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Pharmacokinetics, Safety and Bioequivalence Study of a Single Dose of Two Azithromycin Granules Administered under Fasting and Fed Conditions in Healthy Chinese Volunteers

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Research Article

Keywords: bioequivalence, healthy Chinese volunteers, pharmacokinetics, Azithromycin granules, safety

Posted Date: November 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11430438/v1

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Additional Declarations: No competing interests reported.

Abstract

Purpose

This study aimed to evaluate the pharmacokinetics, safety, and bioequivalence of test and reference Azithromycin granules in healthy Chinese volunteers under fasting and fed conditions.

Methods

This single-center, randomized, open-label, single-dose, 2-period, 2-sequence crossover study enrolled 108 participants. Participants received a 500-mg dose of either formulation under fasting conditions (n = 40) or fed conditions (n = 68), with a 21-day washout. Plasma Azithromycin concentrations were quantified using liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters were calculated noncompartmentally. Bioequivalence was evaluated using 90% confidence intervals (CIs) for test-to-reference geometric mean ratios (GMRs) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$.

Results

Under fasting conditions, GMRs (90% CIs) were 107.07% (99.66%-115.04%) for C_{max}, 102.46% (96.62%-108.65%) for AUC_{0-t}, and 104.35% (97.95%-111.18%) for $AUC_{0-\infty}$. Under fed conditions, values were 102.00% (93.82%-110.91%), 95.00% (88.20%-102.32%), and 96.28% (90.19%-102.79%), respectively. All CIs were within the range of 80.00%-125.00%. One serious adverse event in the fed group (ectopic pregnancy) was assessed as not related to the study drug. No drug-related serious adverse events were observed under fasting or fed condition.

Conclusion

The test and reference Azithromycin granule formulations met bioequivalence criteria under both fasting and fed conditions. No drug-related serious adverse events were identified in this study.

Trial registration

The trial was registered in the Chinese Clinical Trial Registry (ChiCTR2300074532; August 9, 2023)

Introduction

Antibacterial drugs are widely used to prevent and treat bacterial infections caused by Gram-positive and Gram-negative pathogens. Common clinical applications include the treatment of respiratory tract infections, such as pharyngitis, tonsillitis, acute bronchitis, pneumonia, and otitis media, as well as

selected urogenital infections. The selection of an antibacterial drug depends on several factors, including its antimicrobial spectrum, tissue distribution, dosing convenience, tolerability, and pharmacokinetic characteristics. Therefore, the development and evaluation of safe, effective, and interchangeable antibacterial formulations remain important in clinical practice.

Azithromycin is a semisynthetic macrolide antibiotic derived from erythromycin[1, 2]. It contains a 15-membered azalide ring and exerts its antibacterial activity primarily by binding to the 50S subunit of susceptible bacterial ribosomes, thereby inhibiting bacterial protein synthesis[2, 3]. Azithromycin has activity against a broad range of clinically relevant pathogens, including Haemophilus influenzae, Streptococcus pneumoniae, Moraxella catarrhalis, Legionella pneumophila, Mycoplasma pneumoniae, Staphylococcus aureus, Chlamydia trachomatis, Neisseria gonorrhoeae, and Ureaplasma urealyticum[3, 4]. Azithromycin is generally well tolerated, although gastrointestinal symptoms, including diarrhea, nausea, and vomiting, as well as headache and dizziness, may occur[2]. Because food can affect the absorption and systemic exposure of orally administered drugs, the effects of food on pharmacokinetic parameters such as C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ should be considered when evaluating oral Azithromycin formulations[5, 6].

Azithromycin is available in several oral dosage forms, including tablets, capsules, suspensions, and granules. Granule formulations can be dispersed before administration and may provide a practical alternative for pediatric patients and individuals who have difficulty swallowing solid tablets. Because the magnitude and direction of the food effect on Azithromycin exposure have been reported to differ among oral dosage forms, pharmacokinetic and bioequivalence findings obtained with tablets cannot be directly extrapolated to granule or powder-for-suspension formulations[5, 6]. Formulation-specific evaluation under both fasting and fed conditions is therefore warranted. Although the bioequivalence of different Azithromycin tablet formulations has been reported, evidence for Azithromycin granule formulations remains limited[5–7]. Therefore, the present study evaluated the pharmacokinetics, safety, and bioequivalence of a test Azithromycin granule formulation and the corresponding reference formulation under fasting and fed conditions in healthy Chinese volunteers.

Materials and Methods

Drugs and reagents

Test preparation (T): Azithromycin granules, 100 mg/sachet (batch 220103S; assay, 102.9%; Chongqing Lummy Pharmaceutical Co. Ltd., Chongqing, China).

Reference preparation (R): Azithromycin fine granules for pediatric use 10% (Zithromax; 100 mg:1 g per sachet; batch 0140; assay, 102.9%; Pfizer Japan Inc., Japan).

Study Design

This single-center, randomized, open-label, single-dose, two-treatment, two-period, two-sequence crossover bioequivalence study comprised independent fasting and fed cohorts of healthy Chinese adults at the Phase I Clinical Trial Research Center of Guangzhou Panyu Central Hospital. Clinical procedures were conducted from August to November 2023. Healthy individuals who met the eligibility criteria were randomly assigned to the fasting or fed cohort and, within each cohort, were assigned in a 1:1 ratio to the test-reference (T-R) or reference-test (R-T) sequence. Treatment periods were separated by a 21-day washout.

The protocol and informed consent documents were approved by the Drug Clinical Trial Ethics Committee of Guangzhou Panyu Central Hospital (acceptance no. 2023-027-02; opinion no. PYZXYYEC[2023-027]-02; July 5, 2023). The study complied with the Declaration of Helsinki, Good Clinical Practice, and applicable national requirements and was registered in the Chinese Clinical Trial Registry (ChiCTR2300074532; August 9, 2023). Before study procedures, participants were informed of the objectives, procedures, risks, and their rights and provided written informed consent. They could withdraw at any time without reason or disadvantage.

