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Evaluation of pharmacokinetics and safety with bioequivalence of Amlodipine in healthy Chinese volunteers: Bioequivalence Study Findings

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Abstract

Background and Objective: Amlodipine, a main series of L-type calcium channel blockers (CCBs), exerts potent antihypertensive effects. The aim of this trial was to explore the pharmacokinetics (PK) and safety with bioequivalence of orally administered Amlodipine provided by two sponsors in healthy volunteers (HVs).

Methods: Two separate randomized, open-label, single-dose, crossover-design studies were conducted: a fasting study (n = 24) and a fed study (n = 24). In each study, HVs were randomized to Fangming Pharmaceutical Group (Test, T) followed by NORVASC® (Reference, R), or vice versa. Each study subject received a 5-mg Amlodipine tablet with a 15-day washout. The plasma concentrations of Amlodipine were measured using a LC-MS/MS method, and PK parameters were determined by noncompartmental model.

Results: Forty-eight healthy volunteers were enrolled**.** And overall demographics were as follows: the fasting study: female (n = 16/24), age (18-54 years), weight (47-76 kg), and BMI (19.5-26.0). The fed study: female (n = 16/24), age (20-49 years), weight (45.5-69 kg), and BMI (19.1-25.0). All PK endpoints met the pre-specific criteria for PK equivalence. In fasting subjects, the maximum plasma concentration (C_{max}) was 3.881 ± 0.982 ng/mL at 6 hours (median) of sponsor T, and 4.042 ± 1.147 ng/mL at 6 hours (median) of sponsor R. In fed subjects, C_{max} was 3.312 ± 0.789 ng/mL at 6 hours (median) of sponsor T, and 3.392 ± 0.902 ng/mL at 5 hours (median) of sponsor R. Both fasting and fed studies achieved a plausible bioequivalence.

Conclusions: Amlodipine is well tolerated and have a favorable safety profile. The observed adverse events were mild (the severity was assessed according to the Common Terminology Criteria for Adverse Events [version CTCAE4.03]) and all of them were recovered without severe sequences. And the bioequivalence is achieved under fasting and fed conditions, supporting the demonstration of biosimilarity.

KEYWORDS

Amlodipine, bioequivalence, healthy Chinese volunteers, pharmacokinetics, safety

Tongtong Wang and Yannan Wang are contributed equally to this work.

Trial registration: ClinicalTrials.gov YZD-CO-BE-ALDPT-005.

1 | INTRODUCTION

Amlodipine is a main series of long-acting dihydropyridine calcium channel blocker (CCBs), extensively used in the hypertension treatment. It exerts significant cardioprotective effects by regulating endothelial functions and smooth muscle. On account of its high selectivity for peripheral vascular system, it has little effect on atrioventricular node conduction and myocardial contractility.[1] Some studies have revealed that Amlodipine plays an effective role in preventing progression of arteriosclerosis and avoid a stroke,[2-4] as well as ameliorating primary hypertension cardiovascular complications through repressing sympathetic nervous system hyperactivity and aggrandizing parasympathetic activity. Through above effects, Amlodipine decrease the risk for cardiovascular disease (CVD).[5]

Hypertension still remains to be one of the main single factors to global mortality.[6] In addition, most investigation from globe reveals that the blood pressure (BP) of the vast majority of diagnosed as hypertension is not well controlled to BP targets currently recommended.[7,8] Insufficient use of the antihypertensive agent is a contributor to this deficient administration of raised BP.[9]

The prevalence of hypertension has been increasing in China for decades, and reached 18.8% in the year 2002. According to the structure of population in 2006, about 2 millions of hypertensive patients at present, 2 out of every 10 adults have high BP. Accounting for about one-fifth of high BP worldwide. The rates of awareness, treatment and control for hypertension patients remain low compared to high-income countries, in spite of substantial improvements since 1991, less than 50%, 40%, and 10%, respectively.[10] Hypertension incidence in China has a huge increase, which has brought great economic burden on the government, society, and individual patients. So it is imperative to develop own antihypertensive agent.

Amlodipine was first developed by Pifzer Company and listed in the UK in January 1990 and approved by the US Food and Drug Administration (FDA) in 1992 under the trade name NORVASC. In 1991, its tablet was registered in China, registration number is X910016, X910049, X9150050. After the patent protection period of the primary product, bioequivalence (BE) studies have been claimed to compare and research the pharmacological features and safety of the drugs with different sponsors. BE studies date are extremely essential, so as to confirm the treatment similarity between two agents containing similar active ingredients. Normally, bioequivalence is determined by contrast the extent and rate of absorption of different agents under study (Test, T) with the primary product (Reference, R).[11] To this end, investigating the bioequivalence between two products, the FDA claims that the ratio of the two formulation averages (μT/μR) of PK parameters of concern should situate between some rational limits (eg [80, 125%]), with certain guarantee.[11] Fasting and fed studies are recommended to conduct in healthy volunteers by FDA and CFDA for Amlodipine bioequivalence study.

