Differences in tissue drug concentrations following intravenous versus intraperitoneal treatment with amphotericin B deoxycholate or liposomal amphotericin B

T. CHANG *, J. A. OLSON *, R. T. PROFFITT † & J. P. ADLER-MOORE *
*Department of Biological Sciences, California State Polytechnic University, Pomona, and California, †RichPro Associates, Lincoln, California, USA

Amphotericin B formulations were compared in preclinical models by using intraperitoneal (ip) and intravenous (iv) delivery of amphotericin B deoxycholate (DAMB) or liposomal amphotericin B. We examined the effects on drug tissue penetration and retention resulting from different routes of drug administration. Mice were treated with equivalent total doses of AmBisome® (AmBi) or DAMB (i.e., 15 mg/kg) given ip (3 mg/kg/day for 5 days) or iv (3 mg/kg/day AmBi for 5 days or 1 mg/kg/day DAMB for 15 days), with tissues collected 24 h post-treatment. For drug retention studies, mice were given iv or ip total doses of 30 mg/kg AmBi (10 mg/kg/day 3 × /week) or 60 mg/kg AmBi (20 mg/kg/day 3 × /week) with tissue collection 24 h or 7 days post-treatment. Blood samples were collected at 0.5 h, 2 h, 8 h, 12 h and 24 h after ip or iv drug dosing. A Paecilomyces variotii bioassay was used to determine drug concentrations. AmBi and DAMB were detected in the kidneys following iv, but not ip dosing. Significantly more DAMB than AmBi was detected in the lungs with ip dosing (P < 0.008), and more AmBi than DAMB (P = 0.056) was present with iv dosing. Unlike the lungs, the spleen and liver retained the AmBi for up to one week post-treatment regardless of the route of drug administration. Thus, there are significant differences in AmBi and DAMB tissue distribution depending upon the drug route and these differences could effect how the drugs perform in fungal infection models.

Keywords amphotericin B, liposomal amphotericin B, intravenous, intraperitoneal, drug concentrations

Introduction

The use of liposomal amphotericin B (AmBisome®, AmBi), a small unilamellar vesicle (<100 nm) with low toxicity compared with other commercially available amphotericin B (AMB) formulations, is associated with high peak plasma levels and high AUC. In addition there is high penetration into tissues which are often sites of fungal infection [1,2]. Clinical trials [3] and preclinical studies [1,2] have also shown that AmBi retains the broad spectrum of activity of AMB. Because of the interest in using AmBi for prophylaxis [4], intermittent dosing [5], combination therapy with newer antifungal drugs [6,7] and treatment of emerging fungal pathogens [8], it continues to be tested in preclinical infection models, often in comparison to the use of deoxycholate AMB (DAMB). In some animal studies, the intraperitoneal route has been used to compare equivalent doses of AmBi and DAMB because DAMB is much better tolerated by the animals at higher doses when given intraperitoneally (ip) rather than intravenously (iv) [9]. Furthermore, repeated daily dosing of animals with these antifungal drugs is technically easier when the drugs are given ip. The present study was done to characterize the biodistribution of AmBi vs DAMB in mice given therapeutic doses of the drugs either ip or iv to determine if there...
is comparable tissue drug concentrations and retention using these different routes. We selected the mouse in which to do these studies since they are often employed in preclinical models of fungal infection.

