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Title page

Influence of Zuojin Pill on the metabolism of venlafaxine in vitro and in rats and associated herb-drug interaction

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Running Title Page

Influence of Zuojin Pill on venlafaxine

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Abbreviations:

AM, active moiety; AUC, area under drug concentration-time curve; BBR, berberine; C_{max}, maximum blood concentration; CPS, coptisine; CL_{int}, intrinsic clearance; DDI, drug-drug interaction; DMSO, dimethyl sulfoxide; EMs, extensive metabolizers; ESI, electrospray ionization; HLM, human liver microsomes; IC_{50}, half maximal inhibitiory concentration; K_m, Michaelis-Menten constant; KTZ, ketoconazole; LOD, limit of detection; NDV, *N*-desmethylvenlafaxine; NODV, *N*,*O*-didesmethylvenlafaxine; ODV, *O*-desmethylvenlafaxine; PMs, poor metabolizers; QND, quinidine; QNN, quinine; rhCYPs, recombinant human cytochrome P450 isoenzymes; RLM, rat liver microsomes; SNRI, selective serotonin and noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; $T_{1/2}$, half-life; TCM, Traditional Chinese Medicine; V_{max}, maximum velocity of the metabolic reaction; VEN, venlafaxine; ZJP, Zuojin Pill.

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Abstract

Venlafaxine (VEN), a first-line antidepressant, and Zuojin Pill (ZJP), a common Chinese herbal medicine consisting of *Rhizoma Coptidis* and *Fructus Evodiae*, have high likelihood of combination usage in depression patients with gastrointestinal complications. ZJP exhibits inhibitory effects on recombinant human cytochrome P450 isoenzymes (rhCYPs), especially on CYP2D6, while VEN undergoes extensive metabolism by CYP2D6. From this prospective, we for the first time investigated the influence of ZJP on the metabolism of VEN in vitro and in rats. In this study, ZJP significantly inhibited the metabolism of VEN in both rat liver microsomes (RLM) and human liver microsomes (HLM); meanwhile, it inhibited the *O*-demethylation catalytic activity of RLM, HLM, rhCYP2D6*1/*1, and rhCYP2D6*10/*10, primarily through CYP2D6, with IC_{50} values of 129.9, 30.5, 15.4, and 2.3 μg/mL, respectively. Furthermore, the inhibitory effects of ZJP on hepatic metabolism and pharmacokinetics of VEN could also be observed in the pharmacokinetic study of rats. The $AUC_{0\text{-}24h}$ of VEN and its major metabolite *O*-desmethylvenlafaxine (ODV) increased by 39.6% and 22.8%, respectively. The hepatic exposure of ODV decreased by 57.2% 2h after administration (P = 0.014). In conclusion, ZJP displayed inhibitory effects on hepatic metabolism and pharmacokinetics of VEN in vitro and in rats mainly through inhibition of CYP2D6 activity. The human pharmacokinetic interaction between ZJP and VEN and its associated- clinical significance needed to be seriously considered.

Keywords: Zuojin Pill; venlafaxine; depression; metabolism; herb-drug interaction

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Significance Statement

Zuojin Pill, a commonly used Chinese herbal medicine, demonstrates significant inhibitory effects on hepatic metabolism and pharmacokinetics of venlafaxine in vitro and in rats mainly through suppression of CYP2D6 activity. The human pharmacokinetic interaction between Zuojin Pill and venlafaxine and its associated clinical significance needs to be seriously considered.

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Introduction

Venlafaxine (VEN), a selective serotonin and noradrenaline reuptake inhibitor (SNRI), is one of the most efficacious and commonly prescribed antidepressants (Cipriani et al., 2018). VEN undergoes extensive metabolism by cytochrome P450 isoenzymes (CYPs). Its major metabolite *O*-desmethylvenlafaxine (ODV) is predominantly catalyzed by CYP2D6, which has similar efficacy as VEN, while minor metabolites with little pharmacological effect including *N*-desmethylvenlafaxine (NDV) and *N*, *O*-didesmethylvenlafaxine (NODV) are catalyzed by CYP2C9, CYP2C19, and CYP3A4 (Fig. 1) (Otton et al., 1996; Fogelman et al., 1999). The blood (including plasma) concentrations of VEN + ODV (active moiety (AM)) are considered clinically relevant to a relatively narrow therapeutic reference range (100–400 ng/mL) (Hiemke et al., 2018). The factors that may influence the pharmacokinetics of VEN, such as age, disease state, genetic polymorphism of metabolic enzymes, and drug combination, should also be considered seriously (Magalhães et al., 2015). Recently, increasing evidence has suggested that concomitant administration of CYP2D6 inhibitors may incur pharmacokinetic drug-drug interaction (DDI) of VEN (Jiang et al., 2015; Magalhães et al., 2015).

Zuojin Pill (ZJP) is a traditional Chinese herbal formula recorded in the Chinese Pharmacopoeia for treating gastrointestinal disorders (Commission, 2015). It consists of two commonly used herbs, *Rhizoma Coptidis* and *Fructus Evodiae*, following a 6:1 (w/w) ratio. The primary active ingredients in ZJP are supposed to be alkaloid compounds, such as Rhizoma Coptidis alkaloids berberine and coptisine, and Evodiae alkaloids evodiamine and rutaecarpine (Gao et al., 2010; Wang et al., 2013). In previous studies, ZJP and its bioactive

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components, such as berberine and coptisine, exhibit inhibitory effects on CYP2D6, CYP1A2 and CYP3A4 in vitro (Han et al., 2011; Liu et al., 2014). Moreover, while ZJP is co-administrated with dextromethorphan, a specific CYP2D6 prober substrate, the $AUC_{0\text{-}24h}$ of dextromethorphan in healthy CYP2D6*1/*1 carriers increases 3.0-fold (Qiu et al., 2016). Due to the concomitant symptoms of depression itself and the adverse reactions of antidepressants, patients with depression are usually accompanied by several gastrointestinal disorders, and there exists a high likelihood of the combination usage of ZJP and VEN in depression patients with gastrointestinal complications (Qiu et al., 2015; Wang et al., 2020).

Herbs and natural products (including botanical dietary supplements and foods) can produce clinically significant pharmacokinetic interactions with conventional drugs (Johnson et al., 2018). Abnormally increased or decreased drug exposure caused by herbs and natural products will generate potential risks in clinical treatment. In the classic cases, St. John's wort and grapefruit juice can significantly influence the pharmacokinetics of cyclosporine and felodipine, respectively, which will further interfere with the therapeutic efficacy of both drugs (Paine and Oberlies, 2007; Nicolussi et al., 2020).

Therefore, in the present study, we investigated the influence of ZJP on the metabolism of VEN in human liver microsomes (HLM), rat liver microsomes (RLM), and recombinant human CYP enzymes (rhCYPs). Moreover, the potential pharmacokinetic interaction between ZJP and VEN was evaluated by assessing the effects of ZJP on VEN and its metabolites in rats.

Materials and Methods

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Chemicals and materials

Venlafaxine hydrochloride (purity > 99%) and *O*-desvenlafaxine (ODV, purity > 99%) were obtained from Selleck Chemicals (Houston, TX, USA). *N*-desmethyl venlafaxine (NDV, purity > 98%) and *N, O*-didesmethyl venlafaxine (NODV, purity > 98%) were purchased from Toronto Research Chemicals (Toronto, Canada). Berberine hydrochloride (purity ≥ 95%), coptisine hydrochloride (purity ≥ 98%), evodiamine (purity ≥ 99%), and rutaecarpine (purity ≥ 98%) were obtained from Aladdin (Shanghai, China). The pooled liver microsomes of rats (a total of 75 rats of the same gender) and humans (a total of 25 adult donors of the same gender) were obtained from the Research Institute for Liver Diseases Co., Ltd. (Shanghai, China) and BioIVT (Westbury, NY, USA), respectively. Recombinant human CYP2D6*1 and CYP2D6*10 expressed in *E.coli* were obtained from Cypex Ltd. (Scotland, UK). Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was obtained from Solarbio (Beijing, China). Diphenhydramine was purchased from Sigma Aldrich (St. Louis, MO). All other reagents were of the highest quality commercially available.