The purpose of our study was to evaluate the safety and PK parameters of Amlodipine. Meanwhile, to compare the bioequivalence of two 5-mg Amlodipine tablets acquired from one different sponsors (T) and NORVASC® (Reference, R) in both fasting and fed conditions.

TABLE 1 The pharmacokinetic parameters of Amlodipine in each study (geometric mean [CV%])

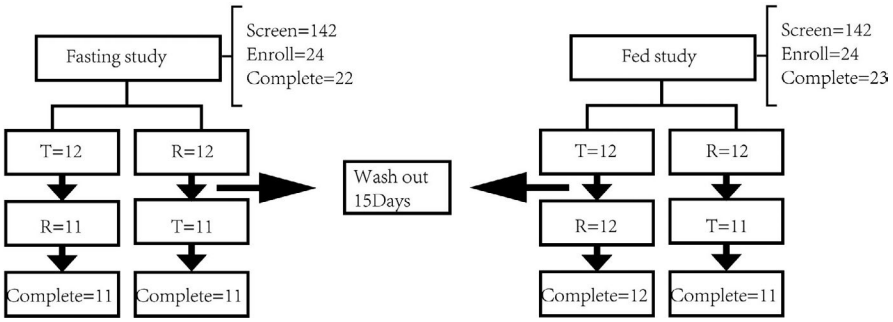
Sponsor	Study	Food	Med	T_{max}[a] (h)	C_{max} (ng/mL)	$AUC_{0\text{-}t}$ (h^ang/mL)	$AUC_{0\text{-}\infty}$ (h^ang/mL)	λz (1/h)	$T_{1/2z}$ (h)
1	1	Fasting	T	6.00 (5.00, 7.00) (14.32%)	3.881 ± 0.982 (25.291)	158.135 ± 57.639 (36.449)	177.053 ± 69.439 (39.219)	0.017 ± 0.004 (20.285)	41.582 ± 8.706 (20.937)
2	1	Fasting	R	6.00 (5.00, 8.00) (16.45%)	4.042 ± 1.147 (28.379)	158.370 ± 49.567 (31.298)[b]	174.555 ± 56.299 (32.253)[b]	0.018 ± 0.004 (20.826)[b]	40.010 ± 8.763 (21.901)[b]
1	2	Fed	T	6.00 (4.00, 16.00) (38.509)	3.312 ± 0.789 (23.824)	145.628 ± 37.418 (25.695)[b]	162.113 ± 44.294 (27.323)[b]	0.017 ± 0.004 (24.956)[b]	42.045 ± 9.807 (23.325)[b]
2	2	Fed	R	5.00 (2.00, 8.00) (32.092)	3.392 ± 0.902 (26.578)	153.699 ± 41.007 (26.680)	171.485 ± 48.360 (28.201)	0.017 ± 0.004 (20.453)	42.143 ± 9.194 (21.816)

Note: $AUC_{0\text{-}t}$, area under the concentration-time curve from 0 h to the last time point; C_{max}, maximum plasma concentration; $AUC_{0\text{-}\infty}$, area under the concentration-time curve from 0 h to infinity; $t_{1/2}$, elimination of terminal half-life; λz, elimination rate constant. The fed study: the $AUC_{\_\%Extrap}$ of 1 subject in the first period was greater than 20%, and the PK evaluation indicators: $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $T_{1/2z}$, and λz were not descriptive statistical analysis.

[a]The Median (The Maximum, the Minimum).

[b]The fasting study: the $AUC_{\_\%Extrap}$ of two subjects in the second period was greater than 20%, and the PK evaluation indicators: $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $T_{1/2z}$, and λz were not descriptive statistical analysis.

FIGURE 1 The flow chart of the study



2 | MATERIALS AND METHODS

2.1 | Study design

Bioequivalence studies of Amlodipine were conducted to compare the bioequivalence of 5-mg Amlodipine tablets from Fangming sponsors (T) and NORVASC® (Reference, R). Two separate trials were conducted. Both fasting and fed studies were single-center, randomized, open-label, single-dose, two–period, and crossover designs. Forty-eight healthy adult volunteers were enrolled and assigned to each study. Fasting study (n = 24) and fed study (n = 24) were conducted to determine the bioequivalence of Amlodipine from T and R products. In each study, half volunteers (n = 12) were randomized to treatment sequences (T-R or R-T), the other way around, based on the randomization plan. Volunteers were taken medicine at the same time on day 1 and 16 in two studies. The plasma clearance of Amlodipine was biphasic, that terminal elimination half-life of Amlodipine in healthy subjects was about 35 ~ 50 hours, the results were the same in our trials (Table 1), in accordance with the demand of not <7 half-lives, so there was a 15-day washout period between each single dosing[12] (Figure 1).

