Effect Of Chinese Herb Danzhi Xiaoyao Pills On Pharmacokinetics Of Venlafaxine In Beagles

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Objective: To investigate the effects of Chinese herb Danzhi Xiaoyao pills on the pharmacokinetics of venlafaxine and its metabolites O-desmethylvenlafaxine (ODV) and N-desmethylvenlafaxine (NDV) in beagles by using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).

Methods: Six beagles (half male, half female) were chosen to test, being fasted before the experiment but having free access to drinking water 1 day before being fed drugs. After oral administration of venlafaxine hydrochloride tablets (10.28 mg/kg), the blood samples were collected in succession at different points in time. After 1-week washout period, Danzhi Xiaoyao pills (0.6g/kg) were given through oral administration to the six beagles every morning until the 7th day, venlafaxine hydrochloride tablets (10.28 mg/kg) were given after feeding Danzhi Xiaoyao pills (0.6g/kg) half an hour and blood samples were collected continuously at different points. All samples were analyzed by UPLC-MS/MS, and the main pharmacokinetic parameters of venlafaxine, ODV and NDV were computed by DAS 2.0.

Results: The $C_{\text{max}}$ of the venlafaxine group (control group) and the combination group (experimental group) were (2267.26±252.89) ng/mL and (1542.64±190.73) ng/mL, respectively. The $\text{AUC}(0-\infty)$ of the two groups were (13,934.79±3609.23) ng·h/mL and (8001.91±2167.58) ng·h/mL, respectively. The ODV $C_{\text{max}}$ of the two groups were (2253.80±215.81) ng/mL and (2721.37±118.20) ng/mL, and $\text{AUC}(0-\infty)$ were (13,974.99±2784.04) ng·h/mL and (17,539.44±1894.29) ng·h/mL, respectively. The NDV $C_{\text{max}}$ of the two groups were (50.98±5.76) ng/mL and (58.74±12.33) ng/mL, and $\text{AUC}(0-\infty)$ were (179.26±34.94) ng·h/mL and (220.68±51.41) ng·h/mL, respectively. After administration of Danzhi Xiaoyao pills, the $C_{\text{max}}$ and $\text{AUC}(0-\infty)$ of venlafaxine decreased significantly, indicating that the plasma exposure of venlafaxine decreased. The increase of $C_{\text{max}}$ and $\text{AUC}(0-\infty)$ of ODV and NDV indicated a rise in plasma exposure.

Conclusion: Danzhi Xiaoyao pills can accelerate the metabolism of venlafaxine in beagles. In clinical, when venlafaxine was co-administrated with Danzhi Xiaoyao pills, dose adjustment of venlafaxine should be taken into account.

Keywords: beagle, UPLC-MS/MS, Danzhi Xiaoyao pills, venlafaxine, ODV, NDV, drug–drug interaction

Introduction

Depressive disorder, also known as major depressive disorder, is one of the symptoms of depression, anxiety, cognitive impairment, hallucination and suicide. According to the World Psychiatric Association (WPA), this disease has become the fourth largest disease in the world and will be the second largest by 2020. Venlafaxine hydrochloride (venlafaxine, molecular formula: $C_{17}H_{23}NO_2\cdot\text{HCl}$) is an important drug for clinical treatment of depression, which was approved by the US Food and Drug Administration (FDA) in 1993 and was the first SNRI (serotonin-norepinephrine reuptake inhibitor).
reuptake inhibitor) for the treatment of major depressive disorder in adults, mainly by inhibiting serotonin (5-HT) and norepinephrine (NE) reuptake dual function. Finally, metabolism is deactivated under the action of cytochrome P450 in liver, and the main metabolite is O-desmethylenlafaxine (ODV, molecular formula: C_{16}H_{25}NO_{2}), which is catalyzed by CYP2D6, while the minor metabolite is N-desmethylenlafaxine (NDV), which is catalyzed by CYP3A4. Both metabolites are eventually metabolized to N, O-didesmethylenlafaxine (DDV), which is possibly catalyzed by CYP2D6. Venlafaxine is a new kind of phenylethylamine antidepressant drug, and its molecular structure (A), ODV structure (B) and NDV structure (C) are shown in Figure 1. Meanwhile, the structure of Diazepam (D) which was regarded as IS is also shown in Figure 1.

