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Safety, Tolerability, and Pharmacokinetics of a Novel Oral Amphotericin B Formulation (iCo-019) following Single-Dose Administration to Healthy Human Subjects: an Alternative Approach to Parenteral Amphotericin B Administration

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ABSTRACT This study evaluated the safety, tolerability, and pharmacokinetics of a novel oral amphotericin B (AmB) formulation (iCo-019) following single doses to healthy humans. The data from this study suggest that iCo-019 has a long circulation time and systemic exposure without the associated gastrointestinal, liver, and kidney toxicity associated with AmB. This novel oral AmB formulation can serve as a new treatment strategy to overcome the limitations of the use of parenterally administered AmB products.

KEYWORDS phase I human clinical trials, oral amphotericin B, safety and tolerability, pharmacokinetics, healthy human subjects, antimicrobial safety

Amphotericin B, which is administered parenterally, has been considered a first-line therapy in the treatment of systemic fungal and parasitic infections, with a broad spectrum of activity and limited drug resistance (1, 2); however, its use has been limited by dose-dependent nephrotoxicity and the requirement for parenteral administration. The latter creates barriers to access, including its expense, the need for supplies and trained personnel for intravenous administration, and the necessity of cold-chain shipping and storage (1, 2). Until very recently, it has been particularly challenging to develop an oral formulation of amphotericin B (3–32) due to its physical and chemical properties, its limited water and lipid solubility, and its very poor oral absorption.

To overcome these challenges, the development of an oral formulation of amphotericin B that is cost effective, easy to administer, stable at ambient temperatures, and nontoxic yet retains excellent pharmacological activity is the ideal and represents the formulation used in the present study.

The primary objective of this study was to evaluate the safety and tolerability of iCo-019 following oral administration of single ascending doses (4 dose levels) in healthy subjects. The secondary objectives of this study were to assess the pharmacokinetic (PK) profile of iCo-019 after single-dose oral administration in healthy subjects and to identify the maximum tolerated dose of iCo-019 and its systemic exposure after a single oral dose in healthy subjects.

Based on the no-observed-adverse-effect level (NOAEL) determined for iCo-019 in dogs (58.8 to 93.75 mg/kg body weight/day for male and female dogs, respectively) in the good laboratory practice (GLP) 14-day toxicology studies (K. M. Wasan, E. K. Wasan, and P. Hnik, unpublished data), the human equivalent dose (HED) was calculated to be 32.7 mg/kg body weight/day (calculations based on 2005 U.S. FDA guidance). Applying a conservative safety factor of 10 to the HED, the maximum safe starting dose in the first-in-human trial is estimated to be 3.3 mg/kg body weight/day (198 mg/day for a

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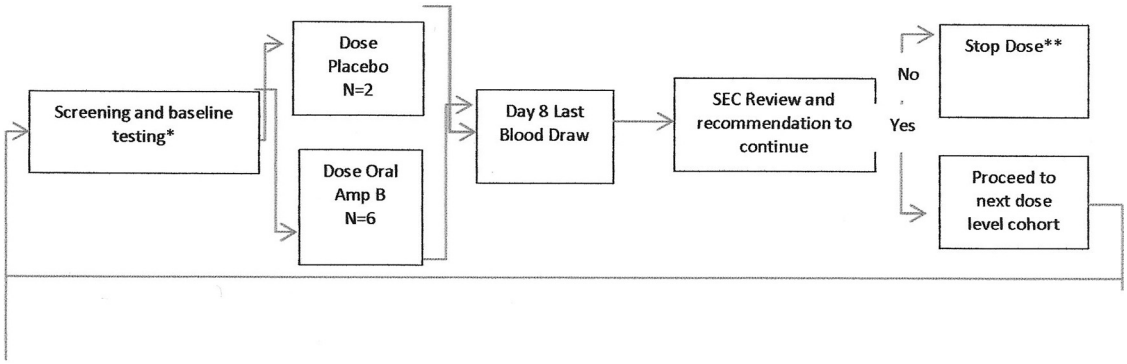

FIG 1 Study diagram. *, each cohort will be screened up to 21 days prior to dosing; therefore, it is possible that screening for the next cohort will begin when the current cohort is being dosed. **, if severe AEs are noted during the first two cohorts, the SEC may stop the study. If severe AEs are noted during the final two cohorts, the SEC may proceed to the next cohort with a reduced dose level.

60-kg individual). A phase I clinical trial was conducted with iCo-019 as a single capsule dose of 100 mg (1.67 mg for a 60-kg subject) which is far below the NOAEL in dogs. Dose escalation proceeded in a doubling approach, i.e., the next cohort received 2 capsules for a 200 mg dose, the next cohort received 4 capsules for a 400 mg dose, and the fourth cohort received 8 capsules for 800 mg and dosing was adjusted based on safety evaluation committee (SEC) review after each cohort.

