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The Effects of Different Administration Regimens of Berberine Hydrochloride on the Pharmacokinetics of Sirolimus in Rats

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ABSTRACT

Sirolimus is a third-generation immunosuppressant with multiple efficacies, including antifungal, antitumor, and immunosuppressive properties. We conducted a pharmacokinetic study to determine the effect of berberine hydrochloride (BBR) on the pharmacokinetics of sirolimus in rats. Thirty-six rats were randomly divided into six groups, and blood samples were collected from the posterior orbital venous plexus at different time points after the last administration to determine the plasma concentration of sirolimus. Liquid chromatography tandem mass spectrometry (LC–MS/MS) was used for detection, and DAS 2.0 software was employed for pharmacokinetic analysis. A single administration of BBR significantly reduced the pharmacokinetic parameter $AUC_{0\text{-}12h}$ of sirolimus. Repeated administration of BBR resulted in an upward trend in C_{max} $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, and CLz all show an upward trend. This study indicates that BBR has a certain impact on the pharmacokinetics of sirolimus in rats. However, further validation of the role of gene polymorphism in pharmacokinetics and clinical efficacy is needed for further research in a wider population.

1 | Introduction

Sirolimus, also known as rapamycin, is a third-generation immunosuppressant with multiple effects such as antifungal, antitumor, and immunosuppressive. As a dual substrate of CYP3A4 and P-gp, sirolimus has shown wide application value in organ transplantation therapy [1, 2]. However, it is prone to complex interactions with multiple drugs [3]. Berberine hydrochloride (BBR) has an effect on CYP3A and P-gp [4, 5].

Berberine can directly inhibit the enzymatic activity of CYP3A4 and may reduce its expression by regulating its transcriptional level. Studies have shown that berberine reduces enzymatic activity by inhibiting the mRNA and protein expression of CYP3A4 [6]. Furthermore, the transcription of CYP3A4 is regulated by nuclear receptors such as PXR and CAR, and berberine may further inhibit the expression of CYP3A4 by interfering with the signaling pathways of these nuclear receptors [7]. Berberine may also cause enzyme inactivation by covalently binding to or irreversibly modifying the active site of CYP3A4 [8]. This inhibitory effect is time-dependent and may prolong the metabolism of other CYP3A4 substrate drugs [8]. Additionally, berberine is an inhibitor of P-glycoprotein (Pgp) and can increase the blood concentrations of other Pgp substrate drugs (such as atorvastatin) by competitively binding to the substrate binding site of Pgp [9]. Long-term use of berberine may indirectly affect Pgp expression levels by modulating nuclear receptors such as PXR, although current mechanistic studies are limited [10].

The combination of berberine with atorvastatin or simvastatin significantly inhibits CYP3A4 activity, leading to increased blood concentrations of statin drugs and an elevated risk of rhabdomyolysis and cardiotoxicity (such as hERG channel inhibition) [11]. When berberine is used concomitantly with calcium channel blockers like verapamil, it may result in increased blood drug concentrations and bradycardia [12]. The co-administration of berberine with immunosuppressants (e.g., cyclosporine) may also increase toxicity risks [13]. The combination of berberine with antineoplastic agents such as paclitaxel and doxorubicin may enhance the efficacy of these antitumor drugs due to Pgp inhibition, but it may also augment toxicity risks, including myelosuppression [9]. For drugs that rely on both CYP3A4 metabolism and Pgp efflux (e.g., digoxin and felodipine), berberine can significantly increase their systemic exposure through dual inhibition, necessitating caution against potential toxic reactions [14].

Therefore, when sirolimus is used in combination with BBR, the possibility of drug interaction increases significantly, which merits further investigation. Hence, this study selected rats as experimental subjects and grouped them during the administration process to receive BBR and sirolimus via gavage, aiming to further explore the impact of BBR on the pharmacokinetics of sirolimus.

2 | Material and Methods

2.1 | Medicinal Materials and Reagents

Sirolimus tablets (1 mg/tablet, Pfizer); berberine hydrochloride tablets (0.1 g/tablet, Northeast Pharmaceutical Group); methanol, formic acid, acetonitrile (Shanghai Aladdin Biochemical); ultrapure water (Nanjing Yipu Yida, EPED-E1-10TJ).

