Metabolic Analysis of Triptolide Microspheres in Human, Dog, Rabbit and Rat Liver Microsomes with UPLC-MS/MS Method

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Abstract: Objectives: Riptolide (TPL) has been shown to have a good clinical effect on rheumatoid arthritis (RA). We designed TPL microspheres (TPL-MS) and investigated its metabolic behavior in human, dog, rabbit and rat liver microsomes (HLM, DLM, RLM and SDRLM) with UPLC-MS/MS method.

Methods: First, a UPLC-MS/MS method was established to measure the concentration of TPL in samples. The sample was separated on a C18 column (2.1×100 mm, 1.8μm) and eluted with a gradient elution. The precursor ions/product ions were m/z 378.1/361.0 for TPL and 260.0/116.2 for the internal standard. Then \( T_{1/2}, V_{max} \) and \( CL_{int} \) were calculated from the above data. Finally, the metabolites of TPL-MS were identified by high-resolution UPLC-MS/MS. The sample was separated on a C18 column (2.1×100 mm, 2.2 μm) and eluted with isocratic elution. Mass spectrometric detection was carried out on a thermo Q-exactive mass spectrometer with HESI. The scanning range of precursor ions was from m/z 50 to m/z 750.

Results: Through several indicators including standard curve, precision, accuracy, stability, matrix effect and recovery rate, the enzymatic kinetics parameters including \( T_{1/2}, V_{max} \) and \( CL_{int} \) were evaluated. Several metabolites of TPL-MS were identified.

Discussion: UPLC-MS/MS method is an accurate and sensitive method for the determination of TPL in liver microsomes samples with good precision, accuracy and stability. The variation of parameters indicated that the microspheres can delay the elimination of TPL in liver microsomes.

Conclusion: The metabolism of TPL-MS varied among species, but no new metabolites appeared.

Keywords: Triptolide, UPLC-MS/MS, high-resolution MS, liver microsomes, microspheres, metabolic analysis.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic and autoimmune disease that is characterized by articular synovitis [1]. Recurrent synovitis leads to the destruction of articular cartilage and bone and ultimately causes joint deformity and dysfunction [2]. RA is known as immortal cancer because of its high morbidity, and it seriously affects the quality of life of patients with no specific treatment and difficult recovery [3, 4].

Triptolide (TPL) is a major active ingredient of the Chinese herbal medicine Tripterygium wilfordii Hook.F [5]. At present, it is believed that the damage of articular and bone tissue in RA is mainly caused by the activation and the proliferation of synovial cells [6]. The pathogenesis of RA is closely related to the abnormal apoptotic process. The excessive proliferation of synovial cells results from the relative deficiency of synovial cell apoptosis [7]. TPL can reduce the activity of the apoptotic process, inhibit the proliferation of synovial cells, induce fibroblast synovial cell apoptosis and prevent bone destruction at low concentrations [8, 9]. It has been reported that TPL not only has immunosuppressive and anti-inflammatory effects but also exhibits antitumor activity, which can inhibit the proliferation of various types of cancer cells in vitro and reduce the growth and metastasis of some solid tumors in vivo [10-12].

Although TPL has been proven to have a good clinical effect on RA, its toxicity and side effects limit its clinical promotion [13-15]. The severity of its toxic side effects is related to the dosage. The larger the dosage, the more obvious the toxic side effects. Common side effects include gastrointestinal irritation (such as nausea, anorexia, vomiting, diarrhea, gastrointestinal ulcers, and bleeding), reproductive system toxicity (female manifestations are amenorrhea and menstrual cycle disorders, and the male manifestation is infertility), skin mucosal reactions (oral ulcers, pigmentation, alopecia), hepatotoxicity and nephrotoxicity. Among them, hepatotoxicity is the most frequently reported side effect. Long-term administration of TPL to individuals of different genders can cause different degrees of liver damage. Moreover, on the cellular level, TPL has been reported to be associated with diverse toxic effects, including membrane damage, mi-
To avoid the systemic toxicity of triptolide, we designed and prepared a TPL-PLGA microsphere sustained-release delivery system (TPL-MS) for the treatment of RA. Microspheres are controlled-release delivery systems that are used to reduce peak concentration in vivo, improve drug bioavailability and reduce side effects [16-18]. Studies have shown that the PLGA microsphere could maintain the slow release of the drug over the entire period of release [19-22].