Danzhi Xiaoyao pill is a classical traditional Chinese medicine, which had been recorded in Jiaozhu Furen Liangfang “Edited and Commented Effective Formulae for Woman” written by Xue Yi (1487–1559) since the Ming Dynasty, to treat the liver stagnation and qi stagnation, and its composition and dose of the prescription are listed in Figure 2. In recent years, many studies have shown that Danzhi Xiaoyao pills have an excellent effect on the treatment of depression. According to Chinese medicinal theory, Danzhi Xiaoyao pills take effect by regulating qi and blood, soothing liver-qi stagnation and adjusting the function of internal organs; however, due to the complex components of traditional Chinese medicine, it is still not clear what the main mechanism of action of its drugs is, which requires further researches in the later stage.

Nowadays, the combination of traditional Chinese medicine (TMC) and venlafaxine has been well applied to clinical practice. The combination method can not only reduce the side effects of western medicines, but also enhance the therapeutic effect. Clinically, Danzhi Xiaoyao pill and venlafaxine are commonly used in the treatment of depression. But most studies have focused on using HPLC methods to determine venlafaxine and its main active metabolite ODV in human plasma. There is no relevant research showing the effect of Danzhi Xiaoyao pill on the metabolism of venlafaxine. Therefore, we selected six healthy beagles and compared the pharmacokinetic differences between control groups (venlafaxine hydrochloride tablets) and experimental groups (venlafaxine hydrochloride tablets after 7 days of oral administration of Danzhi xiaoyao pills) to study the effect of Danzhi Xiaoyao pill on the pharmacokinetics of venlafaxine and its metabolites (ODV and NDV) in beagles, using the UPLC-MS/MS method.

### Materials And Methods

#### Animals

Six beagles (half male, half female, 5–6 kg) were supported by the Laboratory Animal Center of Henan University of Science and Technology (Luoyang, China). They were housed in a room at 16–28°C, with a 12/12-hr light/dark cycle, 40%–70% humidity and were fed twice every day, free access to water. All beagles were forbidden to eat 12hrs before the experiment, but could drink freely. The animals were authorized by Animal Ethics Committee of Henan University of Science and Technology and also were cared on the basis of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

#### Chemicals Materials

Venlafaxine hydrochloride tablets (batch number: 180508) were acquired from Changzhou Siyao Pharmaceuticals Co., Ltd (Changzhou, China). Danzhi Xiaoyao pills (batch number: 280827) were obtained from Kunming Pharmaceutical Corp. (Kunming, China). Venlafaxine (purity >99%, batch number: 100543-200401) were bought from National Institutes for Food and Drug Control (Beijing, China). ODV (purity...
>98%, batch number: M1223A) was bought from Dalian Meilun Biotechnology Co., Ltd (Dalian, China). NDV (purity >97%, batch number: 9-JWA-51-1) was provided by Chem Strong Scientific Co., Ltd (Shenzhen, China). Diazepam (purity > 98%, batch number: 1230-9601) was supplied by National Narcotics Laboratory (Beijing, China) and used as the internal standard (IS). A water purification system (Milli-Q academic reagent grade, Millipore, Bedford, USA) was used to prepare deionized water. Acetonitrile and methanol of liquid chromatography (LC) grade were acquired from Merck Company (Darmstadt, Germany). All other reagents were of analytical grade or better.

Instrumentation And Conditions
The Acquity ultra-performance liquid chromatography (UPLC, Waters Corp., Milford, MA, USA) system was used for analysis. Separation by chromatography was achieved by an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm) at 45 °C. The gradient elution solutions were water with 0.1% formic acid (A) and acetonitrile (B). The following gradient protocol was used: 0–0.5 mins (90-90% A), 0.5–1.0 mins (90-10% A), 1.0–2.0 mins (10-10% A), 2.0–2.1 mins (10-90% A), 2.1–3.0 min (90-90% A). The total run time was set at 3.0 mins and 0.40 mL/min was the flow rate.

