Pharmacokinetics of Liposomal Amphotericin B for Injection in Chinese Healthy Subjects Based on a Bioequivalence Study

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Research Square

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Summary

Purpose: The aim of this study was to evaluate the pharmacokinetic characteristics and safety of Liposomal Amphotericin B for injection in healthy Chinese volunteers based on a pilot bioequivalence clinical trial between a generic formulation and Ambisome®. **Methods:** This randomized, two sequence, open-label, single-dose, two-period crossover study was conducted in healthy volunteers at the dose of 2 mg/kg. Blood samples were collected at pre-defined time points up to 674 h after the start of the 2-h infusion. Plasma concentrations of total, unencapsulated and encapsulated amphotericin B were determined. Pharmacokinetic parameters were calculated using non-compartmental model. The formulations were considered bioequivalent if the 90% confidence intervals (CIs) of the geometric mean ratio of C_{max} and AUC of both products for free and encapsulated amphotericin B were within 80.00%-125.00% for Ln-transformed data. **Results and conclusion:** All the 12 subjects completed the two-period study, no subjects withdrew the study. The plasma pharmacokinetic profile of liposomal amphotericin B based on free, encapsulated and total amphotericin B demonstrated the characteristics of a three compartmental model. The majority drug in the circulating system after IV infusion of liposomal amphotericin B is remained liposomal form. Pharmacokinetic behaviors in Chinese population were consistent with that in western healthy population based on total and unbound amphotericin B concentrations in plasma. The generic liposomal amphotericin B for injection is bioequivalent to Ambisome® in terms of the Pharmacokinetic parameters for free, encapsulated and total amphotericin B. Trial registration number at National Medical Products Administration: CTR20200885. Date of registration: May 22, 2020.

Keywords

Liposomal Amphotericin B for Injection; Pharmacokinetics; Bioequivalence; Healthy Chinese subjects

Declarations

Funding

Not applicable.

Conflicts of interest

Xueyuan Zhang, Huanhuan Qi and Manman Wang are employees of CSPC Zhongqi Pharmaceutical Technology (Shijiazhuang) Co., Ltd. All other authors declare no conflicts of interest.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Authors' contributions

Chunlei Li, Xueyuan Zhang contributed to the study design and conception. Huanhuan Qi contributed to data analysis and manuscript writing. Manman Wang, Yuhuan Ji and Limei Zhao contributed to data collection and analysis.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the principles of the Declaration of Helsinki and the guidance for Good Clinical Practice (GCP).

Consent to participate

All participants provided written informed consent before admission and initiation of the study.

Consent for publication

Not applicable.

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Introduction

Amphotericin B is amacrocyclic, polyene, antifungal antibiotic that is widely used since 1950s for the treatment of systemic fungal infections caused mostly by Candida and Aspergillus [1, 2]. Amphotericin B acts by irreversibly binding its target, the ergosterol components of the fungal cell membrane, leading to cell permeability alterations and therefore resulting in the leakage of the cell contents and eventual cell death. Due to lack of selectivity for fungal versus human cells, the clinical application of Amphotericin B was limited by side effects, such as nephrotoxicity and infusion-related reactions [3]. Three lipid formulations, namely liposomal amphotericin B, amphotericin B lipid complex and amphotericin B colloidal dispersion (ABCD), were approved in the 1990s to overcome these problems [4]. Ambisome®, the liposomal formulation of amphotericin B was developed and manufactured by Gilead Sciences, Inc. The liposomal bilayer membrane contains hydrogenated soy phosphatidylcholine, cholesterol, distearoyl phosphatidyl glycerol and Amphotericin B in a molecular ratio of 2:1:0.8: 0.4. The kidney distribution of amphotericin B is significantly reduced after receiving Ambisome® and the occurrence of nephrotoxicity is therefore lowered, but the potent antifungal activity remains the same as conventional amphotericin B [5].

Circulating liposomes like liposomal amphotericin B can release drug so that free drug (unbound and protein-bound) and liposomal drug pools may exist simultaneously within the body after administration. These drug pools differ in their pharmacokinetic, safety, and efficacy profiles. Especially for free amphotericin B, the active drug, reflects the rate and extent of drug release from the liposomal particles. Therefore it is necessary to develop reliable methods to determine these different types of amphotericin B in plasma to fully characterize the Biopharmaceutical characteristics of liposomal drugs [6].

