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Original Article

Effects of mongolian medicine *Terminalia chebula* Retz. on 6 CYP450 enzymes in rats

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Abstract: *Terminalia chebula* Retz. (TCR) is a medicinal material commonly used in Mongolian medicine. After consulting the literature at home and abroad, current research on TCR focuses on chemical composition, pharmacodynamics, and fingerprints. The pharmacokinetics of TCR has not been reported. Cytochrome P450 (CYP450) is the main drug-metabolizing enzyme, and its activity may be induced or inhibited by certain drugs, resulting in drug interactions in clinical applications. The objective of this study was to establish a high performance liquid chromatography (HPLC) method that can simultaneously detect multiple probe drugs to determine the effect of TCR on the activities of CYP450 enzymes CYP2C19, CYP2E1, CYP2D6, CYP2C9, CYP3A4, and CYP1A2. Wistar rats (male) were divided into 5 groups according to the randomization principle, namely the control group, the positive group, and the high, medium and low dose group. After 15 days of continuous administration, the mixed probe drug was injected into the vein, and then a small amount of blood was collected from the orbital vein at different time points. After the samples were processed, the blood concentration of each probe drug was measured by the established HPLC method. The pharmacokinetic parameters of each probe drug were calculated using DAS software. Compared with the control group, the plasma clearance (CL) of chlorzoxazone and omeprazole decreased, and the maximum plasma concentration (C_{max}) and area under the curve (AUC) increased in the TCR group. The pharmacokinetic parameters of theophylline, midazolam, metoprolol, and tolbutamide did not differ significantly. The results indicated that TCR mainly inhibited the activities of CYP2E1 and CYP2C19, but had no effect on the activities of CYP1A2, CYP2C9, CYP3A4 and CYP2D6. Extra care should be taken when drugs metabolized by CYP2C19 and CYP2E1 enzymes are used in combination with TCR, as drug-herb interactions may occur. These results can guide the clinical application of related drugs and provide valuable information for drug interactions. The main component that affects enzyme activity may be tannins in the water extract.

Keywords: *Terminalia chebula* Retz., CYP450, HPLC, cocktail, herb-drug interaction, rat

Introduction

Traditional herbs have been widely used worldwide, and interactions with herbs have been increasingly reported [1]. At present, there are frequent cases of combination of Chinese medicine and Western medicine, and the resulting drug interaction has attracted attention. The pretreatment with Chinese medicine compound Shaoyao Gancao Decoction (SGD) for 2 weeks significantly reduced AUC (area under the curve) and increased CL (plasma clearance) of paclitaxel [2]. *In vitro* experiments showed that SGD could activate cytochrome P450 3A4 (CYP3A4) and drug transporter multidrug resistance protein 1 (MDR1) promoters and enhance mRNA expression, resulting in accelerated paclitaxel metabolism [3]. *Ginkgo biloba* combined with warfarin or aspirin may increase bleeding. Combined with thiazide diuretics, it may increase blood pressure, and even cause coma with serotonin antagonists [4]. Studies showed that *Ginkgo biloba* extract could significantly increase the expressions and activities of CYP2B, CYP3A, and CYP1A [5, 6]. Studies showed that the reduction of the blood concentration of cyclosporine induced by St. John's wort brought about organ rejection, which revealed that St. John's wort could induce the activities of CYP3A4 and p-glycoprotein [7]. Many studies have shown that Chinese herbal medicines can affect the metabolism associated with the drug by changing the activity of drug metabolism enzymes, which is very risky [1, 8].

Therefore, it is necessary to study the influence of traditional Chinese medicine on CYP450 enzymes to better guide its clinical application.

Terminalia chebula Retz. (TCR), its ripe fruit used as medicine, is native to India and Myanmar, and distributed in Tibet, Yunnan, Guangdong, and Guangxi [9]. The characteristics and taste of TCR are based on traditional Chinese medicine theory. TCR mainly treats long-term diarrhea and dysentery, blood in the stool and prolapse of the anus, lung deficiency and cough, persistent coughing, sore throat and hoarseness [10]. It contains chemical compounds such as steroids, polyphenols, polysaccharides, and volatile oils [11]. TCR has the largest application ratio and the most extensive function in Mongolian medicine, and has the reputation of being the "king of medicine" in Mongolian herbs [12]. TCR is astringent in Mongolian medicine. It has the effects of regulating qi and supplementing qi, digestion and detoxification, and treatment of poisoning and other diseases [13]. Modern pharmacological studies have shown that TCR extracts have a wide range of functions, including antibacterial, antioxidant, hypoglycemic, antiviral, anti-inflammatory, and killing or inhibiting malignant tumor cells. TCR can also be used to relieve the toxicity of aconite [9, 14]. After consulting the literature at home and abroad, the research on Mongolian medicine TCR mainly focuses on the chemical composition, pharmacodynamics, and fingerprint. However, no research has been reported on the pharmacokinetics of TCR, so this project intends to study the effect of TCR on CYP450 enzyme activity in rats, which has practical guiding significance for clinical use.

