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ORIGINAL RESEARCH ARTICLE

Effects of three Chinese patent medicines against cardiovascular diseases on the in vitro activities of rat liver microsomal CYP450 four subtypes

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ABSTRACT

Objective: To evaluate the effects of Shensong Yangxin capsule, Qiliqiangxin capsule and Tongmai Yangxin Pill on the in vitro activities of four subtypes of rat liver microsomal CYP450 enzyme CYP1A2, CYP2C11, CYP2D1 and CYP3A1. **Method:** 15.04 μmol·L^{-1} caffeine, 4.98 μmol·L^{-1} omeprazole, 7.81 μmol·L^{-1} metoprolol and 6 μmol·L^{-1} midazolam is the specific probe substrate of CYP1A2, CYP2C11, CYP2D1 and CYP3A1 respectively. Three Chinese patent medicines against cardiovascular diseases are co incubated with the mixed probe substrate of four CYP450 enzyme subtypes in rat liver microsomes respectively. The remaining probe substrate in rat liver microsomes incubation system is measured by HPLC to calculate the inhibition percentage of corresponding enzyme activity and calculate the IC_{50} value. **Results:** Compared with the blank group, the activities of sub enzymes decreased with the increase of the concentration of three drugs ($P < 0.05$); the IC_{50} values of three drugs on rat liver microsomal CYP1A2, CYP2C11, CYP2D1 and CYP3A1 were 1.896, 31.97, 12.37 and 1.357 respectively μmol·L^{-1}; Qiliqiangxin capsule >100, 1.513, 35.2, 6.669 μmol·L^{-1}; Tongmai Yangxin pill >100, 33.970, 0.566, 14.380 μmol·L^{-1}. **Conclusion:** Shensong Yangxin capsule has moderate inhibitory effect on CYP1A2 and CYP3A1, Qiliqiangxin capsule has moderate inhibitory effect on CYP2C11 and CYP3A1, Tongmai Yangxin pill has strong inhibitory effect on CYP2D1, and other effects are not obvious.

Keywords: rat liver microsomes; Shensong Yangxin Capsule; Qiliqiangxin capsule; Tongmai Yangxin pill; CYP450 enzyme

1. Introduction

With the change of diet structure and the aging of the population, the incidence rate and mortality of cardiovascular diseases have increased significantly, threatening the health of human beings. At present, chemical drugs are mainly used to treat cardiovascular diseases, but the adverse reactions of chemical drugs will limit their use, such as thiazine diuretics may lead to hyperkalemia and hyperuricemia. Renin angiotensin converting enzyme inhibitors are prone to dry cough. Calcium antagonists may cause peripheral edema, constipation, palpitation and facial flushing. β Receptor blockers may cause hypoglycemia and weakness in diabetes patients. Nitrates may cause headache, increased heart rate, radiation and hypotension. Antiplatelet drugs may cause liver damage and myopathy[1], while traditional Chinese medicine and traditional Chinese medicine preparations are widely used in anti cardiovascular diseases because of their mild and lasting

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therapeutic effects and the characteristics of multi-channel and multi-target treatment[2]. The induction/inhibition of CYP450 enzymes (CYPs) is caused by the combination of traditional Chinese medicine and Western medicine, and the drug interaction is significantly increased[3]. Therefore, it is of great significance to evaluate the effect of common Chinese patent drugs on the in vitro activity of cyps for clinical safe drug use and reducing the risk of drug-drug interactionin this study, through extensive literature review and visits to the First Affiliated Hospital of Dali University, Dali Prefecture People's Hospital and Dali first people's Hospital in the early stage, three kinds of Chinese patent medicines commonly used for anti cardiovascular diseases and combined with western medicine are selected: Shensong Yangxin Capsule (SS), Qili Qiangxin capsule (QL) and Tongmai Yangxin pill (TM). However, the effects of the above three drugs on liver cyps have not been reported, and human CYP1A2, CYP2C19, CYP2D6 and CYP3A4 have homology with rat CYP1A2, CYP2C11, CYP2D1 and CYP3A1[4]. In view of this, this subject uses the "cocktail" probe drug method to evaluate the inhibitory activities of three common anti cardiovascular Chinese patent drugs in rat liver microsomes on CYP1A2, CYP2C19, CYP2D6 and CYP3A4 enzymes in vitro. To provide experimental data for predicting the possible interaction between these three common Chinese patent medicines for anti cardiovascular diseases and drugs with cyps 4 sub enzymes as substrates, and lay a theoretical foundation for the rational application of clinical drugs