Zuojin Pill (ZJP, batch number: 20180304) was purchased from Hubei Xianglian pharmaceutical Co., Ltd (Wuhan, Hubei, China). The ZJP dosing solutions were prepared as follows: briefly, Zuojin Pill was ground into powder and dissolved in dimethyl sulfoxide (DMSO) for in vitro study; while for in vivo study, 10-fold volumes of deionized water were subsequently added. The method for determining the concentration of major alkaloid components in ZJP solution was similar to the LC–MS/MS method in a biological matrix described below. The amounts of four major alkaloids in ZJP, namely berberine, coptisine, evodiamine, and rutaecarpine were c*while the rats in blank control g*alculated as 25.5, 3.1,

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0.31, and 0.48 mg/g, respectively (Supplementary Table 1).

Animals

Wistar rats (male, 180-220 g) were purchased from Charles River laboratory animal Co., Ltd. (Beijing, China). Rats were housed in a room at 16-26°C, with a light/dark cycle of 12/12 hours and humidity of 40%-70%. All animal experiments were approved by the Animal Care and Use Committee of Shanghai University of Traditional Chinese Medicine and followed the Guidance for the Care and Use of Laboratory Animals in China.

Metabolic stability assessment of VEN in HLM and RLM

The total volume of the incubation mixture was 200 μL, which included VEN (1 μM), HLM (1.0 mg/mL) or RLM (0.5 mg/mL), and NADPH (10 mM), in 100 mM potassium phosphate-buffered solution (PBS) (pH 7.4). The reaction was conducted in a shaking water bath at 37^{o}C for 60 min. To quench the reaction, 200 μL of ice-cold methanol containing diphenhydramine (100 ng/mL) as an internal standard was added. The mixture was vortexed for 1 min and centrifuged at 15,000 g at 4^{o}C for 5 min. Finally, the supernatant was collected and injected into a high-performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis. The VEN depletion data was used for calculating enzymatic parameters.

Enzymatic kinetics assessment of VEN in RLM, HLM and rhCYPs

Preliminary experiments were conducted to determine the optimal conditions for linear product formation, such as protein concentration and incubation time with RLM, HLM and rhCYPs, respectively. Subsequent experiments were conducted under the conditions of linear product formation. For both HLM and RLM experiments, the reaction mixture containing 0.2

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mg/mL of microsomes, VEN (0.2–750 μM), in 100 mM PBS buffer (pH 7.4) was preincubated at 37°C for 5 min. Then, the mixture was incubated with 10 mM NADPH at 37°C for 15 min. For human recombinant CYP2D6 (rhCYP2D6) *1*1 or *10*10 , the reaction mixture containing 8 pmol/mL of each enzyme and VEN (0.04–150 μM for CYP2D6*1*1, and 2–1500 μM for CYP2D6*10*10) was pre-incubated at 37°C for 5 min, followed by another 15-min incubation after addition of 10 mM NADPH. As described above, all reactions were quenched and treated with ice-cold methanol, and the supernatant was collected for LC–MS/MS analysis. The ODV formation data was used for calculating enzymatic parameters.

Evaluation of the influences of ZJP and its major components on the metabolism of VEN in vitro

The influences of ZJP and its major components, berberine and coptisine, on the metabolism of VEN were conducted with RLM and HLM, respectively. HLM (1.0 mg/mL) or RLMs (0.5 mg/mL) was preincubated with VEN (1 μM), ZJP (150 μg/mL), berberine or coptisine (30 μM), at 37°C for 5 min. Control samples were prepared in the absence of the following inhibitors, quinidine (QND, a human CYP2D6 inhibitor, 2 μM), quinine (QNN, a rat CYP2D inhibitor, 2 μM) and ketoconazole (KTZ, a CYP3A4 inhibitor, 2 μM). All reactions were initiated with 10 mM NADPH, lasted for 15 min at 37°C, and were terminated with 200 μL of ice-cold methanol containing 100 ng/mL diphenhydramine.

To determine the half maximal inhibitory concentration (IC_{50}) of ZJP, and its major components coptisine and berberine towards the metabolism of VEN, a series of concentrations of the above drugs were pre-incubated with RLM, HLM, and rhCYP2D6s,

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respectively. For microsomes studies, HLM (1 mg/mL) or RLM (0.5 mg/mL) was preincubated with VEN (0.02 μM for HLM and 1 μM for RLM), ZJP (0.71–287.56 μg/mL for HLMs and 0.142–575.12 μg/mL for RLMs) or its major components coptisine (0.1–100 μM for HLM and 0.1–200 μM for RLM) and berberine (1–500 μM for HLMs and 1–1000 μM for RLMs), at 37°C for 5 min. For rhCYP studies, rhCYP2D6*1/*1 or rhCYP2D6*10/*10 (8 pmol/mL) was preincubated with 2 μM VEN, ZJP (0.71–287.56 μg/mL for CYP2D6*1/*1 and 0.142–575.12 μg/mL for CYP2D6*10/*10) or its major components coptisine (0.1–100 μM for both CYP2D6s) and berberine (0.1–100 μM for CYP2D6*1/*1 and 0.05–100 μM for CYP2D6*10/*10), at 37°C for 5 min. All reactions were initiated with 10 mM NADPH, lasted for 15 min at 37°C, and were terminated with 200 μL of ice-cold acetonitrile. All reactions were quenched and treated as described above, and the supernatant was collected for LC–MS/MS analysis. The product formation data including ODV, NDV, or NODV was used for calculating enzymatic parameters.

Evaluation of the influences of ZJP on the metabolism and pharmacokinetics of VEN in rats

Twelve Wistar rats (male, 180–220 g) were randomly allocated into 2 groups (n = 6 in each group), which were blank control group and ZJP group. Twelve hours before the experiment, all rats were forbidden from eating except free access to water. Then, each rat was intragastrically administrated with normal saline or ZJP solution (2.52 g/kg, dissolved in saline) at a single dose. Two hours after administration, rats were sacrificed and liver samples were collected immediately on dry ice. All samples were stored at −80°C for further LC–MS/MS analysis.

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Another sixteen Wistar rats (male, 180–220 g) were also randomly divided into 2 groups (*n* = 8 in each group), which were blank control group and ZJP group. Each rat was intragastrically administrated with normal saline or ZJP solution (2.52 g/kg, dissolved in saline) once daily for 9 consecutive days. On the 9th day of administration, after 12-hour fasting but free access to water, the rats in ZJP group received VEN (2.63 mg/kg) as well as ZJP; while the rats in blank control group received the same volume of normal saline. Blood samples were collected in heparinized tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h after VEN administration, then centrifuged at 4000 g 4°C for 10 min, and the plasma was collected and stored at −80°C for LC–MS/MS analysis.

Determination of major bioactive components of ZJP in rat livers

Four major alkaloids (berberine, coptisine, evodiamine, and rutaecarpine) of ZJP in rat livers were quantitatively determined by a validated LC–MS/MS method. The LC–MS/MS system contained an LC20AD liquid chromatography (Shimadzu, Kyoto, Japan) and an API 4000 QTRAP triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed on an Agilent Eclipse XDB C_{18} reversed-phase column (150×4.6 mm, 5 μm) at room temperature. The gradient of the mobile phase consisting of water (0.08% formic acid and 4 mM ammonium acetate) (A) and acetonitrile (B) with a flow rate of 0.8 mL/min was set as follows: 0–3 min: 65% A, 3–4.5 min: 65–20% A; 4.5–9 min: 20% A; 9–11 min: 20–65% A; 11-15 min: 65% A. Positive ionization mode and multiple reaction monitoring mode were selected for quantification. The parameters of mass spectrometry for each analyte were shown in Supplementary Table 2. The intra- and inter-day precision and accuracy of the

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quality control samples for each analyte were < 15% (Supplementary Table 3). All analytes were stable in the measurement circumstances (Supplementary Table 4).

