WITHDRAWN:
Influence of Genetic Polymorphisms on the Pharmacokinetics and Safety of Nifedipine Controlled-release Tablets in Healthy Volunteers

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Abstract

Purpose: Nifedipine is a calcium channel blocker, which is used to treat hypertension and angina pectoris. The present study aimed to evaluate the effects of gene polymorphisms on pharmacokinetics of relevant metabolic genes with inconsistent or incomplete research conclusions and of others that exhibited an impact on pharmacodynamics, so as to provide a scientific basis for the inter-individual variation in nifedipine controlled-release tablet disposition.

Methods: A total of 33 healthy volunteers were administered a single 30 mg oral dose of nifedipine controlled-release tablets, and were genotyped for 17 SNPs within 12 genes, by StepOne Real-Time PCR System and the Matrix-assisted laser desorption ionization-time of flight. The plasma concentration levels were quantified by Ultra-performance liquid chromatography tandem mass spectrometry. Univariate and multivariate analyses were performed and the mean pharmacokinetic variables were compared according to genotypes.

Results: The CYP3A4 rs2242480, ABCB1 rs1045642, CACNA1D rs312481, CACNA1C rs2238032, ACE rs4646994 and SLC14A2 rs3745009 polymorphisms significantly influenced the pharmacokinetics of nifedipine controlled-release tablets with a greater effect being noted on CACNA1D, CACNA1C, and SLC14A2. These results continued to be statistically significant following multivariate correction.

Conclusions: The CYP3A4 rs2242480, ABCB1 rs1045642, CACNA1D rs312481, CACNA1C rs2238032, ACE rs4646994 and SLC14A2 rs3745009 polymorphisms significantly influenced the pharmacokinetics of nifedipine controlled-release tablets . Their pharmacokinetic properties were examined, so as to provide a theoretical basis for individual differences found during treatment.

Key Summary Points

This study aims to evaluate the effects of gene polymorphisms on pharmacokinetics of nifedipine controlled-release tablets .

This study is beneficial in providing a scientific basis for the inter-individual variation in nifedipine controlled-release tablet disposition.

1. Introduction

According to China Cardiovascular Health and Disease Report 2019, the number of hypertensive patients has reached 245 millions in China [1].Five nationwide hypertension sampling surveys have indicated that the overall prevalence of hypertension among residents aged 18 and above in China is increasing year by year [2], including stroke, coronary heart disease, heart failure, kidney disease, and other serious complications of hypertension, which can cause high disability and mortality. These conditions have become a heavy burden on the Chinese families and society. Therefore, prevention and control of hypertension is one of the core strategies to curb the prevalence of cardiovascular and cerebrovascular diseases in China. Dihydropyridine calcium antagonists have shown prominent antihypertensive efficacy, which contributes to the improvement of the treatment and control rates of hypertension. These compounds also play a key role in reducing cardiovascular morbidity and mortality. Their mechanism of action is to block the L-type calcium channel in the smooth muscle tissue and inhibit the influx of calcium ions, thus inducing vasodilation and lowering blood pressure. Moreover, they cause dilatation of the main coronary arteries in normal and ischemic areas for the treatment of chronic stable and vasospastic angina [3]. Intervention as a Goal in Hypertension Treatment (INSIGHT) study [4] demonstrated that nifedipine conferred cardiovascular protection as effectively as diuretics in high-risk patients, with a smaller incidence of adverse metabolic consequences by preventing the progression of carotid atherosclerosis and reducing the worsening of coronary calcifications. This evidence supports the use of calcium channel blockers (CCB) in hypertensive patients, notably those at high cardiovascular risk.

To date, the majority of the studies have demonstrated the effects of gene polymorphisms on the pharmacokinetics of nifedipine conventional or sustained release tablets rather than those of nifedipine controlled-release tablets. However, nifedipine controlled-release tablets provide a constant release rate for approximately 20 to 22 h, and a relatively constant concentration-time profile throughout the 24-h dosing interval compared with that of either formulations, which are more widely used in the clinic [5]. It is important that the maintenance of the relatively constant plasma drug concentrations is sustained so as to maintain smooth control of blood pressure. In this way, large fluctuations in the plasma drug concentrations are avoided, which may improve the efficacy and tolerability of the drug [6]. Nifedipine controlled-release tablets have a rapid and almost complete absorption, and exhibit linear pharmacokinetics. They bind extensively to plasma proteins (95%) [7]. The bioavailability of the controlled-release tablets is relative to an equivalent daily dose of the nifedipine capsule formulation, and is estimated to 55–65% on a single dose, whereas it is increased to 75–85% at the steady-state phase on multiple dosing [8]. It's relative total clearance ranges from 61 to 80 l/h in healthy young volunteers [9, 10]. Following oral administration, nifedipine is metabolized to pharmacodynamically inactive metabolites in the liver by oxidative pathways, which are predominantly mediated by the cytochrome P450 CYP3A enzymes [11]. The majority of adverse events, which are associated with nifedipine controlled-release tablet therapy in patients with hypertension or coronary heart disease, are related to the vasodilatory action of the drug and include peripheral edema, headache, dizziness, and flushing [12]. The incidence of vasodilatory adverse events ranges from 9.7–62.8% [13]. The Modern Approach to the Treatment of Hypertension Trial indicated that approximately 76% of hypertensive patients responded to nifedipine controlled-release tablet monotherapy. The causes of interindividual variation in response to medication have not been conclusively determined, and it is considered that this variation may have a genetic basis [14].

At present, it is known that the genes encoding CYP3A4, CYP3A5, MDR1, PXR, BCRP, CACNA1D, CACNA1C, ADRA1A, ADRB2, ACE, AT1R, and SLC14A2 affect the clinical effects of nifedipine-mediated antihypertensive action. Among them, the genes that encode the enzymes responsible for the metabolism of nifedipine include those for CYP3A4, CYP3A5, MDR1, PXR and BCRP. However, certain conclusions are inconsistent at present. For example, previous studies [15–18] have shown that the average AUC of the CCB in CYP3A5*3/*3 genotype subjects was higher than that noted in CYP3A5*1 allele carriers, whereas the plasma drug peak concentration value was increased to a certain extent. However, the clearance rate indicated no statistically significant changes [16]. Certain studies have shown the opposite conclusions, indicating that carriers of the CYP3A5 homozygous variants exhibit lower oral clearance of CCB [17]. The number of previous studies that have examined the effects of MDR1 on the metabolism of these compounds is low and their dates of publication are early.

