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*Research Article*

# The Pharmacokinetic Effects and Drug-Drug Interaction of Atorvastatin With Danshensu in Rats

Yibin Mei[1,†], Feifei Chen[2,†], Jingjing Guan[3], Qinglian Zhang[3], Abdullah Al Mamun[3], Quan Zhou[3], Peiwu Geng[3], Shuanghu Wang[3,*], Yunfang Zhou[3,*]

[1]Department of Cardiology, Lishui Hospital of Wenzhou Medical University, The First Affiliated Hospital of Lishui University, Lishui People's Hospital, 323000 Lishui, Zhejiang, China
[2]Department of Pharmacy, Ningbo First Hospital, 315010 Ningbo, Zhejiang, China
[3]Central Laboratory of Lishui Hospital of Wenzhou Medical University, The First Affiliated Hospital of Lishui University, Lishui People's Hospital, 323000 Lishui, Zhejiang, China
*Correspondence: wangshuanghu@lsu.edu.cn (Shuanghu Wang); zyf2808@lsu.edu.cn (Yunfang Zhou)
[†]These authors contributed equally.
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**Abstract**

**Background and Objective**: Danshensu and atorvastatin are employed in the management of cardiovascular diseases (CVDs) to lower blood lipid levels and dilate blood vessels. Therefore, this study aimed to examine the potential pharmacokinetic interaction between danshensu and atorvastatin in rats. **Materials and Methods**: Chromatography was performed using a CORTECST UPLC C18 column (2.1 × 1.5 mm, 1.6 µm) at a temperature of 40 °C. Acetonitrile and water were combined in the mobile phase at a rate of 0.4 mL/min via gradient elution. The methodology was validated under the guidance of the FDA and EMA literature. A total of 12 Sprague-Dawley (SD) rats were randomly assigned into two groups: the experimental group (treated with 150 mg/kg/day danshensu) and the control group (treated with 0.5% CMC-Na). Atorvastatin 10 mg/kg was administered after two weeks. The proposed method meets all the standards of bioanalysis technology verification. **Results**: Compared to the control group, the experimental group exhibited significant differences in the area under the curve (AUC), $t_{1/2}$, $CL_{z/F}$, and $C_{max}$ ($p < 0.05$). The AUC, $C_{max}$, and $t_{1/2}$ of the experimental group were higher, yet the clearance rate was significantly slowed. **Conclusions**: This technique was effectively utilized to investigate the pharmacokinetic interaction between danshensu and atorvastatin in rats, which may be of significant clinical importance and contribute to the rationalization of clinical medicine, ultimately facilitating individualized administration.

**Keywords:** danshensu; atorvastatin; drug-drug interaction; pharmacokinetics; cardiovascular diseases

## 1. Introduction

The incidence of cardiovascular diseases (CVDs) such as atherosclerosis, hypertension, hyperlipidemia and stroke is increasing year by year, which is the main cause of death in the world today and its mortality rate is much higher than other types of diseases [1]. CVD has become a predominant cause of mortality among people in recent years, which has sparked a nationwide search for effective treatment [2]. To prevent CVD, it is imperative to lower low-density lipoprotein cholesterol (LDL-C) levels. Moreover, statins reduce the importance of LDL-C in preventing CVD, indicating that clinical use may reduce the risk of cardiovascular disease by using these drugs [3–5].

Atorvastatin is a statin that contains a penta-substituted pyrrole with extremely wide clinical applications [6–9]. The most common disease treated with statins is CVD, which reduces morbidity and mortality of the disease by lowering the concentration of cholesterol in the blood [10–12]. Cholesterol is widely distributed in the cell membrane, and its main synthetic site is in the liver [13]. When CVD occurs, it is an extremely important indicator of whether blood lipids are normal. Therefore, atorvastatin regulates cholesterol biosynthesis by competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) [14]. At present, statins are well tolerated in clinic, and there is no contraindication to atorvastatin. A large amount of literature proves the safety of atorvastatin in the treatment of CVD population, and its clinical tolerance is well tolerated, so it is widely used in the treatment of cardiovascular diseases worldwide [15,16]. The combination of statins may cause various types of myopathy and hemorrhagic stroke (outpatient trends), so the dosage should be limited [17]. Atorvastatin is a lipophilic drug that will prevent cardiovascular disease by reducing plasma endothelin-1 and asymmetric dimethylarginine [18,19]. Furthermore, atorvastatin is a substrate for CYP3A4, a P-glycoprotein (P-gp) and organic anion transporter 1B1 (OATP1B1) [20,21]. The main metabolites of atorvastatin were O-hydroxy atorvastatin and the p-hydroxy atorvastatin, which account for 70% of the HMG CoA reductase activity [3].