The trial design was approved by the Ethics Committee at the Zhejiang provincial people's hospital, Hangzhou City, China. The clinical study (registration No.: YZD-CO-BE-ALDPT-005) was carried out in accordance with the declaration of Helsinki, Good Clinical Practice (GCP) principle, Chinese laws and regulations. All participants signed the informed consent. The trial was conducted at the Zhejiang provincial people's hospital-Phase I Clinical Research Center.

2.2 | Inclusion and exclusion criteria

The volunteers enrolled in this trial were recruited following the eligibility and exclusion criteria strictly. The inclusion criteria were as follows: healthy Chinese individuals of either gender (single sex account for more than one-third of the total), age between 18 and 65 years old, weight ≥50 kg for men, ≥45 kg for women, BMI ranged from 19.0 to 26.0, subjects were willing to use effective contraceptives without pregnancy plan during the trial and within 3 months after the last dose; fully understand the informed consent, test content, process, and possible adverse reactions. The main exclusion criteria were as follows: clinically significant abnormal laboratory examination and special inspection, such as electrocardiogram (ECG); abnormal vital signs and vascular conditions; regular use of alcohol, tobacco, prescription, nonprescription drugs, or citrus fruit juices; previous history of hypotension and allergic to drug ingredients; drug test is positive or alcohol breath test >0 mg/100 mL; participating in other clinical research in the previous three months; lactating and pregnant females.

The eligible volunteers were required to stay in Phase I Clinical Research Center for 24 hours before agent administration and stayed 48 hours after drug administration. Subjects fasted at least 10 hours then taken T or R drug according to the random numbers.

For the fed study, subjects were directed to take high-fat food 30 minutes pre-administration. Volunteers were continuously observed by research investigators throughout the study period. Both fasting and fed study, vital signs (including blood pressure, pulse, and temperature) were monitored at time 0 (within 1 hour before administration) and 2.0, 4.0, 6.0, 12.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0 hours in studies 1 and 2.

2.3 | Estimation of sample size

According to the FDA BE guidelines, Amlodipine is not a highly variable agent. Previous studies have shown that the coefficient of variation (CV) of maximum plasma concentration (C_{max}), area under the concentration-time curve from time zero to last measurable concentration ($AUC_{0\text{-}t}$), and area under the concentration-time curve from time zero to infinity ($AUC_{0\text{-}\infty}$) is about 10% ~ 18%. Conservatively, in this research, the intra-subject variability (intra-CV) is estimated to be 18%, wherein at least 21 valid cases are needed calculating by software. Considering 10% maximum shedding rate, 24 qualified subjects should be enrolled at least.

2.4 | Pharmacokinetic (PK) assessments

Both fasting and fed study, blood samples for PK analysis were collected after oral agent at the following time points: 0, 1.0, 2.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 12.0, 16.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0 hours post-dose. Indwelling needle was placed on subject's forearm and all blood samples were drawn from this. The first 1 mL of blood sample was discarded. 4 mL blood samples were collected into K2-ethylenediaminetetraacetic (EDTA-K2) acid tubes and subsequently chilled on ice water. All blood samples were centrifuged at 2000 *g* and 4°C for 10 minutes. Then, blood samples were divided

into two polypropylene tubes and stored at −70°C ± 10°C for analysis.

2.5 | Safety assessments

Safety and tolerability were evaluated at screening period, administration period, and follow-up period, including adverse events (AEs), vital signs (temperature, blood pressure, and heart rate), clinical laboratory evaluations (blood routine, urine routine, blood biochemistry), 12-lead ECG, and pregnancy tests. All AEs were recorded immediately by the clinical research physician, and the relationship and severity to drug were evaluated for each AE.

2.6 | Pharmacokinetic and statistical analysis

The pharmacokinetic analysis for both study was performed by SAS 9.4 statistical package and using the noncompartmental analysis model. Pharmacokinetic parameters for Amlodipine included C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, time of maximum plasma concentration (T_{max}), $t_{1/2,}$ percentage of residual area ($AUC_{\_\%Extrap}$, calculated by ([$AUC_{0-\infty}$-AUC_{0-t})/$AUC_{0-\infty}$)×100%], apparent gross clearance (CLz/F, calculated by dosage/ $AUC_{0-\infty}$) and apparent volume of distribution (Vd/F, calculated by CL/F/λz). Descriptive statistics were counted for pharmacokinetic parameters. C_{max}, AUC_{0-t} and $AUC_{0-\infty}$were used as criteria for bioequivalence determination. The bioequivalence was defined if the 90% confidence interval (CI) was within the acceptance limits of 80.00%-125.00%.