2.2 | Main Instruments

D3024R high-speed micro-refrigerated centrifuge: Beijing Dalong Xingchuang Experimental Instrument Co. Ltd.; pipettes (2.0–20.0, 20.0–200, and 200–1000 μL, Eppendorf); TSQ Quantum triple quadrupole mass spectrometer: Thermo Fisher Scientific, USA; UltiMate 3000 RS chromatograph: Thermo Fisher Scientific, USA.

2.3 | Animal and Treatment

A total of 36 healthy SD male rats were used in this study and randomly divided into 6 groups ($n=6$). The dosing schedule of each group was as follows: rats in group A were given sirolimus (dissolved in distilled water, 4.17 mg/kg/day; the dose was selected based on human clinical dosage.) only once by gavage; rats in group B were given sirolimus once a day for 7 consecutive days; rats in group C were given BBR (dissolved in distilled water, 200 mg/kg/day) only once by gavage and sirolimus was given once by gavage at 5 min after BBR dosing [15]; rats in group D were given BBR 3 times a day for 7 consecutive days, and sirolimus was given once at 5 min after BBR dosing on the eighth day; rats in group E were given sirolimus once a day for 7 consecutive days, and sirolimus was given once at 5 min after BBR dosing on the eighth day; and rats in group F were given sirolimus and BBR for seven consecutive days (sirolimus was given once a day, BBR was given 3 times a day; sirolimus was given at 5 min after BBR dosing), and BBR was given once again by gavage on the eighth day, and sirolimus was given once at 5 min after BBR dosing. All rats were fasted with free access to water for 12 h before the experiment.

2.4 | Sample Collection

About 0.3 mL of blood was drawn from the retro-orbital venous plexus of rats. Whole blood samples were collected at different time points after the last administration of sirolimus, including 0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 12 h, and stored in anticoagulant tubes and placed in a −80°C refrigerator for analysis.

2.5 | Whole Blood Sample Processing

Accurately measure 100 μL of the sample, add 300 μL of methanol, vortex continuously for 5 min, centrifuge at 13 000 rpm/min for 10 min at 4°C, and extract the supernatant for instrumental analysis.

2.6 | Mass Spectrometry Conditions

Ion source: electrospray ionization source (ESI); Scan mode: positive ion scan; Detection mode: selected reaction monitoring (SRM) Electrospray voltage (Spary Voltage): 4000 V (Positive); Capillary temperature (Capillary Temperature): 350°C; Collision gas: high-purity argon (purity ≥99.999%); Sheath gas: nitrogen (purity ≥99.999%), 650Arb Auxiliary gas (Aux Gus Pressure): nitrogen (purity ≥99.999%), 20Arb; Data acquisition time: 5.0 min.

2.7 | Chromatographic Conditions

Chromatographic column: Welch Ultimate XB-C8 150 × 4.6 mm, 5 μm; Flow rate: 1.0 mL/min; aqueous phase: 0.1% formic acid water; organic phase: methanol; needle wash solution: methanol; column oven temperature: 40°C; autosampler temperature: 8.0°C; injection needle height: 2.00 mm; autosampler cleaning setting: AfterDraw; autosampler needle wash volume: 200.0 μL; autosampler injection volume: 5.00 μL.

2.8 | Methodological Validation

2.8.1 | Specificity Investigation

According to the whole blood sample operation method, 100 μL of blank whole blood and 100 μL of whole blood containing sirolimus control substance were processed separately. 5 μL was taken from each sample for chromatographic analysis, and the corresponding chromatogram was saved.

