin on the metabolic stability of ligustrazine in rat liver microsomes

In rat liver microsomes, the half-life of ligustrazine was 35.26 ± 7.33 min, and the intrinsic clearance rate was calculated to be 39.31 ± 5.67 mL/min/kg protein (Figure 2). Consistent with the pharmacokinetic results, the co-administration of tangeretin significantly shorten the half-life and improved the intrinsic clearance rate. Specifically, in combination with 50, 100, and 150 mg/kg tangeretin, the in vitro half-life of ligustrazine decreased to 15.67 ± 5.12 min, 13.47 ± 4.33 min, and 9.89 ± 2.34 min with the corresponding intrinsic clearance rate of 88.45 ± 13.44 mL/min/kg protein, 102.90 ± 17.69 mL/min/kg protein, and 140.13 ± 24.31 mL/min/kg protein, respectively (Figure 2).

3.3 | Effect of tangeretin on CYP3A4 mRNA expression

In LS180 cells, tangeretin was found to induce the expression of CYP3A4 mRNA, and the induction was enhanced by the increasing concentration of tangeretin ($p < .01$, Figure 3).

4 | DISCUSSION

Co-administration of different herbs with similar indications is commonly applied in the clinic of traditional Chinese medicine.[21–23]

TABLE 1 The pharmacokinetic parameters of ligustrazine with different co-administration strategies.

	ligustrazine	Ligustrazine +50 mg/kg tangeretin	Ligustrazine +100 mg/kg tangeretin	Ligustrazine +150 mg/kg tangeretin
$AUC_{(0\text{-}t)}$ (μg/mL×h)	48.86±12.57	41.02±4.85*	31.47±5.26*	27.55±9.60*
$AUC_{(0\text{-}\infty)}$ (μg/mL×h)	50.41±12.78	43.11±4.52*	32.05±5.79*	27.76±9.77*
$MRT_{(0\text{-}t)}$ (h)	7.05±0.26	6.33±0.48*	5.53±0.68*	5.21±1.31*
$t_{1/2}$ (h)	5.90±1.27	4.84±1.19*	3.48±1.33*	3.09±0.62*
T_{max} (h)	0.83±0.61	1.50±0.84*	0.92±0.21*	1.03±0.17*
CL/F (L/h/kg)	0.31±0.079	0.45±0.036*	0.66±0.48*	0.72±0.28*
C_{max} (μg/mL)	7.45±0.44	6.03±0.44*	5.24±0.47*	5.02±0.56*

*$p < .05$ relative to the single dose of ligustrazine.

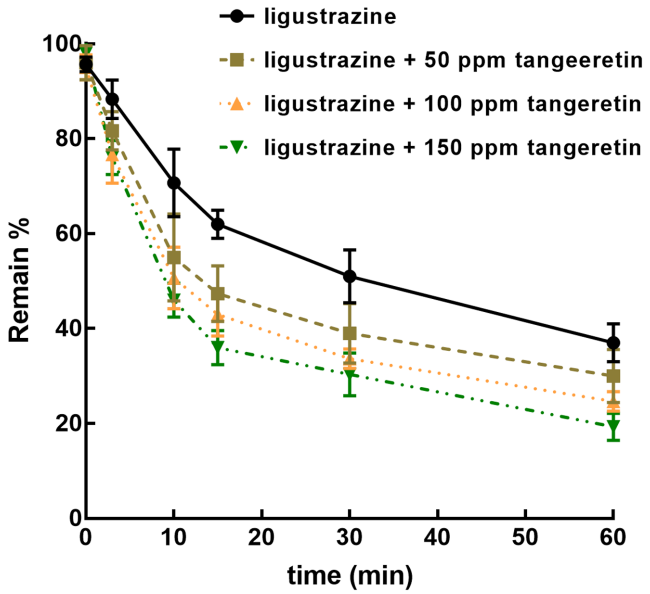

FIGURE 2 In vitro metabolic stability of ligustrazine in the presence of 50, 100, and 150 mg/kg tangeretin in rat liver microsomes.

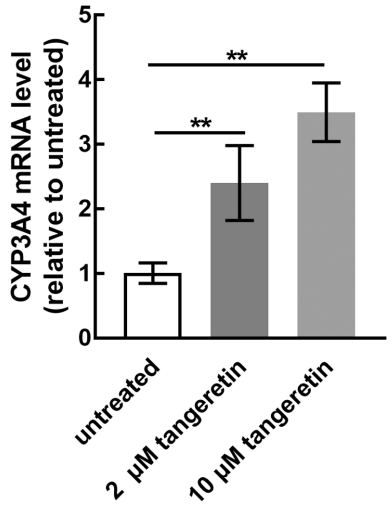

FIGURE 3 Effect of tangeretin on the mRNA expression of CYP3A4 in LS180 cells. **$p < .01$.