Danshensu is derived from the dried roots of *Savia miltiorrhiza* and is the important pharmacological component of traditional Chinese medicine Danshen, which can dilate blood vessels, increase the circulation of blood and reduce the congestion of small pieces [22]. It plays an important role in mediating beneficial antioxidant and anti-apoptotic effects through activating nuclear factor-erythrocyte 2-related factor 2 (Nrf2)/HO-1 signaling [23, 24]. Numerous studies have confirmed that danshensu can treat CVDs including atherosclerosis, coronary heart disease and angina pectoris, etc. It has unique biological activity on the cardiovascular system and can play a protective role [25–27]. The clinical applications of danshensu were investigated in recent years [28,29]. The interaction between danshensu and other conventional drugs has been reported clinically. The combination of rhubarb and danshensu is a drug interaction prescription that treats chronic kidney disease and inhibits oxidative stress [30].

Danshensu has been reported to modulate the activity of cytochrome P450 enzymes, particularly CYP2C9, and to inhibit drug transporters such as OATP1B1, leading to clinically relevant drug-drug interactions (DDIs) [21,31,32]. Furthermore, atorvastatin is a substrate for CYP3A4, a P-gp and OATP1B1 [20,21]. Both DDIs and herbal–drug interactions (HDIs) can alter the pharmacokinetics and therapeutic outcomes of co-administered drugs [33,34]. Compared with DDIs, HDIs are more complex to investigate due to the multicomponent nature of herbal medicines and their diverse pharmacological effects [35]. Moreover, patients with chronic diseases often take multiple medications including herbal products, increasing the potential for clinically significant interactions [36]. The combination of atorvastatin and danshensu is expected to have the effect of dilation of the blood vessels. In this study, a sensitive and reproducible UPLC-MS/MS method was developed for the determination of atorvastatin in rat plasma. This study developed a sensitive and reproducible UPLC-MS/MS method for the quantification of atorvastatin in rat plasma. This work aimed to assess the pharmacokinetic interactions between danshensu and atorvastatin *in vivo* in rats, providing an experimental foundation for their safe clinical application.

## 2. Materials and Methods

### *2.1 Chemicals and Reagents*

Atorvastatin (purity >98%) was purchased from Aladdin (Aladdin, Shanghai, China). Sodium danshensu and simvastatin (IS) were provided by Beijing Sunflower and Technology Development Co., Ltd. (Beijing, China). HPLC-grade formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chromatography grade acetonitrile and methanol were provided by Fisher Scientific Co. (Fair Lawn, NJ, USA). Rat blank plasma samples were obtained from blank rats of the Experimental Animal Center of Wenzhou Medical University. All other chemicals and reagents were of analytical grade.

### *2.2 Equipment and UPLC-MS/MS Analysis*

The determination of atorvastatin was performed by an Acquity UPLC Xevo TQD triple quadrupole mass spectrometer (Waters Co., Milford, MA, USA). Chromatography was carried out using a CORTECST UPLC C18 column (2.1 × 1.5 mm, 1.6 µm) at a constant temperature of 40 °C. The mobile phase consisted of acetonitrile and water at a rate of 0.4 mL/min. The gradient was as follows: 0.0–0.4 min (20% A), 0.4–1 min (rapidly rose from 20% A to 95% A), 1–2 min (maintained at 95% A), 2–2.5 min (reduced to 20% A). The total running time was 3 min. At the end of each injection, the syringe would be washed with a different ratio of methanol-water (50/50, V/V; 10/90, V/V).