2.8.2 | Standard Curve and Quantitative Range

Accurately weigh a certain amount of sirolimus standard, marked as R01, and use pure methanol to prepare a stock solution with

a concentration of 2.00 mg/mL for standby use. Then, using pure methanol as a solvent, R01 was diluted to different concentrations: 50000, 20000, 5000, 2000, 500, 200, 500, 200, and 50 ng/mL for the construction of the standard curve working solution. Subsequently, these solutions were diluted tenfold using blank plasma matrix and processed according to the operating steps of "2.5" for instrumental analysis. The chromatogram acquisition and integration of the compounds were processed by the software Xcalibur 3.0 (Thermo), and linear regression was performed by the 1/×2 weighting coefficient. Studies have shown that rapamycin has a good linear relationship in the range of 2–5000 ng/mL, with $r^2 > 0.99$. The results show that the standard curve meets the detection standards for biological sample analysis.

2.8.3 | Accuracy and Precision Investigation

A certain amount of sirolimus standard stock solution was extracted and mixed with blank whole blood of rats to prepare sirolimus standard whole blood samples with mass concentrations of 20, 200, and 2000 ng/mL, respectively. After processing according to the operating steps of "2.5," the test was carried out according to the test conditions under "2.6." Each sample concentration was tested 5 times on the same day, and the coefficient of variation (CV) was used to express the statistical intra-day precision and accuracy; the sample was injected once a day, and 5 samples were tested and analyzed for each mass concentration for 3 days. The inter-day precision and accuracy were recorded. The accuracy was obtained by calculating the ratio of the measured value to the actual value and multiplying it by 100%, and it was required to be between 85% and 115%.

2.8.4 | Extraction Recovery and Matrix Effect

An appropriate amount of sirolimus standard stock solution was extracted and mixed with blank whole blood of rats in proportion to prepare three groups of sirolimus quality control samples, with mass concentrations of 20, 200, and 2000 ng/mL, respectively, and 5 samples in each group. After the samples were processed according to the operating steps under "2.2," the sirolimus peak area and internal standard peak area were tested according to the detection conditions under "2.3" and recorded separately. Take another 15 polyethylene centrifuge tubes, add 200 μL blank whole blood to each tube, and do not add sirolimus standard stock solution and internal standard solution for the time being. After processing according to the operating steps under "2.5," add sirolimus quality control sample solution and internal standard solution containing 20, 200, and 2000 ng/mL, respectively, and test according to the detection conditions under "2.7." The recorded sirolimus peak area and internal standard peak area are used to calculate the extraction recovery and internal standard normalized matrix effect.

2.9 | Stability Study

Three different concentrations of quality control samples of sirolimus were prepared, with concentrations of 20, 200, and 2000 ng/mL, respectively. After the samples were processed according to the operating steps of item "2.5," they were stored at room temperature for 4 and 12 h, and frozen at −80°C for 3 and 7 days, respectively, and then tested according to the test conditions under item "2.7." Five samples were operated in parallel for each concentration to observe the stability of sirolimus after sample processing.

2.10 | Pharmacokinetic Study

The sirolimus blood concentrations at each time point after administration in the six groups of rats were detected by LC–MS/MS, and the sirolimus blood concentration-time curve was drawn using DAS 2.0 software. The pharmacokinetic parameters such as the apparent elimination half-life (T1/2), peak concentration (C_{max}), clearance (CL), mean residence time (MRT), area under the plasma concentration-time curve (AUC) and time to reach maximum concentration (Tmax) were also statistically analyzed.

2.11 | Statistical Analysis

SPSS22.0 software was used for statistical analysis. The quantitative data were expressed as mean ± standard deviation (SD). The independent sample t test was used for statistical analysis of the normally distributed data, and the nonparametric rank sum test was used for the analysis of the data with obvious skewed distribution. When $p < 0.05$, the difference was considered statistically significant.

3 | Results

3.1 | Methodological Validation Results

As shown in Figure 1, specificity investigation results showed that the detection of sirolimus was not interfered with by endogenous substances in blank whole blood, and the obtained chromatogram had high specificity and met the standards for detection and analysis.

As shown in Table 1, accuracy and precision investigation showed that the intra-day and inter-day accuracy and precision of the three quality control samples of sirolimus in whole blood (20, 200, and 2000 ng/mL) met the requirements for the detection and analysis of biological samples.

The extraction recovery and matrix effect test results are shown in Table 2. The extraction recovery and matrix effect of sirolimus quality control samples meet the requirements for biological sample detection and analysis.

