

Effects of Shaoyao-Gancao-Fuzi Decoction on the Pharmacokinetics of CYP3A4-Mediated Tofacitinib in Rats

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Research

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Abstract

Background: The combination of traditional Chinese medicine and western medicine is commonly accepted in clinics in China. Shaoyao-Gancao-Fuzi decoction (SGFD) has been extensively used to dispel wind, eliminate dampness and treat paralysis. Tofacitinib is approved for the treatment of rheumatoid arthritis. SGFD and tofacitinib could be used together for the treatment of rheumatoid arthritis.

Methods: A cocktail approach was employed to assess the effects of SGFD on the activities of CYP450s. After pretreatment for 2 weeks with SGFD, a cocktail solution was given to rats 24 h after the last dose of saline or SGFD. Additionally, the pharmacokinetic profiles of oral administration of tofacitinib in rats, with or without SGFD pre-treatment were investigated.

Results: The results showed that SGFD could induce the activity of CYP1A2 and inhibit the activity of CYP3A4. Furthermore, SGFD could significantly affect the pharmacokinetics of tofacitinib. Compared with control group, the $AUC_{0-\infty}$ of tofacitinib was increased from 13669.53 ± 4986.83 to 28706.69 ± 9563.13 ng/mL*h ($p < 0.01$), and the C_{max} was increased from 8359.66 ± 1512.22 to 11332.51 ± 2791.90 ng/mL ($p < 0.05$).

Conclusions: The system exposure of tofacitinib was increased by SGFD. The mechanism might be through inhibiting the activity of CYP3A4 and reducing the metabolism of tofacitinib in rats. The study will provide better guidance for the safe clinical use of SGFD and tofacitinib.

Background

Shaoyao-Gancao-Fuzi Decoction (SGFD) is a traditional Chinese medicine formula which consists of three crude herb medicines: Paeoniae Radix Alba (Baishao), Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle (Zhigancao) and Aconiti Lateralis Radix Praeparata (Fuzi) with the ratio of 3:3:1 (*w/w/w*). The prescription was originally described in "Treatise on Febrile Diseases" written by Zhongjing Zhang in the Eastern Han Dynasty. It has been extensively used to dispel wind, eliminate dampness and treat paralysis in China for thousands of years [1, 2]. SGFD exhibits an obvious anti-inflammatory and analgesic effect and achieves good effects on many clinical diseases, such as rheumatoid arthritis and sciatica [3–5].

Tofacitinib is a novel, potent JAK inhibitor with specificity for JAK3 over JAK1 over JAK2, which is approved for the treatment of rheumatoid arthritis with satisfactory effects and acceptable safety in multiple clinical examinations [6, 7]. As rheumatoid arthritis is an incurable disease with complex pathogenesis, it needs long-term medication. The combination of herbal medicine and prescription medicine is very common in clinic. Therefore, tofacitinib might be in combination with SGFD for the treatment of rheumatoid arthritis in clinic. Previously studies showed that the metabolism of tofacitinib is mainly mediated by CYP450 3A4 (53%) with a minor contribution from CYP2C19 (17%) [8–10]. It has been reported that the exposure of tofacitinib increased significantly when co-administered with potent CYP3A4 inhibitors such as ketoconazole, or one or more concomitant medication results in moderate

inhibition of CYP3A4 or potent inhibition of CYP2C19, such as fluconazole, when co-administered with potent CYP3A4 inducers such as rifampin, the exposure of tofacitinib is decreased [11].

Nowadays, with the development of combine therapy of medicines, increasingly importance has been attached to drug-drug interactions (DDIs) and herbal-drug interactions (HDIs). Several studies on the DDIs and HDIs have been reported in the last few years [12–14]. Some ingredients could inhibit or induce the activities of drug-metabolizing enzymes or transporter proteins, which may change the absorption, distribution, metabolism and excretion of drugs, effecting its pharmacological activity and toxicity on concomitant administration. Cocktail approach is a major method to study DDIs and HDIs *in vivo*, consisting of the concomitant administration of various probe substrates followed by the determination of an appropriate pharmacokinetic parameter of probe drugs, which could provide HDIs and DDIs assessment, and the information on real-time activities of cytochrome P450 enzymes (CYP450s) and transporters [15].

Herein, we used a cocktail approach including probe drugs of caffeine, dapsone, coumarin, chlorzoxazone and tolbutamide to assess the effects of SGFD on CYP1A2, 3A4, 2A6, 2E1, and 2C9. The pharmacokinetic parameters of tofacitinib in Sprague-Dawley rats with or without SGFD treatment were investigated by HPLC. The present work provides helpful references to the further clinical efficacy and safety application of SGFD and tofacitinib.

Materials And Methods

Reagents and animals

SGFD is composed of Paeoniae Radix Alba (*Paeonia lactiflora* Pall.), Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle (*Glycyrrhiza uralensis* Fisch.), and Aconiti Lateralis Radix Praeparata (*Aconitum carmichaelii* Debx.). All herbs were purchased from Nanjing Pharmaceutical Baixin Pharmacy (Nanjing, China), which were authenticated by Prof. Yufeng Xia, Department of Pharmacognosy, School of Traditional Chinese Pharmacy, China Pharmaceutical University. Voucher specimens of these herbs were stored in the Department of Pharmacognosy Laboratory of China Pharmaceutical University, labeled as 20170801 (Paeoniae Radix Alba, Baishao), 20170901 (Glycyrrhizae Radix et Rhizoma Praeparata Cum Melle, Zhigancao), and 20170816 (Aconiti Lateralis Radix Praeparata, Fuzi).

