Original Article

Influence of schisantherin A on the pharmacokinetics of lenvatinib in rats and its potential mechanism

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Background: Lenvatinib (LEN) is approved as first-line therapy for advanced hepatocellular carcinoma (HCC). Schisantherin A (STA) can exert hepatoprotective and anti-tumor effects. The clinical combination of LEN and STA is very common, especially for patients with advanced HCC, but the effect of STA on the pharmacokinetics of LEN is unclear. This study aimed to investigate the effects of STA on the pharmacokinetics of LEN in rats and explore its potential mechanism.

Methods: Male Sprague-Dawley (SD) rats were orally administered different doses of STA or vehicle control for 7 consecutive days, and 1.2 mg/kg of LEN was given on day 7. The messenger RNA (mRNA) and protein expression levels in the intestines and liver were investigated by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and western blot.

Results: It was revealed that STA increased the oral bioavailability of LEN. The area under the curve from time 0 to infinity ($AUC_{0-\infty}$) and maximum plasma concentration (C_{max}) of LEN after co-administration with STA (20 mg/kg) increased by 54.3% (3,396.73±989.35 *vs.* 5,240.03±815.49 μg/L/h) and 54.8% (490.64±124.20 *vs.* 759.66±152.75 μg/L), respectively. The clearance decreased from 0.38±0.12 to 0.23±0.04 L/h/kg, and the apparent volume of distribution (Vz) decreased from 10.83±3.19 to 6.35±1.38 L/kg in the presence of 20 mg/kg STA. In addition, the expression of P-glycoprotein (P-gp) mRNA and protein in the intestines was markedly decreased.

Conclusions: This study showed that STA increased the bioavailability of LEN, probably due to inhibition of P-gp in the intestine, thereby increasing systemic absorption of LEN. Thus, there is an interaction between the two drugs, and careful monitoring must be conducted when they are used in combination.

Keywords: Metabolism; CYP3A4; drug-drug interaction; transport

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Introduction

Cancer is one of the leading causes of death among humans worldwide, with primary liver cancer being the third leading cause of all cancer-related deaths. Hepatocellular carcinoma (HCC), which accounts for 75–85%, is the most prevalent form of primary liver cancer, and most patients are already at an advanced stage of HCC at the time of diagnosis. Systemic therapy is an appropriate option for patients with advanced HCC (1-4). One such systemic therapy for HCC is lenvatinib (LEN), which was approved in 2018 by the U.S. Food and Drug Administration as an oral, first-line treatment for advanced HCC after successful completion of a randomized phase III clinical trial (5). Anti-tumor effects are exerted by LEN by not only functioning as a multi-receptor tyrosine kinase inhibitor that selectively targets fibroblast growth factor receptors 1–4, vascular endothelial

growth factor receptors 1–3, and platelet-derived growth factor receptor α, but also by modulating the immune microenvironment of tumors synergistically in combination with immune checkpoint inhibitors (6). In addition, LEN is used to treat other types of tumors, including radioactive iodine-refractory differentiated thyroid cancer, advanced endometrial cancer, and metastatic renal cell carcinoma in clinical practice (7-10). *In vitro* studies have shown that LEN is predominantly metabolized hepatically by CYP3A4 enzymes and is a substrate for breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) (11,12). Therefore, drug interactions may occur based on metabolic enzymes and/or transporters when LEN is used in clinical applications. Treatment with LEN has been shown to cause elevated levels of aspartate aminotransferase (AST) in patients, and most patients with HCC may have underlying liver disease (5,13). Thus, LEN may be combined with hepatoprotective treatments and other therapies that reduce liver enzymes to improve treatment safety and reduce complications.

Schisantherin A (STA) is a dibenzocyclooctadiene lignan derived from *Schisandra chinensis (Turcz.) Baill* and *Schisandra sphenanthera Rehd. et Wils*, and it has a variety of pharmacological effects (14). Studies have shown that STA has an ameliorative effect on liver injury caused by a variety of conditions and can protect liver function (15-18). A recent study has shown that STA induces apoptosis and has anti-tumor activity (19). Therefore, the combined treatment of LEN and STA is hepatoprotective and improves anti-tumor efficacy. However, STA has been shown to inhibit the activities of transporters P-gp and other metabolic enzymes (20-23), and may increase the bioavailability of cyclosporin A and cyclophosphamide (24,25). Moreover, several studies have indicated that pharmacologically active components of *Schisandra* extract may inhibit P-gp or CYP3A4 and increase the bioavailability of tacrolimus, cyclosporin A, and paclitaxel (26-28). Wuzhi capsule (WZC), an ethanol extract herbal preparation of *Schisandra* chinensis comprising various components including STA, can affect the pharmacokinetics of LEN, leading to an increase in systemic LEN exposure (29,30). Based on these studies, we speculated that drug-drug interactions exist between LEN and STA. The combination of STA and LEN is very common in clinical practice, but drug interactions between STA and LEN have been rarely studied.

In this study, we investigated the effects of STA on the pharmacokinetics of LEN and explored the underlying mechanisms by measuring the changes in messenger RNA (mRNA) and protein expression levels of various transporters, including P-gp and BCRP, and metabolic enzymes, such as CYP3A1, in the intestinal and liver tissues of rats after oral administration at various dosages of STA. We present the following article in accordance with the ARRIVE reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-174/rc).

