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Pharmacokinetic interaction of *Forsythia suspensa* extract and azithromycin injection after single and co-intravenous administration in rats

LI Xin-Gang[1], NI Jian[2], SHEN Su[1], WANG Xiao-Ping[3*], TIAN Jing-Chen[4*]

[1] *Department of Pharmacy, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China;*

[2] *School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China;*

[3] *Department of Pharmacy, Shaanxi Provincial Hospital of Traditional Chinese Medicine, Xian 710003, China;*

[4] *Department of Pharmacy, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China*

[ABSTRACT] Azithromycin and Chinese medicine forsythia are often used together to treat pediatric mycoplasma infections in China. We aimed to investigate the pharmacokinetic interaction of *Forsythia suspensa* extract and azithromycin after single and co-intravenous administration in rats. Male Sprague-Dawley rats received single (*Forsythia suspensa* extract or azithromycin) treatment or co-administration of *Forsythia suspensa* extract and azithromycin. Blood samples were collected at scheduled times, and drug concentrations were determined by HPLC-UV or HPLC-MS/MS methods. Both non-compartmental analyses and nonlinear mixed-effects modeling approaches were applied to fit pharmacokinetic data and evaluate the impact of co-administration. Pharmacokinetic analysis showed that the area under the curve of azithromycin and forsythiaside increased, and clearance decreased significantly ($P < 0.05$), after co-administration. The *in vivo* behavior of both azithromycin and forsythiaside could be appropriately described by the two-compartmental model. The final population pharmacokinetic model indicated that co-administration decreased the central volume of azithromycin and forsythiaside clearance significantly. Co-administration of *Forsythia suspensa* extract and azithromycin significantly decreased the clearance and increased exposure for both drugs. Pharmacokinetic data suggest that drug co-administration may increase efficiency.

[KEY WORDS] *Forsythia suspensa*; Azithromycin; Forsythiaside; Co-administration; Non-compartmental analysis; Population pharmacokinetics; Pharmacokinetic interaction

Introduction

The fruit of *Forsythia suspensa* is an important traditional Chinese medicine documented in Chinese Pharmacopeia as an anti-inflammatory, antidotal, and antipyretic agent[1-2]. Many Chinese medicinal preparations containing *Forsythia suspensa* are used clinically, such as *Shuanghuanglian* (SHL) oral solution, *Yinqiao Jiedu* tablet, and *Qinlian* tablet[3-4]. Azithromycin is a macrolide antibacterial drug, and pharmacokinetics analysis in human serum and tissues found that the tissue concentrations of azithromycin were much higher than serum concentrations[5-7]. Therefore, azithromycin can be used for the treatment of several bacterial infections, including middle ear infections, strep throat, pneumonia, traveler's diarrhea, intestinal infections, and sexually transmitted infections.

SHL injection, a Chinese medicine intravenous preparation extracted from honeysuckle, *Scutellaria baicalensis*, and *Fructus forsythia*, has been approved for the treatment of acute respiratory tract infections since 1973 in China[8]. Forsythiaside from *forsythia suspensa* fruit is the primary active compound for infections in the formulation[9-10]. In China, the combination of Chinese and Western medicine is common[11-13], and many hospitals have the Department of Integrated Traditional Chinese and Western Medicine. Azithromycin injection and SHL were often used together to treat pediatric mycoplasma infections in China[12-13]. Compared with azithromycin monotherapy, the combined medicine has certain clinical effects in treating pediatric mycoplasma pneumonia, improving the immunologic function, and it is safe to

use[12].

Understanding the reason why combined medicine is better than a single drug treatment is necessary and essential[14]. This study aimed to investigate the pharmacokinetic interaction of *Forsythia suspensa* extract and azithromycin after single and co-intravenous administration in rats using nonlinear mixed-effects methods[15].

Results

Non-compartment analysis

A total of 24 rats with 204 drug concentrations were collected for the pharmacokinetic analysis. The area under the

curve (*AUC*), clearance (*CL*), and terminal half-life ($t_{1/2}$) were calculated using the non-compartment analysis (NCA) method and are listed in Table 1. All pharmacokinetic parameters are presented as means ± standard deviation (SD). Compared with single administration, AUC increased significantly (forsythiaside $P = 0.001$, azithromycin $P = 0.019$), and CL decreased (forsythiaside $P = 0.003$, azithromycin $P = 0.005$) after coadministration. These results indicated that co-administration could increase forsythiaside and azithromycin exposures through reducing drug elimination. Regarding $t_{1/2}$, there were no obvious differences between the single and co-administration groups ($P > 0.05$).