2. Material

Main instrument: EL-304 electronic analytical balance (Mettler Toledo); PH meter (Shanghai Leici); chromatographic: Agilent 1260 HPLC working system (Agilent company of the United States); Target VX III multi tube vortex oscillator (Beijing step Technology Co., Ltd., China); 315 desktop high-speed centrifuge (sigma, USA).

Reagent and drug caffeine (China Institute for food and drug control, batch No.: 20161014); Omeprazole (batch No. G1325050) and metoprolol (batch No. C1729022) were purchased from Aladdin; Midazolam injection (Jiangsu Enhua Pharmaceutical Co., Ltd., batch No.: 20170328). Diazepam injection (Shanghai Xudong Haipu Pharmaceutical Co., Ltd., batch number ah160602). α Naphthalene brass (batch No. O0506a), quinidine (batch No. A1210a), ketoconazole (batch No. J0620as) and fluconazole (batch No. O0615as) were purchased from Dalian Meilun Biotechnology Co., Ltd. Shensong Yangxin Capsule (Beijing Yiling Pharmaceutical Co., Ltd., batch No. 1703008). Qiliqiangxin capsule (Shijiazhuang Yiling Pharmaceutical Co., Ltd., batch No.: a1704018). Tongmai Yangxin pill (lerentang pharmaceutical factory, batch number 1070275) Experimental animals: 10 male SD rats, weighing (200 ± 20) g, provided by Hunan slake Jingda experimental animal Co., Ltd. (syxk Xiang 20130004).

3. Method

Solution preparation internal standard solution preparation: Accurately measure 2 ml diazepam injection (5 mg·mL^{-1}) and put ultrapure water into 5 ml volumetric flask to obtain 2.00 mg·mL^{-1} internal standard stock solution. Accurately measure an appropriate amount of internal standard stock solution and dilute it with methanol acetonitrile (1:1) to 8 μg·mL^{-1} internal standard working solution. Preparation of three Chinese patent medicine solutions: Accurately weigh 20 mg of SS, QL and TM respectively, add methanol to dissolve and fix the volume in a 50 ml volumetric flask to obtain 400 μg·mL^{-1} drug solution to be tested is prepared as 200, 100, 50, 25, 12.5 and 6.25 by multiple dilution method μg·mL^{-1} solution specific operation[5]: 96 hole plate is adopted, and 100 is added to the first hole first μL 400 μg·mL^{-1} of the drug solution to be tested, suck 50 μL add the second hole and add 50 to the second hole μL methanol, mix well and suck away 50 μL is added to the third hole, and then 50 is added to the third hole μL methanol, mix well and suck away 50 μL is added

to the fourth hole, and so on until the seventh hole, then the drug concentrations from the first hole to the seventh hole are respectively: 400, 200, 100, 50, 25, 12.5, 6.25 μg·mL^{-1}.

Preparation of rat liver microsomes calcium precipitation method[6] preparation of liver microsomes: take out the rat liver, weigh it, cut it into pieces, wash it repeatedly with kcl phosphate buffer (pH = 7.4), add 50 mmol·L^{-1} Tris 1.15% kcl buffer solution at 1:4 (w/V), homogenize it on ice, then 4 ℃, 10,000 R/min, centrifuge for 20 min, take the supernatant, add an appropriate amount of $Cacl_2$ solution (1:1, V:V), then centrifuge at 4 ℃, 14,000 R/min for 40 min, and discard the supernatant. Add 1 ml of the above kcl buffer to precipitate, mix well, 4 ℃, 14,000 R/min, centrifuge for 20 min, and the red precipitate is liver microsomes Suspend it in PBS 20% glycerol buffer, sub pack it into EP tube, determine the content of liver microsomal protein by BCA method, and store it at 80 ℃ for standby.