Before clinical organism analysis, method validation was required. According to “Guiding Principles for Validation of Quantitative Analysis Methods for Biological Samples”(Edition 2015) requirements, International technical guide and laboratory SOP, carry on method validation. The LC-MS/MS method was used to measure the plasma concentrations of Amlodipine.

When unknown test sample was analyzed, an analysis batch included blank sample, zero concentration sample, standard sample (at least six concentration levels), quality control sample (at least three concentration levels), and unknown test sample. All samples were processed and extracted in the same sample batch in sequence, and quality control samples were distributed throughout the whole batches. Ensure the accuracy and precision of entire analysis batches.

Reanalysis of test samples: After completing the detection of the unknown test samples, the test samples were re-analyzed in another analysis batch for evaluation accuracy of actual sample measurement.

The concentration-time curve of Amlodipine was summarized according to the blood concentration of each subject measured in the experiment. The statistical calculations were performed for each sampling time, including sample size, arithmetic mean, standard deviation, and so on. If plasma concentration is lower than Lower Limit of Quantification (LLOQ), before T_{max} is treated as "0," after T_{max}, not involved in the calculation.

3 | RESULTS

3.1 | Subject baseline characteristics

Forty-eight healthy subjects were enrolled, 24 subjects were assigned to fasting study and the rest were fed study. The baseline characteristics and demographic of all subjects are shown in Table 2.

3.1.1 | Fasting study

Twenty-four participants were randomly assigned to take T or R drug. Twenty-two completed the study and two participants were withdrawn due to adverse events (AE) and personal reasons.

3.1.2 | Fed study

Twenty-four participants were randomly assigned to take T or R drug. Twenty-three completed the study and two participants were withdrawn due to adverse events (AE). All participants received high-fat diet pre-administration in this study.

3.2 | Pharmacokinetics

3.2.1 | Plasma concentration-time profiles

Fasting study

The Amlodipine concentrations of plasma samples that represented quite low were recorded as zero before the $C_{max.}$ And the Amlodipine plasma concentration-time profiles and the semi-logarithm figure were illustrated in Figure 2A-B. And the plasma concentration-time profile of each subject after administration was shown in Figure 3A-B. The Amlodipine plasma concentrations increased slowly in all study samples and got to the a C_{max} of 3.881 ± 0.982 ng/mL at 6 hours (median)

TABLE 2 The demographic characteristics (mean ± standard deviation) of participants in this study

Study	n	Age(y)	Body weight (kg)	Height (cm)	BMI	Gender Male [n(%)]	Gender Female [n(%)]
Fast study	24	30.8 (9.41)	61.13 (8.384)	165.54 (6.674)	22.24 (2.09)	16 (66.7)	8 (33.3)
Fed study	24	33.0 (9.13)	61.29 (6.041)	164.54 (6.097)	22.60 (1.548)	16 (66.7)	8 (33.3)

Abbreviations: BMI, body mass index; SD, standard deviation.