Determination of VEN and its three metabolites in in vitro and in vivo samples

VEN and its three metabolites (ODV, NDV, and NODV) from in vitro samples, such as HLMs, RLMs, or hrCYP2D6 reaction samples, and in vivo samples such as rats' plasma and liver samples were quantitatively determined by a validated LC–MS/MS method. The LC–MS/MS system contained an Agilent-1260 liquid chromatograph (Agilent Technologies, Santa Clara, CA) and an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with an electrospray ionization (ESI) source. Chromatographic separation was performed on a Phenomenex Kinetex XB-C18 reversed-phase column (100×4.6 mm, 5 μm) at 40^{o}C. The gradient of the mobile phase consisting of water (0.1% formic acid and 5 mM ammonium acetate) (A) and methanol (B) with a flow rate of 1 mL/min was set as follows: 0–1 min: 60% A, 1–4 min: 60–20% A; 4–5.5 min: 20% A; 5.5–5.6 min: 20–60% A; 5.6-8 min: 60% A. Positive ionization mode and multiple reaction monitoring mode were selected for quantification. The parameters of mass spectrometry for each analyte were shown in Table 1. Linear calibration curves for VEN, ODV, NDV, and NODV were constructed in relative matrices with concentrations ranging 2.5–2000 nM, 0.35–280 nM, 0.35–280 nM, and 0.7–560 nM, respectively. The intra- and inter-day precision and accuracy of the quality control samples for each analyte were < 15% (Supplementary Table 5).

Data analysis

The elimination half-life ($T_{1/2}$) of VEN was estimated as the following:

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$$k = -\ln([S]_t/[S]_0)/t \quad (1)$$

$$T_{1/2} = 0.693/k \quad (2)$$

where t referred to the incubation time, S represented the remaining parent drug concentration and k indicated the first-order rate constant (Obach et al., 1997).

The intrinsic clearance (CL_{int}) of VEN determined in HLM and RLM was directly obtained from k as the following:

$$CL_{int} = k \times mL \cdot incubation / mg \cdot micorosomes \quad (3)$$

Apparent kinetic parameters [e.g. V (reaction velocity), K_m (Michaelis-Menten constant), and V_{max} (maximum reaction velocity)] of the *O*-demethylation metabolism of VEN were obtained by fitting the substrate concentrations and initial reaction rates using Michaelis-Menten equation as the following:

$$V = V_{max}[S]/(K_m+[S]) \quad (4)$$

$$CL_{int} = V_{max}/K_m \quad (5)$$

where S referred to substrate, V, V_{max} and K_m were the same as above.

IC_{50} (concentration with 50% inhibition) was calculated as the following:

$$Y = Bottom + (Top-Bottom)/(1+10\text{^}((LogIC_{50}-X)\times Hill\ Slope))$$

where X referred to log (inhibitor concentration), Y referred to percent inhibition, Top and Bottom referred to maximum and minimum value of Y, and Hill Slope meant curve slope.

Statistics

The data were expressed as mean ± S.D. One-way ANOVA analysis following the least significant difference method was used for the comparisons between control and test groups. A two-sided *P* value < 0.05 was considered statistically significant. Data analysis and graphs

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were generated using GraphPad Prism software (Version 7.0, Graphpad Software Inc., La Jolla, CA).

Results

Metabolic stability of VEN in HLM and RLM

After a 60-min incubation, the remaining VEN in RLM and HLM were 15.6% and 78.3%, respectively, indicating a faster phase I metabolism of VEN in rats than humans (Fig. 2). In both RLM and HLM, ODV was the primary metabolite (30.9% vs. 31.0%), whereas NDV was 6.3-fold higher in RLM than HLM (14.1% vs. 2.2%), indicating a species difference in the metabolism of VEN.

Enzymatic kinetics of VEN in HLM, RLM and rhCYPs

Since ODV was the dominant metabolite of VEN, we explored its generating rate to reflect the enzymatic kinetics of VEN in RLM, HLM and rhCYPs, respectively (Fig. 3, Table 2). In the RLM and HLM experiments, although K_m was very similar, the V_{max} of RLM was 6.6-fold higher than that of HLM. Hence, there was 2.1-fold increase comparing CL_{int} of VEN in RLM to that in HLM (Fig. 3A–3B). In the rhCYPs experiments, the two rhCYP2D6s with different alleles, *1/*1 and *10/*10, showed similar V_{max} values (0.014 *vs.* 0.013 nmol/min/pmol CYP), while the CYP2D6*10/*10 showed a 13.1-fold higher K_m value than that of CYP2D6 *1/*1, resulting in a 14.0-fold lower CL_{int} than CYP2D6*1/*1 (Fig. 3C–3D).

Influence of ZJP and its major components on the metabolism of VEN in HLM and RLM

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To explore whether ZJP could influence the demethylation process of VEN, ZJP and its main components, berberine and coptisine were incubated with VEN (1 μM) in HLM and RLM, respectively (Fig. 4). The results showed that ZJP significantly inhibited VEN depletion (94.5%) and the formation of its demethylation products, ODV, NDV and NODV (91.0%, 69.4%, and 66.5%, respectively), compared with the control group in HLM (all *P* values < 0.001) (Fig. 4A–4D). Coptisine and berberine demonstrated the similar tendency as ZJP. CYP2D6 was primarily responsible for the metabolism of VEN and the generation of ODV in HLM, which were inhibited by quinidine (QND, a specific CYP2D6 inhibitor) at inhibitory degrees of 96.5% and 95.6%, respectively. Meanwhile, NDV increased by 33.7% compared with the control group after QND treatment. In contrast, ketoconazole (KTZ, a specific CYP3A4 inhibitor) significantly inhibited the production of NDV and NODV (both $P < 0.001$), but without obvious effect on ODV ($P = 0.557$).

Similarly, the VEN depletion and formation of ODV and NODV significantly decreased after ZJP (48.3%, 33.0% and 78.9%, respectively) and coptisine (19.8%, 13.5% and 53.8%, respectively) treatment in RLM (all *P* values < 0.05) (Fig. 4E–4H). Meanwhile, NDV increased by 73.5%, 66.8%, and 86.3% in the presence of ZJP, coptisine, and berberine, respectively. Interestingly, ZJP exhibited very similar tendencies in either VEN depletion or its metabolites generation as that of Quinine (QNN), a specific inhibitor of rat CYP2D, and KTZ, indicating that CYP3A4 could also play an important role in the metabolism of VEN in rats.

IC_{50} of ZJP and its major components on the metabolism of VEN in HLM, RLM, and rhCYP2D6s

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To further disclose the inhibitory potency of ZJP and its major bioactive components on the metabolism of VEN, the inhibitory effects, IC_{50}, of ZJP, coptisine, or berberine on ODV production in microsomes or on rhCYP2D6s were investigated subsequently (Fig. 5). The individual IC_{50} values of ZJP, berberine and coptisine on the metabolism of VEN were shown in Table 3. Those results showed that ZJP, coptisine, and berberine could inhibit the *O*-demethylation of VEN, while showing little effect (IC_{50} > 280 μg/mL or 100 μM) on the *N*-demethylation process of VEN. In addition, ZJP and its two bioactive components exhibited higher inhibitory potency in HLM, compared with RLM. Meanwhile, coptisine was more potent than berberine in inhibiting the formation of ODV and NODV as it displayed lower IC_{50} values both in HLM and RLM (7.5% and 41.9%, respectively) than those of berberine. Both coptisine and berberine were potent inhibitors of rhCYP2D6*1/*1 and rhCYP2D6*10/*10 as IC_{50} values did not exceed 2.5 μM. As NDV and NODV were not detected in rhCYP2D6s but in microsomes, other CYPs rather than CYP2D6, were responsible for the *N*-demethylation process of VEN.

Inhibitory effects of ZJP on the metabolism of VEN and pharmacokinetics in rats

VEN and its major metabolites, ODV and NDV, were detected in rat plasma after intragastric administration of VEN alone or co-administration with ZJP (VEN+ZJP). The mean plasma concentration-time curves were plotted in Fig. 6. The pharmacokinetic parameters of VEN and its metabolites were presented in Table 4. Compared with the VEN group, the AUC_{0-24} for VEN and ODV increased obviously by 39.6% and 22.8%, respectively, in the VEN+ZJP group; meanwhile, AUC_{0-24} of NDV increased dramatically ($P < 0.05$) in the VEN+ZJP group.