Fasting-cohort participants fasted overnight for at least 10 hours; food was withheld for 4 hours postdose, with standardized meals at approximately 4 and 10 hours. Fed-cohort participants began a standardized high-fat meal 30 minutes predose, completed it within 30 minutes, and were dosed 30 +/- 0.5 minutes after meal initiation. Water was prohibited before and for 1 hour after dosing except for administration. Each period, participants received five sachets of T or R, equivalent to 500 mg Azithromycin, in divided portions with 240 mL warm water. Protocol-defined restrictions covered alcohol, xanthine-rich foods and beverages, grapefruit juice, smoking, strenuous exercise, and prolonged recumbency. Therapeutic medication was prohibited throughout the study, including washout, unless investigator-approved for an adverse event (AE) or intercurrent illness.

Participants

Eligible participants were healthy men and women aged 18–65 years, weighing at least 50.0 kg for men and 45.0 kg for women, respectively, with body mass index 18.5–26.0 kg/m^2. Health was established by medical history, physical examination, vital signs, clinical laboratory tests, drug screening, 12-lead electrocardiography, and pregnancy testing in women. Participants required no pregnancy plans during screening or the subsequent 3 months, agreed to effective contraception, and had no sperm- or oocyte-donation plans.

Key exclusions were hypersensitivity to Azithromycin, erythromycin, other macrolides, or product components; previous Azithromycin-associated cholestatic jaundice or hepatic dysfunction; clinically relevant gastrointestinal or systemic disease affecting safety, absorption, or disposition; medication within 2 weeks before first dosing; recent vaccination, surgery, another drug trial, blood donation, or substantial blood loss; alcohol or substance misuse; inability to comply with smoking, dietary, or sampling requirements; positive human immunodeficiency virus, hepatitis B surface antigen, hepatitis C

virus, or syphilis screening; and investigator-determined unsuitability. Women were also excluded for oral contraceptive use within 30 days, unprotected intercourse within 14 days, positive pregnancy testing, or breastfeeding.

Blood Sampling and Sample Handling

Each period, approximately 4 mL of venous blood was collected predose and 0.5, 1, 1.5, 1.75, 2, 2.33, 2.67, 3, 3.25, 3.5, 4, 4.5, 5, 6, 8, 12, 24, 48, 72, 96, 120, and 168 hours postdose. K_2-EDTA tubes were gently inverted and temporarily kept at room temperature. Within 1 hour, samples were centrifuged at 1700 x g for 10 minutes at 4 degrees C (allowable range, 2–8 degrees C).

Plasma was divided into two precooled aliquots: approximately 0.8 mL for primary analysis and the remainder as backup. Samples could be temporarily stored below − 20 degrees C within 1 hour after centrifugation and were transferred to -80 degrees C within 24 hours.

Bioanalytical Method

Plasma Azithromycin concentrations were quantified using a validated liquid chromatography-tandem mass spectrometry method. K_2-EDTA plasma underwent protein precipitation with Azithromycin-^{13}C-d_3 as internal standard. An AB SCIEX Exion LC AC coupled to an AB SCIEX Triple Quad 5500 mass spectrometer with electrospray ionization was used with a 2-microliter injection. Samples were processed and analyzed using Analyst 1.6.3.

Calibration was linear from 5.00 to 1000.00 ng/mL, with a lower limit of quantification of 5.000 ng/mL, using 1/x2-weighted linear regression. Quality-control concentrations were 15.000, 150.000, and 800.000 ng/mL. Maximum quality-control precision was 3.285% coefficient of variation (CV) and accuracy deviations were 1.453%-1.714%; corresponding values at the lower limit were 2.401% CV and 0.160%. Selectivity and stability (short-term, long-term, and processed sample stability) were all within acceptable limits according to regulatory guidelines.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic (PK) parameters were calculated by noncompartmental analysis using WinNonlin 8.3.5; descriptive statistics were calculated using SAS 9.4 (SAS Institute Inc. Cary, North Carolina). Primary parameters were maximum observed plasma concentration (C_{max}), area under the plasma concentration-time curve from zero to the last quantifiable concentration (AUC_{0-t}), and AUC from time zero extrapolated to infinity ($AUC_{0-\infty}$). Secondary parameters were time to C_{max} (T_{max}), terminal elimination rate constant (lambda-z), terminal half-life ($T_{1/2}$), and percentage extrapolated AUC ($AUC_{\%Extrap}$).

C_{max} and T_{max} came directly from observed data. AUC_{0-t} used the linear trapezoidal method; $\mathrm{AUC}_{0-\infty}=\mathrm{AUC}_{0-\mathrm{t}}+\frac{\mathrm{C_t}}{\lambda_z}$, where Ct was the last quantifiable concentration and lambda-z the absolute terminal log-linear regression slope. The equations were $\mathrm{t}_{1/2}=\frac{\ln(2)}{\lambda_z}$ and $\mathrm{AUC}_{\backslash\%\mathrm{Extrap}}=100\times\frac{\mathrm{AUC}_{0-\infty}-\mathrm{AUC}_{0-\mathrm{t}}}{\mathrm{AUC}_{0-\infty}}$. Below-quantification concentrations before T_{max} were zero and those after T_{max} missing for PK analysis; all were zero for descriptive concentration summaries. Missing concentrations were not imputed.

Natural log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were analyzed separately by cohort using crossover analysis of variance with sequence, treatment, and period as fixed effects and participant nested within sequence as a random effect. Back-transformed least-squares mean differences provided T/R geometric mean ratios and 90% confidence intervals. Bioequivalence required all intervals within 80.00%-125.00%. T_{max} was summarized descriptively as the arithmetic mean ± standard deviation (SD) and compared using the paired Wilcoxon signed-rank test ($P < 0.05$); other parameters were summarized descriptively. The descriptive statistics included the number of samples (N), arithmetic mean (mean), standard deviation (SD), minimum value (Min), median (median), maximum value (Max), geometric mean (geometric mean), and the geometric coefficient of variation (geometric CV%) and the coefficient of variation (CV%).

