Effects of Xiaochaihu decoction on the expression of cytochrome P450s in rats

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Abstract. Xiaochaihu decoction is one of the most important traditional Chinese medicines that is widely used with other drugs in clinical practice, and may cause drug-drug interactions. However, there is not sufficient experimental evidence for the effects of Xiaochaihu decoction on cytochrome P450s (CYPs). The aim of the present study was to investigate the effects of Xiaochaihu decoction on the mRNA and protein levels of hepatic CYPs. Eighty normal male Sprague-Dawley (SD) rats were randomly divided into two groups based on body weight and duration of drug administration (3 and 6 days). Each group was further divided into subgroups: Control group (2 ml 5‰ CMC-Na); hepatic enzyme inducer group (50 mg/kg/day rifampicin); and experimental groups (Xiaochaihu decoction: Low dose, 1.7 g/kg/day; medium dose, 3.4 g/kg/day; high dose, 6.8 g/kg/day). The effects of Xiaochaihu decoction on *Cyp1a2, Cyp3a1, Cyp2d6,* and *Cyp1b1* mRNA and protein expression in rats were evaluated using reverse transcription quantitative reverse transcription polymerase chain reaction and western blot analysis. After 3 days, medium dose of Xiaochaihu decoction inhibited the mRNA and protein expression of *Cyp1a2, Cyp3a1* and *Cyp1b1*. In addition, after 6 days, Xiaochaihu decoction induced *Cyp3a1* mRNA expression at low and medium doses; *Cyp2d6* mRNA expression at low and high doses; and *Cyp2d6* protein expression at high doses. Nonetheless, the gene and protein expression of *Cyp1b1* was not affected at any dose. The findings of the present study may provide insights into potential drug-drug interactions associated with Xiaochaihu decoction.

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Introduction

Xiaochaihu decoction is a traditional Chinese medicine that is prepared from seven herbs (Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Glycyrrhizae Radix, Zingiberis Rhizoma) (1), which has attracted increasing attention as an alternative treatment and supplement. Xiaochaihu decoction has been widely applied for the treatment of 15 types of 262 diseases, including diseases of the digestive, respiratory and nervous system (2). To facilitate the use of Xiaochaihu decoction in the clinical setting, researchers have developed several dosage forms of Xiaochaihu decoction, including granules, capsules, tablets and effervescent tablets, which have been recorded in the 2015 edition of Chinese Pharmacopoeia (Part I) (3). With continuous experimental and clinical exploration, Xiaochaihu decoction has been co-administered with various synthesised drugs such as rabeprazole, isosorbide mononitrate, and entecavir for digestive, cardiovascular, cerebrovascular and immune diseases, respectively (4-6).

The concomitant administration of two or more drugs may lead to drug-drug interactions (DDIs), which are considered a common cause of adverse drug reactions (ADRs) (7). Previous studies have reported that co-administration of Xiaochaihu decoction with tolbutamide (8), carbamazepine (9) and cyclosporine A (CsA) (10) affects the *in vivo* efficacy of the latter drugs. The interaction between Xiaochaihu decoction and other concomitant drugs is a problem worthy of attention for basic and clinical researchers.

Despite many reasons for DDIs, metabolic interactions account for ~40% of them. Metabolic interactions are often caused by enzymes involved in drug metabolism, mainly hepatic enzymes in the cytochrome P450 (CYP) family. CYPs are mixed functional oxidases involved in the metabolism of endogenous and exogenous substances (11,12), and they can significantly affect drug metabolism (13). Many drugs act as substrates, inducers or inhibitors of CYPs. When a drug induces or inhibits CYPs, it causes changes in the physiological environment of another drug, consequently altering their efficacy and even leading to toxicity (14). Therefore, changes in the content or activity of CYPs by one or more concomitant drugs are one of the main causes of DDIs in the

clinical setting. It is important to study the effects of drugs on CYPs for understanding the interactions between drugs used in combination.

With the continuous development as well as improvements in the knowledge and understanding of traditional Chinese medicine, Xiaochaihu decoction and other formulations would be increasingly used in combination with synthesised drugs. Xiaochaihu decoction in combination with other drugs may alter the metabolism of the concomitant drug and lead to changes in the blood levels of the drugs. It may further decrease the efficacy of drugs or increase the likelihood of ADRs. To effectively obtain the benefits and avoid the disadvantages of combination therapy to the utmost, it is necessary to identify and confirm the mechanisms underlying these interactions at the earliest. At present, there is not sufficient experimental information on the effects of Xiaochaihu decoction on CYPs. Therefore, further research is needed to confirm the existing conclusions. Modern pharmacological toxicology studies have shown that many drugs affect the gene transcription and protein expression of CYPs (14,15). Therefore, rats were selected to investigate the effects of Xiaochaihu decoction on gene transcription and protein expression of different subtypes of CYPs. The findings will help better elucidate the effects of combining Xiaochaihu decoction with other drugs that are metabolised by CYPs.