Liposomal amphotericin B for injection used in our study was the generic product of Ambisome® (the reference formulation) developed by CSPC Zhongnuo Pharmaceutical Co., Ltd. In the preclinical study the same structure and Pharmacokinetic (PK) profiles of Liposomal amphotericin B for injection were

thoroughly proven as the same as Ambisome®. This study was designed to examine the pharmacokinetics of Liposomal amphotericin B in Chinese healthy subjects, and simultaneously to evaluate the bioequivalence of the two preparations. Free amphotericin B, liposomal amphotericin B and total amphotericin B (Free and liposomal amphotericin B) in plasma were determined to fully clarify the PK behaviors of liposomal amphotericin B.

Materials and Methods

Study design and Subjects

Study protocol was approved by Medical Ethics Committee of Wuhan Jinyintan Hospital (also known as Wuhan Infectious Disease Hospital). The study was conducted at Wuhan Jinyintan Hospital (Wuhan Infectious Diseases Hospital), 1 Yintan Road, Dongxihu District, Wuhan, Hubei Province, China in accordance with the principles of the Declaration of Helsinki and the guidance for Good Clinical Practice (GCP). Written informed consent was given by all participants before the study. This was an open, randomized, two sequence, two period, two-way crossover single dose pharmacokinetic study in healthy Chinese males and females. The primary objective was to determine bioequivalence, based on area under the curve (AUC) from time 0 to time of last measurable plasma concentration (AUC_{0-t}), AUC from time 0 extrapolated to infinity ($AUC_{0-\infty}$) and C_{max} of free, encapsulated and total amphotericin B.

Treatments and administration

Eligible participants were fed standard low-fat breakfast about 2 hours prior to the start of drug infusion. Either the test (T, Batch number: 830190703, CSPC Zhongnuo Pharmaceutical Co., Ltd) or reference product (R, Batch number: 015750, Gilead Sciences, Inc.) was injected at a dose of 2.0 mg/kg over2-h infusion. The two treatments were given in two sequence according to the randomization schedule in this study. The wash-period between the two circles was at least 49 days.

Venous blood (about 4mL blood at each sampling time) was collected in K_2EDTA tubes prior to and at 30 min (0.5h), 1.0 h, 1.5 h, 2.0 h, 2.25 h, 2.5 h, 3.0 h, 4.0 h, 6.0 h, 8.0 h, 10.0 h, 14.0 h, 26.0 h, 50.0 h, 74.0 h, 146 h, 218 h, 290 h, 362 h, 434 h, 506 h,

578 h, 674 h after the start of the 2-h infusion. Plasma was separated by centrifugation at 2000 g for 10 minutes at 2~8°C. 1mL of plasma was added to a 2mL tube prefilled with 1.0mL of stabilizer reagents (Treated-Plasma). The use of stabilizer reagents was to prevent the release of free drug from liposomal amphotericin B during the sample handling. Therefore, concentration of encapsulated and unencapsulated amphotericin B (Free amphotericin B) was accurately quantitative determination using two Liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods, respectively. The untreated plasma was to determine the total amphotericin B in plasma with another LC-MS/MS method. All those procedures were conducted at ice-bath and under the yellow right. The treated and untreated plasma was stored at frozen state (< -20℃) until analysis.

Analytical Methods

Concentration of total, encapsulated and unencapsulated amphotericin B was analyzed by three validated LC-MS/MS methods, respectively. All samples were spiked with an internal standard (natamycin; Toronto Research Chemicals). Protein precipitation was used to disrupt the liposome and completely release the amphotericin B to get the total amphotericin B at a linear range of 100-50000 ng/mL. Quantitation of free amphotericin B with a linear range of 10.0-3000 ng/mL from stabilizer treated plasma was performed using solid phase extraction (SPE). Quantitation of encapsulated amphotericin B at a linear range of 100-50000 ng/mL from the stabilizer treated plasma was performed using SPE followed by protein precipitation extraction (PPE) method. The analysis was performed in multiple reaction monitoring (MRM) and positive ionization mode. The mass transitions 924.6→743.6 for Amphotericin B and 666.4→503.3 for Natamycin (Internal Standard) were used. Analyst 1.6.3 software was used for instrument control, data collection and peak integration. The raw data, including peak areas, retention times, acquisition time, etc, were imported to Watson® LIMS software (version 7.5).