The results of the stability test were shown in Table 3. Under this storage condition, the stability of sirolimus meets the requirements for biological sample analysis.

3.2 | Pharmacokinetic Study Results

The drug-time curve was shown in Figure 2A,B, and the pharmacokinetic parameter results were shown in Tables 4 and 5. As

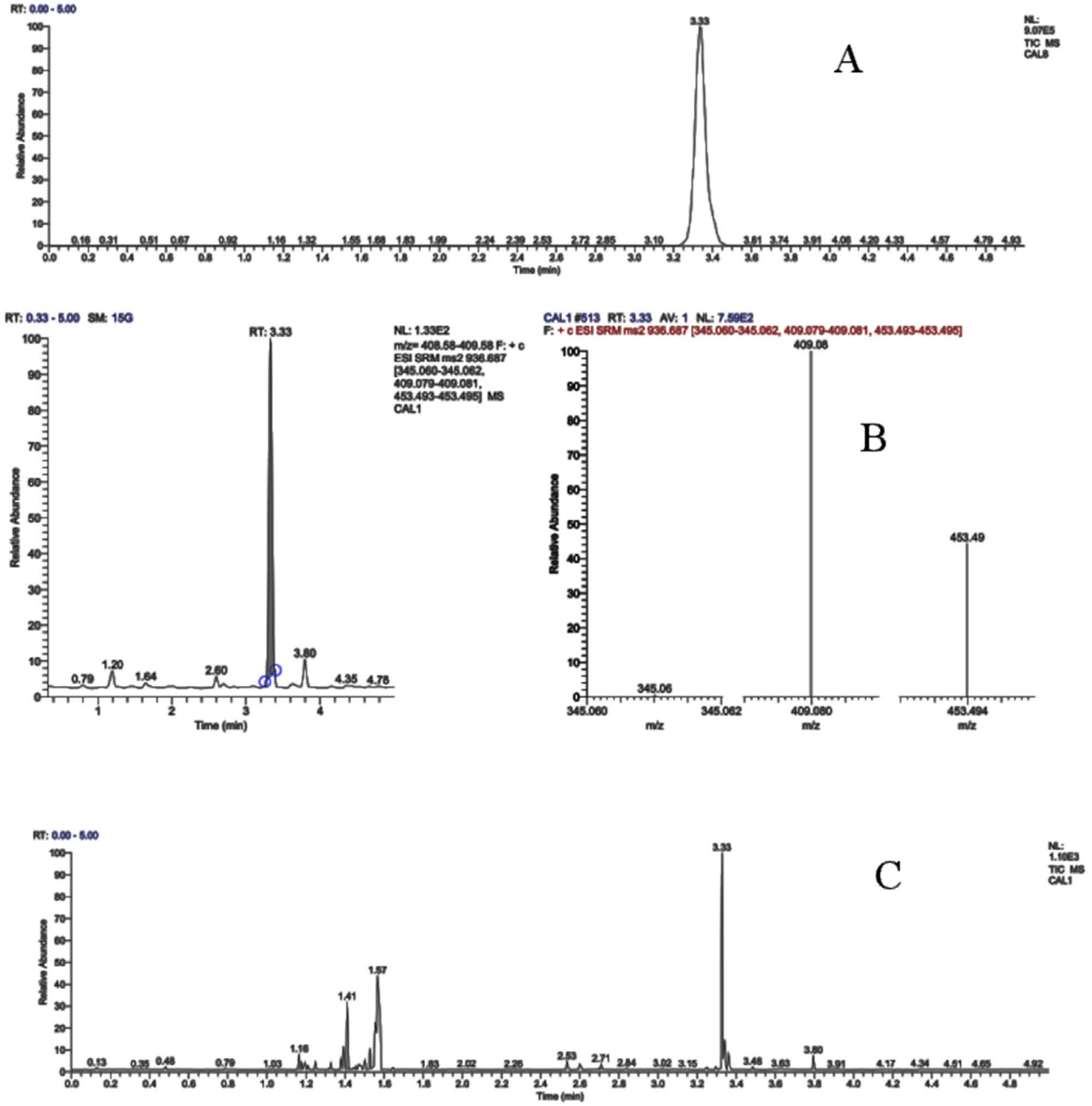

FIGURE 1 | Representative chromatograms of sirolimus. (A) Total ion flow chart with lower limit of quantification (2 ng/mL). (B) Ion flow diagram of rapamycin extraction in the lower limit of quantification (2 ng/mL). (C) Quantitative upper limit (5000 ng/mL) total ion flow chart.

