Pharmacokinetics of a Liposomal Amphotericin B Prepared using Simplified Thin Film Hydration Method

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1. INTRODUCTION

Amphotericin B (AmB) is a potent polyene antifungal agent with dose-limiting nephrotoxicity. Liposomal formulations like AmBisome® have been developed to improve safety and efficacy by altering pharmacokinetics and reducing systemic toxicity. The nanoscale size of the liposomes facilitates prolonged circulation half-life and improved tissue penetration, while the stable bilayer structure minimizes interactions with plasma lipoproteins, thereby significantly reducing toxicity. Given these advantages, the development of generic liposomal amphotericin B formulations has garnered significant interest in the pharmaceutical industry. However, the inherent complexity of the formulation and manufacturing process presents a substantial challenge in generic product development.

In this study, DKF-5122 (Dongkook's Liposomal AmB) was developed using a conventional thin-film hydration method. To enhance formulation and process understanding, Quality by Design (QbD) principles were applied to systematically identify and evaluate the critical quality attributes (CQAs) and critical process parameters (CPPs) associated with DKF-5122. This approach enabled the optimization of key liposomal characteristics, including particle size, encapsulation efficiency, and drug release profile. Furthermore, the QbD enabled improved process robustness, ensuring high reproducibility, industrial scalability, and consistent product quality involving risk assessment.

The overall aim of this study was to develop a complex generic formulation of liposomal AmB and assess the bioequivalence between DKF-5122 and AmBisome®.

2. METHODS

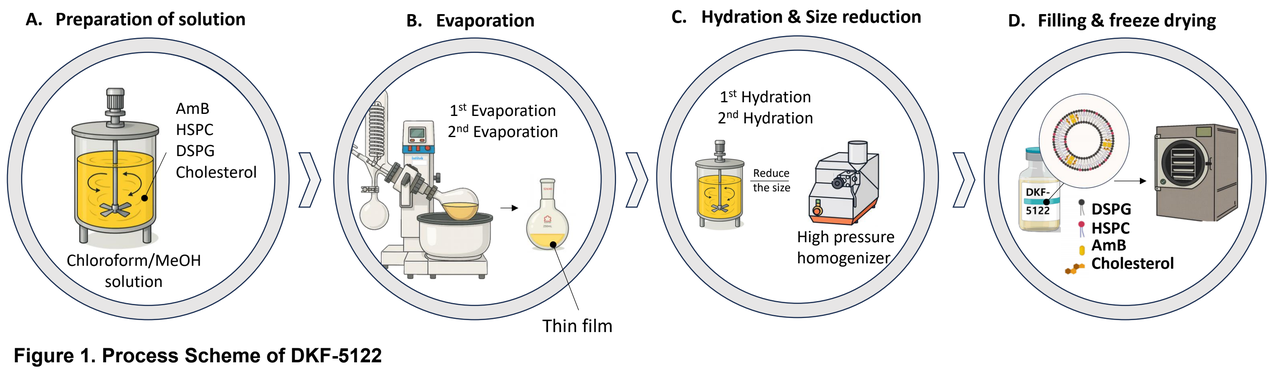

Figure 1. Process Scheme of DKF-5122

DKF-5122 was prepared using the thin-film hydration method, and the formulation was composed of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, distearoylphosphatidylglycerol (DSPG), and amphotericin B (AmB) in a molar ratio of 2:1:0.8:0.4. The hydrated liposomes were further processed to reduce the particle size using a high-pressure homogenization system (Figure 1). CQAs and CPPs were identified through risk assessment. The particle size and zeta potential of DKF-5122 were measured using a dynamic light scattering. The AmB content and total impurities in DKF-5122 were quantified by high-performance liquid chromatography (HPLC). The morphological characteristics of DKF-5122 were analyzed using transmission electron microscopy (TEM). A pharmacokinetic (PK) study was conducted in Sprague-Dawley (SD) rats to compare the profiles between DKF-5122 and the reference drug. Furthermore, the bioequivalence (BE) study in healthy subjects was performed in accordance with the U.S. Food and Drug Administration (FDA) draft guidance for liposomal amphotericin B.