Pharmacokinetic Evaluation

Pharmacokinetic parameters for total, encapsulated and free amphotericin B were

calculated by non-compartmental model using Phoenix WinNonlin 8.3.1 (Certara, Princeton, NJ, USA). The maximum plasma concentrations (C_{max}) and time to reach these maximum concentrations (t_{max}) were the observed values from the concentration-time profiles. The area under the plasma concentration-time curve from 0h to the time point of the last quantifiable plasma concentration C_{last} (AUC_{0-t}) was calculated by the linear trapezoidal rule. The elimination rate constant λ was estimated by log-linear regression of concentrations observed during the terminal phase of elimination. $AUC_{0-\infty}$ was calculated by extrapolating AUC_{0-t} to infinity according to the equation $AUC_{0-\infty} = AUC_{0-t} + C_{last}/\lambda$. The terminal plasma elimination half-life $t_{1/2}$ were determined by the equation $t_{1/2}=0.693/\lambda$.

Safety evaluation

Safety assessments included adverse events (AEs; overall, by severity, and by relation to study treatment which based on clinical observations and laboratory tests). Treatment Emergent Adverse Events (TEAEs) were collected during and after dosing, including all subjective symptoms and objective signs. Vital signs (ear temperature, BP and pulse rate) of the participants were monitored before and 2, 6, 12, 24 hours post administration of drugs and at every follow-up visit.

Statistical Analysis

Statistical analyses were carried out using the SAS 9.4 (SAS Institute, Cary, North Carolina, USA). PK parameters $AUC_{0-\infty}$, AUC_{0-t} and C_{max} data of free and encapsulated amphotericin B were logarithmically transformed (base e) before analysis. The Least Square Mean (LSM) differences of PK parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) and its 90% confidence interval (CI) between the Test preparation and the Reference preparation were determined. The Geometric Least squares mean (GLSM) ratio of the corresponding PK parameters and its 90% confidence interval were estimated. The conventional acceptance limit range of 80%–125% was applied to the GLSM ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ and its 90% CI for two liposomal amphotericin B formulations. Differences were considered to be significant when $P< 0.05$.

Results

Demographic Characteristics

A total of 24 healthy male and female subjects were screened and 12 subjects participated in this trial. All subjects completed the study. Among the recruited subjects, there were 10 males and 2 females. As shown in Table1, there was no significant difference in terms of age, height, weight, or body mass index between sequences A (T/R) and B (R/T) in this study.

Plasma Pharmacokinetics

The mean and Ln-transformed plasma concentration-time profiles after single dose administration of two different liposomal amphotericin B formulations are presented in Fig.1. Resulting pharmacokinetic parameters are summarised in Table 2.

The last point for plasma collection was 674 h after the start of the 2-h infusion, therefore the elimination phase of the liposomal drug was fully depicted. As shown in fig.1, all subjects displayed triphasic plasma amphotericin B concentration profiles with longer half-lives based on the free, encapsulated and total amphotericin B. We also observed a much higher plasma C_{max} and AUC, while a smaller Volumes of distribution compared with amphotericin B deoxycholate even following correction for the higher weight-based dosages that are used for liposomal amphotericin B [7]. Based on the free amphotericin B, the concentration at the last plasma sample was about 60 ng/mL and accounted for 7% of the C_{max}. The half-lives were about 400h which reflected the slower distribution phase of liposomal amphotericin B compared with amphotericin B deoxycholate[4]. The percentage of free drug in total drug was below than 10% between 0.5h and 26h after the start of 2h-infusion, and then slowly rises to 50% at 147h, finally rises to approximately 100% at 218h. Those results were consistent with the liposomal amphotericin B concentrations, which were almost the same as total amphotericin B concentrations. The lipid-based amphotericin B was accounted for 96.2%, 88.0%, 86.8% of the total amphotericin B based on C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ respectively which indicated that during drug infusion period and the early distribution phase, liposomal drug was dominant form in plasma.

Bioequivalence Evaluation

Healthy subjects received a single oral of the test and reference liposomal amphotericin B. The primary evaluation endpoints of the study were geometric least squares mean ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for free and encapsulated amphotericin B after test and reference treatment, which were evaluated for bioequivalence (Table 3). Total amphotericin B in plasma was used as secondary endpoints in this study.

Based on free amphotericin B: The ratios of the LSMs (and 90% geometric CIs) of the test to reference product (A/B) were 100.502% (97.43% - 103.67%) for C_{max}, 98.806% (95.09% - 102.67%) for AUC_{0-t}, 97.931% (91.74% - 104.54%) for $AUC_{0-\infty}$ respectively. The intra-subject CVs for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were 4.201%, 5.187% and 8.839%, respectively.