The reports on the pharmacokinetics of nifedipine were inconsistent [19–20]. Moreover, scarce clinical data were available to evaluate the effects of PXR and BCRP gene polymorphisms on the pharmacokinetics of nifedipine. Other genes also affect the efficacy of nifedipine, although their exact metabolic pathways are not clear. The present study investigated the effects of the aforementioned genes on the metabolism and elimination of nifedipine controlled-release tablets from the perspective of pharmacokinetics, so as to provide a theoretical basis for individual differences in subjects receiving nifedipine controlled-release tablets.

2. Material And Methods

2.1 Subjects

The present study is a continuation of other studies that examined nifedipine the effects of controlled-release tablets in patients from a Class 1 and Grade A Hospital in Shanxi Province. A total of 33 healthy volunteers, who participated in the present pharmacogenetic study were previously enrolled in a study with title 'To evaluate the one-centre, open, randomised, single-dose, two-cycle, two-sequence, crossover bioequivalence of test product nifedipine controlled-release tablets versus reference product nifedipine controlled-release tablets (baixindo ®) in healthy adult subjects in fasting/postprandial conditions' in 2019.09. All of them were authorized by the Research Ethics Committee of the hospital. Every healthy volunteer (n = 62) signed the informed consent form for his/her participation in the clinical trial. A total of 33 subjects consented to participate in this pharmacogenetic study. The volunteers were all from Taiyuan, Shanxi Province. The data from reference preparations were used in the present study. All procedures were performed according to the Declaration of Helsinki and Good Clinical Practice guidelines.

The following inclusion criteria were used for recruitment of volunteers with specific characteristics:

1) Subjects that fully understood the test content, process and possible adverse reactions, voluntarily participated in the test, voluntarily signed the informed consent, and strictly abided by the study process.

2) Males and females aged 18-65.

3) Weight of male subjects ≥50.0 kg, and weight of female subjects ≥45.0 kg;BMI ≥19.0 kg/m^2, ≤27.0 kg/m^2.

4) The results of the physical examination, vital signs, electrocardiogram and specified laboratory tests during screening were normal or without clinical significant abnormalities, and the investigator assessed whether the subjects were in optimal health and mental state.

The following exclusion criteria were applied:

1) Prior known or suspected allergy to nifedipine or its excipients, or allergic constitution (≥2 drugs and ≥3 food allergies).

2) Blood sickness, needle sickness or intolerance of venipuncture blood collection.

3) Patients with a history of diseases of the nervous/mental, respiratory, cardiovascular, digestive, blood and lymphatic, endocrine, skeletal and musculoskeletal systems.

4) The combination of diseases that may affect drug absorption, distribution, metabolism, excretion, and interpretation of safety data, or that may reduce compliance, including but not limited to any of the following: Combination with inflammatory bowel disease, gastric ulcer, duodenal ulcer, gastrointestinal/rectal bleeding, persistent nausea, or other clinically significant gastrointestinal abnormalities; dysphagia; history of gastrointestinal surgery (except for appendicitis and anal prolapse); history or evidence of clinically significant kidney or renal impairment; evidence of liver disease or clinically significant liver function impairment (e.g. active liver disease, including unexplained liver transaminase persistence); obstructive uropathy or difficulty emptying urine at the time of screening.

5) Positive results for detection of hepatitis C antibody (HCV-AB), *treponema pallidum* antibody (anti-TP) or human immunodeficiency virus antibody (HIV-AB).

6) Female subjects who were lactating or exhibited positive serum pregnancy β-HCG during the screening period or the test, or who planned to become pregnant within 6 months following administration (including male subjects' spouse).

7) Any drugs that inhibited or induced the activity of drug metabolism enzymes in the liver and had been used within 4 weeks prior to administration.

8) Therapy with any prescription drug, over-the-counter drug or diet using specific health food supplements within 2 weeks prior to administration.

9) Special diet, including compounds of the *ruttriaceae* citrus subfamily, high xanthine, chocolate diet, caffeinated and alcoholic drinks, and vigorous exercise or smoking cigarettes within 48 h prior to medication and during hospitalization.

10) Smoking more than 5 cigarettes a day within 3 months prior to the trial or smoking during the entire hospital stay.

11) Regular drinkers within 3 months prior to the study, i.e., those who averaged more than 2 units of alcohol per day or who had a positive breath test for alcohol at enrollment.

12) A history of drug use, drug abuse or positive urine drug screening test during screening.

13) Use of experimental drugs or undergoing any surgical procedure in the previous 3 months.

14) Donation of blood or blood loss ≥400 ml within 3 months prior to the trial, or blood donation ≥400 ml during the planned trial and 3 months after the trial.

2.2 Study Design

The bioequivalence trials compared 30 mg nifedipine controlled-release tablets from a pharmaceutical company or Bayer AG, respectively. They were phase I, one-centre, open, randomized, single-dose, two-cycle, two-sequence, crossover bioequivalence clinical trials. In the first period, half of the volunteers received the test formulation and the other half received Bayer®. In the second period, after a 7-day washout period, the groups were exchanged, resulting in two different sequences. In the reference preparations periods, safety was evaluated, and blood samples were obtained for the determination of plasma levels and for genotyping.

During the screening stage, informed consent and screening tests were conducted from 14 to 2 days prior to administration, and the following information was obtained: Personal information, vital signs, physical examination, height and weight measurements, BMI, 12-lead electrocardiogram, routine blood, urine, and blood biochemistry test results, coagulation function test results, erythrocyte sedimentation rate, urine drug abuse screening, alcohol breath test results, infectious disease screening results, and blood pregnancy test results of female subjects.

During the baseline period screening was performed in accordance with the inclusion criteria and did not accord with the standards of exclusion of the subjects in the first cycle for 1 day prior to check in. At baseline inspection, the following items were assessed during the period of examination: Physical examination, height, weight, routine blood tests, routine urine and blood biochemical assessment, and blood sedimentation. These items were not in the case of the baseline phase I repeat. However, stage I and II baseline data required completion of vital signs, urine drug abuse screening, alcohol breath tests, and blood pregnancy tests for female subjects.

The observation period in the hospital was performed as follows: Eligible subjects at baseline were admitted to phase I ward 1 day prior to cycle I administration, and were provided with a light diet, fasting and water restriction overnight for at least 10 h.