Chromatographic conditions chromatographic column: Zorbax SB C18 (250 mm × 4.6 mm, 5 μm); Mobile phase: acetonitrile (a) 0.1% formic acid aqueous solution. (b) Elution procedure: 0~8 min, a is 10%~40%; 8~15 min, a is 40%~45%; 15~30 min, a is 45%~35%; 30~35 min, a is 35%~20%; 35~40 min, a is 20%~10%. Flow rate: 0.5 mL·min^{-1}. Detection wavelength 280 nm. Column temperature 30 ℃. Injection volume 20 μL.

Sample handling: Add 10 μg·mL^{-1} diazepam internal standard 500 μ50. Vortex mixing for 1 min, centrifugation at 12,000 R/min for 10 min, and take the supernatant 200 μ50. Injection 20 μL[7].

In vitro reaction of probe substrate with rat liver microsomes and determination of total volume of incubation system by HPLC μ50. Include 15 μL rat liver microsomal protein (0.5 mg·ML^{-1}), 10 μL NADPH regeneration system (1 mmol·L^{-1} $NADP^+$, 10 mmol·L^{-1} g-6-p, 1 U·mL^{-1} G-6-PDH), 472.5 μL PBS buffer (pH = 7.4), 2.5 μL mixed probe substrate (15.04 μmol·L^{-1} caffeine, 7.81 μmol·L^{-1} metoprolol, 4.98 μmol·L^{-1} omeprazole and 6 μmol·L^{-1} midazolam). Pre incubate in a 37 ℃ water bath for 5 min, the nadhp regeneration system starts the reaction, takes it out after reacting at 37 ℃ for 30 min, processes the sample according to the "2.4" item, and injects the sample according to the chromatographic conditions under "2.3", and makes 3 copies in parallel for each group.

The methodological investigation shall be conducted in accordance with the methodological evaluation guidelines of the U.S. Food and Drug Administration[8], including specificity, linear relationship, recovery rate, precision and stability.

Specificity: Take rat blank liver microsomes, blank liver microsomes + internal standard solution, blank liver microsomes + Standard + internal standard respectively, treat the samples according to "2.4" and inject the samples under chromatographic conditions under "2.3" to investigate the separation and interference of chromatographic peaks.

Linear relationship: Dilute 2.00 mg·mL^{-1} caffeine, metoprolol and midazolam stock solution and 1.00 mg·mL^{-1} omeprazole stock solution into mixed probe solutions of different concentrations with methanol, and the final concentrations are caffeine 1.91, 3.75, 7.52, 15.04, 30.02, 37.59 and 45.16 respectively μmol·L^{-1}, omeprazole 0.64, 1.24, 2.49, 4.98, 9.99, 12.56, 15.14 μmol·L^{-1}, metoprolol 0.98, 1.96, 3.91, 7.81, 15.62, 19.52, 23.42 μmol·L^{-1}, midazolam 0.74, 1.51, 3.02, 6.00, 12.00, 16.49, 18.00 μmol·L^{-1}. Will 25 μL rat liver microsomes added 472.5 μL PBS buffer, heat to inactivate at 60 ℃, place it at room temperature and add 2.5 μL mixed substrate incubation system, incubate according to "2.5" prepare 3 copies of each sample in parallel.

Precision take blank liver microsome25 μ50. Add 472.5 μL PBS buffer, and then add 2.5% respectively μL mixed probe standard solution of low, medium and high concentration (caffeine 3.75, 15.04 and 37.59) μmol·L^{-1}, metoprolol 1.96, 7.81, 19.52 μmol·L^{-1}, omeprazole 1.24, 4.98, 12.56 μmol·L^{-1}, midazolam 1.51,

6.00, 1.49 μmol·L^{-1}), the rest are operated according to the method under "2.4", and the samples are injected for 3 times in the same day for determination to obtain the intraday RSD; Determine three times for three consecutive days to obtain the daytime RSD.

Recovery rate take blank liver microsomes 25 μ50. Add 472.5 μL PBS buffer, and then add 2.5% respectively μL the mixed probe standard solution with low, medium and high concentration prepared according to the method under "2.6.3". After the rest is processed and injected according to the method under "2.4", record the peak area *A*1, then select the reference solution with corresponding concentration for injection, obtain the peak area *A*2, and calculate the absolute recovery (Absolute recovery = *A*1/*A*2 × 100%). Use the standard curve regression equation to calculate the drug concentration, compare it with the theoretical concentration, and calculate the relative recovery (Relative concentration = Measured concentration/Theoretical concentration × 100%).

