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RESEARCH

A multicenter, randomized, open label, two formulation, crossover bioequivalence trial of doxorubicin hydrochloride liposomal injection in Chinese patients with metastatic breast cancer

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Abstract

Purpose The primary objectives of this trial were aimed at exploring the pharmacokinetic profiles and the human bioequivalence of an intravenous liposomal injection of doxorubicin hydrochloride in comparison with a reference formulation in Chinese patients diagnosed with metastatic breast cancer.

Methods To achieve these goals, the trial employed a randomized, open-label, two-formulation crossover dosing strategy among Chinese patients with metastatic breast cancer. Pharmacokinetic (PK) evaluation was conducted through the collection of blood samples, and the liquid chromatography tandem mass spectrometry (LC/MS/MS) method was leveraged to quantify plasma concentrations of both liposome-encapsulated doxorubicin and non-encapsulated doxorubicin in patients. Throughout the trial, all adverse events observed in the patients were meticulously assessed.

Results The results indicated that the maximum concentration (Cmax), AUC from time zero to the last measurable concentration ($AUC_{0\text{-}t}$), and AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$) of in vivo non-encapsulated doxorubicin after administration of both formulations fell within the 80.00%–125.00% range at a 90% confidence interval.

Conclusion These findings strongly indicated that the tested formulations were bioequivalent to the reference formulation. The results also demonstrated that both formulations were well-tolerated, further establishing their safety profile in the context of metastatic breast cancer treatment.

Trial registration Chinadrugtrials.org.cn Identifier: CTR20200878.

Keywords Breast cancer · Doxorubicin hydrochloride liposomes · Pharmacokinetics · Safety · Bioequivalence test

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Introduction

Based on data from the World Health Organization, breast cancer, primarily affecting females, has surpassed lung cancer in terms of incidence rates, emerging as the foremost global malignancy. In China, it holds the position of the most prevalent cancer among women, with an increasing incidence rate that leads globally (Wilkinson and Gathani 2022; CE Noticias Financieras 2024, 2023; Arnold et al. 2022; Gan et al. 2024). Notably, metastatic breast cancer exerts a substantial impact on both the quality and duration of patients' survival (Kim et al. 2020; Arnedos et al. 2015; Frank et al. 2020; Li et al. 2021; Brufsky et al. 2024). In the realm of breast cancer treatment, anthracyclines play a pivotal role and stand as the standard regimen for the initial treatment of breast cancer. They are typically combined with other chemotherapeutic agents and molecularly targeted agents. However, it's important to note that the administration of anthracyclines is associated with common toxicities, including alopecia, myelosuppression, and cardiotoxicity (National Cancer Quality Control Center Breast Cancer Expert Committee et al. 2022; Tan et al. 2017; Wang et al. 2024; Gradishar et al. 2023).

Doxorubicin, an anthracycline antibiotic, falls under the category of cell cycle non-specific drugs. Its mechanism primarily involves intercalating between DNA base pairs and tightly binding to DNA, thereby impeding DNA replication, inhibiting the action of DNA-dependent polymerase, and interfering with the RNA transcription process. Possessing robust anticancer activity and a high chemotherapeutic index, doxorubicin's impact on cell division is non-selective, affecting both tumor and normal cells (National Cancer Institute 2025). Consequently, it leads to common adverse effects such as vomiting, nausea, and alopecia, typical of many chemotherapeutic agents. Notably, doxorubicin exhibits a significantly higher affinity for cardiac muscle compared to other tissues. This heightened affinity can lead to the damage of cardiac myocytes through semiquinone metabolites, resulting in severe dose-dependent cardiotoxicity (Gabizon et al. 2004). To address these challenges and enhance therapeutic outcomes, a novel targeted drug carrier technology involving liposomes for doxorubicin has been developed (Aldughaim et al. 2020). Multiple doxorubicin liposomes, designed with the purpose of synergism and attenuation, have been successfully introduced into clinical applications (Chowdhury, et al. 2020; Younes et al. 2019; Bhowmik et al. 2018a; Jiang et al. 2023; Moskowitz et al. 2021).

Currently, there are two types of liposomal drugs of doxorubicin marketed abroad, which are polyethylene glycol (PEG)—modified long-acting circulating liposomes (PLD) and non PEG modified conventional liposomes (NPLD). Liposomes in which PEG modified doxorubicin (Caelyx ®) approved indications in the EU include metastatic breast cancer with higher cardiac risk (Rivankar 2014). Such liposomes have a low permeability lipoid matrix with an internal aqueous buffering system, both of which synergistically maintain doxorubicin hydrochloride in an encapsulated state in the blood circulation. At the same dose, doxorubicin hydrochloride in its liposomal encapsulated form (approximately 90–95% of the measured amount) is overwhelmingly present in this product, and plasma concentrations and AUC values are significantly higher than those of conventional doxorubicin hydrochloride formulations (Methaneethorn et al. 2023).

Doxorubicin liposomal injection (specification: 10 ml / 20 mg), the test drug, was manufactured by Zhejiang Zhida Pharmaceutical Co., Ltd. and now strictly followed the relevant laws and regulations of GCP specifications and the relevant regulations of the central ethics committee, in accordance with Reference Listed Drug for Generic Drugs issued by National Medical Products Administration (NMPA) (22nd batch) (22–338), the original study drug, doxorubicin liposomal injection (Caelyx®), which has been in the European Union, was selected as a reference formulation and completed a randomized, open label, two formulation, cross-over bioequivalence trial in Chinese metastatic breast cancer patients in accordance with the requirements of the National Medical Products Administration (NMPA) for the quality and efficacy consistency evaluation of generic drugs.