The settings for the multi-reaction monitoring (MRM) scan mode were 150 °C for the source, 500 °C for desolvation, 40 V for the cone voltage, and 20 V and 10 V for collision energy for atorvastatin and IS, respectively. The production for MRM detection was m/z 559.25→440.17 for atorvastatin and m/z 419.22→199.17 for internal standard simvastatin (Fig. 1). All data was processed by Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

### *2.3 Calibration Standards and Quality Control (QC) Samples*

The stock solution of atorvastatin (1 mg/mL) was formulated in methanol. A set of standard working solutions was prepared by diluting the atorvastatin stock solution with methanol. Similarly, the stock solution of simvastatin was prepared in the same way and finally diluted to 2 µg/mL before use. The calibration standard samples for atorvastatin (100, 50, 25, 10, 5, 2.5, 1 ng/mL) were generated by adding 20 µL of the working standard solution to 100 µL of blank rat plasma. Low quality control (LQC) (2 ng/mL), middle quality control (MQC) (8 ng/mL), and higher quality control (HQC) (80 ng/mL) samples were prepared in the same manner. All solutions and samples were stored in a refrigerator at 4 °C until use.

### *2.4 Sample Preparation*

A 10 µL aliquot of IS solution (500 ng/mL simvastatin) and 100 µL of acetonitrile was added to 50 µL plasma sample. The mixture was vortex-mixed for 30 sec, and centrifuged at 13,000 rpm for 5 min. Then 2 µL aliquot of the supernatant was injected into the UPLC-MS/MS system for analysis.

### *2.5 Methodology Verification*

Methodological validation was performed under the guidance of the FDA and EMA literature [37,38], including selectivity, linearity and the lower limit of quantification (LLOQ), linearity, accuracy, precision, matrix effect, extraction recovery and stability. Each set of validations

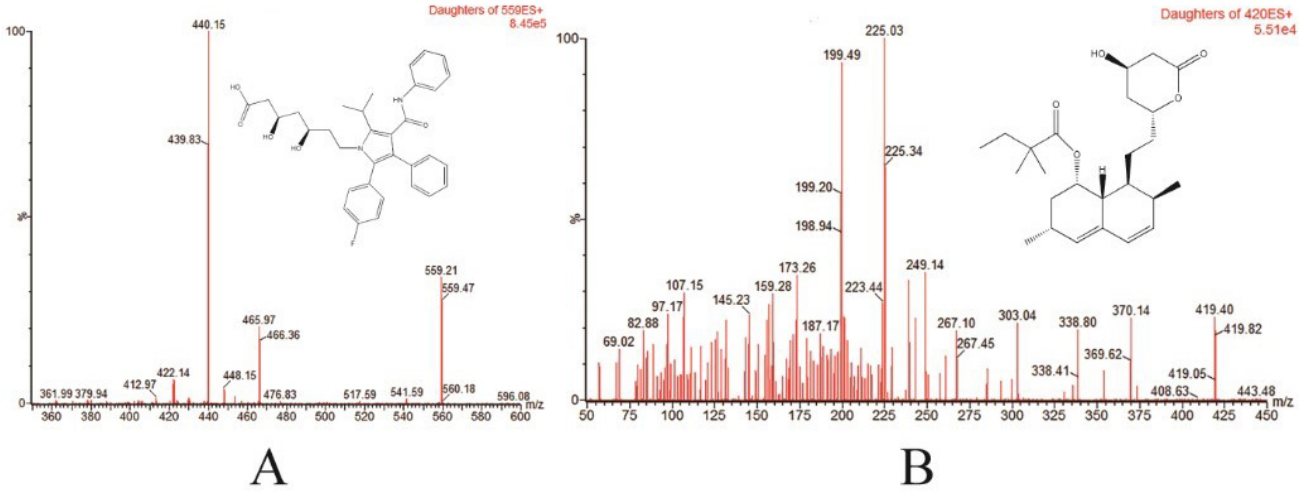


**Fig. 1. Mass spectra of atorvastatin (A) and simvastatin (IS) (B).**

need to be determined for three consecutive days including five sets of four different concentrations of QC samples (LLOQ, LQC, MQC, HQC).

*2.6 Specificity*

To evaluate the specificity, we analyzed five blank biological samples, five biological samples spiked with atorvastatin and IS, and five biological samples following administration of atorvastatin orally or intravenously.

*2.7 Linearity and the Lower Limit of Quantification (LLOQ)*

The standard curve for atorvastatin was within 1 to 100 ng/mL for plasma samples. The reciprocal of concentration (1/X) was used as the weighting factor to evaluate calibration curves using linear regression analysis. LLOQ was defined as the lowest concentration quantified on the scale of calibration curves, where relative standard deviation (RSD) for precision and RE for accuracy were less than 20%.

