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Original Article

Pharmacokinetics changes of ivabradine and N-desmethylivabradine after oral administration with puerarin in rats

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Abstract: The objective of this work was to investigate the effect of orally administered puerarin on the pharmacokinetics of ivabradine and its active metabolite N-desmethylivabradine in rats. Twelve healthy male Sprague-Dawley rats were randomly divided into two groups: the control group (received oral 1.0 mg/kg ivabradine alone) and test group (1.0 mg/kg ivabradine orally coadministered with 250 mg/kg puerarin). The plasma concentration of ivabradine and N-desmethylivabradine were estimated by ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and different pharmacokinetic parameters were calculated using DAS 2.0 software. The pharmacokinetic parameters of $t_{1/2}$, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of ivabradine in test group were significantly higher than those in the control group ($P < 0.01$). However, puerarin had no effect on the pharmacokinetic parameters of N-desmethylivabradine when compared to control. This study demonstrates that puerarin increases plasma concentration of ivabradine only, but not N-desmethylivabradine. Henceforth, the pharmacodynamic influence of this interaction should be taken into consideration while prescribing ivabradine to the patients already taking puerarin.

Keywords: Ivabradine, N-desmethylivabradine, puerarin, drug interaction, pharmacokinetics

Introduction

Herbal medicine is widely used today. A follow-up survey found that about 15 million adults in the US took prescription drugs in combination with herbal remedies [1-3]. But there was less research on the interactions between herbal medicine and chemical drug. Therefore, the research on the interaction between herbs and drugs is important.

Ivabradine is a novel heart rate-lowering agent that selectively and specifically inhibits the depolarizing cardiac pacemaker I_f current in the sinus node. Its activity provides pure heart rate reduction at rest and during exercise, which improves myocardial oxygen balance and increases coronary perfusion, without any relevant influence on conduction, contractility, ventricular repolarization or blood pressure [4-7]. The anti-ischemic efficacy and the safety of ivabradine have been demonstrated in patients with stable angina pectoris [8]. Despite its therapeutic benefit, ivabradine has some important side effects, including bradycardia, atrioventricular block, ventricular extrasystoles and luminous phenomena [9-11]. Because of the high potential of ivabradine to produce adverse reactions on overdosing but also the lack of therapeutic effect on underdosing, is important to understand how other substances can modify this drug's pharmacokinetics.

After oral administration, the metabolic clearance of ivabradine accounts for about 80% of its total clearance, with the other 20% corresponding to renal clearance. Only the cytochrome CYP3A4 isoenzyme is involved in the metabolism of invabradine, so numerous potential interactions can therefore arise with CYP3A4 inhibitors and inducers. To date, only a few pharmacokinetic drug-drug interactions of ivabradine have been published: with omeprazole and lansoprazole [12], azithromycin [13],

clopidogrel [14], phenytoin [15], carbamazepine [16], simvastatin [17], and Hypericum Perforatum [18].

Puerarin (4',7-dihydroxy-8-β-D-glucose isoflavone), an isoflavone glycoside extracted from *Pueraria* plants, can lower blood pressure, resist oxidation, arrhythmia and inflammation, as well as inhibit tumor cell proliferation [19-22]. Recently, the interactions between puerarin and CYPs have been extensively studied, which showed that puerarin has potent inhibitions of CYP3A4 [23, 24]. Therefore, there are potent herb-drug interactions when using puerarin in combination with ivabradine. However, it is not presently clear whether puerarin has the capacity to affect the pharmacokinetics of ivabradine and N-desmethylivabradine.

In this study, the pharmacokinetics of ivabradine and N-desmethylivabradine in rats was investigated after administration of puerarin by simultaneous determination of ivabradine and N-desmethylivabradine in rat plasma with an ultra high performance liquid chromatography-mass spectrometry method (UPLC-MS/MS).

Materials and methods

Chemicals materials

Ivabradine (purity > 98%), N-desmethylivabradine (purity > 98%) and carbamazepine (purity > 98%, IS) were obtained from Sigma (St. Louis, MO, USA). Puerarin (purity > 98%) was purchased from commercial sources (INDOFINE Chemical Company, Inc., Somerville, NJ, USA). Acetonitrile and methanol were HPLC grade and purchased from Merck Company (Darmstadt, Germany). HPLC grade water was obtained using a Milli Q system (Millipore, Bedford, USA).