Based on encapsulated amphotericin B: The ratios of the LSMs (and 90% geometric CIs) of the test to reference product (A/B) were 100.847% (89.82% - 113.23%) for C_{max}, 97.512% (86.40% - 110.06%) for AUC_{0-t}, 97.251% (86.09% - 109.86%) for $AUC_{0-\infty}$, respectively. The intra-subject CVs for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were 15.747%, 16.466% and 16.592%, respectively.

Based on total amphotericin B: The ratios of the LSMs (and 90% geometric CIs) of the test to reference product (A/B) were 95.687% (89.63% - 102.15%) for C_{max}, 95.773% (85.96% - 106.71%) for AUC_{0-t}, 95.101% (85.68% - 105.56%) for $AUC_{0-\infty}$, respectively. The intra-subject CVs for C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were 8.855%, 14.689% and 14.173%, respectively.

The GLSMR and 90% CIs for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ following administration of the test and reference formulations not only for free and encapsulated amphotericin B but also for total amphotericin B were within 80.00%~125.00% (Table 3), which indicated that the bioequivalence criteria were met.

Safety

All 12 subjects received one dose of the test drug and one dose of reference drug, and were included in the safety analysis set (Table 4). There were no serious clinically safety findings in clinical laboratory tests, physical examinations, vital signs, or ECG findings. 8 of the 12 subjects (66.7%) experienced 32 adverse events, 31 of which

were TEAEs. All adverse events were mild in severity and were self-alleviating. 6 of the subjects (50%) reported 18 TEAEs after administration of the test formulation and 8 of the subjects (66.7%) reported 13 TEAEs after administration of the reference formulation. The safety profiles were comparable between these two preparations.

Discussion

Amphotericin B, the polyene class of antifungal agents, is still an important option for the prevention and treatment of invasive fungal diseases due to its broad spectrum and well-documented clinical efficacy. Among the four amphotericin B formulations in market, Ambisome® is widely used in clinical based on its better safety profiles compared with other amphotericin B formulations. Although Ambisome® has established detailed clinical PK/efficacy/safety profiles since its first approved in 1990, there is no research conducted in Chinese subjects. This is the first PK and BE study of Ambisome® (the reference formulation) and the generic product (the test formulation) in healthy Chinese subjects.

Plasma pharmacokinetics of Ambisome® has been reported by other investigators. The plasma pharmacokinetic profile of liposomal amphotericin B based on total amphotericin B in our study revealed a three-compartment model with a longer half-life, consistent with reticuloendothelial uptake and redistribution [8]. As previously reported [9,10], most of the amphotericin B in plasma remained liposome associated (97% at 4 h, 55% at 168 h) after liposomal amphotericin B administration. In our study, the lipid-based amphotericin B was the mainly existence form after 2h-infusion of the test and reference drug between 0.5h and 26h after the start of 2h-infusion. The percentage of free drug in total drug was below than 10% between 0.5h and 26h after the start of 2h-infusion, and then slowly rises to 50% at 147h, finally rises to approximately 100% at 218h, which was consist with other researches. Liposomal drug approximately accounted for 96.2%, 88.0%, 86.8% of the total amphotericin B based on C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ respectively. At least 60 ng/mL of free amphotericin B was determined at 674 hours after drug infusion, therefore a much longer half-life was calculated. This indicated that liposome and deep tissue compartments may act as a “pool” or “sump” of drug, slowly release free drug.

Ihor Bekersky et al reported the PK behaviors based on total amphotericin B in healthy western subjects, and determined the unbound amphotericin B using the same plasma samples. C_{max} and AUC_{0-t} of total amphotericin B in our research were comparable with that in healthy western subjects [8]. Ihor Bekersky [9] reported that unbound amphotericin B concentrations did not exceed 25 ng/mL at any time point in any subject given liposomal amphotericin B, and 20.6 ng/mL of unbound amphotericin B was calculated in our study using free drug (unbound and bound drug) concentration and a protein bind rate 98%. The protein bind rate 98% was made according to protein bind rate- the total amphotericin B concentration in Ihor Bekersky' research. Taking these results together, the similar PK behaviors between East and west populations were demonstrated.

