Pharmacokinetic interaction study between ligustrazine and valsartan in rats and its potential mechanism

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\section*{ABSTRACT}

\textbf{Context:} Ligustrazine and valsartan are commonly used drugs in the treatment of cardiac and cardiovascular disease.

\textbf{Objective:} The interaction between ligustrazine and valsartan was studied to investigate the effect of ligustrazine on the pharmacokinetics of valsartan.

\textbf{Materials and methods:} The pharmacokinetics of valsartan (10 mg/kg) was investigated in Sprague-Dawley rats divided into three groups (with the pretreatment of 4 or 10 mg/kg/day ligustrazine for 10 days and without the pretreatment of ligustrazine as control) of six rats each. The \textit{in vitro} experiments in rat liver microsomes were performed to explore the effect of ligustrazine on the metabolic stability of valsartan.

\textbf{Results:} Ligustrazine changed the pharmacokinetic profile of valsartan. In the presence of 4 mg/kg ligustrazine, the AUC\textsubscript{0-t} (385.37 ± 93.05 versus 851.64 ± 104.26 µg/Lh), t\textsubscript{1/2} (5.46 ± 0.93 versus 6.34 ± 1.25 h), and C\textsubscript{max} (62.64 ± 9.09 versus 83.07 ± 6.15 µg/L) of valsartan were significantly decreased, and the clearance rate was increased from 10.92 ± 1.52 L/h/kg to 25.76 ± 6.24 L/h/kg and similar changes were observed in the group with 10 mg/kg ligustrazine (p < 0.05). The metabolic stability of valsartan was also decreased by ligustrazine as the half-life of valsartan in rat liver microsomes decreased from 37.12 ± 4.06 to 33.48 ± 3.56 min and the intrinsic clearance rate increased from 37.34 ± 3.84 to 41.40 ± 4.32 µL/min/mg protein (p < 0.05).

\textbf{Discussion and conclusions:} Ligustrazine promoted the metabolism of valsartan via activating CYP3A4. The co-administration of ligustrazine and valsartan should be taken into account.

\section*{Introduction}

Ligustrazine is a bioactive ingredient extracted from \textit{Rhizoma Ligustici wallichii} [\textit{Ligustici wallichii} (Apiaceae)]. Ligustrazine has been widely used in the clinic for the treatment of cardiovascular disease because of its function in activating blood circulation (Wu et al. 2012; Qian et al. 2014). Moreover, ligustrazine has been demonstrated to possess antioxidant properties in animal models of ischemic-reperfusion, atherosclerosis, and cerebral vasospasm (Jiang et al. 2011; Lv et al. 2012). Commonly, patients with cardiovascular or cerebral diseases may have received ligustrazine treatment before using other drugs, or it may be used with other drugs together for the treatment of complex chronic disorders (Hockenberry 1991; Phougat et al. 2014).

Valsartan is a commonly used drug in cardiac disease with relatively low absolute bioavailability (Jung et al. 2015). Valsartan is usually applied in the treatment of hypertension due to its properties of lowering blood pressure, and it also co-administrated with other drugs to make the treatment more efficient in the clinic (Cheng et al. 2001; Liu et al. 2019a). Previously, the co-administration of valsartan and quercetin could exert greater cardiac protection and this kind of combination affected the pharmacokinetics of valsartan (Challa et al. 2013). The combination of valsartan and amlodipine has been demonstrated to control blood pressure efficiently and the pharmacokinetic and transport of valsartan were influenced by the administration of amlodipine (Cai et al. 2011). The combination of ligustrazine and valsartan has been reported to have a protective effect on hippocampal neuronal loss with vascular dementia (Qin et al. 2011). However, the drug–drug interaction between ligustrazine and valsartan was still unclear.

This study focussed on the interaction between ligustrazine and valsartan to evaluate the effect of ligustrazine on the pharmacokinetics of valsartan, which can provide more information for the clinical co-administration of ligustrazine and valsartan.

\section*{Materials and methods}

\textbf{Drugs and chemicals}

Standards of valsartan (purity >98%), and ligustrazine (purity >98%) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).
Animals

Experiments were performed with male Sprague–Dawley rats weighing 230–250 g supplied by Sino-British Sippr/BK Lab Animal Ltd (Shanghai, China). The rats were housed in an air-conditioned animal quarter at 22 ± 2°C and 50 ± 10% relative humidity. The animals were acclimatized to the facilities for 5 days, during this period, animals had free access to food and water. Before each experiment, the rats fasted with free access to water for 12 h. The experiment was approved by the Animal Care and Use Committee of The First Affiliated Hospital of Qiqihar Medical University.

Preparation of plasma samples

Plasma sample (100 μL) was mixed with 20 μL methanol and 180 μL internal standard methanol solution and vortexed for 60 s. After centrifugation at 12,000 rpm for 10 min, the supernatant was removed into an injection vial and 5 μL aliquot was injected into LC/MS-MS system for analysis.