Materials and methods

Study design

This study employed a multicenter, randomized, open-label, two-formulation, crossover design. Inclusion criteria encompassed women aged 18–70, weighing ≥ 40.0 kg, with a body surface area < 1.8 m^2, and a confirmed histological or cytological diagnosis of breast cancer. Comprehensive assessment of histology or imaging for metastatic breast cancer was required for eligibility. Exclusion criteria involved individuals with prior cumulative doses of doxorubicin ≥ 450 mg/m^2 or cumulative doses of epirubicin ≥ 600 mg/m^2, or those with a history of anthracycline-induced severe cardiotoxicity.

Cardiac function abnormalities were excluded based on criteria such as an electrocardiogram (ECG) examination with a QTc > 480 ms, left ventricular ejection fraction (LVEF) $< 55\%$, troponin I quantification > upper limit of normal (ULN), N-terminal pro brain natriuretic peptide assay > ULN, creatine phosphate kinase (CK) $> 2.5 \times$ ULN, congestive heart failure, myocardial infarction, or

uncontrolled angina with NYHA functional class ≥ 2 within 6 months before signing informed consent, and a history of cardiac bypass surgery.

Subjects meeting entry criteria were randomly assigned in a 1:1 ratio to the T-R group and the R-T group according to a randomized protocol. They received either the test formulation or the reference formulation through intravenous infusion. Investigators involved in sample analysis remained blinded, and subjects who withdrew or were withdrawn from the trial retained their randomization number, precluding re-entry into the study.

Screening and baseline assessments were conducted from day -14 to day -1 prior to dosing. After confirming compliance with entry criteria, subjects were admitted to the study center one day prior to dosing (day-1). A comprehensive medical history query record, vital signs examination, alcohol and drug abuse screening, concomitant medication records, and a blood pregnancy test for women of childbearing age were completed. Intravenous infusion of doxorubicin liposomes was administered at the prescribed time on Day 1 am.

Close monitoring by experienced physicians occurred during injections, with troponin I quantification and N-terminal pro brain natriuretic peptide monitoring at scheduled times after injection. Pharmacokinetic (PK) blood sample collection took place from Day 1 to Day 4, and subjects could leave the study center with the investigator's consent after PK blood sample collection on Day 4 am. Uniform diet provision by the study center during the stay, scheduled visits for PK blood collection and safety follow-up, including various examinations and adverse effects monitoring, ensured subject safety.

Following a 28-day washout period, subjects underwent a second cycle crossover dosing study, adhering to the study flow from the initial cycle. Subjects were required to avoid vigorous exercise during the trial and complete the last safety examination within 7 days after the end of the last blood collection (Li et al. 2022; Bhowmik et al. 2018b; Prakash et al. 2022).

This bioequivalence study conformed to the requirements of GCP and the declaration of Helsinki, good informed consent was obtained from all patients screened in this study, and those who met the inclusion and exclusion criteria of this study could be enrolled.

Pharmacokinetic assessments

The assessment of bioequivalence was conducted in accordance with the guidelines outlined by the China National Drug Administration technical guidelines for the generic drug study of doxorubicin hydrochloride liposomal injection, issued on October 21, 2020. The primary pharmacokinetic parameters (Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$) of both non-encapsulated doxorubicin and liposome-encapsulated doxorubicin pertaining to the two formulations were thoroughly analyzed. The bioequivalence acceptance criteria were established within the range of 80.00% to 125.00%. As stipulated by the Bioequivalence (BE) guidance document, primary pharmacokinetic parameters (Cmax, $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$) of both non-encapsulated doxorubicin and liposome-encapsulated doxorubicin from both formulations were mandated to be equivalent.

Moreover, secondary measures, such as partial exposure indicators (e.g., $AUC_{0\text{-}48\ h}$ and $AUC_{48h\text{-}last}$) of liposome-encapsulated doxorubicin, were examined with 90% confidence intervals in a similar fashion. The ratio of the geometric means of Cmax, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$, along with their respective 90% confidence intervals, was calculated to assess the primary pharmacokinetic parameters of free doxorubicin and liposome-encapsulated doxorubicin in vivo after the administration of the test formulation compared to the reference formulation. Bioequivalence between the two formulations was deemed acceptable if the 90% confidence interval fell within the specified range of 80.00% to 125.00%.

Throughout the clinical study, all subjects were meticulously monitored for any adverse events. This comprehensive monitoring included the assessment of clinical symptoms, abnormal vital signs, physical examination, blood routine, urine routine, blood biochemistry, troponin I quantitative assay, N-terminal pro brain natriuretic peptide assay, myocardial zymography assay, coagulation routine, blood pregnancy, 12-lead ECG, and cardiac ultrasound. The recorded data encompassed the clinical characteristics, severity, occurrence time, end time, duration, handling measures, and outcome of any observed adverse events. Adjudication of the relatedness of these events to the study drug was also performed as part of the rigorous safety assessment.

Drug administration

Subjects were randomly allocated into two dosing sequence groups (T-R group / R-T group) with a 1:1 ratio. In the first cycle, participants in the T-R group received an intravenous infusion of the test formulation and were later cross-dosed with the reference formulation after a 28-day interval. The study investigators assigned the study drugs for each cycle based on the predetermined randomization scheme.