Stability take blank liver microsome25 μL. Add 472.5 μL PBS buffer, add 2.5 μL samples of low, medium and high concentration under "2.6.3" respectively. Six samples of each mass concentration shall be analyzed. The samples shall be placed at room temperature for 6 h, frozen at 80 ℃ for 2 weeks, and repeatedly frozen and thawed for 3 times. The samples shall be treated and measured according to item "2.4".

Study on the inhibitory effect of positive inhibitors put in an ice bath and add 15 μL rat liver microsomes (0.5 mg·mL^{-1}) were added with 2.5% PBS solution respectively μL different concentrations of positive inhibitors α. The final concentrations of naphthalene brass, quinidine, fluconazole and ketoconazole are: 10, 50, 50, 10 μmol·L^{-1}) and 2.5 μL mix the probe solution. The following operations are the same as those in "2.4". The blank group uses equal volume methanol solution and makes 3 copies of each in parallell.

Inhibition of three Chinese patent medicines against cardiovascular diseases on rat liver microsomes: Replace the positive inhibitor in the incubation system with each Chinese patent medicine to be tested, and the series concentrations of the drug group are 6.25, 12.5, 25, 50, 100 and 200 μg·mL^{-1}, incubate according to the operation under "2.4". The corresponding enzyme inhibition rate is calculated from the residual amount of substrate in different concentration reaction groups: Enzyme activity = (Total substrate concentration, residual substrate concentration)/Total substrate concentration. Use graphpad prism 5.0 software for linear regression, plot the relative activity of the enzyme against the concentration of the drug to be measured, and calculate the half inhibitory concentration (IC_{50}) value of each drug.

SPSS 22.0 software is used for data processing and statistical analysis. The data are described in ($\bar{x} \pm s$). One way ANOVA is used for the drug group to be tested and the blank group. When $p < 0.05$, it can be considered that there is significant difference between the two groups.

4. Results

In vitro reaction of probe substrate with rat liver microsomes and HPLC determination.

The chromatogram of specific probe substrates incubated with rat liver microsomes for 30 min is shown in **Figure 1a–c**. The relative retention times of caffeine, metoprolol, omeprazole, midazolam and internal standard are: 8.006, 9.205, 10.216, 11.678 and 19.120 min. The results show that under the selected chromatographic conditions, other substances in the incubation system do not interfere with the determination of mixed probe substrate and internal standard, and each probe substrate peak is well separated from the internal standard peak, indicating that the method has good specificity.

