Penetration of Amphotericin B Lipid Formulations into Pleural Effusion

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Pages: 12. Word count: 48 (Abstract); 984 (Text); Tables: 2; References: 23.

Running title: Amphotericin B in Pleural Effusion

Key words: target-site concentrations, polyene antifungals, antimycotics, empyema thoracis

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ABSTRACT
The penetration of the Amphotericin B (AMB) lipid formulations (liposomal AMB, AMB colloidal dispersion, AMB lipid complex) into pleural effusion was assessed in 7 critically ill patients. AMB was detected in all pleural effusion samples at concentrations ranging from 0.02 – 0.43 µg/mL. The penetration ratio was 3 – 44%.
Invasive fungal infections are a major cause of morbidity and mortality in immunocompromised patients, particularly when the pleural compartment is affected (9). Although many studies on the penetration of antibacterial agents into pleural effusion have been performed, less attention has been paid to target-site concentrations of antimycotic drugs in pleural effusion (2, 8, 12, 15-17, 22, 23). Amphotericin B (AMB) lipid formulations have been introduced in therapy to reduce the toxicity of amphotericin B. Liposomal amphotericin B (LAMB), amphotericin B colloidal dispersion (ABCD) and amphotericin B lipid complex (ABLC) exhibit different composition, structure and particle size of their lipid moiety, which is reflected in different plasma pharmacokinetics and lung penetration (1, 7, 19, 21). The aim of the present study was to investigate the AMB penetration into pleural effusion during treatment with LAMB, ABCD or ABLC.

The study was approved by the local ethics committee. AMB levels were determined in specimens of seven critically ill patients, who were treated with LAMB (one patient, two samples), ABCD (five patients) or ABLC (one patient) for suspected invasive fungal infection. Demographic and clinical characteristics of the enrolled patients are shown in table 1. LAMB (AmBisome®, Gilead, San Dimas, CA, USA), ABCD (Amphocil®, Torrex Chiesi Pharma, Vienna, Austria) and ABLC (Abelcet®, Elan Pharma International Limited, Athlone, Irland) were dissolved as recommended by the manufacturers and administered intravenously at a dose of 3-5 mg/kg body weight once a day over 4 h. Aliquots of pleural effusion were taken during therapeutic thoracentesis. Blood samples were drawn simultaneously from an arterial line and were centrifuged immediately. The plasma and the pleural effusion samples were stored frozen at –80°C. Samples were purified and concentrated. The lipid-associated fraction of LAMB and ABCD was separated from AMB, that had been liberated from its lipid-encapsulation (comprising free and protein-bound AMB), by C18 solid phase extraction as described previously (with modifications for pleural effusion) (3). For ABLC this separation technique is not feasible. Pleural effusion and plasma specimens were analysed by
reversed-phase high-performance liquid chromatography, using a LiChrosorb-RP-8 column, UV detection ($\lambda = 405$ nm) and a mixture of acetonitrile–methanol–$0.010 \ M \ NaH2PO4$ buffer (41:10:49, v/v) as mobile phase (3). The detection limit was 0.005 µg/mL. The intra-assay coefficient of variation was 2.06%. The concentrations were assessed by means of a linear standard-curve (R=0.998 to R=0.999), obtained by external standards comprising pleural effusion spiked with AMB. Total AMB concentrations were obtained by addition of the liberated and lipid-associated AMB concentrations. The penetration ratio was defined as:

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\text{AMB concentration in pleural effusion} / \text{simultaneous AMB level in plasma} \times 100\%
\]

Statistical calculations were performed using Statistica 5.1–1997® (StatSoft, Inc., Tulsa, USA). Wilcoxon Matched Pairs Test was used to analyse the difference between total AMB concentrations in plasma and in pleural effusion.