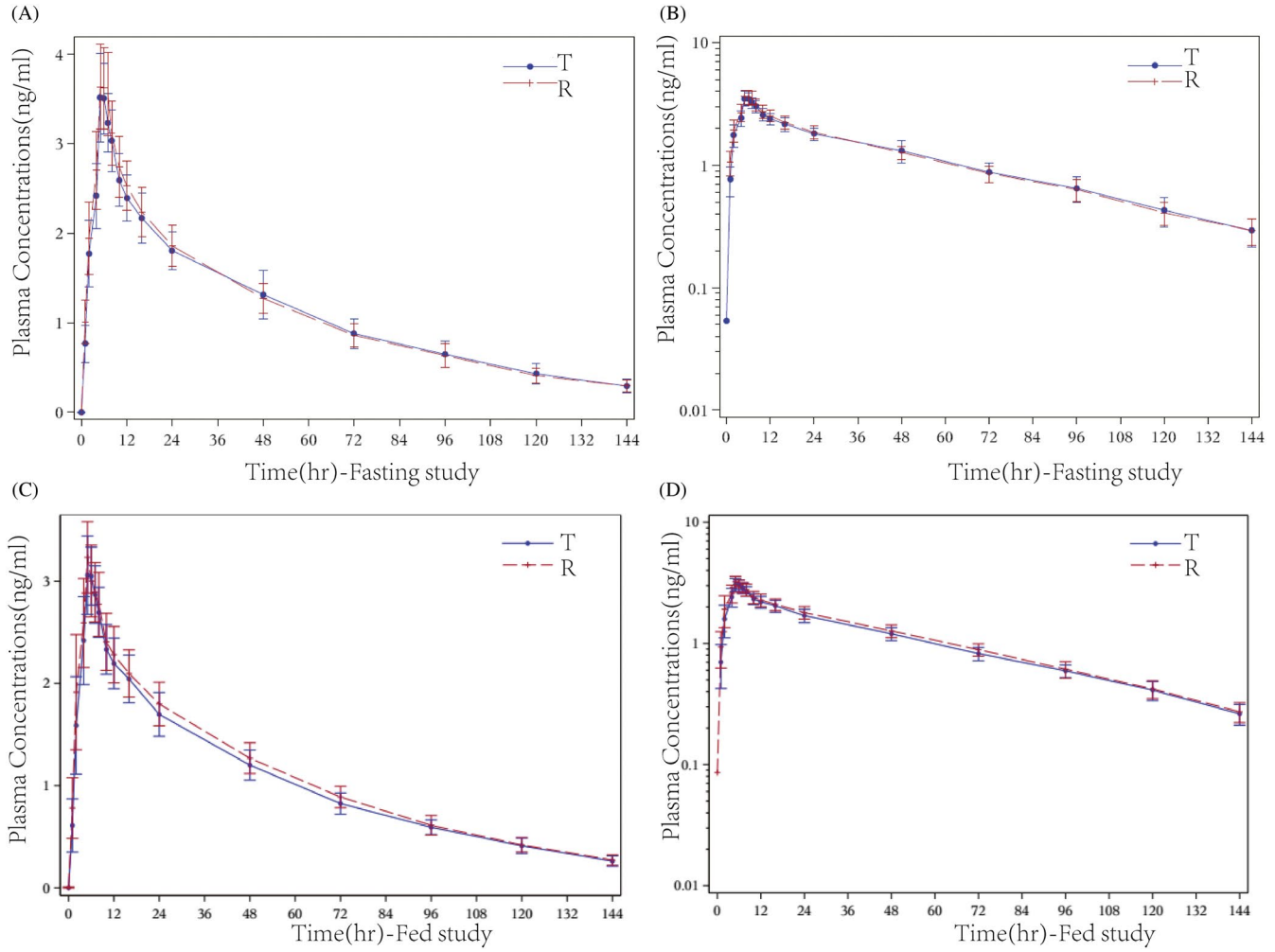


FIGURE 2 Mean plasma concentration-time profiles. A, Mean plasma concentration-time plots for Amlodipine following single oral doses in fasting study. B, Mean plasma concentration-time plots for Amlodipine following single oral doses in fasting study (semi-logarithmic graph). C, Mean plasma concentration-time plots for Amlodipine following single oral doses in fed study. D, Mean plasma concentration-time plots for Amlodipine following single oral doses in fed study (semi-logarithmic graph)

of sponsor T, and 4.042 ± 1.147 ng/mL at 6 hours (median) of sponsor R. The lower limit of plasma concentration was 0.292 ± 0.174 ng/mL at 144 hours of sponsor T, and 0.294 ± 0.163 ng/mL at 144 hours of sponsor R. The plasma concentrations of two sponsors represented a decline in two-mode, that initially declined quickly, then showed a slight decline and the geometric mean of $t_{1/2}$ was arrive at 41.582 ± 8.706 hours of sponsor T, 40.010 ± 8.763 hours of sponsor R.

From Figure 3A-B, Plasma Concentration-Time Profile of 1015 was different from others. According to original data, the plasma concentration of 1015 at every time point was higher than others in both T and R trails. No special operation in the whole process. This slight deviation is mainly due to the certain subject variation, and the results were taken into the per-protocol set (PPS).

3.2.2 | Fed study

The Amlodipine plasma concentration-time profiles and the semi-logarithm figure were also illustrated in Figure 2C-D. And the plasma concentration-time profile of each subject after administration was shown in Figure 3C-D. Plasma concentrations increased slowly and reached C_{max} of 3.312 ± 0.789 ng/mL at 6 hours (median) of sponsor T, and 3.392 ± 0.902 ng/mL at 5 hours (median) of sponsor R. The lower limit of plasma concentration was 0.262 ± 0.117 ng/mL at 144 hours of sponsor T, and 0.273 ± 0.121 ng/mL at 144 hours of sponsor R. Plasma concentrations represented a decline in a bi-phasic mode. $t_{1/2}$ was arrive at 42.045 ± 9.807 hours of sponsor T, 42.143 ± 9.194 hours of sponsor R.

3.3 | Assay validation results

In our experiment, the LOQ was 0.05 ng/mL. Assay validation investigated the cycle stability of freezing-thawing (five times, −60°C refrigerator storage, wet ice yellow light melting) and long-term stability (for 25 and 60 days), the results were stable.

In fasting study, assay validation revealed that the range of quality control samples precision for each concentration (%CV) was ≤9.3%, the accuracy deviation range of each quality control samples (%) was −6.7% ~ −3.7%.