Mass spectrometric measurement was achieved on a XEVO TQ-S triple quadrupole mass spectrometer with an electrospray ionization (ESI) interface in positive ionization mode. Quantification was achieved by multiple reaction monitoring (MRM) mode with transitions of m/z 278.1 → 58.1 for venlafaxine, m/z 264.2 → 58.1 for ODV, m/z 264.2 → 147.0 for NDV and m/z 285.0 → 154.0 for IS, respectively. Data acquisition and control of instrument were done by Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

Standard Solutions, Calibration Standards, And Quality Control (QC) Samples
The standard venlafaxine solution (1 mg/mL) was obtained by accurately weighing 10 mg of standard venlafaxine in a 10-mL
volumetric flask and dissolving it with methanol to a constant volume of 10 mL. The ODV reserve solution (1 mg/mL) was obtained by accurately weighing 1 mg and adding 1 mL methanol in a 2-mL EP tube. The NDV reserve solution (1 mg/mL) was also obtained by accurately weighing 1 mg and adding 1 mL methanol in a 2-mL EP tube. The standard substance of diazepam, 25 mg, was accurately weighed in a 25-mL volumetric flask, dissolved in methanol and then in a constant volume to 25 mL. The final concentration was 1 mg/mL, which was diluted with acetonitrile to form an internal standard working solution with a concentration of 50 ng/mL. All solution samples were stored at 4°C.

Study Design
Six beagles were given venlafaxine hydrochloride tablets (10.28 mg/kg) through oral administration and then given normal saline (NS, 10~20 mL) immediately through gavage administration; the blood samples (2 mL) were collected from foreleg vein or hind vein into heparinized polythene tubes at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hrs. All samples were centrifuged at 10,000×g for 10 mins. The acquired plasma (100 μL) was saved at −20°C until the test. After the 1-week washout period, six beagles were given Danzhi Xiaoyao pills (0.6 g/kg) through oral administration in the everyday morning until the 7th day, giving venlafaxine hydrochloride tablets (10.28 mg/kg) after feeding Danzhi Xiaoyao pills for half an hour. The blood samples (2 mL) were also collected from foreleg vein or hind vein into heparinized polythene tubes at the same point in time and were handled with the same conditions.

Method Validation
Before using this method to confirm venlafaxine, ODV and NDV in plasma, the method was totally validated for specificity, linearity, precision, accuracy, recovery, and stability. The UPLC-MS/MS method in this experiment was validated according to the United States Food and Drug Administration (FDA) guidelines.22

Preparation Of Samples
Samples were prepared by protein precipitation. In brief, 10 μL IS working solution (50 ng/mL) was added to 50 μL plasma in a 1.5-mL Eppendorf tube and followed by vortexing for 15 s. The mixture was precipitated by addition of 200 μL acetonitrile and then vortexed for 1 min. Finally, the plasma was centrifuged at 15,000×g for 15 mins to obtain the supernatant, and a 2 μL supernatant was injected for analysis by the UPLC-MS/MS system.

Specificity
To estimate the methodological specificity, three groups samples were chosen to be analysed by the UPLC-MS/MS. A blank plasma which included venlafaxine, ODV and NDV standard substance (a). A blank plasma with internal standard substance (b). A plasma collected after oral administration of venlafaxine hydrochloride tablets after 2 hrs (c). All plasma samples were collected from beagles. The three groups samples chromatograms were compared to judge the existence of endogenous substances in plasma.

Linearity
Concentration of venlafaxine for 1, 5, 10, 50, 100, 500, 1000, 2000 ng/mL, concentration of ODV for 1, 5, 10, 50, 100, 500, 1000, 2000 ng/mL and concentration of NDV for 0.1, 0.5, 1, 5, 10, 50, 100, 200 ng/mL were prepared, and after treatment, the linearity of each standard calibration curve was computed by plotting the peak areas rations (Ai/As) versus concentration of analytes.

Precision And Accuracy
The plasma solutions of venlafaxine (2.5, 100, 1500 ng/mL), ODV (2.5, 100, 1500 ng/mL) and NDV (0.25, 10, 150 ng/mL) at low, medium and high concentrations (6 portions at each concentration) were prepared and then were processed before entering the chromatographic system for detection. In the same day, the intra-day precision was calculated. Standard curves were measured and followed for 3 consecutive days to calculate the inter-day precision.

Recovery And Matrix Effects (ME)
Venlafaxine (2.5, 100, 1500 ng/mL), ODV (2.5, 100, 1500 ng/mL) and NDV (0.25, 10, 150 ng/mL) plasma reference solutions at low, medium and high concentrations were prepared, and 6 samples were prepared in parallel for each concentration, and then the peak area (A) was obtained after treatment. The supernatant was taken from 6 portions of blank beagle plasma, and the peak area (B) was obtained by adding three matrix reference substances with the same concentration as the theoretical sample injection of quality control samples. The ratio of A to B was the extraction recovery rate of the above three mass concentrations, and the extraction recovery rate of the internal standard was calculated by the same method.