Cytochrome P450s (CYP450s) are the most important Phase I metabolic enzymes. They are able to metabolize many endogenous substances as well as many exogenous substances and they participate in drug interaction [15]. CYP450 activity can be induced or inhibited by exogenous substances, which can change the effectiveness and safety of other drugs, causing severe clinical drug-drug interaction (DDI) [8, 16]. The main regulator of herbal-drug interaction (HDI) is thought to be the inhibition or induction of specific enzymes, and this effect will occur when herbs and western medicine are administered in combination [17, 18]. Therefore, in order to infer potential HDI, it is important to understand whether herbs can inhibit and induce the activity of CYP450 enzymes. Six important CYP isoenzymes (CYP3A4, CYP2C9, CYP2E1, CYP2C19, CYP1A2, and CYP2D6) were selected in this study because they metabolize many drugs and are major contributors to most known drug metabolism [19]. Studies show that CYPs (1a2, 2c11, 2e1, 2d1, 3a1/2, and 2d2) of rats are homologous to CYPs (1A2, 2C9, 2E1, 2C19, 3A4, and 2D6) of humans [20, 21]. Therefore, the results in rats can be inferred to have clinical application in humans.

In order to evaluate the safety of TCR in clinical application, theophylline, chlorzoxazone, tolbutamide, omeprazole, metoprolol and midazolam were selected as CYP1A2, CYP2E1, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 specific probe substrates. In this experiment, the effects of TCR on the activities of six CYP450 isoforms in rats were investigated using a probe-drug method combined with HPLC.

Materials and methods

Drug and standard substances

Terminalia chebula Retz. (TCR) was purchased from the Chinese and Mongolian Medical Hospital of Baotou, Inner Mongolia. Theophylline, metoprolol, omeprazole, midazolam, chlorzoxazone, tolbutamide, and tinidazole (all >98%) were obtained from Dalian Meilun Biotechnology Co., Ltd (Dalian, China).

Animals

Male Wistar rats (200±20 g) were provided by Beijing Si Beifu Biotechnology Co., Ltd. All rats lived in the Cardiovascular Research Laboratory of Baotou Medical College. First they were acclimated for one week, and then the experiment was started. The room temperature was 22°C, and the light and dark were alternated. All experimental procedures were ethically approved by the Experimental Animal Management Committee of Baotou Medical College.

Instrumentation and conditions

In order to analyze mixed probe drugs, this experiment used Ultimate 3000 HPLC, which is equipped with a diode array detector. Tinidazole (the internal standard, IS), theophylline, metoprolol, omeprazole, midazolam, chlorzoxazone, and tolbutamide were separated using Eclipse

XDB-C18 (4.6 × 150 mm, 5 μm, Agilent, USA). The column temperature was 35°C. The mobile phase was acetonitrile (A) and an aqueous solution containing 0.1% phosphoric acid (B), the flow rate was 1.0 mL/min and the injection volume was 10 μL. The gradient elution procedure is as follows: 0-14 min (12%-31% A); 14-15 min (31%-38% A); 15-17 min (38%-43% A); 17-18 min (43%-50% A); 18-20 min (50%-53% A); 20-25 min (53% A).

Preparation of standard solutions

Theophylline, metoprolol, omeprazole, midazolam, chlorzoxazone, tolbutamide and IS were dissolved in methanol to prepare 0.1485, 0.047, 0.057, 0.03, 0.057, 0.152, and 0.0416 mg/mL of stock solution. The stock solution was serially diluted with methanol to prepare a working solution for each analyte. All solutions were stored at 4°C and allowed to return to room temperature before use. In order to quantify the blood concentrations of the six probe drugs, a standard curve was established for each drug. Concentrations of each probe drug were diluted 4, 10, 20, 40, 100, 200, 1000, and 2000 times.

Pharmacokinetic study

Wistar rats (male, 200±20 g) were used for pharmacokinetic analysis. Forty Wistar male rats were divided into 5 groups according to the randomization principle, the control group, the positive group, the TCR high-dose group, the middle-dose group, and the low-dose group (n = 8). Continuous intragastric administration was provided for 15 days. After 15 days, a cocktail solution containing six probe drugs was injected through the tail vein: theophylline (10 mg/kg), metoprolol (10 mg/kg), omeprazole (10 mg/kg), chlorzoxazone (5 mg/kg), tolbutamide (2.5 mg/kg), and midazolam (5 mg/kg).