Caffeine (purity > 98%) was obtained from Push Bio-technology Co., Ltd. (Chengdu, China). Antipyrine (IS, purity > 98%) was purchased from National Institutes for Food and Drug Control (Beijing, China). Dapsone and chlorzoxazone (purity > 98%) were obtained from Shanghai Guangrui Biological Technology Co., Ltd. (Shanghai, China). Coumarin (purity \geq 98%) was purchased from Nanjing Jingzhu Bio-technology Co., Ltd. (Nanjing, China). Heparin sodium salt (194 USP units/mg) was purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany), and HPLC-grade formic acid was purchased from Shanghai Linen Science and Technology Development

Co., Ltd. (Shanghai, China). Ultrapure water was prepared from a Milli-Q water purification system (Millipore, Milford, MA, USA). All other reagents were of analytical grade.

Male Sprague-Dawley rats (180–220 g) were supplied by Nanjing Qinglongshan Laboratory Animal Breeding Center (Nanjing, China). The animal study was performed according to the guideline of current ethical regulations for institutional animal care and use at China Pharmaceutical University. All animals were housed at controlled temperature 22 ± 2 °C, relative humidity $55 \pm 10\%$ in an SPF environment. The animals were provided tap water ad libitum and fed with standard rodent chow for five days. Before being orally administrated with SGFD extract, the rats were fastened with access to water for 12 hours.

Preparation of SGFD extracts

To prepare of the SGFD extract, 225 g Baishao, 225 g Zhigancao and 75 g Fuzi were mixed together and then macerated in 10-fold volume of double-distilled water for 30 min, and after boiling with high heat, keep slight boiling with gentle fire for 1 h followed by filtering with absorbent cotton. The decoction step was repeated two times. The filtrates were combined and concentrated under reduced pressure. The powder of SGFD (125 g) was obtained by freeze-drying machine and stored at -20 °C for further use. The assay of contents was calculated by the external standard method according to the LC-MS/MS method established by our laboratory. The average contents of the main components in SGFD were 29.06 mg/g for peoniflorin, 9.16 mg/g for liquiritin, 0.31 mg/g for benzoylmesaconine, 0.0029 mg/g for mesaconitine, and 16.99 mg/g for glycyrrhizic acid, respectively.

Effects of SGFD on activities of CYP 450 s

Ten rats were randomly divided into two groups (SGFD-treated and control), and administered SGFD (10 g/kg) or physiological saline for consecutive fourteen days. On day fifteen, all rats were orally administrated cocktail drugs containing caffeine (10 mg/kg), dapsone (20 mg/kg), coumarin (30 mg/kg), chlorzoxazone (20 mg/kg) and tolbutamide (5 mg/kg) and dissolved in an aqueous solution of 0.5% sodium carboxymethyl cellulose as probe substrates for CYP1A2, 3A4, 2A6, 2E1, and 2C9, respectively. Food intake was restricted 12 h before cocktail drugs administration. The blood samples were collected into heparinized polythene tubes at 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h after administration of probe drugs. The plasma samples for chromatographic analysis were obtained by centrifugation (8000 r/min for 10 min at 4 °C) of the blood samples and stored at -20 °C until analysis.

Plasma samples were thawed at room temperature, 100 μ L of the plasma was mixed with 10 μ L internal standard (antipyrine, 50 μ g/mL) by vortexing for 30 s, extracted with 1.5 mL trichloromethane, the mixture was vortexed for 5 min and centrifuged at 12000 r/min for 10 min, the organic phase (1.4 mL) was transferred into another tube and evaporated to dryness in vacuum concentrator at 37 °C. The residue was reconstituted with 50 μ L methanol-water (50:50, v/v), vortex for 5 min and centrifugation at 12000 r/min for 10 min, and then the supernatant was used for chromatographic analysis.

The chromatographic separation was performed on a C_{18} column (4.6 mm \times 150 mm, 5 μ m, Dubhe). The flow rate was 1 mL/min and the injection volume was 20 μ L. The mobile phase consisted of water

(containing 0.1% phosphate acid, A) and acetonitrile (B). The gradient was set as follows: 0–12 min, a linear gradient of 5–22% B; 12–15 min, a linear gradient to 33% B; 15–30 min, a linear gradient to 45% B. The detect wavelength was set as follows: 0–22 min, 280 nm; 22–30 min, 220 nm. The column compartment was set at 40 °C and the automatic sample was held at room temperature.

Stock standards of caffeine, dapson, coumarin, chlorzoxazone, tolbutamide and antipyrine (IS) were precisely weight and separately dissolved in methanol to achieve a concentration of 2.100 mg/mL, 2.010 mg/mL, 2.040 mg/mL, 5.100 mg/mL, 10.100 mg/mL and 2.000 mg/mL, respectively. Standard solutions were precisely in blank rat plasma to obtain final concentrations of 21, 105, 210, 1050, 2100, 10500 and 21000 ng/mL for caffeine, 20.1, 100.5, 201, 1005, 2010, 10050 and 20100 ng/mL for dapson, 51, 102, 201, 1020, 2040, 10200 and 20400 ng/mL for coumarin, 102, 510, 1020, 5100, 10200, 20400 and 51000 ng/mL for chlorzoxazone, 101, 505, 1010, 5050, 10100, 50500, 101000 ng/mL for tolbutamide. The QC samples were prepared in blank plasma at final concentrations of 21, 1050, 21000 ng/mL for caffeine, 20.1, 1005, 20100 ng/mL for dapson, 51, 1020, 20400 ng/mL for coumarin, 102, 5100, 51000 ng/mL for chlorzoxazone, 101, 5050, 101000 ng/mL for tolbutamide.

Methods were validated for specificity, linearity, accuracy, precision, extract recovery and stability, according to USA FDA guidelines [16].

Effects of SGFD on the pharmacokinetics of tofacitinib

For orally administration solution, tofacitinib was dissolved in an aqueous solution of 0.5% sodium carboxymethyl cellulose. Twelve rats were randomly divided into two groups, and were orally administrated SGFD (10 g/kg) or physiological saline fourteen days before given tofacitinib. On day fifteen, both groups were orally administrated tofacitinib (50 mg/kg). Food intake was restricted 12 h before the tofacitinib administration. The plasma concentrations were collected at prior to dosing and 0.083, 0.167, 0.25, 0.75, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after dosing. The plasma was separated from the blood by centrifugation (8000 r/min for 6 min at 4 °C) and stored at -20 °C until analysis.