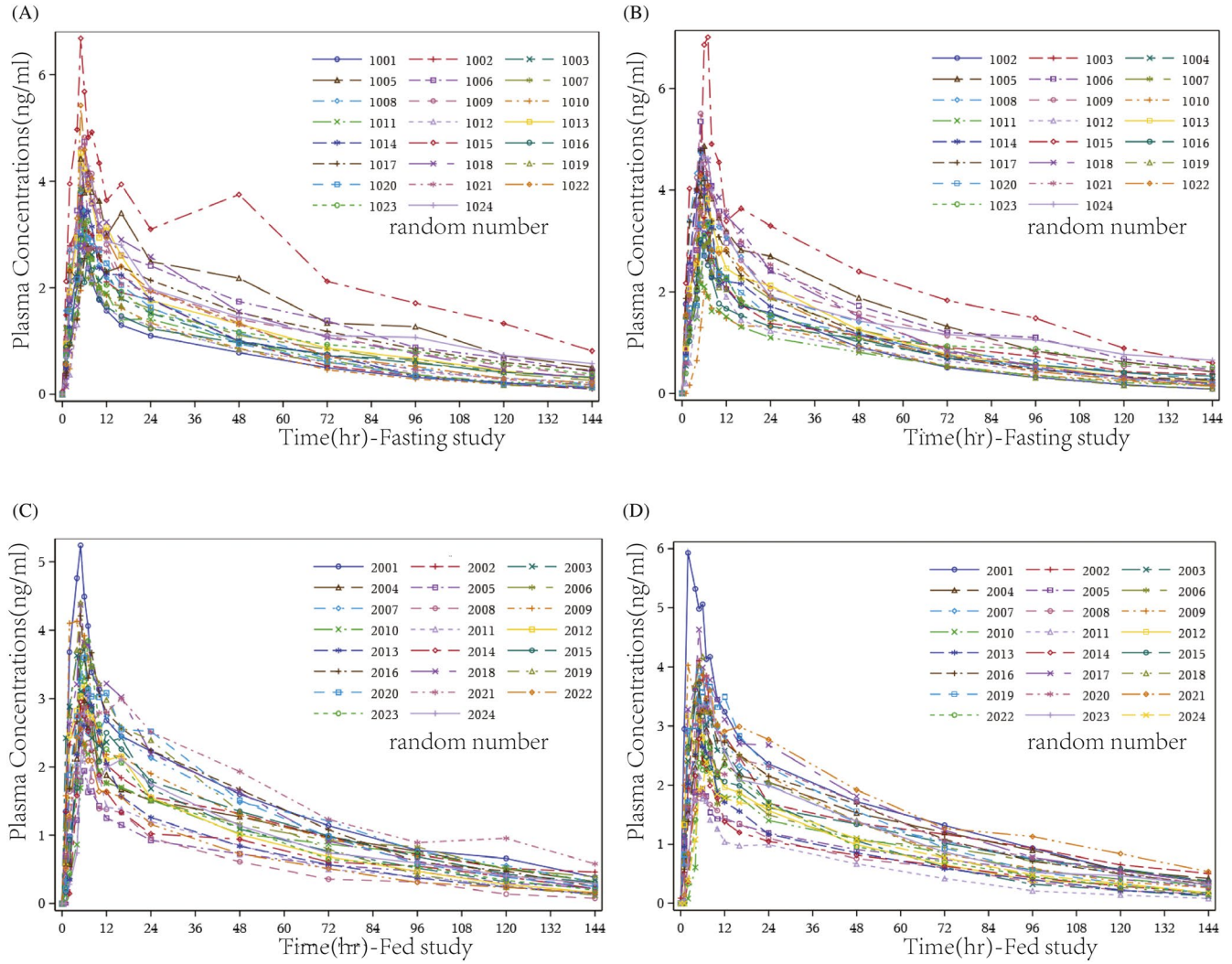


FIGURE 3 All subjects enrolled mean plasma concentration-time profiles. A, All subject plasma concentration-time plots for T following single oral doses in fasting study. B, All subjects plasma concentration-time plots for R following single oral doses in fasting study. C, All subjects plasma concentration-time plots for T following single oral doses in fed study. D, All subjects plasma concentration-time plots for R following single oral doses in fed study

In fed study, assay validation revealed that the range of quality control samples precision for each concentration (%CV) was ≤9.1%, the accuracy deviation range of each quality control samples (%) was −6.2% ~ −3.3%.

The order of quality control samples were evenly distributed among samples.

