Pharmacokinetics and biodistribution of amphotericin B in rats following oral administration in a novel lipid-based formulation

Pavel Gershkovich¹*, Ellen K. Wasan¹,², Molly Lin¹, Olena Sivak¹, Carlos G. Leon¹, John G. Clement³ and Kishor M. Wasan¹

¹Faculty of Pharmaceutical Sciences, The University of British Columbia, 2146 East Mall, Vancouver, BC, Canada V6T 1Z3; ²School of Health Sciences, British Columbia Institute of Technology, 3700 Willingdon Avenue, Burnaby, BC, Canada V5G 3H2; ³iCo Therapeutics Inc., 760-777 Hornby, Vancouver, BC, Canada V6Z 1S4

Objectives: To assess the pharmacokinetics and biodistribution of amphotericin B (AmB) following oral administration in a novel mono/diglyceride–phospholipid formulation and to compare with intravenous (iv) administrations using commercial formulations.

Methods: Rats were allocated into the following treatment groups: oral gavage of AmB dispersed in mono/diglyceride–phospholipid formulation at doses of 4.5 and 10 mg/kg; iv bolus administration of 0.8 mg/kg Fungizone™; iv bolus of 5 mg/kg Abelcet® and iv bolus of 5 mg/kg AmBisome™. Blood was sampled from jugular vein cannula at certain time points. The animals were sacrificed 72 h following administration of AmB and multiple tissues were harvested. The concentration of AmB in plasma and tissues was determined by means of HPLC. The plasma creatinine concentrations were determined using an enzymatic kit.

Results: The pharmacokinetics and tissue distribution of AmB following iv administrations of the commercial formulations were found to be highly formulation dependent. The terminal half-life and biodistribution of orally administered AmB in a mono/diglyceride–phospholipid formulation resembled those of Fungizone™. The larger volume of the co-administered lipid-based formulation in the case of the higher dose of orally administered AmB resulted in flip-flop kinetics and in preferential distribution into the kidneys. No nephrotoxicity was detected for any formulation and route of administration.

Conclusions: Oral administration of AmB in a mono/diglyceride–phospholipid formulation to rats resulted in significant intestinal absorption into the systemic circulation with pharmacokinetic and biodistribution properties similar to a micellar iv preparation.

Keywords: intestinal absorption, tissue distribution, polyene antibiotics, systemic fungal infections

Introduction

Amphotericin B (AmB) is a polyene antibiotic that was first isolated in 1955 from cultures of Streptomyces nodosus.¹–⁴ AmB is used for treating serious systemic fungal infections caused by different fungal microorganisms.⁵ In addition, AmB is an important drug for treating cases of visceral and cutaneous leishmaniasis caused by flagellated protozoa of the genus Leishmania.⁶ Even after 30 years of clinical use, only a few reports exist on the development of resistance to AmB.⁷ The major drawbacks of AmB treatment are its acute (fever, chilling, rigor) and subacute (mainly nephrotoxicity) toxicity and the need to administer the drug intravenously (iv). The need for parenteral delivery results in high costs of treatment and limits the use of AmB in developing countries.

Thus, oral administration of AmB is an appealing idea, since it has the potential to eliminate acute toxicity associated with iv delivery of the drug, to reduce and control subacute side effects (renal toxicity), to decrease substantially the associated costs of the treatment, to improve the quality of life for patients and to allow therapy to reach developing nations.
Unfortunately, due to its amphiphilic nature, AmB has low solubility in both water at physiological pH and most organic solvents. According to the Biopharmaceutics Classification System, AmB belongs to class IV, i.e. it has low solubility and low membrane permeability. These two properties, aggravated by poor stability of the molecule in acidic gastric environment, are primary factors responsible for the poor bioavailability of AmB when it is administered orally. Although the poor stability in the acidic gastric environment could be relatively easily addressed by, for example, enteric coated dosage forms, low solubility and poor membrane permeability of AmB present a significant challenge for pharmaceutical scientists. There are multiple reports that plasma concentrations of AmB in humans following oral administration are very low. Poor oral absorption of AmB was also reported in rats, mice and dogs. The common confidence in low oral bioavailability is so high that this drug is given orally in clinical practice to eradicate susceptible microorganisms from the oral cavity and gastrointestinal tract with negligible risk of significant systemic exposure.