Methods

Materials

The LEN used in this study was kindly provided by Shijiazhuang Pharmaceutical Group (Q75191201, Shijiazhuang, China). Internal standard (IS) ($^{2}H_{5}$-LEN, ZZS-20-624-A9) was purchased from Shanghai Zhen Zhun Biological Technology Co., Ltd. (Shanghai, China). The STA was purchased from Shanghai Macklin Biochemical Co., Ltd. (S886029). High-performance liquid chromatography (HPLC)-grade acetonitrile, ethyl acetate, methanol, and formic acid were purchased from Fisher Scientific (Pittsburgh, PA, USA). An RNA simple Total RNA Kit, Fastking RT Kit, and SuperReal PreMix Plus (SYBR Green) were acquired from Tiangen Biotech Co., Ltd. (Beijing, China). Radioimmunoprecipitation assay (RIPA) buffer was acquired from Beijing Solarbio Science Technology Co., Ltd. (Beijing, China). The antibody anti-P-gp (ab170904) was procured from Abcam (Shanghai, China); rabbit anti-BCRP (27286-1-AP) was purchased from Proteintech (Wuhan Sanying, Hubei, China); and rabbit anti-β-actin (AF7018) and goat anti-rabbit secondary antibodies (S0001) were purchased from Affinity Biosciences (Cincinnati, OH, USA).

Animals

Sprague-Dawley (SD) rats (male, body weight: 220–280 g, approximately 7 weeks of age) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (Beijing, China). All animal experimental protocols were approved by the Ethics Committee of Hebei General Hospital, Shijiazhuang, China (No. 2021131) and were in accordance with Hebei General Hospital guidelines for the care and use of animals. The rats were fed in a controlled environment with a temperature of 23–27 ℃, humidity of 50%±10%, and a 12-hour light/dark cycle for 7 days before starting the experiment. The animals had free access to adequate water and food. The rats were fasted for 12 hours prior to experimentation, but were allowed sufficient access to water.

Pharmacokinetics

A total of 18 male SD rats were divided into three groups (n=6 in each group, consistent with previous pharmacokinetic studies). The rats were randomized by a random number table according to their weight. We administered two different dosages of STA to 2 of the 3 groups (12 rats total). We administered STA (5.5 mg/kg) to the rats in Group I (LS) by oral gavage for 7 consecutive days; 15 minutes after administration of STA on day 7, the rats were administered 1.2 mg/kg of LEN by gavage. Group II (HS) was given 20 mg/kg of STA by oral gavage for 7 consecutive days, and 15 minutes after administration of STA on day 7, the rats were administered 1.2 mg/kg of LEN by gavage. The control group (C) was given 0.5% sodium carboxymethyl cellulose (CMC-Na) for 7 consecutive days, and was given 1.2 mg/kg of LEN by gavage on day 7. Gavage administration of STA and the blank solvent was performed independently by 1 investigator (who was not involved in the follow-up analysis), and the other investigators were unable to distinguish the experimental group from the control group. Approximately 0.3 mL of blood samples were collected from the orbital venous plexus 0, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, and 96 hours after oral administration of LEN. After 4 hours, the rats were allowed free access to water. The blood was added to heparinized centrifuge tubes, which were centrifuged at 3,500 rpm for 10 minutes, and the supernatant (blood plasma) was transferred to a new tube and stored at –20 ℃ until ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis. No animals were excluded unless the animal died during experiments.

Blood plasma analysis

A previously developed and validated UPLC-MS/MS method was utilized to analyze the blood plasma samples (30). An LC-30A Nexera ultra-high performance liquid chromatography (UPLC, Shimadzu, Kyoto, Japan) equipped with an API 5500 triple quadrupole mass spectrometer (AB Sciex, Framingham, MA, USA) was employed for the analyses of the plasma samples. Prior to UPLC-MS/MS analysis, the plasma samples underwent liquid-liquid extraction with ethyl acetate. A 150 μL aliquot of ethyl acetate was used to extract 50 μL of the plasma sample, and 20 μL of a 50 ng/mL solution of IS, then 130 μL of supernatant was dried under a stream of N_2 gas after centrifugation. Finally, 100 μL of 50% methanol in water was used to redissolve the dried residue, and the resulting solution was injected into the UPLC-MS/MS for analysis. Chromatographic separation was achieved on a ZORBAX SB-C18 column (2.1×100 mm, 3.5 μm, Agilent, Santa Clara, CA, USA) using a gradient elution of 0.1% formic acid in water (A) and acetonitrile (B) as the mobile phase (flow rate: 0.3 mL/min). The gradient elution method was set to 0–2 minutes from 20% to 60% B, 2–3 minutes at 60% B, 3–3.5 minutes from 60% to 20% B, and 3.5–4 minutes at 20% B. The range of calibration curve for LEN was 0.2 to 1,000 ng/mL, and the lower limit of quantification (LLOQ) was 0.2 ng/mL. The temperature of the column was set to 40 ℃, and the injection volume was 3 μL. For mass spectrometry analysis, the methods were run in positive-ion mode using multiple reaction monitoring (MRM) by monitoring the transitions between the precursor ion and the product ion of LEN and the IS (m/z 427.1 → 370 and m/z 432.1 → 370, respectively). All blood samples were measured by UPLC-MS/MS, and points that were below the LLOQ of the method were excluded.