Table 1 Pharmacokinetic parameters of azithromycin and forsythiaside obtained from noncompartmental analysis (mean ± SD, *n* = 6)

	$AUC/(\mu g \cdot mL^{-1} \cdot h^{-1})$	$CL/(mL \cdot h^{-1})$	$t_{1/2}/(h)$
	Azithromycin		
Single	23.21 ± 4.95	525.88 ± 105.09	6.79 ± 3.14
Coadministration	37.91 ± 10.79	290.52 ± 116.87	12.14 ± 5.55
Significance (*P*-value)	0.019*	0.005*	0.074
	Forsythiaside		
Single	6.51 ± 0.90	515.62 ± 78.05	0.47 ± 0.09
Coadministration	9.29 ± 1.21	355.65 ± 49.23	0.54 ± 0.03
Significance (*P*-value)	0.001*	0.003*	0.150

AUC: area under the plasma concentration-time curve from time zero to time of last concentration area; *CL*: clearance; $t_{1/2}$: terminal half-life; *P < 0.05

Population pharmacokinetic analysis

Compared with one- and three-compartment models, the two-compartment model could best describe the azithromycin and forsythiaside pharmacokinetic data[14]. For azithromycin, co-administration markedly decreased the central volume (V_{CA}), and the final model is described as follow:

Single administration

$$V_{CAi}(\text{mL}) = 503.2 \cdot \exp(\eta_i) \qquad (1)$$

Co-administration

$$V_{CAi}(\text{mL}) = 503.2 \cdot \exp(-0.573) \cdot \exp(\eta_i) \qquad (2)$$

Where V_{CAi} is the individual central volume, and 503.2 mL is the typical value when single azithromycin was administrated. −0.573 is the coefficient indicating the relationship between co-administration and V_{CAi}. After co-administration, V_{CA} will decrease.

The typical value of forsythiaside clearance (CL_F) is 492.9 mL·h^{-1}, and this parameter could be significantly decreased by co-administration:

Single administration

$$CL_{Fi}(\text{mL/h}) = 492.9 \cdot \exp(\eta_i) \qquad (3)$$

Co-administration

$$CL_{Fi}(\text{mL/h}) = 492.9 \cdot \exp(-0.297) \cdot \exp(\eta i) \qquad (4)$$

Coefficient −0.297 suggests the influence of co-adminis-

tration. Estimated parameters of azithromycin and forsythiaside final pharmacokinetic models, inter-individual variability (IIV) and residual errors are presented in Table 2. All parameters were estimated with an acceptable precision [relative standard error (RSE)% < 30%]. The lower IIV may be due to rats having similar physiological features.

Model evaluation

The objective function value (OFV) decreased by 9.12 in the final azithromycin population model compared with the base model. For forsythiaside pharmacokinetic model, the inclusion of co-administration as a covariate decreased the OFV by 10.65. Goodness-of-fit plots (GOF) of base and final model are displayed in Fig. 1 (azithromycin) and Fig. 2 (forsythiaside). No systematic bias for both base (Figs. 1A−1D and Figs. 2A−2D) and final models (Figs. 1A'−1D' and Figs. 2A'−2D') were observed from these plots. After co-administration was incorporated into the final model, the predictions were closer to observations, and the diagnostic plots improved significantly. A significant improvement in the predictive performance of the final model was achieved compared to the base model.

Model validation

A total of 976/1000 runs (97.6%) converged successfully in the bootstrap analysis. The medians of the parameter values estimated from the bootstrap were in good agreement with the estimated pharmacokinetic parameter values based

Table 2 Estimated parameters of azithromycin and forsythiaside final pharmacokinetic models

