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Article

CYP3A4 inducer and inhibitor strongly affect the pharmacokinetics of triptolide and its derivative in rats

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Abstract

Triptolide is the most active ingredient of *Tripterygium wilfordii* Hook F, which is used to treat rheumatoid arthritis. (5*R*)-5-Hydroxytriptolide is a hydroxylation derivative of triptolide with a reduced toxicity. To investigate the metabolic enzymes of the two compounds and the drug-drug interactions with enzyme inducers or inhibitors, a series of *in vitro* and *in vivo* experiments were conducted. *In vitro* studies using recombinant human cytochrome P450 enzyme demonstrated that cytochrome P450 3A4 (CYP3A4) was predominant in the metabolism of triptolide and (5*R*)-5-hydroxytriptolide, accounting for 94.2% and 64.2% of the metabolism, respectively. Pharmacokinetics studies were conducted in male SD rats following administration of triptolide or (5*R*)-5-hydroxytriptolide (0.4 mg/kg, *po*). The plasma exposure to triptolide and (5*R*)-5-hydroxytriptolide in the rats was significantly increased when co-administered with the CYP3a inhibitor ritonavir (30 mg/kg, *po*) with the values of $AUC_{0-\infty}$ (area under the plasma concentration-time curve from time zero extrapolated to infinity) being increased by 6.84 and 1.83 times, respectively. When pretreated with the CYP3a inducer dexamethasone (50 mg·kg^{-1}·d^{-1}, for 3 d), the $AUC_{0-\infty}$ values of triptolide and (5R)-5-hydroxytriptolide were decreased by 85.4% and 91.4%, respectively. These results suggest that both triptolide and (5R)-5-hydroxytriptolide are sensitive substrates of CYP3a. Because of their narrow therapeutic windows, clinical drug-drug interaction studies should be carried out to ensure their clinical medication safety and efficacy.

Keywords: triptolide; (5*R*)-5-hydroxytriptolide; CYP3A4; ritonavir; dexamethasone; pharmacokinetics; immunosuppressant; traditional Chinese medicine

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Introduction

Tripterygium wilfordii Hook F is a traditional Chinese medicine that has a range of pharmacological activities and is widely used clinically to treat rheumatoid arthritis, systemic lupus erythematosus, nephrotic syndrome, and encephalomyelitis[1, 2]. Triptolide is the most active component of *Tripterygium wilfordii* Hook F, and its relevant titer is approximately 100- to 200-fold higher than that of the total glycosides of *Tripterygium*[3]. However, its narrow therapeutic window which results from its high toxicity, has limited its clinical applications[4, 5]. To improve its druggability, a range of structure modifications was generated, during which (5R)-5-hydroxytriptolide was synthesized and was shown to have reduced toxicity[6, 7]. Both triptolide and (5R)-5-hydroxytriptolide are diterpene triepoxide compounds, and the chemical structures of triptolide and (5R)-5-hydroxytriptolide are shown in Figure 1.

Previous studies[6] have shown that the CC_{50} (cytotoxic concentration of the compound that reduces cell viability by 50%, which indicates the cytotoxicity of a drug) value of triptolide was 2.1 nmol/L and that of (5R)-5-hydroxytriptolide was 256.6 nmol/L. The IC_{50} (inhibitory concentration of the compound that reduces cell proliferation by 50%, which indicates the activity of a drug) value of triptolide was close to or even higher than its CC_{50} value. Additionally, the LD_{50} (median lethal dose) of triptolide was only 0.86 mg/kg (ip) in mice[6]. Furthermore, the LD_{50} of (5R)-5-hydroxytriptolide was 9.3 mg/kg (ip), approximately ten times higher than that of triptolide[6]. The above-mentioned information indicated that (5R)-5-hydroxytriptolide might be relatively safer than triptolide in clinical applications.

It was reported that triptolide was mainly metabolized through oxidation[8, 9] in rats. Cytochrome P450 3A4 (CYP3A4)

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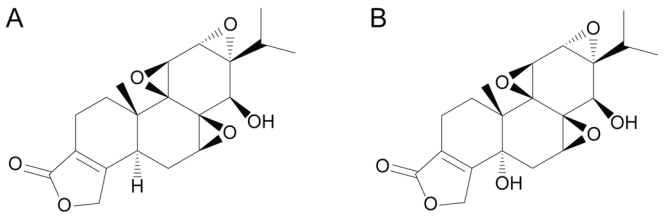

Figure 1. Structures of triptolide (A) and (5R)-5-hydroxytriptolide (B).

and CYP2C19 were involved in the metabolism of triptolide in human liver microsomes and that CYP3A4 was the primary isoform responsible for its hydroxylation[10]. It was reported that glycyrrhizin could significantly accelerate the metabolism of triptolide by inducing CYP3a in rats[11]. Additionally, the activity of CYP3a significantly affected the toxicity of triptolide in rats[12]. Co-administration with dexamethasone, a strong CYP3a inducer in rats, significantly attenuated triptolide-induced rat liver and kidney toxicity and maintaining normal levels of serum aspartate aminotransferase (AST) and alanine transaminase (ALT)[13]. Thus, we speculated that the influence by CYP3a induction or inhibition on plasma exposure of triptolide might be closely linked to triptolide-related tissue toxicity.