T_{max} was compared between the test and reference formulations separately under fasting and fed conditions using a paired Wilcoxon signed-rank test. Within-subject differences were defined as reference minus test. Zero differences were excluded from the rank calculations, and tied absolute differences were assigned average ranks. Two-sided conditional exact P values were calculated from the observed ranks. The centered signed-rank statistic was defined as $S = (W^+ - W^-)/2$, where W^+ and W^- denote the sums of ranks for positive and negative differences, respectively. A two-sided P value < 0.05 was considered statistically significant for these secondary comparisons.

Safety Assessment

Safety assessments included AEs, serious AEs, concomitant medications, physical examinations, vital signs, 12-lead electrocardiograms, hematology, serum chemistry, coagulation, and urinalysis. Seated temperature, pulse, and blood pressure were measured predose; 2.0 +/- 0.5, 8.0 +/- 0.5, 24.0 +/- 1.0, 48.0 +/- 1.0, 72.0 +/- 1.0, 96.0 +/- 1.0, and 120.0 +/- 1.0 hours postdose; and at exit. Exit assessments also included physical examination, laboratory tests, pregnancy testing in women, and electrocardiography.

Results

Participants

A total of 108 subjects participated in this trial. All 40 participants in the fasting arm and 65 of the 68 participants in the fed arm completed the study. Three participants in the postprandial group withdrew: one subject in the T-R group (N = 34) voluntarily withdrew from the trial, and two subjects in the R-T group (N = 34) due to poor compliance with the protocol (N = 2). The demographic and baseline characteristics of the enrolled subjects are presented in Table 1. The grouping and treatment of the subjects under fasting and fed conditions are shown in Fig. 1.

Table 1
Demographic Characteristics of Enrolled Subjects

Characteristic	**Fasting (n = 40)**	**Fed (n = 68)**
Sex, n (%)		
Male	28 (70.0)	55 (80.9)
Female	12 (30.0)	13 (19.1)
Age (years)		
Mean ± SD	29.08 ± 5.29	30.50 ± 5.76
Range	19.00–42.00	18.00–42.00
Height (cm)		
Mean ± SD	165.41 ± 6.94	165.65 ± 6.36
Range	151.50–183.00	149.50–179.50
Weight (kg)		
Mean ± SD	60.48 ± 6.35	60.81 ± 7.27
BMI (kg/m²)		
Mean ± SD	22.08 ± 1.49	22.11 ± 1.82
Ethnicity, n (%)		
Han Chinese	36 (90.0)	59 (86.8)
Other*	4 (10.0)	9 (13.2)

* Other ethnicities — Fasting arm: Zhuang (n = 2), Dong (n = 1), Yao (n = 1); Fed arm: Zhuang (n = 5), Bouyei (n = 1), Manchu (n = 1), Miao (n = 1), Tujia (n = 1). BMI, body mass index.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters are summarized in Table 2. Under fasting conditions, the mean T_{max} ± SD was 1.55 ± 0.84 h for the test formulation (T) and 2.00 ± 0.91 h for the reference formulation (R),

while the mean C_{max} values were 760.05 ± 256.99 and 727.17 ± 265.24 ng/mL, respectively. The mean AUC_{0-t} values were 6194.27 ± 1448.89 ng·h/mL for T and 6131.96 ± 1674.89 ng·h/mL for R, and the corresponding $AUC_{0-\infty}$ values were 7103.09 ± 1766.70 and 6855.43 ± 1802.44 ng·h/mL. Under fed conditions, the mean T_{max} ± SD was 2.86 ± 1.03 h for T and 2.82 ± 1.09 h for R. The mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values were 638.93 ± 266.91 ng/mL, 5047.80 ± 2054.65 ng·h/mL, and 5721.65 ± 2245.80 ng·h/mL for T, compared with 611.48 ± 231.15 ng/mL, 5227.67 ± 1967.19 ng·h/mL, and 5901.15 ± 2200.82 ng·h/mL for R, respectively. The mean plasma concentration–time profiles of Azithromycin were generally comparable between the two formulations under both fasting and fed conditions (Figs. 2&3). Interestingly, the mean plasma concentration–time profiles showed an apparent biphasic pattern, with a secondary rise in plasma Azithromycin concentrations after the initial peak under both fasting and fed conditions (Figs. 2&3). This double-peak phenomenon was observed for both the test and reference formulations, although the timing and magnitude of the secondary peaks varied between formulations and nutritional conditions. Because this pattern was observed in the mean profiles, it should be regarded as a descriptive feature of the concentration–time data rather than evidence of a formulation-specific difference or a distinct absorption mechanism.

Table 2
Pharmacokinetic Parameters

Parameters	**Mean ± SD**			
	Fasting		**Fed**	
Test	**Reference**	**Test**	**Reference**	
T_{max} (h)	1.55 ± 0.84	2.00 ± 0.91	2.86 ± 1.03	2.82 ± 1.09
C_{max} (ng/mL)	760.05 ± 256.99	727.17 ± 265.24	638.93 ± 266.91	611.48 ± 231.15
AUC_{0-t} (ng·h/mL)	6194.27 ± 1448.89	6131.96 ± 1674.89	5047.80 ± 2054.65	5227.67 ± 1967.19
$AUC_{0-\infty}$ (ng·h/mL)	7103.09 ± 1766.70	6855.43 ± 1802.44	5721.65 ± 2245.80	5901.15 ± 2200.82
λ_z (h^{-1})	0.0105 ± 0.0029	0.0106 ± 0.0028	0.0144 ± 0.0154	0.0123 ± 0.0041
$AUC_{\%Extrap}$ (%)	12.38 ± 4.36	10.78 ± 2.38	12.62 ± 6.36	11.69 ± 3.36
$T_{1/2}$ (h)	69.37 ± 16.60	69.37 ± 16.60	61.96 ± 21.64	61.56 ± 16.93