**Materials and methods**

Female Swiss Webster mice (8 weeks old) (Harlan, Indianapolis, IN, USA) were maintained in microisolator cages with standard rodent diet (Taklad Laboratory Rodent diet #2918 (18% protein), HarlanTeklad, Madison, Wisconsin) and water *ad libitum*. All animal research procedures were approved by the Institutional Animal Care and Use Committee of California State Polytechnic University, Pomona, CA, USA. AmBi (AmBisome®, Gilead Sciences, Inc., San Dimas, CA) and DAMB (Amphotericin B for Injection USP; X-Gen Pharmaceuticals Inc., Northport, NY) were reconstituted per the manufacturer’s instructions and diluted further with 5% dextrose (Baxter Healthcare Corp., Deerfield, IL). The drugs were administered iv via the tail vein or ip. To compare drug concentrations in the lungs, kidneys, spleens and livers of animals given equivalent total doses of AmBi or DAMB (i.e., 15 mg/kg), mice (*n* = 5/mice/group) were treated for 5 days with 3 mg/kg/day AmBi given ip or iv or 3 mg/kg/day DAMB given ip. For the group receiving DAMB iv, 1 mg/kg/day was given daily for 15 days because higher iv doses would be lethal [1]. To determine the effect of iv vs ip dosing on tissue maintenance of AmBi over time (measured as amphotericin B/g tissue), mice were given iv or ip total doses of 30 mg/kg AmBi (10 mg/kg/day Mon., Wed., Fri.) or 60 mg/kg AmBi (20 mg/kg/day Mon., Wed., Fri.). Tissues were collected 24 h or 7 days after the last drug treatment, weighed, frozen and subsequently analyzed for AMB concentration. Thawed tissues were homogenized on ice in 1.0 ml phosphate buffered saline (PBS) with a Tissue Tearor mechanical homogenizer, diluted 1:1 with methanol, heated for 10 min at 65°C, diluted with PBS, and duplicate aliquots of each dilution were used to determine the AMB concentration/g tissue using a previously described *P. variotii* bioassay [10,11] (lower limit of detection = 0.03 μg amphotericin B/ml). In this assay the recovery of amphotericin B from the tissues was 91.3% from lungs, 92.7% from kidneys, 89.6% from spleen and 91.6% from liver. The intra-day coefficient of variation ranged from 3.4% to 6.3% and the inter-day coefficient of variation ranged from 9–10% [11]. To evaluate the serum drug levels over time, following iv or ip dosing, mice (*n* = 25/group) were treated iv or ip with 3 mg/kg/day AmBi for 5 days or 10 mg/kg/day AmBi every other day for one week (Mon, Wed, Fri.). DAMB was given ip at 3 mg/kg/day for 5 days or iv at 1 mg/kg/day for 15 days. At 0.5 h, 2 h, 8 h, 12 h and 24 h following the last treatment, cardiac punctures were performed on 5 mice/timepoint. Serum was collected, frozen and subsequently analyzed for AMB concentration using the *P. variotii* bioassay.

Statistics for comparisons of tissue drug concentrations were performed using GraphPad Prism, version 4.0 (GraphPad Software, Inc., San Diego, CA). Individual group comparisons were carried out using the two-tailed Mann–Whitney test. A *P* value of ≤0.05 was considered significant.

**Results**

The studies were done to compare how the route of drug administration (iv vs ip) affected the serum clearance and tissue distribution in mice. We observed that the serum level of AmBi following a dose of 10 mg/kg/day or 3 mg/kg/day, was highest at 0.5 h, the first sampling point following iv administration (Table 1). In contrast, the serum level peaked at 2 h following ip administration. With either route of injection, the peak serum levels with the 10 mg/kg/day AmBi dose were significantly higher than those observed with the 3 mg/kg/day AmBi dosing (*P* = 0.008). By 8 h after injection, the serum levels had dropped and were similar (6 μg/ml–12 μg/ml) regardless of the dose or route of administration. Detectable levels of AmBi (1.2–2.0 μg/ml) were still present in the serum at 24 h post treatment. Like the AmBi, the peak level of DAMB was detected.

**Table 1** Comparison of serum levels of AMB from mice (*n* = 5/group) receiving a total dose of 30 mg/kg AmBi (10 mg/kg × 3) or 15 mg/kg AmBi (3 mg/kg × 5) by intravenous or intraperitoneal administration; 15 mg/kg DAMB (1 mg/kg × 15, iv administration) or DAMB (3 mg/kg × 5, ip administration)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>AmBi 10 mg/kg, iv</th>
<th>AmBi 10 mg/kg, ip</th>
<th>AmBi 3 mg/kg, iv</th>
<th>AmBi 3 mg/kg, ip</th>
<th>DAMB 1 mg/kg, iv</th>
<th>DAMB 3 mg/kg, ip</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>47.1 (4.1)</td>
<td>33.5 (5.5)</td>
<td>15.7 (3.0)</td>
<td>11.0 (1.7)</td>
<td>2.0 (1.2)</td>
<td>2.1 (0.3)</td>
</tr>
<tr>
<td>2</td>
<td>35.6 (7.4)</td>
<td>58.1 (4.5)</td>
<td>14.4 (1.6)</td>
<td>16.6 (2.8)</td>
<td>1.1 (0.1)</td>
<td>3.5 (0.6)</td>
</tr>
<tr>
<td>8</td>
<td>11.1 (4.0)</td>
<td>12.1 (0.6)</td>
<td>6.7 (1.3)</td>
<td>10.6 (1.2)</td>
<td>0.6 (0.3)</td>
<td>1.6 (0.2)</td>
</tr>
<tr>
<td>12</td>
<td>11.2 (3.1)</td>
<td>11.0 (1.1)</td>
<td>4.4 (1.5)</td>
<td>7.2 (1.3)</td>
<td>0.5 (0.3)</td>
<td>1.6 (0.4)</td>
</tr>
<tr>
<td>24</td>
<td>2.0 (0.6)</td>
<td>1.5 (0.4)</td>
<td>1.2 (0.5)</td>
<td>1.8 (0.2)</td>
<td>0.0 (0.0)</td>
<td>0.2 (0.2)</td>
</tr>
</tbody>
</table>
at 0.5 h following iv administration and at 2 h with ip delivery. By 8 h, the drug levels had dropped to about 30–45% of their peak levels. However, unlike AmBi, almost no DAMB was detected in the serum at 24 h post treatment. It is important to note that we found with comparable ip dosing of DAMB and AmBi (i.e., 3 mg/kg/day), marked differences in the levels of the drug over time. With ip dosing, AmBi levels at all time points were significantly higher ($P = 0.008$) than DAMB levels and the AmBi levels remained at least 4.5-fold higher than the DAMB levels throughout the study.