The test or reference preparations were provided following fasting at approximately 8:00 a.m. of the same day according to the corresponding drug administration sequence, which was randomly coded by the subjects. A total of 240 ml warm water was provided to the subjects. With the exception of drinking water, it was forbidden to drink water from 1 to 2 h following administration, and 100 ml water was used for 2 h following administration. Water intake was not allowed for 4 h after administration. Fasting was performed within 4 h following drug administration, and standard lunch and dinner were provided at 4 and 10 h following drug administration. The dietary standards during hospitalization were consistent.

2.3 Pharmacokinetic analysis

The pharmacokinetic analysis was performed using blood samples, which were obtained in EDTA K2 tubes immediately before (0 h) and after 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 20, 24, 26, 28, 30, 36, 48, and 72 h of dosing. Approximately 3 ml venous blood was collected into vacuum collection vessels containing EDTA-K2 anticoagulant, mixed gently and allowed to stand at room temperature, followed by centrifugation at 2-10℃, 1,700 g for 10 min to separate the plasma. Following separation, the plasma was transferred to a refrigerator with a temperature of -20℃ and left no more than 48 h. Subsequently, the samples were transferred to a -70℃ refrigerator until transshipment. The plasma concentration levels of nifedipine controlled-release tablets were quantified by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) in an external laboratory.

The pharmacokinetic parameters were calculated by non-compartmental analysis. For this purpose, the Phoenix WinNonLin Professional Software was used (version 8.0). The area under the curve (AUC) between the pre-dose and the last observed time point was calculated according to the linear trapezoidal standard. Several parameters were calculated as derived from the AUC. Firstly, total drug clearance was adjusted for bioavailability (Cl/F), which was calculated as the dose divided by $AUC_{0-\infty}$ and weight. Secondly, the volume of distribution was calculated by dividing Cl/F by the terminal rate constant (ke). The ke was calculated as the slope of the line traced over the log-linear part of the concentration-time curve, which was estimated by linear regression. Other parameters were observed directly in the time-concentration graph as follows: C_{max} and T_{max}. Finally, $t_{1/2}$ was calculated as $-\ln 2/ke$. The parameters were logarithmically transformed for statistical analysis to obtain a normal distribution (except for T_{max}, $t_{1/2}$), which was confirmed with a Shapiro-Wilks normality test.

2.4 Safety

In the clinical trials, safety was assessed by measurements of vital signs and of blood, biochemical, and urine parameters and by evaluating the 12-lead electrocardiogram. Physical examination was established at pre-dose and during the last observation. The vital signs were scheduled at pre-dose and at several occasions in the following 72 h, depending on the clinical trial design (e.g. 2, 12, 24, 48, and 72 h post-dose). The subjects' subjective feelings and possible adverse events were observed and questioned during hospitalization. Only the AEs with a definite, probable or possible relationship with nifedipine controlled-release tablet intake were considered adverse drug reactions (ADRs).

2.5 Genotyping

Genomic DNA was isolated from the whole blood samples using a MagMAXTM DNA Multi-Sample Ultra Kit, following the manufacturer's instructions. The following genes: CYP3A4 rs2242480, CYP3A5 rs776746, ABCB1 rs1045642, rs2032582, BCRP rs2231142, CACNA1D rs312481, CACNA1C rs2239050, ACE rs4646994, and AT1R rs5186 was performed following the commendatory protocols with measurement of charge-to-mass ratio. The matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) was used for genotyping on the Sequenom MassARRAY iPLEX (Sequenom, USA) platform. TYPER 4.0 software was subsequently applied to assess the output of the genotyping results. The remaining of the gene polymorphisms, namely CYP3A4 rs28371759,

PXR rs1523127, CACNA1C rs2238032, ADRA1A rs1048101, ADRB2 rs1042713, rs1042714, SLC14A2 rs1123617, and rs3745009 were genotyped by a StepOne Real-Time PCR System (Nanjing Bioengineering sequencing service network).

2.6 Statistical analysis

Statistical analysis was performed with SPSS 16.0 software. All data are expressed as mean ± standard deviation unless otherwise indicated. The Hardy-Weinberg equilibrium was used for all analyzed variants. The pharmacokinetic parameters AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were divided by the Dose/Weight (DW) ratio to eliminate the effect of weight and dose. For statistical analysis, a logarithmic transformation was applied to all pharmacokinetic variables to normalize the distributions. Firstly, a univariate analysis was performed. The mean pharmacokinetic variables were compared according to sex and genotypes. The following statistical tests were used: a t-test (variables with two categories) or an ANOVA test (variables with three or more categories). In addition, a Bonferroni post-hoc analysis was performed when ANOVA was applied. All factors that demonstrated significant differences in the univariate analysis were included as independent variables. Multiple linear regression models were used to study the factors related to all the pharmacokinetic-dependent variables. Univariate analysis was combined with the Fisher exact test to assess the incidence of ADRs.

3. Results

3.1 Demographic characteristics

In the studies examined, 14 women and 19 men were enrolled. Women had lower weight and height than men ($p < 0.01$). However, no significant differences were noted in the age or body mass index (BMI) (Table 1). Following correction for DW, the C_{max} of men was significantly higher than that of women (56.07 ± 19.56 vs. 42.44 ± 16.25 kg*ng/ml*mg, p = 0.014). The $t_{1/2}$ and Vd/F of women were significantly higher than those of men (p = 0.029, non-standardized β coefficient = 0.779, R^2 = 0.352; p = 0.035, respectively). No significant differences were observed in the other parameters examined between the two sexes (Table 1).

Table 1
Volunteer's demographic characteristics and pharmacokinetic parameters by gender.

Parameters	Women(n = 14)	Man(n = 19)
Age(years)	26.50(5.73)	25.89(4.05)
Weight(kg)	**54.01(5.81)##**	64.31(6.31)
Height(m)	**155.82(5.04)##**	167.50(5.83)
BMI(kg/m2)	22.22(1.74)	22.94(2.13)
T_{max}(h)	18.57(8.59)	16.21(9.22)
C_{max}/DW(kg*ng/mL*mg)	**42.44(16.25)#**	56.07(19.56)
AUC_{0-t}/DW(kg*ng*h/mL*mg)	1101.10(469.09)	1269.10(574.20)
$AUC_{0-\infty}$/$DW_{(kg*ng*h/mL*mg)}$	1129.04(479.98)	1305.5(578.73)
$t_{1/2}$(h)	7.6(1.84)#	6.3(2.02)
Cl/F (mL/h*kg)	1025.1(409.19)	924.85(445.39)
Vd/F (L/kg)	**10.69(3.17)#**	8.32(4.52)
The data are shown as mean (standard deviation). Bold: statistically significant.		
# p < 0.05,## p < 0.01, against man. Underlined: p < 0.05 in multivariate analysis.		