mRNA expression analysis by reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The total RNA was isolated from the intestine and liver using the RNA simple Total RNA Kit. Ultra violet (UV) spectrophotometry (Thermo NanoDrop 2000c; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to determine the concentration of the total RNA and the quantity and purity of the RNA based on the ratio of the absorbance between 260 and 280 nm. The total RNA (2 μg) was reverse-transcribed into complementary DNA (cDNA) using the FastKing RT kit. Next, 380 μL of RNase-free water was added to 20 μL of the cDNA solution. RT-qPCR was performed using a two-step amplification method according to the manufacturer's instructions in an ABI Prism TM 7500 Real-Time qPCR system (Thermo Fisher Scientific, Inc.) using primers ordered from Seville according to the primer sequences listed in *Table 1*. The amplification procedure was conducted as follows: step 1 consisted of denaturation at 95 ℃ for 15 minutes, while step 2 consisted of 40 cycles of 95 ℃ for 10 seconds and 60 ℃ for 32 seconds. The relative expression level of the RNA was computed using the $2^{-\Delta\Delta Ct}$ method, with β-actin serving as the endogenous control.

Protein expression analysis by western blot

Western blot was used to analyze the expression of protein in the intestines and liver. The total protein in the two

Table 1 Primer sequences for RT-qPCR analysis

Gene	Forward	Reverse
β-actin	5'-TGCTATGTTGCCCTAGACTTCG-3'	5'-GTTGGCATAGAGGTCTTTACGG-3'
Abcb1a	5'-TCTGGTATGGGACTTCCTTGGT-3'	5'-TCCTTGTATGTTGTCGGGTTTG-3'
Abcg2	5'-TGAAGAGTGGCTTTCTAGTCCG-3'	5'-TTGAAATTGGCAGGTTGAGGTG-3'
CYP3A1	5'-TGCATTGGCATGAGGTTTGC-3'	5'-TTCAGCAGAACTCCTTGAGGG-3'

RT-qPCR, reverse transcription quantitative polymerase chain reaction.

tissues was extracted using RIPA buffer containing 1 μg/mL protease inhibitors (Solarbio, Beijing China), and the protein concentration was measured using a bicinchoninic acid (BCA) Protein Assay Kit. The total protein (40 μg) was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; 8%). After separation, the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. Primary antibodies against P-gp (1:2,000), BCRP (1:1,000), and β-actin (1:5,000) were used to detect the presence of the respective proteins after the membranes were blocked with 5% skimmed milk. After incubating with appropriate primary antibodies at 4 ℃ overnight, the membranes were washed 3 times with tris-buffered saline containing 0.1% Tween-20 (TBST). Then, the membranes were incubated with the secondary antibody for 1 hour at room temperature and washed again 3 times with TBST. Finally, the blots were visualized using a hyper signal electrochemiluminescence (ECL) substrate. The relative expression levels of the target proteins in each sample were analyzed by comparing the gray value of target protein with β-actin bands with the ImageJ software (v1.8.0; National Institutes of Health, Besthesda, MD, USA).

Statistical analysis

The mean plasma concentration (mean ± standard deviation) *vs.* time curves for LEN were fitted using GraphPad Prism 8.0.1 (GraphPad Software, Inc., La Jolla, CA, USA). The pharmacokinetic parameters of LEN were calculated using the DAS 2.1.1 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The parameters were analyzed by a *t*-test or a nonparametric rank-sum test using the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA). The RT-qPCR and western blot results were statistically analyzed by SPSS 25.0. A P value <0.05 was considered a statistically significant difference.

Results

Effects of STA on the pharmacokinetics of LEN

The main pharmacokinetic parameters of LEN are provided in *Table 2* as the mean ± standard deviation (n=6 for each group). All animals were included in the analysis as no animals died. We excluded 6 points that were below the LLOQ of the method. As shown in the mean plasma concentration-time curves of LEN in *Figure 1*, an increase in the plasma concentration of LEN was observed in the lower STA-dosed rat group compared to the control group, but this result was not statistically significant; however, a significant increase in systemic exposure of LEN was observed in the higher STA-dosed group. The area under the curve from 0 to 96 hours (AUC_{0-96} area) of LEN after the higher oral dosage of STA (20 mg/kg) increased from 3,369.02±976.35 to 5,223.48±804.96 μg/L/h (P<0.05) compared to the control group; at the same time, the area under the curve from 0 to infinity ($AUC_{0-\infty}$) of LEN increased from 3,396.73±989.35 to 5,240.03±815.49 μg/L/h (P<0.05). Administration of STA (20 mg/kg) led to an increase in the maximum plasma concentration (C_{max}; 490.64±124.20 *vs.* 759.66±152.75 μg/L) of LEN compared to the control group. Meanwhile, the apparent volume of distribution (V_z) of LEN reduced from 10.83±3.19 to 6.35±1.38 L/kg, and the volume of plasma cleared per time unit (CL) decreased from 0.38±0.12 to 0.23±0.04 L/h/kg, after oral administration (20 mg/kg) of STA.

mRNA expression in the intestines and liver

To investigate the mechanism of effects of STA on the pharmacokinetics of LEN, RT-qPCR was employed to quantify the mRNA expression levels of P-gp (encoded by Abcbla in rats) and BCRP (encoded by Abcg2 in rats) in the intestine as well as the mRNA expression levels of CYP3A1 (which is homologous to human CYP3A4, and product by