Each subject received a single intravenous (IV) infusion at the specified morning time on the dosing day. The dose of doxorubicin liposomal injection, calculated by the investigator using the subject's body surface area at enrollment with the recommended dose of 50 mg / m^2, was administered through IV infusion after dilution with 250 ml of 5% dextrose injection. This process occurred approximately 90 min after the initiation of the IV infusion. The administration

process was closely monitored by the investigator, with relevant first aid measures prepared. Additionally, reasonable dietary and lifestyle management were implemented based on the subject's physical condition during the study.

Stability

Venous plasma samples, Blood samples were collected for each subject within 1 h before medication (0 h) and 30 min, 60 min, 75 min, 90 min, 95 min, 105 min, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, 240 h, 336 h, 504 h after intravenous infusion, a total of 20 blood collection points, two cycles of a total of 40 blood collection points. Approximately 4 ml of whole blood samples were taken at each time point and transferred to a vacuum container containing the EDTA-K2 anticoagulant.

Blood samples were collected and immediately centrifuged at 4 °C for 10 min (2000 g) to separate the plasma. The entire volume of plasma was then transferred into a 2-ml centrifuge tube using a pipette, followed by precise pipetting of 1.00 ml of plasma into a cryovial containing 0.100 ml of stabilizer. Subsequently, the cryovial was gently inverted at least fifteen times to ensure thorough mixing of the plasma and stabilizer the stabilized plasma samples were kept in an ice bath for a minimum of 10 min and subsequently transferred to an ultra-low temperature freezer at -60 ℃ within 24 h. It is important to note that the doxorubicin hydrochloride liposomal injection has a red color, thus it is expected that the plasma sample would exhibit a light red hue. The samples were shipped using dry ice as a coolant by a designated cold chain shipping company to the testing unit within the specified timeframe.

Concentration detection

The validated liquid chromatography-tandem mass spectrometry (LC–MS/MS) method, which is extensively utilized for the quantitative assessment of drug concentrations in biological samples, was employed to ascertain the concentration of both free doxorubicin and liposome-encapsulated doxorubicin in K2EDTA human plasma samples. The mobile phases consisted of 5 mM aqueous ammonium acetate (designated as mobile phase A) and 100% acetonitrile (mobile phase B).

For the analysis of free doxorubicin, human plasma samples underwent solid phase extraction (SPE) as a pretreatment step. Following the re-dissolution of the extracts via nitrogen blow-drying, the samples were subjected to LC–MS/MS analysis. This analysis incorporated the use of electrospray ionisation (ESI) in the positive ionisation mode, along with multiple reaction monitoring (MRM).

On the other hand, the pretreatment of human plasma samples for liposome-encapsulated doxorubicin involved both solid phase extraction (SPE) and protein precipitation (PP). After the dilution of the obtained extract, the samples were analyzed using LC–MS/MS. This analysis similarly employed ESI in the positive ionisation mode and MRM.

Statistical method

All subjects who signed informed consent to participate in the pilot screening were entered into the screening set (SCN) analysis. Subjects with documented safety indicators who were randomized and received the study drug were included in the analysis of the safety set (SS); all randomized subjects were included in the analysis of the full analysis set (FAS). All randomized, and who received study drug, subjects with at least 1 plasma concentration data during the trial entered the PK concentration set (PKCs) analysis; All randomized subjects who received at least one dose of study drug and had at least one valid PK parameter data were included for PK parameter set (PKPs) analysis. The bioequivalence assessment is primarily based on the be set (BES), which typically comprises an analysis set belonging to the PK parameter set and encompassing completed clinical trials with at least one evaluable pharmacokinetic parameter. The above datasets were utilized to perform analyses on subject distribution, demographic data and baseline characteristics, medication adherence and concomitant medication usage, as well as pharmacokinetic and safety evaluations.

Results

Background characteristics of the volunteers

A total of 40 female patients with metastatic breast cancer were screened, and 31 subjects, aged between 33 and 68 years, with heights ranging from 146.5 to 165.0 cm, weights ranging from 41.5 to 64.6 kg, and body surface areas (BSA) ranging from 1.40 to 1.77 m2 participated in the study. Among them, there were thirty Han ethnicity subjects and one Zhuang ethnicity subject. Among them, 24 subjects completed the two cycle study while seven withdrew prematurely. Among the 7 withdrawn subjects 5 of them withdrew themselves from the study during the first cycle, 1 withdrew because of adverse events during the first cycle, and 1 withdrew themselves from the study during the second cycle. (Table 1).

Pharmacokinetics

Thirty one subjects enrolled in the study, with 25 subjects using the test formulation and 25 subjects using the reference formulation, with at least one valid PK parameter data, were included in the PK parameter set(PKPS) for analysis.