LC-MS/MS condition

Chromatographic analysis was performed by using an Agilent 1290 series liquid chromatography system (Agilent Technologies, Palo Alto, CA) as previously reported (Deng et al. 2017; Liu et al. 2019b). The sample was separated on Waters Xbridge C18 column (100 × 3.0 mm, i.d.; 3.0 μm, Waters Corporation, Milford, MA) and eluted with an isocratic mobile phase: solvent A (Water containing 0.1% formic acid) – solvent B (acetonitrile) (65:35, v/v). The column temperature was set at 25°C, the flowing rate at 0.4 mL/min, and the injection volume at 5 μL. Mass spectrometric detection was carried out on an Agilent 6460 triple-quadruple mass spectrometer (Agilent Technologies, Palo Alto, CA) with Turbo Ion spray, which is connected to the liquid chromatography system. The mass scan mode was MRM positive. The precursor ion and product ion were m/z 434.2→179.0 for valsartan and m/z 357.9→321.8 for methyclothiazide as IS according to the previous study (Wei et al. 2019). The collision energy for valsartan and methyclothiazide was 30 and 20 eV, respectively. The MS/MS conditions were optimized as follows: fragmentor, 110 V; capillary voltage, 3.5 kV; nozzle voltage, 500 V; nebulizer gas pressure (N2), 40 psig; drying gas flow (N2), 10 L/min; gas temperature, 350 °C; sheath gas temperature, 400 °C; sheath gas flow, 11 L/min. Agilent MassHunter B.07 software was used for the control of the equipment and data acquisition. Agilent Quantitative analysis software was used for data analysis.

Pharmacokinetic study

The rats were randomly divided into three groups of six animals each, including 10 mg/kg valsartan only group (A), 10 mg/kg valsartan + 4 mg/kg ligustrazine group (B), and 10 mg/kg valsartan + 10 mg/kg ligustrazine (C). The administration of ligustrazine was prior to valsartan for 10 days. Blood samples (0.25 mL) were collected into a heparinized tube via the oculi choroidal vein before drug administration and at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. After centrifuging at 3500 rpm for 10 min, the supernatant was obtained and frozen at −80°C until analysis.

The metabolic stability of valsartan in rat liver microsomes

According to previous studies, the reaction mixture was pre-incubated at 37°C for 5 min, after that 1 μM valsartan was added (Qi et al. 2014; Li et al. 2016). For the effect of ligustrazine on the metabolic stability of valsartan, ligustrazine was added into rat liver microsomes and pre-incubated for 30 min at 37°C, and then valsartan was added. After incubating for 0, 1, 3, 5, 15, 30, and 60 min, 30 μL samples were collected from volumes and the reaction was terminated by ice-cold acetonitrile. Then samples were prepared according to the above methods and analyzed by LC-MS/MS.

The half-life (t1/2) in vitro was obtained using equation: \( t_{1/2} = \frac{0.693}{k} \). V (μL/mg) = volume of incubation (μL)/protein in the incubation (mg); Intrinsic clearance (Clint) (μL/min/mg protein) = V × 0.693/t1/2.

Statistical analysis

All data were represented as mean ± SD obtained from triplicate experiments and analyzed with one-way analysis of variance (ANOVA) with SPSS 20.0 (SPSS, Inc., Chicago, IL). The pharmacokinetic parameters of valsartan were obtained with DAS 3.0 pharmacokinetic software (Chinese Pharmacological Association, Anhui, China). Differences were considered to be statistically significant when p < 0.05.

Results

Effect of ligustrazine on the pharmacokinetics of valsartan

The plasma concentration of valsartan was analyzed by LC-MS/MS (Supplementary Figure 1) and the plasma concentration–time curves of valsartan in rats with or without treatment of ligustrazine are shown in Figure 1. The pharmacokinetic parameters, including area under curves (AUC(0→t)), half-life (t1/2), time to reach maximum concentration (Tₘₐₓ), maximum concentration (Cₘₐₓ), and clearance (Clz/F), are summarized in Table 1.

The administration of ligustrazine significantly changed the pharmacokinetic profile of valsartan. In the presence of 4 mg/kg ligustrazine, the AUC(0→t) of valsartan decreased from 851.64 ± 104.26 to 385.37 ± 93.05 μg·h/L and the Cₘₐₓ reduced from 83.87 ± 6.15 to 62.64 ± 9.09 μg/L (p < 0.05). Similarly, the oral administration of 10 mg/kg ligustrazine also dramatically decreased the AUC(0→t) (249.85 ± 41.27 versus 851.64 ± 104.26 μg·h/L) and Cₘₐₓ (51.28 ± 2.67 versus 83.87 ± 6.15 μg/L). The t1/2 of valsartan reduced from 6.34 ± 1.25 to 5.46 ± 0.93 h after co-administrated of 4 mg/kg ligustrazine and to 5.15 ± 0.88 h in the presence of 10 mg/kg ligustrazine, and the difference was significant (p < 0.05). Additionally, the clearance rate of valsartan was significantly enhanced by both dose of ligustrazine (from 10.92 ± 1.52 to 25.76 ± 6.24 L/h/kg for 4 mg/kg ligustrazine, and
neuronal loss with vascular dementia (Qin et al. 2011). Previous and valsartan can exert protective effects on the hippocampal cardiac and cardiovascular disease. A combination of ligustrazine Both ligustrazine and valsartan are widely used in the therapy of

### Discussion

Effect of ligustrazine on the metabolic stability of valsartan

In rat liver microsomes, the obtained half-life of valsartan was 37.12 ± 4.06 min, which was significantly shortened to 33.48 ± 3.56 min after the administration of ligustrazine \( (p < 0.05) \). Moreover, the intrinsic clearance rate of valsartan significantly increased from 37.34 ± 3.84 to 41.40 ± 4.32 \( \mu \text{L/min/mg protein} \) by ligustrazine indicating the significant decrease in the metabolic stability of valsartan after the co-administration with ligustrazine \( (p < 0.05) \).

Conclusions

The co-administration of valsartan and ligustrazine induced drug–drug interaction, which decreased the system exposure of valsartan by activating CYP3A4. Therefore, the dose of ligustrazine and valsartan should be carefully considered in the clinic, when they are co-administred.

Disclosure statement

The authors declare that there are no conflicts of interest.
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