However, in recent years, there has been a substantial progress towards potential formulations of AmB that can be successfully administered orally. These include a formulation of AmB as a nanosuspension for oral administration, lipid-based co-coclates and lipid nano spheres. Our group has previously reported significant concentrations of AmB in plasma and tissues of rats, as well as antifungal activity of orally administered AmB when it is dispersed in a mixture of monoglycerides and diglycerides (Peceol®; Gattefosse, Saint Priest Cedex, France). This preliminary formulation had, however, significant drawbacks, i.e. lack of uniformity and low solubility of AmB. As a consequence, unrealistically high volumes of lipids had to be co-administered. We have recently shown that antifungal activity (along with drug solubility and stability) was significantly increased when the mixture of monoglycerides and diglycerides was further developed to a novel oral formulation of AmB in Peceol/distearoylphosphatidylethanolamine-poly(ethylene glycol)2000 (DSPE-PEG2000) (iCo-009). It is noteworthy that the improved activity (including eradication of susceptible microorganisms from the kidney tissue) was achieved without related kidney toxicity. In addition, the oral formulation of AmB in Peceol/DSPE-PEG2000 was recently found to be highly effective in a murine visceral leishmaniasis model. It was unclear in these studies whether the improved activity of the novel lipid-based formulation of AmB was related to increased intestinal absorption, improved tissue distribution to the target organs or a combination of these factors.

Thus, the aim of this work was to assess the pharmacokinetics and tissue distribution of AmB following oral administration of AmB in Peceol/DSPE-PEG2000 formulation to rats and to compare the obtained plasma concentration and tissue distribution profiles with iv administered AmB using commercially available formulations.

Materials and methods

Materials

AmB powder (from Streptomyces sp., ~80% purity) was purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received. Fungizone® (AmB micelle dispersion, Bristol-Myers Squibb, Montreal, Canada), Abelcet® (AmB lipid complex, Enzon Pharmaceuticals Inc., Bridgewater, NJ, USA) and AmBisome® (liposomal AmB, Astellas Pharma Canada Inc., Markham, Ontario, Canada) were purchased from Vancouver General Hospital pharmacy. All other chemicals were of analytical reagent grade, and solvents were of HPLC grade.

Preparation of Peceol/DSPE-PEG2000 formulation of AmB (iCo-009)

The preparation of Peceol/DSPE-PEG2000 formulation of AmB (iCo-009) was performed as recently reported. Briefly, AmB was mixed with ethanol, Peceol® and DSPE-PEG2000. The solution was stirred for 1 h (protected from light), followed by solvent evaporation under vacuum (65 mbar) over 4 h in a rotary evaporator. Ethanol was considered to be completely removed by achieving the original weight of the sample containing AmB, Peceol and lipids measured immediately prior to the addition of ethanol. A translucent yellow mixture without particulates was formed. The final concentration of AmB in a formulation in this study (validated by means of HPLC) was 2.5 mg/mL.

Surgical procedures

The animal protocols used in this study were approved by the University of British Columbia’s Animal Care Committee and conform to the Canadian Council on Animal Care guidelines. Male Sprague Dawley rats (UBC animal care centre (Vancouver, BC, Canada)) weighing 330–350 g were used in this study. The rats were kept under a 12 h light/dark cycle with free access to water and food (regular rat chow).

The right external jugular vein was cannulated with a two-part catheter consisting of PE-50 connected to silastic tubing. The surgery was performed under general anaesthesia induced by intra-peritoneal injection of 90 mg/kg ketamine and 9 mg/kg xylazine. The cannula was exteriorized at the dorsal part of the neck, which allowed the investigation in non-anaesthetized and unrestrained rats to be carried out. Following the surgery, animals were housed individually and allowed to recover for 24 h until the experiment.