(5R)-5-Hydroxytriptolide is currently under clinical investigation in China to evaluate its suitability for use in the treatment of rheumatoid arthritis. To the best of our knowledge, information concerning the metabolism of (5R)-5-hydroxytriptolide has not been reported.

The purpose of the current work was to investigate the potential risk of the possible drug-drug interactions (DDI) of these two compounds combined with other drugs. In the present work, CYP450 isozymes that metabolize triptolide and (5R)-5-hydroxytriptolide were determined using recombinant human CYP450 enzymes *in vitro*. Furthermore, male Sprague-Dawley (SD) rats were used as experimental animals to evaluate the effect of induction or inhibition of major metabolic enzymes on the pharmacokinetics of triptolide and (5R)-5-hydroxytriptolide.

Materials and methods

Chemicals and materials

Triptolide (99.53%) and ritonavir were purchased from Dalian Meilun Biotech Co, Ltd (Dalian, China). (5R)-5-Hydroxytriptolide (99.11%, measured by HPLC-UV) was provided by the Investment and Development Department of Shanghai Pharmaceuticals Holding Co, Ltd (Shanghai, China). Dexamethasone was purchased from Shanghai Yuanye Bio-technology Co, Ltd (Shanghai, China). Recombinant human cytochrome P450s (rCYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) were purchased from BD Gentest (Woburn, MA, USA). NADPH and CMC-Na were purchased from Sigma-Aldrich (St Louis, MO, USA). Formic acid and concentrated aqueous ammonia, AR, were purchased from Fluka Corporation (St Louis, MO, USA). Acetonitrile and methanol of HPLC grade were purchased from Merck (Darmstadt, Germany). Benzylamine, anhydrous ethanol, Tween 80, dipotassium, and potassium dihydrogen phosphate, AR, were purchased from Sinopharm Group Co, Ltd (Shanghai, China). Ammonium acetate was purchased from Alfa Aesar (Shanghai, China). Purified water was generated using a Milli-Q Gradient Water Purification System (Millipore, Molsheim, France).

Enzyme phenotype

A stock solution of triptolide was prepared in DMSO. rCYP450s (rCYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5) were co-incubated with triptolide to examine its metabolic enzyme phenotypes. The mixture containing triptolide (3 μmol/L), phosphate-buffered saline (PBS; 100 mmol/L, pH 7.4), rCYP450s (50 pmol P450/mL), and NADPH (2 mmol/L) had a final volume of 100 μL. The final DMSO concentration was 0.1%. Each incubation was performed in duplicate. Incubation without NADPH served as the negative control. After incubation at 37 °C for 1 h, the reaction was quenched with an equal volume of ice-cold acetonitrile (100 μL) containing an internal standard ((5R)-5-hydroxytriptolide, 1 μmol/L). After the mixture was vortexed, all samples were centrifuged at 14 000×*g* for 5 min. The supernatant was dried at 40 °C under a stream of nitrogen. The residues were reconstituted with 100 μL of water/acetonitrile (90:10, *v*/*v*). Next, a 10-μL aliquot of the reconstituted solution was injected into a liquid chromatography–tandem mass spectrometry (LC-MS/MS) system for analysis.

The same experimental protocol was applied to (5R)-5-hydroxytriptolide (3 μmol/L) in parallel with triptolide (1 μmol/L, ice cold acetonitrile) as the internal standard.

Animal experiments

Male SD rats (200–300 g, 7–8 weeks) were purchased from Shanghai SLAC Experimental Animal Co, Ltd (Shanghai, China). All animals were kept in a clean-grade animal room and were acclimated for 7 d before the experiments were conducted. All procedures in animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Twelve SD rats were fasted overnight and were randomly divided into three groups (n=4). Dexamethasone was prepared with 0.5% CMC-Na and was orally administered at a dose of 50 mg/kg to the first group, qd, for three consecutive days[13] before administration of triptolide. Ritonavir, prepared with Tween 80: anhydrous ethanol (1:1, *v*/*v*, 18 mg/mL), stored at –20 °C, and diluted with deionized water 1: 5 (*v*/*v*) to the desired concentration when used, was orally administered to the second group at a dose of 30 mg/kg 1 h before administration of triptolide[14]. The third group was set as the control.

All rats were orally administered triptolide at a dose of 0.4 mg/kg (saline containing 1% DMSO). Blood samples (~300 μL) were collected from the orbital vein at pre-dosing as well as 10, 20, and 40 min and 1, 2, 4, 6, and 10 h post-dosing. Blood samples were placed in EDTA-K_2-containing tubes and were immediately centrifuged at 11 000×*g* for 5 min. Plasma

samples were stored at –80 °C until analysis.