After the injection of the mixed probe drug solution, 0.5 ml of blood samples were obtained from eyes at 0.083, 0.167, 0.333, 0.667, 1.5, 2.5, 3.5, 4.5, 6, 8, 10, 12, 24, 36, and 48 h. Each sample was centrifuged at 3500 r/min for 10 min, and 200 μL plasma was obtained from the sample.

HPLC was used to detect drugs extracted from plasma samples. To 200 μL of plasma sample, was added 100 μL IS working solution (0.0416 mg/mL) and 2 mL of dichloromethane. After vortexing for 5 minutes, the samples were centrifuged at 3500 r/min for 10 minutes. Then, we carefully transferred 1.2 mL of the organic phase to another glass tube and blowed it dry with nitrogen. The dried material was re-dissolved in 200 μL of methanol and analyzed by HPLC.

Preparation and content determination of TCR

The common method of TCR administration is oral water decoction, so this experiment used distilled water to decoct TCR powder, and then detected the tannin content and gallic acid content in the extract according to the method in the Chinese Pharmacopoeia (Part Four) [22].

Statistical analysis

Pharmacokinetic parameters of each probe drug were calculated using DAS software (version 3.0), such as $T_{1/2}$, CL, AUC and C_{max}. The SPSS 17.0 statistical software was used to analyze the pharmacokinetic parameters of each probe drug. Analysis of variance was used to compare between groups. $P<0.05$ was considered to be a significant difference. All data were expressed as mean ± standard deviation.

Results

Method validation

HPLC chromatograms and retention times of six probe drugs: HPLC chromatograms of probe drugs are shown in **Figure 1**. In **Figure 1**, the retention times were 4.978 min (theophylline), 8.988 min (IS), 10.588 min (metoprolol), 11.208 min (omeprazole), 16.770 min (midazolam), 19.230 min (chlorzoxazone), 22.663 min (tolbutamide). These samples had good separation effect.

Calibration curve: **Table 1** shows the regression types, correlation coefficients, and calibration values for each analyte over a range. The calibration curve shows good linearity and good correlation coefficients at selected concentrations in all analyte samples.

Precision and extraction efficiency: The precision of intraday and interday samples were measured to be 7.51% and 7.96% or less at each level, respectively. The value of mean extraction efficiency was measured to be in the