Table 1 Subject demographic data. Full analysis set

				T-R group N = 15			R-T group N = 16			Summation N = 31
Sex, number of cases (%)										
Male				0 (0.0)			0 (0.0)			0 (0.0)
Female				15 (100.0)			16 (100.0)			31 (100.0)
Ethnic group, number of cases (%)										
Han Chinese				14 (93.3)			16 (100.0)			30 (96.8)
Other				1 (6.7)			0 (0.0)			1 (3.2)
Age(years)										
Number of cases (%)				15 (100.0)			16 (100.0)			31 (100.0)
Mean (standard deviation)				50.5 (7.64)			53.4 (9.94)			52.0 (8.88)
	Cycle 1 (D-1)	Cycle 2 (D-1)	Last safety examination	Cycle 1 (D-1)	Cycle 2 (D-1)	Last safety examination	Cycle 1 (D-1)	Cycle 2 (D-1)	Last safety examination	
Height[cm]										
Number of cases (%)	15 (100.0)	11 (73.3)	14 (93.3)	16 (100.0)	14 (87.5)	15 (93.8)	31 (100.0)	25 (80.6)	29 (93.5)	
Mean (standard deviation)	156.33 (5.573)	155.41 (5.481)	156.18 (6.201)	153.44 (3.750)	153.43 (3.807)	153.17 (4.039)	154.84 (4.867)	154.30 (4.623)	154.62 (5.325)	
Weight[kg]										
Number of cases (%)	15 (100.0)	11 (73.3)	14 (93.3)	16 (100.0)	14 (87.5)	15 (93.8)	31 (100.0)	25 (80.6)	29 (93.5)	
Mean (standard deviation)	56.85 (5.914)	57.90 (6.328)	56.06 (5.973)	51.63 (7.655)	51.66 (7.289)	51.30 (7.785)	54.15 (7.257)	54.40 (7.448)	53.60 (7.261)	
BSA[m^2]										
Number of cases (%)	15 (100.0)	11 (73.3)	0 (0.0)	16 (100.0)	14 (87.5)	0 (0.0)	31 (100.0)	25 (80.6)	0 (0.0)	
Mean (standard deviation)	1.651 (0.0987)	1.659 (0.0988)	0 (0.0)	1.566 (0.1091)	1.566 (0.1013)	0 (0.0)	1.607 (0.1112)	1.607 (0.1089)	0 (0.0)	

The number of acquired samples was 1003 in two cycles, and the number of completed assays was 1003. The specific pharmacokinetic parameters are shown in Table 2. The semilog curve of free doxorubicin mean blood concentration (C) to time (T) is shown in Fig. 1. The semilog curve of mean blood concentration (C) to time (T) of doxorubicin encapsulated in liposome is shown in Fig. 2.

Assessment of bioequivalence

The ABE method was used to evaluate the bioequivalence of non-encapsulated doxorubicin in vivo after administration of the two formulations: the geometric mean ratio (T / R) of Cmax was 109.15% with a 90% confidence interval of (101.78%–117.05%); The geometric mean ratio (T / R) of $AUC_{0\text{-}t}$ was 105. 91%, with a 90% confidence interval of (100.07%–112.08%); The geometric mean ratio (T / R) of $AUC_{0\text{-}\infty}$ was 107.37% with a 90% confidence interval of (102.14%–112.87%). The 90% confidence intervals for Cmax, $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ were in the range (80.00%–125.00%) with a confidence of 94.30%, 99. 93%, 99. 97% (Table 3).

The ABE method was used to evaluate bioequivalence of liposome encapsulated doxorubicin in vivo after administration of the two formulations: the geometric mean ratio (T / R) of Cmax was 100.00%, and the 90% confidence interval was (97.16%–102. 92%); The geometric mean ratio (T / R) of $AUC_{0\text{-}t}$ was 102.50%, with a 90% confidence interval of 97.54%–107.71%; The geometric mean ratio (T / R) of AUC $_{0\text{-}\infty}$ was 104.30%, with a 90% confidence interval of (99.

Table 2 Pharmacokinetic parameters of free doxorubicin, liposome encapsulated doxorubicin PKPS

PK parameters (units)	Count means ± SD	
	T (N = 25)	R (N = 25)
Free doxorubicin		
T_{max}* (h)	8.02	1.75
C_{max} (ng/mL)	339.84 ± 74.87	308.24 ± 47.79
$AUC_{0\text{-}t}$ (h·ng/mL)	48,832.07 ± 12,353.89	45,655.55 ± 11,164.83
$AUC_{0\text{-}\infty}$ (h·ng/mL)	51,091.08 ± 11,755.65	46,938.50 ± 11,937.87
$T_{1/2}$ (h)	80.95 ± 17.30	85.33 ± 18.94
λ_z (h^{-1})	0.009 ± 0.0016	0.009 ± 0.0018
Vd (mL)	193,239.79 ± 46,525.73	215,488.98 ± 39,327.78
$AUC_{\_\%Extrap}$ (%)	3.32 ± 5.70	2.53 ± 1.68
Liposomal encapsulation of doxorubicin		
T_{max}* (h)	4	4
C_{max} (ng/mL)	35,452.00 ± 3150.15	35,520.00 ± 3409.91
$AUC_{0\text{-}t}$ (h·ng/mL)	4,529,858.51 ± 925,968.24	4,360,541.94 ± 952,540.08
$AUC_{0\text{-}\infty}$ (h·ng/mL)	4,710,461.34 ± 927,914.08	4,448,959.21 ± 1,022,515.04
$AUC_{0\text{-}48}$ (h·ng/mL)	1,332,410.28 ± 124,195.24	1,297,241.67 ± 111,138.49
$AUC_{48\text{-}last}$ (h·ng/mL)	3,197,448.23 ± 856,850.36	3,063,300.27 ± 868,129.45
$T_{1/2}$ (h)	81.20 ± 18.95	77.65 ± 19.99
λ_z (h^{-1})	0.009 ± 0.0020	0.010 ± 0.0025
Vd (mL)	2018.31 ± 344.47	2025.17 ± 285.10
$AUC_{\_\%Extrap}$ (%)	2.81 ± 4.07	1.77 ± 1.33