The same experimental protocol was applied to the pharmacokinetic study of (5R)-5-hydroxytriptolide (0.4 mg/kg, saline containing 1% DMSO) in parallel.

Plasma sample preparation

Plasma sample preparation was based on a previously described method[15], with some modifications. According to previous studies in our laboratory[8, 16], (5R)-5-hydroxytriptolide was not a metabolite of triptolide in rats *in vitro* and *in vivo*. Therefore, choosing triptolide and (5R)-5-hydroxytriptolide as internal standards for each other was reasonable. The linear range was set as 0.03–100 ng/mL for both compounds. The plasma sample preparation procedures are described below. A rat plasma sample of 100 μL was added into a 2-mL Eppendorf tube. The plasma sample was then spiked with 20 μL of internal standard (20 ng/mL, methanol: water, 1:1, *v*/*v*) and 400 μL of a 6 mol/L ammonium acetate aqueous solution. After vortexing for 10 s, 1000 μL of acetonitrile was added. The mixture was then slightly shaken for 10 min. The two phases were separated by centrifugation at 14 000×*g* for 10 min. The upper organic layer of 900 μL was transferred into a glass tube and was completely evaporated at 40 °C under a stream of nitrogen. The dry residue was reconstituted with 200 μL of a 10% derivatization reagent (anhydrous ethanol: benzylamine, 9:1, *v*/*v*) and was incubated at 80 °C for 1 h. After incubation, the sample was evaporated at 40 °C under a stream of nitrogen and was reconstituted with 200 μL of acetonitrile-water (30:70, *v*/*v*). After further centrifugation at 14 000×*g* for 10 min, 10 μL of the supernatant was taken for analysis.

LC–MS/MS equipment and conditions

Samples from the enzyme phenotyping experiment were measured without derivatization. Rat plasma samples were subjected to derivatization and were then analyzed. All of the samples were analyzed using a QTRAP 5500 tandem mass spectrometer (AB Sciex, Canada) equipped with a LC-30AD liquid chromatograph (Shimadzu, Kyoto, Japan). The analysis conditions are described separately as follows.

Analysis conditions for the enzyme phenotypic sample

Separation of the analytes was achieved using an Agilent XDB-C18 system (50 mm×2.1 mm, 1.8 μm; CA, USA). The mobile phase was a mixture of a 5 mmol/L ammonium acetate aqueous solution containing 0.1% (*v*/*v*) formic acid (A) and acetonitrile (B) at a flow rate of 0.6 mL/min. The following gradient elution was used: 0.00–0.50 min, 10% B; 0.50–1.50 min, 10%–60% B; 1.50–2.00 min, 60% B; 2.00–2.50 min, 60%–10% B; and 2.50–3.00 min, 10% B. The autosampler and column temperature were set at 15 °C and 40 °C, respectively.

The MS parameters were set as follows: an electrospray ionization source was used. In the negative ion mode, the ion spray voltage was –4500 V, and the source temperature was 500 °C. The multiple reaction monitoring (MRM) mode was chosen, and the related parameters were as follows: *m/z* 405.0 → 45.0, with a declustering potential (DP) of –80 V and collision energy (CE) of –38 eV for triptolide, and *m/z* 421.0 → 45.0, with a DP of –130 V and a CE of –47 eV for (5R)-5-hydroxytriptolide. The dwell time for each transition was 100 ms. The nebulizer gas (Gas 1) was set at 15 psi. The heater gas (Gas 2) was set at 20 psi. The curtain gas was set at 20 psi.

Plasma sample analysis conditions

After derivatization, chromatographic separation of analytes was conducted using a Gemini C18 system (50 mm×2.0 mm; 5 μm; Phenomenex, USA). The mobile phase was a mixture of 0.003% ammonia aqueous solution (A) and acetonitrile (B) at a flow rate of 0.6 mL/min. The following gradient elution was used: 0.00–0.30 min, 32% B; 0.30–2.00 min, 32%–75% B; 2.00–2.50 min, 75% B; 2.50–3.00 min, 75%–32% B; and 3.00–4.00 min to maintain 32% B. The autosampler and column temperature were set at 4 °C and 40 °C, respectively.

An electrospray ionization source was chosen for MS detection. The ion spray voltage was 5500 V, and the source temperature was 550 °C. The scanning mode was set as MRM, and the related parameters were as follows: *m/z* 468.5→192.0 for the derivative of triptolide and *m/z* 484.3→192.1 for the derivative of (5R)-5-hydroxytriptolide with a DP of 80 V and a CE of 30 eV for both analytes. The dwell time for each transition was 100 ms. Gas 1 was set at 55 psi. Gas 2 was set at 60 psi. Curtain gas was set at 35 psi.