In our study, total, encapsulated, and free drug were all determined to show the similarity of the test and reference products in Chinese healthy subjects. Bioequivalence was consistently established based on free, encapsulated and total amphotericin B. All CIs for comparisons of C_{max} values and AUC ratios were within the 80.00-125.00% confidence bounds. In addition to PK bioequivalence, the test and reference products were well tolerated, and there were no significant differences between the safety profiles of the test and reference products. Those results showed the test produce manufactured by CSPC Zhongnuo Pharmaceutical Co., Ltd might be an alternative for Ambisome® for the treatment of invasive fungal infection in China.

Conclusion

The data in the study show plasma pharmacokinetic profile of liposomal amphotericin B based on free, encapsulated and total amphotericin B in our study revealed a three-compartment model with a longer half-life. The majority drug in the circulating system after IV infusion of liposomal amphotericin B is remained the liposomal form. PK behaviors in Chinese population was consistent with that in western healthy populations based on total and unbound amphotericin B concentrations in plasma. In addition, the generic liposomal amphotericin B for injection manufactured by CSPC Zhongnuo Pharmaceutical Co., Ltd is bioequivalent to Ambisome® in terms of the PK

parameters for free, encapsulated and total amphotericin B and with similar safety profiles as Ambisome®.

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Tables and Figures

Table 1 Demographic profile and baseline clinical characteristics of subjects

Characteristic	Sequence A(T/R) N=12	Sequence B(R/T) N=12
Sex, No. (%)	**Men:** 10(83.33%) **Women:** 2(16.67%)	**Men:** 10(83.33%) **Women:** 2(16.67%)
Age, years (mean ± SD)	30.00±5.64	30.00±5.64
Height, cm (mean ± SD)	166.42±7.31	166.42±7.31
Weight, kg (mean ± SD)	63.48±7.38	63.73±7.47
Body mass index, kg/m^2 (mean ± SD)	22.89±1.83	22.98±1.90

Table 2 The main pharmacokinetic parameters of free, encapsulated and total amphotericin B after IV infusion of the test or reference drugs.

Free PK Parameters (Units)	Mean ± SD (CV%) (N^*=12)			
	n^*	Test Drug (T)	n^*	Reference Drug (R)
C_{max} (ng/mL)	12	905.00±93.59(10.3%)	12	900.50±95.77(10.6%)
T_{max}(h) *	12	2.00(2,3)	12	2.00(2,3)
AUC_{0-t} (h*ng/mL)	12	87262.33±9344.90(10.7%)	12	88603.23±12260.26(13.8%)
$AUC_{0-\infty}$(h*ng/mL)	12	122593.43±22548.95(18.4%)	12	125902.37±26056.93(20.7%)
$\%AUC_{ex}$	12	27.67±8.39(30.3%)	12	28.26±8.98(31.8%)
λ_z(1/h)	12	0.002±0.001(28.8%)	12	0.002±0.001(31.5%)
$t_{1/2}$(h)	12	393.99±113.59(28.8%)	12	408.63±117.29(28.7%)
V_d (L)	12	590.14±165.26(28.0%)	12	602.41±170.64(28.3%)
CL (L/h)	12	1.06±0.18(16.7%)	12	1.04±0.18(17.3%)
F(%)	12	99.09±8.17(8.2%)	/	/
Encapsulated PK Parameters (Units)	**Mean ± SD(CV%)（N^*=12）**			
	n^*	Test Drug (T)	n^*	Reference Drug (R)
C_{max} (ng/mL)	12	23233.33±4033.80(17.4%)	12	23366.67±5609.30(24.0%)
T_{max}(h) *	12	2.00(2,2.5)	12	2.00(2,2.5)
AUC_{0-t} (h*ng/mL)	12	365781.47±117361.61(32.1%)	12	377628.18±128271.58(34.0%)
$AUC_{0-\infty}$(h*ng/mL)	12	373858.46±117392.44(31.4%)	12	387051.50±128837.05(33.3%)
$\%AUC_{ex}$	12	2.32±0.88(37.8%)	12	2.58±1.04(40.3%)
λ_z(1/h)	12	0.021±0.003(14.6%)	12	0.018±0.004(20.9%)
$t_{1/2}$(h)	12	34.21±6.36(18.6%)	12	39.12±8.63(22.1%)
V_d (L)	12	18.20±6.94(38.1%)	12	19.85±5.97(30.1%)
CL (L/h)	12	0.37±0.11(29.6%)	12	0.36±0.09(26.0%)
F (%)	12	99.66±20.97(21.0%)	/	/
Total PK Parameters (Units)	**Mean ± SD (CV%)（N^*=12）**			
	n^*	Test Drug (T)	n^*	Reference Drug (R)
C_{max} (ng/mL)	12	22991.67±3791.01(16.5%)	12	24291.67±5444.17(22.4%)
T_{max}(h) *	12	2.00(2,2)	12	2.00(2,2)
AUC_{0-t} (h*ng/mL)	12	409097.85±122843.29(30.0%)	12	429165.03±132355.52(30.8%)
$AUC_{0-\infty}$(h*ng/mL)	12	420859.73±122514.13(29.1%)	12	445682.07±137897.15(30.9%)
$\%AUC_{ex}$	12	3.01±1.40(46.5%)	12	3.68±1.51(41.0%)