Cmax peak concentration, *AUC0-t0* area under the curve for time on drug to end of blood collection, *AUC0-0* area under the curve at drug time to infinity, *T1/2* plasma elimination half-life, *λz* apparent rate constants, *Vd* apparent volume of distribution, *AUC_%Extrap (%)* AUC from the last point onwards to the theoretical extrapolation to infinity as a proportion of aucinf

*Tmax time to peak was expressed as median (minimum, maximum)

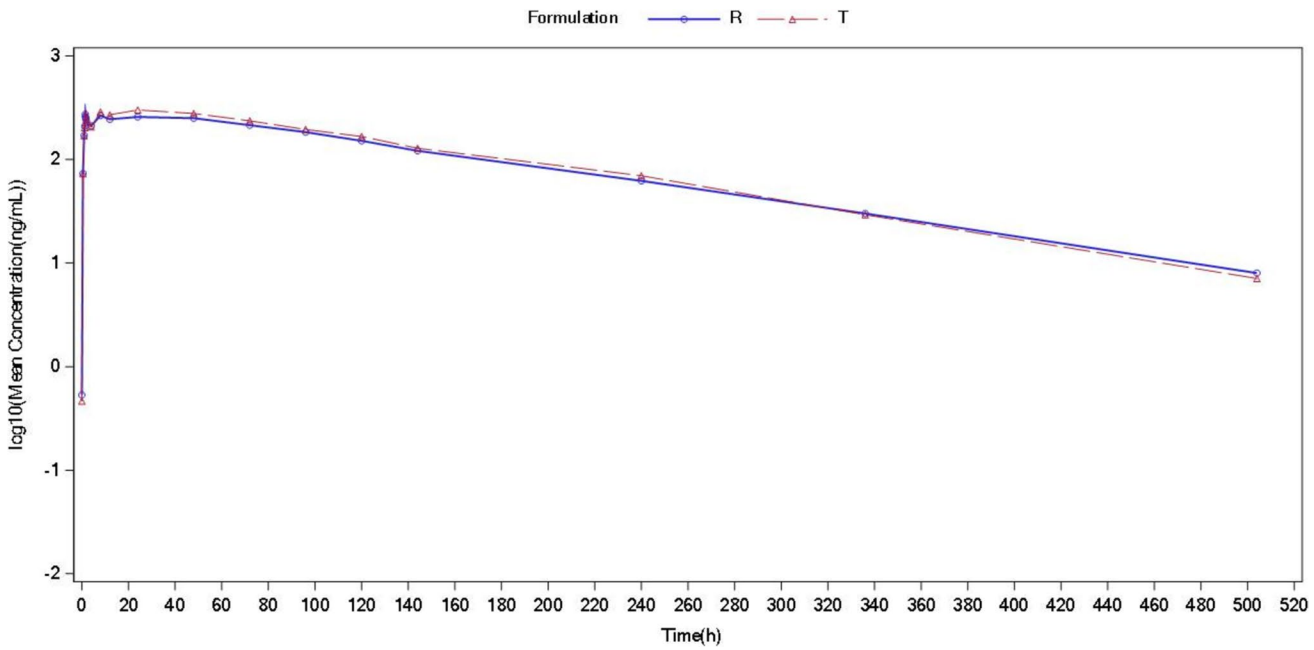

Fig. 1 Free doxorubicin mean plasma concentration (C)–time (T) semilogarithmic curve

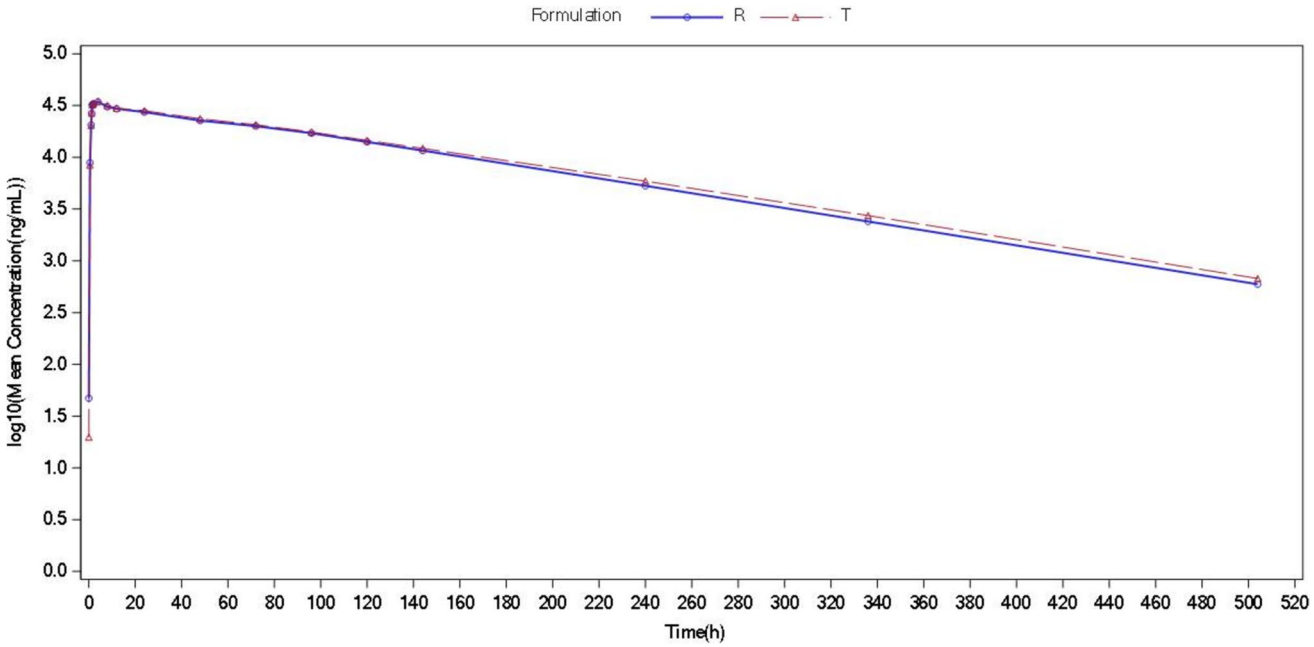

Fig. 2 Mean plasma concentration of liposome encapsulated doxorubicin (C)–time (T) semilogarithmic curve