Materials and methods

Medicinal materials and reagents. The crude components of Xiaochaihu decoction, including Bupleuri Radix, Pinelliae Tuber, Scutellariae Radix, Zizyphi Fructus, Ginseng Radix, Glycyrrhizae Radix, and Zingiberis Rhizoma, were all purchased from the Department of Pharmacy in the Affiliated Hospital of Zunyi Medical University (Guizhou, China) and identified as quality Chinese herb medicines by Professor Jianwen Yang in Department of Pharmacognosy, Zunyi Medical University. Rifampicin was purchased from Chengdu ALFA Biotechnology Co., Ltd. Baicalin was purchased from Guizhou Dida Technology Co., Ltd. All other reagents were obtained from local reagent companies.

Preparation of Xiaochaihu decoction. Xiaochaihu decoction was prepared according to the proportion of Xiaochaihu granules in the Pharmacopoeia of the People's Republic of China 2015 edition (3) and the optimal extraction scheme of Xiaochaihu decoction reported by Cai *et al* (16). Briefly, 125 g Bupleuri Radix, 45 g Pinelliae Tuber, 45 g Scutellariae Radix, 45 g Zizyphi Fructus, 45 g ginger, 45 g Codonopsis Pilosula and 45 g Glycyrrhizae Radix were mixed with an 8-fold volume of distilled water evenly. After the herbs were moistened thoroughly, the mixture was boiled at 100˚C for 40 min and filtered through a gauze to obtain the filtrate. Subsequently, the dregs were boiled with an 8-fold volume of distilled water at 100˚C for 40 min and filtered again. Subsequently, the filtrates were mixed together and concentrated to a brown sticky extract (1 g/ml) in a rotary evaporator to obtain the decoction for experiments. The decoctions were stored at 4˚C.

Rifampicin solution. Rifampicin powder was suspended in distilled water and mixed in a vortex mixer to obtain rifampicin at a concentration of 5 mg/ml prior to each administration.

Animals. Eighty specific pathogen-free (SPF) male SD rats (220±20 g) for the experiments were provided by Liaoning Changsheng Biotechnology Co., Ltd. [Animal license number of the rats was SCXK: (Liaoning) 2015-0001]. All the rats were housed in environmentally controlled conditions (temperature, 20-24˚C; and relative humidity, 40-60%) with a 12-h light/dark cycle. Rats were acclimated to the environment for 7 days before the experiments. All animal experiments were strictly carried out in accordance with the NIH guidelines for the Care and Use of Laboratory Animals (NIH publications no. 80-23; revised 1996). The study protocol was approved by the Animal Experimentation Ethics Committee of Zunyi Medical University, China (approval no. ZMUER2014-2-069).

Experiment grouping. Eighty normal SPF SD male rats were randomly divided according to their body weight into two groups corresponding to the duration of administration (3 and 6 days). Each group of rats was further divided into blank control (2 ml 5% sodium carboxymethyl cellulose solution), positive control of CYP inducer (50 mg/kg/day rifampicin), and experimental groups (Xiaochaihu decoction: Low dose, 1.7 g/kg/day; medium dose, 3.4 g/kg/day; and high dose, 6.8 g/kg/day), with eight rats in each group. These drug doses given to animals were selected primarily in reference to previous studies (17,18) and pre-experimental results.

Intragastric administration and sample extraction. The corresponding treatments were administered to the rats in each group every morning. After the last dose, the rats were fasted for 24 h and euthanised by cervical dislocation. Animals were anesthetized via intraperitoneal injection with pentobarbital sodium (60 mg/kg) prior to cervical dislocation. The livers of the rats were excised, frozen in liquid nitrogen, and stored in a refrigerator at -80˚C.

Quality control of Xiaochaihu decoction by HPLC. Quality control of the Xiaochaihu decoction was carried out by using high pressure liquid chromatography (HPLC) to determine baicalin content in Xiaochaihu decoction. HPLC method was adapted from previously established method (19). Briefly, by using an Agilent-1260 HPLC system (Agilent Technologies, Inc.) with a photodiode array detector, the analysis was achieved through a TSK gel ODS C18 (250x4.6 mm; 5 μm; Tosoh) column with mobile phase of methanol-water phosphoric acid (65:35:0.7) at a flow rate of 1.0 ml/min. The detection wavelength was set as 280 nm, the column temperature as 30˚C, and the injection volume as 5 μl.

Total RNA isolation and reverse transcription-quantitative (RT-q)PCR assays. Total RNA was isolated from rat liver tissue using RNAiso Plus (Berkeley), and the concentration was quantified by spectrophotometer (A260/A280). The RNA was reverse transcribed into complementary DNA (cDNA) using a FlexCycler and the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Target gene expression was analysed via qPCR system (Bio-Rad Laboratories, Inc.) with SYBR Green PCR kit (Berkeley). The reactions for each of the target mRNAs consisted of 25-μl volumes including 12.5 μl TB Green Premix Ex Taq II (Takara Bio, Inc.), 1 μl specific

Table I. Primer sequences used for reverse transcription-quantitative PCR reactions.

Gene	Forward (5' to 3')	Reverse (5' to 3')
CYP1A2	aggtcaaccatgatgagaagcagtg	aggaggatggctaagaagaggaagac
CYP1B1	gagagttggtggcagtgttggtg	ctcggcatcgtcgtggttgtac
CYP2D6	gtgctgccttcgctgaccatag	tccagattcctcctcaagagtgtcc
CYP3A1	cgttcaccagtggaagactcaagg	acttctttcacagggacaggtttgc
β-actin	cacccgcgagtacaaccttc	cccatacccaccatcacacc

forward primer, 1 μl specific reverse primer, 2 μl cDNA and 8.5 μl sterile water. A standard PCR amplification procedure was performed as follow: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec. Analysis of each specimen for each target was repeated three times. Gene transcription was analyzed by the $2^{-\Delta\Delta Cq}$ method (20) using β-actin expression as a reference. The forward and reverse primer sequences used in this study are shown in Table I.