3.4 | Pharmacokinetic parameters of Amlodipine in two studies

The pharmacokinetic parameter analysis for both studies was conducted via noncompartmental analysis module. The main pharmacokinetic parameters of Amlodipine such as C_{max}, T_{max},

TABLE 3 Factors affecting pharmacokinetic parameters (analysis of variance after logarithmic transformation)

	P					
	Fasting			Fed		
Main Factors	Ln (C_{max}) (ng/mL)	Ln (AUC0-t) (h*ng/mL)	Ln (AUC0-∞) (h*ng/mL)	Ln (C_{max}) (ng/mL)	Ln (AUC0-t) (h*ng/mL)	Ln (AUC0-∞) (h*ng/mL)
Administration sequence	0.8443	0.7241	0.5109	0.9082	0.4187	0.3639
Administration period	0.0010	0.1522	0.1283	0.2114	0.0612	0.0868
Formulation factor	0.5400	0.7958	0.9291	0.9792	0.3613	0.4842

$t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$ and elimination rate constant (λz) are represented in Table 1. AUC_{0-t} occupied more than 90% of the $AUC_{0-\infty}$ in both studies, revealing that the plasma concentration-time profiles were well described. The coefficient of variation (CV) values of pharmacokinetic parameters for R product and T product were similar.

No significant differences were found in either absorption or elimination proportion of R or T formulation of Amlodipine, as shown by analogous values for different pharmacokinetic parameters in both fasting and fed studies. In addition, we analyzed the relevant factors that affect pharmacokinetic parameters including administration sequence, administration period and formulation factor both in fasting and fed studies. In fed study, as to administration sequence, administration period and formulation factor, *P* value were all above .05, no statistical difference was found. However, in fasting study, the *P* value of administration period about C_{max} was .001, with statistical significance.

We comprehensively analyzed the entire test process, and there were no obvious abnormalities in subjects' medication cycle, administration sequence, medication order, medication method, sampling sequence, blood sample processing, transportation, and sample analysis. After review data, we found the plasma concentration of subject 1015 at 0 hour of the second period (before administration) was 0.054 ng/mL, which was higher than LOQ. This means subject 1015 had residues after the washout period, leading to period effect. This also suggests it is necessary to prolong washout period in follow-up experiments. Results are shown in Table 3 (Analysis of variance after logarithmic transformation).

3.5 | Bioequivalence analysis

Both fasting and fed studies achieved bioequivalence. Table 4 represented the 90% confidence intervals (CIs) for the rate of the logarithmical conversion pharmacokinetic parameters of Amlodipine. Fortunately, both studies, all 90% CIs satisfied the bioequivalence criteria.

3.6 | Tolerability and safety analysis

Amlodipine was generally well tolerated in both fasting and fed studies. In fasting study, two subjects withdrew from the program, one for personal reasons and the other withdrew for upper respiratory tract infection. In fed study, one subject withdrew for upper respiratory tract infection. A total 23 AEs were recorded during execution of studies. Eight of these AEs were found to be related to fasting study, and 15 of these AEs were found to be related to fed study. In fasting study, 5 of these AEs were related to T formulations, and the other were related to R formulations. Only 1 AE was definitely related to the T product. The rest AEs were considered unrelated to the drug. In fed study, 4 of these AEs were related to T formulations, and the other were related to R formulations. All AEs were considered unrelated to the drug.

TABLE 4 Bioequivalence assessment summary

	Fasting							Fed					
Pharmacokinetic parameters	T	R	(T/R)%	In vivo variative (%CV)	90% Confidence interval (CI)	Power%		T	R	(T/R)%	In vivo variative (%CV)	90% Confidence interval (CI)	Power%
C_{max}(ng/mL) (N = 22)	3.786	3.871	97.807	11.836	91.988~103.994	>99.9		3.225	3.228	99.903	11.093	94.619 ~ 105.869	>99.9
AUC_{0-t} (h*ng/mL) (N = 20)	151.339	151.782*	99.708	8.146	94.970 ~ 103.876	>99.9		140.800	144.169	97.663	8.409	93.492 ~ 102.021	>99.9
$AUC_{0-\infty}$ (h*ng/mL) (N = 20)	167.177	166.835*	100.205	9.340	94.740 ~ 104.989	>99.9		156.199	159.503	97.929	9.760	93.094 ~ 103.015	>99.9

TABLE 5 Summary of adverse events in the study arms

	Fasting		Fed	
Adverse events	T (n = 23) 4(17.4%)	R (n = 23) 2(8.7%)	T (n = 23) 17.4% (4/23)	R (n = 24) 29.2% (7/24)
Total				
Upper respiratory tract infection	2	0	2	1
serum creatinine increased	0	1	0	0
Epistaxis	1	0	0	0
Hemobilirubin increased	0	1	0	1
Serum sodium increased	0	1	0	0
Nausea	1	0	0	0
Hypotension	1	0	0	0
Leukocyte increased	0	0	0	2
Neutrophil increased	0	0	0	2
Urine protein positive	0	0	1	2
Numbness of arm	0	0	0	1
Fainting during acupuncture	0	0	0	1
Cervical pain	0	0	1	0
Urinary occult blood positive	0	0	0	1
SAEs	0	0	0	0
Deaths	0	0	0	0
TEAEs leading to withdrawal of study	2		1	
Drug				
Treatment-related TEAEs	1(definitely relevant)		1(possible related)	

Note: Data are expressed as number of participants (%).