λ_z(1/h)	12	0.011±0.004(35.9%)	12	0.009±0.005(55.7%)
$t_{1/2}$(h)	12	71.44±33.99(47.6%)	12	102.27±64.66(63.2%)
V_d (L)	12	32.43±14.42(44.5%)	12	41.19±17.83(43.3%)
CL (L/h)	12	0.32±0.08(25.9%)	12	0.31±0.07(24.0%)
***F* (%)**	12	97.48±18.54(19.0%)	/	/

*: T_{max}: Median (Max, Min); N: Number of subjects involved in the study; n: Number of subjects involved in the statistical analysis.

Table 3 Geometric least squares mean ratios (GLSMR) and 90% CIs for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ following administration of IV infusion of 2mg/kg of test or reference drugs in healthy subjects.

Analyte form	PK Parameters (Units)	GLS Mean				T/R Ratio (%)	Ratio 90% CI (%)	CV (%)	Power (%)
		n	T	n	R				
Free (Unencapsulated)	C_{max}(ng/mL)	12	900.092	12	895.592	100.502	(97.43,103.67)	4.201	>99.999
	AUC_{0-t} (h*ng/mL)	12	86822.971	12	87871.757	98.806	(95.09,102.67)	5.187	>99.999
	$AUC_{0-\infty}$(h*ng/mL)	12	120811.415	12	123364.134	97.931	(91.74,104.54)	8.839	99.980
Encapsulated	C_{max}(ng/mL)	12	22917.401	12	22724.968	100.847	(89.82,113.23)	15.747	88.734
	AUC_{0-t} (h*ng/mL)	12	349244.825	12	358154.867	97.512	(86.40,110.06)	16.466	83.128
	$AUC_{0-\infty}$(h*ng/mL)	12	357559.485	12	367666.755	97.251	(86.09,109.86)	16.592	81.937
Total	C_{max}(ng/mL)	12	22713.785	12	23737.659	95.687	(89.63,102.15)	8.855	99.842
	AUC_{0-t} (h*ng/mL)	12	393179.897	12	410533.998	95.773	(85.96,106.71)	14.689	87.046
	$AUC_{0-\infty}$(h*ng/mL)	12	405402.314	12	426284.208	95.101	(85.68,105.56)	14.173	87.131

Table 4 TEAEs receiving the test or refer drug in healthy subjects

TEAEs	Test (No. of TEAEs; No. of subjects, %)		Reference (No. of TEAEs; No. of subjects, %)		Total (No. of TEAEs; No. of subjects, %)	
Total	**18**	**6(11.43)**	**13**	**8(13.89%)**	**31**	**8(66.67)**
Elevated alanine aminotransferase	1	1(8.33)	2	2(16.67)	3	2(16.67)
Elevated cholesterol	1	1(8.33)	1	1(8.33)	2	1(8.33)
Elevated blood triglycerides	2	1(8.33)	0	0	2	1(8.33)
Chest tightness	4	4(33.33)	5	5(41.67)	9	7(58.33)
nausea	2	2(16.67)	0	0	2	2(16.67)
Lower blood pressure	1	1(8.33)	0	0	1	1(8.33)
Palpitations	0	0	1	1(8.33)	1	1(8.33)
Purulent tonsillitis	1	1(8.33)	0	0	1	1(8.33)
Head numbness	0	0	1	1(8.33)	1	1(8.33)
Difficulty breathing	1	1(8.33)	0	0	1	1(8.33)
Gingivitis	1	1(8.33)	0	0	1	1(8.33)
dizziness	0	0	1	1(8.33)	1	1(8.33)
anemia	1	1(8.33)	0	0	1	1(8.33)
Meditation	1	1(8.33)	1	1(8.33)	2	2(16.67)
Elevated blood uric acid	2	1(8.33)	1	1(8.33)	3	1(8.33)