The blank plasma was replaced by pure water, and the peak area (C) was obtained by adding three matrix reference substances with the same concentration as that in
the quality control sample theory. The ME of venlafaxine, ODV, NDV and internal standard were computed with B/C ×100%.

**Stability**
To test the plasma stability of venlafaxine, ODV and NDV, three different concentrations (2.5, 100, 1500 ng/mL for venlafaxine, 2.5, 100, 1500 ng/mL for ODV and 0.25, 10, 150 ng/mL for NDV) were disposed. Each sample was prepared 6 portions and tested the stability in 4 conditions: room temperature for 4hrs, 4°C for 24hrs, three cycles of freezing and thawing (−20~25°C), 4 weeks storage at −20°C.

**Plasma Sample Detection**
The concentrations of the three substances in all samples were tested by the method established in this experiment. For samples with a concentration higher than the upper limit of the standard curve, the samples were diluted with blank plasma and tested, and the drug concentration was calculated according to the dilution factor.

**Statistical Analysis**
The drug concentration data of plasma were processed by DAS (Drug And Statistics, version 2.0) and then were expressed as mean ± SD. Meanwhile, the group data were analyzed to make the Independent-Samples T Test by SPSS 20.0. P values less than 0.05 were considered statistical difference, and P values less than 0.01 were considered statistically significant difference.

**Results**

**Specificity**
As Figure 3 shows, the chromatographic peaks of venlafaxine, ODV, NDV and IS in plasma were well separated. Endogenous substances in plasma did not interfere with the determination of analytes and IS. The retention times of venlafaxine, ODV, NDV and IS were 1.2, 1.08, 1.15 and 1.44 min, respectively. The chromatographic peak shape was better and the baseline was stable, meaning that the method had a high specificity.

**Linearity**
Linearity for each analyte was established over the concentration range of 0.25–1500 ng/mL. The typical regression equations and correlation coefficient were as follows: y = 0.0168x + 0.0643, r = 0.999 3 for venlafaxine, y = 0.0178x−0.0381, r = 0.999 4 for ODV, and y = 0.0214 x + 0.0162, r = 0.999 3, respectively. The lower limit of quantification (LLOQ) was 1 ng/mL for venlafaxine, 1 ng/mL for ODV, and 0.1 ng/mL for NDV, respectively.

**Precision And Accuracy**
The low, medium and high quality concentrations of venlafaxine, ODV and NDV were prepared at 2.5, 100, 1500 ng/mL, 2.5, 100, 1500 ng/mL and 0.25, 10, 150 ng/mL, respectively. Each of the concentrations was prepared by 6 sections and entered into the UPLC-MS/MS chromatographic system for detection after processing. In the same day, the intra-day precision was calculated. Inter-day precision was calculated by measuring and following the standard curve for 3 consecutive days. The results are shown in Table 1.

**Recovery And ME**
Table 2 shows the recoveries and ME at low, medium and high concentrations of venlafaxine, ODV and NDV. The recoveries rang of three substances were 80.53 ± 2.39% ~ 98.48 ± 2.96% (RSD<15%, n = 6), while IS (50 ng/mL) was 91.03 ± 5.88% (RSD = 6.46%, n = 6). At the same time, the ME rang of three substances was 98.91 ± 2.46% ~ 101.88 ±3.11% (RSD<15%, n = 6), which indicated that the ME did not affect the determination of three substances.

**Stability**
Tables 3–5 shows the stability for venlafaxine, ODV and NDV in 4 different storage and temperature conditions. The RE of samples all ranged from −8.13% to 4.27%. It indicated that concentrations of three substances in beagle plasma samples were stable under the above four preservation conditions, and no significant degradation was observed.