UPLC-MS/MS conditions

Liquid chromatography was performed on an Acquity ultra performance liquid chromatography (UPLC) unit (Waters Corp., Milford, MA, USA) with an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm) and inline 0.2 μm stainless steel frit filter (Waters Corp., Milford, MA, USA). A gradient elution program was conducted for chromatographic separation with mobile phase A (acetonitrile), and mobile phase B (0.1% formic acid) as follows: 0-1.0 min (10-90% A), 1.0-1.9 min (90-90% A), 1.9-2.0 min (90-10% A), 2.0-3.0 min (10-10% A). The flow rate was 0.40 mL/min. The overall run time was 3.0 min. A XEVO TQD triple quadrupole mass spectrometer equipped with an electro-spray ionization (ESI) source was used for mass spectrometric detection. The detection was operated in the multiple reaction monitoring (MRM) mode under unit mass resolution in the mass analyzers. The MRM transitions were *m/z* 469.2 → 177.2 for ivabradine, *m/z* 455.2 → 262.2 for N-desmethylivabradine and *m/z* 237.1 → 194.2 for IS, respectively. Mass spectrometry was operated with the capillary voltage set at 3.50 kV, the cone and source offset set at 35 and 50 V, respectively. The desolvation temperature and desolvation gas flow rate set at 600°C and 500 L/h, respectively. And the argon flow rate and collision set at 150 L/h and 7.0 Bar, respectively. The Masslynx 4.1 software (Waters Corp., Milford, MA, USA) was used for data acquisition and instrument control.

Sample preparation

Before analysis, frozen plasma sample was thawed to room temperature. In a 1.5 mL centrifuge tube, an aliquot of 200 μL of the internal standard working solution (30 ng/mL) was added to 0.1 mL of plasma sample. The tubes were vortex mixed for 1.0 min. After centrifugation at 15,000 g for 10 min, the supernatant (2 μL) was injected into the UPLC-MS/MS system for analysis.

Method validation

The method was considered valid according to the following criteria: selectivity, linearity, accuracy, precision, percent recovery and stability. The concentration ranges of the calibrators of ivabradine and N-desmethylivabradine were 0.1-100, 0.05-10 ng/ml for plasma, respectively. The plasma sample concentrations were calculated from the calibration curves. Intra- and inter-day precision and accuracy were evaluated by assaying six replicates of each spiked QC sample at the low, middle, and high concentrations on three separate days. Precision is expressed as relative standard deviation. Accuracy was calculated as the percent error in the calculated mean concentration relative to the nominal concentrations. The short-term stored stabilities of analytes after being processed

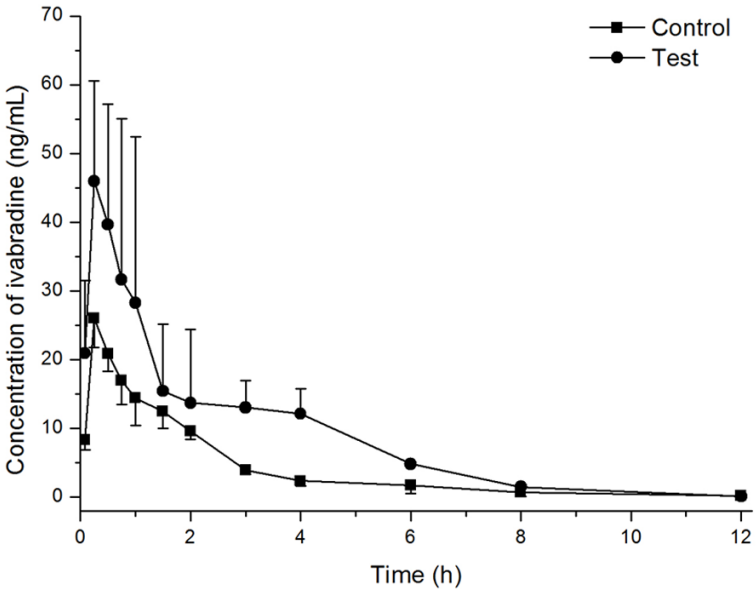

Figure 1. Mean plasma concentrations of ivabradine versus time after single oral administration of ivabradine 1.0 mg/kg alone or coadministration with 250 mg/kg puerarin in rats.