*2.8 Precision and Accuracy*

Five replicate samples at four concentrations (LLOQ, LQC, MQC, HQC) of 1, 2, 8, and 80 ng/mL, respectively, were determined on one day to calculate accuracy and precision. The validation procedure was carried out over three consecutive days. RSD and relative error (RE) were employed for evaluating precision and accuracy, respectively. Intra-day and inter-day precision were verified by measuring concentration changes over the same day and for three consecutive days. Accuracy was achieved by comparing the results to the prepared concentration, with a lower than 15% error and a variation within $\pm$ 15%, respectively.

*2.9 Extraction Recovery and Matrix Effect*

The recovery rate is the ratio of the response value of the test substance recovered from the sample to the response value of the standard substance. Four different concentrations of LLOQ, LQC, MQC, HQC (1, 2, 8, 80 ng/mL, respectively) were prepared according to the method of the sample preparation, and each concentration was made in five parallel groups and the corresponding peak area of atorvastatin was measured as A1. 500 µL of blank plasma and 1 mL of acetonitrile were added to a 2 mL centrifuge tube, vortexed for 30 s, and centrifuged for 5 min (13,000 rpm). Unextracted atorvastatin samples were prepared from 145 µL of supernatant, IS and atorvastatin standard solutions to obtain the average A2 of the corresponding peak area of atorvastatin and calculated the recovery rate by A1/A2.

Significant interferences frequently occur throughout the analyte analysis procedure, impacting the accuracy of the results. These influences and disturbances are referred to as matrix effects. The standard solution and IS were added to 45 µL aliquots of methanol to make four different concentrations of atorvastatin solution (1, 2, 8, 80 ng/mL, respectively). The matrix effect was calculated as A2/A3 based on the average value of the area A3.

*2.10 Stability*

Long-term stability and short-term stability indicate the reliability and reproducibility of results. Five sets of parallel experiments were carried out for each of the four sample concentrations of LLOQ, LQC, MQC and HQC (1, 2, 8, 80 ng/mL, respectively).

*2.11 Animals and Treatment*

Sprague-Dawley (SD) male rats (280 $\pm$ 20 g) from the Experimental Animal Center of Wenzhou Medical University (Wenzhou, China) were housed in a laboratory with appropriate temperature and humidity (Laboratory Animal Center of Wenzhou Medical University). Rats were acclimated to the laboratory environment for approximately 10 days to minimize any potential animal suffering before the experiment commenced. All experimental procedures were approved by the Institutional Animal Ethics Committee of

**Table 1. Precision, accuracy, recovery and matrix effect for atorvastatin of QC samples in rat plasma (n = 5).**

| Concentration (ng/mL) | RSD | | Accuracy | | Recovery (%) | Matrix effect (%) |
|---|---|---|---|---|---|---|
| | Intra-day (%) | Inter-day (%) | Intra-day (%) | Inter-day (%) | | |
| 1.00 | 10.93 | 13.31 | 12.84 | 9.67 | 95.36 | 89.58 |
| 2.00 | 7.43 | 2.35 | 6.95 | 7.46 | 92.46 | 90.47 |
| 8.00 | 5.81 | 5.27 | 5.86 | 3.20 | 85.32 | 91.87 |
| 80.00 | 4.58 | 2.27 | 5.98 | 7.04 | 85.28 | 87.06 |

QC, Quality Control; RSD, relative standard deviation.