Both ligustrazine and tangeretin have been reported to possess heart-protective effects, which improves the possibility of their co-administration. In former studies, adverse interactions between co-administered drugs or herbs, which has been considered to be the major reason for most clinical adverse reactions.[24,25] A previous clinical trial reported that over one third of patients suffered severe drug interactions and about half outpatients also showed adverse drug interactions due to inappropriate medication usage.[26] For instance, the co-administration of pyrotinib and rifampicin in healthy individuals induced significant changes in the metabolism of rifampicin.[27] Zhang et al. employed berberine as the pharmacokinetic marker to investigate the potential interaction between Gancao and Huanglian. It was found that the intestinal absorption and exposure of Huanglian were suppressed by Gancao caused by the formation of sediment, which reduced the solubility and extracted amount of berberine.[28] The co-consumption of Gancao with glycyrrhetinic acid induced the sodium-water retention side effects, while the combination of Kushen with Gancao could slow down the absorption and enhance the metabolism of glycyrrhetinic acid.[29]

The co-administration of ligustrazine with different concentrations of tangeretin was conducted in rats in the present study. To avoid the chemical interaction between tangeretin and ligustrazine, a pretreatment with tangeretin at different dosages was carried out. Tangeretin was found to significantly affect the pharmacokinetics of ligustrazine behaved as the decreasing *AUC*, *MRT*, and $t_{1/2}$, and the increasing clearance rate. The *AUC*, C_{max}, and T_{max} of the in vivo absorbance rate and degree of ligustrazine, while the $t_{1/2}$, MRT, and clearance rate are critical indicators for the retention and clearance of ligustrazine. On the other hand, the pharmacokinetic profiling could also indicate the bioavailability of ligustrazine. The observed changes in the pharmacokinetic parameters indicated the promotion of ligustrazine metabolism and bioavailability in vivo. In vitro, the metabolic stability of ligustrazine was observed to reduce in the presence of various concentrations of tangeretin. Specifically, tangeretin increased the intrinsic clearance rate and decreased the half-life of ligustrazine in rat liver microsomes, which is consistent with the in vivo results. Hence, it can be seen that the co-administration of ligustrazine with tangeretin shortened the system exposure and reduced the metabolic stability of ligustrazine. Although in vivo and

in vitro evidence has been revealed in the present study, the specific situation of the interaction and the clinical prescription dosage of tangeretin, ligustrazine, and their source herbs need further clinical validations, which was considered as future investigation directions.

Previously, cytochrome P450 enzymes have been considered as the critical mediator of herb-herb or herb-drug interactions, which catalyzed the metabolism of endogenous substances, such as fatty acids, steroids, and bile acids, as well as the exogenous substances, including herbs or drugs.[30,31] In previous herb–herb interaction studies on ligustrazine, CYP3A4 was demonstrated to play an important role, which was also revealed to be involved in the metabolism of ligustrazine.[8,18,32] A previous study focused on the effect of clementine juice disclosed the induced effect of tangeretin on the activity of CYP3A4. Herein, the induction of CYP3A4 by tangeretin was also observed in a human colon adenocarcinoma cell. Therefore, the decreasing system exposure and the metabolic stability of ligustrazine were speculated to result from the induction of CYP3A4 by tangeretin during their co-administration. However, there was a lack of direct evidence on the involvement of CYP3A4 in the pharmacokinetics of ligustrazine, which can be verified by recombinant CYP450 supersomes according to previous reports, which can be considered an outlook of the present study.[33–35]

Taken together, the co-consumption of ligustrazine with tangeretin significantly accelerates the in vivo pharmacokinetics and reduces in vitro metabolic stability of ligustrazine. The potential mechanism might be associated with the induction of CYP3A4. These findings provides theoretical reference for the prescription of tangeretin- and ligustrazine-containing herbs, which needs further clinical validation.

AUTHOR CONTRIBUTION

D.D. L, Y.J. L, L.F. Z and J. Z designed the research study. X. Q, J.W. Y and C.J. L performed the research. D.D. L and Y.J. L analyzed the data. L.F. Z and J. Z wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Yueyue Li participated in the design of the experimental scheme. We sincerely thank Yueyue Li for his important contributions to the research.

DISCLOSURE

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Shanghai Baoshan Luodian Hospital.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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