Statistical analysis

The LC–MS/MS data were collected by Analyst software (version 1.6.2; AB Sciex, Foster City, CA, USA). Pharmacokinetic data analysis was performed according to a non-compartmental model using Phoenix WinNonlin 6.4 (Pharsight, Mountain View, CA, USA) software. All data are expressed as the mean±SD (tests in duplicate are expressed as the means only). The treatment effects were evaluated by unilateral, unpaired *t*-test by Excel (Microsoft, USA). $P<0.05$ was considered to be statistically significant.

Results

Enzyme phenotypes of triptolide and (5R)-5-hydroxytriptolide

The results of the triptolide co-incubated with different rCYP450s are shown in Figure 2A. The remaining amount of triptolide in the CYP3A4 incubation system was the lowest among all of the incubation systems. The relative contribution of the CYP450 subtypes to the metabolism of triptolide (Figure 2C) was determined after normalization according to the relative amount of each CYP450 in human liver[17]. Among them, CYP3A4 accounted for 94.2% of triptolide metabolism. CYP2E1 was also involved in the metabolism of triptolide, but only accounted for 5.6%. CYP3A4 was the main metabolic enzyme of triptolide, as reported previously[10].

When (5R)-5-hydroxytriptolide was co-incubated with different rCYP450s, the results showed that various CYP450s (Figure 2B, 2D) were involved in the metabolism of (5R)-5-hydroxytriptolide. Among the CYP450s, CYP3A4 was predominant, accounting for 64.2% of the total contribution.

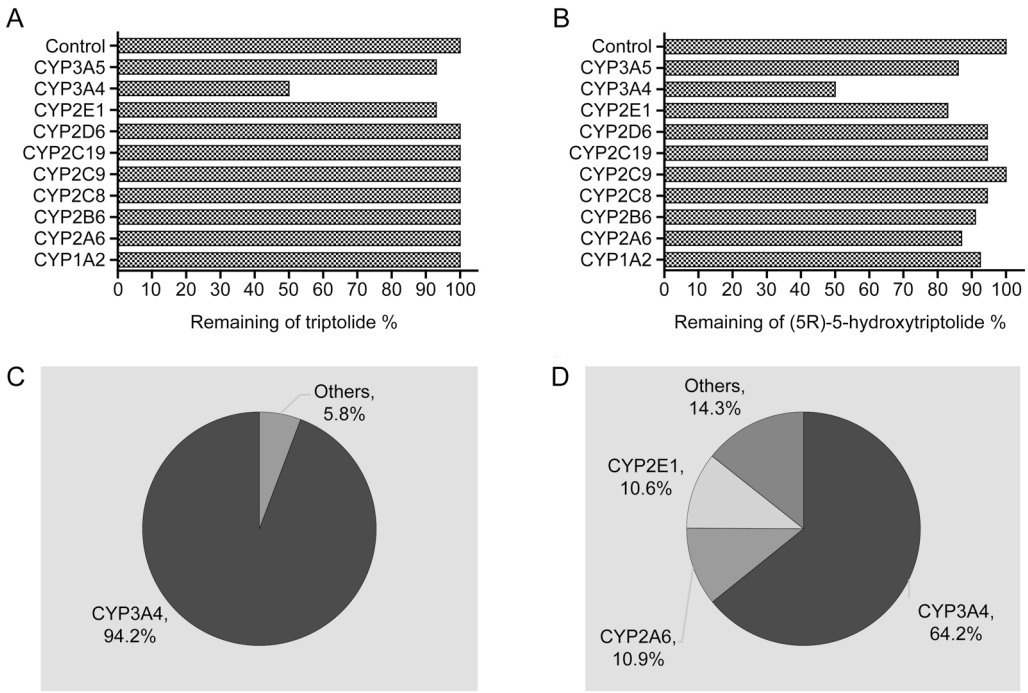

Figure 2. Enzyme phenotypes of triptolide and (5R)-5-hydroxytriptolide. The incubation system containing triptolide (or (5R)-5-hydroxytriptolide, 3 μmol/L), PBS (100 mmol/L, pH 7.4), CYP450s (50 pmol P450/mL), and NADPH (2 mmol/L) was at a final volume of 100 μL. Each incubation was performed in duplicate. (A) Remaining triptolide. (B) Remaining (5R)-5-hydroxytriptolide. (C) Contribution of CYP450s to the metabolism of triptolide. (D) Contribution of CYP450s to the metabolism of (5R)-5-hydroxytriptolide.

Effects of dexamethasone and ritonavir on the pharmacokinetics of triptolide in rats

Considering the significant contribution of CYP3A4 to the metabolism of triptolide and (5R)-5-hydroxytriptolide *in vitro*, we further investigated the effect of a CYP3A4 specific inhibitor and inducer on the pharmacokinetics of triptolide and (5R)-5-hydroxytriptolide in rats *in vivo*. The drug's concentration-time curves are shown in Figure 3, and the triptolide-related pharmacokinetic parameters are listed in Table 1.