Table 2 The main pharmacokinetic parameters of LEN with and without administration of two dosages of STA

Parameters (unit)	Drug combination group, LEN (1.2 mg/kg)		Alone, LEN (1.2 mg/kg)
	STA (5.5 mg/kg)	STA (20 mg/kg)	Control group
AUC_{0-t}, μg/L/h	4,361.77±1,016.83	5,223.48±804.96**	3,369.02±976.35
$AUC_{0-\infty}$, μg/L/h	4,387.57±1,012.19	5,240.03±815.49*	3,396.73±989.35
C_{max}, μg/L	545.37±106.32	759.66±152.75*	490.64±124.20
T_{max}, h	2.33±0.82	2.25±1.33	1.75±0.88
$t_{1/2z}$, h	18.58±5.72	19.11±4.71	20.23±5.74
CL, L/h/kg	0.29±0.07	0.23±0.04*	0.38±0.12
V_z, L/kg	8.09±4.75	6.35±1.38*	10.83±3.19

*P<0.05, **P<0.01, means that the difference is statistically significant compared to the control group with LEN alone. LEN, lenvatinib; STA, schisantherin A; AUC_{0-t}, area under the curve from time point 0 to time; $AUC_{0-\infty}$, area under the curve from time point 0 to infinity; C_{max}, maximum plasma concentration; T_{max}, maximum time; CL, volume of plasma cleared per time unit; V_z, apparent volume of distribution.

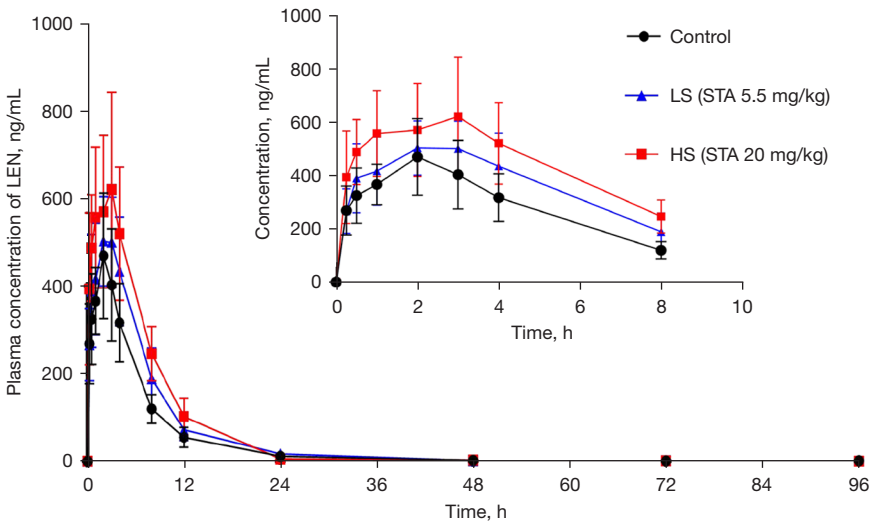

Figure 1 Mean plasma concentration-time curves of LEN in rats after the oral administration of 1.2 mg/kg LEN (n=6). Control: 1.2 mg/kg of LEN; LS: 1.2 mg/kg of LEN after 7 consecutive days of oral treatment with 5.5 mg/kg STA; HS: 1.2 mg/kg of LEN after 7 consecutive days of oral treatment with 20 mg/kg STA. LEN, lenvatinib; STA, schisantherin A.

CYP3A1 in rats), P-gp, and BCRP in the liver; the results are shown in *Figure 2*. The mRNA expression levels of P-gp and BCRP decreased by 69% (P<0.01) and 67.5% (P<0.01), respectively, in the intestine after treatment with a high dosage of STA (*Figure 2A*). It was shown that STA could inhibit the mRNA expression of P-gp and BCRP in the intestine, but there was no statistical difference between the low-dosed STA group and the control group. Meanwhile, there was no significant influence of both dosage of STA on the mRNA expression levels of CYP3A1, P-gp, and BCRP in the liver when co-administered to the rats (*Figure 2B*).

Protein expression in the intestine and liver

To further investigate the effects of STA on the expression of P-gp and BCRP transporters, the expression of P-gp and BCRP proteins in the intestine and liver were quantified by western blot, and the results as shown in *Figure 3*. The protein expression results were consistent with the mRNA RT-qPCR results, which further demonstrated that high