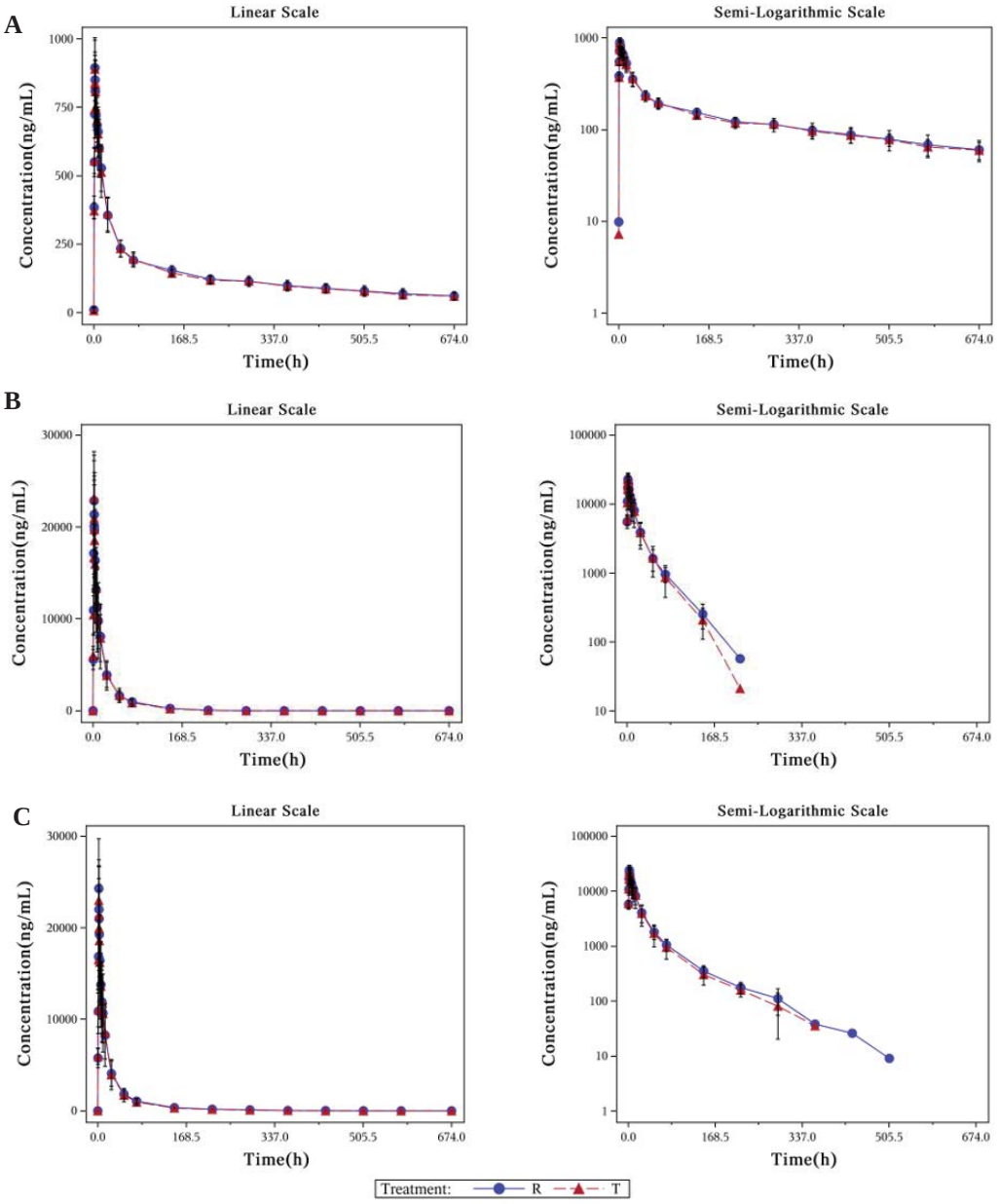

Fig.1 Mean plasma concentration-time curves of Free (A), encapsulated (B) and total (C) amphotericin B after IV infusion of the test and refer drug in healthy subjects (Mean ± SD).