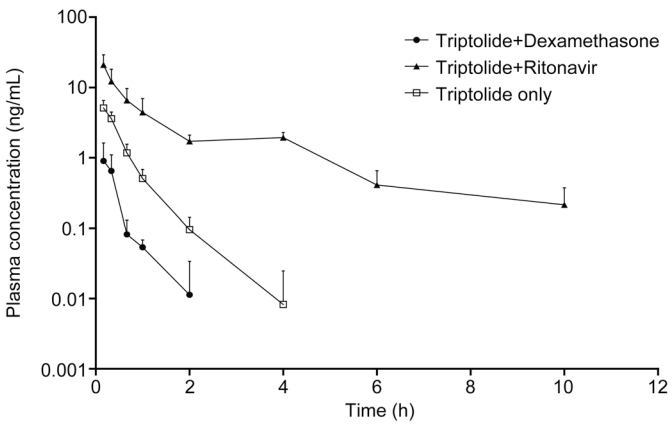

Figure 3. Concentration-time curves of triptolide in rats. Triptolide+dexamethasone (n=4): rats were orally administered dexamethasone (50 mg/kg, qd) for three consecutive days and then orally administered triptolide (0.4 mg/kg); Triptolide+ritonavir (n=4): rats were orally administered ritonavir (30 mg/kg) 1 h before triptolide (0.4 mg/kg); triptolide only (n=4): rats were orally administered triptolide (0.4 mg/kg) only.

When pretreated with a CYP3a inducer, dexamethasone (50 mg/kg, qd, 3 d) significantly reduced the exposure of triptolide, with the C_{max} and $AUC_{0-\infty}$ decreased by 81.0% and 85.4%, respectively. When pretreated with ritonavir (30 mg/kg) 1 h before treatment with triptolide, exposure of the latter was increased significantly, with the C_{max} and $AUC_{0-\infty}$ increased by 4.08 and 7.84 times, respectively, compared with those of the group control.

Effects of dexamethasone and ritonavir on the pharmacokinetics of (5R)-5-hydroxytriptolide in rats

Plasma exposure of (5R)-5-hydroxytriptolide was tremendously decreased when co-administered with dexamethasone, as reflected in the C_{max} and $AUC_{0-\infty}$ decreasing by 94.0% and 91.4%, respectively. While orally pretreated with ritonavir, the $AUC_{0-\infty}$ was increased 2.83 times. Remarkably, no significant difference occurred between the control and ritonavir-pretreated groups according to their C_{max} values. The concentration-time curves of (5R)-5-hydroxytriptolide are shown in Figure 4. The corresponding pharmacokinetic parameters are listed in Table 2.

Discussion

In the present study, we examined the metabolic enzyme phenotypes of triptolide and (5R)-5-hydroxytriptolide as well as

Table 1. Pharmacokinetic parameters of triptolide (details see the illustration under **Figure 3**).

Pharmacokinetics parameters	Triptolide+dexamethasone	Triptolide+ritonavir	Triptolide only
$t_{1/2}$ (h)	0.28±0.13*	1.80±0.39***	0.44±0.08
T_{max} (h)	0.25±0.10	0.17±0.00	0.21±0.08
C_{max} (ng/mL)	0.98±0.67***	21.0±8.1**	5.16±1.36
MRT_{0-t} (h)	0.36±0.07**	2.01±0.48***	0.47±0.05
$MRT_{0-\infty}$ (h)	0.48±0.24	2.33±0.62***	0.52±0.07
AUC_{0-t} (ng·h/mL)	0.36±0.25***	19.8±5.8***	2.55±0.58
$AUC_{0-\infty}$ (ng·h/mL)	0.38±0.25***	20.4±6.1***	2.60±0.58

*P<0.05, **P<0.01, ***P<0.001 versus values in control group.

Abbreviations: $t_{1/2}$, half-life; T_{max}, time to maximum concentration; C_{max}, maximum plasma concentration; MRT_{0-t}, mean residence time from time zero extrapolated until the end of the dosing interval; $MRT_{0-\infty}$, mean residence time from time zero extrapolated to infinity; AUC_{0-t}, area under the plasma concentration–time curve from time zero extrapolated until the end of the dosing interval; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero extrapolated to infinity.

obtained the relative contribution of each isozyme involved. Next, we investigated the effect of a CYP3A4-related strong inducer and inhibitor on the pharmacokinetics of these two compounds in male SD rats.

Several studies[18] have shown large differences among species in regard to CYP450s. Of CYP450s, the activity of CYP3a in mice and male rats is closest to that in humans[19, 20]. However, the most commonly used CYP3A inducer (rifampicin) in the clinic cannot induce the activity of CYP3a in rats[21]. In rats, dexamethasone is commonly used and is able to induce the activity of both CYP2b and CYP3a *in vivo*[22]. Nonetheless, different isoforms have been found in the species of CYP2b for ADME studies with different substrate specificities[18]. Because we lacked the recombinant rat CYP2b isozyme, we did not determine whether the CYP2b subfamily was involved in the metabolism of triptolide and (5R)-5-hydroxytriptolide in rats.