This was a randomized, double-blind, placebo-controlled, single-dose ascending study to assess the safety, tolerability, and pharmacokinetics of iCo-019 in healthy male and female subjects between 18 and 55 years of age. Subjects were randomized into 4 cohorts in a 3:1 ratio, each representing an ascending single dose of treatment (Fig. 1) or placebo. Cohorts were dosed sequentially in ascending fashion. Each cohort consisted of eight subjects, where six subjects were randomized to receive the investigational product (IP) and two subjects were randomized to receive placebo. A sentinel group consisting of two subjects (one subject receiving the IP and one subject receiving the placebo) was dosed before the other subjects in each cohort. When the sentinel group completed a 24-h follow-up after dosing in the study, the safety profile of these two subjects was reviewed by the investigator (or delegate) to determine whether it was safe to dose the remaining subjects. All subjects were followed for 7 days after dosing.

Subjects were dosed on day 1 and remained fasted for 4 h after the study drug administration. No other food or water restrictions were applied during the study. Blood samples for pharmacokinetic testing were taken at 0 h (prior to dosing) and at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 h after dosing. Subjects were required to report to the clinic at least 12 h before dosing (day −1) and required to stay overnight. Dosing was performed on day 1 of the study, and subjects were required to stay in the clinic until the last blood draw at 72 h on day 4 for a total confinement period of 3.5 days. Subjects were evaluated for safety by measuring vital signs, electrocardiography (ECG), clinical laboratory parameters, and physical exam, and subjects were monitored for adverse events (AEs) throughout the study. Subjects returned to the clinic on day 6 for clinical laboratory testing, safety evaluation, and review of adverse events. Vital signs (blood pressure and heart rate) were measured, and end of study procedures were performed on day 8 postdose. After the last subject in the cohort completed the day-8 visit, the safety profile for each subject treated in that cohort was reviewed by the safety evaluation committee (SEC). The SEC met to discuss safety findings and determined the next step in the dose escalation schedule using guidelines prespecified in the protocol. Adverse events were graded according to the Common Toxicity Criteria for Adverse Events version 4.0 (NIH). Adverse events considered associated with oral Amp B and of a high severity would have resulted in immediate cessation of treatment at that dose level.

TABLE 1 Summary of pharmacokinetic parameters for amphotericin B in human plasma in subjects that were administered a single oral dose of iCo-019 at 100 mg, 200 mg, 400 mg, and 800 mg of amphotericin B

	Median (range) values[a]			
Dose (mg)	T_{max} (h)	C_{max} (ng/ml)	$AUC_{0-Tlast}$ (h·ng/ml)	$t_{1/2}$ (h)
100	6.0 (6–6)	28.0 (22.7–43.6)	759.7 (635.8–1606.4)	27.3 (14.4–55.1)
200	6.0 (6–8)	28.6 (18.8–42.5)	804.0 (596.1–1344.9)	24.6 (15.3–68.8)
400	6.0 (6–10)	28.4 (20.2–41.1)	1,089.2 (461.8–1,856.8)	39.0 (13.7–142.1)
800	7.0 (6–10)	32.1 (29.9–42.8)	1,345.5 (915.1–1,854.7)	25.6 (23.1–32.7)

[a]$n = 6$ subjects per dosing cohort. T_{max}, time to reach the maximum observed concentration; C_{max}, maximum observed amphotericin B plasma concentration; $AUC_{0-Tlast}$, area under the concentration-time curve from hour 0 to the last measurable concentration, estimated by the linear trapezoidal rule; $t_{1/2}$, elimination half-life.

Thirty-two volunteers (men, 43.8%; women, 56.2%) participated in the study. Their mean age was 26.6 years (18 to 52 years), mean body weight was 68.1 kg (48.4 to 87.7 kg), mean height was 170.5 cm (152 to 188 cm), and mean body mass index (BMI) was 23.26 kg/m^2 (18.1 to 29.6 kg/m^2). No volunteers had clinically significant renal, liver, cardiac, pulmonary, gastrointestinal, or hematological diseases. The study was performed in Australia based on ethics committee approval and under the supervision of an independent safety evaluation committee (SEC). The primary endpoints of safety and tolerability of iCo-019 following oral administration of all single ascending doses were met, showing no serious adverse events and no drug-related adverse events, including no signs of kidney, liver, or gastrointestinal (GI) toxicity of note. A safety summary dose escalation review form assessing any clinically relevant abnormal vital signs, acute ECG changes observed in P wave, PR interval, QRS complexes, ST segments, QTc, and T waves, relevant changes in laboratory safety data, and clinically relevant changes in the physical examination was completed at each dose and reviewed by the safety evaluation committee (SEC). No clinically relevant abnormal vital signs, acute ECG changes observed in P wave, PR interval, QRS complexes, ST segments, QTc, and T waves, relevant changes in laboratory safety data, and clinically relevant changes in the physical examination were observed at all doses administered. The adverse events reported within each dosing group were classified as mild and not related to the administration of iCo-019.