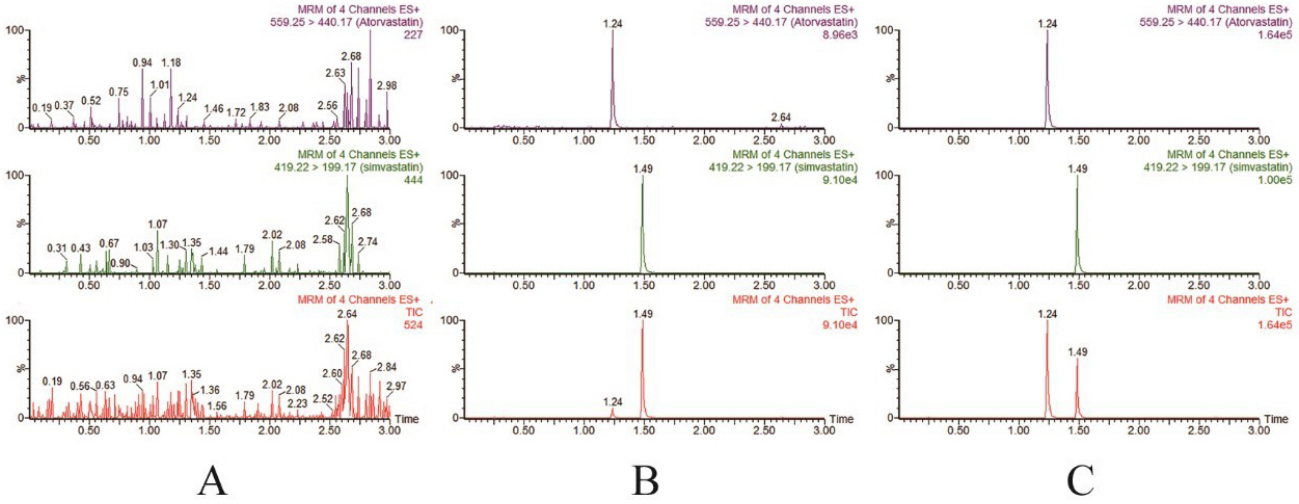


**Fig. 2. Typical ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) chromatograms of atorvastatin and simvastatin (IS) include: (A) blank plasma; (B) blank plasma spiked with IS (2 µg/mL) and atorvastatin (1 ng/mL); and (C) rat plasma samples obtained orally two hours after receiving a dosage of 10 mg/kg atorvastatin.**

Wenzhou Medical University and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (ID No: xmsq2021-0408). According to the AVMA Guidelines for the Euthanasia of Animals (2020), all animals were humanely euthanized at the end of the blood sampling period by 200 mg/mL pentobarbital sodium, 60 mg/kg, i.p, deep anesthesia was confirmed by loss of pedal withdrawal and corneal reflexes. After confirming deep anesthesia, thoracotomy (open-chest procedure) was performed as the terminal procedure to ensure rapid and irreversible cessation of cardiac function. Death was confirmed by absence of respiration, heartbeat, and reflexes.

### *2.12 Effects of Danshensu on the Pharmacokinetics of Atorvastatin in Rats*

Twelve SD rats were randomly allocated into two groups: the experimental group (Group A, n = 6, receiving a dosage of 150 mg/kg/day of danshensu for two weeks) and the control group (Group B, n = 6, administered 0.5% CMC-Na). Subsequent to the last administration of danshensu or 0.5% CMC-Na (control group), both cohorts were administered a single oral dosage of atorvastatin at 10 mg/kg. The rats underwent a 12-hour fasting period before to injection. The 100 µL blood samples were obtained by the tail vein at intervals of 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours into a 1.5 mL centrifuge tube. The samples underwent centrifugation at 4000 rpm for 10 minutes and were promptly separated. The plasma samples were subsequently transferred to a different centrifuge tube and frozen at –80 °C until analysis, following the removal of the supernatant.

## 3. Results and Discussion

### *3.1 Method Development*

Electrospray ionization (ESI) was the most common method, which enhanced the sensitivities and reproducibility of UPLC-MS/MS. The second impact of the analysis results was to take into account the type of column and the choice of mobile phase. The CORTECST UPLC C18 column (2.1 × 1.5 mm, 1.6 µm) was selected for this column due to its smoother chromatogram and more satisfactory peak shape. Acetonitrile water was chosen for elution due to its appropriate retention time. The retention times of atorvastatin and simvastatin were 1.24 and 1.49 min, respectively. The removal of proteins and biological samples was a critical step before performing LC-MS analysis. This study demonstrated that protein precipitation using acetonitrile yielded superior matrix effects and enhanced recovery compared to liquid-liquid extraction.

### 3.2 Specificity

Standard chromatograms for blanks, pooled samples, and individual biological samples are shown in Fig. 2. The outcomes demonstrated that atorvastatin and IS were not hampered by any endogenous molecules.

### 3.3 Calibration Curve and LLOQ

The standard curve of atorvastatin in rat plasma had good linearity, ranging from 1–100 ng/mL, with the linear regression equation of $y = 0.0370389 \times x + 0.0616107$, $R^2 = 0.9964$. The LLOQ of atorvastatin in plasma was determined to be at least 1 ng/mL. The RSD of precision and accuracy at LLOQ was less than 20%, suggesting that this method was rather sensitive to our analysis.