Bioequivalence Assessment

As shown in Table 3 and Table 4, under fasting conditions, the geometric mean ratios of the test formulation to the reference formulation were 107.07% for C_{max}, 102.46% for AUC_{0-t}, and 104.35% for $AUC_{0-\infty}$. The corresponding 90% confidence intervals were 99.66%-115.04%, 96.62%-108.65%, and

97.95%-111.18%, respectively. Analysis of variance of the log-transformed primary pharmacokinetic parameters showed no statistically significant treatment or period effects on C_{max}, AUC_{0-t}, or $AUC_{0-\infty}$ under fasting conditions (all $p > 0.05$). No significant sequence effects were observed for C_{max} or AUC_{0-t} ($p = 0.3898$ and $p = 0.0611$, respectively), whereas a statistically significant sequence effect was detected for $AUC_{0-\infty}$ ($p = 0.0358$).

Under fed conditions, the geometric mean ratios for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 102.00%, 95.00%, and 96.28%, respectively, with corresponding 90% confidence intervals of 93.82%–110.91%, 88.20%–102.32%, and 90.19%–102.79%. The analysis of variance showed no statistically significant sequence, treatment, or period effects on any of the three log-transformed primary pharmacokinetic parameters under fed conditions (all $p > 0.05$). All 90% confidence intervals were within the prespecified bioequivalence range of 80.00%-125.00%. Therefore, the test and reference Azithromycin formulations met the bioequivalence criteria under both fasting and fed conditions.

Although all 40 participants finished the fasting study, only 39 were included in the paired T_{max} analysis. For participant K013, the period 1 data after taking the test formulation were left out of the bioequivalence analysis because the first sample after dosing was already at C_{max}, and no early samples (5–15 minutes after dosing) were collected. However, the reference formulation data from period 2 were kept. In the fed study, 65 of the 68 randomized subjects completed the study; however, evaluable T_{max} data for both formulations were available for 67 subjects, including two subjects who withdrew during the second period. Subject C062 had test-formulation data only and was excluded from the paired analysis. Under fasting conditions, T_{max} differed significantly between the test and reference formulations (S = 134.5, two-sided conditional exact $p = 0.0045$), with a median within-subject difference of 0.50 h for reference minus test. Under fed conditions, no statistically significant difference was detected (S = 6.0, two-sided conditional exact $p = 0.9534$(Table 5).

Table 3
Bioequivalence statistics for the PK parameters of Azithromycin under fed conditions

PK parameters		C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)
Intraindividual standard deviation		0.2907	0.2578	0.2269
intra-CV%		29.70	26.21	22.99
Geometric mean of T		580.17	4547.24	5220.75
Geometric mean of R		568.77	4786.75	5422.32
Geometric mean ratio (GMR) (T/R), %		102.00	95.00	96.28
90% CIs, %		[93.82, 110.91]	[88.20, 102.32]	[90.19, 102.79]
Power, %		99.02	98.52	99.88
Point estimate of the geometric mean ratio (GMR), %		102.00	95.00	96.28
Bioequivalence methods		ABE	ABE	ABE
ANOVA P value	**Sequence**	0.6089	0.1566	0.2060
	Period	0.5500	0.3724	0.1157
	Formulations	0.6792	0.2647	0.3520

Table 4
Bioequivalence statistics for the PK parameters of Azithromycin under fasting conditions

PK parameters		C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)
Intraindividual standard deviation		0.1880	0.1538	0.1660
intra-CV%		18.97	15.47	16.72
Geometric mean of T		722.88	6013.33	6867.17
Geometric mean of R		675.13	5868.97	6580.61
Geometric mean ratio (GMR) (T/R), %		107.07	102.46	104.35
90% CIs, %		99.66, 115.04	96.62, 108.65	97.95, 111.18
Power, %		97.31	100.00	99.89
Point estimate of the geometric mean ratio (GMR), %		107.07	102.46	104.35
Bioequivalence methods		ABE	ABE	ABE
ANOVA P value	**Sequence**	0.3898	0.0611	0.0358
	Period	0.3423	0.3210	0.1515
	Formulations	0.1060	0.4369	0.2258

Table 5
Paired comparisons of Azithromycin T_{max} under fasting and fed conditions

Group	PK parameters	S	P
Fasting	**T_{max} (R vs. T)**	134.5	0.0045
Fed	**T_{max} (R vs. T)**	6.0	0.9534

Safety Assessment

Adverse events and adverse reactions are summarized in Table 6. In the fasting trial, 29 adverse events occurred in 10 of the 40 subjects after oral administration of the test preparation, with an adverse event rate of 25.0% (10/40); 24 adverse events occurred in 12 volunteers after oral administration of the reference preparation, with an adverse event rate of 30.0% (12/40). No participant experienced serious adverse events or died, and no subject withdrew from the trial due to adverse events. In the postprandial trial, 80 adverse events occurred in 25 of the 68 subjects after taking the test preparation orally after meals, with the incidence rate of adverse events being 36.8% (25/68). Eighty-two adverse events

occurred in 22 of the 67 subjects after taking the reference preparation orally after meals, with the incidence rate of adverse events being 32.8% (22/67). One participant experienced an ectopic pregnancy, which was classified as a serious adverse event. The event was assessed by the investigator as unrelated to the study drug. No serious adverse reactions or deaths were reported.