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VEN and its metabolites ODV, NDV, and NODV, were detected in the liver 2h after intragastric administration of VEN alone or co-administration with ZJP (VEN+ZJP) (Fig. 7). Compared with the VEN group, after co-administration with ZJP, the ODV exposure in the liver decreased significantly by 57.2% (P = 0.014), while NDV exposure increased significantly by 72.7% in VEN+ZJP group (P = 0.014). Meanwhile, the ratio of ODV/VEN in the liver also decreased significantly by 37.7% (P = 0.018) upon ZJP administration.

Discussion

Depression is the leading cause of psychiatric disorders with an estimated 350 million people affected worldwide (Cipriani et al., 2018). Depression can be long-lasting or recurrent, and can dramatically affect people's ability to live a rewarding life. Venlafaxine (VEN), approved in 1995, is a classic antidepressant, but still stands up as one of the most efficacious and prescribed antidepressants, particularly for the treatment of selective serotonin reuptake inhibitors (SSRIs)-resistant depression (Cipriani et al., 2018). VEN undergoes extensive phase I metabolism and is primarily catalyzed by CYP2D6 to generate an active metabolite, *O*-desmethylvenlafaxine (ODV). The recommended therapeutic reference range for VEN is from 100 ng/mL to 400 ng/mL of active moiety (AM), which is calculated from the total amount of VEN and ODV (Hiemke et al., 2018). The metabolic ratio ODV/VEN is usually used as a tool to distinguish patients as poor metabolizers (PMs) or extensive metabolizers (EMs) of CYP2D6 (Lobello et al., 2010). When VEN is co-administrated with a CYP2D6 inhibitor such as paroxetine, both C_{max} and AUC of VEN are significantly elevated in patients, which might explain VEN responses/adverse events (Jiang et al., 2015). Although the

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discrepancy on the clinical implication of exposure changes to VEN and/or ODV is great still, a good knowledge of the mechanisms of potential DDI between VEN and a drug with CYP2D6 inhibition is necessary for assessing and minimizing clinical risks (Schoretsanitis et al., 2019).

Traditional Chinese medicines (TCMs) belong to herbal medicines and have a long history of usage in clinical practice. In Asia, TCMs are prescribed concomitantly with conventional medications (prescription and non-prescription) for treatments. However, the risk assessment of the pharmacokinetic interaction between TCMs and conventional drugs is insufficient, leading to safety and efficacy issues in contemporary pharmacotherapy. Zuojin Pill (ZJP) is a wildly used traditional Chinese herbal formula recorded in the Chinese Pharmacopoeia for routine treatments of gastrointestinal disorders (Commission, 2015). It is commonly prescribed to patients with indications of gastritis, such as gastric ulcer, pyloric obstruction, gastroesophageal reflux disease, etc. It is also beneficial for depression with maladjusted gastrointestinal function (Wang et al., 2020). ZJP consists of two commonly used herbs, *Rhizoma Coptidis* and *Fructus Evodiae* following a 6:1 (w/w) ratio. *Rhizoma Coptidis* is reported to inhibit CYP2D6 activity with an IC_{50} value of 5.8 μg/mL of its extracted powder (approximately equals to 30.4 μg/mL of crude herbs) in HLM (Han et al., 2011). The inhibitory effect might be mostly attributable to Coptis alkaloid components, especially coptisine, with an IC_{50} value of 4.4 μM (Han et al., 2011). Moreover, while ZJP is co-administrated with dextromethorphan, a known CYP2D6 substrate, the $AUC_{0\text{-}24h}$ of dextromethorphan in healthy volunteers with dominant CYP2D6 phenotype (CYP2D6*1/*1) increases 3.0-fold higher than those administrated with dextromethorphan only (Qiu et al.,

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2016). With the high likelihood of combination usage of the two conventional prescriptions ZJP and VEN for treating depression patients with gastrointestinal disorders in China and potential herb-drug interaction (Qiu et al., 2015; Wang et al., 2020), we for the first time investigated the pharmacokinetic influences of ZJP on VEN. In this study, we found that ZJP significantly inhibit the metabolism of VEN in vitro. After co-incubation of VEN and ZJP for 1 h, the VEN depletion and ODV formation decreased significantly by 94.5% and 91.0%, respectively, compared with the blank control in HLM, and similarly, by 48.3% and 33.0% respectively, compared with the blank group in RLM. It was supported by IC_{50} values of ZJP determined by ODV formation with 30.5 μg/mL and 129.9 μg/mL for HLM and RLM, respectively. Given that ignoring the minor inhibitory effects of *Fructus Evodiae* on CYP2D6, the IC_{50} values of ZJP (approximately equals to 26.2 μg/mL of crude herbs of *Rhizoma Coptidis*) on VEN is similar to that reported on dextromethorphan (30.4 μg/mL of crude herbs of *Rhizoma Coptidis*) (Han et al., 2011). We also observed that ZJP inhibited the pharmacokinetics and tissue distribution of VEN in rats. ZJP increased AUC_{0-24} of VEN by 39.6% in rats, and two hours after co-administration of VEN with ZJP, the hepatic exposure of ODV was reduced by 57.2% ($P = 0.014$). Those findings suggested a potential human DDI between ZJP and VEN, although not firmly supported by some DDI data in rats. In addition, it was worth mentioning that due to focuses on the plasma exposure of VEN (between 100 ng/mL and 400 ng/mL) in clinical practice which was far below the K_m values obtained both in HLM and RLM, we selected a clinically relevant VEN concentration, e.g. 0.5μM or 1 μM, for IC_{50} experiments.

Species difference is probably the reason for weak evidence of DDI potential between ZJP

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and VEN in rats. CYP2D isoforms between rats and humans share a good homology (> 70%) (Venhorst et al., 2003). Rat CYP2D1 is known as an ortholog (approximately 83% homology) of human CYP2D6 (Martignoni et al., 2006). However, when comparing the metabolic capability of VEN, the two species are obviously different. In literatures, the C_{max} and $AUC_{0-\infty}$ of VEN in PMs increased by approximately 70–80% and 200–300%, respectively, compared with those of EMs (Lessard et al., 1999; Lobello et al., 2010). In contrast, the C_{max} and $AUC_{0-\infty}$ of VEN in CYP2D1-null rats only increased by 24% and 59% respectively, compared with those of wild type rats, indicating an apparently weaker inhibitory effect involved in CYP2D6 inhibition in rats than humans (Zhou et al., 2019). Similarly, in the study, we observed that the capability and magnitude of inhibitory effects of ZJP were not comparable between HLM and RLM. First, the metabolic characteristics of VEN were different between the two species. CYP2D6 was the sole principle CYP enzyme responsible for VEN metabolism in HLM, but not in RLM. After 1 h incubation of VEN, ODV (generated primarily by CYP2D6) was the major metabolite (31.0% of spiked VEN) and NDV (generated primarily by CYP3A4 and CYP2C19) was minor metabolite (2.2% of spiked VEN) in HLM. In contrast, ODV and NDV were both major metabolites in RLM, with 30.9% and 14.1% of formation rate percentages, respectively. Second, the inhibitory effects of CYP2D6 inhibitor on ODV formation in rats were weaker than those in humans. Quinidine, a known human CYP2D6 inhibitor, inhibited VEN depletion (96.5%) and ODV formation (95.6%) completely in HLM. In contrast, quinine, the quinidine enantiomer and a known rat CYP2D6 inhibitor, inhibited VEN depletion (58.4%) and ODV formation (50.6%) partly in RLM. The inhibitory efficacy (IC_{50} values obtained from ODV formation) of the potent

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CYP2D6 inhibitor in HLM was greater than that in RLM (10.0 μM *vs* 31.5 μM) (Supplementary Figure 1). Taken together, CYP2D6 (in fact CYP2D1 in rat) did not play a dominant role in VEN metabolism in RLM as it did in HLM, and there were possibly other CYPs involved in ODV formation in RLM. In the study, we observed that ketoconazole, a known CYP3A4 inhibiter, could also significantly inhibit ODV formation in RLM but it showed negligible inhibition in HLM. Therefore, the underestimated risks of predicting the magnitude of DDI between ZJP and VEN in human through rat DDI data based only on the enzymatic activity of CYP2D6 should be seriously noted. Considering that ZJP could increase the $AUC_{0\text{-}24h}$ of dextromethorphan, a sensitive index substrate of CYP2D6, in healthy CYP2D6*1/*1 carriers by 3.0-fold greater, further studies were merited to explore ZJP and VEN interaction in humans (Qiu et al., 2016).