Parameter (unit)	Model estimate			Bootstrap	
	Estimate	RSE%	IIV (CV%)	Median	95%CI
Azithromycin					
V_{CA} (mL)	503.2	13.3	15.3	516.8	371.3−650.0
CL_A (mL·h^{-1})	371.6	10.5	31.5	364.2	276.5−443.7
V_{PA} (mL)	3372.3	23.8	22.1	3141.7	2171.6−4577.0
Q_A (mL·h^{-1})	332.1	27.2	43.6	298.9	226.4−491.2
$f_{CO\text{-}v}$	−0.573	23.9	−	−0.575	−0.875−0.329
Residual variability					
σ_1	0.393	11.3	−	0.384	0.306−0.471
Forsythiaside					
V_{CF} (mL)	92.8	1.6	0.0	93.4	89.7−96.2
CL_F (mL·h^{-1})	492.9	3.4	10.8	494.7	469.2−537.7
V_{PF} (mL)	91.6	10.2	0.0	91.3	78.7−119.8
Q_F (mL·h^{-1})	214.2	13.5	0.0	208.8	154.0−284.9
$f_{CO\text{-}Cl}$	−0.297	23.2	−	−0.300	−0.468−−0.188
Residual variability					
σ_2	0.197	13.3	−	0.193	0.143−236

CL: apparent clearance of the central compartment; V_C: volume of distribution for the central compartment; V_P: volume of distribution of the peripheral compartment; *Q*: intercompartmental clearance; *f*: coefficient between coadministration and pharmacokinetic parameters; RSE: relative standard error; IIV: inter-individual variability; CV: coefficient of variation; 95%CI: 95% confidence interval
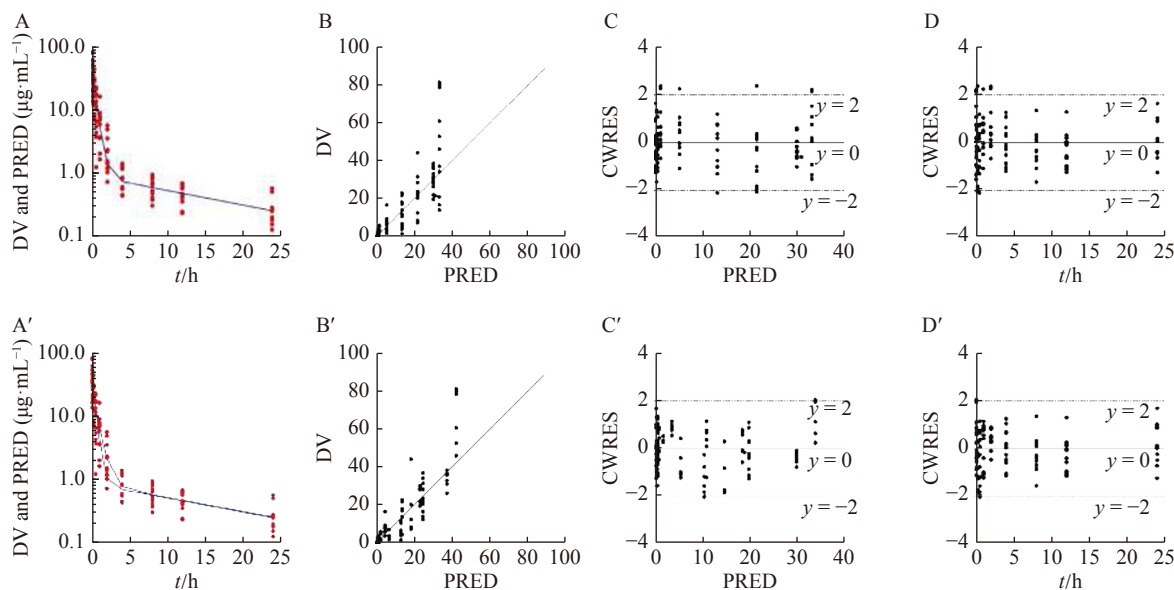

Fig. 1 The scatter plots of model evaluation of azithromycin base (A, B, C and D) and final (A', B', C' and D') pharmacokinetic models. A and A': observation (DV, dots) and prediction (PRED, solid lines) versus time after dose; B and B': observation (DV) against prediction (PRED), the lines are the lines of unity *y* = *x*; C and C': conditional weighted residual (CWRES) versus prediction (PRED); D and D': CWRES versus time. All the data processing and plots were generated using Phoenix NLME software