Plasma (100 µL) was mixed with 10 µL strychnine (200 µg/mL, IS). The mixture was vortexed for 30 s and added 1 mL ethyl acetate, and then was vortexed 5 min and centrifuged at 12000 r/min for 10 min. The supernatant (900 µL) was transferred into another tube and evaporated to dryness in vacuum concentrator at 37 °C. The residue was reconstituted with 100 µL acetonitrile-water (25:75, v/v), vortex for 2 min and centrifugation at 12000 r/min for 10 min, and then the supernatant was used for chromatographic analysis.

The chromatographic separation was performed on a C₁₈ column (4.6 mm × 150 mm, 5 µm, Dubhe). The flow rate was 1 mL/min and the injection volume was 20 µL. The mobile phase consisted of water (containing 0.1% phosphate acid, A) and acetonitrile (B). The gradient was set as follows: 0–1 min, a linear gradient of 10–13% B; 1–4 min, a linear gradient to 25% B; 4–10 min, an isocratic gradient at 25% B. UV detection was at wavelength of 287 nm, the column compartment was set at 40 °C and the automatic sample was held at room temperature.

To prepare the samples of the calibration curve, the blank plasma (90 μ L) added various concentrations of tofacitinib to obtain final concentrations of 54.5, 109, 218, 545, 1090, 2180, 5450, 10900 and 21800 ng/mL. The QC samples were prepared in blank plasma at final concentrations of 54.5, 1090 and 21800 ng/mL for tofacitinib.

Methods were validated for specificity, linearity, accuracy, precision, extract recovery and stability, according to USA FDA guidelines [16].

Data analysis

Pharmacokinetic parameters were determined by non-compartmental modeling with the PK Slover software. The PK parameters including maximum plasma concentration (C_{max}), the time to reach C_{max} (T_{max}), area under the curve (AUC), half-time $t_{1/2}$, oral clearance per unit time (CL/F) and mean residence time (MRT). The differences were considered to be significant at $p < 0.05$ or extremely significant at $p < 0.01$ as determined by independent-sample t -tests.

Results

Method validation

The performance of the method for cocktail drugs detection was evaluated in terms of specificity, linearity, accuracy, precision, extract recovery and stability. All cocktail drugs exhibited good linear relationship within the select linearity range with correlation coefficients (r^2) between 0.9995 and 0.9999 (Table S1). The overall recoveries ranged from 93.36–110.25% with the relative standard deviations (RDSs) of 2.55%-12.60% for all analytes. The intra- and inter-batch accuracy ranged from 88.40–110.07% and 90.24–105.36%, respectively. The intra- and inter-batch precision ranged from 1.31–9.78% and 1.07–9.66%, respectively. The results of all stability tests are shown in Table S2. These data demonstrated that the developed method was accurate, sensitive and reliable for the detection of cocktail drugs in rat plasma.

Under optimized experimental condition, the tofacitinib calibration curve was linear over the concentration range of 5.45–21800 ng/mL. A typical regression equation from nine concentrations was $y = 0.0004x - 0.0026$ ($r^2 = 0.9999$). The intra-day accuracy was in the range of 98.58% – 103.06%, while the inter-day accuracy ranged from 99.89% – 104.97%. The maximum intra- and inter-day precision were 5.12% and 7.62%, respectively. Besides, the stability under all the evaluated conditions ranged from 95.59–108.50% (Table S3). The results indicated the assay was a precise and reliable method for the analysis of tofacitinib in pharmacokinetic studies.

The effects of SGFD on activities of CYP1A2, 3A4, 2A6, 2E1 and 2C9 *in vivo*

The plasma concentration-time curves and main pharmacokinetics parameters of caffeine, dapson, coumarin, chlorzoxazone and tolbutamide in the control group and SGFD-treated group are presented in Table 1–2 and Fig. 1, respectively. There are no significant differences in the main pharmacokinetics parameters of coumarin, chlorzoxazone, and tolbutamide between the control group and the SGFD group. While the $AUC_{0-\infty}$ value of caffeine (-28.52%) was decreased ($p < 0.05$) by SGFD. In addition, SGFD caused a significant increase ($p < 0.01$) in the $AUC_{0-\infty}$ value of dapson (+ 58.88%). The results indicated that SGFD could induce the activity of CYP1A2 and inhibit the activity of CYP3A4.

Table 1
Effect of SGFD exposure on the pharmacokinetics of caffeine and dapson (Mean \pm SD, n = 5)

Parameters	Caffeine		Dapson	
	Control group	SGFD group	Control group	SGFD group
$t_{1/2}$ (h)	3.25 \pm 1.68	2.15 \pm 1.38	7.71 \pm 0.94	7.65 \pm 1.24
T_{max} (h)	0.50 \pm 0.31	0.18 \pm 0.09	0.75 \pm 0.35	2.7 \pm 2.28
C_{max} (ng/mL)	3458.74 \pm 685.66	2988.96 \pm 853.82	1869.84 \pm 410.19	2144.72 \pm 598.83
AUC_{0-t} (ng/mL*h)	12308.77 \pm 2356.99	8774.89 \pm 934.20*	21329.54 \pm 4708.50	34031.56 \pm 6672.84**
$AUC_{0-\infty}$ (ng/mL*h)	12440.70 \pm 2312.84	8892.32 \pm 869.61*	21683.44 \pm 4885.48	34451.56 \pm 6615.41**
MRT (h)	3.12 \pm 0.56	3.13 \pm 1.32	10.86 \pm 1.27	13.18 \pm 2.26
V_z/F (L/kg)	3.95 \pm 2.01	3.60 \pm 2.51	10.58 \pm 1.90	6.68 \pm 1.95*
Cl/F (L/kg/h)	0.82 \pm 0.14	1.13 \pm 0.12**	0.98 \pm 0.30	0.60 \pm 0.11*
* $p < 0.05$, ** $p < 0.01$				

