Biodistribution and tissue toxicity of amphotericin B in mice following multiple dose administration of a novel oral lipid-based formulation (iCo-009)

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Objectives: The purpose of this study was to assess the biodistribution and toxicity of amphotericin B (AMB) following multiple dose administration of an oral lipid-based formulation (iCo-009).

Methods: BALB/c female mice were used. ICo-009 was administered twice daily for 5 days at doses of 2.5–20 mg/kg. Untreated animals, oral vehicle or intravenous Fungizone® (1 or 2 mg/kg) served as control groups. The animals were sacrificed 12 h following the last administration of AMB, and blood and multiple tissues were harvested for drug analysis and histopathological evaluation. Plasma or tissue samples were analysed for concentrations of AMB or creatinine by means of HPLC–UV.

Results: A dose-dependent accumulation of AMB in liver, spleen, kidney and lung tissues was found. The concentration of the drug in all these organs exceeded the corresponding concentrations in plasma at the same dose. The concentrations of AMB in heart and brain were similar to the corresponding concentrations in plasma. Creatinine concentrations were elevated above normal concentrations in the 2 mg/kg Fungizone® group only. Histopathological analysis of kidney and liver tissues revealed a normal pattern in all treated groups, except the 2 mg/kg Fungizone® group. No gastrointestinal toxicity was found in this study.

Conclusions: A multiple dose treatment regimen with iCo-009 in mice results in a gradual accumulation of AMB in tissues. Despite significant concentrations of AMB, no kidney or liver toxicity of orally administered AMB was detected in this study. Furthermore, multiple oral administration of iCo-009 or of vehicle control did not induce gastrointestinal toxicity.

Keywords: oral administration, nephrotoxicity, histopathology

Introduction

Amphotericin B (AMB) is a potent antibiotic that is used for the treatment of systemic fungal infections and some deadly parasitic diseases. Although AMB is considered a very effective drug with only a few reports of resistance, the toxicity (mainly dose-dependent nephrotoxicity) and the need to administer the drug intravenously (iv) significantly limit the benefits associated with AMB therapy.1 AMB has been administered orally to different laboratory animals1–3 as well as to humans;3–6 however, only very low plasma concentrations were obtained.

We have recently shown that a novel lipid-based oral formulation of AMB, based on Peceol® and polyethylene glycol (PEG)-phospholipids (iCo-009), was effective in the treatment of systemic aspergillosis and candidiasis in a rat model,7 as well as in the treatment of visceral leishmaniasis in a mouse model.8 It should be noted that a multiple dose twice-daily regimen was required in fungal as well as in leishmaniasis animal models for treatment success. The pharmacokinetics and tissue distribution pattern of AMB following single oral administration of iCo-009 suggested that AMB was relatively homogeneously distributed among tissues, similar to a micellar...
formulation (Fungizone®) administered iv. Moreover, AMB remained in the tissues for a prolonged time and was still detectable in the majority of tissues for as long as 72 h following oral administration, while the corresponding concentrations in plasma were below the level of detection. Based on this pattern of biodistribution, we hypothesized that repeated administration of oral AMB would potentially result in a gradual accumulation in the tissues, eventually leading to concentrations high enough for eradication of the pathogen, but without the toxic ‘peak’ concentrations in plasma and tissues that are usually associated with iv administration.

The purpose of the current study was to test the above hypothesis by assessing the biodistribution and toxicity of AMB in mice following multiple dose administration of an oral lipid-based formulation (iCo-009).

Materials and methods

The lipid-based oral formulation of AMB (iCo-009) was prepared as recently reported. AMB powder and 1-amino-4-nitronaphthalene were purchased from Sigma–Aldrich (St Louis, MO, USA), and used as received. Fungizone® (AMB micellar dispersion, Bristol-Myers Squibb, Montreal, Canada) was purchased from Vancouver General Hospital pharmacy. All other chemicals were of analytical reagent grade and solvents of HPLC grade.