Western blot analysis. The protein levels of *Cyp1a2*, *1b1, 2d6* and *3a1* in rat livers were evaluated using western blotting assay. Briefly, total protein was extracted from rat liver tissue using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.) containing with 1% proteinase inhibitor phenylmethylsulfonyl fluoride (PMSF). After centrifugation, the supernatant of the lysates was obtained. The protein concentration was quantified using a bicinchoninic acid (BCA) assay kit (Beijing Solarbio Science & Technology Co., Ltd.). The protein samples (50 μg) were added to per lane, and separated on 10% sodium dodecyl sulphate-polyacrylamide gels by electrophoresis and then transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% non-fat milk at room temperature for 2 h. The primary antibodies GAPDH (1:5,000, cat. no. BL006B; Biosharp life sciences Co., Ltd), CYP1A2 antibody (1:4,000, cat. no. bs-2589R; Biosynthesis Biotechnology Co., Ltd.), CYP1B1 antibody (1:4,000; cat. no. bs-12926R; Biosynthesis Biotechnology Co., Ltd.), CYP3A1 antibody (1:4,000, cat. no. bs-20586R; Biosynthesis Biotechnology Co., Ltd.) and CYP2D6 (1:4,000, cat. no. bs-1725R; Biosynthesis Biotechnology Co., Ltd.) were incubated at 4˚C overnight. Goat anti-rabbit IgG secondary antibody (1:5,000; cat. no. BL003A; Biosharp Life Sciences) was added to the membranes and incubated for 1 h at room temperature. The protein bands were visualized using the ECL prime detection reagent (cat. no. KGP1121-KGP1123; Nanjing KeyGen Biotech Co., Ltd.) and using a ChemiDoc MP imaging system (Bio-Rad Laboratories, Inc.). Image Lab version 6.0 gel imaging system software (Bio-Rad Laboratories, Inc.) was used to analyze the gray value of the target strip.

Statistical analysis. SPSS20.0 (IBM Corp.) was used for statistical analysis of all data; and the Kolmogorov-Smirnov test was used as a distribution normality test. The comparison between groups was evaluated using one-way analysis of variance (ANOVA). When variance was equal, the last significant difference method was used for multiple comparisons between groups. When assessed variance was not equal, the Dunnett's T3 method was used for multiple comparisons between groups.

Table II. Content of baicalin in Xiaochaihu decoction (n=3).

Batch no.	Content, mg/ml	Average content, mg/ml
1	3.21	
2	3.27	3.26
3	3.30	

Results are presented as mean ± SE. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Quality control of Xiaochaihu decoction. The HPLC chromatograms of reference substance and baicalin in Xiaochaihu decoction are presented in Fig. 1, and the retention time of baicalin in reference substance and in Xiaochaihu decoction were 4.87 and 4.85 min, respectively. As shown in Table II, the average content of baicalin in Xiaochaihu decoction was 3.26 mg/ml, which was consistent with previous results (3.39 mg/ml) (19), and the decoction could be used for follow-up research.

Effects of Xiaochaihu decoction on the mRNA and protein expression of Cyp1a2 in the liver. Fig. 2 shows the relative expression of *Cyp1a2* mRNA in the liver tissue of each group. Compared with that in the control, *Cyp1a2* mRNA was downregulated in the rifampicin group after 3 days of administration ($P<0.05$) but slightly upregulated after 6 days of administration. The results showed that rifampicin had no significant effects on the expression of *Cyp1a2* when administered for a relatively longer time. Liver-specific *Cyp1a2* mRNA and protein expression levels were significantly downregulated in the medium-dose treatment groups after 3 days compared with those in the control group ($P<0.01$). In the high-dose treatment group, there was a slight but insignificant increase in *Cyp1a2* mRNA and protein expression after 3 days ($P>0.05$). After 6 days, *Cyp1a2* was upregulated in the medium-dose group and upregulated slightly in other groups ($P>0.05$). These results showed that short-term administration of Xiaochaihu decoction can slightly inhibit *Cyp1a2* expression in rats; however, this inhibitory effect disappeared with prolonged administration.

Effects of Xiaochaihu decoction on mRNA and protein expression of Cyp3a1 in the liver. Fig. 3 shows the relative expression of *Cyp3a1* mRNA in the livers of each group. *Cyp3a1* mRNA and protein levels in the liver were increased in