Table 6
Occurrences of adverse events in participants receiving the test and reference formulations

Safety outcomes	Fed		Fasting	
	Test (N = 68)	Reference (N = 67)	Test (N = 40)	Reference (N = 40)
Participants with any adverse event, n (%)	25 (36.8)	22 (32.8)	10 (25.0)	12 (30.0)
Number of adverse events	80	82	29	24
Participants with serious adverse event, n (%)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)
Number of serious adverse events	0	1	0	0
Participants with serious adverse drug reaction, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Participants who died, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Participants who withdrew because of an adverse event, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Serious adverse event reported	None	Ectopic pregnancy (1)	None	None

Discussion

In this single-dose, two-period crossover study, the test and reference Azithromycin formulations met the prespecified bioequivalence criteria under both fasting and fed conditions. The 90% confidence intervals for the test-to-reference geometric mean ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were all within the acceptance range of 80.00%-125.00%. No drug-related serious adverse events were observed; the single serious adverse event reported in the fed cohort (ectopic pregnancy) was assessed as not related to the study drug.

The observed T_{max} and elimination half-life were broadly consistent with previously reported pharmacokinetic characteristics of oral Azithromycin[8, 9]. In this study, mean T_{max} ranged from 1.55 to 2.86 h, while the mean elimination half-life was approximately 62–71 h across formulations and study conditions. Previous studies of Azithromycin tablets, granules, capsules, and powders for suspension have reported T_{max} values generally within the first several hours after administration, although the

magnitude of C_{max} and AUC has varied according to dose, formulation, study population, and feeding condition[5, 6, 8, 10].

Consistent with the formulation-dependent food effects reported for oral Azithromycin products, the present study showed lower systemic exposure under fed conditions than under fasting conditions[5, 6]. Based on the arithmetic mean values, C_{max} decreased by 15.9% for both the test formulation and the reference formulation after food intake. The T_{max} was delayed from 1.00 to 2.67 h for the test formulation and from 1.75 to 2.33 h for the reference formulation. In addition, the mean AUC_{0-t} decreased by 18.5% and 14.7%, and the mean $AUC_{0-\infty}$ decreased by 19.4% and 13.9% for the test and reference formulations, respectively. Reductions of this magnitude are modest and, given the wide therapeutic index of Azithromycin, are unlikely to be of clinical significance.

In this study, an apparent secondary peak was observed in the mean concentration–time profiles; this phenomenon has not been systematically characterised or quantified in recent bioequivalence studies of Azithromycin granules: in both the fed and fasting arms, the mean plasma concentration–time curves for the test and reference formulations showed a suspected double-peak phenomenon after a single oral administration. In the fasting arm, the mean plasma concentration continued to decrease after the first peak until the mean plasma concentration–time curve rose slightly again at 4 hours and reached the second peak between 4.5 and 5 hours. The shape of the second peak was flatter than the first peak, but it was still visible intuitively. After the second peak, the mean plasma concentration–time curve continued to decrease gradually and no longer showed a noticeable upward trend. A similar pattern was seen in the fed arm. When the first peaks of the test and reference preparations occurred at 2 h and 2.33 h, respectively, the mean plasma concentration began to decrease for the first time. The curve turned at 4 h and had an obvious uptrend, with a second peak appearing at 4.5 h. As in the fasting arm, the second peak was less pronounced than the first peak. The upwards trend of the former is also not as pronounced as that of the latter. Several mechanisms could theoretically contribute to a genuine secondary peak. First, enterohepatic recirculation: Azithromycin is excreted predominantly as unchanged drug into bile and can be reabsorbed from the intestine, a recognized source of secondary concentration peaks[2, 11] Hepatoenteric and enterogastric recycling have been characterised as distinct contributors to multiple peaking, alongside dual absorption pathways, lymphatic uptake, and formulation-dependent factors[12, 13]. Second, two-absorption-compartment models, in which the dose is absorbed sequentially from different gastrointestinal regions, describe double-peaked profiles of several orally administered compounds[14, 15]; multiple peaks limited to the absorption phase have likewise been interpreted as formulation-dependent absorption[12, 13]. For Azithromycin specifically, PBPK analysis of oral absorption indicates that absorption within the therapeutic dose range is limited by membrane permeation (passive and active processes) rather than solubility[7]. So formulation-related differences in dissolution and dispersion-including precipitation and redissolution behaviour described for other lipophilic drugs[16] -could plausibly contribute to a secondary absorption phase. Granule-to-granule differences in dispersion are a formulation-consistent explanation that does not require an unestablished gastric absorption pathway.

From a clinical perspective, this pattern suggests that the timing of peak concentration may be variable and that food and formulation characteristics may influence the rate of Azithromycin absorption. Nevertheless, because the prespecified bioequivalence criteria based on $C_{max,}$ AUC_{0-t}, and $AUC_{0-\infty}$ were met under both fasting and fed conditions, the observed double-peak pattern did not prevent the test and reference formulations from being considered bioequivalent in this single-dose study. The finding does not by itself justify a change in dosing frequency or dose. Where clinically relevant, this phenomenon may be further evaluated using individual concentration–time profiles, formulation dissolution data, gastric-transit information, or mechanistic metabolite studies.

A statistically significant difference in T_{max} was observed under fasting conditions, with a median paired difference of 0.50 h indicating earlier attainment of peak concentration for the test formulation. No statistically significant difference in T_{max} was detected under fed conditions. T_{max} was a secondary pharmacokinetic endpoint in this study. The bioequivalence conclusion was based on the 90% confidence intervals for the test-to-reference geometric mean ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$, all of which were within the prespecified acceptance range of 80.00%–125.00%. The clinical relevance of the observed fasting T_{max} difference cannot be determined from the statistical comparison alone.