TABLE 1 | Results of precision and accuracy tests ($n = 5$).

	Intraday			Daytime		
Sample mass concentration (ng/mL)	**Measured mass concentration (ng/mL)**	**Accuracy (%)**	**CV (%)**	**Measured mass concentration (ng/mL)**	**Accuracy (%)**	**CV (%)**
20	20.07 ± 0.47	100.35	2.34	19.80 ± 0.57	99.0	2.87
200	199.44 ± 1.11	99.72	0.55	199.39 ± 1.30	99.69	0.65
2000	1998.01 ± 4.92	99.90	0.24	1999.04 ± 2.61	99.95	0.13

shown in Table 4, compared with group C, $AUC_{0\text{-}12h}$ in group D decreased significantly ($p < 0.05$). Compared with group C and group D, there were no significant differences in the pharmacokinetic parameters of sirolimus in group A ($p > 0.05$); as shown in Table 5, compared with group B, C_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, and CLz in group E increased significantly ($p < 0.05$);

C_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, and CLz in group F increased significantly ($p < 0.05$), and there were no significant differences in the pharmacokinetic parameters of sirolimus between group E and group F ($p > 0.05$). The above results show that when BBR is administered once, the pharmacokinetic parameter AUC of sirolimus decreases; while when BBR is administered multiple times, the pharmacokinetic parameters C_{max}, $AUC_{0\text{-}12h}$, $AUC_{0\text{-}\infty}$, and CL of sirolimus increase.

TABLE 2 | Results of extract recovery rate and matrix effect (%, $n = 5$).

Sample mass concentration (ng/mL)	Extraction recovery		Matrix effects	
	Mean ± SD	CV	Mean ± SD	CV
20	88.41 ± 1.45	2.21	86.71 ± 2.54	2.69
200	89.32 ± 2.85	4.51	87.65 ± 3.34	4.24
2000	93.40 ± 2.53	1.46	92.14 ± 2.27	1.81

4 | Discussion

The rapid rise of pharmacogenomics has attracted further attention from researchers on the molecular genetic mechanisms of differences in pharmacokinetic and pharmacodynamic kinetics between different patients [16]. The development and research of pharmacogenomics have demonstrated the importance of the rational use of clinical drugs in guiding clinicians to shift from empirical medication to personalized medication. At present, pharmacogenomics research focuses more on the cytochrome P450 enzyme system [17, 18]. Cytochrome P450 is an oxidase present in the endoplasmic reticulum, mitochondria, and microsomes of hepatocytes. It mainly oxidizes and modifies drugs and other metabolites in the body [19–21]. At the same time, in order to avoid the shortcomings of insufficient efficacy of single medication and the susceptibility to drug resistance, the current clinical practice is increasingly inclined to adopt combination therapy. Combination therapy can not only exert the targeting properties of each drug, but also exert a synergistic effect, thereby reducing the dependence of drugs on dosage and avoiding the development of drug resistance to a certain extent [22].

TABLE 3 | Results of stability tests ($n = 5$).

Storage conditions	Sample mass concentration (ng/mL)	Measured mass concentration (ng/mL)	Deviation (%)
Room temperature 4 h	20	19.72 ± 0.70	−1.40
	200	197.97 ± 1.47	−1.01
	2000	1998.92 ± 1.91	−0.05
Room temperature 12 h	20	19.61 ± 1.02	−1.95
	200	196.53 ± 1.65	−1.73
	2000	1999.09 ± 1.61	−0.04
Store at −80°C; 3 days	20	19.70 ± 0.86	−4.92
	200	198.72 ± 1.88	−0.06
	2000	1989.32 ± 1.08	−0.05
Store at −80°C; 7 days	20	19.62 ± 0.62	−4.90
	200	199.46 ± 1.67	−1.90
	2000	1997.68 ± 2.11	−0.12