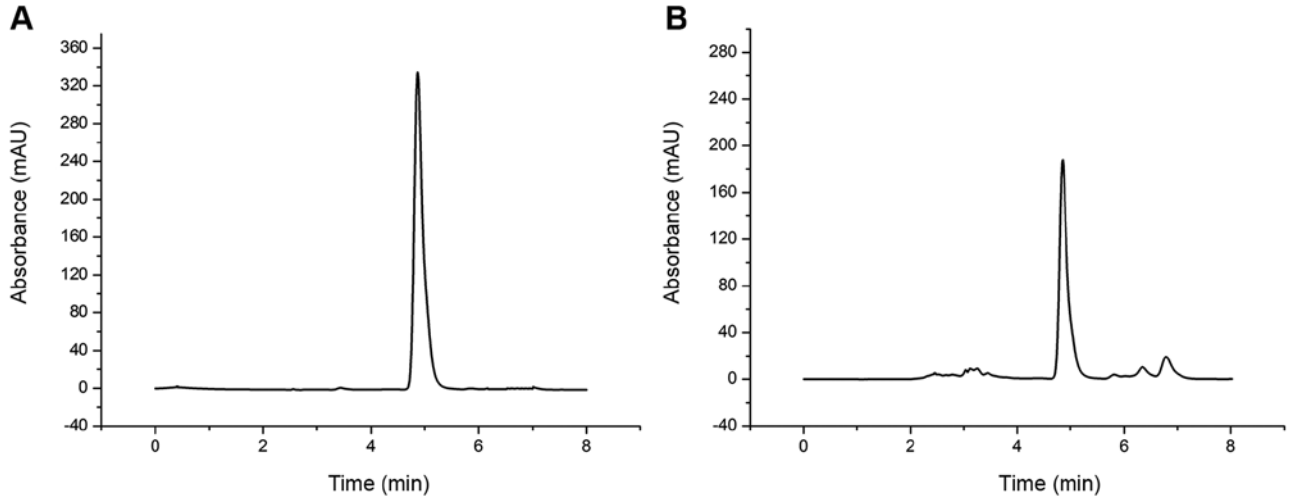

Figure 1. HPLC chromatogram of baicalin. Representative HPLC chromatogram of baicalin reference substance (A) and baicalin in Xiaochaihu decoction (B), with a retention time of 4.87 and 4.85 min, respectively. HPLC, high-performance liquid chromatography.

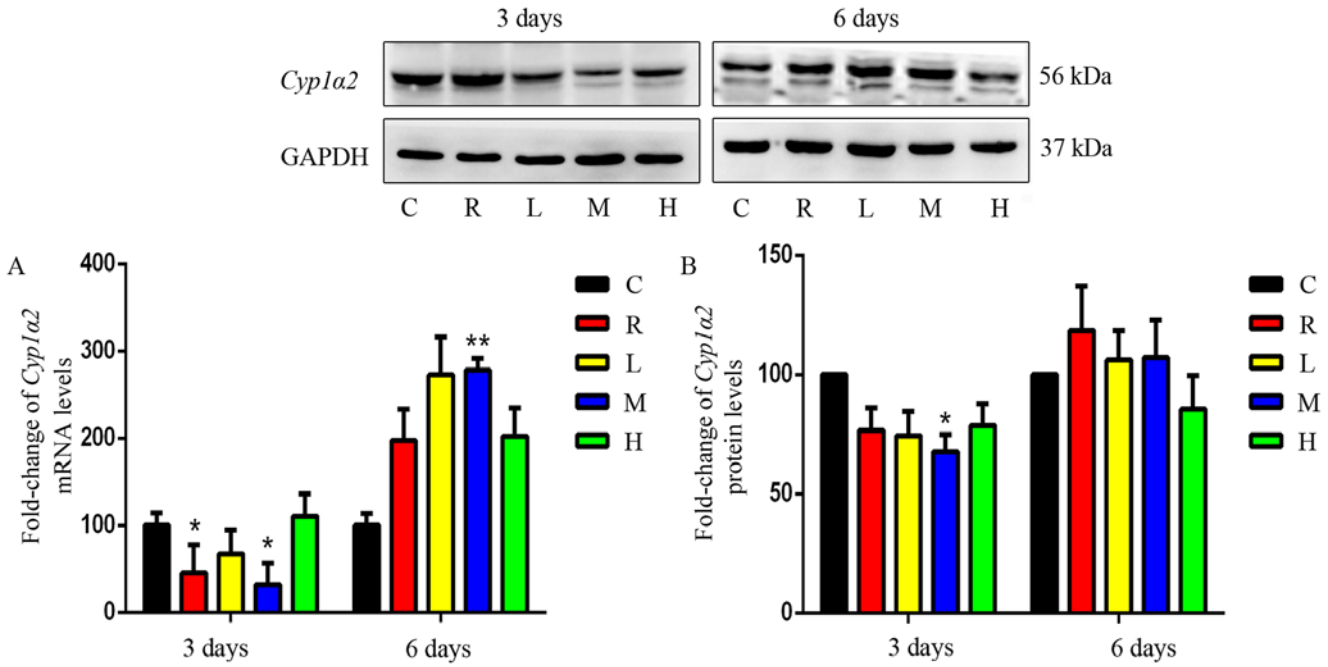

Figure 2. Relative expression level of *Cyp1a2* mRNA and protein level. The relative expression level of *Cyp1a2* mRNA (A) and protein (B) in the liver of rats which were treated with Xiaochaihu decoction once daily for 3 and 6 days. The data are expressed as mean ± SE (n=8). *P<0.05; **P<0.01 vs. control. C, control; R, rifampicin; L, low-dose; M, medium-dose; and H, high-dose group.

the rifampicin group after 6 days of administration compared with those in the control. There was a significant increase in *Cyp3a1* mRNA expression in the medium-dose group compared with that in the low-dose group. Furthermore, there was a significant increase in *Cyp3a1* mRNA expression in the high-dose group compared with that in the medium-dose group. *Cyp3a1* protein levels were significantly higher in the high-dose group than in the low- and medium-dose groups. Thus, Xiaochaihu decoction was shown to induce *Cyp3a1* mRNA and protein expression in a dose-dependent manner.