TPL is mainly eliminated by cytochrome P450 (CYP450) metabolism [23]. CYP3A-mediated metabolism is one of the detoxification pathways of TPL. TPL is converted into three monohydroxy metabolites and dihydroxy metabolites in human and rauwolfia microsomes [24, 25]. The ability of the liver to metabolize TPL is closely related to hepatotoxicity [26, 27]. Pretreatment with the CYP broad-spectrum inhibitor 1-aminobenzotriazole (ABT) and glutathione depletion can reduce the metabolism of TPL and enhance its hepatotoxicity, while CYP enzyme inducers, such as dexamethasone and glycyrrhizin, can reduce the hepatotoxicity of TPL in rats [28]. The release of the drug from the microsphere matrix may cause some changes in metabolic behavior.

The detection limit of TPL is relatively high with a UV detector because of terminal absorption at 220nm. UPLC-M-S/MS method is an accurate and sensitive method for the determination of TPL, especially in biological samples (such as plasma, serum, organs, cells, tissues, microsomal enzymes, etc.). Studies have shown that UPLC-MS/MS has the advantages of high sensitivity, low detection limit, less interference and fast time in biological sample analysis and metabolite detection [29-32]. Therefore, the metabolic elimination behaviors of TPL-MS and crude TPL in human, dog, rabbit and rat liver microsomes were studied with the UPLC-MS/MS method. The difference in the metabolic behavior between TPL-PLGA microspheres and the crude drug may be evidence that microspheres slow down drug release. The differences between species and dosage forms were compared, which provided a scientific basis for the preclinical study of TPL-MS.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Standards of TPL (the purity was above 98.5% determined by HPLC-UV, National Institutes for Food and Drug Control); Reduced nicotinamide adenine dinucleotide phosphate (NADPH, 100mg, Real-Times(Beijing) Biote-chnology Co., Ltd); Invitro CYP 150-donor pooled human liver microsomes (HLM, 20 g/L), male pooled beagle dog liver microsomes (DLM, 20 g/L), male pooled New Zealand white rabbit liver microsomes (RLM, 20 g/L) and male pooled SD rat liver microsomes (SDRM, 20 g/L); PLGA (molecular weight=15000 Da, Shandong institute of medical devices); methanol (Merck Co., Gradient Grade), ammonium formate (Merck Co., Gradient Grade), were included in the study. All kinds of liver microsomes were purchased from Research Institute for Liver Diseases (Shanghai), Co. Ltd, and stored at -80°C until use.

2.2. Preparation of TPL-MS

A self-emulsifying solvent evaporation method was used to prepare TPL-MS. A moderate amount of PLGA and TPL (the mass ratio of TPL and PLGA is 1:15) was dissolved in methylene chloride/methanol as the oil phase. The oil phase was added into the water phase, which contained a certain amount of PVA, under mixing conditions for 5-10 min until the formation of an oil/water (O/W) emulsion was achieved. The same volume of ultrapure water was added into the oil/water (O/W) emulsion. The mixing speed was reduced and the emulsion was continued to stir for approximately 4-6 hours until the organic solvent completely evaporated. Ultrafast freezing centrifugation (12000 r/min, 4°C) was carried out, and the microspheres were washed and collected. After freeze-drying, a dried microsphere powder was obtained.