**Pharmacokinetics Of Venlafaxine, ODV And NDV**
After the beagles were given venlafaxine hydrochloride tablets alone, and combining with venlafaxine hydrochloride tablets and Danzhi Xiaoyao pills, the average plasma drug concentration–time curves of venlafaxine are shown in Figure 4, and the average plasma drug concentration–time curves of its metabolites (ODV, NDV) in plasma are shown in Figures 5–6, respectively. DAS 2.0 was used for analysis and processing. Meanwhile, Tables 6–8 show the pharmacokinetic parameters of three substances, respectively.
Compared with control groups, the experimental groups' results showed that the $t_{1/2}$ of venlafaxine was mildly decreased by 24.83%, which indicated that the metabolism was accelerated in the experimental groups. AUC$_{(0-t)}$ and AUC$_{(0-\infty)}$ of venlafaxine were also decreased by 42.39% and 42.57%, respectively, and the $C_{\text{max}}$ was decreased by 31.96%. In addition, the $T_{\text{max}}$ was significantly declined by 46.39%, while the CLz/F was sharply increased by 73.68%. All figures showed that after the combined administration of Danzhi Xiaoyao pills, the plasma exposure of venlafaxine decreased. After the combined administration of Danzhi Xiaoyao pills, the AUC$_{(0-t)}$ and AUC$_{(0-\infty)}$ of ODV were increased by 25.68% and 25.51%, respectively. Meanwhile, the $C_{\text{max}}$ of ODV had an opposite trend compared to $C_{\text{max}}$ of venlafaxine, jumped by 20.75%, which indicated that the plasma exposure of ODV increased. As for NDV, the trend was similar to ODV. AUC$_{(0-t)}$ and AUC$_{(0-\infty)}$ were also increased by 21.91% and 23.11%, respectively.

**Discussion**

At present, there are a number of methods to analyze the active metabolite in plasma such as HPLC, \(^{23}\) HPLC-PDA, \(^{24}\) RP-HPLC, \(^{25}\) etc. But according to the reports, this method has a low sensitivity, narrow linear range

![Figure 3 Chromatograms of venlafaxine and its metabolite. (A) Blank plasma; (B) blank plasma spiked with venlafaxine, ODV, NDV and internal standard; (C) beagle sample.](3348)

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Zhu et al

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Drug Design, Development and Therapy 2019:13

3348
and long analysis time (can only detect venlafaxine and its main active metabolite ODV). This study adopts the method of UPLC-MS/MS and measures the concentration of venlafaxine, ODV and NDV, which is verified by the methodology. This method is in full compliance with the bioassay standard and has a high sensitivity and fast analysis speed, greatly improving the standard of test and reliability, which meets the pharmacokinetic study of venlafaxine. As for IS substance, diazepam was chosen after many preliminary experiments, and venlafaxine, ODV, NDV and diazepam have a good separation and peak shape. At the same time, the endogenous substances in beagle plasma samples do not interfere with them, and so diazepam was chosen as an internal standard to determine the content of venlafaxine and its metabolites. In this experiment, methanol, acetonitrile and ultra-pure water solution were respectively investigated in solvent selection. After UPLC-MS/MS analysis, it was found that the separation degree of methanol after dissolution was the best. Therefore, venlafaxine, ODV and NDV were separated by methanol.

As Tables 6–8 show, the difference between the three groups was statistically significant (P<0.05). Danzhi Xiao Yao pills promoted the metabolism of venlafaxine, and so the
blood concentration of its metabolite ODV (the major active metabolite of venlafaxine) and NDV (the minor metabolite of venlafaxine) was also increased. A study has shown that the interaction of traditional Chinese medicine on western medicine mainly affects CYP450. CYP450, which is a superfamily of enzymes, is the main phase 1 enzyme system for the metabolism of herbal substance. In previous studies, CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 (all belong to CYP450) have been proven that they are the major actors to metabolize a vast majority of widely known drugs. In recent years, a number of studies have shown that the active ingredients in Danzhi Xiaoyao pill can affect the activity of CYP2D6 or CYP3A4. Ying Cheng et al showed that Radix Bupleuri and vinegar-baked Radix Bupleuri had strong induction effects on the CYP2E1, CYP2D6 and CYP3A4. Jing Wang et al found that high-dose bupleurum injection can induce the expression of CYP3A4 protein in rats. Venlafaxine is metabolized to ODV by CYP2D6 and NDV by CYP3A4, and therefore we speculate that Danzhi Xiaoyao pill may induce venlafaxine metabolism by affecting the activity of CYP2D6 or CYP3A4.