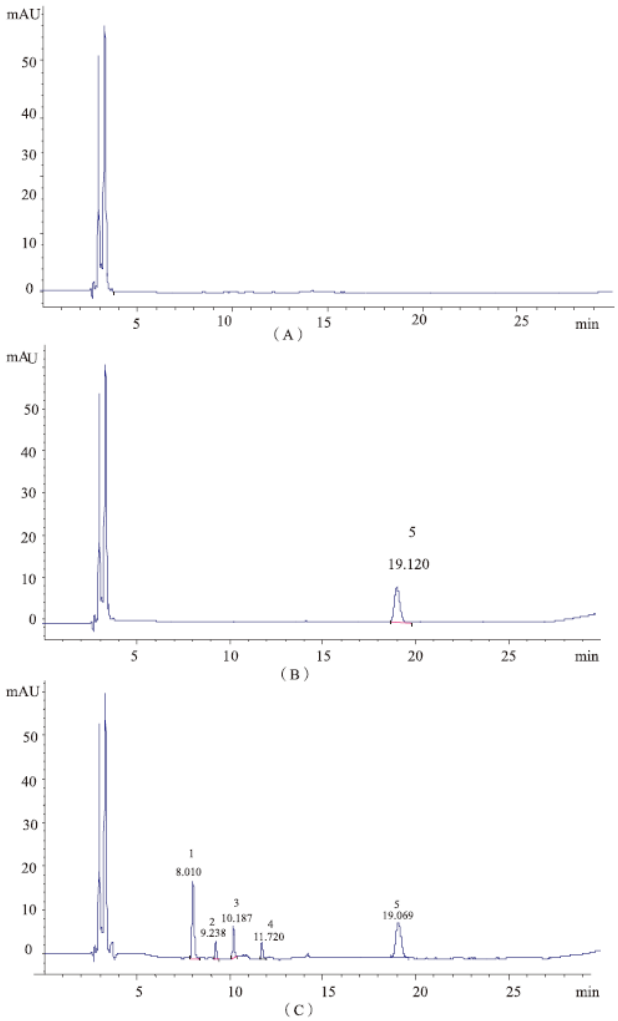

Figure 1. (a) HPLC spectra of blank liver microsomes; **(b)** blank liver microsomes + internal standard solution; **(c)** blank liver microsomes + Standard + internal standard.
1: Caffeine; 2: Metoprolol; 3: Omeprazole; 4: Midazolam; 5: Diazepam.

The linear relationship takes the probe substrate concentration as the abscissa, and the ratio of the peak area of the object to be measured to the internal standard peak area as the ordinate. The weighted least square method is used for linear regression, as shown in **Table 1**. The results show that the regression equation of each probe drug has a good linear relationship in the concentration range, indicating that the method has good sensitivity and is accurate and reliable when determining samples in the linear range.

Table 1. Standard curve equation of each probe drug (n = 3).

Substrate	**Linear range/μmol·L^{-1}**		**Regression equation**	***R***
Caffeine	1.90	~45.16	$Y = 0.0928x - 0.1209$	0.9956
Metoprolol	0.98	~23.42	$Y = 0.0182x + 0.0072$	0.9964
Omeprazole	0.64	~15.14	$Y = 0.0589x + 0.0137$	0.9960
Midazolam	0.74	~18.00	$Y = 0.0352x + 0.0051$	0.9971

Precision the intra day and intra day precision of the four probe substrates in liver microsomes are RSD <10%, and the precision is good. See **Table 2** for the results.

Recovery rate the range of relative recovery rate is (88.18 ± 0.32)%~(108.92 ± 0.34)%, and the range of absolute recovery rate is (75.85 ± 6.09)%~(93.37 ± 1.40)%, which meets the detection requirements. See **Table 2** for the results.

Table 2. Test results of drug precision and recovery of each probe (n = 5, $\bar{x} \pm s$).

Substrate	Addition amount/μmol·L^{-1}	Intra day precision		Daytime precision		Rate of recovery	
		Measured quantity μmol·L^{-1}	RSD/%	Measured quantity μmol·L^{-1}	RSD/%	Relative recovery/%	Absolute recovery/%
Caffeine	3.75	4.01 ± 0.19	4.78	4.02 ± 0.37	9.28	100.50 ± 2.58	86.28 ± 10.20
	15.04	13.04 ± 0.04	0.34	12.29 ± 1.17	9.51	90.82 ± 0.21	86.32 ± 4.82
	45.16	44.57 ± 0.14	0.30	49.55 ± 3.02	6.10	109.00 ± 0.34	93.37 ± 1.40
Metoprolol	1.96	1.57 ± 0.02	1.27	1.71 ± 0.12	6.72	107.80 ± 0.99	87.82 ± 5.72
	7.81	5.10 ± 0.03	0.54	6.85 ± 0.61	8.89	90.93 ± 0.55	79.68 ± 4.07
	23.42	21.28 ± 0.31	1.44	25.93 ± 2.45	9.43	94.98 ± 0.48	80.44 ± 1.43
Omeprazole	1.24	1.06 ± 0.08	7.59	1.18 ± 0.12	9.94	91.06 ± 6.68	75.85 ± 6.09
	4.98	4.59 ± 0.02	0.48	4.49 ± 0.37	8.34	95.14 ± 0.05	87.38 ± 0.51
	15.14	16.12 ± 0.10	0.63	14.89 ± 0.91	6.09	104.20 ± 0.24	78.56 ± 1.91
Midazolam	1.51	1.37 ± 0.11	7.76	1.35 ± 0.11	8.26	90.93 ± 2.33	80.94 ± 5.33
	6.00	5.47 ± 0.07	1.36	5.26 ± 0.22	4.12	88.18 ± 0.32	87.05 ± 1.36
	18.00	16.11 ± 0.22	1.36	15.83 ± 0.50	3.14	89.97 ± 0.40	80.61 ± 0.16

Stable liver microsomal samples with high, medium and low concentrations are placed at room temperature for 6 h and 80 ℃ for 3 times, and frozen for 14 days. Each probe has good stability and meets the requirements of biological sample analysis.