AmBi levels in various mouse tissues collected 24 h after the last iv or ip treatment resulting in a cumulative dose of 15 mg/kg, are shown in Fig. 1. There was no significant difference in AmBi levels in the livers between the two routes of delivery (Fig. 1D), but the ip route delivered more AmBi to the spleens than the iv administration although the difference did not reach significance ($P = 0.095$) (Fig. 1C). Intraperitoneal dosing delivered significantly less AmBi to the lungs ($P = 0.032$), and kidneys ($P = 0.008$) (Fig. 1B and 1A). In comparison, mice given total doses of 15 mg/kg DAMB, had significantly more drug in

![Fig. 1](https://academic.oup.com/mmy/article-abstract/48/2/430/1017373/210733)
their spleens ($P = 0.016$) and lungs ($P = 0.016$) following ip versus iv dosing (Fig. 1C and 1B), and significantly less in their kidneys ($P = 0.008$) and livers with ip dosing ($P = 0.032$) (Fig. 1A and 1D).

When AmBi and DAMB were compared with one another using the same routes of administration, ip but not iv dosing resulted in higher drug levels in the livers of mice treated with AmBi compared to DAMB ($P = 0.03$). In the spleens, both ip and iv dosing gave significantly higher drug levels with AmBi vs DAMB ($P = 0.008$ for both). In comparison, the drugs levels in the kidneys for both AmBi and DAMB were comparable with iv dosing, and following ip dosing there was no detectable AmBi or DAMB in this tissue. In the lungs, significantly more DAMB than AmBi was detected with ip dosing ($P = 0.008$), and conversely, iv dosing produced markedly more AmBi than DAMB ($P = 0.056$) in the lungs although the level did not quite reach significance.

We also examined the tissue drug concentrations with increasing total doses of AmBi (i.e., 30 mg/kg and 60 mg/kg) (Fig. 2). Overall, the drug levels in all the tissues rose with increasing total doses of drug and this is consistent with other reports in the literature [11]. In general, the lungs and kidneys at a given dose of AmBi were about $15 \times$ and $2.5 \times$ lower than the drug levels in the spleens and livers, respectively (Fig. 2). Notably, however, the level of drug in the tissues differed depending upon the route of administration. At 24 h post-treatment with 60 mg/kg, we observed lower drug levels in the lungs ($P = 0.008$), kidneys ($P = 0.008$) and spleen ($P = 0.095$) with ip vs iv AmBi dosing. In contrast, in the livers of mice given 60 mg/kg AmBi ip had higher drug levels than when iv dosing ($P = 0.008$) was employed. With 30 mg/kg of ip AmBi, but not 30 mg/kg iv AmBi, there was no detectable drug in the kidneys.

Fig. 2 Tissue AMB concentrations (μg/g tissue) in organs collected 24 h (d1) or one week (d7) after the last drug treatment from female Swiss Webster mice ($n = 5$ mice/group) treated iv or ip with a total dose of 30 mg/kg AmBi [open symbols] (10 mg/kg/day $3 \times$ /week) or 60 mg/kg AmBi [filled symbols]. (20 mg/kg/day $3 \times$ /week). The organs analyzed for AMB concentration included lungs (a), kidneys (b), spleens (c) and livers (d). The groups were as follows: 30 mg/kg AmBi d1 iv, □, or ip, □ and d7 iv, △, or ip, △; 60 mg/kg AmBi d1 iv, ● or ip, ■ and d7 iv, ▲, or ip, ▲. Bar = median value.
Retention of AmBi was also examined at 7 days post-treatment (Fig. 2). AmBi was retained for a much longer period of time in the tissues as compared to the blood, where the drug reached low levels by 24 h. Comparison among the tissues showed that the lungs retained the least amount of drug (Fig. 2). One week post-treatment, only 60 mg/kg AmBi, given iv, gave any detectable drug levels in the lungs. In contrast, one week after iv AmBi dosing, there was still detectable levels of drug in the livers, spleens and kidneys and the levels showed a dose-dependent response. One week following ip dosing, high levels of AmBi were also seen in the livers and spleens, but only the 60 mg/kg ip dose gave detectable drug levels in the kidneys.