In conclusion, based on the results of our research, we suspect that the mechanism of action of Danzhi Xiaoyao pill on venlafaxine may affect CYP450. This study can provide a
### Table 1 Precision And Accuracy Of Venlafaxine, ODV And NDV In Beagle Plasma (n = 6, Mean±SD)

| Analytes | Added (ng/mL) | Detected (ng/mL) | RSD(%) | RE(%) | Detected (ng/mL) | RSD(%) | RE(%) |
|----------|---------------|------------------|--------|-------|------------------|--------|-------|
|          |               |                   |        |       |                   |        |       |
|          |               | Intra-Day Precision |      |        | Inter-Day Precision |        |       |
|          |               |                   |        |       |                   |        |       |
|          | 2.5           | 2.42±0.17         | 6.87   | −3.07 | 2.42±0.13         | 5.23   | −3.07 |
|          | 100           | 98.94±6.73        | 6.80   | −1.06 | 99.94±1.92        | 1.92   | −0.06 |
|          | 1500          | 1498.87±14.54     | 0.97   | −0.08 | 1503.00±8.54      | 0.57   | 0.20  |
|          | 100           | 2.49±0.14         | 5.59   | −0.40 | 2.47±0.08         | 3.10   | −1.12 |
|          | 1500          | 100.65±0.97       | 0.96   | 0.65  | 101.42±0.51       | 0.51   | 1.42  |
|          | 0.25          | 0.25±0.01         | 2.97   | −0.53 | 0.24±0.01         | 4.53   | −5.24 |
|          | 10            | 10.32±0.48        | 4.66   | 3.20  | 10.07±0.25        | 2.50   | 0.69  |
|          | 150           | 152.29±3.66       | 2.40   | 1.53  | 150.71±1.59       | 1.05   | 0.47  |
|          | 100           | 100.65±0.97       | 0.96   | 0.65  | 101.42±0.51       | 0.51   | 1.42  |
|          | 1500          | 1501.75±6.81      | 0.45   | 0.12  | 1495.23±7.46      | 0.50   | −0.32 |
|          | 0.25          | 0.25±0.01         | 2.97   | −0.53 | 0.24±0.01         | 4.53   | −5.24 |
|          | 10            | 10.32±0.48        | 4.66   | 3.20  | 10.07±0.25        | 2.50   | 0.69  |
|          | 150           | 152.29±3.66       | 2.40   | 1.53  | 150.71±1.59       | 1.05   | 0.47  |
|          | 100           | 100.65±0.97       | 0.96   | 0.65  | 101.42±0.51       | 0.51   | 1.42  |
|          | 1500          | 1501.75±6.81      | 0.45   | 0.12  | 1495.23±7.46      | 0.50   | −0.32 |

### Table 2 Recovery And Matrix Effects For Venlafaxine, ODV, NDV And IS Of Quality Control Samples In Beagle Plasma (n = 6, Mean±SD)

| Analytes | Added (ng/mL) | Recovery (%) | ME(%) |
|----------|---------------|--------------|-------|
|          |               | Detected (ng/mL) | RSD(%) | Detected (ng/mL) | RSD(%) |
|          | 2.5           | 85.76±2.64   | 3.08  | 100.94±4.45      | 4.40  |
|          | 100           | 84.47±4.30   | 5.09  | 101.53±3.01      | 2.96  |
|          | 1500          | 80.53±2.39   | 2.97  | 99.49±2.21       | 2.22  |
|          | 2.5           | 87.43±4.90   | 5.60  | 101.30±4.32      | 4.18  |
|          | 100           | 84.92±3.62   | 4.26  | 99.83±3.67       | 3.67  |
|          | 1500          | 98.48±2.96   | 3.00  | 98.91±2.46       | 2.49  |
|          | 0.25          | 83.45±4.18   | 5.01  | 99.49±4.15       | 4.17  |
|          | 10            | 81.66±3.85   | 4.72  | 101.88±3.11      | 3.06  |
|          | 150           | 85.24±2.56   | 3.01  | 99.83±2.53       | 2.53  |
|          | 50            | 91.03±5.88   | 6.46  | 100.10±3.69      | 3.68  |