Table 1. The main pharmacokinetic parameters of ivabradine after single oral administration of ivabradine 1.0 mg/kg alone or coadministration with 250 mg/kg puerarin in rats

Parameters	Control	Test
$t_{1/2}$ (h)	1.71 ± 0.25	2.20 ± 0.14**
T_{max} (h)	0.26 ± 0.03	1.06 ± 0.31**
CL_z/F (L/h/kg)	20.70 ± 2.47	11.95 ± 6.49**
C_{max} (ng/mL)	26.03 ± 4.25	46.67 ± 13.82**
$AUC_{(0-t)}$ (ng·h/mL)	48.12 ± 5.16	104.24 ± 26.45**
$AUC_{(0-\infty)}$ (ng·h/mL)	48.88 ± 6.35	104.51 ± 26.35**

**Significantly different from control, P < 0.01.

were evaluated by testing their stabilities after being protein precipitated and stored for 3 h at room temperature. Long-term stability was examined for 31 days at -25°C. Freeze-thaw stability testing was determined after freezing at -25°C and thawing to room temperature three times.

Animals

Male Sprague-Dawley rats with body weights of 220 ± 20 g were purchased from Laboratory Animal Center of Henan University of Science and Technology. The rats were acclimatized for a week in laboratory conditions to minimize all efforts of any animal suffering before initiating the experiment. Necessary approval from the Institutional Animal Ethics Committee was obtained to carry out the experiments.

Pharmacokinetic study

Twelve Sprague-Dawley male rats were divided into 2 groups: the control group (received oral 1.0 mg/kg ivabradine alone) and test group (1.0 mg/kg ivabradine orally coadministered with 250 mg/kg puerarin). Diet was prohibited for 12 h before the experiment but water was freely available. Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after oral administration. The samples were immediately centrifuged at 4000 g for 8 min. The plasma obtained (100 μL) was stored at -20°C until analysis.

Statistical analysis

The results are given as mean standard deviation (SD). The noncompartmental analysis was used to calculate the pharmacokinetic parameters by DAS (Drug and statistics) software (Version 2.0, Shanghai University of Traditional Chinese Medicine, China). The statistical analyses were evaluated by unpaired *t*-test (SPSS 19.0, Chicago, IL). A value of $P < 0.05$ was considered to be statistically significant.

Results

UPLC-MS/MS method validation

No interference can be observed in the UPLC chromatograms. The calibration curve was linear over the concentration range of 0.1-100 ng/ml for ivabradine and 0.05-10 ng/mL for N-desmethylivabradine, both with a correlation coefficient $r > 0.996$. The LLOQ of ivabradine and N-desmethylivabradine was 0.1 and 0.05 ng/mL, respectively. The precision evaluation revealed that all coefficients of variation were below 15% and the accuracy analysis showed that the relative errors to the true concentra-

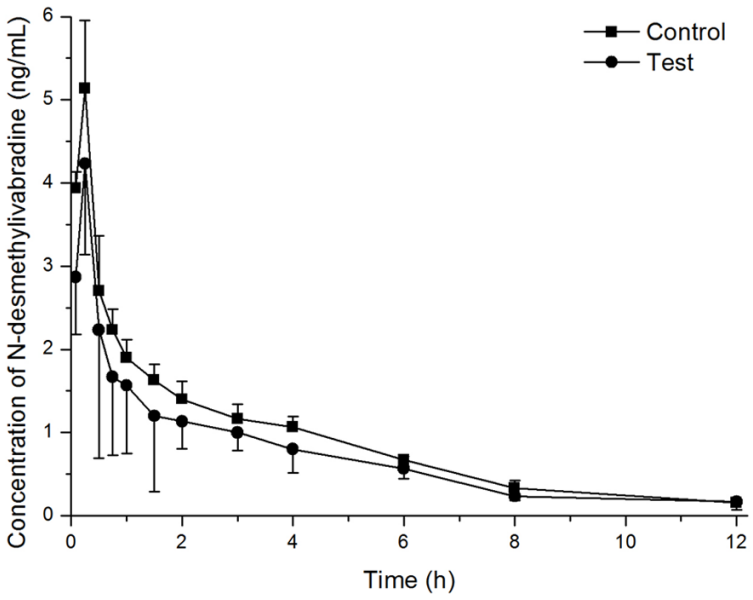

Figure 2. Mean plasma concentrations of N-desmethylivabradine versus time after single oral administration of ivabradine 1.0 mg/kg alone or coadministration with 250 mg/kg puerarin in rats.