3. RESULTS & DISCUSSION

Physicochemical Evaluations

Table 1: Physicochemical Comparison of Reference & Test Drugs

	AmBisome®	DKF-5122
Assay	102.5%	101.1%
Free amphotericin B content (%)	0.44 ± 0.05	0.60 ± 0.01
Appearance	Vial containing yellow, liposomal lyophilized	Vial containing yellow, liposomal lyophilized
Impurity(%)		
Impurity A	N/D	N/D
Impurity B	1.0%	0.5%
Total	10.7%	5.8%
Liposome size distribution (Z-average)	90.46 ± 0.55 nm	84.7 ± 0.80 nm
Number of lamella	Small unilamellar vesicles (SUVs)	Small unilamellar vesicles (SUVs)
Electrical surface potential	-50.06 ± 1.17 mV	-48.66 ± 1.08 mV
Lipid bilayer phase transition	61.43 ± 0.44 ℃	59.6 ± 0.08 ℃
Osmolality	297 ± 2.1	289 ± 2.8
pH (reconstitution, WFI)	5.5	5.34

Table2. Summary of Population Bioequivalence Results for Reference & Test Drugs

Parameter	Geometric Mean (nm)		Geometric Mean Ratio (T:R ratio)	Standard Deviation		σT/σR ratio
	Test (DKF-5122)	Reference (AmBisome)		σT	σR	
Z-average	86.870	89.163	0.974	0.04398888	0.04185918	1.051
Scaled	Linearized Point Estimate (Eq)		95% Upper Confidence Bound (Hη1 or Hη2)		Pass or Fail PBE	
Constant-scaled	-0.02002932		-0.01814581		**Pass**	
Parameter	Geometric Mean (nm)		Geometric Mean Ratio (T:R ratio)	Standard Deviation		σT/σR ratio
	Test (DKF-5122)	Reference (AmBisome)		σT	σR	
D50%	0.853	0.923	0.924	0.05876185	0.07994877	0.735
Scaled	Linearized Point Estimate (Eq)		95% Upper Confidence Bound (Hη1 or Hη2)		Pass or Fail PBE	
Constant-scaled	-0.01986269		-0.0178061		**Pass**	
Parameter	Geometric Mean (nm)		Geometric Mean Ratio (T:R ratio)	Standard Deviation		σT/σR ratio
	Test (DKF-5122)	Reference (AmBisome)		σT	σR	
Span	90.447	93.583	0.966	0.04212722	0.04368922	0.964
Scaled	Linearized Point Estimate (Eq)		95% Upper Confidence Bound (Hη1 or Hη2)		Pass or Fail PBE	
Constant-scaled	-0.01752307		-0.01105894		**Pass**	

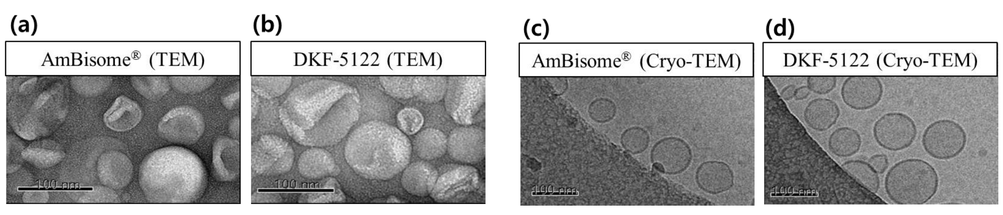

Figure 2. Morphology of (a) AmBisome® and (b) DKF 5122 by TEM at 100 nm Scale. Cryo-TEM Images of (c) AmBisome® and (d) DKF-5122 at 100 nm Scale.

- The original formulation was prepared using a spray-drying process, whereas the DKF-5122 was developed via a thin-film hydration method that offers superior operational efficiency and a more environmentally sustainable approach through the use of reduced quantities of organic solvents.
- A comprehensive comparative evaluation of liposomal quality attributes was conducted in accordance with the USFDA's PSG for generic liposomal products.
- All quality attributes of DKF-5122 met the acceptance criteria for the liposomal AmB generic. Importantly, total impurities were significantly lower in the DKF-5122 (5.8 ± 0.6%) compared to the AmBisome® (10.7 ± 0.7%).