Table 2

Effect of SGFD exposure on the pharmacokinetics of coumarin, chlorzoxazone and tolbutamide (Mean \pm SD, n = 5)

Parameters	Coumarin		Chlorzoxazone		Tolbutamide	
	Control group	SGFD group	Control group	SGFD group	Control group	SGFD group
$t_{1/2}$ (h)	6.43 \pm 1.85	3.43 \pm 1.68*	0.75 \pm 0.34	1.00 \pm 0.49	11.00 \pm 2.86	10.76 \pm 2.35
T_{max} (h)	0.30 \pm 0.11	0.22 \pm 0.08	0.30 \pm 0.11	0.25 \pm 0.00	3.40 \pm 1.95	3.20 \pm 2.59
C_{max} (ng/mL)	1194.43 \pm 535.95	1039.58 \pm 203.96	15029.28 \pm 1157.30	17232.72 \pm 3648.29	11164.74 \pm 2281.31	10481.29 \pm 1269.90
AUC_{0-t} (ng/mL*h)	1538.74 \pm 504.66	1359.77 \pm 157.87	22902.73 \pm 5239.75	24367.97 \pm 6021.98	220768.52 \pm 51639.59	219199.89 \pm 40770.48
$AUC_{0-\infty}$ (ng/mL*h)	1926.47 \pm 530.77	1565.86 \pm 209.56	22965.61 \pm 5246.74	24406.45 \pm 6041.21	234465.67 \pm 65076.43	231010.93 \pm 49049.10
MRT (h)	5.85 \pm 2.12	3.29 \pm 1.12	1.10 \pm 0.22	0.98 \pm 0.11	16.52 \pm 3.77	16.38 \pm 2.59
V_z/F (L/kg)	151.74 \pm 56.60	92.18 \pm 39.71	0.98 \pm 0.55	1.21 \pm 0.60	0.34 \pm 0.058	0.34 \pm 0.068
Cl/F (L/kg/h)	16.41 \pm 3.90	19.50 \pm 3.17	0.91 \pm 0.19	0.85 \pm 0.16	0.023 \pm 0.0055	0.022 \pm 0.0041
* $p < 0.05$						

Effect of SGFD on the pharmacokinetics of tofacitinib

The calibration curve for tofacitinib was linear over concentration range of 54.5 to 21800 ng/mL. The plasma concentration of tofacitinib measured by HPLC had good precision (98.58-104.97%), recovery (92.72-101.96%), and stability (95.59–108.50%). Results showed that the values of C_{max} and $AUC_{0-\infty}$ of tofacitinib were significantly increased after treatment with SGFD for 14 days, which were 1.36 ($p < 0.05$) and 2.10 times ($p < 0.01$) higher than those of tofacitinib alone (Fig. 2 and Table 3).

Table 3
Pharmacokinetic parameters of tofacitinib (Mean \pm SD, n = 6)

Parameters	Control group	SGFD group
$t_{1/2}$ (h)	1.86 \pm 0.73	2.05 \pm 0.82
T_{max} (h)	0.26 \pm 0.12	0.46 \pm 0.19
C_{max} (ng/mL)	8359.66 \pm 1512.22	11332.51 \pm 2791.90*
AUC _{0-t} (ng/mL*h)	13651.71 \pm 4977.27	28681.15 \pm 9555.88**
AUC _{0-∞} (ng/mL*h)	13669.53 \pm 4986.83	28706.69 \pm 9563.13**
MRT (h)	1.59 \pm 0.31	2.33 \pm 0.65*
Vz/F (L/kg)	11.71 \pm 7.56	5.41 \pm 2.45
Cl/F (L/kg/h)	4.33 \pm 2.36	1.90 \pm 0.58*
* $p < 0.05$, ** $p < 0.01$		

Discussion

The combination of medicine is very common in Chinese clinical practice, which may greatly raise the potential risk of DDIs and HDIs. The DDIs and HDIs are generally achieved by the pharmacokinetic properties, and may occur in the phase of absorption, distribution, metabolism or excretion. The CYP450 system is crucial for drug metabolism which is related to the metabolism of endogenous substances, detoxification of exogenous substances and activation of precarcinogens [13]. CYP450 superfamilies include CYP1, CYP2 and CYP3. CYP3A4 is the most abundant enzyme in CYP subtype and accounts for approximately 34% of drug metabolism, which is followed by 2D6, 2C8, 2C9, 2C19 and 1A2 [17]. Inhibition or induction of any isoforms of CYP450 has been recognized as the pivotal cause of DDIs and HDIs. SGFD has no effects on the activities of CYP2A6, 2E1 and 2C9 *in vivo*. However, SGFD did affect the activities of CYP3A4 and 1A2 which indicated that there is a potential HDI between SGFD and the drugs metabolized by CYP3A4 and 1A2. We speculated that SGFD might increase the plasma concentration of tofacitinib through inhibiting the activity of CYP3A4 which may lead to the enhancement of drug efficacy, occurrence of adverse reactions, reduction of efficacy, or failure of treatment.

The metabolism of tofacitinib is mainly mediated by CYP3A4 (53%) with a minor contribution from CYP2C19 (17%) [8–10]. It has been reported that when tofacitinib was co-administered with ketoconazole or fluconazole, the AUC and C_{max} values of tofacitinib were increased, while when co-administered with rifampin, the AUC and C_{max} values were decreased [11]. Report indicated that high dose of tofacitinib might increase the risk of infection, pulmonary thrombosis and even death in clinic [18]. However, there are few studies about HDI between tofacitinib and traditional Chinese medicine.

The present study indicated that when the rats were pre-treated with SGFD for 14 days, the pharmacokinetic parameters of tofacitinib were significantly changed. After oral administration of SGFD for 14 consecutive days, the C_{\max} ($p < 0.05$), $AUC_{0-\infty}$ ($p < 0.01$) and MRT ($p < 0.05$) values of tofacitinib increased significantly, and the Cl/F values ($p < 0.05$) decreased. Therefore, SGFD could increase the efficacy and toxicity of tofacitinib. Accordingly, when patients were given SGFD together with tofacitinib in clinics, the dosage of tofacitinib might be appropriately reduced to avoid adverse reactions.