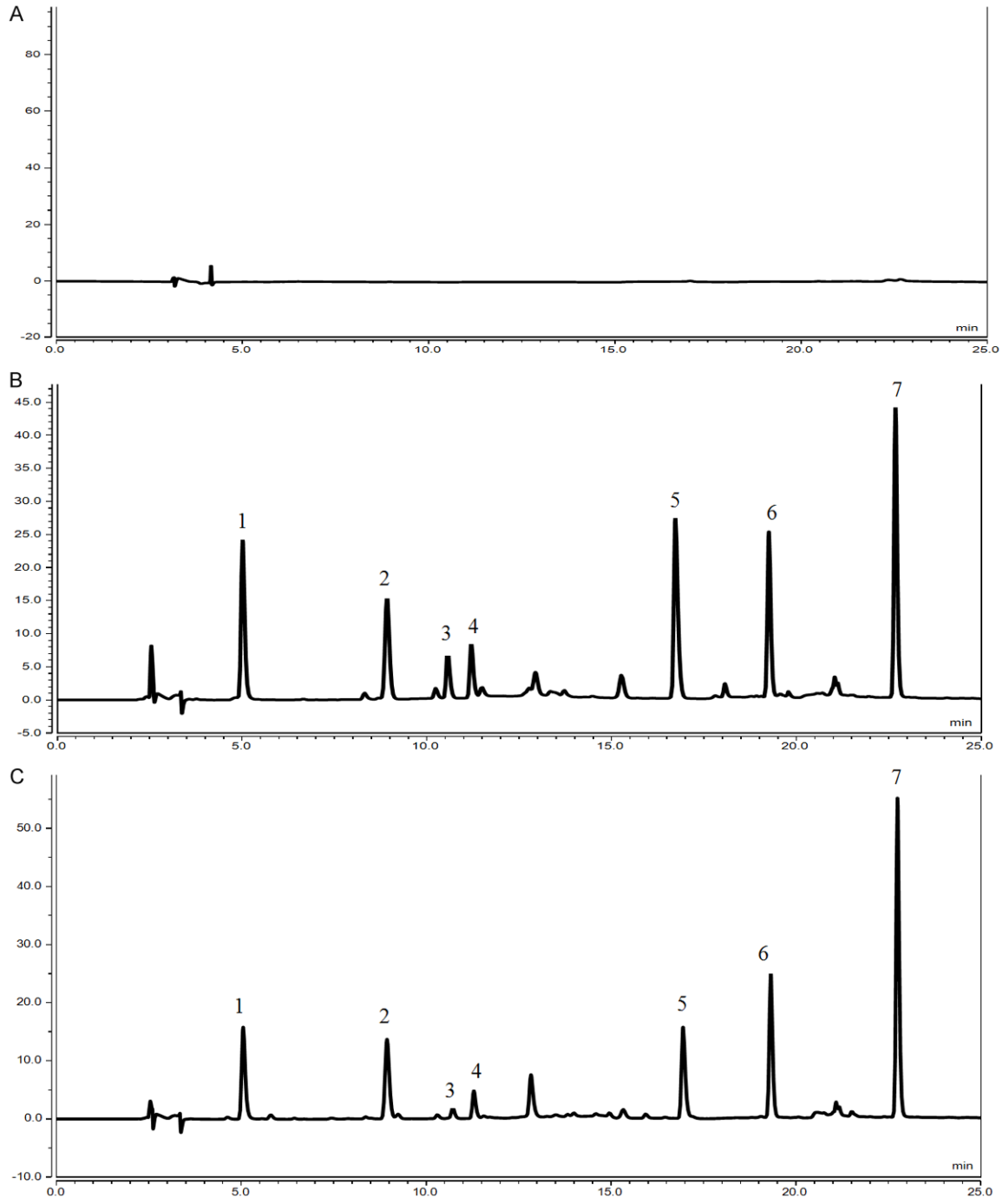

Figure 1. HPLC chromatograms: A. Blank plasma; B. Blank plasma spiked with the cocktail probe drugs and the IS; C. Plasma sample obtained from a rat after intravenous injection of the cocktail probe drugs spiked with the IS; 1. theophylline; 2. IS; 3. metoprolol; 4. omeprazole; 5. midazolam; 6. chlorzoxazone; 7. tolbutamide.

range of 86.59-104.58%. Assay performance data are shown in **Table 2**.

Stability: The plasma samples were placed at room temperature and frozen for 1 week and 3 weeks for stability studies. The results showed that the relative errors of these probe drugs in plasma were <10%, indicating that the plasma sample was stable.

Effects of TCR on CYP450 activity in rats

The probe substrate concentrations in blood samples at different time points were mea-

Table 1. Regression equation and correlation coefficient for six probe drugs

Probe drugs	Regression equation	Correlation coefficient	Linear range (μg/mL)
Theophylline	y = 0.0342x - 0.0021	0.9996	0.07~37.13 μg/mL
Metoprolol	y = 0.0324x + 0.0066	0.9998	0.02~11.8 μg/mL
Omeprazole	y = 0.0297x + 0.0092	0.9982	0.02~14.3 μg/mL
Midazolam	y = 0.2168x + 0.0259	0.9998	0.01~7. 5 μg/mL
Chlorzoxazone	y = 0.0806x + 0.0603	0.9994	0.02~14.3 μg/mL
Tolbutamide	y = 0.0531x + 0.0581	0.9949	0.07~38.0 μg/mL

y = peak area ratio of probe drugs vs IS; x = concentration of probe drugs.

sured by HPLC. The pharmacokinetic parameters of theophylline, metoprolol, omeprazole, midazolam, chlorzoxazone and tolbutamide were calculated by using DAS software. According to the changes of pharmacokinetic parameters, the effects of TCR on the activities of six CYP450 enzymes were assessed. Pharmacokinetic parameters included $T_{1/2}$, C_{max}, $AUC_{(0\sim t)}$, $AUC_{(0\sim\infty)}$, and CL. The pharmacokinetic parameters and mean plasma concentration time curves of the six probe drugs are shown in **Table 3** and **Figure 2**.

Effect of TCR on CYP1A2 activity in rats

The activity of CYP1A2 is described by the pharmacokinetic parameters of theophylline. No significant difference in pharmacokinetic parameters was found between the TCR group and the control group (P>0.05), which indicates that TCR has no effect on CYP1A2 activity *in vivo*.

Effect of TCR on CYP2D6 activity in rats

CYP2D6 activity was evaluated with metoprolol's pharmacokinetic parameters in different groups. The high and medium dose groups *of TCR* tended to increase $AUC_{(0\sim t)}$ and $AUC_{(0\sim\infty)}$, decrease CL, but these pharmacokinetic parameters had no significant differences with the control group (p>0.05). Compared with the control group, the C_{max} of the high-dose group was significantly increased (P<0.01). These data indicate that TCR may not affect the activity of CYP2D6.

Effect of TCR on CYP2C19 activity in rats

The pharmacokinetic parameters of omeprazole in different dose groups were used to evaluate the activity of CYP2C19. The high dose group of TCR significantly increased C_{max}, $AUC_{(0\sim t)}$, and $AUC_{(0\sim\infty)}$, and decreased CL (P<0.05). The medium dose group also significantly decreased CL (P<0.05). These data indicated that *TCR* could inhibit the activity of CYP2C19.