Experimental design

The rats were fasting during the post-surgery recovery period and until 12 h after administration of AmB with free access to drinking water. Twenty-four hours after surgery, animals were allocated into the following five treatment groups: oral gavage of AmB dispersed in Peceol/DSPE-PEG2000 at a dose of 4.5 mg/kg (n=6); oral gavage of AmB dispersed in Peceol/DSPE-PEG2000 at a dose of 10 mg/kg (n=8); iv bolus administration of 0.8 mg/kg Fungizone® (n=5); iv bolus of 5 mg/kg Abelcet® (n=6) and iv bolus of 5 mg/kg AmBisome® (n=6). The doses chosen for iv administration of commercial formulations of AmB in this study are in the range for which there is no clinical nephrotoxicity for each formulation, based on previously published reports. All iv administrations were given as a slow (over 1 min) bolus to minimize the acute toxic reaction to AmB. Systemic blood (0.25 mL) was sampled at 10 min pre-dose and 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48 and 72 h post-dose in the case of oral gavage administration of AmB or at 10 min pre-dose and 5, 10, 15 min and 0.5, 1, 2, 4, 7, 12, 24, 48 and 72 h post-dose in the case of iv bolus administrations of AmB. The withdrawn blood was replaced by an equal volume of normal saline to prevent hypovolaemia. Plasma was separated by centrifugation (5000 rpm, 10 min, 15°C). The animals were sacrificed 72 h following administration of
AmB, and liver, right kidney, spleen, right lung, heart and brain were harvested for drug analysis. Plasma and tissue samples were stored at −80°C until analysis.

Analytical procedures

Plasma samples were analysed using a Waters 2695 Separation Module HPLC system with Waters 996 Photodiode Array Detector (Waters Corporation, Milford, MA, USA). Tissue samples were analysed using a system comprising a Waters 600 pump, a Waters 717plus autosampler and a Waters 486 UV detector (Waters Corporation, Milford, MA, USA). The analysis of AmB in plasma and tissues samples was based on a previously published validated isocratic analytical method,29 using the same column [BDS Hypersil C18, 5 μm, 250×4.6 mm (Thermo Scientific)] and mobile phase [ACN/AC/2H2O (52:4.3:43.7, v/v/v)]. A slight modification included reduction of the flow rate to 0.8 mL/min and increase in the column temperature to 40°C to improve separation efficiency. Tissues were homogenized in normal saline (2 g of normal saline/1 g of tissue) using a Polytron® PT 10/35 for 3–4 min over an ice bath. The aliquots of 70 μL of homogenized tissues were used for the HPLC analysis. Calibration curves of AmB were linear in the range of 0–120 ng/mL for plasma samples and 0–120 μg/g for tissue samples. The limit of quantification of AmB was 20 ng/mL for plasma samples and 20 ng/g for tissue samples (except 10 ng/g for brain samples).

Determination of plasma creatinine levels

Creatinine concentrations in plasma (one of the indirect methods for assessment of potential renal toxicity) were determined using a commercially available kit (Thermo Fisher Scientific Inc., Middletown, VA, USA), as previously reported.30 A baseline at 0 h (pre-dose) was compared with plasma creatinine concentration at 24, 48 and 72 h following administration of AmB.

Pharmacokinetic methodology

WinNonlin 5.0.1 Professional was used for pharmacokinetic analysis of the data. The non-compartmental approach was applied for analysis of the data following iv and oral administrations of AmB.

Statistical analysis

The data in this paper are presented as means ± SEM, if not specified otherwise. Differences were assessed for significance using ANOVA followed by Tukey–Kramer or Dunnett post-test, or Student’s t-test, where appropriate. A P value of <0.05 was considered statistically significant.

Results

Pharmacokinetic profile of AmB following iv bolus administration in Fungizone®, Abelcet® and AmBisome®

The semi-logarithmic plasma concentration–time profiles of AmB following iv bolus administration of 0.8 mg/kg Fungizone®, 5 mg/kg Abelcet® and 5 mg/kg AmBisome® are shown in Figure 1. The pharmacokinetic parameters derived from plasma concentration–time profiles are summarized in Table 1. As seen in Figure 1 and Table 1, the formulations demonstrate pronounced differences in pharmacokinetics of AmB following iv administration. The apparent volume of distribution of AmB administered as Abelcet® is 16-fold larger than that of Fungizone® and 292-fold larger than that of AmBisome®. The clearance of AmB administered as Abelcet® is 7-fold higher than that of Fungizone® and 73-fold higher than that of AmBisome®. As a result, the half-life of Abelcet® is three times greater than that of Fungizone® and almost five times greater than that of AmBisome®.