2.3. Instrumentation and Conditions of UPLC-MS/MS

Chromatographic analysis was performed using an Agilent 1290 Infinity II ultrahigh-performance liquid chromatography system (UPLC), including a binary pump, an online vacuum degasser, a surveyor autosampling system, a column temperature controller and a diode-array detector. The sample was separated on an Agilent ZORBAX SB C18 column (2.1×100 mm, 1.8μm) and eluted with a gradient elution of solvent A (water containing 5 mmol/L ammonium formate) and solvent B (acetonitrile) as follows: 30% B (0-3.7 min), 95% B (3.7 min-4.0 min), 95% B (4.0 min-10.0 min), 30% B (10.1 min) and 30% B (10.1 min-15 min). The column temperature was set at 40°C, the flow rate was set at 0.4 mL/min, and the injection volume was set at 2 μL. Mass spectrometric detection was carried out on an Agilent 6460 triple-quadrupole mass spectrometer with positive electro-spray ionization, which was connected to the liquid chromato-graphy system. The MRM mass scan mode was implemented. The precursor ions/product ions were m/z 378.1/361.0 for TPL and m/z 260.0/116.2 for the internal standard (IS, propranolol hydrochloride). The MS/MS conditions were optimized as follows: fragmentor, 100 V; capillary voltage, 8000 V; nebulizer gas pressure (N2), 35 psi; drying gas flow rate (N2), 10 L/min; and gas temperature, 350°C. Agilent MassHunter software was used for the control of the equipment and data acquisition. Agilent Quantitative Analysis software was used for data analysis.

2.4. Preparation of Calibration Standards, Quality Control Standards and the Internal Standard

A stock solution of TPL was prepared in methanol at a concentration of 1.97 mg/mL. A stock solution of the IS was prepared in methanol at a concentration of 1.50 mg/mL, and the IS solution was diluted to 25 ng/mL before use. A series
of standard working solutions were obtained by further diluting the stock solution of TPL with methanol. The calibration standard samples for TPL (5, 25, 50, 125, 250, and 500 ng/mL) were prepared by mixing TPL standard working solutions and the IS solution of the same volume. The quality control (QC) samples for TPL were prepared at low (5 ng/mL), medium (50 ng/mL), and high (500 ng/mL) concentrations in the same way as the calibration standard samples. The QC samples were stored at 4°C until analysis.

2.5. Method Validation

2.5.1. Linearity and Sensitivity

For the calibration curve, six concentrations of calibration standards (5, 25, 50, 125, 250, and 500 ng/mL) were processed and determined as described above. The calibration curves were constructed as the peak area ratios of TPL to IS against the TPL concentrations. The lower limit of quantification (LLOQ) was determined as the concentration of TPL with a signal-to-noise ratio of 10.

2.5.2. Precision and Accuracy

To determine intraday and interday precision and accuracy, three replicates of QC samples at low, medium and high concentration levels were prepared and analyzed on the same day and on three different days. The intra- and interday precisions are expressed as the mean ± RSD value.

2.5.3. Extraction Recovery and Stability

The extraction recovery was determined by calculating the ratio of QC samples obtained at low, medium and high concentration levels (5, 50 and 500 ng/mL) against those originally spiked in the liver microsomes (n=3). The short-term stability was evaluated by determining QC samples at room temperature for 12 h. The freeze-thaw stability was determined through three freeze-thaw cycles on consecutive days. The extraction recovery and stability were expressed as the mean ± RSD value.

2.5.4. Matrix Effect

The matrix effect was determined by calculating the ratio of QC samples obtained at low, medium and high concentration levels (5, 50 and 500 ng/mL) against those originally spiked in the mobile phase (n=3). The detection of the matrix effect can exclude the influence of endogenous components in those liver microsomes, resulting in ion suppression of the analyte signal.