Effect of TCR on CYP3A4 activity in rats

Midazolam's Pharmacokinetic parameters were used to describe CYP3A4 activity. The high dose group of TCR significantly increased C_{max} (P<0.05). However, there were no significant differences in AUC, $T_{1/2}$, and CL between the TCR group and the control group (P>0.05). These results indicated *TCR* might have no influence on the activity of CYP3A4.

Effect of TCR on CYP2E1 activity in rats

The pharmacokinetic parameters of chlorzoxazone were used to analyze the activity of CYP2E1. Compared with the control group, C_{max}, $AUC_{(0\sim t)}$, and $AUC_{(0\sim\infty)}$ of the TCR high-dose group were significantly increased, and CL decreased significantly (P<0.05). Compared with the control group, medium dose group had significantly increased C_{max} (P<0.05) but $AUC_{(0\sim t)}$, $AUC_{(0\sim\infty)}$ increased and CL decreased, with no significant differences. These data indicated that *TCR* might inhibit the activity of CYP2E1.

Effect of TCR on CYP2C9 activity in rats

The activity of CYP2C9 was depicted by pharmacokinetic parameters of tolbutamide in different dose groups. There were no significant differences in the various pharmacokinetic parameters between the TCR group and the control group. These data indicated that CYP2C9 activity might not be influenced.

Preparation and content determination of TCR

According to the literature method, the tannin content in TCR extract was 23.2%, and the gallic acid content was 1.67%.

Discussion

The use of herbal medicines as an important component of multi-component therapy has

Table 2. Precision and extraction efficiency of six probe drugs in rat plasma (n = 5)

Compound	Concentration (μg/mL)	Intraday precision		interday precision		Extraction efficiency	RSD%
		x±s	RSD%	x±s	RSD%		
Theophylline	37.13	36.95±0.48	1.29	37.17±0.05	1.41	100.67±2.14	2.13
	1.49	1.49±0.04	2.43	1.49±0.04	2.43	100.50±2.44	2.43
	0.74	0.72±0.03	3.88	0.72±0.03	3.88	96.46±3.74	3.88
	0.07	0.08±0.00	3.54	0.08±0.00	3.54	104.58±3.70	3.54
Metoprolol	11.75	11.75±0.09	0.74	11.76±0.08	0.70	101.63±2.97	2.92
	1.18	1.15±0.01	0.85	1.16±0.00	0.64	98.05±2.37	2.42
	0.24	0.23±0.01	6.32	0.23±0.01	5.11	97.48±4.98	5.11
	0.02	0.02±0.00	4.09	0.02±0.00	7.96	99.62±7.93	7.96
Omeprazole	14.25	14.22±0.07	0.52	14.32±0.22	1.53	100.47±1.54	1.53
	1.43	1.39±0.06	4.37	1.46±0.04	2.41	102.22±2.46	2.41
	0.29	0.28±0.01	4.72	0.28±0.01	4.72	99.11±4.68	4.72
	0.03	0.03±0.00	4.69	0.03±0.00	7.14	92.85±6.63	7.14
Midazolam	7.5	7.45±0.11	1.52	7.52±0.05	0.61	100.56±1.01	1.01
	1.5	1.49±0.01	0.69	1.56±0.01	0.42	103.24±1.96	1.90
	0.75	0.72±0.01	0.91	0.72±0.01	0.95	97.21±3.34	3.43
	0.02	0.01±0.00	7.51	0.01±0.00	7.51	94.11±7.07	7.51
Chlorzoxazone	14.25	14.23±0.05	0.32	14.35±0.05	0.33	100.51±1.24	1.23
	1.43	1.36±0.02	1.26	1.39±0.05	3.38	97.23±3.29	3.38
	0.29	0.28±0.01	1.90	0.28±0.00	1.53	99.40±1.52	1.53
	0.03	0.03±0.00	7.13	0.03±0.00	7.14	88.25±6.30	7.14
Tolbutamide	38	37.49±0.17	0.44	37.81±0.11	0.29	101.12±2.16	2.14
	3.8	3.78±0.06	1.60	3.80±0.08	1.86	99.89±1.86	1.86
	0.76	0.75±0.02	2.10	0.76±0.02	3.01	100.29±3.02	3.01
	0.08	0.07±0.00	5.43	0.08±0.00	2.35	100.81±2.37	2.35

increased steadily over the past decade. Eighty percent of the population in Asian countries use herbs to promote health and treat common diseases, such as inflammation, pain, heart disease, cirrhosis, and central nervous system disease. However, as the use of herbal medicines increased, so did the risk for herb and drug interactions [23]. Herb-drug interactions include pharmacodynamic and pharmacokinetic interactions. In pharmacokinetic interactions, approximately 65% of drug interactions occur at metabolic sites. Drug-metabolizing enzymes are considered to be the most important interaction sites [24]. Increasing evidence demonstrated that the activity of CYP450 enzymes (including induction and inhibition) was one of the risk factors for drug combination in a patient's life. As a result, the number of pharmacokinetics studies on safe drug treatments is increasing [25].