AMB concentrations in plasma and pleural effusion and the penetration ratios are displayed in table 2. Liberated AMB could be detected in all samples of pleural effusion and plasma. In pleural effusion total AMB concentrations were significantly lower than the total plasma levels ($p=0.03$). Concentrations of the lipid-associated fraction of ABCD and LAMB, measured in pleural effusion, were very low (<0.03 µg/mL, in four samples even below the detection limit). The highest AMB concentration in pleural effusion was found in Patient 1 (0.43 µg/mL), who had been treated with LAMB at a daily dose of 300 mg for four days. AMB concentrations in pleural effusion correlated positively with the administered cumulative dose in patients treated with ABCD ($R=0.96$, $p=0.01$). In Patient 7, who had received a cumulative dose of 12,750 mg of ABLC, an AMB concentration of 0.18 µg/mL (penetration ratio 44%) was reached in pleural effusion. The respective plasma level was as low as 0.4 µg/mL.

Since several mycoses such as candidiasis, aspergillosis, blastomycosis, histoplasmosis, cryptococcosis and coccidioidomycosis can cause pleural manifestations (5, 6, 9, 11, 13, 20), AMB concentrations in pleural effusion can be crucial for clinical response. After treatment
with AMB lipid formulations, AMB concentrations were substantially lower in pleural effusion than in plasma and in lung tissue (19). However, the small number of patients, the different underlying clinical conditions of the patients and the differences in cumulative doses are clear limitations of our study, and preclude a comparison between the lipid formulations.

Median intraperitoneal levels of AMB amounted 0.12 µg/mL in critically ill patients during intravenous treatment with AMB deoxycholate (18), which is comparable to our results in pleural effusion. In non-infected rabbits total AMB concentrations were much lower in alveolar epithelial lining fluid than in lung tissue (7). By the separation of lipid-associated and liberated AMB, we could show, that only AMB that has been liberated from its lipid-encapsulation penetrates into pleural effusion.

The MIC of AMB has been reported to range from 0.125 to 1 mg/L for Candida spp. (14), from 0.25 to 4 mg/L for Aspergillus spp. and from <0.03 to 2 mg/L for relevant dimorphic fungi, such as Blastomyces dermatitidis or Histoplasma capsulatum (4). Thus the MIC may exceed in some cases the pleural AMB concentrations that are achieved by therapeutic dosage. The criteria of an exudate (10) are met in Patient 1 and Patient 6, exhibiting the highest levels of AMB in the pleural effusion. This might indicate that the presence of local inflammation increases drug penetration of AMB into pleural effusion. However, none of our patients suffered from a fungal empyema thoracis. Recently, voriconazole was found to achieve penetration ratios of 45 – 95 % into a pleural empyema caused by A. fumigatus (15).

In conclusion, AMB levels in pleural effusion were in the range of - or even below - its MIC for most relevant pathogens after administration of AMB lipid formulations. Therefore long-term treatment with high doses of AMB lipid formulations will be required for eradication of fungal infection from pleural effusion, and alternative therapeutic strategies have to be considered. Further clinical studies are required to elucidate the penetration of antimycotics into pleural effusion.
This study was supported by the Tiroler Wissenschaftsfonds and Torrex-Chiesi Pharma, Austria.
References


Legend to Tables:

TABLE 1. Demographic and clinical characteristics of patients: Two samples (sA and sB) were obtained from Patient 1. AMB LF: Amphotericin B lipid formulation; age in [years]; Cumulative Dose [mg], Interval: duration between last administration and sampling in hours, Creat: plasma creatinine [mg/dL], normal range 0.70 - 1.20 mg/dL; protein: protein concentration [g/dL], normal range in serum 6.30 – 8.20 g/dL; LDH [U/L]: plasma lactate dehydrogenase, normal range 100 – 250 U/L; RBC: erythrocytes, ++: many, +: some; Cells: number of cells per µL [no./µL]; n.a. not available; DM: diabetes mellitus; KidneyTx: status post kidney transplantation.