2.6. \( T_{1/2} \) Determination of TPL-MS in HLM, DLM, RLM and SDRLM

The incubation system, with a total volume of 250 μL, consisted of phosphate buffer, TPL-MS, liver microsomes, and NADPH. All incubation samples were carried out in triplicate. The mixed solution of liver microsomes and TPL were preincubated at 37°C for 5 mins, and then NADPH, which was incubated at 37°C for 5 minutes, was added to initiate the reaction, and incubation continued at 37°C in a constant temperature oscillator. Aliquots (40 μL) were collected from the incubation system at 5, 15, 30, 45, 60 and 120 minutes after the reaction started, and 400 μL iced acetonitrile containing the IS (final concentration of 25 ng/mL) was added to terminate the reaction. The samples were vortexed for 1 min and centrifuged for 10 min (15000 r/min). The supernatant was used to analyze the contents of TPL with the UPLC-MS/MS method described. A blank control group without NADPH, a zero-time reaction group and a positive control group with midazolam were prepared.

2.7. Enzymatic Kinetics of TPL-MS in HLM, DLM, RLM and SDRLM

Different concentrations of TPL-MS were added to the above incubation system. The final concentrations of TPL in the incubation systems were 0.5, 1, 2, 4, 10, 25, and 50 μmol/L. According to the results of the metabolic stability experiment, the reaction times of the HLM, DLM, RLM and SDRLM groups were set at 120, 120, 120 and 5 minutes, respectively, and the corresponding zero-time control groups were set at the same times. All protein concentrations of HLM, DLM, RLM and SDRLM were 1.0 g/L. The reaction was terminated by adding iced acetonitrile containing the IS (final concentration of 25 μg/mL). Samples were diluted when needed in order to fit the linear range of the method. The sample treatment methods and detection methods were the same as the above metabolic stability experiment.

2.8. Accurate Molecular Weight Identification of Metabolites

Accurate molecular weight identification of metabolites was performed using UPLC including a binary pump, an online vacuum degasser, an autosampling system, a column temperature controller and a diode-array detector. The sample was separated on a C18 column (2.1×100 mm, 2.2 μm) and eluted with isocratic elution of solvent A (water containing 0.1% formic acid, 1%) and solvent B (acetonitrile). The column temperature was set at 30°C, the flow rate was set at 0.2 mL/min, and the injection volume was set at 100 μL. Mass spectrometric detection was carried out on a thermo Q-exactive mass spectrometer with HESI, which was connected to the liquid chromatography system. The mass scan mode was that of a full scan. The scanning range of precursor ions was from m/z 50 to m/z 750. The MS conditions were optimized as follows: spray voltage, 3800 V; capillary temperature, 320°C; nebulizer gas pressure (N₂), 40 psi; drying gas flow rate (N₂), 40 L/min; and probe heater temperature, 350°C. Thermo Xcalibur Qual Browser software was used for the control of the equipment and data analysis.

2.9. Statistical Analysis

The remaining percentage of TPL was the ratio of the remaining concentration at each incubation time to the average concentration at zero-time. The metabolic elimination curves were plotted with the incubation time as the abscissa and the remaining percentage of TPL at each time point as the ordinate. The remaining percentage of TPL after natural
logarithm conversion at each time point was mapped with the corresponding incubation time, and the slope ($k$) was obtained by linear regression. The half-life ($T_{1/2}$) of elimination in four kinds of liver microsomal metabolism was obtained from formula 1. The enzyme kinetics of TP in HLM, DLM, RLM and SDRLM was investigated by the substrate elimination method. The enzymatic kinetic parameters were estimated by nonlinear regression using GraphPad Prism software, version 8.0. The enzyme kinetic parameters, including the $K_m$ and $V_{max}$ values, were calculated from nonlinear regression analysis of experimental data according to the Michaelis-Menten equation. The internal clearance rate ($CL_{int}$) was calculated with formula 2.

$$T_{1/2}=-0.693/k \quad \text{formula 1}$$

$$CL_{int} = \frac{V_{max}}{K_m} \quad \text{formula 2}$$

Experimental values are expressed as the mean ± SD. Statistical analysis of the results obtained from the clinical study was performed using an independent-samples t-test. Differences were considered statistically significant and very significant when $p$ values calculated by SPSS 13.0 software were <0.05 and <0.01, respectively.