Similar to previous researches, we had determined extremely low systemic exposures for the four major alkaloid constituents，berberine，coptisine, evodiamine and rutaecarpine, in rats after intragastric administration of ZJP. Only berberine could be detected in some time intervals, and the average of C_{max} was below 50 ng/mL; while the other three alkaloids, coptisine, evodiamine and rutaecarpine, were detected below the limit of detection (LOD) of 1.0 ng/mL during the whole blood sampling period (data not shown). The observation showed strong consistency with previous researches that those alkaloids possessed the characteristics of low bioavailability either in animals or humans (Yan et al., 2011; Qian et al., 2017; Wang et al., 2018; Zhang et al., 2018). For example, after single oral administration of *Rhizoma Coptidis* granules in healthy volunteers (which contains 20 mg/kg of berberine and is approximately 1.8-fold higher than rat dosage in the animal experiment when converted

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equivalently refers to body surface area), the average plasma C_{max} of berberine is still as low as 360.9±46.1 ng/mL (Huang et al., 2011). Thus, an interesting question will be raised as to why there is increasing evidence that many herbs containing active alkaloids with low bioavailability have pharmacokinetic interactions with other co-administrated drugs. The underling mechanism may be multiple principles involving one or more processes of absorption, disposition, metabolism, and excretion (ADME). In this study, we found that the concentrations of Coptis alkaloids berberine and coptisine in liver tissue were much higher (> 10.0-folder) than those in plasma, which was in accordance with the literature (Liu et al., 2010). Although the concentration of single alkaloids in the rat liver (e.g. 25.5 and 3.1 mg/g for berberine and coptisine, respectively) for CYP2D6 mediated metabolism of VEN was slightly lower than the IC_{50} (e.g. 21.7 and 8.6 mg/g for berberine and coptisine, respectively), considering the structural and mechanistic similarities of the alkaloids in ZJP, whether or not determined, the synergistic effects should be considered seriously. Furthermore, several Coptis alkaloids (such as berberine) might have cumulative effects with multiple dosing (Ma and Ma, 2013). This might be one of the main reasons for the herb-drug interaction induced by the herbs containing Coptis alkaloids.

In conclusion, Zuojin Pill has significant inhibitory effects on hepatic metabolism and pharmacokinetics of venlafaxine in vitro and in rats mainly through suppression of CYP2D6 activity. In view of the species differences in the role of CYP2D6 involved with venlafaxine metabolism, the human pharmacokinetic interaction between Zuojin Pill and venlafaxine and its related clinical significance need to be seriously considered.

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Authorship Contributions

Participated in research design: Tan, Qiu.

Conducted experiments: Tan, Y. Li, J. Li, Yan, Wang, and Jin.

Performed data analysis: Tan, Y. Li, and Qiu.

Wrote or contributed to the writing of the manuscript: Y. Li, Tan, and Qiu.

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Reference

Cipriani A, Furukawa TA, Salanti G, Chaimani A, Atkinson LZ, Ogawa Y, Leucht S, Ruhe HG, Turner EH, Higgins JPT, Egger M, Takeshima N, Hayasaka Y, Imai H, Shinohara K, Tajika A, Ioannidis JPA, and Geddes JR (2018) Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. *Lancet* **391:**1357-1366.

Chinese Pharmacopoeia Commission (2015) Zuojin Wan, in: *The Pharmacopoeia of the People's Republic of China* pp 763-764, China Medical Science Press, Beijing.

Fogelman SM, Schmider J, Venkatakrishnan K, von Moltke LL, Harmatz JS, Shader RI, and Greenblatt DJ (1999) O- and N-demethylation of venlafaxine in vitro by human liver microsomes and by microsomes from cDNA-transfected cells: effect of metabolic inhibitors and SSRI antidepressants. *Neuropsychopharmacology* **20:**480-490.

Gao X, Yang X, and Marriott P (2010) Simultaneous analysis of seven alkaloids in Coptis-Evodia herb couple and Zuojin pill by UPLC with accelerated solvent extraction. *J Sep Sci* **33:**2714-2722.

Han Y, Yu H, Li D, Meng X, Zhou Z, Yu Q, Zhang X, Wang F, and Guo C (2011) In vitro inhibition of Huanglian [Rhizoma coptidis (L.)] and its six active alkaloids on six cytochrome P450 isoforms in human liver microsomes. *Phytother Res* **25:**1660-1665.

Hiemke C, Bergemann N, Clement HW, Conca A, Deckert J, Domschke K, Eckermann G, Egberts K, Gerlach M, Greiner C, Grunder G, Haen E, Havemann-Reinecke U, Hefner G, Helmer R, Janssen G, Jaquenoud E, Laux G, Messer T, Mossner R, Muller MJ, Paulzen M, Pfuhlmann B, Riederer P, Saria A, Schoppek B, Schoretsanitis G, Schwarz M, Gracia MS, Stegmann B, Steimer W, Stingl JC, Uhr M, Ulrich S, Unterecker S, Waschgler R, Zernig G, Zurek G, and Baumann P (2018) Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry* **51:**e1.

Huang Z, Lu F, Dong H, Xu L, Chen G, Zou X, and Lei H (2011) Effects of cinnamon granules on pharmacokinetics of berberine in Rhizoma Coptidis granules in healthy

male volunteers. *J Huazhong Univ Sci Technol Med Sci* **31:**379-383.

Jiang F, Kim H, Na H, Lee S, Seo D, Choi J, Ha J, Shin H, Kim Y, and Chung M (2015) The influences of CYP2D6 genotypes and drug interactions on the pharmacokinetics of venlafaxine: exploring predictive biomarkers for treatment outcomes. *Psychopharmacology (Berl)* **232:**1899-1909.

Johnson EJ, Gonzalez-Perez V, Tian DD, Lin YS, Unadkat JD, Rettie AE, Shen DD, McCune JS, and Paine MF (2018) Selection of Priority Natural Products for Evaluation as Potential Precipitants of Natural Product-Drug Interactions: A NaPDI Center Recommended Approach. *Drug Metab Dispos* **46:**1046-1052.

Lessard E, Yessine MA, Hamelin BA, O'Hara G, LeBlanc J, and Turgeon J (1999) Influence of CYP2D6 activity on the disposition and cardiovascular toxicity of the antidepressant agent venlafaxine in humans. *Pharmacogenetics* **9:**435-443.

Liu S, Qiu F, He M, Miu P, Zeng J, and Jiang J (2014) Research of Constituents Metabolism and Effect for CYP450 of Zuojin Pill. *Acta Chinese Medicine* **29:**1622-1625.

Liu Y, Hao H, Xie H, Lai L, Wang Q, Liu C, and Wang G (2010) Extensive intestinal first-pass elimination and predominant hepatic distribution of berberine explain its low plasma levels in rats. *Drug Metab Dispos* **38:**1779-1784.

Lobello K, Preskorn S, Guico-Pabia C, Jiang Q, Paul J, Nichols A, Patroneva A, and Ninan PJJCP (2010) Cytochrome P450 2D6 phenotype predicts antidepressant efficacy of venlafaxine: a secondary analysis of 4 studies in major depressive disorder. *J Clin Psychiatry* **71:**1482-1487.

Ma B and Ma Y (2013) Pharmacokinetic properties, potential herb-drug interactions and acute toxicity of oral Rhizoma coptidis alkaloids. *J Expert Opin Drug Metab Toxicol* **9:**51-61.

Magalhães P, Alves G, LLerena A, and Falcão A (2015) Clinical drug-drug interactions: focus on venlafaxine. *Drug Metab Pers Ther* **30:**3-17.

Martignoni M, Groothuis G, and de Kanter R (2006) Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. *Expert Opin Drug Metab Toxicol* **2:**875-894.