### 3.4 Precision, Accuracy, Extraction Recovery and Matrix Effect

Table 1 shows the results of precision, accuracy, extraction recovery, and matrix effects of atorvastatin in rat plasma. The results showed that the precision and accuracy were within 15% as required by the FDA biological sample analysis method. The extraction recovery rate ranged from 85.28% to 95.36%, and the matrix effect ranged from 87.06% to 91.87%. The analytical results meet the requirements. The results of the matrix effect also indicated that plasma has no significant impact on the matrix. Fig. 2 illustrates the representative chromatograms of a blank plasma sample, a plasma sample spiked with atorvastatin and internal standard, and a plasma sample collected 2 hours post-oral administration of 10 mg/kg atorvastatin.

### 3.5 Stability

The results of Table 2 indicated the stability of atorvastatin LLOQ, LQC, MQC and HQC (1, 2, 8, 80 ng/mL, respectively) in rat plasma. There was no difference in stability between samples, whether they were stored at room temperature for 24 hours in a refrigerator at 4 °C for 6 hours or at –20 °C for repeated freeze-thaw cycles three times.

### 3.6 Effects of Danshensu on the Pharmacokinetics of Atorvastatin in Rats

The pharmacokinetic character of atorvastatin in combination with danshensu (150 mg/kg) were profiled by DAS software (Version 3.2.8, The People's Hospital of Lishui, China) and the statistical results were shown in Table 3. When the two drugs were administered simultaneously, it may significantly affect the atorvastatin pharmacokinetic parameters including AUC, $C_{max}$, $t_{1/2}$ and $CL_{z/F}$ ($p < 0.05$). The highest blood concentration of atorvastatin in rats was reached about 0.35 hours after oral administration. The changes in AUC, $C_{max}$, $t_{1/2}$ and $CL_{z/F}$ in rats after the combination treatment are sufficient to demonstrate an interaction between the two types of treatment. The AUC of the experimental group was about 3-fold that of the control group, and the $C_{max}$ and $t_{1/2}$ of the experimental group were about 2-fold that of the control group. The results showed that combined with drugs, atorvastatin slowed down elimination in rats and inhibited metabolism, resulting in significantly increased drug accumulation. $CL_{z/F}$ was three times higher in the control group than in the experimental group. The lower clearance rate for the experimental group may also be related to its slower metabolism and longer residence time in the body, resulting in a slower clearance of the drug. Therefore, the results show that danshensu and atorvastatin interact in rats. The mean plasma concentration-time curve for atorvastatin was shown in Fig. 3. The peak plasma concentration of the experimental group was higher than that of the control group, but its peak time was slower. This phenomenon may be caused by the interaction of danshensu and atorvastatin, which increases the accumulation of atorvastatin in the body and inhibits its metabolism.

**Table 2. Summary of stability of atorvastatin and IS under various storage conditions (n = 6).**

| Conditions | Concentration (ng/mL) | RSD (%) | Accuracy (%) |
|---|---|---|---|
| Room temperature, 24 h | 1 | 10.77 | 5.44 |
| | 2 | 10.19 | 4.84 |
| | 8 | 4.75 | 1.18 |
| | 80 | 5.08 | 6.96 |
| Freeze-thaw | 1 | 5.91 | 12.9 |
| | 2 | 9.29 | 7.78 |
| | 8 | 4.45 | 5.23 |
| | 80 | 6.4 | 11.28 |
| 4 °C, 6 h | 1 | 9.91 | 7.52 |
| | 2 | 10.82 | –2.32 |
| | 8 | 7.6 | 3.38 |
| | 80 | 5.09 | 3.92 |
| –20 °C, 2 weeks | 1 | 10.04 | 14.72 |
| | 2 | 6.74 | 8.57 |
| | 8 | 9.05 | 8.04 |
| | 80 | 5.66 | 7.35 |