Pharmacokinetic profile of AmB following oral administration in Peceol/DSPE-PEG2000 (iCo-009)

The plasma concentration–time profiles of AmB following oral administration in Peceol/DSPE-PEG2000 at doses of 4.5 and 10 mg/kg are shown in Figure 2. The pharmacokinetic parameters derived from plasma concentration–time profiles are summarized in Table 1. As seen in Figure 2, the oral administration of a lower dose of AmB (4.5 mg/kg) resulted in a higher initial concentration of AmB in plasma at 2 h following the administration. However, at 3 and 4 h after the administration, plasma concentrations of AmB were equal for lower and higher (10 mg/kg) doses and after 5 h the plasma concentrations after higher dose exceeded those after a lower dose. At 48 h following the administration, the concentration of AmB could still be detected in the case of the higher dose (10 mg/kg) but not for 4.5 mg/kg (Figure 2). The differences in plasma concentrations of AmB starting from 5 h following the administration of 10 and 4.5 mg/kg doses are reflected by a statistically significant difference in the calculated half-life (Table 1) between the doses.

Tissue distribution of AmB following oral administration in Peceol/DSPE-PEG2000 (iCo-009) and iv administration in Fungizone®, Abelcet® and AmBisome®

The results of tissue distribution of AmB 72 h following oral gavage administration in Peceol/DSPE-PEG2000 at doses of 4.5 and 10 mg/kg and following iv administration in 0.8 mg/kg Fungizone®, 5 mg/kg Abelcet® and 5 mg/kg AmBisome® are summarized in Table 2. The vast majority of AmB is concentrated in the spleen and liver following iv administration of Abelcet® and AmBisome®. In addition, Abelcet® (but not
Table 1. Summary of pharmacokinetic parameters of AmB following iv administration of commercial formulations or oral administration in Peceol/DSPE-PEG2000 formulation (means ± SEM)

| Treatment group | PK parameter | n   | AUC_{0-24} (h·ng/mL) | AUC_{0-48} (h·ng/mL) | AUC_{0-72} (h·ng/mL) | C_{max} (ng/mL) | T_{max} (h) | t_{1/2} (h) | CL (mL/h/kg) | V_z (L/kg) |
|-----------------|--------------|-----|----------------------|----------------------|----------------------|----------------|-------------|------------|-------------|-------------|------------|
| Abelcet® iv bolus, 5 mg/kg | 6            | 1780 ± 148 | 2761 ± 239 | 3334 ± 346 | — | — | 42.5 ± 9.8 | 1134 ± 196 | 58.5 ± 8.2 |
| Fungizone® iv bolus, 0.8 mg/kg | 5            | 3454 ± 198 | 4601 ± 274 | — | — | — | 15.2 ± 0.6 | 161 ± 10 | 3.5 ± 0.2 |
| AmBisome® iv bolus, 5 mg/kg | 6            | 322341 ± 44276 | 346594 ± 50018 | 70077 ± 10251 | — | — | 8.8 ± 0.3 | 15.5 ± 1.8 | 0.2 ± 0.02 |
| Oral AmB in Peceol/DSPE-PEG_{2000}, 4.5 mg/kg | 6            | 991 ± 170 | — | — | 71 ± 10 | 6.3 ± 0.9 | 13.2 ± 3.1 | — | — |
| Oral AmB in Peceol/DSPE-PEG_{2000}, 10 mg/kg | 8            | 1534 ± 229 | 2695 ± 433 | — | 96 ± 15 | 12.5 ± 2.7 | 24.7 ± 4.8 | — | — |

V_z, volume of distribution.

AmBisome® is statistically significantly different from other iv treatment groups (P < 0.001); one-way ANOVA followed by Tukey–Kramer post-test. There is a statistically significant difference between two oral treatment groups (P < 0.05); unpaired one-tailed Student’s t-test.

AmBisome® is statistically significantly different from other iv treatment groups (P < 0.001); one-way ANOVA followed by Tukey–Kramer post-test.

AmBisome® is statistically significantly different from Abelcet® (P < 0.0001); unpaired Student’s t-test.

No statistically significant difference between oral treatment groups; unpaired one-tailed Student’s t-test.