Inhibition of cyps by positive inhibitors. The activity of cyps subenzymes is inhibited by positive inhibitors, as shown in **Table 3**. After adding the positive inhibitor, the metabolism of each sub enzyme slowed down significantly, indicating that α. Naphthalene brass, quinidine, fluconazole and ketoconazole significantly inhibited the activities of CYP1A2, P2D1, P2C11 and P3A1 respectively, with significant difference compared with the control group ($P < 0.05$), so as to further verify the rationality of the test method. Comparing the IC_{50} value of positive inhibitors with the literature[9], it meets the scope of the literature, and the experimental system meets the requirements of cyps inhibitory activity evaluation.

Table 3. Inhibition of cyps enzymes by positive inhibitors (n = 3, $\bar{x} \pm s$).

CYPs	Positive inhibitor	Inhibition rate/%	Inhibition rate of control group/%
CYP1A2	α-Naphthalene brass	29.22 ± 0.54*	39.26 ± 1.31
CYP2D1	Quinidine	53.60 ± 0.25*	64.56 ± 1.74
CYP2C11	Fluconazol	29.30 ± 0.50*	34.98 ± 1.70
CYP3A1	Tong Kangzuo	16.71 ± 0.58*	30.47 ± 0.46

Note: compared with the blank control group, *$P < 0.05$.

Inhibitory effect of three Chinese patent medicines against cardiovascular diseases on cyps.

The inhibition curves of four cyps enzymes in different concentration drug groups are shown in **Figures 2–4**. It can be seen from the figure that with the increase of drug concentration, the activity of sub enzymes decreased significantly The IC_{50} value calculated from the suppression curve is shown in **Table 4**. According to the general classification rules of cyps enzyme inhibition intensity[10]: $IC_{50} < 1$ μmol·L^{-1} is a strong inhibitor;

one μmol L^{-1} < IC_{50}< 10 μmol·L^{-1} was a medium inhibitor. IC_{50} > 10 μmol·L^{-1} is a weak inhibitor. Therefore, the IC_{50} values of Shensong Yangxin Capsule for CYP1A2 and P3A1 and Qiliqiangxin capsule for CYP2C11 and P3A1 are respectively: 1.896, 1.357, 1.513, 6.669 μmol·L^{-1}, all moderate inhibitors. The IC_{50} value of Tongmai Yangxin pill for CYP2D1 was 0.566 μmol·L is its strong inhibitorr. The IC_{50} values of Shensong Yangxin Capsule for CYP2D1 and p2c11, Qiliqiangxin capsule for CYP2D1 and p1a2 and Tongmai Yangxin pill for CYP2C11, P3A1 and p1a2 were greater than 10 μmol·L^{-1}, the possibility of inhibiting cyps is small, and it is not easy to produce metabolic drug interaction.

Table 4. IC50 values of three anti cardiovascular Chinese patent medicines (μmol·L^{-1}).

CYPs	Substrate	IC_{50} value of SS	IC_{50} value of QL	IC_{50} value of TM
CYP1A2	Caffeine	1.896	>100.000	>100.000
CYP2D1	Metoprolol	31.970	35.200	0.566
CYP2C11	Omeprazole	12.370	1.513	33.970
CYP3A1	Midazolam	1.357	6.669	14.380