Cytochrome P450 (CYP450) is an important metabolic enzyme and is involved in most drug metabolism [26]. Drugs are absorbed, distributed, metabolized, and excreted in the body. CYP450 is the major enzyme in drug metabolism. It is a superfamily gene, and includes CYP1, CYP2, CYP3 gene family, their subtypes, CYP1A2, CYP2E1, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 are the main enzymes in drug metabolism [27, 28].

CYP2C19 is a clinically important enzyme and is considered to be a major contributor in the formation of active metabolites [29]. It plays a vital role in the metabolism of many therapeutic agents which include proton pump inhibitors (such as pantoprazole and omeprazole), antiepileptic agents, antiplatelet drugs (clopidogrel), antidepressants (citalopram), and antifungal agents (voriconazole) [30, 31]. As shown in **Table 3** and **Figure 2**, the medication group had a significant influence on omeprazole's pharmacokinetic parameters. Compared with the control group, the $AUC_{(0\sim t)}$, $AUC_{(0\sim\infty)}$, C_{max} increased, and the CL decreased ($P<0.05$) in

Table 3. Pharmacokinetic parameters of six probe drugs (mean ± SD, n = 8)

Probe drug	Group	$AUC_{(0\sim t)}$/ug·mL^{-1}·h	$AUC_{(0\sim\infty)}$/ug·mL^{-1}·h	$T_{1/2}$/h	C_{max}/ug·mL^{-1}	Cl/L·h^{-1}·Kg^{-1}
Theophylline	Control	196.77±39.11	222.98±32.56	8.26±2.18	28.32±4.31	0.05±0.01
	Positive	159.22±22.94	209.08±80.39	5.72±2.04	28.55±5.50	0.05±0.01
	High	199.47±44.61	231.12±34.53	9.79±2.15	30.92±3.29	0.04±0.01
	Medium	197.40±36.91	200.37±38.76	7.05±3.06	27.29±1.64	0.05±0.01
	Low	195.61±58.30	211.81±48.64	7.83±3.64	25.96±4.34	0.05±0.01
Metoprolol	Control	2.36±0.62	2.50±0.69	0.57±0.09	2.31±0.31	4.21±0.91
	Positive	2.39±0.31	2.50±0.27	0.50±0.15	2.75±0.63	4.04±0.45
	High	2.88±0.51	2.98±0.52	0.49±0.09	3.35±0.80**	3.45±0.60
	Medium	2.73±0.30	3.00±0.52	0.68±0.26	2.71±0.42	3.42±0.52
	Low	2.16±0.75	2.27±0.75	0.52±0.16	2.43±0.58	4.92±1.82
Omeprazole	Control	2.37±0.91	2.60±0.88	0.22±0.08	7.18±2.05	4.60±1.68
	Positive	2.27±0.58	2.44±0.57	0.19±0.04	7.17±2.01	4.28±0.78
	High	3.85±1.08*	4.06±1.11*	0.22±0.12	11.12±3.58*	2.63±0.70*
	Medium	3.26±0.98	3.46±0.90	0.21±0.05	9.72±2.71	3.01±0.59*
	Low	2.36±0.71	2.59±0.78	0.17±0.04	7.42±2.20	4.31±1.77
Midazolam	Control	2.26±0.65	2.28±0.65	0.36±0.07	2.41±0.37	2.32±0.53
	Positive	1.70±0.37	1.77±0.36	0.37±0.09	2.32±0.56	2.93±0.58
	High	2.51±0.54	2.66±0.46	0.32±0.05	3.23±0.69*	1.93±0.33
	Medium	2.34±0.50	2.38±0.51	0.40±0.08	2.72±0.60	2.18±0.42
	Low	2.45±0.64	2.48±0.64	0.39±0.05	2.57±0.59	2.14±0.56
Chlorzoxazone	Control	17.84±4.42	20.80±4.33	1.06±0.27	12.29±3.21	0.26±0.05
	Positive	15.69±2.82	16.41±2.75	0.66±0.27	13.67±1.86	0.31±0.05
	High	21.24±5.74	26.90±5.08*	1.16±0.77	16.21±3.66*	0.19±0.04*
	Medium	20.50±4.63	22.50±4.91	1.05±0.35	15.15±3.52*	0.23±0.05
	Low	18.24±4.88	19.73±5.46	0.95±0.26	12.61±2.46	0.27±0.08
Tolbutamide	Control	405.52±95.47	459.59±124.09	13.32±4.31	38.92±8.85	0.006±0.002
	Positive	141.53±35.85	167.24±51.39	6.91±3.13	44.03±11.04	0.017±0.006
	High	406.23±69.92	560.42±138.27	15.17±5.05	49.70±9.90	0.004±0.001
	Medium	431.05±77.86	521.37±190.74	11.34±2.35	42.30±6.45	0.005±0.001
	Low	454.91±111.05	495.22±140.86	12.57±4.56	39.83±6.28	0.006±0.002