Table 2. The main pharmacokinetic parameters of N-desmethylivabradine after single oral administration of ivabradine 1.0 mg/kg alone or coadministration with 250 mg/kg puerarin in rats

Parameters	Control	Test
$t_{1/2}$ (h)	3.10 ± 0.24	2.92 ± 0.64
T_{max} (h)	0.27 ± 0.05	0.29 ± 0.06
CL_z/F (L/h/kg)	86.88 ± 7.50	102.90 ± 46.89
C_{max} (ng/mL)	5.13 ± 0.82	4.28 ± 0.23
$AUC_{(0-t)}$ (ng·h/mL)	10.79 ± 1.08	8.62 ± 3.63
$AUC_{(0-\infty)}$ (ng·h/mL)	11.58 ± 1.01	9.11 ± 3.54

tions were below 11%. The recovery of ivabradine and N-desmethylivabradine from plasma was 78.1-85.4% and 75.8-84.3%, respectively. The RSDs of the mean test responses were within 15% in all stability tests of ivabradine and N-desmethylivabradine in plasma. The above results demonstrated that the values were within the acceptable range and the method was accurate and precise.

Effect of puerarin on the pharmacokinetic study of ivabradine and N-desmethylivabradine

The mean plasma concentration-time profiles of ivabradine administered (1.0 mg/kg) alone or in combination with puerarin (250 mg/kg) orally in rats are shown in **Figure 1**. Absorption of ivabradine was rapid; ivabradine was detected in plasma from the first blood sampling time (5 min) for both without and with puerarin. **Table 1** summarizes the pharmacokinetic parameters of ivabradine. The presence of puerarin significantly increased the $AUC_{(0-\infty)}$ (113.8%) and C_{max} (79.3%) of orally administered ivabradine ($P < 0.01$). In addition, statistical comparison of pharmacokinetic parameters of ivabradine showed that coadministration with puerarin caused significant increase in $t_{1/2}$ and T_{max} of ivabradine in comparison to the control group. Further, CL_z/F of ivabradine was significantly decreased in puerarin coadministration group in comparison with that of control group (**Table 1**).

The mean plasma concentration-time profiles of N-desmethylivabradine are shown in **Figure 2**, and the pharmacokinetic parameters are summarized in **Table 2**. However, coadministered puerarin did not alter $t_{1/2}$, T_{max}, CL_z/F, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of N-desmethylivabradine significantly ($P > 0.05$) when compared to control (**Table 2**).

Discussion

Flavonoids are the most abundant polyphenolic compounds present in most plants and are frequently consumed in human diet [25]. With the increasing usage of botanical supplement, herb-drug interactions have become a growing medical concern [26]. Many flavonoids are reported to alter bioavailability of many conventional medications due to their modulatory effect on drug transporters or various cytochrome P450 enzymes [27].

For safety reason, it is thus important to evaluate the potential pharmacokinetic interaction between herbal drug and oral pharmaceutics in animals before conducting studies in human. In this study, we investigated the interaction of

puerarin with a CYP3A4 substrate, ivabradine, and its major metabolite N-desmethylivabradine in rats. A statistical comparison of data against the control group was performed to determine the influence of puerarin on the pharmacokinetics of ivabradine and N-desmethylivabradine.

Surprisingly, in the present study, oral administration of puerarin at the dose of 250 mg/kg significantly increased the $AUC_{(0-\infty)}$ and C_{max} of ivabradine, which is a CYP3A4 substrate; even though puerarin is known to inhibit the function of CYP3A4 in rats [24]. However, the $t_{1/2}$, T_{max}, CL_z/F, C_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of N-desmethylivabradine were not altered significantly in puerarin treated rats in comparison to the control, suggesting that puerarin has no effect on the formation of N-desmethylivabradine. So the results suggested that puerarin may mainly inhibit CYP3A4 *in vivo*, but it also needs to be studied further.

From the analysis, the results also indicated that a single dose administration of puerarin could reduce the metabolism rate of ivabradine, increase the bioavailability, and extend the resistance time of prototype drug *in vivo*. Those changes might be related to a potential interaction between puerarin and CYP3A4 enzyme.

The co-administration of herbal drug puerarin significantly increased the plasma concentration of ivabradine. However the formation of N-desmethylivabradine was not affected. Further studies using clinical trials will be needed to determine if the results obtained in this study can be extrapolated to humans. If the results obtained from the rats' model is confirmed in the clinical trials, the ivabradine dose should be adjusted for potential drug interactions when ivabradine is used with puerarin or the puerarin-containing herbal drugs.

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Disclosure of conflict of interest

None.

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