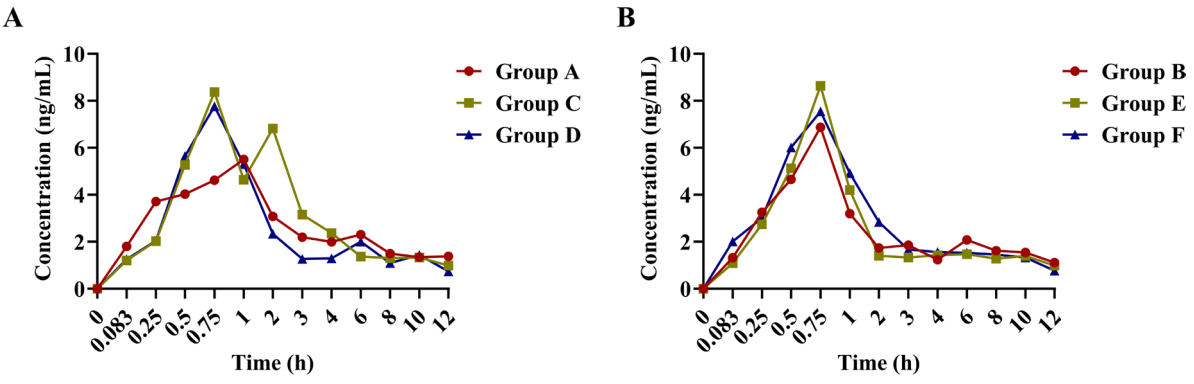

FIGURE 2 | The concentration-time curves of sirolimus. (A) Group A, Group C, and Group D. (B) Group B, Group E, and Group F.

TABLE 4 | Pharmacokinetic parameters of sirolimus after a single gavage of berberine in rats ($n = 6$).

Parameters	Group A	Group C	Group D	P_1	P_2	P_3
t_{max} (h)	1.333 ± 0.516	1.958 ± 0.954	1.083 ± 0.465	0.136	0.275	0.039
C_{max} (ng/mL)	5.724 ± 0.601	6.343 ± 1.026	6.109 ± 1.032	0.318	0.553	0.247
$t_{1/2}$ (h)	6.971 ± 3.445	4.461 ± 1.394	6.288 ± 2.712	0.091	0.701	0.100
$AUC_{0\text{-}12h}$ (h.ng/mL)	31.225 ± 6.001	35.364 ± 3.345	30.192 ± 6.163	0.071	0.393	0.015
$AUC_{0\text{-}\infty}$ (h.ng/mL)	42.006 ± 6.565	42.739 ± 3.084	39.918 ± 9.631	0.808	0.663	0.421
CL_z (L/(h.g))	101.491 ± 17.217	97.988 ± 6.985	109.689 ± 26.487	0.651	0.552	0.262
$MRT_{0\text{-}12h}$ (h)	4.46 ± 0.214	4.58 ± 0.348	4.626 ± 0.32	0.529	0.264	0.681
$MRT_{0\text{-}\infty}$ (h)	9.73 ± 4.26	6.603 ± 1.256	8.876 ± 2.427	0.072	0.685	0.125

Note: P_1 is the statistical value for comparing groups A and C; P_2 is the statistical value for comparing groups A and D; P_3 is the statistical value for comparing groups C and D.

TABLE 5 | Pharmacokinetic parameters of sirolimus after multiple gavages of berberine in rats ($n = 6$).