The use of animals for this study was approved by the University of British Columbia’s Animal Care Committee and all experimental protocols conform to the Canadian Council on Animal Care guidelines. BALB/c female mice with a body weight of 18–20 g (Charles River Laboratories, Wilmington, MA, USA) were used in this study. Animals were allocated and placed immediately in 10% neutral buffered formalin. Sections of jejunum and liver, a portion of jejunum 5 cm in length and a piece of median lobe of liver were removed from each mouse and a piece of organ was bisected and embedded in paraffin. Sections of 5 μm thickness were cut from each kidney, and stained with haematoxylin and eosin. For histopathological analysis, one kidney from each mouse was fixed in neutral buffered 10% formalin for ≥48 h, bisected and embedded in paraffin. Sections of 5 μm thickness were cut from each kidney, and stained with haematoxylin and eosin.

Table 1. Plasma concentration (ng/mL) and tissue concentration (ng/g) of AMB in mice 12 h following the completion of a 5 day multiple dose (twice daily) treatment course of oral AMB (iCo-009)

<table>
<thead>
<tr>
<th>Oral AMB</th>
<th>Oral AMB</th>
<th>Oral AMB</th>
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<tr>
<td>20 mg/kg</td>
<td>10 mg/kg</td>
<td>5 mg/kg</td>
<td>2.5 mg/kg</td>
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<tr>
<td>twice daily for 5 days</td>
<td>twice daily for 5 days</td>
<td>twice daily for 5 days</td>
<td>twice daily for 5 days</td>
</tr>
<tr>
<td>Plasma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>519 ± 53</td>
<td>390 ± 51</td>
<td>168 ± 11</td>
</tr>
<tr>
<td>Liver&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4728 ± 527</td>
<td>3151 ± 179</td>
<td>957 ± 134</td>
</tr>
<tr>
<td>Spleen&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4194 ± 801</td>
<td>1920 ± 285</td>
<td>751 ± 263</td>
</tr>
<tr>
<td>Kidney&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1589 ± 177</td>
<td>928 ± 70</td>
<td>323 ± 75</td>
</tr>
<tr>
<td>Lung&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5048 ± 195</td>
<td>4076 ± 810</td>
<td>1449 ± 265</td>
</tr>
<tr>
<td>Heart&lt;sup&gt;f&lt;/sup&gt;</td>
<td>621 ± 24</td>
<td>626 ± 41</td>
<td>289 ± 69</td>
</tr>
<tr>
<td>Brain&lt;sup&gt;g&lt;/sup&gt;</td>
<td>184 ± 16</td>
<td>160 ± 6</td>
<td>112 ± 5</td>
</tr>
</tbody>
</table>

<sup>a</sup>The 20 mg/kg group is statistically significantly different from the 5 and 2.5 mg/kg groups (P < 0.01), and the 10 mg/kg group is statistically significantly different from the 2.5 mg/kg group (P < 0.05) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

<sup>b</sup>The 20 mg/kg group is statistically significantly different from the 5 mg/kg group (P < 0.01) and 2.5 mg/kg (P < 0.001) groups, and the 10 mg/kg group is statistically significantly different from the 2.5 mg/kg group (P < 0.01) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

<sup>c</sup>The 20 mg/kg group is statistically significantly different from the 5 mg/kg group (P < 0.01) and 2.5 mg/kg (P < 0.001) groups, and the 10 mg/kg group is statistically significantly different from the 2.5 mg/kg group (P < 0.05) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

<sup>d</sup>The 20 mg/kg group is statistically significantly different from the 5 mg/kg group (P < 0.01) and 2.5 mg/kg (P < 0.001) groups, and the 10 mg/kg group is statistically significantly different from the 2.5 mg/kg group (P < 0.01) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

<sup>e</sup>The 20 mg/kg group is statistically significantly different from the 5 mg/kg group (P < 0.01), and the 10 mg/kg group is statistically significantly different from the 2.5 mg/kg group (P < 0.001) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).