TABLE 2. Concentrations of Amphotericin B in Plasma and Pleural Effusion: Two samples (sA and sB) were obtained from Patient 1. AMB concentration [µg/mL]. n.a. not available. For ABLC the chromatographic separation of lipid-associated and liberated AMB was not feasible.
TABLE 1. Demographic and clinical characteristics of patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>AMB</th>
<th>Cumulative Dose</th>
<th>Interval</th>
<th>Serum Protein</th>
<th>LDH</th>
<th>Protein</th>
<th>LDH</th>
<th>RBC Cells no/µL</th>
<th>Diagnosis</th>
</tr>
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<tbody>
<tr>
<td>1 sA</td>
<td>LAMB</td>
<td>1,050</td>
<td>22.5</td>
<td>0.60</td>
<td>5.73</td>
<td>425</td>
<td>2.57</td>
<td>281</td>
<td>700</td>
<td>Sepsis, pneumonia, pancytopenia</td>
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</tr>
<tr>
<td>1 sB</td>
<td>M</td>
<td>76</td>
<td>LAMB</td>
<td>1,650</td>
<td>4</td>
<td>0.40</td>
<td>5.53</td>
<td>417</td>
<td>2.12</td>
<td>n.a.</td>
<td>Sepsis, lymphoma, DM, renal failure</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>66</td>
<td>ABCD</td>
<td>150</td>
<td>14.5</td>
<td>0.87</td>
<td>4.62</td>
<td>272</td>
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<td>n.a.</td>
<td>Sepsis, pneumonia, KidneyTx, Kaposi sarcoma</td>
</tr>
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<td>3</td>
<td>M</td>
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<td>ABCD</td>
<td>1,300</td>
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<td>2.21</td>
<td>5.00</td>
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<td>1.63</td>
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<td>290</td>
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<tr>
<td>4</td>
<td>M</td>
<td>68</td>
<td>ABCD</td>
<td>1,350</td>
<td>21.5</td>
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<td>5.06</td>
<td>247</td>
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<tr>
<td>5</td>
<td>F</td>
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<td>ABCD</td>
<td>1,500</td>
<td>2</td>
<td>2.07</td>
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<td>286</td>
<td>1.23</td>
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<tr>
<td>6</td>
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<td>44</td>
<td>ABCD</td>
<td>7,250</td>
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<td>4.74</td>
<td>194</td>
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<td>134</td>
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</tr>
<tr>
<td>7</td>
<td>M</td>
<td>53</td>
<td>ABLC</td>
<td>12,750</td>
<td>5</td>
<td>0.39</td>
<td>6.65</td>
<td>246</td>
<td>2.66</td>
<td>128</td>
<td>+ 220</td>
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TABLE 2. Concentrations of Amphotericin B in Plasma and Pleural Effusion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample</th>
<th>Pleural Effusion</th>
<th>Plasma</th>
<th>Penetration Ratio</th>
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<tr>
<td></td>
<td></td>
<td>liberated</td>
<td>lipid-</td>
<td>total</td>
</tr>
<tr>
<td></td>
<td></td>
<td>associated</td>
<td></td>
<td>total</td>
</tr>
<tr>
<td>1</td>
<td>sA</td>
<td>0.40</td>
<td>0.03</td>
<td><strong>0.43</strong></td>
</tr>
<tr>
<td>1</td>
<td>sB</td>
<td>0.13</td>
<td>0.02</td>
<td><strong>0.15</strong></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.02</td>
<td>0.00</td>
<td><strong>0.02</strong></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.05</td>
<td>0.00</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.04</td>
<td>0.02</td>
<td><strong>0.06</strong></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.12</td>
<td>0.00</td>
<td><strong>0.12</strong></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>0.25</td>
<td>0.00</td>
<td><strong>0.25</strong></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>n.a.</td>
<td>n.a.</td>
<td><strong>0.18</strong></td>
</tr>
</tbody>
</table>