3. RESULTS

3.1. Preparation of TPL-MS

The preparation method of TPL-MS is shown in Fig. (1). Freeze-dried microspheres are shown in Fig. (1B). When the microspheres were dispersed in water, they were in the state of a uniform suspension emulsion which is shown in Fig. (1C). The particle size of the microspheres shown in Fig. (1D) measured by transmission electron microscopy (TEM) was approximately 5 μm. The encapsulation efficiency (EE%) measured by the HPLC method was approximately 74.37±2.30%.

3.2. Method Validation of the UPLC-MS/MS Method

A quantitative analysis method utilizing UPLC-MS/MS was developed to accurately determine the concentration of TPL in the different liver microsomal incubation systems. The mass-to-charge ratio of TPL and the IS is shown in Fig. (2A). The mass transitions of TPL and the IS were 378.0→361.1 and 260.0→142.8, respectively. Mass spectra of a blank liver microsome and a liver microsome with TPL and the IS are shown in Fig. (2B). No significant interference substances were observed at the retention times of TPL.

Fig. (1). The preparation and characterization of TPL-MS. (A) the preparation of TPL-MS; (B) the freeze-dried TPL-MS; (C) the uniform suspension emulsion of TPL-MS; (D) the TEM photo of TPL-MS (×12000).
and the IS in the liver microsomal samples. The calibration curves for TPL were constructed by plotting the peak area ratios of the analyte to the IS against the TPL concentrations in the liver microsomal matrix using a linear least-squares regression model. The calibration curves were obtained with squared correlation coefficients ($r^2$) greater than 0.99 for concentrations between 5 and 500 ng/mL TPL.

The precision (intra- and inter-day precision), accuracy (recovery efficiency) and stability (12 h at room temperature
and after three freeze-thaw cycles) of the analytical method were also tested at three concentration levels in triplicate and verified by determining the ratios of the peak areas of TPL to the IS with relative standard deviation (RSD), as listed in Table 1. The precision of this method was no more than 5% RSD for TPL, indicating satisfactory precision and accuracy.

Table 1. The precision, accuracy and stability of the UPLC-MS/MS method.

<table>
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<tr>
<th>Concentration of TPL (ng/mL)</th>
<th>Intra-day Precision</th>
<th>Inter-day Precision</th>
<th>Recovery Efficiency</th>
<th>Short-term Stability in 12h</th>
<th>Three Times Freeze-thaw</th>
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<td>RSD (%)</td>
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Fig. (3). The metabolic stability and enzyme kinetics curves of TPL-MS in HLM, DLM, RLM and SDRLM. (A) the metabolic elimination curves of blank control groups without NADPH; (B) the metabolic elimination of TPL-MS was similar in HLM, DLM, RLM and SDRLM; (C) the half-life of elimination in HLM, DLM, RLM and SDRLM; (D) the enzyme kinetics curves, D-(1) means HLM, D-(2) means DLM, D-(3) means RLM, D-(4) means SDRLM; "ns" means that there were no significant differences, p>0.05; "**" means that there were very significant differences, p<0.01.
accuracy of the instrumentation. The direct precipitation method with acetonitrile was used to achieve high recovery efficiency. The extraction efficiency of TPL exceeded 95%. The stability data indicated that TPL, under these conditions, was stable in all liver microsomal samples (RSD <5%). The results of the matrix effect showed that no matrix effect or interferences from endogenous compounds were detected for assays of four kinds of liver microsomes.