3.2 Pharmacokinetic analysis

The significant differences noted in pharmacokinetic parameters were associated with genetic polymorphisms, as shown in Table 2. Subjects with CYP3A4rs2242480 C/C genotypes exhibited a lower C_{max}/DW than C/T and T/T individuals (p = 0.049; p = 0.02, respectively) and a higher Vd/F than the T/T subjects (p = 0.039). The presence of polymorphic variants reduced the rate of metabolism. The CYP3A5 rs776746 and BCRP rs2231142 polymorphisms accelerated the metabolic activity of the corresponding enzymes encoded by these genes. ABCB1rs1045642 A/A subjects exhibited a lower T_{max} (p = 0.04) than G/G carriers. The Vd/F of the CACNA1D rs312481 G/G subjects was lower than that of the A/G carriers (p = 0.002, non-standardized β coefficient = -0.298, R^2 = 0.408), whereas the $t_{1/2}$ followed the same pattern (p = 0.002, non-standardized β coefficient = -3.326, R^2 = 0.352). The C_{max}/DW of CACNA1C rs2238032 T/G carriers was lower than that of the T/T carriers (p = 0.027), whereas the $t_{1/2}$ followed a similar tendency (p = 0.049, non-standardized β coefficient = 1.817, R^2 = 0.352). ACE rs4646994, I/I subjects presented a higher C_{max}/DW (59.87 ± 21.23 vs. 34.93 ± 10.80 kg*ng/ml*mg, p = 0.047), a higher AUC_{0-t}/DW and $AUC_{0-\infty}$/DW (1410.00 ± 530.75 vs. 730.30 ± 234.92 kg*ng*h/ml*mg, p = 0.041; 1445.60 ± 530.29 vs. 742.96 ± 235.72 kg*ng*h/ml*mg, p = 0.037, respectively), and a lower Cl/F (770.60 ± 238.96 vs. 1456.30 ± 527.07 ml/h*kg, p = 0.037) than D/D carriers. I/I subjects exhibited a higher T_{max} (19.75 ± 8.35 vs. 12.72 ± 8.48 h, p = 0.031), and a higher C_{max}/DW (59.87 ± 21.23 vs. 42.63 ± 11.84 kg*ng/ml*mg, p = 0.034) than D/I carriers.

Table 2
Pharmacokinetic parameters according to genotypes

Genotype		N	T_{max}(h)	C_{max}/DW(kg*ng/mL*mg)	AUC_{0-t}/DW(kg*ng*h/mL*mg)	$AUC_{0-\infty}$/DW(kg*ng*h/mL*mg)	$t_{1/2}$(h)	Cl/F (
CYP3A4	C/C	12	15.42(9.35)	40.61(9.73)	1005.60(231.70)	1049.50(249.98)	7.25(2.01)	1030.
rs2242480	C/T	20	17.95(8.82)	**54.69(21.12)#**	1282.00(626.57)	1309.50(637.21)	6.65(2.10)	950.7
	T/T	1	24.00	**78.37#**	1820.50	1825.90	6.20	547.6
CYP3A4	T/T	32	17.57(8.81)	50.01(19.46)	1177.50(526.34)	1210.90(534.72)	6.84(2.05)	980.8
rs28371759	C/T	1	6.00	59.23	1847.10	1861.90	7.40	537.0
CYP3A5	A/A	2	24.00	54.98(33.08)	1300.40(735.48)	1333.20(696.83)	7.65(2.05)	868.7
rs776746	A/G	21	17.38(8.99)	54.02(20.77)	1274.80(609.18)	1306.00(618.65)	6.74(2.22)	941.0
	G/G	10	15.50(9.42)	41.51(10.47)	1015.70(254.42)	1051.80(273.69)	6.94(1.73)	1042.
ABCB1	G/G	10	20.60(8.83)	49.11(20.41)	1191.00(459.88)	1227.40(470.18)	6.59(1.88)	952.7
rs1045642	A/G	18	17.17(8.59)	52.01(20.36)	1215.50(566.23)	1242.60(567.64)	6.87(1.79)	966.1
	A/A	5	**10.61(7.82)#**	46.44(14.80)	1147.80(642.35)	1193.90(670.46)	7.66(3.19)	1001.
ABCB1	C/C	5	18.80(9.09)	45.52(27.83)	1144.10(563.74)	1168.40(573.50)	6.69(1.59)	1073.
rs2032582	C/T C/A	19	15.85(9.08)	49.66(16.21)	1128.60(491.32)	1155.70(494.26)	6.57(2.00)	1012.
	A/T A/A T/T	9	19.23(8.91)	54.26(21.58)	1373.90(614.63)	1423.40(622.63)	7.56(2.32)	813.2
PXR	G/G	1	12.00	54.34	1183.60	1207.50	5.30	828.1
rs1523127	G/T	18	17.78(8.83)	46.45(17.46)	1097.20(493.51)	1128.20(497.38)	6.71(2.04)	1063.
	T/T	14	16.86(9.51)	54.94(21.57)	1328.20(585.87)	1363.90(596.85)	7.15(2.09)	853.4
BCRP	C/C	20	16.75(9.17)	50.13(20.42)	1262.10(522.87)	1304.00(534.17)	7.27(2.09)	899.2
rs2231142	C/A	11	16.82(9.17)	51.55(19.52)	1125.20(596.38)	1139.60(594.91)	6.01(1.90)	1084.
	A/A	2	24.00	44.95(3.11)	954.08(109.79)	988.30(85.15)	7.30(0.85)	1005.
CACNA1D	A/A	1	26.01	31.60	1094.10	1113.20	8.50	898.2
rs312481	A/G	6	12.67(10.42)	45.76(16.00)	1079.00(652.23)	1150.80(691.22)	8.60(3.23)	1162.
	G/G	26	17.93(8.43)	52.05(19.98)	1229.30(520.56)	1253.60(522.30)	6.39(1.45)**	924.9
CACNA1C	G/G	29	18.00(8.78)	49.71(18.97)	1156.70(477.04)	1190.60(488.32)	6.83(2.15)	981.8
rs2239050	C/G	4	11.52(8.75)	54.46(23.54)	1496.10(865.23)	1521.20(858.98)	7.03(0.68)	862.4
CACNA1C	T/T	24	17.71(8.85)	53.71(21.04)	1269.80(608.60)	1304.30(616.28)	6.97(1.95)	965.3
rs2238032	T/G	8	17.13(9.28)	**39.53(7.64)#**	1012.20(80.15)	1043.30(88.890	6.71(2.41)#	964.3
	G/G	1	6.00	54.33	955.68	960.72	5.30	1040.
ADRA1A	C/T	6	19.17(8.45)	48.82(14.60)	1142.90(412.35)	1167.40(406.96)	6.22(1.55)	947.5
rs1048101	C/C	27	16.78(9.09)	50.62(20.32)	1210.00(559.89)	1244.70(568.98)	7.00(2.11)	971.7
ADRB2	G/G	8	16.50(9.29)	47.97(15.32)	1274.00(366.34)	1294.80(358.74)	6.68(1.46)	818.6
rs1042713	A/G	18	16.67(9.00)	50.13(22.73)	1183.70(650.15)	1225.80(664.56)	7.21(2.33)	1053.
	A/A	7	19.43(9.23)	53.36(14.60)	1147.00(371.76)	1169.70(366.65)	6.14(1.74)	914.9
ADRB2	G/G	1	12.00	54.34	1183.60	1207.50	5.30	828.1
rs1042714	C/G	8	16.88(9.10)	55.75(18.10)	1420.90(596.00)	1459.20(585.17)	7.14(1.87)	778.1
	C/C	24	17.54(9.15)	48.30(19.97)	1124.10(511.07)	1155.40(524.35)	6.82(2.12)	1036.
ACE	D/D	3	24.67(1.15)	34.93(10.80)	730.30(234.92)	742.96(235.72)	5.53(1.55)	1456.
rs4646994								