Effects of Xiaochaihu decoction on mRNA and protein levels of Cyp2d6 in the liver. As shown in Fig. 4, the differences in *Cyp2d6* mRNA or protein expression between the rifampicin or each Xiaochaihu group and the control after 3 days of administration were unchanged. After 6 days, *Cyp2d6* mRNA and protein levels were mildly upregulated in the livers of the rifampicin group. *Cyp2d6* mRNA was significantly upregulated in the livers of the low- and high-dose groups (P<0.05). *Cyp2d6* protein levels were significantly upregulated in the livers of the high-dose group (P<0.05). Therefore, Xiaochaihu decoction induced *Cyp2d6* mRNA and protein expression after administration for 6 days.

Effects of Xiaochaihu decoction on mRNA and protein expression of Cyp1b1 in the liver. As demonstrated by Fig. 5,

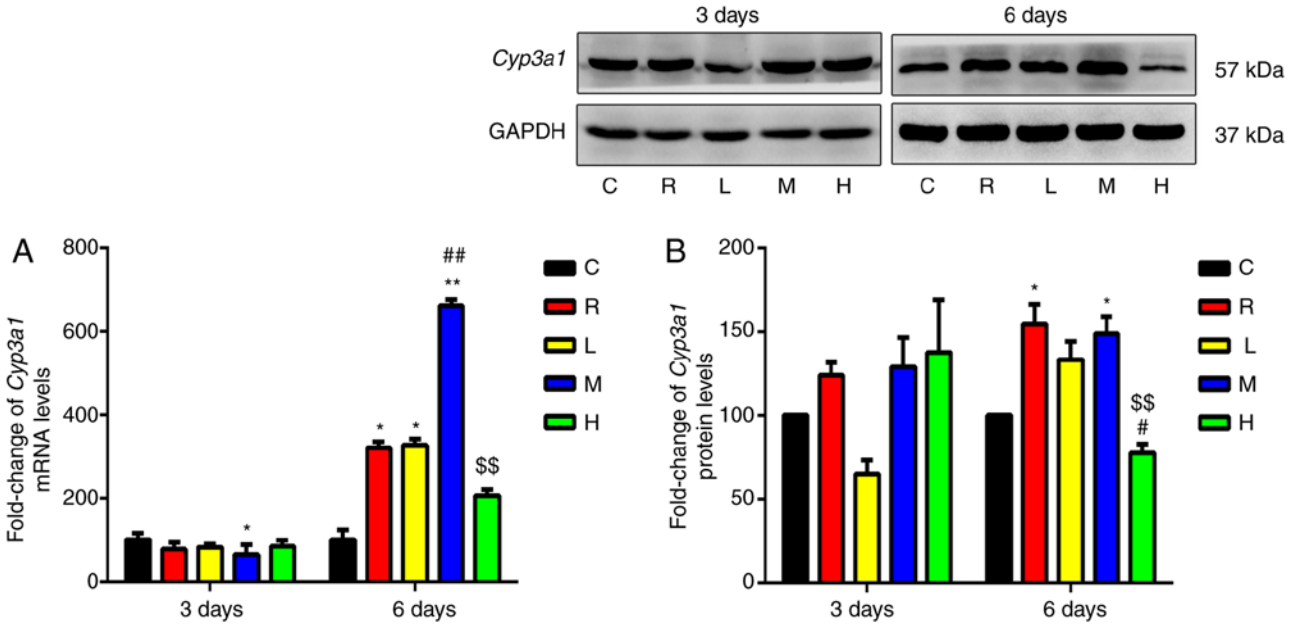

Figure 3. Relative expression level of *Cyp3a1* mRNA and protein. Relative expression level of *Cyp3a1* mRNA (A) and protein (B) in the liver of rats that were treated with Xiaochaihu decoction once daily for 3 and 6 days. The data are expressed as mean ± SE (n=8). *P<0.05; **P<0.01 vs. control rats; $^{\#}$P<0.05; $^{\#\#}$P<0.01 vs. Xiaochaihu decoction low-dose group; $^{\$\$}$P<0.01 vs. Xiaochaihu decoction medium-dose group rats. C, control; R, rifampicin; L, low-dose; M, medium-dose; and H, high-dose group.