3.3. \( T_{1/2} \) Determination of TPL-MS in HLM, DLM, RLM and SDRLM

The metabolic elimination curves of the blank control groups shown in Fig. (3A) indicate that TPL was not significantly eliminated without the addition of NADPH. The metabolic elimination of TPL-MS was similar in HLM, DLM and RLM. The remaining percentages of TPL-MS in HLM, DLM, RLM at 15 min and 120 min were 89.26% ± 1.37%, 91.32% ± 2.38%, and 92.01% ± 2.22% and 74.22% ± 4.05%, 78.11% ± 4.44%, and 77.35% ± 2.87%, respectively (p>0.05). However, there was a significant difference, as shown in Fig. (3B), between the SDRLM and other liver microsomes. The remaining percentages of TPL-MS in SDRLM at 15 min and 120 min were 77.48% ± 3.88% and 23.29% ± 3.39% (p<0.01), respectively. The half-lives (\( T_{1/2} \)) of elimination in HLM, DLM, RLM and SDRLM as shown in Fig. (3C) were 315.0, 364.7, 346.5 and 57.8 min, respectively, indicating that there was a significant difference between SDRLM and other liver microsomes.

3.4. Enzymatic Kinetics of TPL-MS in HLM, DLM, RLM and SDRLM

The enzyme kinetics curves of TPL-MS in HLM, DLM, RLM and SDRLM are shown in Fig. (3D). They were fitted with the concentration of TPL as the abscissa and the elimination rate of TPL as the ordinate. The elimination rate of TPL is the amount of TPL removed per microgram of liver microsome per minute. The concentrations of all four liver microsomes were 1.0 g/L. The incubation time of TPL-MS in HLM, DLM, RLM and SDRLM was determined to be 120, 120, 120 and 30 minutes, respectively, according to the metabolic stability experiment. The enzyme kinetic parameters, including \( K_m \), \( V_{max} \) and \( CL_{int} \) are shown in Table 2.

3.5. Accurate Molecular Weight Identification of Metabolites

TPL and four of its metabolites were found in the HLM, DLM, RLM and SDRLM incubation systems, including m/z 361.1646 (TPL, [M+H]), m/z 377.1595 (metabolite, [M1+H]), m/z 377.1595 (metabolite, [M2+H]), m/z 391.1387 (metabolite, [M3+H]) and m/z 391.1387 (metabolite, [M4+H]), whose extraction ion chromatograms and secondary mass spectra are shown in Fig. (4). Their retention times are approximately 11.4-11.7 min, 7.9-8.4 min, 10.0-10.4 min, 3.4-4.3 min and 7.1-8.2 min, respectively. During the experiment, we found that TPL can convert to m/z 359.1489 (TPL, [M-H]) in addition to the [M+H] peak. M1 and M2 can both convert to m/z 375.1438 (dehydrogenation of the metabolites, [M1/M2-H]) in addition to the [M1/M2+H] peak. M3 and M4 can both convert to m/z 389.1244 (dehydrogenation of metabolite, [M3/M4-H]) in addition to the [M3/M4+H] peak. This may be due to the full scan mode of MS. Therefore, it needs to be evaluated in combination with the retention time to verify whether it is a metabolite. The specific metabolism results of TPL-MS in the four liver microsomes are shown in Table 3.

4. DISCUSSION

Due to the low concentration of TPL and more interfering substances in liver microsomes samples, it was very important to choose a sensitive measurement method [33-35]. The product ions of TPL were found to be very few and unstable in the secondary mass spectrometry. We added ammonium formate into the mobile phase to produce a strong excimer ion peak of added ammonia m/z 378.1. The second-
Fig. (4). The extraction ion flow diagrams and secondary mass spectograms of TPL and its four metabolites in different liver microsome incubation systems. (A)HLM; (B)DLM; (C)RLM; (D)SDRLM (*refers to the extraction ion flow diagrams; # refers to the secondary mass spectograms; ▼ means the location of TPL and its metabolites). (A higher resolution / colour version of this figure is available in the electronic copy of the article).
The metabolism of TPL in the liver microsomes was dependent on NADPH [36-38]. TPL was metabolized by the liver microsomes after it was released from the microspheres. TPL-MS was eliminated to varying degrees in the different liver microsomal incubation systems with NADPH. At the same time, the highest metabolic elimination amount of TPL-MS was observed in SDRLM. After incubation for 60 minutes, the remaining percentage of TPL was 39.39% in the SRLM, which showed a significant difference (p<0.05) compared to the remaining 82.55%, 83.86% and 82.12% observed in the HLM, DLM and RLM, respectively. However, there was no significant difference between the remaining percentage of TPL-MS in the HLM, DLM and RLM. It has been reported that the remaining percentage of TPL that was not encased in microspheres was 75.3% and 12.3% during the same incubation time (60 mins) in the HLM and SRLM. The T_{1/2} of elimination measured in our study (315.0 mins in HLM and 57.8 mins in SRLM) was 2-3 times longer than the reported value in the literature (154.4 mins in HLM and 19.9 mins in SRLM) [39].