Parameters	Group B	Group E	Group F	P_1	P_2	P_3
t_{max} (h)	1.083 ± 0.465	0.75 ± 0	0.75 ± 0	0.140	0.140	—
C_{max} (ng/mL)	5.552 ± 0.714	7.864 ± 0.526	7.879 ± 0.285	0.00	0.001	0.963
$t_{1/2}$ (h)	6.038 ± 2.114	5.923 ± 2.711	8.689 ± 5.509	0.916	0.305	0.179
$AUC_{0\text{-}12h}$ (h.ng/mL)	27.126 ± 4.659	37.469 ± 9.171	37.193 ± 8.555	0.013	0.005	0.842
$AUC_{0\text{-}\infty}$ (h.ng/mL)	36.603 ± 9.498	49.262 ± 11.068	55.888 ± 11.487	0.002	0.011	0.324
CL_z, (L/(h.g))	119.687 ± 27.371.	88.070 ± 18.760	77.926 ± 19.725	0.011	0.005	0.368
$MRT_{0\text{-}12h}$ (h)	4.526 ± 0.344	4.693 ± 0.061	4.488 ± 0.251	0.253	0.838	0.074
$MRT_{0\text{-}\infty}$ (h)	8.083 ± 2.062	8.034 ± 3.032	9.524 ± 4.094	0.969	0.253	0.245

Note: P_1 is the statistical value compared to groups B and E; P_2 is the statistical value for comparing groups B and F; P_3 is the statistical value for comparing groups E and F; "-" is 0.

This study observed opposing trends in the effects of single and repeated administrations of berberine hydrochloride on the pharmacokinetic parameters of sirolimus, suggesting a possible time-dependent mechanism of action. The significant reduction in $AUC_{0\text{-}12h}$ after a single administration may be related to the short-term induction of CYP3A4 or P-glycoprotein (P-gp) activity by BBR [3, 23]. However, the increase in parameters such as C_{max} and AUC after repeated administrations could reflect the dominant inhibitory effect of berberine on CYP3A4/P-gp upon long-term exposure.

According to Cui et al.'s research, berberine hydrochloride has an inductive effect on the metabolism of lovastatin, possibly by upregulating CYP3A activity to accelerate drug clearance [24]. If a similar mechanism applies to sirolimus, it could lead to a decrease in its AUC and C_{max}, and an increase in clearance rate (CLz). However, Wang et al. found that the oral clearance rate of berberine hydrochloride was significantly reduced in diabetic model rats, suggesting that it may prolong its own exposure time by inhibiting metabolic enzymes such as CYP3A or P-glycoprotein [25]. If sirolimus is also regulated by such enzymes, the combination of the two drugs may increase the blood concentration and toxicity risk of sirolimus.

Furthermore, Deng et al. showed that in rats with depleted gut microbiota, the AUC of a certain drug increased by 1.3 times, and CLz/F decreased by 0.6 times, suggesting that the microbiota may affect drug exposure through metabolism or first-pass effect [26]. If the absorption or metabolism of sirolimus also depends on microbiota activity, berberine hydrochloride may indirectly interfere with its pharmacokinetics by altering the composition of the microbiota [27]. The formulation of berberine hydrochloride may also affect its pharmacokinetic properties. Li et al. compared the pharmacokinetic differences between pure berberine and compound preparations, finding that the compound preparations significantly increased the AUC by improving intestinal absorption [28]. If the absorption of sirolimus is also affected by the formulation, attention should be paid to the influence of formulation selection on the interaction during combination therapy.

In clinical practice, the current sirolimus dosage is different for patients with different gene expressions, different organ transplants, and different age stages [29, 30]. In view of this situation, this study believes that by adjusting the different dosages of sirolimus and BBR, the interaction between the two can be further explored.

There are also some limitations in this study. This study is only conducted in rats. The number of experimental rats is small and the results obtained had certain limitations. The impact of gene polymorphism on pharmacokinetics and clinical efficacy needs to be further verified on a larger group in the future.

5 | Conclusion

BBR has a certain impact on the pharmacokinetics of sirolimus in rats. However, further validation of the role of gene polymorphism in pharmacokinetics and clinical efficacy is needed for further research in a wider population.

Author Contributions

Yuanxia Yang designed the experiments. Yue Zhang and Yingdong Qu performed the experiments. Yingdong Qu and Liwan Cui collected and analyzed the data. Yuanxia Yang and Liwan Cui drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors have nothing to report.

Ethics Statement

All animal experiments were in line with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was permitted by the Committee on the Ethics of Animal Experiments of Shenzhen Longhua District People's Hospital.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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