The data are shown as mean (standard deviation). Bold: statistically significant.

# p < 0.05,## p < 0.01,against wild type individuals; *p < 0.05, **p < 0.01, against heterozygous type individuals; Underlined: p < 0.05 in multivariate analysis.

Genotype		N	T_{max}(h)	C_{max}/DW(kg*ng/mL*mg)	AUC_{0-t}/DW(kg*ng*h/mL*mg)	$AUC_{0-\infty}$/DW(kg*ng*h/mL*mg)	$t_{1/2}$(h)	Cl/F (
	D/I	14	12.72(8.48)	42.63(11.84)	1055.50(482.58)	1089.40(497.18)	7.20(2.45)	1087.
	I/I	16	**19.75(8.35)***	**59.87(21.23)#***	**1410.00(530.75)#**	**1445.60(530.29)#**	6.80(1.66)	**770.6**
AT1R	A/A	31	17.68(8.93)	49.66(19.46)	1169.40(510.22)	1201.10(513.61)	6.76(1.70)	984.2
rs5186	A/C	2	10.01(5.64)	59.96(15.87)	1639.20(893.48)	1687.80(957.04)	8.35(6.29)	705.9
SLC14A2	C/C	13	18.08(8.62)	55.41(22.13)	1269.40(496.49)	1305.80(507.20)	6.40(1.79)	867.8
rs1123617	C/T	16	14.57(9.23)	48.39(18.56)	1204.50(617.50)	1233.30(624.25)	7.37(2.24)	1033.
	T/T	4	25.00(1.15)	41.21(3.80)	938.45(53.77)	975.57(53.38)	6.25(1.76)	1027.
SLC14A2	C/C	16	17.00(9.09)	50.00(20.20)	1231.10(530.71)	1265.20(523.35)	7.13(1.69)	896.9
rs3745009	C/T	11	16.27(8.59)	40.95(10.55)	894.57(288.80)	909.64(292.68)	6.20(1.36)	1248.
	T/T	6	19.51(10.15)	68.18(18.46)#*	1665.20(569.96)* **#**	1727.00(579.44)* **#**	7.32(3.54)	640.8 **#**

The data are shown as mean (standard deviation). Bold: statistically significant.

# p < 0.05,## p < 0.01,against wild type individuals; *p < 0.05, **p < 0.01, against heterozygous type individuals; Underlined: p < 0.05 in multivariate analysis.

The data indicated that these polymorphisms reduced the metabolic activity of these enzymes. SLC14A2 rs3745009 T/T subjects were associated with higher C_{max}/DW (p = 0.034, non-standardized β coefficient = 0.166; p = 0.025, non-standardized β coefficient = 0.216, R^2 = 0.370), higher AUC_{0-t}/DW (p = 0.04; p = 0.016, non-standardized β coefficient = 0.325, R^2 = 0.142), higher $AUC_{0-\infty}$/DW (p = 0.049; p = 0.014, non-standardized β coefficient = 0.326, R^2 = 0.148), lower Cl/F (p = 0.049; p = 0.014, non-standardized β coefficient = -0.326, R^2 = 0.148), and lower Vd/F (p = 0.006, non-standardized β coefficient = -0.273; p = 0.011, non-standardized β coefficient = -0.301, R^2 = 0.408) compared with C/C and C/T carriers, respectively as shown in Fig. 1. Following analysis of these results in a concentration-time curve, it was observed that CT subjects indicated a significant lower AUC. Heterozygous mutations accelerated nifedipine controlled-release tablet metabolism while homozygous mutations reduced the rate of metabolism.

3.3 Adverse drug reactions

During the development of these studies, 15 volunteers (45.5%) suffered at least one adverse reaction, whereas 7 volunteers (21.2%) exhibited two. The most frequent ADRs were nervous system-associated disorders, such as the following:Headache and dizziness (27.3%). Following group analysis, the most frequent ADRs were heart-related problems, including palpitation and T wave abnormalities (15.2%). ADRs also involved the respiratory system, nasal congestion and bleeding, which accounted for 9.1% of the total ADRs. Nausea, hypotension, and hypoleucocytosis accounted for 3.0% of the total ADRs. Women exhibited a higher rate of adverse reactions than men (50% vs. 42.1%). The number of volunteers who suffered at least one adverse reaction was associated with the B2 rs1042713 polymorphism (12.5% of G/G subjects, 61.11% of A/G subjects and 42.8% of A/A subjects; p = 0.038). An association between CYP3A5 rs776746 and the development of headache and dizziness was also noted (50% of A/A subjects, 14.3% of A/G subjects and 50% of G/G subjects; p = 0.002).