In vitro & In vivo Study

- ***In Vitro Study (Dissolution Test)***

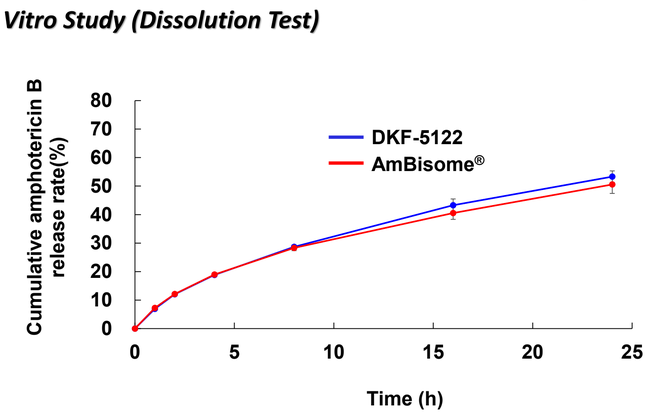

Figure 3. The Cumulative Amphotericin B Release of DKF-5122 and AmBisome®

- ***In Vivo Rat Study***

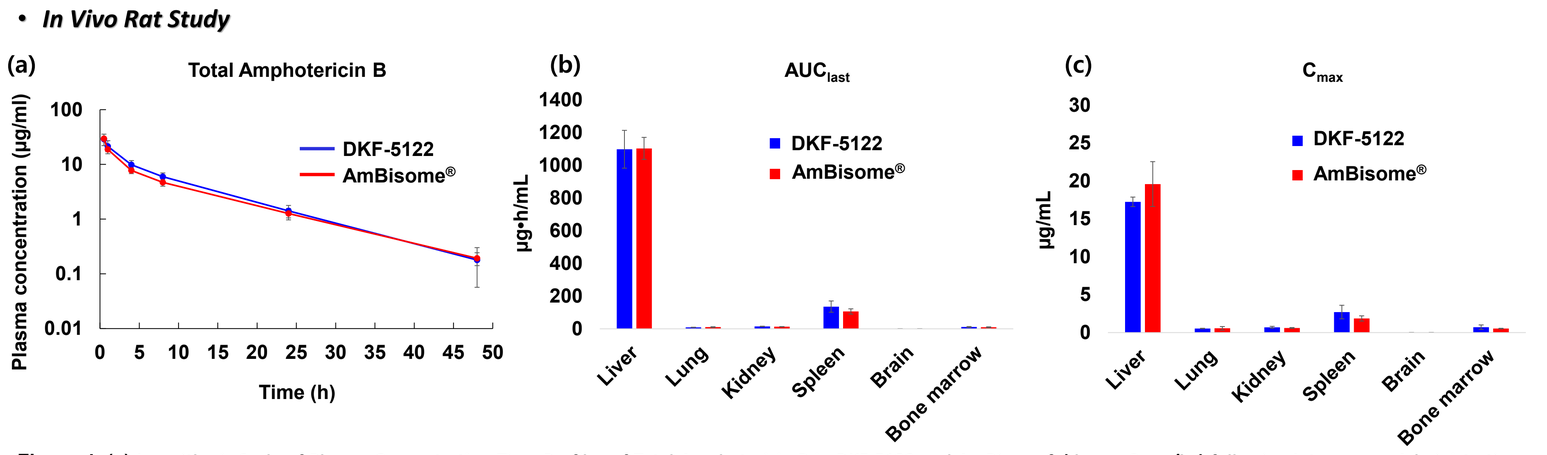

Figure 4. (a) Logarithmic Scale of Plasma Concentration-Time Profiles of Total Amphotericin B in DKF-5122 and AmBisome® (dose = 3 mg/kg) following Intravenous Administration (10-minute infusion) in SD Rats (n = 6, mean ± SD) (b) AUC_{last} of Total Amphotericin B in Various Tissues after Intravenous Administration (10-minute infusion) of DKF-5122 and AmBisome® (dose = 3 mg/kg) in SD rats (n = 21, mean ± SD) (C) C_{max} of Total Amphotericin B in Various Tissues after Intravenous Administration (10-minute infusion) of DKF-5122 and AmBisome® (dose = 3 mg/kg) in SD rats (n = 21, mean ± SD)

- DKF-5122 and AmBisome® showed similar dissolution profiles, with increasing release over time. The similarity factor (f_2) was found to be 84.52, confirming comparable dissolution behavior.
- Comparable pharmacokinetic profiles and tissue distribution of total amphotericin B were observed in Sprague-Dawley rats for both DKF-5122 and the reference listed drug (RLD), supporting equivalent drug exposure and efficacy in preclinical models.