In this study, adverse effects in the gastrointestinal tract and central and peripheral nervous systems are relatively common among Azithromycin-related adverse events[2]. In this trial, the most common systemic adverse reactions related to Azithromycin were those involving the digestive system and the central and peripheral nervous systems. Among them, 6 subjects in the fasting group experienced adverse reactions related to the digestive system (including abdominal pain, nausea, diarrhea, constipation, and difficulty in defecation), with an incidence rate of 15%, and 1 subject experienced an adverse reaction related to the nervous system (headache), with an incidence rate of 2.5%. In the postprandial group, the number of subjects with digestive system-related adverse reactions (including diarrhea and abdominal pain) and nervous system-related adverse reactions were 3 (4.4%) and 1 (1.5%), respectively. Other adverse reactions, including shortness of breath, dermatitis, rash, chest discomfort, and itching, occurred in less than 5% of participants. Compared with previous research, this finding indicates that the safety profile of Azithromycin is within the acceptable range. Both the test and reference formulations were well tolerated by the healthy Chinese volunteers studied under fasting and fed conditions.

One serious adverse event, assessed as not related to the study drug, occurred in the fed cohort: ectopic pregnancy. The subject had negative serum β-hCG pregnancy tests at screening on August 10, 2023, and immediately before the second dosing period on September 4, 2023. The subject reported no use of any contraceptive method during the study. Therefore, contraceptive adherence was not applicable. Serum β-hCG was elevated at the end-of-study assessment on September 12, 2023, and a suspected ectopic pregnancy was identified by transvaginal ultrasonography on September 26, 2023, 21 days after the second and final study dose administered on September 5, 2023. The event was assessed by the investigator as not related to the study drug, given the absence of a plausible biological mechanism linking Azithromycin to tubal implantation and the lack of evidence from the available data that

Azithromycin increases the risk of ectopic pregnancy. The subject received conservative management. Follow-up on October 4, 2023, showed no apparent adnexal mass or pelvic fluid collection, consistent with resolution of the ultrasound abnormality; no persistent sequelae were documented in the available follow-up records. The event was reported to the institutional ethics committee and regulatory authorities within the required timelines.

This study has several limitations. First, it was a single-center study conducted in healthy Chinese volunteers after a single oral dose; therefore, the findings may not be generalizable to patients, other ethnic populations, repeated-dose conditions, or routine clinical use. Second, the sample sizes were relatively small, particularly for detecting uncommon safety events, and the study was designed primarily to assess bioequivalence rather than rare adverse events or the mechanisms underlying food effects. Third, although the fed and fasting conditions were compared descriptively, the available data did not allow a comprehensive mechanistic evaluation of the apparent double-peak concentration–time profiles. Information on individual profiles, gastrointestinal transit, formulation dissolution, and relevant metabolites was not available. Finally, further clarification of the pregnancy-related safety case, including the exact timing of pregnancy detection, contraceptive method and adherence, and documentation of protocol-compliant pregnancy testing, would be necessary for a complete safety assessment.

In short, the test and reference Azithromycin formulations demonstrated comparable pharmacokinetic exposure and met the prespecified bioequivalence criteria under both fasting and fed conditions. Both formulations were generally well tolerated. The descriptive differences between the fasting and fed cohorts and the apparent double-peak pattern provide directions for further formulation-specific and mechanistic investigation but do not alter the bioequivalence conclusion of the present study.

Conclusion

In this study, the pharmacokinetic parameters of two different Azithromycin granule preparations administered orally to healthy Chinese volunteers were bioequivalent under both fasting and postprandial conditions. The above two medications (test drug and reference drug) were well tolerated among Chinese volunteers.

Abbreviations

AE
Adverse event
ABE
Average bioequivalence
AUC
Area under the plasma concentration-time curve
AUC_{0-t}
Area under the plasma concentration-time curve from time zero to the last quantifiable concentration

$AUC_{0-\infty}$
Area under the plasma concentration-time curve from time zero extrapolated to infinity
$AUC_{\%Extrap}$
Percentage of the area under the plasma concentration-time curve extrapolated beyond the last quantifiable concentration
BMI
Body mass index
CI
Confidence interval
C_{max}
Maximum observed plasma concentration
Ct
Last quantifiable plasma concentration
CV
Coefficient of variation
CV%
Coefficient of variation (percentage)
Geometric CV%
Geometric coefficient of variation (percentage)
GM
Geometric mean
GMR
Geometric mean ratio
K_2-EDTA
Dipotassium ethylenediaminetetraacetic acid
LC
Liquid chromatography
ln
Natural logarithm
Max
Maximum
Min
Minimum
PBPK
Physiologically based pharmacokinetic
PK
Pharmacokinetic
SAS
Statistical Analysis System
SEM

Standard error of the mean
SD
Standard deviation
$T_{1/2}$
Terminal elimination half-life
T_{max}
Time to reach maximum plasma concentration
λ_z
Terminal elimination rate constant
β-hCG
Beta subunit of human chorionic gonadotropin

Declarations

Author contributions

Yang Zhang: Writing original draft, Methodology, Data curation, Formal analysis. Jianfen Su: Writing review & editing, Methodology, Data curation, Supervision. Xihua Fu: Methodology, Investigation, Data curation. Zuoheng Xu: Investigation, Data curation, Formal analysis. Bingna Wang: Investigation, Data curation. Wenzhao Gu: Investigation, Data curation. Jianqing Jian: Project administration, Resources, Investigation. Shuhua Chen: Project administration, Supervision, Resources. Xinsheng Peng: Conceptualization, Methodology, Supervision, Project administration, Writing review & editing.

Funding

This study received no specific funding from any public, commercial, or not-for-profit funding agency.

Data availability

Researchers interested in accessing the data supporting this study's findings are welcome to contact the corresponding author upon reasonable request.

Ethics approval and informed consent

This single-center clinical trial was conducted between August and December 2023 at the Phase I Clinical Trial Research Center of Panyu Central Hospital. The study protocol was reviewed and approved by the Ethics Committee of Panyu Central Hospital (PYZXYYEC2023-027-02). The study was performed in accordance with the ethical principles of the Declaration of Helsinki, applicable Good Clinical Practice requirements, and relevant national laws and regulations. The trial was registered with the Chinese Clinical Trial Registry (ChiCTR2300074532; registration date: August 9, 2023). Before enrollment and initiation of any study-specific procedures, all participants received an explanation of the study objectives, procedures, potential risks, and their rights, and provided written informed consent.