Statistically significant difference between oral treatment groups (P < 0.05); unpaired one-tailed Student’s t-test.

Abelcet® is statistically significantly different from Fungizone® (P < 0.05) and AmBisome® (P < 0.01); one-way ANOVA followed by Tukey–Kramer post-test. There is a statistically significant difference between two oral treatment groups (P < 0.05); unpaired one-tailed Student’s t-test.

Abelcet® is statistically significantly different from other iv treatment groups (P < 0.001); one-way ANOVA followed by Tukey–Kramer post-test.
AmBisome® showed preferential disposition into the lung tissue. Fungizone® shows more homogeneous distribution between tissues, with, however, a significant shift towards the kidney. Similarly to Fungizone®, orally administered AmB shows homogeneous distribution between tissues, with a significant shift towards the kidneys in the case of the 10 mg/kg dose, but not in the case of the lower dose (4.5 mg/kg). Both Fungizone® and the oral formulation of AmB (at both doses) demonstrate negligible (below the limit of quantification) distribution into heart tissue. AmBisome® demonstrated higher affinity to heart tissue than all other oral or iv formulations. Penetration of AmB into brain tissue was low for all modes of administration and all formulations.

**Plasma creatinine concentrations**

There was no statistically significant change in the levels of creatinine in plasma at any timepoint and in any treatment group relative to the baseline pre-dose concentrations (one-way ANOVA followed by Dunnett post-test, data not shown).

**Discussion**

In this study, we have compared the pharmacokinetics and tissue distribution of AmB in rats following iv administration in commercially available preparations with oral administration in a Peccol/DSPE-PEG2000 lipid-based formulation (iCo-009).

The pharmacokinetics of AmB was found to be highly formulation dependent. As can be seen in Figure 1 and Table 1, there are pronounced differences in plasma concentration–time profiles and the calculated pharmacokinetic parameters derived from iv bolus administration of each commercial formulation tested in this study. Both Abelcet® and AmBisome® are particulate systems that are recognized by the mononuclear phagocyte system (MPS) (primarily liver, spleen, bone marrow, lymph nodes and, to a lesser extent, lungs). However, while AmBisome® consists of spherical unilamellar liposomes with a mean diameter of <100 nm, Abelcet® is composed of ribbon-like structures with particle sizes between 1600 and 11000 nm.31 Abelcet®, being composed of larger particles, is recognized faster by the MPS, which results in rapid initial clearance from the bloodstream. On the other hand, smaller

AmB (4.5 mg/kg) showed preferential disposition in Peceol/DSPE-PEG 2000 (2.5 mg/mL) at doses of 4.5 mg/kg (n=6) and 10 mg/kg (n=8).

**Figure 2.** Plasma AmB concentration–time plot (mean±SEM) following oral administration in Peccol/DSPE-PEG2000 (2.5 mg/mL) at doses of 4.5 mg/kg (n=6) and 10 mg/kg (n=8).

![Plasma AmB concentration–time plot](image)

**Table 2.** Tissue distribution of AmB 72 h following iv bolus administration of commercial formulations and oral administration in Peccol/DSPE-PEG2000 formulation (ng/g, means±SEM).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tissue</th>
<th>0.8 mg/kg, n=6</th>
<th>5 mg/kg, n=6</th>
<th>10 mg/kg, n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abelcet® iv bolus</td>
<td>Liver</td>
<td>20874±332</td>
<td>5652±508</td>
<td>33±8</td>
</tr>
<tr>
<td>Fungizone® iv bolus</td>
<td>Kidney</td>
<td>203±3</td>
<td>17±3</td>
<td>32±8</td>
</tr>
<tr>
<td>AmBisome® iv bolus</td>
<td>Spleen</td>
<td>6567±199</td>
<td>29±565</td>
<td>2±8</td>
</tr>
<tr>
<td>Oral AmB in Peccol/DSPE-PEG2000</td>
<td>Brain</td>
<td>102±24</td>
<td>2±10</td>
<td>1±8</td>
</tr>
</tbody>
</table>

BLQ, below the limit of quantification.

Statistically significantly different: liver versus kidney (P<0.05) and spleen versus all other tissues (P<0.001) within the treatment group; one-way ANOVA followed by Tukey–Kramer post-test.