Nicolussi S, Drewe J, Butterweck V, and Meyer Zu Schwabedissen H (2020) Clinical

relevance of St. John's wort drug interactions revisited. *Br J Pharmacol* **177:**1212-1226.

Obach RS, Baxter JG, Liston TE, Silber BM, Jones BC, MacIntyre F, Rance DJ, and Wastall P (1997) The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. *J Pharmacol Exp Ther* **283:**46-58.

Otton SV, Ball SE, Cheung SW, Inaba T, Rudolph RL, and Sellers EM (1996) Venlafaxine oxidation in vitro is catalysed by CYP2D6. *Br J Clin Pharmacol* **41:**149-156.

Paine M and Oberlies N (2007) Clinical relevance of the small intestine as an organ of drug elimination: drug-fruit juice interactions. *Expert Opin Drug Metab Toxicol* **3:**67-80.

Qian P, Zhang Y-B, Yang Y-F, Xu W, and Yang X-W (2017) Pharmacokinetics Studies of 12 Alkaloids in Rat Plasma after Oral Administration of Zuojin and Fan-Zuojin Formulas. *Molecules* **22:**214.

Qiu C, Cui Y, Qi X, Jiang H, and Wang Q (2015) Advance in modern studies on compatibility of Coptidis Rhizoma and Evodiae Fructus. *Zhongguo Zhong Yao Za Zhi* **40:**582-587.

Qiu F, Liu S, Miao P, Zeng J, Zhu L, Zhao T, Ye Y, and Jiang J (2016) Effects of the Chinese herbal formula "Zuojin Pill" on the pharmacokinetics of dextromethorphan in healthy Chinese volunteers with CYP2D6*10 genotype. *Eur J Clin Pharmacol* **72:**689-695.

Schoretsanitis G, Haen E, Grunder G, Hiemke C, Endres K, Ridders F, Correll CU, and Paulzen M (2019) Pharmacokinetics of venlafaxine in treatment responders and non-responders: a retrospective analysis of a large naturalistic database. *Eur J Clin Pharmacol* **75:**1109-1116.

Venhorst J, ter Laak A, Commandeur J, Funae Y, Hiroi T, and Vermeulen N (2003) Homology modeling of rat and human cytochrome P450 2D (CYP2D) isoforms and computational rationalization of experimental ligand-binding specificities. *J Med Chem* **46:**74-86.

Wang L, Tang Y, Liu X, Ge Y, and Li W (2013) Research on Chinese medicine pairs (VI)--Coptidis Rhizoma-Euodiae fructus. *Zhongguo Zhong Yao Za Zhi* **38:**4214-4219.

Wang T, Yan Y, Yang L, Huang Y, Duan X, Su K, and Liu W (2020) Effects of Zuojin pill on depressive behavior and gastrointestinal function in rats with chronic unpredictable mild stress: Role of the brain-gut axis. *J Ethnopharmacol* **254:**112713.

Wang Y, Zhang Y, Xiao J, Xu R, Wang Q, and Wang X (2018) Simultaneous determination of baicalin, baicalein, wogonoside, wogonin, scutellarin, berberine, coptisine, ginsenoside Rb1 and ginsenoside Re of Banxia xiexin decoction in rat plasma by LC-MS/MS and its application to a pharmacokinetic study. *Biomed Chromatogr* **32:**e4083.

Yan R, Wang Y, Shen W, Liu Y, and Di X (2011) Comparative pharmacokinetics of dehydroevodiamine and coptisine in rat plasma after oral administration of single herbs and Zuojinwan prescription. *Fitoterapia* **82:**1152-1159.

Zhang Q, Wang G, Han Z, Chen X, Na R, Jin H, Li P, and Bu R (2018) Metabolic profile of Rhizoma coptidis in human plasma determined using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry. *Rapid Commun Mass Spectrom* **32:**63-73.

Zhou H, Yang L, Wang C, Li Z, Ouyang Z, Shan M, Gu J, and Wei Y (2019) CYP2D1 Gene Knockout Reduces the Metabolism and Efficacy of Venlafaxine in Rats. *Drug Metab Dispos* **47:**1425-1432.

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Footnotes

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Figure Legends

Fig. 1. The primary metabolic pathway of venlafaxine (VEN) in human

Fig. 2. The remaining VEN and generated demethylation metabolites, ODV, NDV and NODV, after 60-min incubation of VEN in HLM (A) or RLM (B). Data for each component were normalized to spiked VEN concentration in reaction mixtures, and represented as mean ± S.D. in triplicates.

Fig.3. Michaelis-Menten plots for formation of ODV by RLM (A), HLM (B), CYP2D6*1/*1 (C), and CYP2D6*10/*10 (D). Data were presented as mean ± S.D. in triplicates.

Fig.4. Inhibitory effects of ZJP (150 μg/mL), coptisine (CPS, 30 μM), berberine (BBR, 30 μM), quinine (QNN, 2 μM), quinidine (QND, 2 μM) and ketoconazole (KTZ, 2 μM) on the metabolism of VEN and formation of ODV, NDV and NODV in HLM (A–D) and RLM (E–H). Each column represented the remaining VEN (% control) or product formation (% spiked VEN). Data were presented as mean ± S.D. in triplicates. * represents $P < 0.05$ compared with the control.

Fig.5. Inhibitory effects (IC_{50}) of ZJP (A–D), coptisine (E–H), and berberine (I-L) on ODV formation in HLM, RLM, CYP2D6*1/*1, and CYP2D6*10/*10, respectively. Data were presented as mean ± S.D. in triplicates. The curve represented the fitting of the observed

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ODV formation rate (% control) (y) versus the inhibitor concentration (x).

Fig.6. Mean plasma concentration-time profiles of VEN (A), ODV (B), and NDV (C) after intragastric administration of VEN alone or co-administration with ZJP (VEN+ZJP) in rats (n = 6). Data were presented as mean ± S.D.

Fig.7. Hepatic exposures of VEN (A), ODV (B), NDV (C), NODV (D), and ODV/VEN ratio (E) at 2h after intragastric administration of VEN alone or co-administration with ZJP (VEN+ZJP) in rats (n = 6). Data were presented as mean ± S.D. * represented $P < 0.05$ compared with the VEN group.

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Tables

Table 1. Mass spectrometry parameters for measuring VEN and its metabolites, ODV, NDV, and NODV

Analytes	Transition (*m/z*)	Spray voltage (V)	Temp. (^{o}C)	DP (V)	CE (V)	CXP (V)	EP (V)
VEN	278.2>58.1	5500	400	46	46	12	10
ODV	264.2>58.1	5500	400	46	46	12	10
NDV	264.2>246.1	5500	400	35	14	12	10
NODV	250.2>232.1	5500	400	30	14	12	10
diphenhydramine	256.0>167.1	5500	400	46	25	12	10

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Table 2. Enzymatic kinetics parameters of VEN in RLM, HLM, and rhCYP2D6s

subject	K_m (μM)	V_{max} (nmol/min/mg protein or nmol/min/pmol CYP)	CL_{int} (μL/min/mg protein or μL/min/pmol CYP)
RLM	41.2±23.4	0.480±0.013	11.7±5.5
HLM	39.9±1.1	0.182±0.004	5.7±0.2
rhCYP2D6*1/*1	11.3±3.2	0.014±0.003	1.2±0.3
rhCYP2D6*10/*10	148.2±26.5	0.013±0.002	0.088±0.016

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Table 3. IC_{50} of ZJP, berberine and coptisine towards VEN metabolism in microsomes and rhCYP2D6

	Inhibitors	IC_{50} value		
		ODV	NDV	NODV
RLM	ZJP (μg/mL)	129.9±2.6	>575	42.8±8.0
	Berberine (μM)	64.5±11.5	>1000	59.5±18.6
	Coptisine (μM)	27.0±6.2	>200	16.9±0.1
HLM	ZJP (μg/mL)	30.5±5.5	>288	11.5±10.2
	Berberine (μM)	30.5±9.6	>500	>500
	Coptisine (μM)	2.3±1.3	>100	>100
rhCYP2D6*1/*1	ZJP (μg/mL)	15.4±4.5	N/A	N/A
	Berberine (μM)	2.5±0.9		
	Coptisine (μM)	0.7±0.2		
rhCYP2D6*10/*10	ZJP (μg/mL)	2.3±1.0	N/A	N/A
	Berberine (μM)	1.5±0.2		
	Coptisine (μM)	2.2±0.2		

N/A represented not applicable.