4. Discussion

As a calcium antagonist, nifedipine is widely used in the treatment of hypertension. Its efficacy and toxicity have shown variations among different genotypes in previous studies. However the PK parameters of different genotypes have not been adequately addressed in the Chinese population. The results of the present study indicated that the genotypes may be one of the major factors that affect the PK parameters of nifedipine controlled-release. The prediction may provide evidence for the adoption of nifedipine controlled-release dosage in the Chinese population.

The values of the pharmacokinetic parameters varied according to the corresponding sex. The women presented a lower weight and height than men, which was often associated with higher exposure to drugs and therefore with a greater risk for the development of ADRs. In addition, no consensus was reached on the impact of sex on nifedipine pharmacokinetics. A previous study [21]demonstrated that clearance was significantly slower in men compared with women, whereas a different study [22] reported no evidence of association between the gender and the pharmacokinetics of nifedipine. In the present study, women exhibited lower C_{max}/DW (p = 0.014) and higher $t_{1/2}$ (p = 0.029) and Vd/F (p = 0.035) than men.

The influence of genotypes on nifedipine controlled-release pharmacokinetics was also examined. Currently, CYP3A is known to be a very important metabolizing enzyme of nifedipine. The CYP3A4 rs2242480 polymorphism is located in the 10th intron of the CYP3A4 gene and has a mutation rate of 18–19%, which is the highest mutation rate found currently [23]. In the present study, the effects of this gene polymorphism were investigated on nifedipine controlled-release tablet metabolism. The study indicated that the CYP3A4 rs2242480 mutant TT increased C_{max}/DW (78.37 vs. 40.61 kg*ng/ml*mg) and reduced CL/F (547.68 vs. 1030.10 ml/h*kg) compared with the wild-type CC genotype. However, the CYP3A4 rs28371759 polymorphism did not affect the metabolism of nifedipine controlled-release in the present study, which was consistent with previous research studies [24, 25]. The current study indicated that the CYP3A5 gene mutation accelerated the metabolism with increased CL/F (1042.30 ± 414.68 vs. 868.77 ± 454.09 ml/h*kg) compared with the wild-type genotype, which was consistent with the study of Kim et al [16–18]. The findings regarding the association of CYP3A5 and nifedipine controlled-release metabolism exhibited no significant differences probably due to the low sample size of wild-type carriers.

P-glycoprotein (P-gp) is encoded by the multidrug resistance gene (MDR1) and affects the uptake and metabolism of various drugs. Nifedipine belongs to the P-gp substrate [17]. In addition, exon 21 (G2677T/A, rs2032582) and exon 26 (C3435T, rs1045642), are two of the most common coding region single nucleotide polymorphisms (SNP) with low allele frequency [26]. In the present study, the rs1045642 polymorphism reduced the T_{max} (10.61 ± 7.82 vs. 20.60 ± 8.83 h), whereas other pharmacokinetic data were not affected. These results were consistent with Yanxia Qi and Jiang-feng Li 's research studies [19, 20]. With regard to the rs2032582 polymorphism, no relevant study has been published at present. Therefore, the current study indicated that this gene polymorphism exhibited no significant effects on nifedipine controlled-release metabolism.

The functional variation of the human progesterone receptor (PXR) may affect the removal of specific drugs. Its genetic polymorphisms affects the activity of the cytochrome P450 enzymes, which directly affect drug metabolism [27]. To the best of our knowledge, current reports that examined the PXR polymorphism and nifedipine pharmacokinetics are not available. Assayas's study indicated no significant effects on the main pharmacokinetics parameters (C_{max} AUC and $t_{1/2}$) of oral amlodipine. The present study indicated that genetic specific polymorphisms had no significant effects on the main pharmacodynamic parameters of nifedipine controlled-release tablets.

In vitro and in vivo experiments have shown that nifedipine was confirmed as the substrate of the BCRP transporter [28–32]. However, no clinical data have been published to evaluate the effects of BCRP polymorphism on the pharmacokinetics of nifedipine controlled-release, and only studies on felodipine have been conducted. The current study indicated that the BCRP rs2231142 homozygous variant exhibited lower AUC_{0-t}/DW (954.08 ± 109.79 vs. 1262.10 ± 522.87 kg*ng*h/ml*mg), $AUC_{0-\infty}$/DW (988.30 ± 85.15 vs. 1304.00 ± 534.17 kg*ng*h/ml*mg) compared to the wild-type genotype.

The in vivo binding receptor of the CCB is located at the α1 subunit of the voltage-dependent L-type calcium channel of the cell membrane. α1C and α1D are widely expressed in excitable cells of the cardiovascular system, such as the myocardium, vascular smooth muscle, brain and other tissues. In the cardiovascular system, α1C is the most common α1 subtype [33], and previous studies have suggested that it is the main target of CCB to regulate cardiovascular function and reduce blood pressure. α1D may control certain physiological processes [34].The SNPs of the voltage-dependent L-type calcium channel α1C gene, CACNA1C rs2238032 [35–37], rs2239050 [17, 37] and of the α 1D gene CACNA1D rs312481 [37] exhibited a significant correlation with the antihypertensive efficacy of CCB [38]. The L-type calcium channel α1 subunit is the main target, and its gene polymorphism has been shown to be correlated with the antihypertensive efficacy of CCB. The present study explored whether the latter was correlated with the pharmacokinetics of nifedipine controlled-release. The data indicated that the Vd/F of the CACNA1D rs312481 G/G carriers was lower than that of the A/G subjects (8.40 ± 3.33 vs. 12.99 ± 5.67 L/kg), whereas the $t_{1/2}$ followed the same pattern (6.39 ± 1.45 vs. 8.60 ± 3.23 h). The t1/2 of the CACNA1C rs2238032 T/G subjects was lower than that of the T/T subjects (6.71 ± 2.41 vs. 6.97 ± 1.95 h). Therefore, it was speculated that the CACNA1D rs312481 and CACNA1C rs2238032 polymorphisms may affect nifedipine controlled-release pharmacodynamics by affecting its pharmacokinetics. Additional research is required to confirm these findings.