Conclusion

The effects of long-term administration of SGFD on CYP450s in rats were investigated using a cocktail approach to explore its potential HDIs. Moreover, the co-administration of SGFD with tofacitinib was studied to verify the HDI. The current study provides valuable information for the potential HDI between tofacitinib and SGFD, and is helpful to guide the clinical application of SGFD and tofacitinib.

Abbreviations

AUC, area under the plasma concentration-time curve; CID, collision-induced dissociation; CL/F, apparent clearance; C_{\max} , maximum plasma concentration; CYP450s, cytochrome P450 enzymes; DDIs, drug-drug interactions; HDIs, herbal-drug interactions; HPLC-MS/MS, high performance liquid chromatography tripe quadrupole mass spectrometry; IS, internal standard; MRM, multiple reaction monitoring; PK, pharmacokinetic; QC, quality control; SD, standard deviation; SGFD, Shaoyao-Gancao-Fuzi decoction; TCMP, traditional Chinese medicine prescription; T_{\max} , maximum concentration

Declarations

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Authors' contributions

LL designed the analysis, wrote and reviewed the manuscript. YW performed the experiments and collected the data. SNS, WL, and DH performed the experiments. RHP provided the conception, obtained funding and supervised the study. YFX designed the experiments, reviewed the manuscript and supervised the study. All authors read and approved the final manuscript.

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Availability of data and materials

All data included in this article are available from the corresponding author upon request.

Ethics approval and consent to participate

The animal study was performed according to the guideline of current ethical regulations for institutional animal care and use at China Pharmaceutical University.

Consent for publication

All authors have provided consent for publication in the journal of Chinese Medicine.

Conflicts of interests

The authors report no conflicts of interest in this work.

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Figures

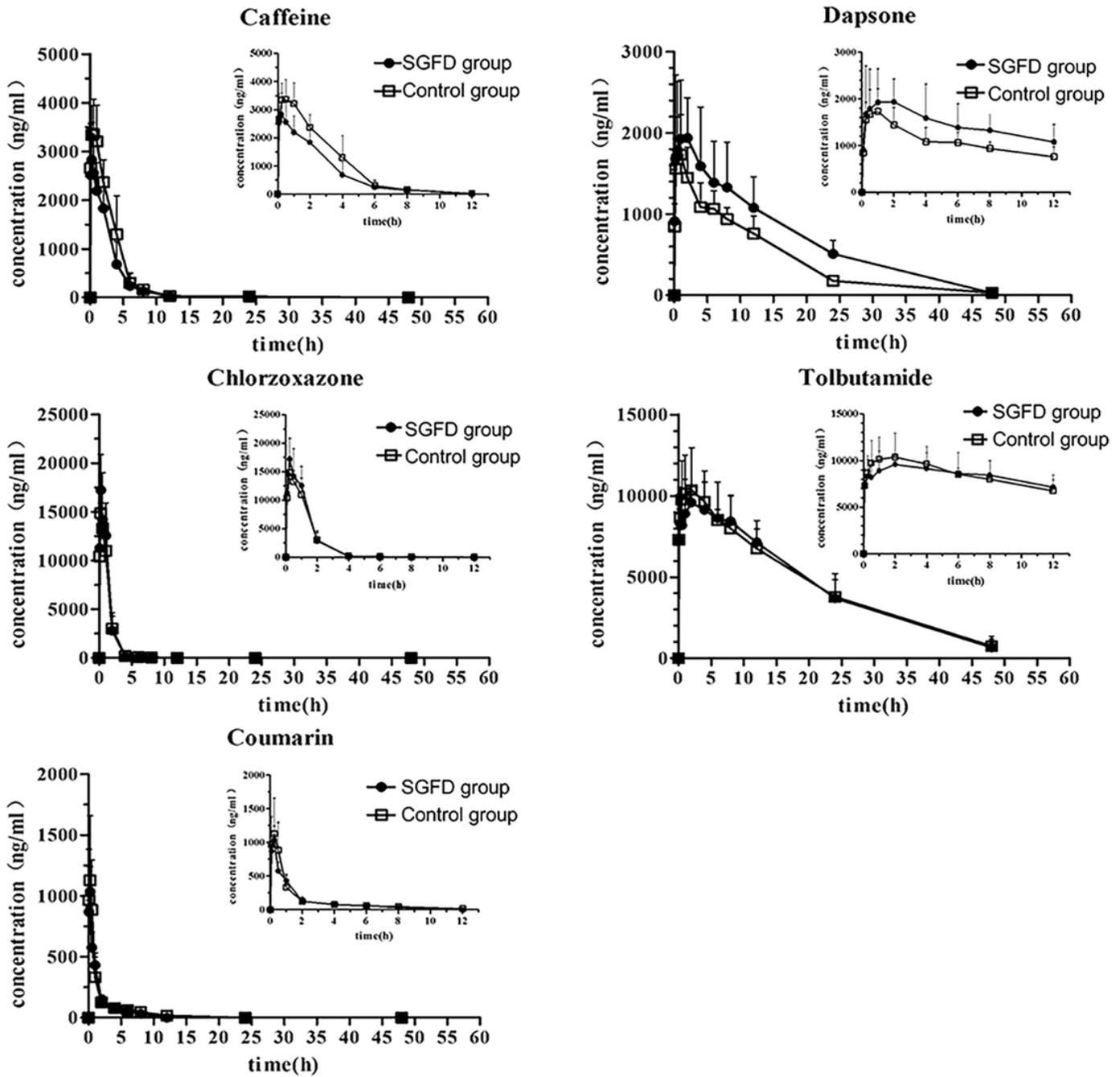


Figure 1

Mean concentration-time curves of five probe drugs in rat plasma (n = 5).