### Table 3 Stability For Venlafaxine In 4 Different Storage And Temperature Conditions (n = 6, Mean±SD)

| Storage Conditions | Added (ng/mL) | Detected (ng/mL) | RSD(%) | RE(%) |
|--------------------|---------------|------------------|--------|-------|
| Room temperature, 4hrs | 2.5           | 2.54±0.17        | 6.54   | 1.40  |
|                    | 100           | 98.15±2.72       | 2.77   | −1.85 |
|                    | 1500          | 1512.03±41.36    | 2.74   | 0.80  |
| 4°C, 24 hrs        | 2.5           | 2.48±0.15        | 6.15   | −0.80 |
|                    | 100           | 102.08±4.28      | 4.20   | 2.08  |
|                    | 1500          | 1490.78±38.56    | 2.59   | −0.61 |
| Three freeze-thaw cycles | 2.5           | 2.35±0.15        | 6.23   | −5.87 |
|                    | 100           | 101.46±5.30      | 5.22   | 1.46  |
|                    | 1500          | 1479.61±46.91    | 3.17   | −1.36 |
| −20°C, 4 weeks     | 2.5           | 2.30±0.12        | 5.05   | −8.13 |
|                    | 100           | 96.35±2.60       | 2.70   | −3.65 |
|                    | 1500          | 1468.51±36.93    | 2.52   | −2.10 |
Table 4 Stability for ODV in 4 Different Storage and Temperature Conditions (n = 6, Mean±SD)

| Storage Conditions          | Added (ng/mL) | Detected (ng/mL) | RSD(%) | RE(%) |
|-----------------------------|---------------|------------------|--------|-------|
| Room temperature, 4 hrs     | 2.5           | 2.54 ± 0.07      | 2.95   | 1.53  |
|                             | 100           | 100.86 ± 2.41    | 2.39   | 0.86  |
|                             | 1500          | 1504.01 ± 11.07  | 0.74   | 0.27  |
| 4 °C, 24 hrs                | 2.5           | 2.56 ± 0.09      | 3.42   | 2.27  |
|                             | 100           | 102.07 ± 5.81    | 5.69   | 2.07  |
|                             | 1500          | 1493.00 ± 25.55  | 1.71   | -0.47 |
| Three freeze–thaw cycles    | 2.5           | 2.56 ± 0.14      | 5.31   | 2.47  |
|                             | 100           | 95.60 ± 3.89     | 4.07   | -4.40 |
|                             | 1500          | 1503.85 ± 11.20  | 0.75   | 0.26  |
| -20°C, 4 weeks              | 2.5           | 2.61 ± 0.15      | 5.81   | 4.27  |
|                             | 100           | 102.61 ± 5.28    | 5.15   | 2.61  |
|                             | 1500          | 1490.73 ± 20.42  | 1.37   | -0.62 |

Table 5 Stability for NDV in 4 Different Storage and Temperature Conditions (n = 6, Mean±SD)

| Storage Conditions          | Added (ng/mL) | Detected (ng/mL) | RSD(%) | RE(%) |
|-----------------------------|---------------|------------------|--------|-------|
| Room temperature, 4 hrs     | 0.25          | 0.25 ± 0.02      | 6.65   | -2.00 |
|                             | 10            | 9.54 ± 0.40      | 4.17   | -4.62 |
|                             | 150           | 150.23 ± 7.45    | 4.96   | 0.16  |
| 4 °C, 24 hrs                | 0.25          | 0.24 ± 0.01      | 5.04   | -3.47 |
|                             | 10            | 10.28 ± 0.51     | 4.93   | 2.76  |
|                             | 150           | 148.52 ± 6.18    | 4.16   | -0.99 |
| Three freeze–thaw cycles    | 0.25          | 0.25 ± 0.01      | 5.87   | -0.67 |
|                             | 10            | 9.74 ± 0.80      | 8.18   | -2.58 |
|                             | 150           | 148.33 ± 9.07    | 6.12   | -1.12 |
| -20°C, 4 weeks              | 0.25          | 0.25 ± 0.02      | 7.16   | -0.73 |
|                             | 10            | 10.30 ± 0.71     | 6.93   | 3.62  |
|                             | 150           | 147.79 ± 5.35    | 3.62   | -1.47 |

Figure 4 Mean plasma concentration–time curves of venlafaxine after oral administration of 10.28 mg/kg venlafaxine hydrochloride tablets in beagles (n = 6, mean±SD).
scientific basis for clinical treatment of depression with Danzhi Xiaoyao pill and venlafaxine. But due to the complex composition of traditional Chinese medicine and a lot of targets, the mechanism needs further research.

**Conclusion**

This experiment successfully established an UPLC-MS/MS method for simultaneous determination of venlafaxine and its major active metabolite ODV and minor metabolite NDV in beagles. The UPLC-MS/MS method was used for the determination of venlafaxine and its metabolites in beagle plasma with high specificity, sensitivity and rapid detection time, which met the requirements of pharmacokinetic guidelines.