The sympathetic nervous system plays an important role in the pathogenesis of essential hypertension, mainly through the action of catecholamines on G protein-coupled α and β adrenergic receptors, which stimulates adrenergic receptors in blood vessels and leads to vasoconstriction [39, 40]. Both calcium ions released from intracellular storage and flowing through membrane calcium channels are involved in activation of the α1A adrenergic receptor. α1A-adrenergic receptors have been shown to selectively activate Ca^{2+} influx through dihydropyridine-sensitive channels in smooth muscle cells [41, 42]. It has also been shown that nifedipine can inhibit 85% of α 1A-adrenaline-receptor-mediated contraction of the smooth muscle ring of the porcine renal artery [43]. A previous study [44] conducted multivariable linear regression analysis on 447 patients. It was found that the ADRA1A rs1048101 polymorphism influenced the hypotensive effect of nifedipine controlled-release. In view of the association between adrenoceptor and nifedipine controlled-release, the effects of the adrenoceptor gene polymorphism on the pharmacokinetics of nifedipine controlled-release were investigated. The present results indicated that the ADRA1A rs1048101 polymorphism did not affect the metabolism of nifedipine controlled-release. Therefore, it is not assumed that gene polymorphisms affect pharmacodynamics by influencing pharmacokinetics, and further research is required to confirm this hypothesis.

Angiotensin converting enzyme (ACE) is a key enzyme in the renin-angiotensin-aldosterone system (RAS), which catalyzes the conversion of angiotensin to angiotensin with angioconstrictive activity, and plays an important role in the regulation of blood pressure. The ACE rs4646994 polymorphism, which is located in region 16 of the intron of the gene can significantly affect ACE levels in vivo. The effects of the CCB on RAS have been previously reported. It is believed that the vasoconstriction mechanism of Ang depends on the cellular calcium level, and its blood pressure boosting reaction requires calcium. Therefore, CCB blocks the vasoconstriction effect of Ang by reducing cellular calcium, and may inhibit RAS activity, and directly reduce the levels of Ang [45].Cross therapy with enalapril and felodipine was performed on essential hypertension patients [46], and it was found that the antihypertensive effect of ACEI on subjects with the D allele was more apparent than that of the CCB, whereas the antihypertensive effect of the calcium antagonist on type II patients was more apparent than that of ACEI. Seremak-Mrozikiewicz et al. [47] demonstrated that the angiotensin receptor type 1, AT1R rs5186 polymorphism was associated with the effects of the calcium antagonists in alleviating arteriosclerosis. The present study [47] compared the decrease of pulse wave conduction velocity in the posterior carotid femoral artery and indicated that ACEI antihypertensive drugs were more sensitive to individuals carrying the C than the A allele, while CCB demonstrated only antihypertensive effects on AA patients. In the present study, the genetic polymorphisms ACE rs4646994 and AT1R rs5186 reduced the metabolic activity of ACE, which was manifested as a higher C_{max}/DW, AUC_{0-t}/DW, and $AUC_{0-\infty}$/DW, and as a lower Cl/F. Therefore, it was speculated that the ACE rs4646994 and the AT1R rs5186 polymorphisms may affect nifedipine controlled-release pharmacodynamics by affecting its pharmacokinetics.

Nifedipine exhibits diuretic and natriuretic effects, which can enhance its antihypertensive effect [48–53]. It is important to evaluate the polymorphism of the urea transporter A gene (SLC14A2) in order to assess the inter-individual variation of nifedipine controlled-release pharmacokinetics, while no relevant study has examined these associations to date. The results of the present study indicated that the SLC14A2rs3745009 polymorphism was involved in nifedipine metabolism. Specifically, heterozygous carriers could accelerate drug metabolism, while homozygous carriers could reduce the rate of drug metabolism. Statistically significant effects were noted on the main pharmacokinetics parameters (C_{max}, AUC, $t_{1/2}$ and Cl/F). The SLC14A2 rs1123617 polymorphism

exhibited a tendency to accelerate the metabolism, as determined by Cmax, AUC and Cl/F. This is the first study that explored the association of the SLC14A2 variant with the pharmacokinetics of nifedipine controlled-release tablets. Hong's study [54] investigated 405 subjects receiving a single oral administration of nifedipine sustained-release tablets, and found that the SLC14A2 polymorphisms were significantly correlated with the pharmacodynamics of nifedipine. However, this effect was not noted with the pharmacokinetic parameters. The present study further investigated the effects of SLC14A2 gene polymorphisms on drug pharmacokinetics. We speculated that the SLC14A2 rs1123617, rs3745009 polymorphisms may affect nifedipine pharmacodynamics by affecting its pharmacokinetics.

In terms of safety, no severe ADRs were noted, probably due to the administration of a single dose of nifedipine, which was lower than that used in clinical practice. The results obtained for the ADRB2 rs1042713 polymorphism were related to the ADR. The volunteers who suffered at least one adverse reaction were associated with the ADRB2 rs1042713 polymorphism (12.5% of G/G subjects, 61.11% of A/G subjects and 42.8% of A/A subjects; $p = 0.038$). An association between the CYP3A5 rs776746 polymorphism and the development of headache and dizziness was noted (50% of A/A subjects, 14.3% of A/G subjects and 50% of G/G subjects; $p = 0.002$).

The main limitation of our study is the low sample size that limits the adequate evaluation of the main genes, such as CYP3A5, ABCB1, and BCRP. Moreover, the current study was performed after a single-dose administration to healthy volunteers, that did not allow the assessment of long-term effectiveness and safety. Further basic experiments are required to verify the conclusion drawn on the most significant gene polymorphism SLC14A2 rs3745009.