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Table 4. Pharmacokinetic parameters of VEN, ODV, and NDV in rats after oral administration of VEN alone or VEN combined with ZJP

Analyte	Groups	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	CLz/F (L/h/kg)	AUC_{0-24} (μg/L×h)
VEN	VEN	3.47±0.80	1.0±0.6	2.4±2.2	231.7±18.6	11.1±0.9
	VEN+ZJP	2.01±0.85*	3.1±1.8*	4.0±2.2	198.7±151.3	15.5±7.6
ODV	VEN	4.82±4.18	1.1±0.9	6.2±3.3	172.7±123.5	19.3±11.3
	VEN+ZJP	3.41±2.00	2.1±1.4	10.9±6.5	116.8±66.8	23.7±12.8
NDV	VEN	1.24±0.90	2.0±1.8	5.1±2.9	468.0±208.7	6.45±4.34
	VEN+ZJP	1.91±1.02	3.6±2.2	6.1±4.5	215.4±132.1*	13.5±5.7*

* represented $P < 0.05$ compared to VEN group.

Figure 1

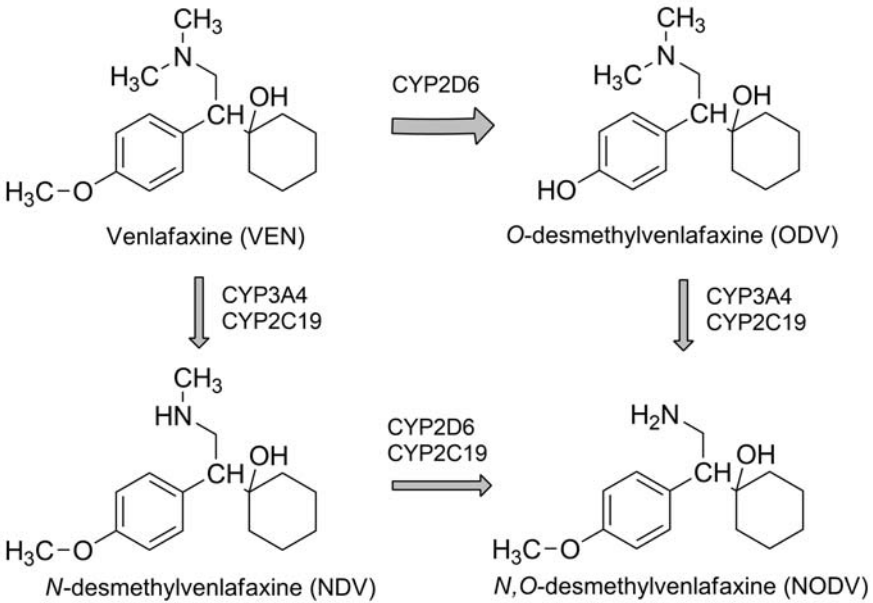

CH3
N
H3C
CH
OH
H3C–O
Venlafaxine (VEN)
CYP2D6
HO
O-desmethylvenlafaxine (ODV)
CYP3A4
CYP2C19
HN
N-desmethylvenlafaxine (NDV)
CYP2D6
CYP2C19
H2N
N,O-desmethylvenlafaxine (NODV)