Table 3 Determination of bioequivalence index for free doxorubicin, liposome-encapsulated doxorubicin and liposome-encapsulated doxorubicin partial exposure

PK parameters	Preparation	N	T-R		F(%)	90% CI	Power(%)
			GM LS Mean	95% CI			
Free doxorubicin							
C_{max} (ng/mL)	T	25	331.644	(305.93, 359.52)	109.15	(101.78,117.05)	94.3
	R	25	303.851	(280.29, 329.39)			
$AUC_{0\text{-}t}$ (h·ng/mL)	T	25	47,336.901	(42,416.24,52,828.40)	105.91	(100.07,112.08)	99.93
	R	25	44,695.934	(40,049.80,49,881.06)			
$AUC_{0\text{-}\infty}$ (h·ng/mL)[#]	T	24	49,250.73	(44,374.24,54,663.12)	107.37	(102.14,112.87)	99.97
	R	25	45,869.741	(41,346.10,50,888.31)			
Liposomal encapsulation of doxorubicin							
C_{max} (ng/mL)	T	25	35,164.134	(33,968.44, 36,401.92)	100	(97.16,102.92)	100
	R	25	35,164.248	(33,968.55, 36,402.04)			
$AUC_{0\text{-}t}$ (h·ng/mL)	T	25	4,418,580.61	(4,059,935.35,4,808,907.76)	102.5	(97.54,107.71)	100
	R	25	4,310,923.46	(3,961,016.46,4,691,740.42)			
$AUC_{0\text{-}\infty}$ (h·ng/mL)[#]	T	24	4,578,981.18	(4,214,630.06,4,974,830.14)	104.3	(99.95,108.84)	100
	R	25	4,390,309.74	(4,042,585.36,4,767,943.76)			
Indicators of partial exposure of liposome encapsulated doxorubicin							
$AUC_{0\text{-}48}$ (h·ng/mL)	T	25	1,317,786.97	(1,275,028.29,1,361,979.59)	101.69	(98.54,104.95)	100
	R	25	1,295,825.06	(1,253,778.98,1,339,281.17)			
$AUC_{48\text{-last}}$ (h·ng/mL)	T	25	3,071,661.55	(2,738,259.11,3,445,658.08)	102.66	(95.96,109.83)	99.93
	R	25	2,991,950.59	(2,667,200.09,3,356,241.75)			

- sequence, *T* test preparation, *R* reference preparation, *LS* least square, *CI* confidence interval

Geometric LS means ratio of PK parameters of T to R

[#]If the AUC of the subject Extrac > 20%, and the $AUC_{0\text{-}\infty}$ of the corresponding cycle were not included in the bioequivalence analysis

95%–108.84%). The 90% confidence intervals for Cmax, AUC_{0-t} and $AUC_{0-\infty}$ were in the range (80.00%–125.00%) with 100.00%, 100.00%, 100.00% confidence limits (Table 3).

The ABE method was used to evaluate bioequivalence of some of the exposure indicators of liposome encapsulated doxorubicin in vivo after administration of the two formulations: the geometric mean ratio (T / R) of AUC_{0-48} was 101.69%, with 90% confidence interval (CI) of 98.54%–104. 95%. The geometric mean ratio (T / R) of the $AUC_{48-last}$ was 102.66%, with a 90% confidence interval of (95. 96%–109.83%). The 90% confidence intervals of AUC_{0-48} and $AUC_{48-last}$ were in the range (80.00%–125.00%) with 100.00% confidence and 99. 93% confidence (Table 3).

Safety evaluation

Safety indicators including adverse events, physical examination, laboratory tests and vital signs were monitored according to the protocol, and safety was analysed and assessed by calculating the incidence of adverse effects and system classification. A total of 28 adverse events occurred in subjects who received the test formulation in the formal trial and 27 in subjects who received the reference formulation (90.3% vs 87.1%). Among them, serious adverse events occurred in 3 (9.7%) subjects who received the test formulation and in 1 (3.2%) subject who received the reference formulation; The severity of the adverse events in the subjects who received the reference preparation was grade I (173 events in 28 patients), grade II (90 events in 25 patients), grade III (8 events in 5 patients), and grade IV (1 event in 1 patient), and the severity of the adverse events in the subjects who received the reference preparation was grade I (158 events in 27 patients), grade II (93 events in 24 patients), and grade III (11 events in 8 patients) (AES of grade III or higher are listed below); A total of 1 adverse event leading to withdrawal from the trial occurred in a subject receiving the subject's formulation (25%), and no adverse event leading to withdrawal from the trial occurred in a subject receiving the reference formulation. The investigator conducted close medical monitoring of the subject during administration, provided prompt medical attention to adverse events that emerged, and laboratory tests after completion of the clinical phase study confirmed the absence of a significant impact of the investigational medicinal product on the health status of the subject. Adverse events of grade III or above are shown in Table 4.