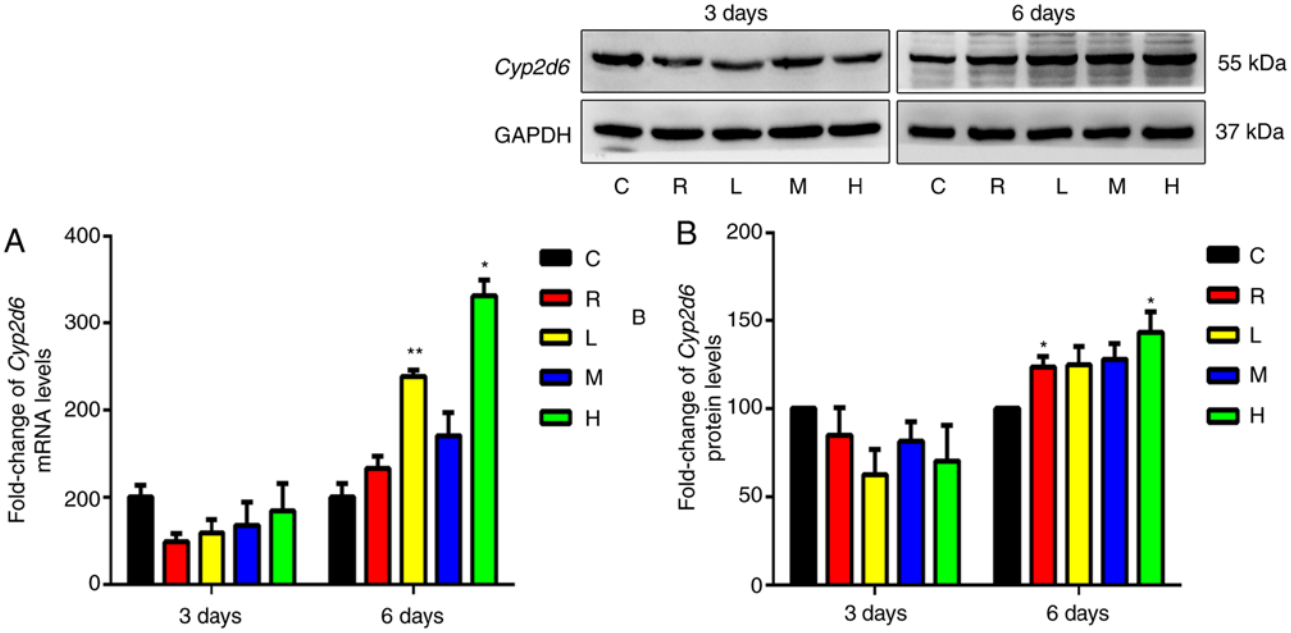

Figure 4. Relative expression level of *Cyp2d6* mRNA and protein. The relative expression level of *Cyp2d6* mRNA (A) and protein (B) in the liver of rats that were treated with Xiaochaihu decoction once daily for 3 and 6 days. The data are expressed as the mean ± SE (n=8). *P<0.05; **P<0.01 vs. control. C, control; R, rifampicin; L, low-dose; M, medium-dose; and H, high-dose group.

the differences in *Cyp1b1* mRNA or protein levels between the rifampicin for each Xiaochaihu group and the control were not significant (P>0.05). Xiaochaihu decoction may not affect the mRNA and protein expression of *Cyp1b1*.

Discussion

Although several modern formulations of Xiaochaihu, including granules and tablets, have been developed from Xiaochaihu decoction, and are widely used in clinical practice, the concentrated decoction is still administered in many cases. To better mimic the clinical use of drugs and consider the reference value of the research results for different forms of dosage, concentrated Xiaochaihu decoction was selected in the present study.

The main reasons for selecting the drug doses given to animals were selected primarily in reference to previous studies (17,18); and pre-experimental results found that

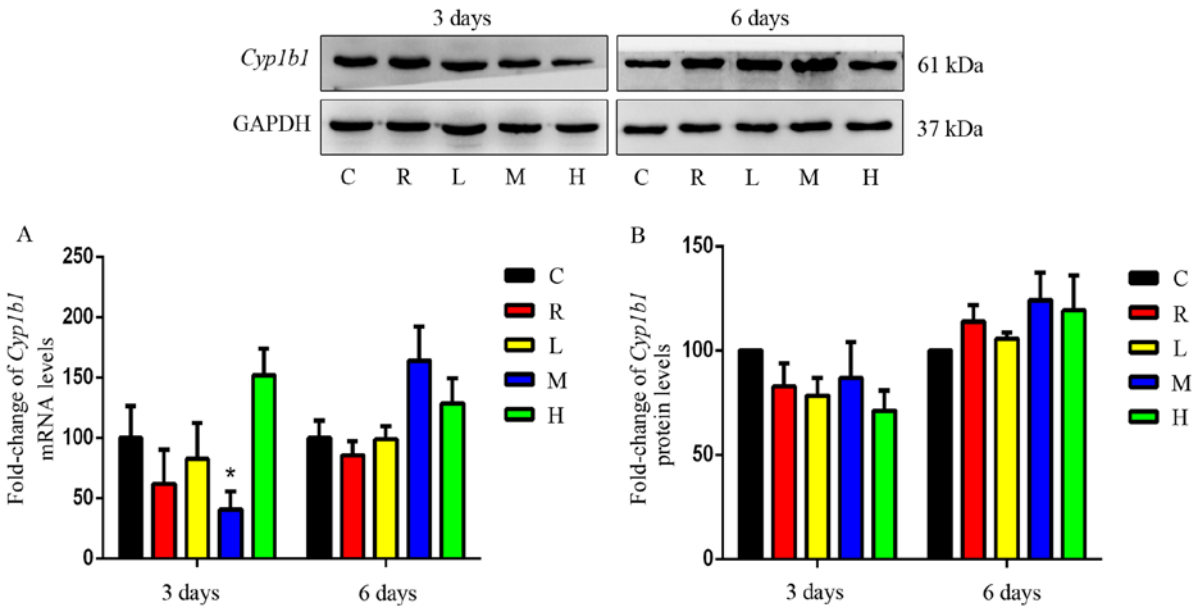

Figure 5. Relative expression level of *Cyp1b1* mRNA and protein. The relative expression level of *Cyp1b1* mRNA (A) and protein (B) in the liver of rats that were treated with Xiaochaihu decoction once daily for 3 and 6 days. The data are expressed as the mean ± SE (n=8). *P<0.05 vs. control. C, control; R, rifampicin; L, low-dose; M, medium-dose; and H, high-dose group.