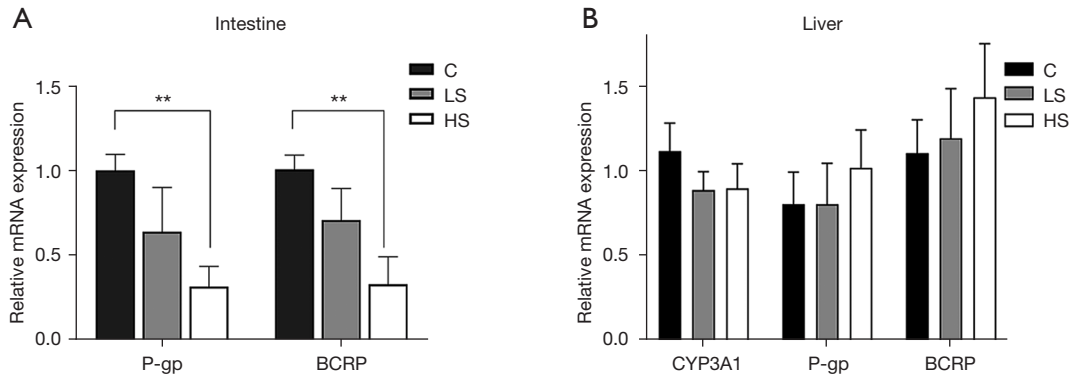

Figure 2 Relative mRNA expression ratio of P-gp and BCRP in the intestines (A) and CYP3A1, P-gp, and BCRP in the liver (B). C: LEN alone; LS: LEN combined with 5.5 mg/kg STA; HS: LEN combined with 20 mg/kg STA (n=3; **P<0.01). mRNA, messenger RNA; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; LEN, lenvatinib; STA, schisantherin A.

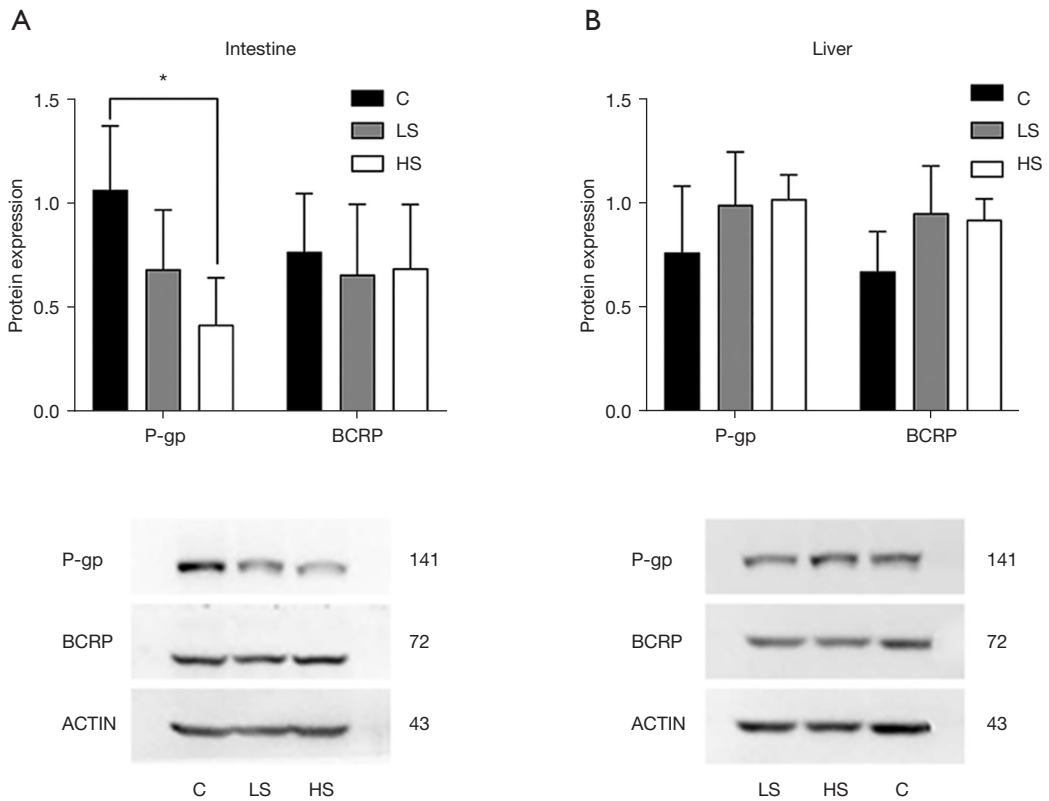

Figure 3 Protein expression ratio of P-gp and BCRP in the intestines (A) and liver (B). C: LEN alone; LS: LEN combined with 5.5 mg/kg STA; HS: LEN combined with 20 mg/kg STA (n=3; *P<0.05). P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; LEN, lenvatinib; STA, schisantherin A.

oral dosage of STA inhibited P-gp protein expression in the intestines (P<0.05) (*Figure 3A*). Although the low dosage of STA also inhibited expression of P-gp in the intestines, there was no statistically significant difference compared to the control group, while there was no effect on the expression of BCRP in the intestine after treatment with

different dosages of STA. Comparably, both dosages of STA had no significant effect on the expression of P-gp and BCRP in the liver after administration of both dosages of STA (*Figure 3B*).

Discussion

As STA is a lignin-like small molecule and the main active ingredient in the *Schisandra* species, a variety of different clinical preparations based on Schisandra that contain STA have been formulated. The ethanol extract herbal preparation of Schisandra sphenanthera, WZC, can be used to reduce serum levels of transaminases and total bilirubin levels as well as to protect liver function (31,32). Wuzhi tablet, an ethanol extract of Schisandra sphenanthera, has also demonstrated excellent hepatoprotective effects (33). Although LEN is a first-line drug for the systemic treatment of HCC, it can elevate transaminase levels in the liver and cause hepatic encephalopathy and other adverse effects (5,13). Some studies have shown that LEN is more likely to cause adverse liver reactions when used for the treatment of HCC, which might have been attributed to the specific pathophysiological changes in the HCC patients (5,9,34), and most patients need hepatoprotective therapy. Therefore, the combination of STA and LEN is very common in clinical practice when administering herbal preparations based on Schisandra. This provided an impetus to explore the effects of different dosages of STA on the rat pharmacokinetics of LEN after oral administration, and the mechanisms were explored at the molecular level by RT-qPCR and western blot. In our previous study, we demonstrated that WZC engendered a significant increase in the AUC and C_{max} of LEN after oral administration, which may increase the efficacy or toxicity of LEN (30). Previous studies have shown that WZC contains a variety of ingredients, and STA is one of the main components of WZC absorbed into the blood (26,29). Therefore, it is necessary to investigate the effect of STA on the pharmacokinetics of LEN.