Discussion

Doxorubicin stands out as a crucial and extensively utilized anthracycline antitumor drug. The liposomal formulation of doxorubicin enhances the drug's solubility and stability by encapsulating the active pharmaceutical ingredients within lipid nanostructures. The biodegradable nature of the lipid structures in vivo ensures a better safety profile and enhances tumor-targeting capabilities. Consequently, the liposomal formulation of doxorubicin demonstrates improved efficacy, stability, and minimal toxic side effects. Furthermore, the utilization of generic versions of doxorubicin liposomes offers pharmacoeconomic advantages, alleviating the financial burden on Chinese oncology patients undergoing doxorubicin liposomal

Table 4 Grade III or higher adverse events

Adverse event	T n (%)			R n (%)		
	(N = 31)			(N = 31)		
Severity	Total	Grade III	Grade IV	Total	Grade III	Grade IV
Various types of examinations	26(83.9)	3(9.7)	0(0)	23(74.2)	7(22.6)	0(0)
Decreased neutrophil count	13(41.9)	2(6.5)	0(0)	16(51.6)	4(12.9)	0(0)
Decreased white blood cell count	16(51.6)	1(3.2)	0(0)	19(61.3)	3(9.7)	0(0)
Lymphocyte count decreased	12(38.7)	1(3.2)	0(0)	12(38.7)	1(3.2)	0(0)
Elevated alanine aminotransferase	4(12.9)	1(3.2)	0(0)	3(9.7)	0(0)	0(0)
Elevated aspartate aminotransferase	4(12.9)	1(3.2)	0(0)	3(9.7)	0(0)	0(0)
Platelet count decreased	2(6.5)	0(0)	0(0)	3(9.7)	1(3.2)	0(0)
Gastrointestinal disorders	14(45.2)	1(3.2)	0(0)	16(51.6)	0(0)	0(0)
Oral mucositis	6(19.4)	1(3.2)	0(0)	6(19.4)	0(0)	0(0)
Respiratory, thoracic and mediastinal disorders	4(12.9)	1(3.2)	0(0)	7(22.6)	2(6.5)	0(0)
Pleural effusion	1(3.2)	1(3.2)	0(0)	2(6.5)	2(6.5)	0(0)
Immune system disorders	4(12.9)	0(0)	1(3.2)	4(12.9)	0(0)	0(0)
Anaphylactic shock	1(3.2)	0(0)	1(3.2)	0(0)	0(0)	0(0)

therapy. This approach not only benefits a broader spectrum of Chinese tumors but also holds crucial clinical and market value.

Assessing the bioequivalence of two liposomal drugs in patients with advanced tumours may present some challenges when conducting bioequivalence trials.Liposomal drugs enter the body in various forms, such as free drugs, drug-laden liposomes, and the polymeric components constituting the liposomes, all undergoing dynamic transformations. Consequently, the pharmacokinetic profile of these drugs exhibits complexity and potential variability. This profile can be influenced by several factors, including the clearance of the liposomal drug encapsulating the active ingredient, the rate of active ingredient release from the liposome, the metabolism and clearance of the unencapsulated active ingredient, the distribution of liposomal drugs within the body, and the interactions between liposomes, active ingredients, and plasma or serum proteins, blood cells, or vascular endothelium. Furthermore, disparities in the formulation and processing of the subject drug compared to the reference drug can result in altered pharmacokinetic behavior in vivo, ultimately leading to variations in efficacy and safety. These potential variations pose challenges in assessing the bioequivalence of liposomal dual-agent formulations.

To minimise the influence of various factors on bioequivalence test bias, we have devised a rigorous management plan. This plan involves several key steps: ensuring that both the subject preparation and the reference preparation have the same batch number, maintaining consistency in the dosing timeframe for both cycles of the subject, using the same drug combination for both cycles, strictly adhering to the blood collection time points, and centrifuging blood samples to separate the plasma right after collection. Additionally, we precisely measure and transfer the plasma into a stabiliser-containing freezing tube for further testing. We are confident that these precautions will significantly decrease the pharmacokinetic variability of liposomal drugs.

Our study focused on patients with metastatic breast cancer, taking into account the significant impact of both the biological heterogeneity of the tumour and individual subject differences on the pharmacokinetic profile of the drug. Tumour heterogeneity, referring to the diverse genotypic and phenotypic characteristics among cells or cell populations within a single tumour, can result in notable variations in drug distribution and metabolism within the tumour tissue. Specifically, hypoxic regions within the tumour microenvironment have the potential to alter drug activity and metabolism. Additionally, individual differences, including genetic background, physiological state, and pathological conditions such as tumour size, location, liver function, renal function, and other relevant factors, can influence the entire process of drug absorption, distribution, metabolism, and excretion. Despite our rigorous inclusion and exclusion criteria aimed at controlling potential effects, these factors objectively persist and influence bioequivalence outcomes.

High dropout rates often pose a challenge for researchers conducting clinical trials involving patients with advanced tumours. Similarly, our study encountered dropouts midway, but fortunately, the final sample size was adequate for statistical analysis, and the dropout rate fell within our predictions. In our case, some participants voluntarily withdrew after consulting with the study physician, primarily due to infusion reactions during dosing or other medical considerations. Subjects' adverse feelings, stemming from concerns about their health condition and potential drug reactions, may have influenced their decision to opt-out. We have thoroughly discussed the study's limitations and implemented corresponding measures. To enhance the subjects' understanding of the study process and potential risks, we improved communication with them. Furthermore, we implemented rigorous medical monitoring during drug administration to ensure timely detection and management of any adverse reactions.