on the original dataset (Table 2). The bootstrap indicated the stability and robustness of the final model. Visual predictive check (VPC) with 1000 replicates for azithromycin (Fig. 3A) and forsythiaside (Fig. 3B) concentrations plotted versus time showed good agreement between simulations and observations. About 90% observed data were within the 90%
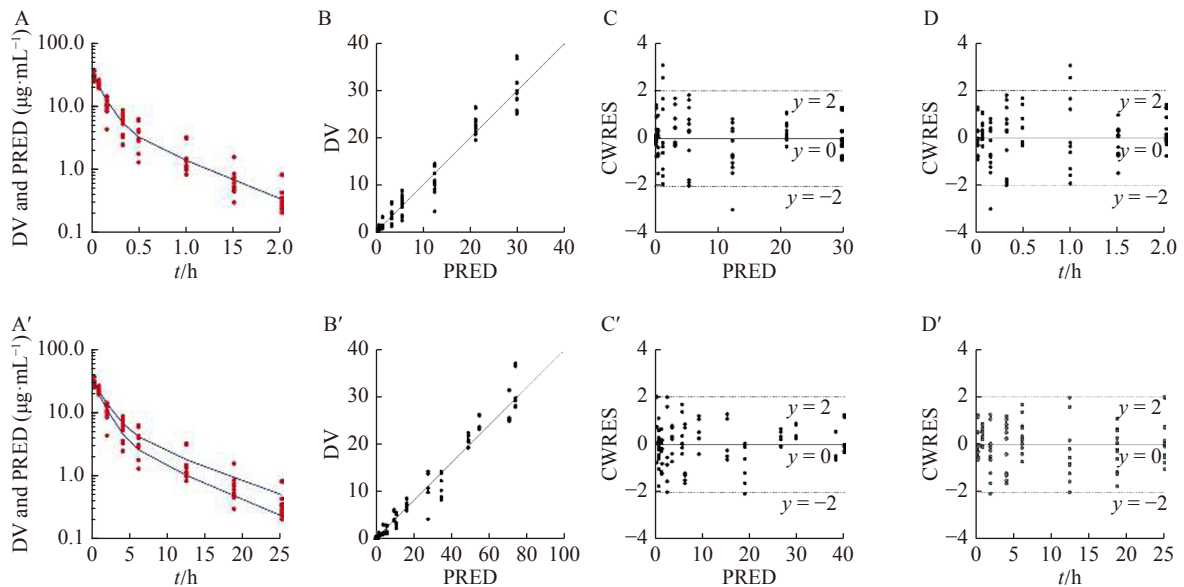
Fig. 2 The scatter plots of goodness-of-fit of base (A, B, C and D) and final (A', B', C' and D') forsythoside pharmacokinetic model. A and A': observation (DV, dots) and prediction (PRED, solid lines) versus time; B and B': observation (DV) against prediction (PRED), the lines are the lines of unity $y = x$; C and C': CWRES versus prediction (PRED); D and D': CWRES versus time. All the data processing and plots were generated using Phoenix NLME software
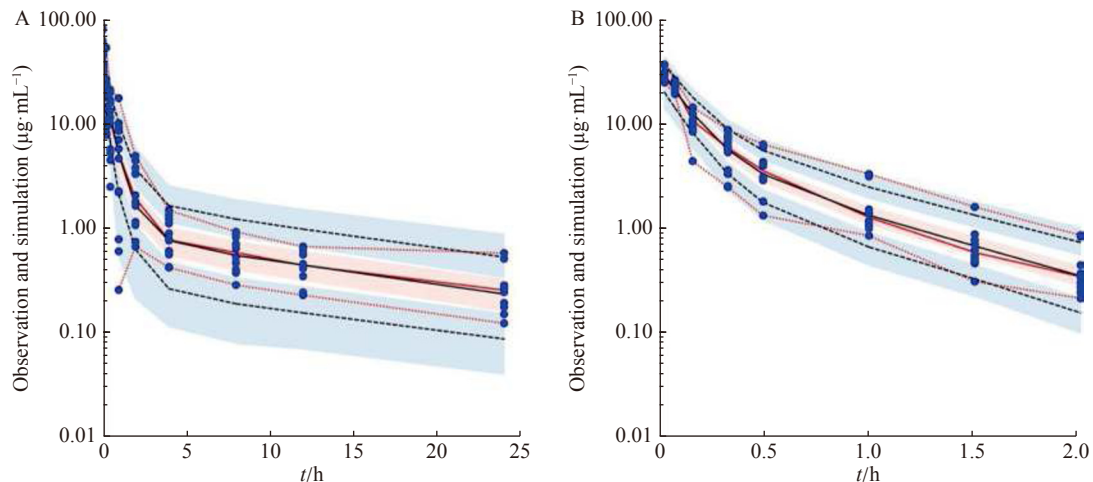