- ***In Vivo Bioequivalence Study in Heathy Human Subjects***

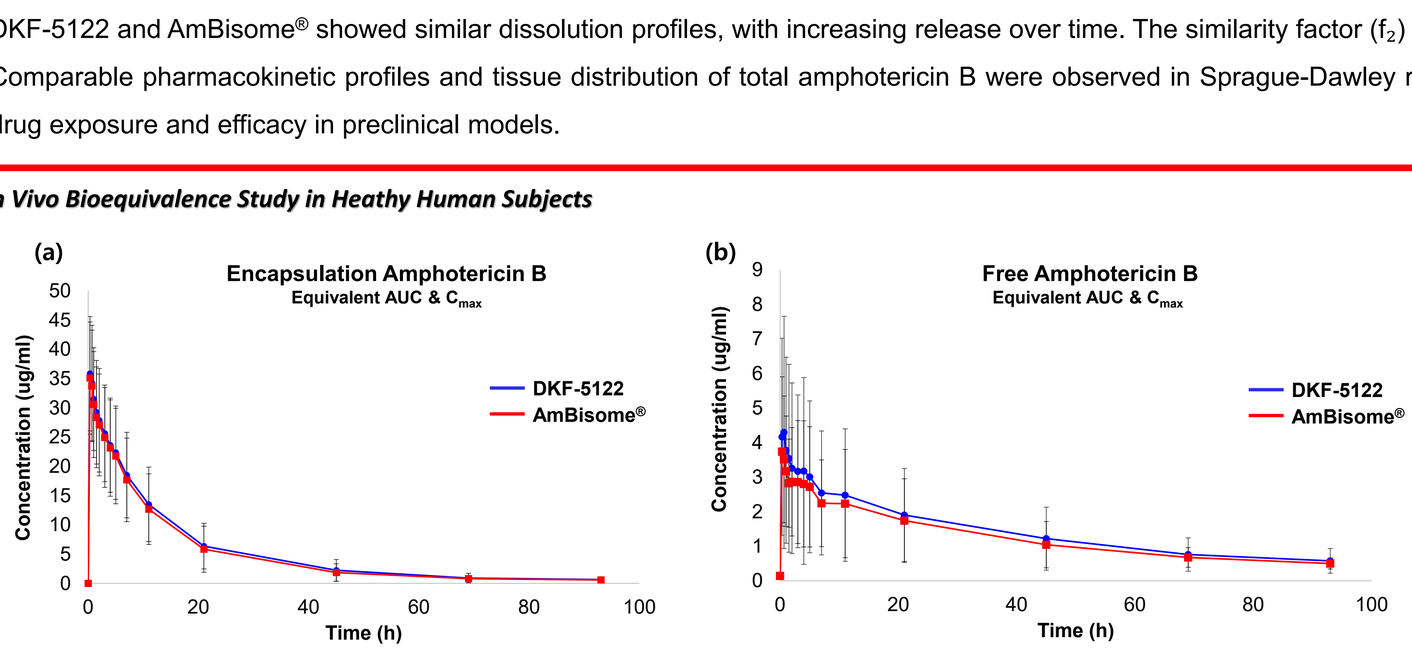

Figure 5. (a) Mean Plasma Concentration-Time Curves of (a) Encapsulated Amphotericin B (b) Free Amphotericin B after Intravenous Administration of DKF-5122 and AmBisome® (dose = 3 mg/kg)

- A BE study was conducted as per the USFDA's guidance, using a single-dose, two-treatment, two-period crossover design in healthy subjects, with a 3 mg/kg dose of the 50 mg/vial formulations of DKF-5122 and AmBisome®.
- BE studies in healthy human subjects demonstrated that the test/reference (T/R) ratios and the corresponding 90% confidence intervals (CIs) for peak plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC_{last}) met the bioequivalence criteria, confirming the pharmacokinetic equivalence between DFK-5122 and the AmBisome®.

4. CONCLUSION

In this study, we developed DKF-5122, a liposomal AmB formulation produced by a simplified thin-film hydration method that generates fewer impurities than the originator's spray-drying process. The physicochemical characteristics and pharmacokinetic profiles of DKF-5122 were demonstrated to be comparable to those of AmBisome® in human subjects. As the first complex generic liposomal AmB product approved in Korea (March 2025), DKF-5122 offers a cost-effective alternative to AmBisome®, which addresses the scarcity of liposomal generics and significantly improving treatment accessibility for life-threatening fungal infections. Given that only a limited number of generics have been approved to date, DKF-5122 is expected to capture a substantial share of the global AmB market in the coming years.

5. ACKNOWLEDGMENT

This work was supported by customized QbD consulting project (KIMCo 21-84 ho), provided by the Ministry of Food and Drug Safety (MFDS).