Discussion

As reported by other investigators, iv dosing with AmBi or DAMB produces peak serum levels within 0.5 h after treatment [12], while these peak levels are not reached with ip dosing until 1.5 h later. This delay is probably related to the time it takes for the drug to leave the intraperitoneal cavity, enter the lymph and gain access to the blood circulation via the thoracic duct [13]. Small, cholesterol-rich liposomes, like AmBi get into the blood circulation intact from the lymph following intraperitoneal administration [14] with little lymph node retention of the small liposomes (<100 nm) [15]. Based on published studies of AmBi incubated with plasma for up to 72 h, 95% of the amphotericin B remains associated with the liposome [16]. Once in the blood, AmBi remains in circulation longer than DAMB, resulting in higher drug levels for AmBi, which is most likely related to differences in composition and structure, as well as drug interactions with other components in the blood. AmBi does not bind with serum proteins while DAMB does [17]. This binding could contribute to more rapid DAMB removal from the blood circulation, even when DAMB is administered at the same dose and via the same route as AmBi.

In our studies we observed that regardless of the route of administration drug delivery to the spleen with either AMB formulation was higher than for any other organ per gram of tissue, followed by the liver. Total doses of 30 or 60 mg/kg AmBi were also retained in the spleen and liver for at least one week post-treatment. These results are probably a consequence of marked liposome uptake by the phagocytic cells in these tissues [11,18]. In comparison, in the lungs, there was little AmBi retention over time. This could be related to differences in drug localization within the lungs vs the spleen and liver. Groll et al. [19] reported that in the lungs, AmBi localized primarily in the endothelial lining fluid in rabbits with less uptake by the alveolar macrophages. Thus, more frequent dosing with AmBi could potentially be better for treating lung infections, while treatment of infections in the spleen and liver may require a less intense dosing regimen.

The present studies showed that with 3 mg/kg, detectable levels of AmBi and DAMB were seen in the kidneys 24 h after iv dosing, but not with ip administration. This latter observation could have important implications if one were evaluating the therapeutic index of the drug. There have been reports of antifungal DAMB activity in Candida-infected kidneys following iv dosing and this is likely the result of antifungal drug levels in the kidneys remaining for several hours post drug treatment [20,21]. These same authors reported that following a 20 mg/kg bolus dose of DAMB, but not a 5 mg/kg bolus dose, antifungal drug levels could still be detected in the kidneys 24 h after treatment [21]. The differences between these studies and those reported here are probably related to the variations in dosing regimen.

In the clinic, the drug is given iv and the development of nephrotoxicity with iv administration is dose limiting for amphotericin B [22]. Differences in amphotericin B nephrotoxicity following iv administration have also been reported to markedly effect survival in animal infection models. A comparison between iv AmBi and ABLC treatment of murine pulmonary aspergillosis demonstrated that both lipid formulations significantly reduced the lung fungal burden [23]. However, only iv AmBi significantly enhanced survival, with blood chemistry (BUN and creatinine) and histopathology demonstrating significant nephrotoxicity in the iv ABLC treated mice, but not in mice treated with AmBi. Similarly, when iv AmBi treatment was compared with another, amphotericin B liposome formulation (i.e., Anfogen®, Genpharma, S.A., Argentina) for murine pulmonary aspergillosis, survival was significantly better with iv AmBi and acute nephrotoxicity was only observed with the iv Anfogen formulation [24].

Our data also showed that ip dosing with DAMB delivered more drug to the lungs than ip AmBi, whereas iv dosing with AmBi produced higher drug levels in the lungs than iv DAMB. Consequently, ip dosing, with DAMB might be favored over iv AmBi if the efficacy of these drugs was being compared in a lung infection model. Conversely, AmBi might be more effective than DAMB if the drug was given by the iv route [13]. Given these differences, efficacy comparisons between these drugs in murine pulmonary infection models have to be interpreted carefully.

In summary, there are important differences in AmBi and DAMB tissue distribution depending upon whether the drug is given by the ip or the iv route. By using the ip route for administering these drugs, one can reduce the nephrotoxicity since in our studies, no or very little drug was detectable in the kidneys 24 h after ip dosing. However,
because these drugs are given iv in the clinic, iv delivery of the drugs in animal models will more closely simulate possible complications associated with drug nephrotoxicity.

Acknowledgements
Support for this research was provided by a Research Grant from Gilead Sciences, Inc.

Disclosure of conflict of interest: J.P.A.-M has received funds for speaking at symposia organized on behalf of Gilead Sciences Inc. All of the authors have received funds for research support from Gilead Sciences Inc.

References

This paper was first published online on Early Online on 01 February 2010.