Fig. 3 Visual predictive check plots of final azithromycin (A) and forsythoside (B) population pharmacokinetic models. One thousand Monte Carlo simulations of the final pharmacokinetic model were performed. Summary measures of the distribution of predictions and observations are compared visually. Dots represent the actual observations. The observed 50th percentiles are red solid lines, and the 5th and 95th percentiles are the red dotted lines. The black solid lines are predicted 50th percentile and the black dashed lines are 5th and 95th percentiles from the simulated observations (shadow means 95% confidence band). The 90% prediction interval is the area between the 5th and 95th percentiles. All the data processing and plots were generated using Phoenix NLME software

prediction interval (90% PI), suggesting adequate predictive properties of the final population pharmacokinetic model.

Discussion

Both NCA and population pharmacokinetic analysis indicate there are significant interactions between the *Forsythia suspensa* extract and azithromycin. Due to an increased exposure for both drugs after co-administration, lower doses can provide sufficient exposure to obtain antibac-

terial activity. From pharmacokinetics, the study demonstrates that drug co-administration may increase efficiency.

In a previous study, we evaluated the pharmacokinetic interaction of SHL and azithromycin in rats, using forsythiaside as the pharmacokinetic marker of SHL. Both forsythiaside and azithromycin exposures increased after co-administration[14]. *Forsythia suspensa* is the major ingredient of SHL. To exclude interference from other ingredients, the pharmacokinetic analysis focused on the *Forsythia suspensa* extract.

This result is in accordance with our previous study[14].

In pharmacokinetic analysis, NCA is a widely used and accepted analytical method[16-18]. Compared with the compartmental model analysis, the NCA method does not have to consider the drug *in vivo* compartmental model characteristics but to directly calculate parameters using actual drug concentration measurements. Therefore, results are more objective, and the calculated *AUC* is more reliable. After a combination of the two drugs, *AUC* increased significantly (azithromycin increased by 63.3%, forsythiaside increased by 42.7%) and the *CL* decreased significantly (azithromycin decreased by 44.8%, forsythiaside decreased by 31.0%). *CL* reflects the overall clearance rate of a drug in the body, which is obtained by dividing the dose of a drug with the *AUC* of the drug, and *AUC* has a significant correlation with *CL*. As can be seen from Table 1, the $t_{1/2}$ of the two drugs did not change significantly. This is because the NCA method calculates the $t_{1/2}$ of a drug using only the last several drug concentration-time data points in the drug elimination phase. Because the drug concentration value is near the lower limit of the detection method, a slight deviation in test results can lead to significant differences in the $t_{1/2}$ calculations. This bias is more pronounced for drugs that meet the multi-compartment model. Therefore, in the NCA results of this study, we mainly focused on drug *AUC* and *CL*. The main disadvantage of the NCA method is that there is no fixed model, and the parameters of the NCA cannot reflect the details of the drug concentration-time curve. This deficiency can be compensated by using compartment models.

The population pharmacokinetic model analyzes the *in vivo* behavior of a drug, but more importantly, it can identify factors affecting the behavior of the drug *in vivo*. A drug population pharmacokinetic model can be developed through quantitatively estimating the degree of influence of these factors[19-20]. The *in vivo* behavior of both drugs was slow-distributed, and finally, the two-compartment model was used for fitting. By screening covariates, we found that the combined drug significantly reduced the central compartment distribution volume of azithromycin and significantly slowed the *CL* of forsythiaside (Fig. 4). This indicates that azithromycin increases the *in vivo* exposure of forsythiaside by slowing the *CL* of forsythiaside. Forsythiaside increases the exposure of azithromycin in the blood by reducing the volume of distribution of azithromycin in the body, allowing it to be more dis-

tributed in the central compartment.