**Table 3. Primary pharmacokinetic parameters after oral administration of atorvastatin in rats (n = 6 each group).**

| Parameters | Atorvastatin+danshensu | Atorvastatin |
|---|---|---|
| $AUC_{(0-t)}$ (μg/L·h) | 225.71 ± 64.51* | 76.12 ± 17.47 |
| $AUC_{(0-\infty)}$ (μg/L·h) | 248.49 ± 87.96* | 76.91 ± 17.76 |
| $MRT_{(0-t)}$ (h) | 2.52 ± 0.25 | 2.16 ± 0.29 |
| $MRT_{(0-\infty)}$ (h) | 3.91 ± 1.53 | 2.29 ± 0.33 |
| $t_{1/2}z$ (h) | 4.45 ± 2.02* | 1.89 ± 0.39 |
| $T_{max}$ (h) | 0.58 ± 0.20 | 0.35 ± 0.18 |
| $V_{z/F}$ (L/kg) | 267.17 ± 112.74 | 369.45 ± 102.24 |
| $CL_{z/F}$ (L/h/kg) | 43.58 ± 11.75* | 136.84 ± 35.93 |
| $C_{max}$ (μg/L) | 122.62 ± 36.19* | 52.06 ± 26.53 |

Notes: *$p < 0.05$ indicates statistical difference between Group A and Group B.

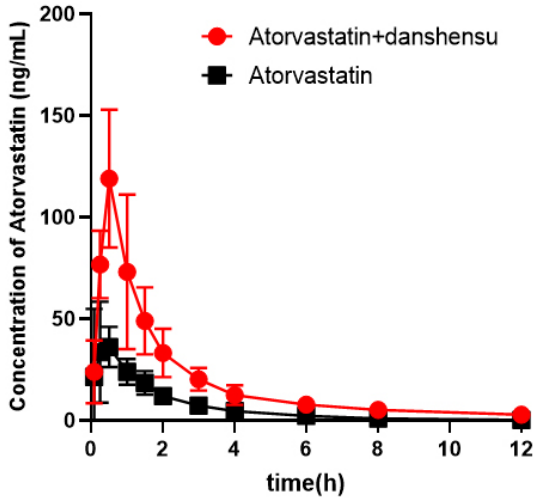


**Fig. 3. Mean plasma concentration-time curve of atorvastatin in rats (n = 6, mean ± SD).**

Danshensu has been reported to act as a competitive inhibitor of CYP2C9, while it does not significantly affect CYP3A4-mediated metabolism [31]. Atorvastatin is metabolized primarily by CYP3A4, while P-gp and OATP1B1 modulate its hepatic uptake and excretion [20, 22]. Danshensu has also been reported to inhibit OATP1B1-mediated transport of rosuvastatin, providing a mechanistic basis for the pharmacokinetic interactions observed *in vivo* [39]. Therefore, the *in vivo* increase in atorvastatin exposure observed in the presence of danshensu may result from reduced efflux via P-gp and/or altered hepatic uptake via OATP1B1, rather than altered CYP3A4 metabolism. Understanding these transporter-mediated interactions is important for predicting potential herb–drug interactions and optimizing individualized therapy. Both danshensu and atorvastatin are used for the regulation of blood lipids and the treatment of cardiovascular diseases. In this study, a sensitive and reproducible UPLC-MS/MS method was developed to simultaneously quantify danshensu and atorvastatin in rat plasma. The findings provide a foundation for future studies to evaluate the efficacy and safety of the combined use of these two drugs in cardiovascular therapy.

## 4. Conclusion

In this study, a highly sensitive and selective UPLC-MS/MS method was successfully established for the quantification of atorvastatin in rat plasma, and the pharmacokinetic interaction between atorvastatin and danshensu was systematically investigated. The co-administration of danshensu significantly altered the pharmacokinetic parameters of atorvastatin, suggesting the occurrence of a potential herb-drug interaction. Based on previous studies, danshensu has been reported to inhibit OATP1B1-mediated hepatic uptake, suggesting that the pharmacokinetic interaction observed with atorvastatin is more likely transporter-mediated rather than metabolism-mediated. These findings highlight the importance of considering transporter systems when evaluating the pharmacokinetic interactions between herbal components and conventional drugs. Further *in vitro* and clinical studies are needed to elucidate the precise mechanisms underlying the interaction between danshensu and atorvastatin and to ensure their safe and rational co-administration in clinical practice.

## Availability of Data and Materials

The data generated in this study can be requested from the corresponding author.

## Author Contributions

YM, FC and JG conceived the study, performed the experiments, wrote the manuscript, and designed the research. QZ, QLZ, PG and AAM performed data acquisition, analysis and interpretation. SW, YZ conceptualized, funded, supervised and confirmed the authenticity of all the raw data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

All experimental procedures were approved by the Institutional Animal Ethics Committee of Wenzhou Medical University and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals (ID No: xmsq2021-0408).

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Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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