Participants were informed that they could withdraw from the study at any time without providing a reason or incurring any disadvantage.

Consent for publication

Written informed consent has been obtained from the participants to publish their related experimental results, and the privacy of the subjects is strictly protected.

Conflicts of interests

The authors declare that there are no conflicts of interest.

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Figures

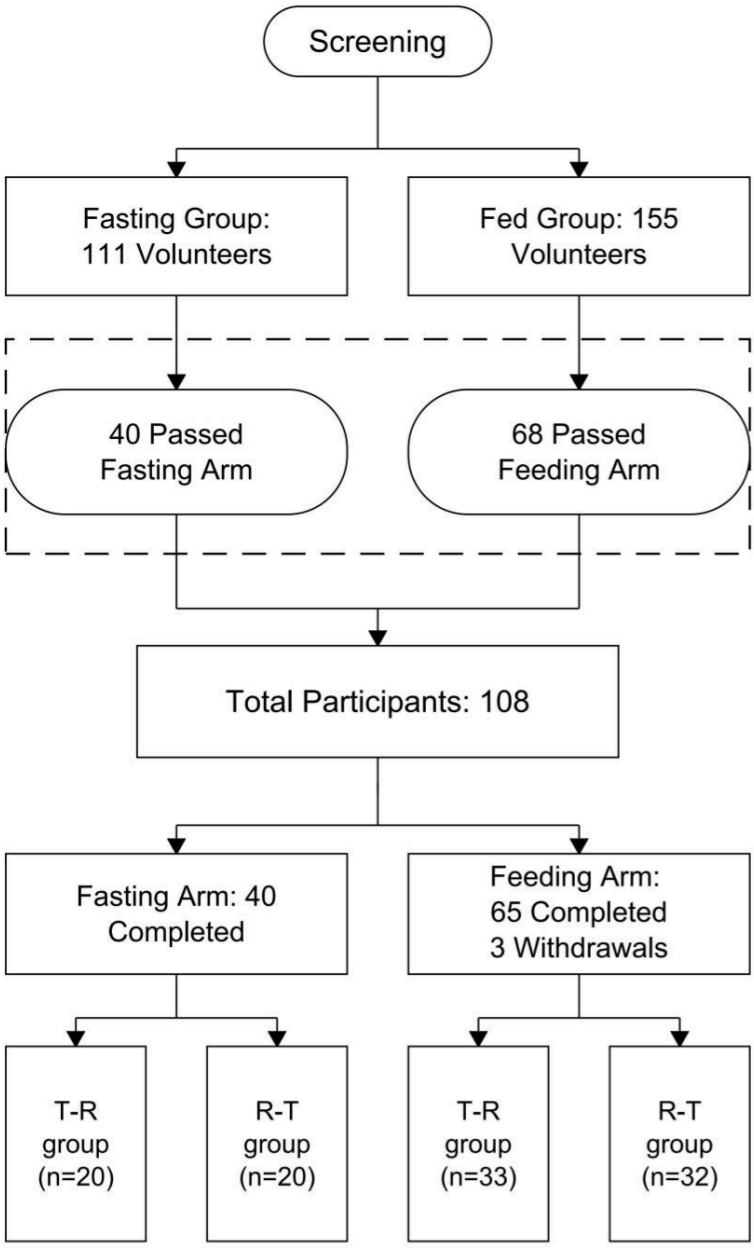


Figure 1

Dispositions for the TR group and the RT group of Subjects

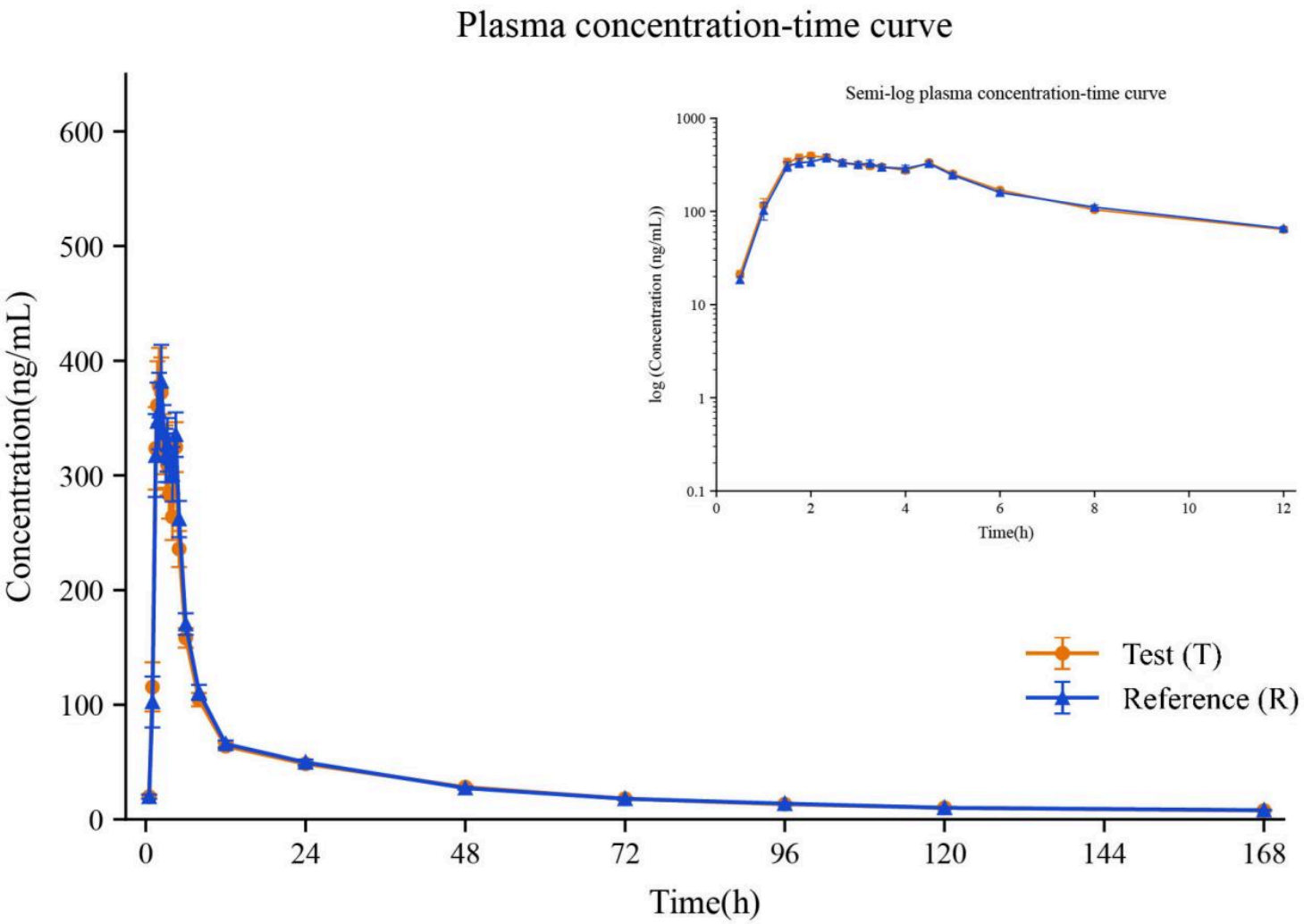