In line with the Chinese expert consensus (2020 Edition) on the management of adverse reactions with pegylated liposomal doxorubicin, myelosuppression and infusion reactions are anticipated adverse reactions during liposomal doxorubicin treatment. Among the frequent adverse events noted during the study, myelosuppression-related issues, such as decreased white blood cell count, decreased neutrophil count, thrombocytopenia, and anemia, were observed (Yuan and Xu 2020). Furthermore, proactive preventive and symptomatic measures were implemented to mitigate potential infusion reactions, given the risk of hypersensitivity reactions (HSR) triggered by the polyethylene glycol on the surface of the liposomes. These included pre-treatment with an appropriate glucocorticoid dose (10 mg dexamethasone) via intravenous infusion 15 to 30 min before administering liposomal doxorubicin. Other preventive measures encompass the dilution of the drug solution during its preparation, rigorous infusion rate control, and continuous patient monitoring by a nurse. In case symptoms such as chest pain, itching, flushing, chills, and fever arise in the patient, prompt symptomatic treatment should be administered. Moreover, in severe scenarios, the infusion must be halted immediately to optimize the benefit-risk ratio. Furthermore, patients were instructed to discontinue drug intake immediately upon experiencing an instillation reaction. In the event of an infusion reaction, the infusion set was changed, and normal saline drops were initiated. Various assessments, such as ECG monitoring, temperature measurement, oxygen absorption, and close observation of the patient's blood pressure, heart rate, respiration, and oxygen saturation, were conducted. Timely administration of dexamethasone, phenagen, cimetidine, and diphenhydramine was carried out based on symptoms, with a prompt assessment of the response.

Infusion was restarted at a slower rate once the patient's discomfort was relieved. The subjects' safety was diligently ensured throughout the study through close observation and aggressive symptomatic management (Mao et al. 2019; Zhao et al. 2019; Rossi and Osborn 2020; Wenande and Garvey 2016).

It is widely acknowledged that bioequivalence trials aim to assess the human bioequivalence of the test formulation compared to the reference formulation, as well as the safety of the former. However, one of the limitations of this trial type is that subjects only participate in two dosing cycles, which results in a shorter duration of potential benefit from the treatment of the disease.

Conclusion

Based on the comprehensive results of this study, it has been established that intravenous administration of the trail-blazing doxorubicin hydrochloride liposome injection (10 ml: 20 mg) produced by Zhejiang Zhida Pharmaceutical Co. Ltd. in combination with the reference formulation doxorubicin hydrochloride liposome injection (10 ml: 20 mg) (Caelyx ®), in Chinese metastatic breast cancer subjects, showed bioequivalence to the reference formulation. The 90% confidence intervals (CIs) for the geometric mean ratios (GMR) of Cmax, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$, based on the primary PK parameters of free doxorubicin and liposomally encapsulated doxorubicin, fell within the acceptable range of 80.00% to 125.00%. Furthermore, the test formulation was well tolerated alongside the reference formulation, demonstrating a comparable safety profile.

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Author contributions All authors have full access to all the data in the study and take responsibilityfor integrity of the data and the accuracy of the data analysis.Jingshu Wang,Xiuping Lai are co-first authors. Junyan Wu and Suiwen Ye are co-corresponding authors. Concept and design: Suiwen Ye, Heuri Yao and Junyan Wu. Acquisition: All authors. Analysis, or interpretation of data: Suiwen Ye, Jingshu Wang, Xiuping Lai, Hui Yang, Xiaolong Cao, Xiaochen Wang, Ying Wang, Weiqi Nian, Xiaodong Zheng,Qingxiu Mai, Xiaozhi Lv, XiaoYing Bi, Junyi Chen, YeHerui Yao. Drafting of the manuscript: All authors. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Suiwen Ye, Jingshu Wang, Xiuping Lai, Qingxiu Mai, Xiaozhi Lv, XiaoYingBi, YeHerui Yao and JunyiChen. Obtained funding: Suiwen Ye, Herui Yao and Junyan Wu. Administrative, technical, or material support: All authors. Supervision: Suiwen Ye,Junyan Wu and Herui Yao. Concentration detection: Anding Liu.

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Data availability The data are governed by data usage policies specified by the data controller (SunYat-sen Memorial Hospital, Sun Yat-sen University). To protect the privacy of the patients, all datawill be available for non-commercial research purposes upon approval by the corresponding author Junyan Wu and Herui Yao for 10 years upon reasonable request approved by the Ethics Committee.

Declarations

Conflicts of interest All authors were investigators of this clinical study and signed a conflict of interest statement.

Ethical approval The protocol of this study, any protocol amendment, the final approved informed consent form, associated supporting documents, and all types of subject recruitment documents were submitted by the investigators to ethics committees and approved by the ethics committees and regulatory authorities prior to study initiation. The ethical approval process complied with the requirements of GCP, the declaration of Helsinki and relevant laws and regulations of China. Written consent was obtained before any study specific procedures were performed. Subjects received an informed consent form from the investigator and an oral explanation of the nature, purpose, procedures, expected duration and benefits and risks of participating in the trial, expected duration and benefits and risks of participating in the trial. The subject's informed consent form (appropriate form as approved by IEC / IRB) documented the signature and date of the subject and investigator (or authorized person).

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