Due to experimental limitations, we were unable to conduct a mechanism study of drug interactions *in vivo*. When *Forsythia suspensa* extract and azithromycin injection were mixed, a fine flocculent precipitate was visible. In order to avoid the vascular embolism, which may be caused by the indirect mixing of the two drugs, the combined drug group was administered sequentially. Whether it is sequential or simultaneous administration, the two drugs will meet in the body and produce more or less insoluble substances. This may affect drug distribution and elimination, resulting in slowing down elimination and narrowing drug distribution, and lead to an increase in drug exposure. Changes in drug distribution in the body reduce adverse reactions caused by widespread distribution, and this may be one of the reasons for an increased efficacy yet reduced toxicity after the combination use of Chinese and Western medicines.

Adverse drug reactions are important and common in everyday medical practice. Our study is an animal trial, and adverse drug reactions cannot be observed. Theoretically, there are benefits and risks when drugs are co-administered: smaller drug doses are possible due to an increased *in vivo* exposure of drugs. However, more factors may affect drug interactions such as the dosing ratio of drugs, and the timing and the frequency of drug administration. Further studies are needed to identify these factors to achieve optimal clinical efficacy. Improper co-administration may result in over- or underexposure of drugs, an increased risk of adverse reactions or poor efficacy.

In this study, the interaction between azithromycin and forsythiaside was found in rats from the perspective of pharmacokinetics. However, the following problems still need to be further explored: 1) the interaction between drugs is directly related to drug doses. The effects of drug interactions at different dose ratios are still unknown, and it is necessary to conduct interaction studies at various doses; 2) the $t_{1/2}$ of forsythiaside in this study is very short, only about half an hour. In clinical practice, this drug is administered three times daily, and we do not know if this dosing frequency is reasonable. It is necessary to study the pharmacokinetics of *Forsythia suspensa* extract in the human body to establish dosing frequency based on clinical data, or develop sustained release formulations; 3) in clinical practice, the combination of the two drugs and the time of administration depends on the personal experience of physicians. It is necessary to conduct drug interaction studies to provide dosing regimens, and 4) the mechanism of drug interaction needs to be further explored.

Methods

Study design

All experimental procedures with animals used in this study were according to the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the Animal Ethics Committee of the Capital Medical University. A
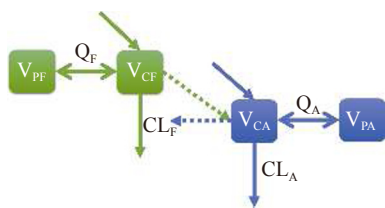

Fig. 4   The schematic diagram of forsythoside (left panel), azithromycin (right panel), and pharmacokinetic models and their interaction. The definition of pharmacokinetic parameters could be found in Table 2.

total of 24 male Sprague-Dawley rats (weight 240−260 g, Vital River Laboratories, Beijing, China) were randomly divided into four groups, single azithromycin group, single *Forsythia suspensa* extract group, co-administration group 1 and co-administration group 2. All rats were housed at least one week (water and food were available) before the experiment and fasted for 12 h (with free access to water) before drug administration.

In single azithromycin and *Forsythia suspensa* extract groups, a single dose of 13.0 mg of azithromycin and *Forsythia suspensa* extract (containing 3.4 mg of forsythiaside) in 5% glucose solution was given intravenously. In the two co-administration groups, all rats received sequential administration of *Forsythia suspensa* extract (containing 3.4 mg of forsythiaside) and 13.0 mg azithromycin. After administration, blood samples (−0.4 mL) were obtained *via* the retro-orbital sinus at scheduled times: forsythiaside, 2, 5, 10, 20, 30 min, 1, 1.5 and 2 h; azithromycin, 4, 15, 30 min, 1, 2, 4, 8, 12 and 24 h. Blood samples were separated by centrifugation (10 000 r·min$^{−1}$ for 5 min), and the plasma was frozen at −70 °C.

Drug determination

The forsythiaside concentration was determined by HPLC-UV (Shimadzu liquid chromatographic system, Tokyo, Japan) method using hesperidin (Shanghai Source Leaf Bio-Tech Co., Ltd., lot number: 20130820, Purity ≥ 98.0%) as an internal standard[21]. The mobile phase consisted of A, acetonitrile, and B, aqueous solution containing 0.4% acetic acid. Plasma samples were separated at a flow-rate of 1.0 mL·min$^{−1}$, using gradient elution: 0−10 min, 10% A; 10−20 min, 10%−30% A; 20−25 min, 90% A; and 25−32 min, 10% A. The column eluate was monitored at 284 nm and 330 nm.