The apparent K_{m} and V_{max} of TPL-MS in the HLM, DLM and RLM exhibited no significant differences (p>0.05). The apparent K_{m} and V_{max} of TPL-MS in SRLM were significantly different from those in the HLM, DLM and RLM (p<0.05). The V_{max} and CL_{int} values in the SRLM were the largest compared to those in the other three liver microsomes, which suggests that the removal of TPL in rats is faster than in humans, dogs and rabbits. The V_{max} values measured in our study (83.89 pmol/min•mg in HLM and 725.7 pmol/min•mg in SRLM) were reduced by 40% and 67% compared with the reported value in the literature (139 pmol/min•mg in HLM and 2194 pmol/min•mg in SRLM), respectively [39, 40]. The CL_{int} values measured in our study (3.19 μL/min•μmol in HLM and 21.06 μL/min•mg in SRLM) were reduced by 76% and 82% compared with the reported value in the literature (13.1 μL/min•mg in HLM and 118.3 μL/min•mg in SRLM), respectively [39, 40]. The extension of T_{1/2} and the decrease of V_{max} and CL_{int} indicate that microspheres can delay the elimination of TPL in liver microsome incubation systems. This suggests that we might use a lower dose of TPL-MS, which could reduce systemic adverse reactions while achieving the same therapeutic effect.

TPL is mainly metabolized by the liver cytochrome P450 enzyme 3A4 (CYP3A) [41-44], and the main metabolic pathway is that of oxidation. The main metabolites in rats and humans reported in the literature are mono- and double-hydroxylated metabolites [38, 40]. In our research, four metabolites were found. M1 and M2 were mono-hydroxy triptolides, which could possibly be a pair of isomers. M3 and M4 were mono-hydroxy and mono-carbonyl triptolides, which could also possibly be a pair of isomers. M, M2 and M4 were found in all of the tested liver microsome incubation systems. M1 was found in the RLM and SRLM incubation systems. M3 was found in the HLM and SRLM liver microsome incubation system. The detection conditions described in this paper. The metabolism of TPL-MS varied among species. However, compared with the types of metabolites reported in the literature, there were no new metabolites detected, indicating that the administration of TPL-MS would not introduce new toxicity. Metabolites in the HLM were also found in the other liver microsomes, indicating that there are no human-specific metabolites of TPL-MS.

CONCLUSION

UPLC-MS/MS method is an accurate and sensitive method for the determination of TPL in liver microsome samples with good precision, accuracy and stability. The results showed that the microspheres changed the metabolic behavior of TPL in vitro to a certain extent, such as the extension of T_{1/2} and the decrease of V_{max} and CL_{int}. This confirms the feasibility of prolonging drug release in clinical practice with the use of TPL-MS. No new metabolites or human-specific metabolites appeared, indicating that the administration of TPL-MS would not introduce new toxicity.

ETHICS APPROVAL AND CONSENT TO PARTICIATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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L.W. performed all experiments and acquisition of the data. Y.L. established the UPLC-MS/MS method. R.L. participated in the metabolic experiment. D.H. provided the laboratory instruments. L.W. drafted the manuscript.
REFERENCES


Metabolic Analysis of Triptolide Microspheres