Figure 2

Plasma concentration–time profiles of Azithromycin following a single oral dose of 500 mg of the test (T) and reference (R) granule formulations under fed conditions (mean ± SEM). The inset shows the corresponding semi-logarithmic plasma concentration–time profiles over the first 12 h.

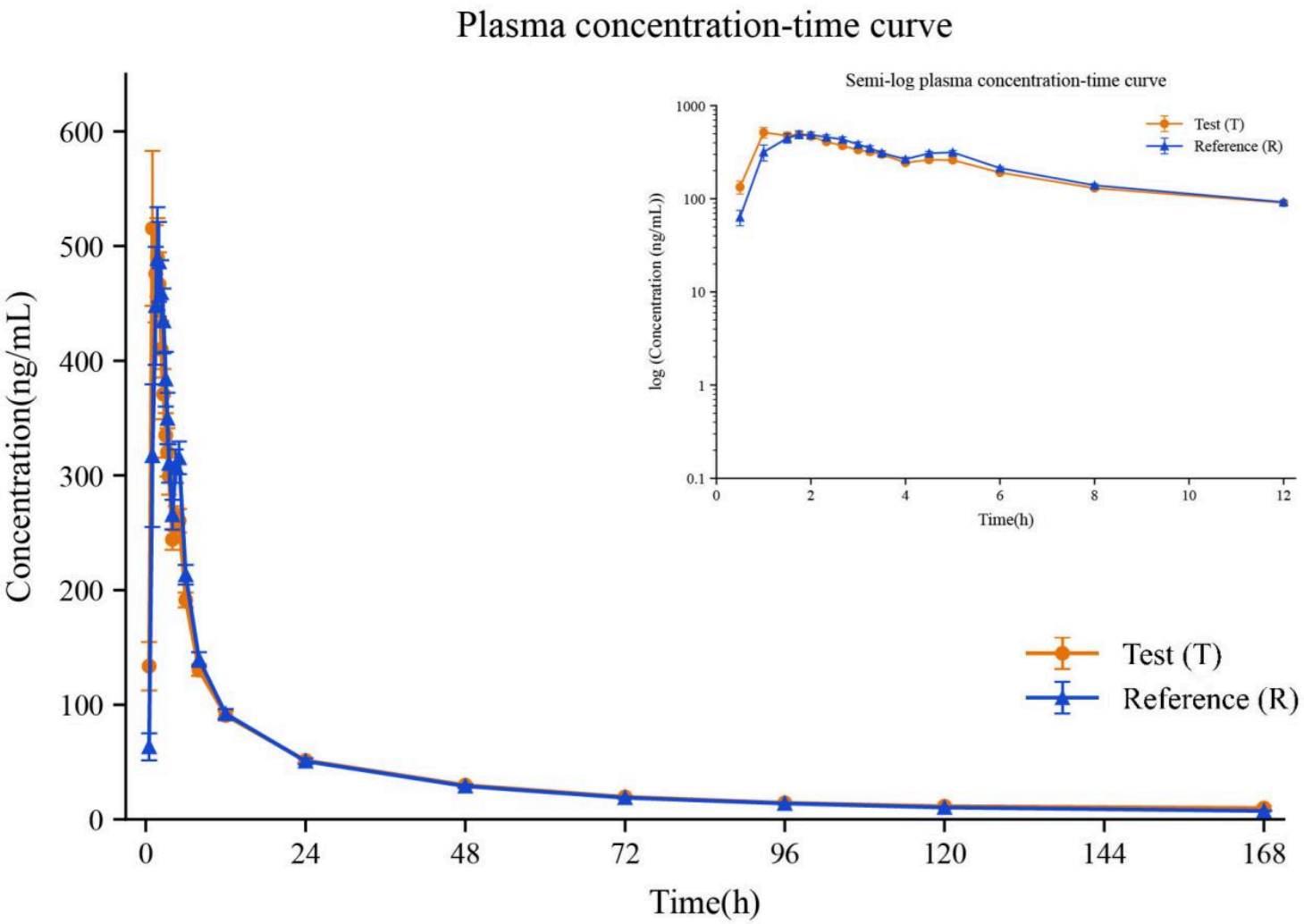


Figure 3

Plasma concentration–time profiles of Azithromycin following a single oral dose of 500 mg of the test (T) and reference (R) granule formulations under fasting conditions (mean ± SEM). The inset shows the corresponding semi-logarithmic plasma concentration–time profiles over the first 12 h.