5. Conclusion

The pharmacokinetics of nifedipine controlled-release tablets is affected by sex and the presence of gene polymorphisms. The CYP3A4 rs2242480, ABCB1 rs1045642, CACNA1D rs312481, CACNA1C rs2238032, ACE rs4646994, and SLC14A2 rs3745009 genetic polymorphisms significantly influenced the pharmacokinetics of nifedipine controlled-release tablets, with a greater effect noted on CACNA1D, CACNA1C, and SLC14A2. These results were statistically significant following multivariate correction. It was speculated that the CACNA1D rs312481, CACNA1C rs2238032, ACE rs4646994 and SLC14A2 rs3745009 polymorphisms may influence the pharmacodynamics by affecting the pharmacokinetics of this drug. In addition, a tendency was noted to present more differences in pharmacokinetics between different genotypes within the CYP3A5 rs776746, BCRP rs2231142, AT1R rs5186 and SLC14A2 rs1123617 polymorphisms. However, the results were not statistically significant. Following adjustment for the dosage of the nifedipine controlled-release tablets according to the pharmacogenetic profile of each subject, the influence of CACNA1D CACNA1C, ACE, SLC14A2 genetic effects should be considered in addition to the polymorphisms of the traditional metabolic enzymes CYP3A4/5, ABCB1, and BCRP. Among them, the most important gene was SLC14A2, which could accelerate drug metabolism in its heterozygous state, while the homozygous carrier could reduce the rate of metabolism. The incidence of adverse reactions was related to several genes as follows: At least one ADR was associated with the ADRB2 rs1042713 polymorphism, whereas headache and dizziness were associated with the CYP3A5 rs776746 polymorphism. Therefore, certain pharmacogenetic factors must be taken into account in order to improve nifedipine controlled-release tablets therapy, thus enabling a better treatment outcome and safety profile.

Declarations

Ethics approval and Consent to participate

Ethical approval document no. :(2020) YX (104). All volunteers signed informed consent.

Consent for publication

Not applicable

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Authorship

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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Tingting Zhi: Blood collection, Project administration; Hongxia Wang: Blood collection, Project administration; Yuanyuan Zhu: Formal analysis-Equal, Supervision-Equal; Zhiqing Yao: Datacuration-Equal, Writing-original; Yanhui Wang: Datacuration-Equal, Writing-original; Huizi Zhang: Datacuration-Equal, Writing-original; Ruiqin Zhang(Corresponding author ：Conceptualization-Equal, Formal analysis-Equal, Writingreview &editing-Supporting; Xiaoru Wang Corresponding author Conceptualization-Equal, Formal analysis-Equal, Writingreview & editing-Supporting.

Competing Interests

The authors declare that there are no conflicts of interest.

Disclosures

Zhen Li, Siyang Wang, Jian Ren, Tingting Zhi, Hongxia Wang, Yuanyuan Zhu, Zhiqing Yao, Yanhui Wang, Huizi Zhang, Ruiqing Zhang, Xiaoru Wang declare that they have no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this published article.

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Figures

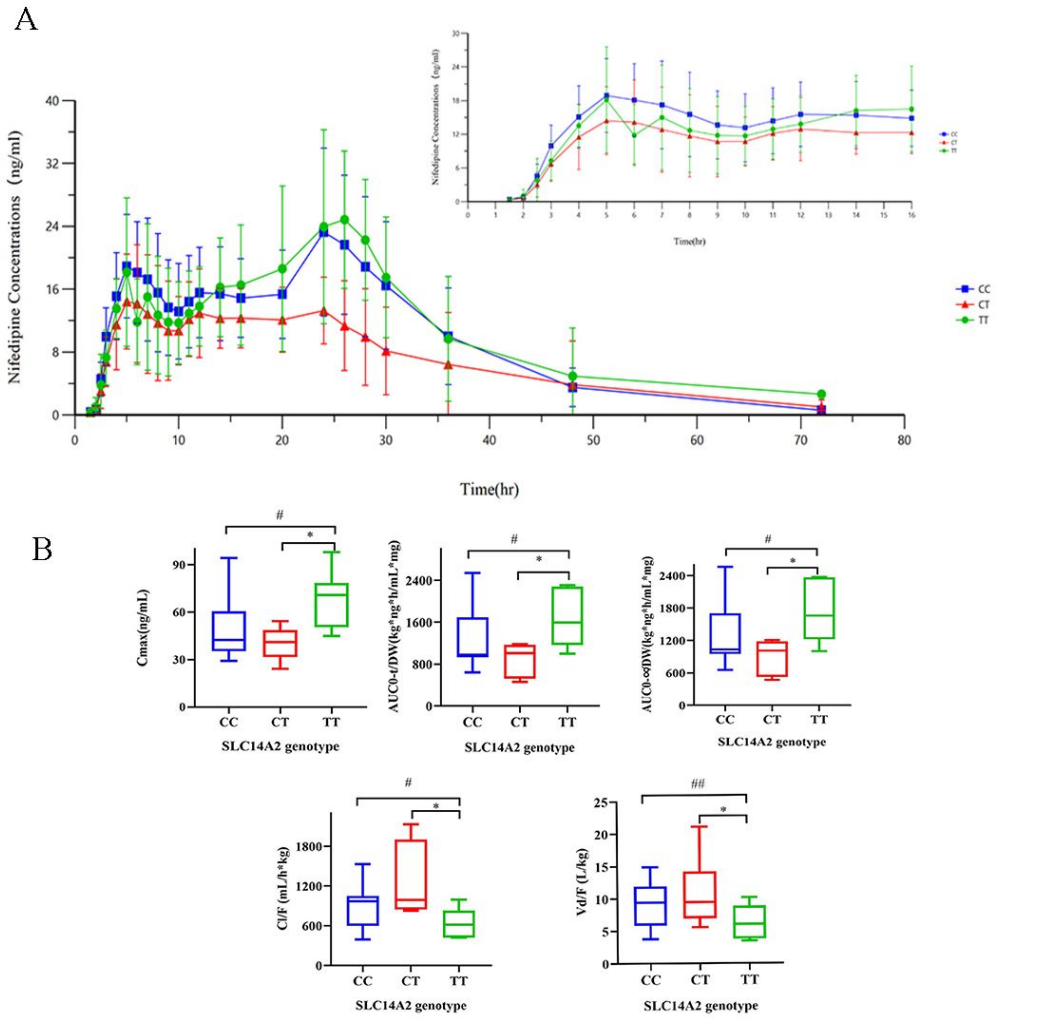

Figure 1

(A) Nifedipine controlled-release tablets concentration-time curve according to the SLC14A2 rs3745009 genotypes. (B) Pharmacokinetic parameters in different SLC14A2 rs3745009 genotypes. The bottom and top of each box represent the first and third quartiles, and the band inside the box corresponds to the second quartile (the median). Whiskers extend to the maximum and minimum values of the series. The differences between TT with CC, and CT are plotted with a warning or a star, respectively in order to highlight statistical significance.