<sup>f</sup>The 20 and 10 mg/kg groups are both statistically significantly different from the 2.5 mg/kg group (P < 0.001) (Kruskal–Wallis test followed by Dunn’s multiple comparisons test).
5 μm thickness were cut and stained with haematoxylin and eosin similarly to kidney tissue.

**Results and discussion**

The biodistribution of AMB in mice 12 h following completion of the multiple dosing regimens of oral lipid-based formulation (iCo-009) is summarized in Table 1. As can be seen, there is a dose-dependent accumulation of AMB in liver, spleen, kidney and lung tissues, with the concentration of the drug in all these organs exceeding the corresponding concentrations in plasma at the same dose. The concentrations of AMB in heart and brain are similar to the corresponding concentrations in plasma. All the concentrations (in tissues and in plasma) are significantly higher than previously reported after single dose administration in rats. This pattern of biodistribution supports the hypothesis that a multiple dosing regimen of oral iCo-009 results in the gradual accumulation of AMB in tissues. The high concentrations of AMB obtained in liver and spleen tissues explain the recently reported excellent activity of oral iCo-009 against murine visceral leishmaniasis.

Creatinine concentrations were in the range of 0.11–0.18 mg/dL, which is within the normal range for mouse serum, in all treatment groups (data not shown) except the 2 mg/kg Fungizone®

![Figure 1](image-url). (a) Representative kidney histopathology of a mouse from the 20 mg/kg oral AMB group, which shows a normal pattern. (b) Representative kidney histopathology of a mouse from the 2 mg/kg iv Fungizone® group, which shows necrotic tubules that are interspersed with viable ones (an arrow indicates a necrotic tubule and arrowheads indicate some of the tubules with necrotic debris). (c) Representative jejunum histopathology of a mouse from the oral vehicle control group, which shows a normal villus pattern. (d) Representative jejunum histopathology of a mouse from the 20 mg/kg oral AMB group, which shows a normal villus pattern with no necrosis or inflammatory infiltrate. (e) Representative liver histopathology of a mouse from the 20 mg/kg oral AMB group, which shows normal morphology. (f) Representative liver histopathology of a mouse from the 2 mg/kg iv Fungizone® group, which shows a focal collection of necrotic hepatocytes with surrounding inflammatory cells (as indicated by arrows). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
group, in which the mean creatinine concentration reached concentrations consistent with moderate renal toxicity (0.28 ± 0.08 mg/dL, mean ± SEM). The acute toxicity and mortality associated with this dose of iv Fungizone® (25% of animals in this group died within 3 min post-injection) prevented further escalation of the dose. In accordance with plasma creatinine results, the histopathological analysis of kidney tissue revealed a normal pattern in all treated groups except the 2 mg/kg Fungizone® group, which showed patchy tubular epithelial necrosis that varied in extent from focal to extensive (Figure 1a and b).

No gastrointestinal toxicity was found in this study following multiple dose oral administration of the vehicle control or iCo-009, including at the highest dose (20 mg/kg) (Figure 1c and d). These results are important in light of previously reported serious gastrointestinal toxicity (nausea, vomiting and diarrhoea) when AMB sodium desoxycholate preparation was administered orally to humans.5

The analysis of liver tissue revealed normal histopathology in all treatment groups except the 2 mg/kg Fungizone® group, which showed focal lesions consisting of inflammatory cells surrounding a cluster of necrotic hepatocytes, isolated foci of necrotic hepatocytes, hepatocytes in the centrilobular zone showing degenerative features and bile duct damage (Figure 1e and f).

In conclusion, it has been shown in the current study that a multiple dose treatment regimen with oral AMB (iCo-009) in mice results in a gradual accumulation of AMB in tissues. Of equal importance, despite significant concentrations of AMB in kidney and liver tissues, no kidney or liver toxicity of orally administered AMB was detected in this study. Furthermore, according to histopathological evaluation, multiple oral administration of iCo-009 or of vehicle control did not induce gastrointestinal toxicity in mice. Further studies will be needed to confirm that the pattern of lack of toxicity and of gradual accumulation of the drug in target tissues repeats itself in other laboratory animals and in humans.

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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