In this study, the results of the pharmacokinetic analysis indicated that STA increased systemic LEN exposure, resulting in a significant increase in the AUC and C_{max} as well as a significant decrease in the CL and V_z. We selected 2 dosages of STA: the low dosage of STA was 5.5 mg/kg, and the high dosage of STA was 20 mg/kg. Previous study found that 450 mg/kg of WZC significantly increased the systemic exposure of LEN, tacrolimus, and methotrexate. To investigate the contribution of STA on the effect of WZC on the pharmacokinetics of LEN, a dose of 5.5 mg/kg of STA was determined to be administered, which was equivalent to the STA contained in 450 mg/kg of WZC (35). On the other hand, the effect of STA on LEN may be related to dosage, and the blood concentrations of STA were significantly higher in hepatic dysfunction (36). Therefore, it is necessary to investigate the effect of high dose STA on the pharmacokinetics of LEN, and the high dose of STA was determined to be 20 mg/kg with reference to previous studies (36,37). The results showed that the 5.5 mg/kg dosage of STA demonstrated a weaker effect on the pharmacokinetics of LEN compared to WZC at 450 mg/kg. This phenomenon suggested that other components of WZC also had effects on the pharmacokinetics of LEN, and changes in pharmacokinetics of LEN may occur as a result of the combined effect of multiple components, and the underlying mechanisms need to be further explored.

The mechanism of STA-induced changes in the pharmacokinetics of LEN was further explored by measuring the changes in expression levels of mRNA and proteins, such as P-gp and BCRP. As an efflux transport protein that is widely distributed in the intestines, kidneys, liver, and brain, P-gp can affect the pharmacokinetics of drugs, including absorption, distribution, excretion. In the intestines, P-gp inhibits the absorption of the substrate into the blood stream through efflux mechanisms. However, P-gp inhibitors can increase drug concentrations by inhibiting P-gp and, therefore, reducing drug efflux (38-40). Studies have shown that LEN is a substrate of P-gp, and rifampicin may influence the pharmacokinetics of LEN by regulating the activity of P-gp (11,12,41). At the same time, previous studies have reported that STA is a potent inhibitor of P-gp (20,23). Thus, changes in the expression of P-gp mRNA and protein in response to STA were measured by RT-qPCR and western blot. Our results indicated that both P-gp mRNA and protein expression levels in the intestines decreased after oral administration of a high dosage of STA over the course of 7 days. This may be an important reason for the change in LEN pharmacokinetics due to STA. In addition, the changes in mRNA and protein expression levels of BCRP in response to STA administration were investigated because LEN is also a substrate for BCRP. Like P-gp, BCRP is an efflux transporter that mediates the unidirectional transport of drugs across cell membranes (39). However, our findings indicated that the protein expression levels of BCRP were not affected by STA administration. Therefore, we speculated that the main reason for the change in LEN pharmacokinetics and increased exposure may be the inhibition of P-gp in intestine rather than BCRP. Approximately 25% LEN is

excreted by the kidneys (42), and it is uncertain whether STA interferes with the pharmacokinetics of LEN by affecting renal excretion. To date, LEN is a relatively new drug and there are only several studies on drug interactions with LEN due to limited research time. A study showed that a P-gp inhibitor single-dose rifampicin can increase the system exposure of LEN (41); At the same time, ABCB1 (codes P-gp) genetic polymorphism could affect the pharmacokinetics of LEN (11). The above studies indicate that there may be transporter-mediated drug interactions in LEN, which are consistent with our study.

The efficacy and safety of LEN correlate with its plasma drug concentration (43-45). Our results showed that STA caused an increase in blood plasma levels of LEN, which might be deleterious because it could lead to an increase in the number of adverse drug reactions. However, this study had some limitations. The disease state affects drug disposition, and there are species differences not only between humans and rats but also between animals of the same species. In this study, we used normal SD rats, differences exist between individual rats, so the results might not be fully consistent with the human disease state. However, hepatic diseases are considered a major factor affecting the disposition of many drugs, patients with HCC have poor functioning livers and, therefore, a reduced ability to dispose of some drugs, making them more susceptible to drug interactions (46-48). Therefore, adjusting the dosage of LEN when used in combination with herbal preparations containing STA might provide an appropriate reference, but further clinical studies are needed.