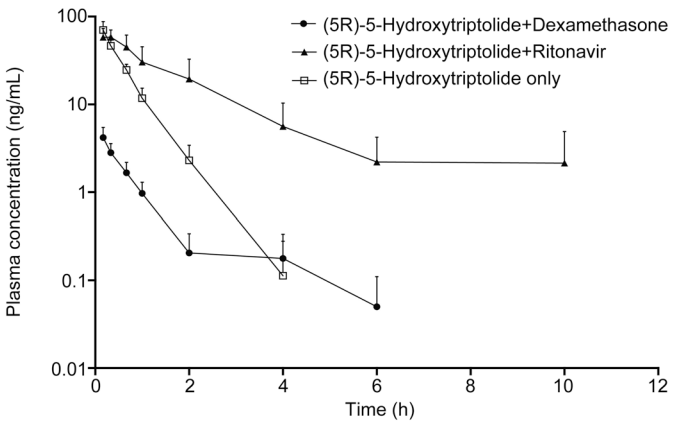

Figure 4. Concentration-time curves of (5R)-5-hydroxytriptolide in rats. (5R)-5-Hydroxytriptolide+dexamethasone (n=4): rats were orally administered dexamethasone (50 mg/kg, qd) for three consecutive days and then were orally administered (5R)-5-hydroxytriptolide (0.4 mg/kg); (5R)-5-Hydroxytriptolide+ritonavir (n=4): rats were orally administered ritonavir (30 mg/kg) 1 h before (5R)-5-hydroxytriptolide (0.4 mg/kg); (5R)-5-Hydroxytriptolide only (n=4): rats were orally administered (5R)-5-hydroxytriptolide (0.4 mg/kg) only.

Dexamethasone significantly increased the elimination rate of triptolide and (5R)-5-hydroxytriptolide *in vivo* with a dramatically decreased $AUC_{0-\infty}$. These findings suggested that the metabolic extent of triptolide and (5R)-5-hydroxytriptolide was strongly (>5-fold) induced[23, 24]. The accelerated metabolism of triptolide and (5R)-5-hydroxytriptolide is thought to be a detoxification route[13, 25, 26].

Ritonavir is not only an inhibitor of CYP3A4 but also an inhibitor of P-gp[27]. Triptolide has been reported to be a substrate of P-gp[25] and BCRP[28]. Inhibition of P-gp in the intestine significantly improves the absorption of orally administered drugs, which are substrates of P-gp[29-31]. Verapamil, a P-gp inhibitor, increases absorption of triptolide[32]. Thus, as a potent P-gp inhibitor, ritonavir may also promote absorption of triptolide in the intestine. Moreover, triptolide has been shown to be extensively metabolized in rats, and the excretion recovery of triptolide by bile and feces was less than 4%[9], indicating that inhibition of the liver efflux transporter may minimally contribute to the plasma exposure of triptolide. Therefore, the effect of ritonavir on the plasma exposure of triptolide was mainly due to its increased absorption in the intestine and decreased metabolism.

By contrast, (5R)-5-hydroxytriptolide is a highly permeable compound (data not shown), suggesting that transporters contribute little to its clearance. Because various CYP450s are involved in (5R)-5-hydroxytriptolide metabolism, inhibition of CYP3a may result in compensation by other enzymes. This condition may be the cause of the more pronounced increase of the $AUC_{0-\infty}$ of triptolide than that of (5R)-5-hydroxytriptolide due to ritonavir. Additionally, the involvement of P-gp may explain why there was no significant change in the C_{max} of (5R)-5-hydroxytriptolide between the control and ritonavir-pretreated groups, but an increase was noted with triptolide. In conclusion, ritonavir can strongly inhibit the elimination of triptolide in rats (the AUC increased more than five times). However, the metabolism of (5R)-5-hydroxytriptolide was only moderately inhibited (the AUC increased two to five times)[23, 24]. From this perspective, it can be concluded that (5R)-5-hydroxytriptolide has a lower risk than triptolide when

Table 2. Pharmacokinetic parameters of (5R)-5-hydroxytriptolide (details see the illustration under **Figure 4**).

Pharmacokinetics parameters	(5R)-5-Hydroxytriptolide+dexamethasone	(5R)-5-Hydroxytriptolide+ritonavir	(5R)-5-Hydroxytriptolide only
$t_{1/2}$ (h)	1.51±1.07*	1.53±1.02*	0.39±0.13
T_{max} (h)	0.21±0.08	0.25±0.10	0.17±0.00
C_{max} (ng/mL)	4.20±1.27***	63.8±13.3	70.2±17.4
MRT_{0-t} (h)	1.04±0.31	1.71±0.96*	0.56±0.16
$MRT_{0-\infty}$ (h)	1.68±0.82	2.23±1.61*	0.59±0.15
AUC_{0-t} (ng*h/mL)	3.18±0.32***	110±48*	40.7±6.2
$AUC_{0-\infty}$ (ng*h/mL)	3.56±0.32***	118±55*	41.7± 5.3

*P<0.05, **P<0.01, ***P<0.001 versus values in control group.