The results showed that Danzhi Xiaoyao pill could reduce the plasma exposure of venlafaxine and increase

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**Table 6** Pharmacokinetic Parameters Of Venlafaxine After Oral Administration Of 10.28 mg/kg Venlafaxine In Beagles (n = 6, Mean±SD)

| Parameters                          | Control Groups       | Experimental Groups       |
|-------------------------------------|----------------------|---------------------------|
| $t_{1/2}$ (hrs)                     | 4.35 ± 0.82          | 3.27 ± 0.63*              |
| $T_{max}$ (hrs)                     | 0.97 ± 0.30          | 1.42 ± 0.49**             |
| MRT$_{0-\infty}$ (hrs)             | 6.16 ± 0.96          | 6.29 ± 1.08               |
| MRT$_{0-t}$ (hrs)                   | 6.29 ± 1.08          | 5.17 ± 1.24               |
| $C_{max}$ (ng/mL)                   | 2267.26 ± 252.89     | 1542.64 ± 190.73*         |
| CL$_z$/F (L/h/kg)                   | 0.76 ± 0.22          | 1.32 ± 0.31**             |
| AUC$_{0-\infty}$ (ng h/mL)         | 13,875.79 ± 3569.69  | 7993.16 ± 2157.76**       |
| AUC$_{0-t}$ (ng h/mL)              | 13,934.79 ± 3609.23  | 8001.91 ± 2167.58**       |

Notes: Compared with venlafaxine group: *P<0.05, **P<0.01.
the content of ODV and NDV, meaning that Danzhi Xiaoyao pills can influence the metabolism of venlafaxine in beagles. Based on the possibility of this result, it is recommended to adjust the dose when the two drugs were combined in the clinical practice, and this study should be considered significant in guiding clinical treatment.

Acknowledgments

We appreciate the contribution of the members participating in this study. We also would like to thank The First Affiliated Hospital of WenZhou Medical University for supporting the equipment in this work.

Author Contributions

Xiang-jun Qui conceived and designed the experiments. Yong-liang Zhu conducted the experiments and drafted the paper. Shuang-long Li, Ke-li Chen, Kun-peng Ma, De-qian Wu conducted part experiments and analyzed the data. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Table 7 Pharmacokinetic Parameters Of ODV After Oral Administration Of 10.28 mg/kg Venlafaxine In Beagles (n = 6, Mean±SD)

| Parameters          | Control Groups | Experimental Groups |
|---------------------|----------------|---------------------|
| t1/2 (hrs)          | 4.56 ± 0.89    | 4.51 ± 0.69         |
| T_max (hrs)         | 1.33 ± 0.26    | 1.03 ± 0.27*        |
| MRT_{0-1} (hrs)     | 6.28 ± 0.98    | 6.38 ± 0.57         |
| MRT_{0-∞} (hrs)     | 6.51 ± 1.17    | 6.61 ± 0.59         |
| C_{max} (ng/mL)     | 2253.80 ± 215.81 | 2721.37 ± 118.20*  |
| CL/F (L/h/kg)       | 0.76 ± 0.16    | 0.59 ± 0.06*        |
| AUC_{0-1} (ng h/mL)| 13,888.24 ± 2714.73 | 17,454.69 ± 1854.58* |
| AUC_{0-∞} (ng h/mL)| 13,974.99 ± 2784.04 | 17,539.44 ± 1894.29* |

Notes: Compared with venlafaxine group: *P<0.05.

Table 8 Pharmacokinetic Parameters Of NDV After Oral Administration Of 10.28 mg/kg Venlafaxine In Beagles (n = 6, Mean±SD)

| Parameters          | Control Groups | Experimental Groups |
|---------------------|----------------|---------------------|
| t1/2 (hrs)          | 4.07±0.88      | 4.75±1.52           |
| T_max (hrs)         | 0.73±0.14      | 1.03±0.27**         |
| MRT_{0-1} (hrs)     | 4.06±0.44      | 4.15±0.75           |
| MRT_{0-∞} (hrs)     | 4.35±0.53      | 4.75±1.21           |
| C_{max} (ng/mL)     | 50.98±5.76     | 58.74±12.33         |
| CL/F (L/h/kg)       | 57.45±10.35    | 47.27±10.10         |
| AUC_{0-1} (ng h/mL)| 177.36±34.78   | 216.22±51.85*       |
| AUC_{0-∞} (ng h/mL)| 179.26±34.94   | 220.68±51.41*       |

Notes: Compared with venlafaxine group: *P<0.05, **P<0.01.
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