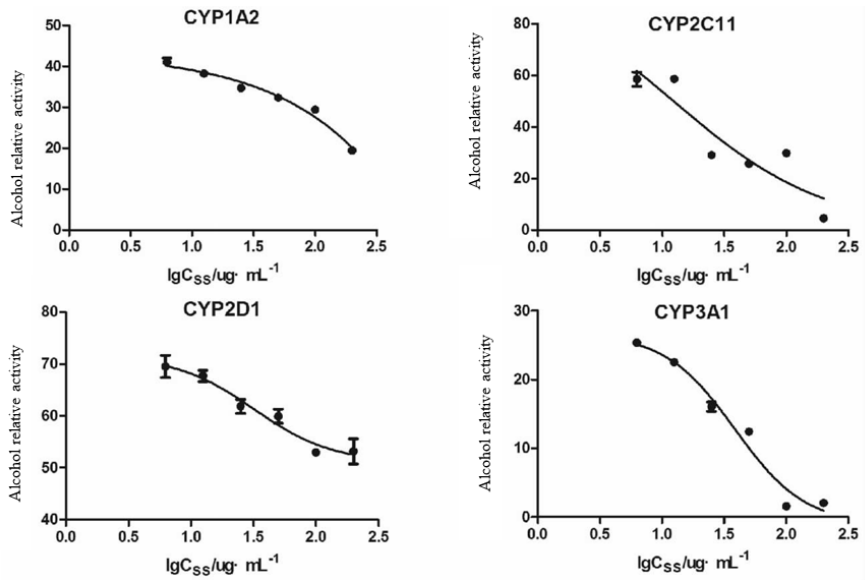

Figure 2. Inhibition curve of SS on CYP enzyme activities.

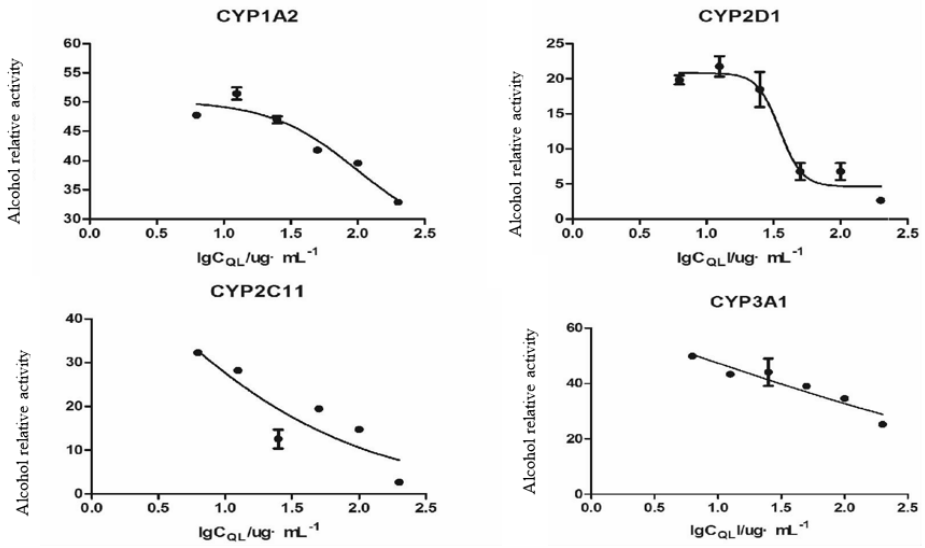

Figure 3. Inhibition curve of QL on CYP sub enzyme activity.

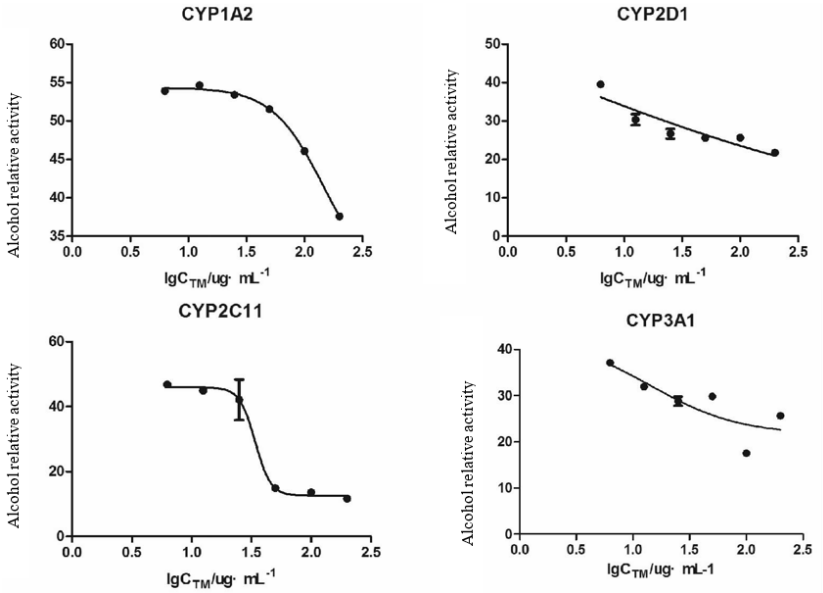

Figure 4. Inhibition curve of TM on CYP sub enzyme activity.