Statistically significantly different: liver versus kidney (P<0.05) and spleen versus all other tissues (P<0.001) within the treatment group; one-way ANOVA followed by Tukey–Kramer post-test.

Statistically significantly different: liver versus kidney (P<0.01) and spleen versus all other tissues (P<0.001) within the treatment group; one-way ANOVA followed by Tukey–Kramer post-test.

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liposome particles in AmBisome® are recognized more slowly by the MPS, which results in longer circulation time of the administered formulation in the bloodstream. Interestingly, despite the differences in the timing and extent of recognition by the MPS and in the resultant differences in pharmacokinetic parameters (Table 1), the final concentrations of AmB in tissues 72 h following iv bolus administration of Abelcet® and AmBisome® share common features. In both cases, the vast majority of the drug is found in major tissues of the MPS, i.e. liver and spleen (Table 2). Following iv administration of Abelcet®, AmB is more concentrated in the spleen, while after administration of AmBisome®, there is a higher concentration of the drug in the liver. In addition, Abelcet® shows preferential disposition into another organ of the MPS (lung), while AmBisome® is distributed equally between lung, kidney and heart.

Fungizone® is a micellar preparation of AmB and there is much more homogeneous distribution of AmB between body tissues 72 h following administration, with no preferential distribution into tissues of the MPS (Table 2). In the absence of the effect of the MPS, the preferential distribution into the kidney tissue, however, becomes evident. Indeed, Fungizone® was reported in multiple studies to be more nephrotoxic than lipid-based formulations of AmB.28,32–34

The distribution of AmB into the brain tissue was low in all the treatment groups, which is in agreement with previously published works.28,35,36

Given the marked differences in the pharmacokinetics and tissue distribution of AmB administered iv in different formulations, it is interesting to observe the profile of AmB within an in vivo model following oral administration in Peceol/DSPE-PEG2000. As we previously reported, our Peceol/DSPE-PEG2000 formulation of AmB creates a particulate system with a bimodal distribution of ~150 nm (30%) and ~1600 nm (70%) (median diameter) in simulated fasted-state intestinal fluid (pH 6.8).25 Although micro- and nano-particulate systems have been reported to be absorbed intact into the systemic circulation via the Peyer’s patches (M-cells) route, the net absorption of the material via this transport was reported to be extremely low.37 This is, apparently, not the case in the issue of the Peceol/DSPE-PEG2000 formulation of AmB because considerable concentrations in the plasma are achieved following both 10 and 4.5 mg/kg oral doses. Thus, it is likely that microparticles and nanoparticles that are created in the gastrointestinal tract following oral administration of AmB in Peceol/DSPE-PEG2000 promote the absorption of AmB by protecting the drug from the acidic environment in the stomach, by enhancing the solubility of AmB in the intestinal lumen, and perhaps by affecting the fluidity of the enterocyte membrane thereby improving the permeability of this amphiphilic drug, rather than by absorption of the intact particles by endocytosis via the M-cells route.

As can be seen in Figure 2, the plasma concentrations of AmB in the first hours following oral administration of the higher dose (10 mg/kg) are slightly lower than in the case of the smaller dose (4.5 mg/kg). This suggests a slower absorption rate as a result of the higher volume of the co-administered lipids, which may delay gastric emptying and slow peristaltic movements in a rat model. Starting from 6 h following administration, the concentrations after a higher dose of AmB exceed those of a lower dose and eventually AmB could still be detected in the plasma 48 h following administration of 10 mg/kg but not in the case of 4.5 mg/kg. When the terminal half-life of orally administered AmB is compared with the terminal half-lives of the iv formulations (Table 1), the marked similarity between Fungizone® and the dose of 4.5 mg/kg (but not the dose of 10 mg/kg) can be seen. The terminal half-life of the 10 mg/kg oral dose administered with the higher volume lipid-based formulation is, however, significantly longer, as can be seen in Table 1. This is an example of ‘flip-flop’ kinetics due to a prolonged absorption phase caused by higher volume of lipids given with 10 mg/kg dose in a rat model. It should be noted that since rats do not have a gall bladder, they are unable to digest rapidly and efficiently larger volumes of lipids. Thus, because of the differences in the lipid absorption and metabolism, a prolonged absorption phase and flip-flop kinetics might not be seen in other species.