low-dose Xiaochaihu decoction has slightly stronger induction effect on *Cyp1A2* and *Cyp3A1* gene expression compared with medium and high dose at the doses of 13.6, 6.8 and 3.4 g/kg/day. It was assumed that a lower dose of Xiaochaihu decoction may also have a certain induction effect on some CYPs, and the doses of Xiaochaihu decoction given to animals were decided as low dose of 1.7, medium dose of 3.4 and high dose 6.8 g/kg/day for subsequent experiments.

Scutellariae Radix is a main herb in the prescription of Xiaochaihu decoction. As a traditional Chinese herbal medicine, Scutellariae Radix exhibits important effects in the treatment of various diseases, including emesis, hepatitis and high blood pressure. Baicalin, the main bioactive ingredient in Scutellariae Radix, exerts antidepressant (21), anti-inflammatory and antioxidant effects (22). Therefore, baicalin content is determined for quality control of many traditional Chinese medicines containing Scutellariae Radix (23). In the Pharmacopoeia of the People's Republic of China 2015 edition, baicalin content was specified as a quality control parameter of Xiaochaihu granules (3). A quality control method for Xiaochaihu decoctions in clinical use and experimental research was established in the present study by referring to the quality control methods used for Xiaochaihu granules in the Pharmacopoeia of the People's Republic of China 2015 edition, and improving the relevant chromatographic conditions (19). Xiaochaihu decoction prepared using this method was uniform and quality was controllable.

Rifampicin is a broad-spectrum, classical, non-specific inducer of hepatic enzymes, including CYP3A, 1A2, 2B6, 2C9, 2C19 and 2D6, and rifampicin has the greatest effects on the expression of CYP3A in the liver (24). According to the US Food and Drug Administration, rifampicin can be used as a strong index inducer of CYP2B6 (moderate inducer), 2C8 (moderate inducer), 2C9 (moderate inducer), 2C19, and 3A when evaluating drug interactions (25). For the consideration of animal welfare (research on animals should involve the fewest number of animals) and simplicity in interpretation of results, only rifampicin was used as a positive drug for the different types of CYPs in the experiments. This experiment design can be found in some similar studies, in which only rifampicin or phenobarbital was used as a positive control for different CYPs (26,27). However, it is reasonable that using a positive control for inhibition of hepatic enzymes may better reveal the different effects of Xiaochaihu decoction on different CYPs. The lack of a positive control for CYP inhibition is a limitation to the present study.

The present results revealed that rifampicin induced the gene and protein expression of *Cyp3a1* after 6 days, and the difference was significant. This is consistent with the findings of a previous study in which rifampicin increased CYP3A4 expression (CYP3A4 in human was equivalent to CYP3A1 in rats) (28). After 3 days of rifampicin administration, the expression of *Cyp1a2* gene was inhibited; however, rifampicin had little effect on protein expression. The uncoordinated regulation of *Cyp1a2* gene and protein expression in rats by rifampicin may be due to the regulation of protein expression at the transcriptional and translational levels. After 6 days of administration, rifampicin had little effect on the expression of *Cyp2d6* gene and protein. These results were consistent with the findings by Rae *et al* (29) who showed that rifampicin had little effect on CYP2D6 gene expression in primary human hepatocytes. A previous study also found that rifampicin does not affect the gene and protein expression of *Cyp1b1* in rats (29). The present results indicated that rifampicin had varying degrees of effects on the genes and proteins of different subtypes of CYPs in rats, and that induction effects varied with the duration of administration.

Effect of Xiaochaihu decoction on the gene and protein expression of Cyp1a2 in rats. CYP1A2 accounts for ~13%

of the total CYP enzyme content in the liver, making it the third most abundant CYP in the liver (28,30). CYP1A2 gene and its human counterpart show a high homology of 80%. Additionally, amino acid homology between CYP1A2 enzymes in humans and rats is 70% (31). CYP1A2 mediates 10% of clinical drug metabolism (32), including *in vivo* elimination of propranolol, clomipramine, phenacetin, mexiletine, propanol, β-fluoroamine, verapamil and nifedipine (13,33,34). As shown in Fig. 2, short-term administration of Xiaochaihu decoction inhibited the gene and protein expression of *Cyp1a2* in rats. This was consistent with the result of the study by Saruwatari *et al* (35), in which Xiaochaihu decoction was found to have a slight inhibitory effect on human CYP1A2 activity using the probe drug method. It has also been reported that astragalus, baicalein and baicalin have an inhibitory effect on CYP1A2 activity (34-39). The inhibitory effect of Xiaochaihu decoction on the gene and protein expression of *Cyp1a2* weakened with prolonged administration. It may be speculated that some components in Xiaochaihu decoction can slowly induce the expression of *Cyp1a2* gene and protein. Liquorice (a component in Xiaochaihu decoction) can significantly increase the activity of CYP1A2 (39-41). The results showed that Xiaochaihu decoction had different effects on *Cyp1a2* gene and protein expression at different durations of continuous administration. However, the inhibitory effects of short-term administration of Xiaochaihu decoction need to be considered when co-administered with the metabolic substrates of CYP1A2, such as clomipramine, phenacetin, and theophylline, since this inhibitory effect may increase the blood concentration of concomitant drugs, as well as the risks of poisoning and other ADRs.