5. Discussion

Cyps, as a kind of important drug metabolizing enzymes, participate in 75% of clinical drug metabolism. Traditional Chinese medicine preparations will inhibit or induce enzymes while undergoing metabolic transformation under the action of metabolic enzymes in vivo, resulting in metabolic interaction[10]. The clinical significance of drug interaction caused by the inhibition of cyps (about 70% of all interactions) is much greater than that of induction (23%)[11]. "Cocktail" probe drug method refers to a method to obtain the phenotypic information of multiple metabolic enzymes, first give a variety of relatively low-dose probe drugs at the same time, and then determine the metabolic rate or other metabolic typing indicators of each probe drug in biological samples. It has the advantages of fast, high efficiency and good reproducibility[12]. The author evaluated the effects of Shensong Yangxin capsule, Qiliqiangxin capsule and Tongmai Yangxin Pill on the activities of cyps in liver microsomes by "cocktail" probe drug method, so as to predict the drug interaction and provide clinical medication reference According to the literature[10], Shensong Yangxin capsule has moderate inhibitory effects on CYP1A2 and P3A1 and Qiliqiangxin capsule has moderate inhibitory effects on CYP2C11 and P3A1. Tongmai Yangxin pill has strong inhibitory effect on CYP2D1 The inhibitory effect of drugs on CYP1A2, CYP2C11 and CYP3A1 enzymes will reduce the enzyme activity accordingly, which may lead to the slow metabolism of other drugs metabolized by the above sub enzymes, the increase of blood drug concentration or drug accumulation in the body, and the action time is relatively prolonged. It should be used with caution in clinic The function of traditional Chinese medicine is the interaction between two complex systems caused by the organic combination of multiple effective components and the comprehensive influence of multiple disease-related targets[13]. It is reported in literature[14,15] that the main component of Shensong Yangxin capsule, water extract of jujube kernel, and gallic acid, the main active component of Cornus officinalis and mulberry parasitism, can inhibit CYP3A activity Piperin II, the main active component of Astragalus membranaceus in Qiliqiangxin capsule, can inhibit the activity of human cyp2c16, and the main pharmacodynamic substance of ginseng in the component, total ginsenoside, can down regulate the activity of human CYP3A4 enzyme and prolong the drug action time[16,17]. Both Shensong Yangxin capsule and Qiliqiangxin capsule contain Salvia miltiorrhiza. Wang et al.[18] showed that the alcohol extract of Salvia miltiorrhiza significantly inhibited the activity of CYP1A2 in human and rat liver particles, as well as the enzyme activity of CYP3A4 in human liver particles and CYP3A1 in rat liver particles Glycyrrhizic acid

(glycyrrhizin), glycyrrhetinic acid, glycyrrhizin and glycyrrhizin, the effective components of licorice in Tongmai Yangxin pill, can inhibit the activity of CYP2D1[19,20]. These research results are not inconsistent with the experimental resultstherefore, the inhibitory effect of Shensong Yangxin capsule, Qiliqiangxin capsule and Tongmai Yangxin Pill on cyps sub enzymes may be related to the active components of its main components.

In this study, the three drugs act directly on rat liver microsomes, which is quite different from the traditional way of administration, and the composition of traditional Chinese medicine is complex. The effect of oral administration and absorption on cyps sub enzyme activity of liver microsomes is quite different from that in vitro. In vitro research or animal research can not completely replace human in vivo research. Therefore, whether the three drugs have inhibitory effect in clinical application needs to be further verified by human liver microsomes or human combined drugs.

Conflict of interest

The authors declare no conflict of interest.

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