The final distribution of AmB into the tissues 72 h following oral administration in Peceol/DSPE-PEG2000 also shows the similarity between the biodistribution behaviour of the iv bolus of Fungizone® and the oral AmB formulation. The homogeneous disposition of AmB following oral administration is similar to that of Fungizone®. There is no preferential distribution into the tissues associated with the MPS as was seen in the case of particulate formulations like Abelcet® or AmBisome®, which is additional evidence that micro- and nanoparticulate systems created in the gastrointestinal tract following oral administration of AmB in Peceol/DSPE-PEG2000 most likely are not absorbed intact. Interestingly, the larger dose (10 mg/kg) co-administered with a higher volume of Peceol/DSPE-PEG2000 shows a preferential distribution into the kidney tissue, similar to Fungizone®, but not the lower dose (4.5 mg/kg) co-administered with a lower volume of the lipid-based formulation. On the other hand, concentrations in the spleen and lung are actually lower when the higher dose of oral AmB (and larger volume of lipid) is administered. It is conceivable that co-absorbed components of the lipid-based formulation affect the final biodistribution of AmB in a similar way that co-administered deoxycholate can affect the biodistribution of the drug following iv administration of Fungizone®. It was previously proposed that different components of investigational new formulations of AmB, such as poloxamer 188 or poly(e-caprolactone), affect the distribution characteristics of the drug in the body.38 It should also be noted that AmB is highly bound to plasma proteins and lipoprotein and thus relatively small changes in binding might significantly affect the biodistribution pattern of the drug.

Given the similarities between the pharmacokinetics and tissue distribution of AmB following iv bolus administration of Fungizone® and oral administration of AmB in Peceol/DSPE-PEG2000, one would suspect that orally administered AmB would lead to an increased chance of nephrotoxicity, similar to Fungizone®. This, however, may not be an issue with orally administered AmB. As can be seen in Table 2, the concentration of AmB in most tissues is still significant 72 h following administration, while the concurrent concentration in plasma is below the limit of detection or very low. This suggests that once AmB reaches the tissues, it remains there for a prolonged time, slowly ‘leaking’ back to the bloodstream and subsequent slow elimination from the body. The hypothesis of prolonged storage of AmB in the tissues is also supported by previously published works and multicompartmental analysis of the
Pharmacokinetics of AmB administered orally and intravenously

data. Thus, with repeated administrations of oral AmB, increasing concentrations in target tissues will be achieved, to levels high enough for eradication of the pathogen. This is completely different from iv administration, in which high initial concentrations in the target tissues are achieved, resulting in a higher chance of related toxicity. We have previously shown that a multiple dosing regimen (10 mg/kg twice for 2 days) significantly reduced fungal infection in target tissues in rats infected with Candida albicans or Aspergillus fumigatus. No changes in plasma creatinine levels were detected in these studies despite multiple and frequent oral administration of AmB in PecoeI/DSPE-PEG2000.

Although a longer treatment regimen will possibly be required in the case of oral administration, gradual accumulation of the drug in the target tissues may lead to an improved safety profile of the therapy. Moreover, it was proposed before that since AmB is retained in the tissues for an extended period of time, it is conceivable that it can be used prophylactically in high-risk patients. Oral administration in a lipid-based formulation, such as PecoeI/DSPE-PEG2000, may provide an important platform for prophylactic/therapeutic AmB regimens, especially in areas with limited access to iv therapy in hospital settings.

In conclusion, oral administration of AmB in PecoeI/DSPE-PEG2000 to rats resulted in considerable intestinal absorption into the systemic circulation. The plasma pharmacokinetics and tissue distribution of orally administered AmB resemble those of a micellar commercial iv formulation (Fungizone®). According to the pharmacokinetics and tissue distribution data, the oral PecoeI/DSPE-PEG2000 formulation of AmB has the potential for improving current therapeutic treatment and prophylaxis of systemic fungal infections and some fatal parasitic diseases.

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Transparency declarations

J. G. C. is an employee/co-founder/shareholder and director of iCo Therapeutics Inc. All other authors: none to declare.

References

23. Risovic V, Rosland M, Sivak O et al. Assessing the antifungal activity of a new oral lipid-based amphotericin B formulation following