Abbreviations: $t_{1/2}$, half-life; T_{max}, time to maximum concentration; C_{max}, maximum plasma concentration; MRT_{0-t}, mean residence time from time zero extrapolated until the end of the dosing interval; $MRT_{0-\infty}$, mean residence time from time zero extrapolated to infinity; AUC_{0-t}, area under the plasma concentration–time curve from time zero extrapolated until the end of the dosing interval; $AUC_{0-\infty}$, area under the plasma concentration–time curve from time zero extrapolated to infinity.

co-administered with CYP3A inhibitors *in vivo*.

The above-mentioned results suggest that a specific inducer (dexamethasone) and inhibitor (ritonavir) strongly alter the pharmacokinetic characteristics of triptolide and (5R)-5-hydroxytriptolide in rats. Based on these findings, we strongly suggest that clinical trials be conducted to clarify these effects in humans. Dose adjustment and therapeutic drug monitoring might be required for possible DDI.

Conclusions

In-vitro-enzyme phenotype experiments showed that CYP3A4 was the primary metabolic enzyme for both triptolide and (5R)-5-hydroxytriptolide. *In vivo* pharmacokinetic studies showed that dexamethasone and ritonavir significantly affected the pharmacokinetic characteristics of triptolide and (5R)-5-hydroxytriptolide in rats. Due to the narrow therapeutic index of both drugs, this drug combination should be emphasized. The present work suggested that a corresponding clinical drug-drug interaction study should be carried out to clarify the effects of CYP3A4 inhibitors and inducers on triptolide and (5R)-5-hydroxytriptolide.

Acknowledgements

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Author contribution

Ye XU and Da-fang ZHONG were responsible for the research design; Ye XU conducted the experiments; Da-fang ZHONG and Xiao-yan CHEN contributed new reagents or analytical tools; Ye XU, Yi-fan ZHANG and Da-fang ZHONG performed data analysis and wrote the manuscript.

References

1 Lin N, Liu CF, Xiao C, Jia HW, Imada K, Wu H, *et al.* Triptolide, a diterpenoid triepoxide, suppresses inflammation and cartilage destruction in collagen-induced arthritis mice. Biochem Pharmacol 2007; 73: 136–46.

2 Panichakul T, Intachote P, Wongkajorsilp A, Sripa B, Sirisinha S. Triptolide sensitizes resistant cholangiocarcinoma cells to TRAIL-induced apoptosis. Anticancer Res 2016; 26: 259–66.

3 Luo YW, Shi C, Yuan Y, Zhang M, Liao MY. Research progress on the toxicology of triptolide. Dulixue Zazhi 2009; 23: 74–7.

4 Yang F, Ren L, Zhuo L, Ananda S, Liu L. Involvement of oxidative stress in the mechanism of triptolide-induced acute nephrotoxicity in rats. Exp Toxicol Pathol 2012; 64: 905–11.

5 Wang JY, Jiang ZZ, Ji JZ, Wang XZ, Wang T, Zhang Y, *et al.* Gene expression profiling and pathway analysis of hepatotoxicity induced by triptolide in Wistar rats. Food Chem Toxicol 2013; 58: 495–505.

6 Zhou R, Zhang F, He PL, Zhou WL, Wu QL, Xu JY, *et al.* (5R)-5-hydroxytriptolide (LLDT-8), a novel triptolide analog mediates immunosuppressive effects *in vitro* and *in vivo*. Int Immunopharmacol 2005; 5: 1895–903.

7 Tang W, Zuo JP. Immunosuppressant discovery from *Tripterygium wilfordii* Hook F: the novel triptolide analog (5R)-5-hydroxytriptolide (LLDT-8). Acta Pharmacol Sin 2012; 33: 1112–8.

8 Liu J, Li L, Zhou X, Chen XY, Huang HH, Zhao SB, *et al.* Metabolite profiling and identification of triptolide in rats. J Chromatogr B Analyt Technol Biomed Life Sci 2013; 939: 51–8.

9 Liu J, Zhou X, Chen XY, Zhong DF. Excretion of [^{3}H]triptolide and its metabolites in rats after oral administration. Acta Pharmacol Sin 2014; 35: 549–54.

10 Li W, Liu Y, He YQ, Zhang JW, Gao Y, Ge GB, *et al.* Characterization of triptolide hydroxylation by cytochrome P450 in human and rat liver microsomes. Xenobiotica 2008; 38: 1551–65.

11 Tai T, Huang X, Su YW, Ji J, Su YJ, Jiang ZZ, *et al.* Glycyrrhizin accelerates the metabolism of triptolide through induction of CYP3A in rats. J Ethnopharmacol 2014; 152: 358–63.

12 Xue X, Gong LK, Qi XM, Wu YF, Xing GZ, Yao J, *et al.* Knockout of hepatic P450 reductase aggravates triptolide-induced toxicity. Toxicol Lett 2011; 205: 47–54.