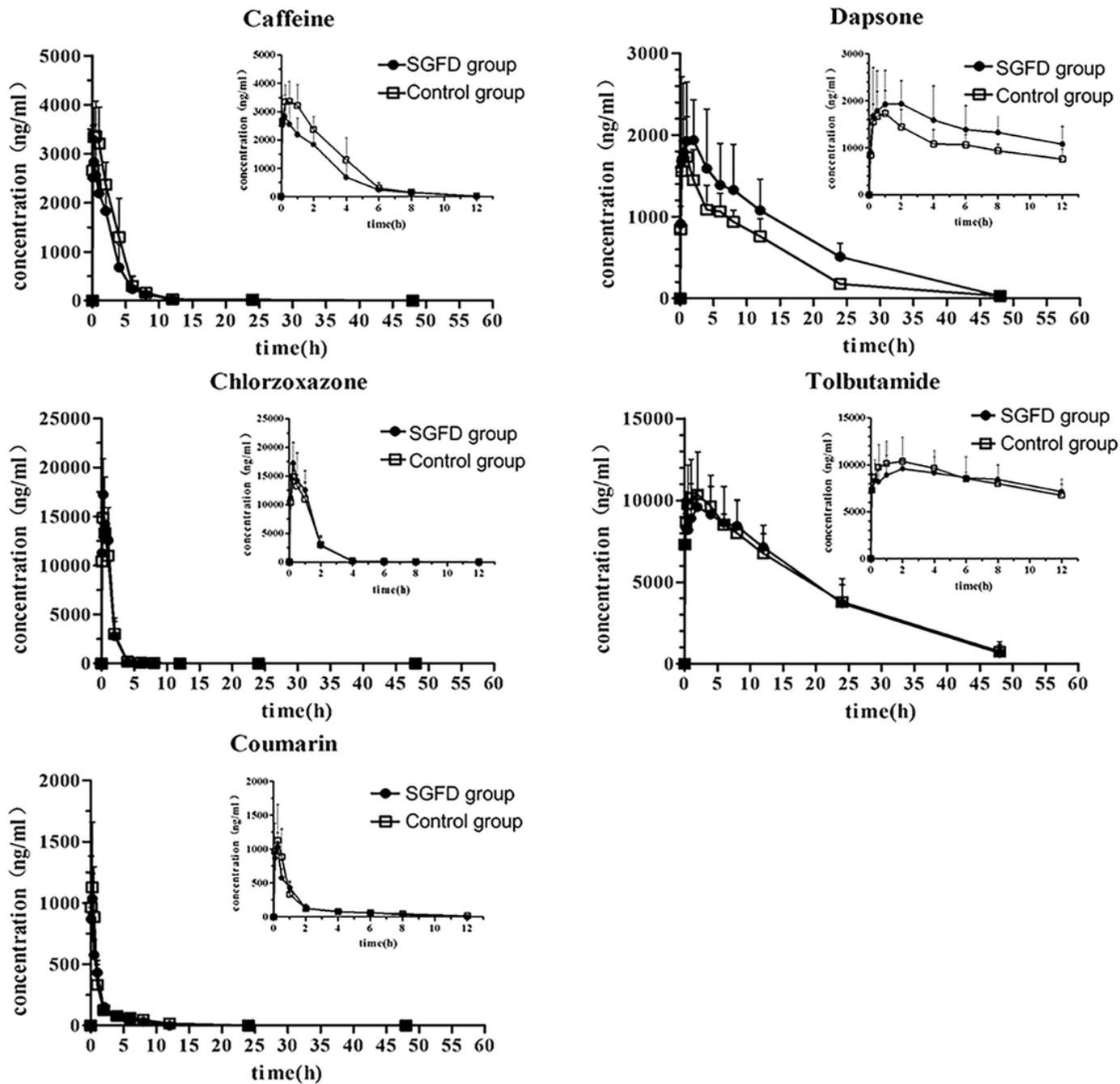


Figure 1

Mean concentration-time curves of five probe drugs in rat plasma (n = 5).