Conclusions

In this work, we investigated the effects of oral administration of STA on the rat pharmacokinetics of LEN and detected mRNA and protein expression involved in LEN pharmacokinetics after exposure to STA by using RT- qPCR and western blot analyses. Oral administration of STA altered the pharmacokinetic profile of LEN by increasing the bioavailability of LEN, as demonstrated by a significant increase in the AUC and C_{max}, as well as a significant decrease in the CL, of the drug. Further studies revealed that STA possibly affected LEN exposure by regulating the expression of P-gp. Drug interactions between STA and LEN may have led to increased blood plasma concentrations of LEN, resulting in enhanced efficacy or increased toxicity. Therefore, when co-administered with STA, the dosage of LEN should be closely monitored to avoid possible serious side effects in clinical practice.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-174/rc

Data Sharing Statement: Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-174/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-174/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. 2021131) granted by the Ethics Committee of Hebei General Hospital, in compliance with the Hebei General Hospital guidelines for the care and use of animals.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. McGlynn KA, Petrick JL, El-Serag HB. Epidemiology of Hepatocellular Carcinoma. Hepatology 2021;73

Suppl 1:4-13.
3. Rimassa L, Pressiani T, Merle P. Systemic Treatment Options in Hepatocellular Carcinoma. Liver Cancer 2019;8:427-46.
4. Llovet JM, Kelley RK, Villanueva A, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6.
5. Kudo M, Finn RS, Qin S, et al. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391:1163-73.
6. Zhao Y, Zhang YN, Wang KT, et al. Lenvatinib for hepatocellular carcinoma: From preclinical mechanisms to anti-cancer therapy. Biochim Biophys Acta Rev Cancer 2020;1874:188391.
7. Hao Z, Wang P. Lenvatinib in Management of Solid Tumors. Oncologist 2020;25:e302-10.
8. Motzer R, Alekseev B, Rha SY, et al. Lenvatinib plus Pembrolizumab or Everolimus for Advanced Renal Cell Carcinoma. N Engl J Med 2021;384:1289-300.
9. Schlumberger M, Tahara M, Wirth LJ, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015;372:621-30.
10. Makker V, Rasco D, Vogelzang NJ, et al. Lenvatinib plus pembrolizumab in patients with advanced endometrial cancer: an interim analysis of a multicentre, open-label, single-arm, phase 2 trial. Lancet Oncol 2019;20:711-8.
11. Li J, Wang X, Ning C, et al. Influences of ABC transporter and CYP3A4/5 genetic polymorphisms on the pharmacokinetics of lenvatinib in Chinese healthy subjects. Eur J Clin Pharmacol 2020;76:1125-33.
12. Ozeki T, Nagahama M, Fujita K, et al. Influence of CYP3A4/5 and ABC transporter polymorphisms on lenvatinib plasma trough concentrations in Japanese patients with thyroid cancer. Sci Rep 2019;9:5404.
13. Draper A. A concise review of the changing landscape of hepatocellular carcinoma. Am J Manag Care 2020;26:S211-9.
14. Yang K, Qiu J, Huang Z, et al. A comprehensive review of ethnopharmacology, phytochemistry, pharmacology, and pharmacokinetics of Schisandra chinensis (Turcz.) Baill. and Schisandra sphenanthera Rehd. et Wils. J Ethnopharmacol 2022;284:114759.
15. Wang H, Che J, Cui K, et al. Schisantherin A ameliorates liver fibrosis through TGF-β1mediated activation of TAK1/MAPK and NF-κB pathways in vitro and in vivo. Phytomedicine 2021;88:153609.
16. Fan S, Liu C, Jiang Y, et al. Lignans from Schisandra sphenanthera protect against lithocholic acid-induced cholestasis by pregnane X receptor activation in mice. J Ethnopharmacol 2019;245:112103.
17. Jiang Y, Fan X, Wang Y, et al. Hepato-protective effects of six schisandra lignans on acetaminophen-induced liver injury are partially associated with the inhibition of CYP-mediated bioactivation. Chem Biol Interact 2015;231:83-9.
18. Zhai J, Zhang F, Gao S, et al. Schisandra chinensis extract decreases chloroacetaldehyde production in rats and attenuates cyclophosphamide toxicity in liver, kidney and brain. J Ethnopharmacol 2018;210:223-31.
19. Wang Z, Yu K, Hu Y, et al. Schisantherin A induces cell apoptosis through ROS/JNK signaling pathway in human gastric cancer cells. Biochem Pharmacol 2020;173:113673.
20. Pan Q, Lu Q, Zhang K, et al. Dibenzocyclooctadiene lingnans: a class of novel inhibitors of P-glycoprotein. Cancer Chemother Pharmacol 2006;58:99-106.
21. Zhao J, Sun T, Wu JJ, et al. Inhibition of human CYP3A4 and CYP3A5 enzymes by gomisin C and gomisin G, two lignan analogs derived from Schisandra chinensis. Fitoterapia 2017;119:26-31.
22. He Q, Bu F, Zhang H, et al. Investigation of the Impact of CYP3A5 Polymorphism on Drug-Drug Interaction between Tacrolimus and Schisantherin A/Schisandrin A Based on Physiologically-Based Pharmacokinetic Modeling. Pharmaceuticals (Basel) 2021;14:198.
23. Adiwidjaja J, Boddy AV, McLachlan AJ. Potential for pharmacokinetic interactions between Schisandra sphenanthera and bosutinib, but not imatinib: in vitro metabolism study combined with a physiologically-based pharmacokinetic modelling approach. Br J Clin Pharmacol 2020;86:2080-94.
24. Fan J, Chen L, Lu X, et al. The Pharmacokinetic Prediction of Cyclosporin A after Coadministration with Wuzhi Capsule. AAPS PharmSciTech 2019;20:247.
25. Chen L, Ji N, Zhang M, et al. The Influence of Wuzhi Capsule on the Pharmacokinetics of Cyclophosphamide. Recent Pat Anticancer Drug Discov 2021. [Epub ahead of print]. doi: 10.2174/1574892816666211110152119.
26. Wei H, Tao X, Di P, et al. Effects of traditional chinese medicine Wuzhi capsule on pharmacokinetics of tacrolimus in rats. Drug Metab Dispos 2013;41:1398-403.
27. Xue XP, Qin XL, Xu C, et al. Effect of Wuzhi tablet (Schisandra sphenanthera extract) on the pharmacokinetics of cyclosporin A in rats. Phytother Res 2013;27:1255-9.
28. Jin J, Bi H, Hu J, et al. Effect of Wuzhi tablet (Schisandra sphenanthera extract) on the pharmacokinetics of paclitaxel in rats. Phytother Res 2011;25:1250-3.