Abbreviations: ALT, alanine aminotransferase; SAEs, serious adverse events; TEAEs, treatment-emergent adverse events.

There were no specific AEs, and all AEs were mild and slight with good prognosis. Only 1 volunteer received additional medical treatment for fever. Other AEs did not receive any other medical treatment. No SAEs were found during the study. Date is represented at Table 5.

4 | DISCUSSION

Our studies (fasting and fed studies) pertaining to Amlodipine bioequivalence were conducted by drug from two different sponsors. Each study was characterized by randomized, open-label,

TABLE 6 Specific analysis of the three subjects withdrew from the trials

Subject	Food	period	Med	Cause of drop out	Treat	drug combination	prognosis
1001	Fasting	1	T	Upper Respiratory Tract Infection	Physical cooling	Cefuroxime Axetil tablets Acetaminophen Compound Caplets tablets	Recovery
1004	Fasting	2	R	Personal reasons	NA	NA	NA
2017	Fed	1	R	Upper respiratory tract infection	Physical cooling	Cefuroxime Axetil tablets Acetaminophen Compound Caplets Tablets	Recovery

Abbreviation: NA, Not Applicable.

single-dose, crossover, and two-period designs. Two studies were conducted to study the single-dose pharmacokinetic profile and bioequivalence of Amlodipine tablets in healthy Chinese subjects. Of note, Amlodipine was well tolerated and no clinically significant changes in vital signs, laboratory inspection, and ECGs after oral drugs. Throughout the studies, there were no deaths or SAEs in either part of the research (Table 6).

Biosimilarity is demonstrated by totality of the testimony from laboratory, non-clinical, clinical, and analytical researches to reveal the proposed generic drug is highly similar in metabolism, absorption, function, effects, purity, and safety to the reference product.[13] The purpose of a biosimilarity clinical research is to resolve remaining uncertainties about biosimilarity after analytical assessments and non-clinical evaluations.[13] With the evolving of biosimilars regulatory landscape, detailed and effective analytical researches, with clinical PK/PD researches in healthy volunteers may be enough to fulfill the regulatory approval of generic agent without the demand to carry out a relatively effect and safety study in corresponding patients.[14] FDA bioequivalence guidance claimed that clinical pharmacology studies apply either a parallel devise or a crossover devise to assess PK and PD similarity and the studies design was chosen due to agent half-life period as well as the duration of PD reaction and immunogenicity.[15] Based on the US FDA Draft Guidance on Amlodipine Besylate (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm082471.pdf), the CV% of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were about 10%-18% in vivo. Our studies meet the FDA's demands and exhibit less subject variability, more stable metabolism. The slight difference in different studies might be due to sample size and racial difference.

Amlodipine was absorbed slow with the peak plasma degree occurring at 5-6 hours after oral dose, the T_{max} was 6 hours at fast study and 5, 6 hours at fed study which is similar to literatures.[16,17] The plasma concentrations decreased slowly in a biphasic mode and the measured $T_{1/2z}$ was 30-50 hours which is trifle shorter than record previously, and the literatures $T_{1/2}$ is 40-60 hours.[18,19] Two sponsors were considered bioequivalent and the criterion developed previously was that 90% CI for parameters, such as C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ fall within 80-125%. In our studies, both fast and fed studies, T and R met the above criteria and gain bioequivalent. However, at fast state, Amlodipine bioequivalence was easier to achieve. This is a significant consideration from a patient adherence and convenience perspective, patient could take medicine regardless of meals. The results of these clinical studies provide foundation for subsequent clinical studies. In further studies, the clinical efficiency of Amlodipine will be evaluated in confirmed hypertensive patients.

Certainly, there are still some potential study limitations: randomization is flawed, in this study, the randomization is single-blind, that is, the researchers informed; we did not further compare the effects of gender on pharmacokinetic parameters; the active molecules of Amlodipine metabolism are not further tested. In subsequent tests, we will further address the above issues.

5 | CONCLUSIONS

The results collected from the two studies (fast and fed studies) revealed that the drug Amlodipine is well tolerated in healthy Chinese male subjects. The date exhibited that Amlodipine is orally bioavailable in healthy test subjects under fasting and fed state.

AUTHOR CONTRIBUTIONS

QG Yang, YW, TT Wang designed the experiment. YN Wang, SS Li, LF, SL performed the clinic trials. WG Zhang and DZ analyze the data. TT Wang and YN Wang wrote the paper and drew figures.

ETHICAL APPROVAL/INFORMED CONSENT

The trial design was approved by the Ethics Committee at the Zhejiang provincial people's hospital, Hangzhou City, China. The clinical study (registration No.: YZD-CO-BE-ALDPT-005) was carried out accordance with the declaration of Helsinki, Good Clinical Practice (GCP) principle and Chinese laws and regulations. All participants signed the informed consent.

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