Effect of Xiaochaihu decoction on the gene and protein expression of Cyp3a1 in rats. CYP3A4 is one of the most important CYPs and accounts for 60% of the total weight of CYP enzymes in human liver. It participates in the metabolism of 50% of all drugs (13), including macrolide antibiotics, imidazoles (antifungal medication), antivirals, rifamycins, selective serotonin reuptake inhibitors, calcium antagonists, β-hydroxy-β-methylglutaryl-CoA reductase inhibitors and benzodiazepines. *Cyp3a1* in rats is equivalent to CYP3A4 in human. In the present study, short-term administration of low, medium and high doses of Xiaochaihu decoction showed slight inhibitory effects of varying degrees on the gene and protein expression of *Cyp3a1*. Zhou *et al* (10) reported that a patient who had kidney transplantation in the previous year experienced CsA poisoning when Xiaochaihu granules were ingested concomitantly. Xiaochaihu granules were assumed to inhibit the gene and protein expression of CYP3A4, thus interfering with the metabolism of CsA and increasing its blood concentration. Xiaochaihu decoction may inhibit the expression of *Cyp3a1* gene and protein because of the inhibitory effects of some of its components on *Cyp3a1*. It has been reported previously that both baicalein and astragalus membranaceus extracts have inhibitory effects on CYP3A4 activity (37,38).

After 6 days of continuous administration, low and medium doses of Xiaochaihu decoction induced *Cyp3a1* gene expression, whereas the medium dose induced *Cyp3a1* protein expression. The long-term use of Xiaochaihu decoction was found to induce *Cyp3a1* gene and protein expression. This finding was consistent with the study by Nose *et al* (42), who showed the induction of *Cyp3a1* gene by Xiaochaihu decoction in female SD rats. A possible reason for this induction is that Xiaochaihu decoction contains components that can induce the expression of *Cyp3a1* gene and protein. As previously reported, saponins from Bupleurum can induce *Cyp3a1* in mice. A high-dose Bupleurum injection (0.72 ml/kg) can induce the protein expression of CYP3A4 in mouse liver (43), and liquorice can significantly increase the enzymatic activity of CYP3A1/2 (37,41,44). Therefore, when Xiaochaihu decoction is combined with the metabolic substrates of CYP3A1, such as macrolide antibiotics and imidazoles, the induction effects of Xiaochaihu decoction on CYP3A1 gene and protein expression should be considered.

Effect of Xiaochaihu decoction on the gene and protein expression of Cyp2d6 in rats. Although CYP2D6 only accounts for 2-4% of the total CYP content in the liver, it is involved in 20-25% of drug metabolism (45,46). It can metabolise more than 30 drugs, such as dextromethorphan, statin, propranolol, codeine, selective serotonin reuptake inhibitors, antiarrhythmic drugs and antipsychotic drugs (47). In the present study, after 6 days of continuous administration, high-dose Xiaochaihu decoction induced *Cyp2d6* gene and protein expression. It is suggested that Xiaochaihu decoction may slowly and dose dependently induce *Cyp2d6* expression in rats through its ingredients. Consistent with this finding, a previous study reported that Xiaochaihu decoction can induce the expression of *Cyp2d6* (48). Thus, when Xiaochaihu decoction is combined with the metabolic substrates of CYP2D6, such as isoquinoline, dextromethorphan, propranolol, statin, metoprolol and codeine, the induction of CYP2D6 expression should be considered.

Effect of Xiaochaihu decoction on the gene and protein expression of Cyp1b1 in rats. CYP1B1 accounts for 3% of drug metabolism and is involved in the metabolic activation of 11% of precancerous carcinogens, such as polycyclic aromatic hydrocarbons (32). It can affect the tumour sensitivity of several chemotherapeutic drugs, such as paclitaxel, docetaxel and cyclophosphamide (49). The results of the present study showed that Xiaochaihu decoction does not affect the expression of *Cyp1b1* gene and protein in normal rats. Thus, the effects of Xiaochaihu decoction on metabolic enzymes may be not be relevant for drugs metabolised by CYP1B1.

Conclusions. The study revealed that Xiaochaihu decoction can affect the expression of C*yp1a2, Cyp2d6* and *Cyp3a1* in rats in a dose- and/or time-dependent manner. Moreover, it was found that there may be potential drug interactions when Xiaochaihu decoction is concomitantly administered with substrates of C*yp1a2, Cyp2d6* and *Cyp3a1* (CYP1A2, CYP2D6 and CYP3A4 in humans).

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FT, YT and HL conceived and designed the experiments; YT, HL, WW performed the experiments; HL, FT, CY analyzed the data; and FT, HL and YT prepared the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Animal Experimentation Ethics Committee of Zunyi Medical University of China (approval no. ZMUER2014-2-069).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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