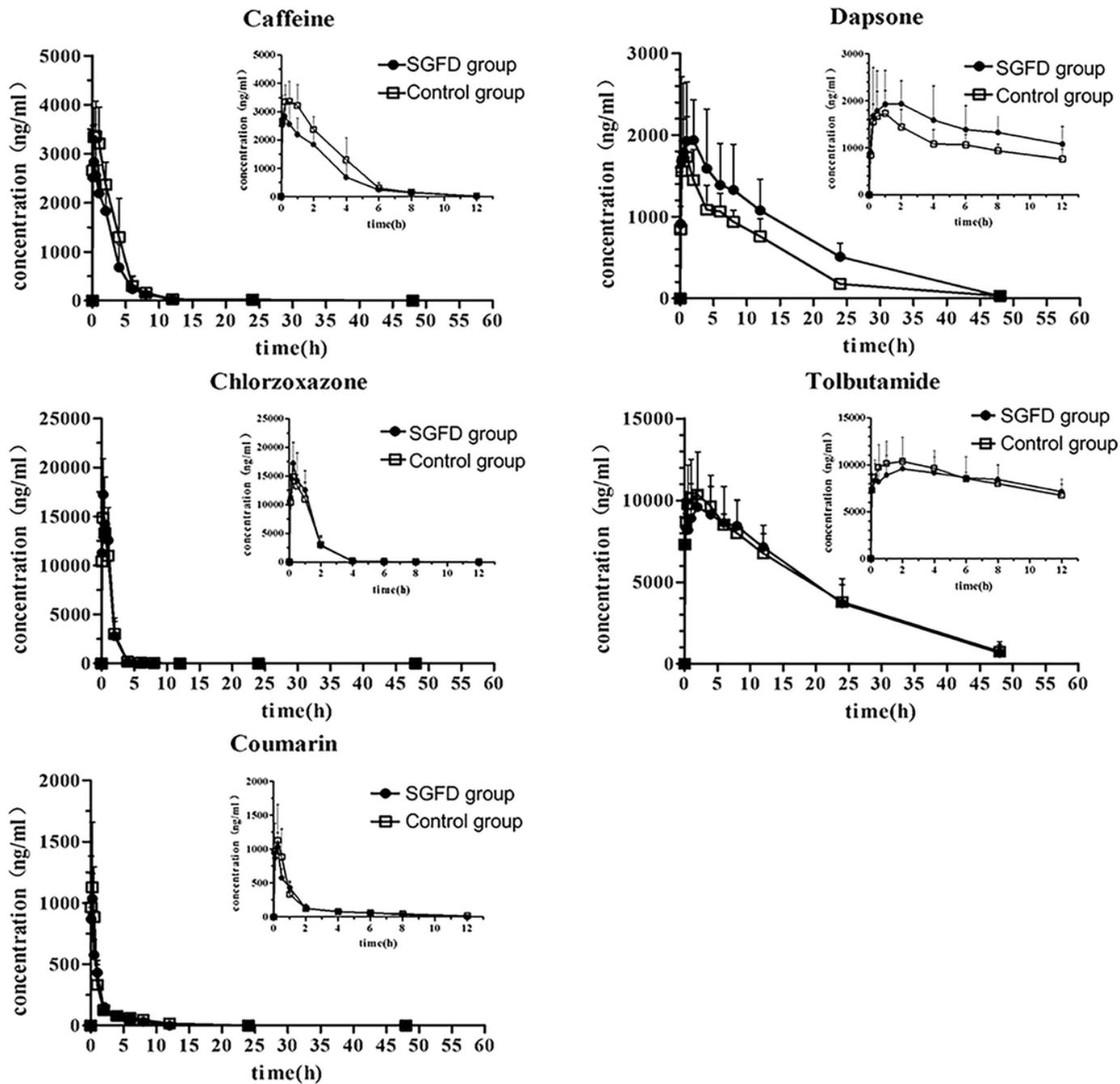


Figure 1

Mean concentration-time curves of five probe drugs in rat plasma (n = 5).

Tofacitinib

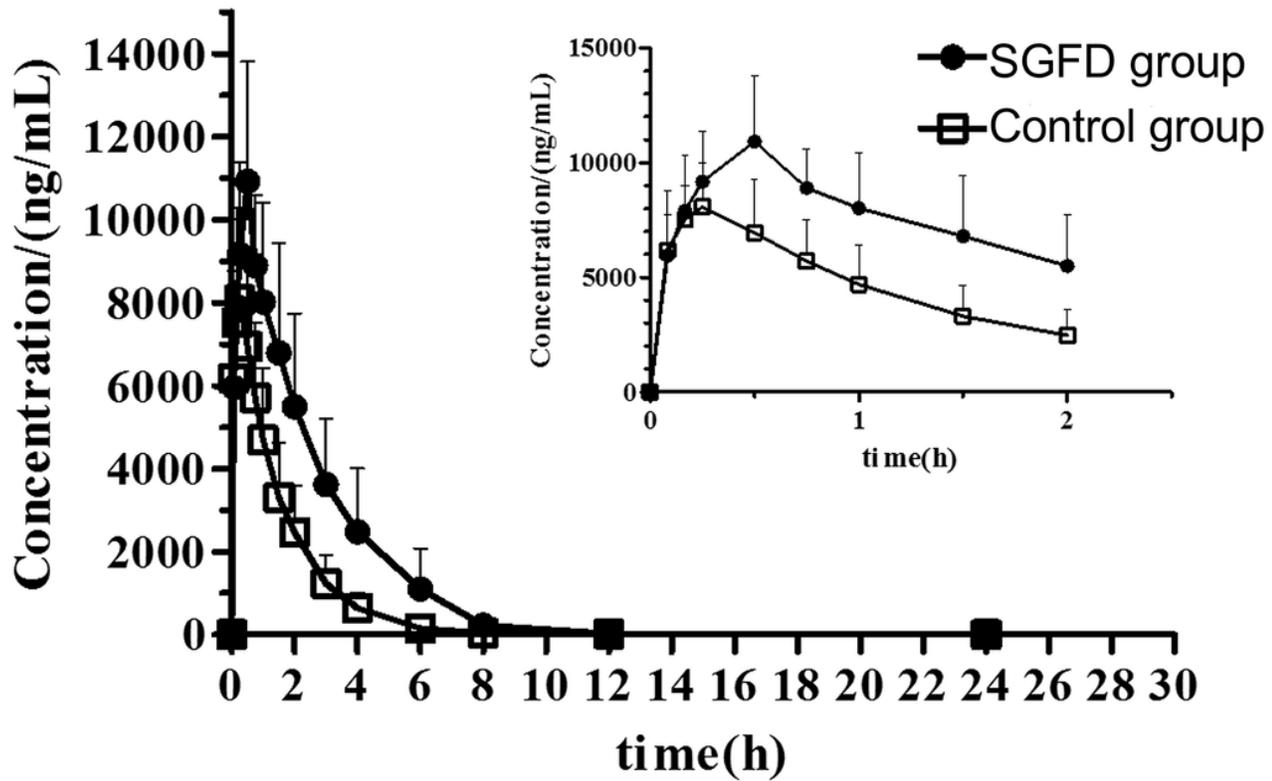


Figure 2

Mean plasma concentration-time profile of tofacitinib (n = 6).