Comparing medication groups with the control group, *P<0.05, **P<0.01. ($T_{1/2}$, half-life; C_{max}, maximum plasma concentration; AUC, the area under the plasma concentration-time curve; CL, plasma clearance).

the medication group. These results indicated that TCR may inhibit the activity of CYP2C19. Herb-drug interactions may occur if TCR is combined with drugs metabolized by CYP2C19, for example, if it is used in combination with clopidogrel. It is unclear whether TCR will increase the adverse effects of clopidogrel (such as bleeding) due to the slower metabolism of clopidogrel in the body and an increase in blood plasma concentration. In clinical applications, if adverse reactions increase, this can be avoided by adjusting the dosing schedule.

The expression of CYP2E1 in the liver is higher than in other organs [32]. Some endogenous substrates are metabolized by CYP2E1, especially acetone and fatty acids (they are rich in the brain). CYP2E1 is also involved in the metabolism of many exogenous compounds, which include acetaminophen, aspartame, anesthetics, oxazolone, nicotine, acetone, phenobarbital, tetrachloride ethanol, and chloroform [33, 34]. According to **Table 3** and **Figure 2**, compared with the control group, the AUC, C_{max} increased, and CL decreased in the high-dose group (P<0.05). The result indicated that TCR can inhibit the activity of the CYP2E1 enzyme and thereby slow down the metabolism of chlorzoxazone. When drugs metabolized by CYP2E1 are used in combination with TCR, TCR will also slow down the metabolism of these drugs and lead to excessive drug concentra-