iCo-019 achieved a median maximum concentration in plasma (C_{max}) of 28 ng AmB/ml and an area under the concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) of 1,030 h·ng/ml at the lowest dose of iCo-019 of 100 mg (Table 1). At the 400-mg dose of iCo-019, a median $AUC_{0-\infty}$ of 2,029 h·ng/ml was achieved, representing an approximate doubling of the AUC measure at an increased dose. On further analysis median AUC from 0 to the last measurable concentration ($AUC_{0-Tlast}$) increased from 759.7 h·ng/ml at the 100-mg dose to 1,345.5 h·ng/ml at the 800-mg dose (Table 1 and Fig. 2).

Unlike other drugs, AmB, due to its lack of water and lipid solubility, has a very unusual pharmacokinetic and pharmacodynamic profile. The absolute bioavailability of AmB from iCo-019 administration can be approximated as 2% to 3%. However, it is the accumulation of drug within infected tissues as a function of time (i.e., depot effect) and the systemic exposure of the drug as a function of time (AUC) and not blood levels (C_{max}) that is correlated with its pharmacological activity (1–8). The clearance of the drug from systemic circulation is faster than the clearance of the drug from the tissues, leading to an increased tissue accumulation and enhanced pharmacological effect. This type of pharmacokinetic profile is fundamentally different from parenteral liposomal amphotericin B, the most used commercially available form of the drug; the liposomal form is long circulating, which results in a large AUC. However, iCo-019 exhibits prolonged tissue levels that are the most important factor for efficacy against leishmaniasis and systemic fungal infections. In this study, we report a prolonged AmB half-life and sustained systemic drug exposure as measured by AUC. Furthermore, the

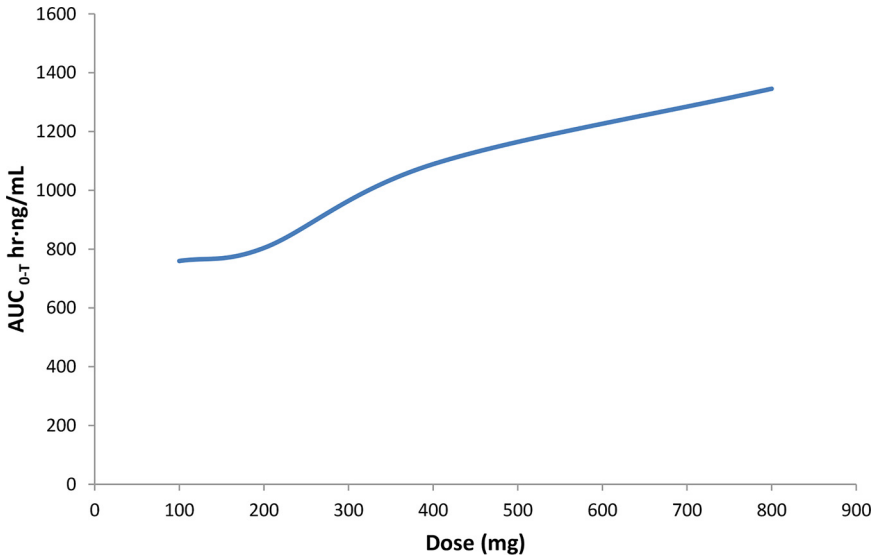

FIG 2 Increase in iCo-019 dose is associated with an increase in AUC0–Tlast.

AUC observed in this trial is superior to that of the cochleate AmB formulation that recently completed human clinical trials (30, 31), suggesting pharmacological activity can be expected at the doses tested in this study (1–8).

In conclusion, we have reported that all single doses of iCo-019 were well tolerated with no serious adverse events and no drug-related adverse events, including no signs of gastrointestinal, kidney, and liver toxicities. iCo-019 achieved favorable AmB pharmacokinetics with a prolonged AmB half-life and increasing area under the concentration-time curve as the dose increased. These data suggest that iCo-19 has a long circulation time which may result in the ability of the formulation to increase and sustain amphotericin B tissue concentrations within infected tissues without the associated GI, liver, or kidney toxicity typically associated with this drug. This novel oral formulation may also serve as a new treatment strategy to overcome the limitations of and barriers to the use of parenterally administered amphotericin B products.

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