Figure 2

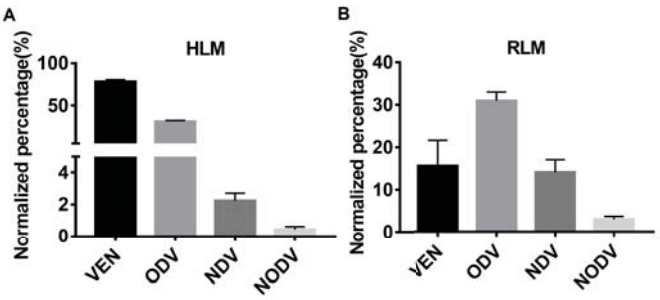


Figure 3

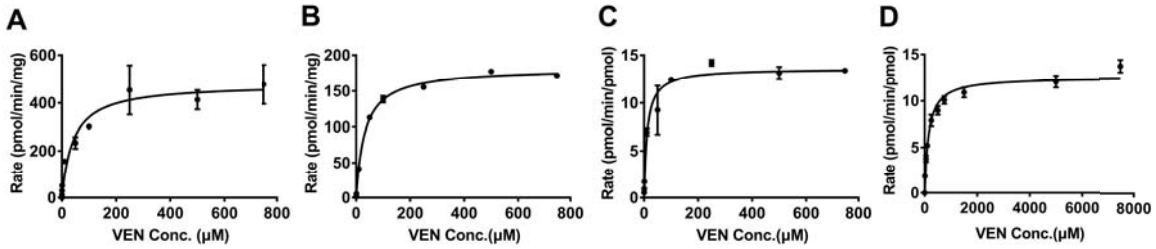


Figure 4

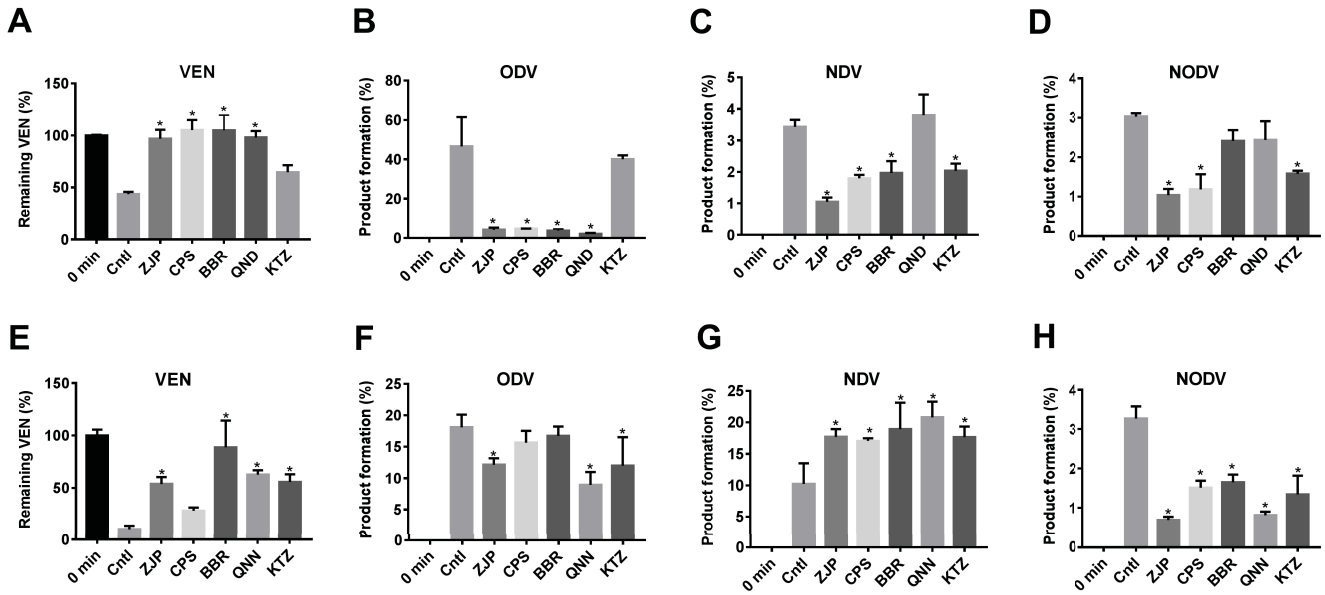


Figure 5

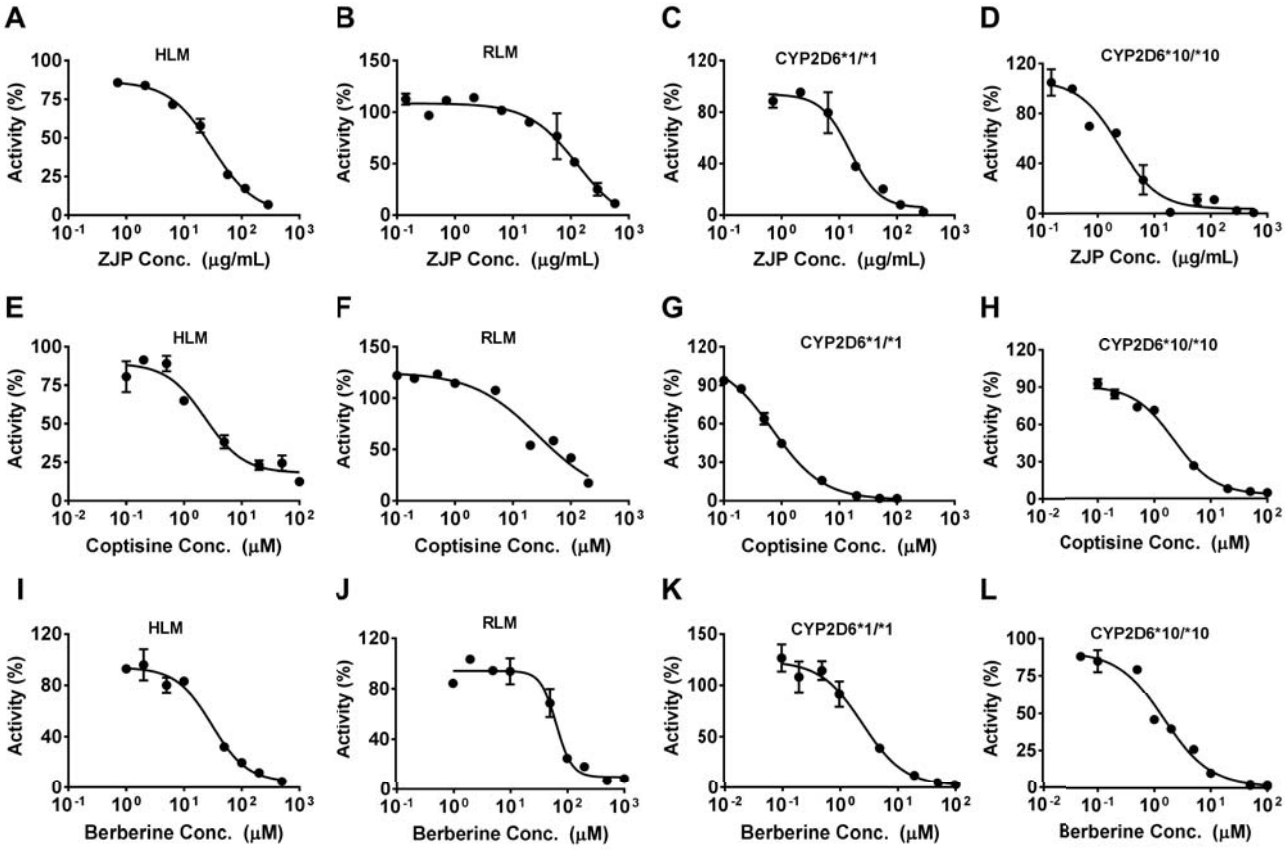


Figure 6

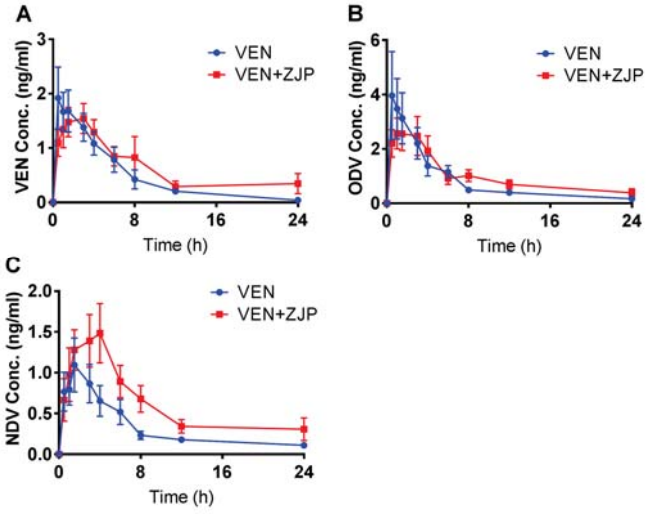
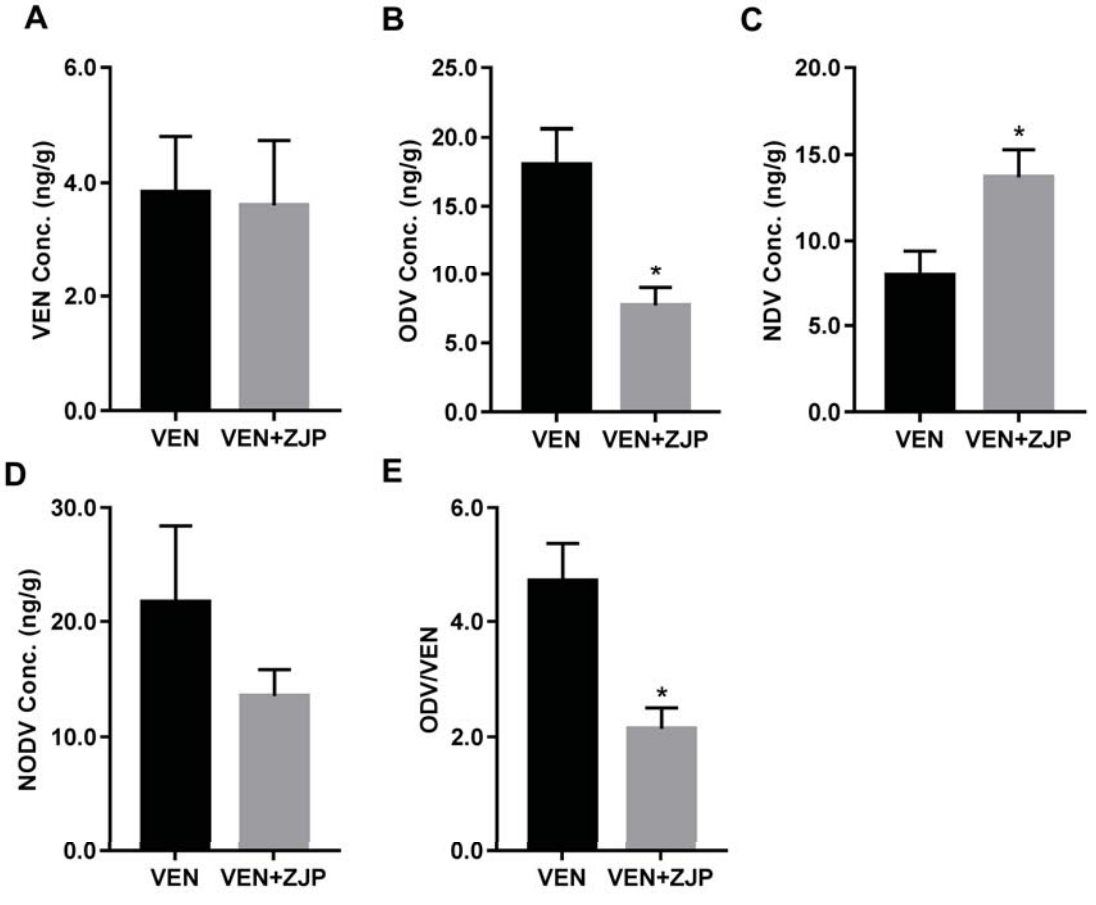


Figure 7


A
VEN Conc. (ng/g)
6.0
4.0
2.0
0.0
VEN
VEN+ZJP
B
ODV Conc. (ng/g)
25.0
20.0
15.0
10.0
5.0
0.0
*
VEN
VEN+ZJP
C
NDV Conc. (ng/g)
20.0
15.0
10.0
5.0
0.0
*
VEN
VEN+ZJP
D
NODV Conc. (ng/g)
30.0
20.0
10.0
0.0
VEN
VEN+ZJP
E
ODV/VEN
6.0
4.0
2.0
0.0
*
VEN
VEN+ZJP