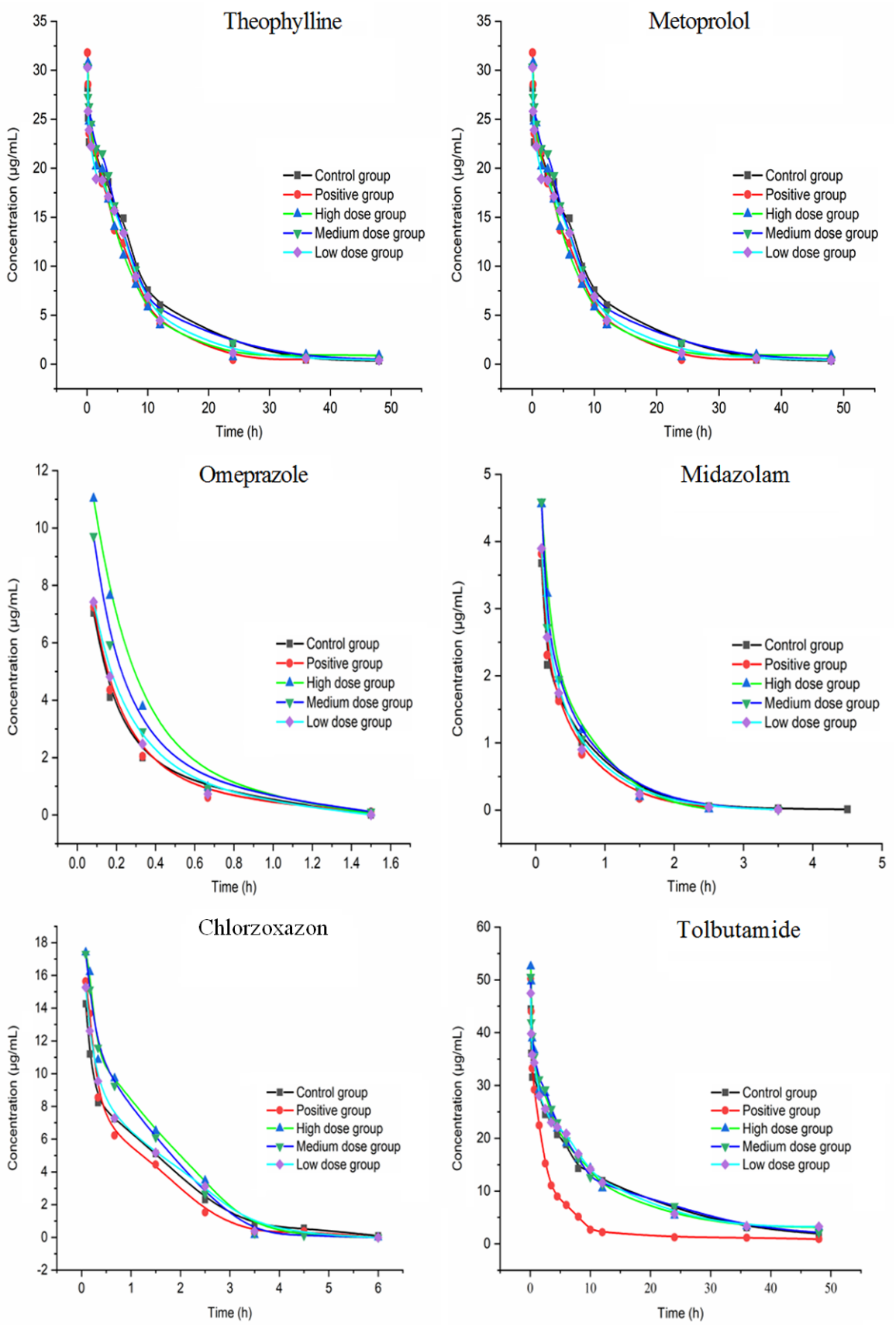

Figure 2. Mean plasma concentration-time curves for theophylline, tolbutamide, metoprolol, chlorzoxazone, omeprazole, and midazolam.

tions *in vivo*, which will cause adverse reactions and even toxic effects. Hence, TCR should be carefully used in clinical practice if it is combined with drugs metabolized by CYP2E1 enzymes. If combined use is needed, the dosing regimen should be adjusted to avoid adverse effects from high concentrations.

CYP1A2 is a very important enzyme in the CYP1A subfamily [35]. It is involved in the metabolism of some xenobiotics in the body and it also plays an important role in the synthesis of cholesterol, steroids, and other lipids [36]. For example, some analgesics and antipyretics, antipsychotics, and cardiovascular drugs require CYP1A2 for metabolism [32, 37]. CYP2C9 accounts for about 20% of the total liver CYP content [38]. It can metabolize over 15% of clinically administrated drugs (>100 drugs) and several endogenous compounds [39]. CYP2D6 participates in approximately 15-20% the metabolism of all drugs which include β-blockers, antipsychotics, most selective serotonin reuptake inhibitors, and antitumor agents [40, 41]. CYP3A4 can metabolize 40%-50% of drugs in clinical use, such as lipid-lowering statins (simvastatin, atorvastatin and lovastatin), calcium channel blockers, anticoagulant factor Xa inhibitors (rivaroxaban and apixaban), and macrolide antibiotics [42]. According to **Table 3** and **Figure 2**, the pharmacokinetic parameters of theophylline, tolbutamide, metoprolol, and midazolam were not significantly different between the control group and the TCR dose group ($P>0.05$). These results showed TCR did not influence the activities of CYP1A2, CYP2C9, CYP2D6, and CYP3A4. That is to say, the combination of TCR with drugs metabolized by CYP1A2, CYP2C9, CYP2D6, and CYP3A4 enzymes may not cause herbal-drug interactions. Therefore, drugs metabolized by CYP1A2, CYP2D6, CYP3A4, and CYP2C9 enzymes are safe to use in combination with TCR.

The chemical composition of TCR is rich and diverse, including phenolic acids, tannins, triterpenoids, aliphatics, flavonoids, volatile oils, amino acids, trace elements, carbohydrates, and so on. Tannins are the main active components of TCR, accounting for about 23.60% to 37.36%, and are the main source of polyphenols [43]. Studies have shown that the content of active ingredients such as gallic acid, chebulinic acid, chebulagic acid, ellagic acid, and 5-O-galloyl shikimic acid are higher in TCR, among which gallic acid is the highest. These ingredients can be absorbed into the blood [44]. In this experiment, the tannin content in the water decoction of TCR was 23.2% and gallic acid content was 1.67%. Many traditional medicines work because of a group of ingredients. It is speculated that tannin may be the main component group affecting enzyme activity.

Conclusion

In this study, the HPLC method was developed to simultaneously measure the concentration of six probe drugs in rat plasma to evaluate the effect of TCR on the activity of six CYP450 isoforms. The results indicated that TCR may inhibit the activities of CYP2C19 and CYP2E1, and had no effect on the activities of CYP1A2, CYP2D6, CYP3A4, and CYP2C9. When drugs metabolized by CYP2C19 and CYP2E1 enzymes are used in combination with TCR, extra care should be taken as herb -drug interactions may occur. These results can guide the clinical application of related drugs and provide valuable information for drug interactions. The main component that affects enzyme activity may be tannins in the water extract.

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Disclosure of conflict of interest

None.

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