13 Ye XC, Li WY, Yan Y, Mao CW, Cai RX, Xu HB, *et al.* Effects of cytochrome P4503A inducer dexamethasone on the metabolism and toxicity of triptolide in rat. Toxicol Lett 2010; 192: 212–20.

14 Kumar S, Kwei GY, Poon GK, Iliff SA, Wang YF, Chen Q, *et al.*

Pharmacokinetics and interactions of a novel antagonist of chemokine receptor 5 (CCR5) with ritonavir in rats and monkeys: Role of CYP3A and P-glycoprotein. J Pharmacol Exp Ther 2003; 304: 1161–71.

15 Liu J, Chen XY, Zhang YF, Miao H, Liu K, Li L*, et al.* Derivatization of (5R)-hydroxytriptolide from benzylamine to enhance mass spectrometric detection: application to a Phase I pharmacokinetic study in humans. Anal Chim Acta 2011; 689: 69–76.

16 Zhao SB, Huang HH, Chen XY, Li XL, Zhong DF. Biotransformation of triptolide to (5R)-hydroxytriptolide. Zhongguo Xinyao Zazhi 2012; 21: 7.

17 Rodrigues AD. Integrated cytochrome P450 reaction phenotyping: attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. Biochem Pharmacol 1999; 57: 465–80.

18 Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol 2006; 2: 875–94.

19 Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ*, et al.* Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiotica 2000; 30: 1131–52.

20 Zuber R, Anzenbacherova E, Anzenbacher P. Cytochromes P450 and experimental models of drug metabolism. J Cell Mol Med 2002; 6: 189–98.

21 Lu C, Li AP. Species comparison in P450 induction: effects of dexamethasone, omeprazole, and rifampin on P450 isoforms 1A and 3A in primary cultured hepatocytes from man, Sprague-Dawley rat, minipig, and beagle dog. Chem-Biol Interact 2001; 134: 271–81.

22 Meredith C, Scott MP, Renwick AB, Price RJ, Lake BG. Studies on the induction of rat hepatic CYP1A, CYP2B, CYP3A and CYP4A subfamily form mRNAs *in vivo* and *in vitro* using precision-cut rat liver slices. Xenobiotica 2003; 33: 511–27.

23 European medicines agency (Europe). Guideline on the investigation of drug interactions. London: Committee for human medicinal products; 2012.

24 Food and administration (US). Services. Guidance for industry: drug interaction studies — study design, data analysis, implications for dosing, and labeling recommendations. Washington: Center for drug evaluation and research; 2012.

25 Zhuang XM, Shen GL, Xiao WB, Tan Y, Lu C, Li H. Assessment of the roles of P-glycoprotein and cytochrome P450 in triptolide-induced liver toxicity in sandwich-cultured rat hepatocyte model. Drug Metab Dispos 2013; 41: 2158–65.

26 Du FY, Liu ZH, Li XX, Xing J. Metabolic pathways leading to detoxification of triptolide, a major active component of the herbal medicine *Tripterygium wilfordii*. J Appl Toxicol 2014; 34: 878–84.

27 Vermeer LM, Isringhausen CD, Ogilvie BW, Buckley DB. Evaluation of ketoconazole and its alternative clinical CYP3A4/5 inhibitors as inhibitors of drug transporters: the *in vitro* effects of ketoconazole, ritonavir, clarithromycin, and itraconazole on 13 clinically-relevant drug transporters. Drug Metab Dispos 2016; 44: 453–9.

28 Li CZ, Xing GZ, Maeda K, Wu CY, Gong LK, Sugiyama Y*, et al.* The role of breast cancer resistance protein (BCRP/ABCG2) in triptolide-induced testis toxicity. Toxicol Res 2015; 4: 1260–8.

29 Jia Y, Liu Z, Wang C, Meng Q, Huo X, Liu Q*, et al.* P-gp, MRP2 and OAT1/OAT3 mediate the drug-drug interaction between resveratrol and methotrexate. Toxicol Appl Pharmacol 2016; 306: 27–35.

30 Wang L, Wang C, Peng J, Liu Q, Meng Q, Sun H*, et al*. Dioscin enhances methotrexate absorption by down-regulating MDR1 *in vitro* and *in vivo*. Toxicol Appl Pharmacol 2014; 277: 146–54.

31 Rengelshausen J, Goggelmann C, Burhenne J, Riedel KD, Ludwig J, Weiss J*, et al.* Contribution of increased oral bioavailability and reduced nonglomerular renal clearance of digoxin to the digoxin-clarithromycin interaction. Br J Clin Pharmacol 2003; 56: 32–8.

32 Zhang YC, Li J, Lei XL, Zhang TY, Liu GX, Yang MH*, et al.* Influence of verapamil on pharmacokinetics of triptolide in rats. Eur J Drug Metab Pharmacokinet 2016; 41: 449–56.