29. Wei H, Xu W, Cai F, et al. Simultaneous determination of five lignan constituents of Wuzhi capsule in rat plasma by LC-MS/MS: application to pharmacokinetic study. J Pharm Biomed Anal 2010;52:741-6.
30. Cui Y, Li Y, Fan L, et al. UPLC-MS/MS method for the determination of Lenvatinib in rat plasma and its application to drug-drug interaction studies. J Pharm Biomed Anal 2021;206:114360.
31. Kang D, Shao Y, Zhu Z, et al. Systematically identifying the hepatoprotective ingredients of schisandra lignan extract from pharmacokinetic and pharmacodynamic perspectives. Phytomedicine 2019;53:182-92.
32. Zhu P, Li J, Fu X, et al. Schisandra fruits for the management of drug-induced liver injury in China: A review. Phytomedicine 2019;59:152760.
33. Zeng X, Li X, Xu C, et al. Schisandra sphenanthera extract (Wuzhi Tablet) protects against chronic-binge and acute alcohol-induced liver injury by regulating the NRF2-ARE pathway in mice. Acta Pharm Sin B 2017;7:583-92.
34. Ikeda M, Okusaka T, Mitsunaga S, et al. Safety and Pharmacokinetics of Lenvatinib in Patients with Advanced Hepatocellular Carcinoma. Clin Cancer Res 2016;22:1385-94.
35. Chen L, Xiong X, Hou X, et al. Wuzhi capsule regulates chloroacetaldehyde pharmacokinetics behaviour and alleviates high-dose cyclophosphamide-induced nephrotoxicity and neurotoxicity in rats. Basic Clin Pharmacol Toxicol 2019;125:142-51.
36. Wu R, Xiao Z, Zhang X, et al. The Cytochrome P450-Mediated Metabolism Alternation of Four Effective Lignans From Schisandra chinensis in Carbon Tetrachloride-Intoxicated Rats and Patients With Advanced Hepatocellular Carcinoma. Front Pharmacol 2018;9:229.
37. Wang B, Yang S, Hu J, et al. Multifaceted interaction of the traditional Chinese medicinal herb Schisandra chinensis with cytochrome P450-mediated drug metabolism in rats. J Ethnopharmacol 2014;155:1473-82.
38. Nguyen TT, Duong VA, Maeng HJ. Pharmaceutical Formulations with P-Glycoprotein Inhibitory Effect as Promising Approaches for Enhancing Oral Drug Absorption and Bioavailability. Pharmaceutics 2021;13:1103.
39. Estudante M, Morais JG, Soveral G, et al. Intestinal drug transporters: an overview. Adv Drug Deliv Rev 2013;65:1340-56.
40. Lund M, Petersen TS, Dalhoff KP. Clinical Implications of P-Glycoprotein Modulation in Drug-Drug Interactions. Drugs 2017;77:859-83.
41. Shumaker RC, Aluri J, Fan J, et al. Effect of rifampicin on the pharmacokinetics of lenvatinib in healthy adults. Clin Drug Investig 2014;34:651-9.
42. Dubbelman AC, Rosing H, Nijenhuis C, et al. Pharmacokinetics and excretion of (14)C-lenvatinib in patients with advanced solid tumors or lymphomas. Invest New Drugs 2015;33:233-40.
43. Gupta A, Jarzab B, Capdevila J, et al. Population pharmacokinetic analysis of lenvatinib in healthy subjects and patients with cancer. Br J Clin Pharmacol 2016;81:1124-33.
44. Noda S, Iida H, Fujimoto T, et al. Exploratory analysis of target concentration of lenvatinib in the treatment of hepatocellular carcinoma. Cancer Chemother Pharmacol 2021;88:281-8.
45. Nagahama M, Ozeki T, Suzuki A, et al. Association of lenvatinib trough plasma concentrations with lenvatinib-induced toxicities in Japanese patients with thyroid cancer. Med Oncol 2019;36:39.
46. Drożdzik M, Oswald S, Drożdzik A. Extrahepatic Drug Transporters in Liver Failure: Focus on Kidney and Gastrointestinal Tract. Int J Mol Sci 2020;21:5737.
47. Drozdzik M, Czekawy I, Oswald S, et al. Intestinal drug transporters in pathological states: an overview. Pharmacol Rep 2020;72:1173-94.
48. Shumaker R, Aluri J, Fan J, et al. Influence of hepatic impairment on lenvatinib pharmacokinetics following single-dose oral administration. J Clin Pharmacol 2015;55:317-27.

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