LC-MS/MS (Agilent 6460 Triple Quad LC/MS/MS with 1260 HPLC) methods were applied for quantification of azithromycin. Roxithromycin was selected as the internal standard. The mobile phase consisted of A, methanol (containing 0.1% formic acid) and B, 0.1% formic acid solution. The flow rate was 0.2 mL·min$^{−1}$, using gradient elution: 0−1 min, 30% A; 1−4 min, 30%−100% A; 4−6 min, 100% A; and 6−10 min, 30% A. The injection volume was 10 μL and the oven temperature was set at 25 °C. The negative electrospray ionization (ESI) was operated at 350 °C, and the ion spray voltage was 4000 V. Multiple reaction monitoring (MRM) transitions were performed. The intra- and inter-day accuracy for azithromycin in rat plasma were 93.0%−111.0% and 82.0%−121.0%. The intra- and inter-day precision (relative standard deviation, RSD%) were 3.4%−7.1% and 3.8%−11.1%. The internal standard normalized recovery and matrix factor was 91.3%−110.3% and 81.4%−101.4%, respectively (Chromatogram data are not shown). Blood samples from co-administration of groups 1 and 2 were used to determine azithromycin and forsythiaside concentrations, respectively.

Non-compartment analysis

NCA could compute pharmacokinetic parameters of a drug from the time course of measured drug concentrations. It is often used to determine the degree of exposure following administration of a drug, such as *AUC*, and other parameters such as *CL* and $t_{1/2}$. Phoenix's NCA engine computes derived measurements from raw data by using methods appropriate for serially-sampled data. NCA was performed on each rat and then averaged the results. The pharmacokinetic parameters were compared between single and co-administration groups (*t*-test).

Population pharmacokinetic model

Phoenix NLME (Certara, Inc., Princeton, New Jersey, USA) software using the first-order conditional estimation method with the η-ξ interaction (FOCE-ELS) was used to build the population model[22]. Pharmacokinetic data were fitted using one-, two- and three-compartmental models, respectively. Based on GOF plots and OFV, proper models were selected to characterize drug *in vivo* behavior. The IIV of population pharmacokinetic parameters was described by exponential model:

$$P_i = P \cdot \exp(\eta_i) \qquad (5)$$

Where P and P_i represent the typical and individual value of parameters. η_i is normally distributed with a mean of 0 and a variance of ω^2. Multiplicative error model was selected describing the residual error:

$$C_i = C \cdot (1 + \varepsilon_i) \qquad (6)$$

Where C_i and C respectively account for determination and prediction. ε_i is the residual error of prediction, which is normally distributed with zero mean and variance of σ^2.

Based on a structural pharmacokinetic model for both azithromycin and forsythiaside, the influence of co-administration on pharmacokinetic parameters was assessed using forward-inclusion (ΔOFV > 3.84, $P < 0.05$) and backward-exclusion (ΔOFV > 6.63, $P < 0.01$) methods. Coadministration (coadministration = 1 and none = 0) was incorporated using indicator variables:

$$P_i = P \cdot \exp(f_{CO}) \cdot \exp(\eta_i) \qquad (7)$$

The f_{CO} represents the impact of co-administration on parameter P_i and indicates the relationship between P_i and P.

Goodness-of-fit

GOF plots play a key role in checking the data fitting of pharmacokinetic models. These plots give an overall perspective of model performance, including scatter plots for observation and prediction against time, observation versus prediction, conditional weighted residuals (CWRES) versus prediction, and CWRES versus time.

Model validation

Bootstrap and VPC were used to validate the final model. One thousand bootstrap replicates were constructed by randomly sampling (with replacement) 24 rats from the original dataset. Model parameters were estimated for each bootstrap replicate, and the resulting values were used to estimate medians and 95% confidence intervals (95%CIs, the range from the 2.5th to the 97.5th percentiles of the results

from individual replicates). Final model parameters were compared with bootstrap results. If no significant difference between the data was observed, the estimates for the final model were precise and stable. For VPC, 1000 Monte Carlo simulation of the pharmacokinetic dataset was generated using Phoenix NLME software. The simulations were compared with the observations by superimposing the median, 90%PI, (5th and 95th percentiles) of the observed data with the median and 90% PI of the simulations. The model deemed to be precise if the observed concentration data were approximately distributed within 90% PI.

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