Tofacitinib

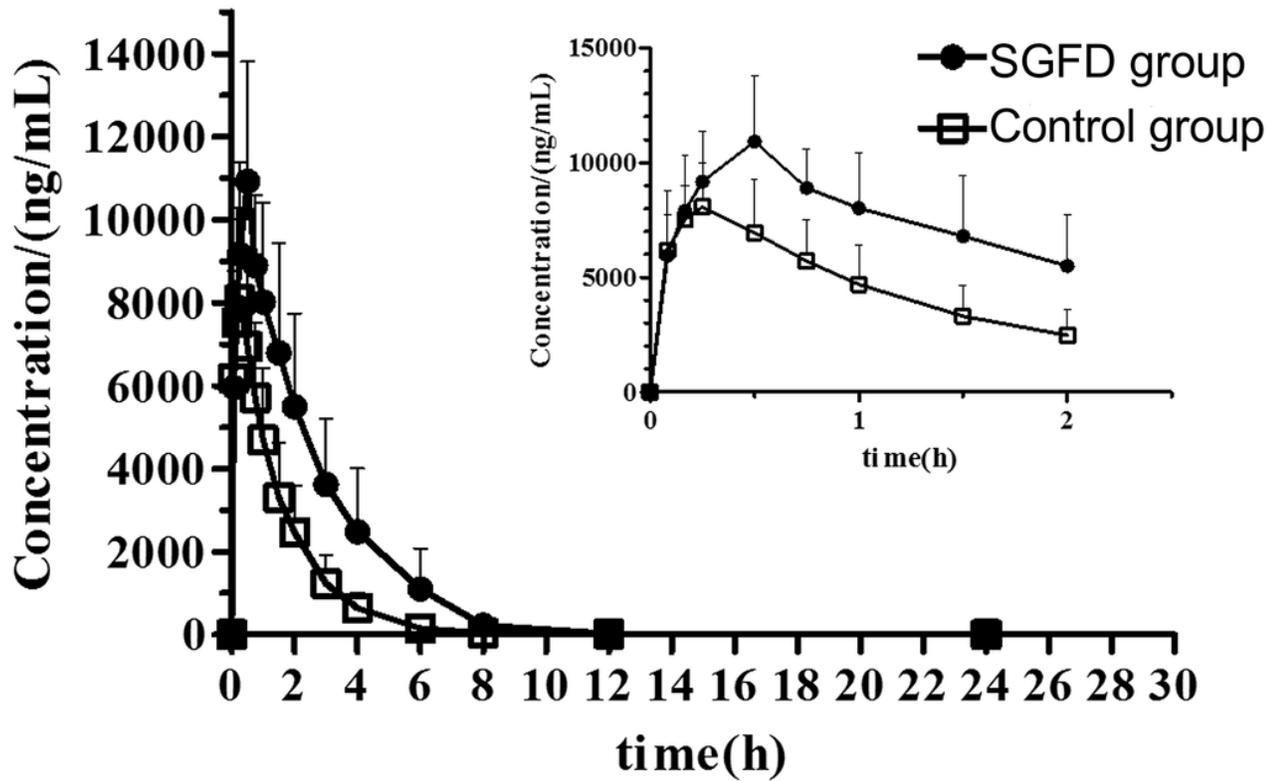


Figure 2

Mean plasma concentration-time profile of tofacitinib (n = 6).

Tofacitinib

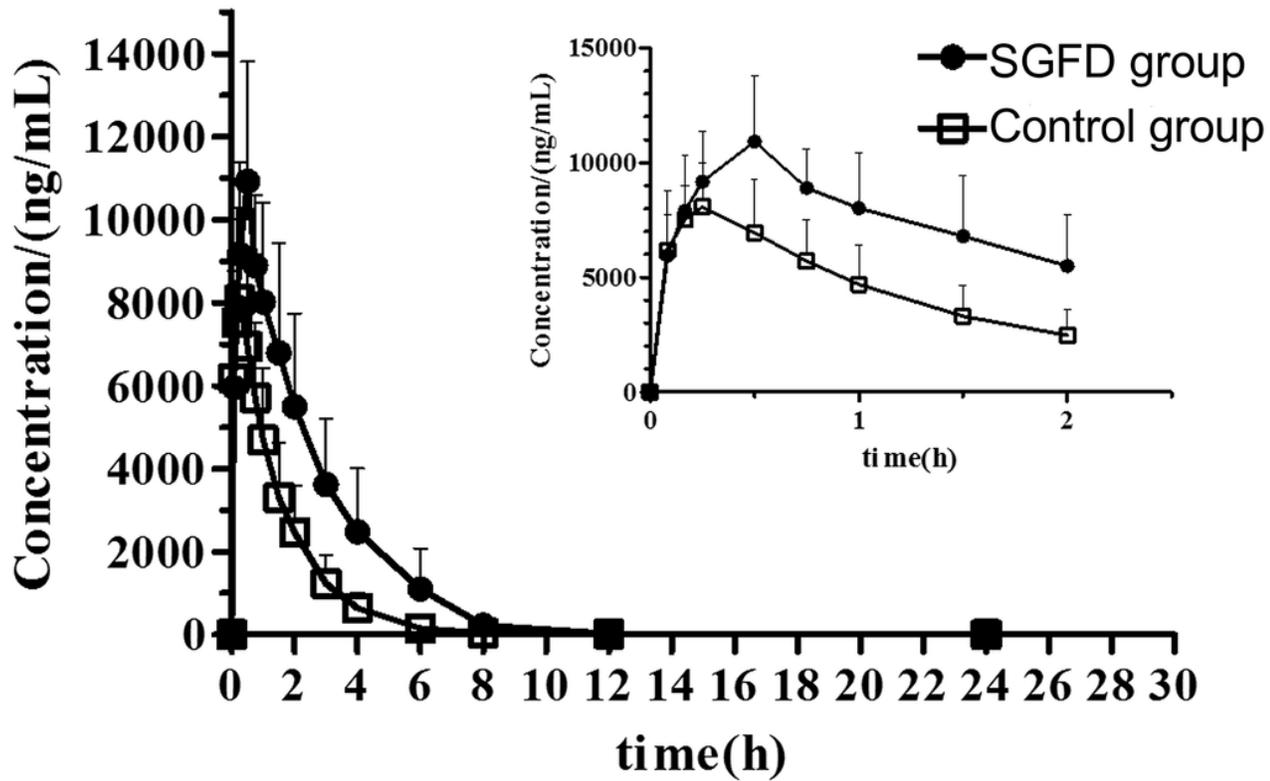


Figure 2

Mean plasma concentration-time profile of tofacitinib (n = 6).

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