Brief reports

Amphotericin B intralipid formulation: stability and particle size


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Amphotericin B remains the drug of choice for treating systemic fungal infections, although toxicity limits its clinical use. Some studies reported the use of Intralipid deoxycholate amphotericin B as an alternative delivery system. An in-vitro study was performed to assess the compatibility of deoxycholate amphotericin B in Intralipid. With two types of dilution of deoxycholate amphotericin B (in 5% dextrose and Intralipid or in Intralipid alone) the solution stability was not constant with a clear yellow precipitate. We observed an increase in the size of the particles (1.5-4-fold). In light of these results Intralipid deoxycholate amphotericin B should not be routinely administered.

Introduction

In recent years, the population at risk of severe disseminated infections has grown (Bodey et al., 1992). The reasons for this are, among other things, associated with an increased number of AIDS patients, more advanced medical treatments such as solid organ and bone marrow transplants, intensive chemotherapy for cancer, the use of broad spectrum antibiotics and modern intensive care. Although an increasing number of new drugs are available for treatment of fungal infections, including imidazoles and triazoles, amphotericin B is still the drug of choice in patients with systemic fungal infections (Perfect et al., 1991). However, its use is considerably hampered by the high incidence of toxicity (Warnock, 1991).

Efforts to reduce the toxicity of amphotericin B without compromising antifungal potency have continued for more than a decade. Commercial amphotericin B is complexed with sodium deoxycholate. Other formulation that have been prepared include N-acyl, N-glycosyl, N-aminoacyl derivatives, amides and guanidino derivatives. Recently, lipid based formulations have shown greater potential, including liposomal amphotericin B, amphotericin B lipid complex, small unilamellar vesicle, and amphotericin B colloidal dispersion. In an experimental model (Kirsh et al., 1988), an alternative emulsion based delivery system was described using a lipid solution available.
for parenteral nutrition. The efficacy and reduced toxicity of the amphotericin B emulsion had been shown in experimental models. This formulation was prepared with amphotericin B diluted in 20 mg Na deoxycholate (Na Doc)/mL of dimethylacetamide (DMA). The Na Doc/DMA solution was used to solubilize the amphotericin B (Sigma, St Louis, USA) by gentle mixing at 30°C. A concentration of 45 mg of amphotericin B/mL was achieved, and the solution was filtered through a 0.22 μm Acrodisc CR filter (Gelman Sciences, Ann Arbor, USA) and then added to a vial containing 20% Intralipid (Kabi-Pharmacia, Stockholm, Sweden), with a final DMA concentration not exceeding 3% v/v. Following this publication some authors reported that a combination of amphotericin deoxycholate (Fungizone, Bristol-Myers-Squibb, Paris, France) diluted in 20% Intralipid was less nephrotoxic and clinically better tolerated than Fungizone (given in 5% dextrose), when given to neutropenic and HIV infected patients with candidiasis (Caillot et al., 1994). In another study Fungizone was diluted in 5% dextrose and then mixed with 250 mL of 20% Intralipid (Moreau et al., 1992). In neither of these cases was a study of the stability of the formulation carried out. We report here an in-vitro study of Fungizone diluted in 20% Intralipid in order to identify whether enlarged lipid particles are formed, which may cause pulmonary embolism.

**Materials and methods**

In study A, 50 mg of Fungizone were diluted in 10 mL of Intralipid, and 13 mL of this mixture (65 mg Fungizone) were added to 35 mL of Intralipid, to give a total volume of 48 mL in a 50 mL syringe. Infusion was simulated by emptying a syringe driver at a rate of 9 mL/h, collecting the solution in a receptacle. The tests performed were microscopic examination using a Thoma cell (x 500) (Bioblock, Paris, France) to evaluate this modification of Intralipid, a study of particle size using a Coulter Counter TA II (Coulter Electronics, Luton, UK) with 50 μm orifice; two dilutions were studied, 10⁻⁵ for particles 0.79–5.04 μm and 10⁻³ for particles >1.59 μm. Visual examination of the solution was also recorded. Measurements were made at times 0, 1, 2, 3, 4 and 5 h, and after homogenization of the emulsion at 5 and 24 h. The same experiment was performed with Endolipide (lipid emulsion manufactured by Laboratoire Bruneau, Paris, France).

In study B, two concentrations of Fungizone were prepared in 5% dextrose (75 mg Fungizone in 15 mL dextrose 5%, and 150 mg in 30 mL), then diluted by injecting the solution through a Millex filter 0.22 μm (Millipore, Paris, France) into 50 mL or 500 mL of Intralipid for each dosage. The tests performed were visual inspection of the solution, pH (Orion pH meter, Orion Research, Kusnacht, Switzerland), osmolality (Roebling, Osmometer, Messtechnik, Berlin, Germany), mean particle diameter (Nanosizer, Coulter Electronics, Luton, UK) and further study of particle size (Coulter Counter TA II) using a 50 μm probe. Measurements were made at times 0, 3 and 24 h. In both studies, the same measurements were performed for 20% Intralipid alone, and all determinations were carried out in quadruplicate.

**Results**

In study A, the solution stability was not constant with a clear yellow precipitate being formed in the lower part of the syringe, beginning immediately and increasing with time. The microscopic study did not show a modification of the lipid emulsion, but there were
many aggregates of large particles. The study of the particle size (Table I) showed an increase in the larger particles seen in the $10^{-3}$ dilution. The particle size data were normalized to report the percentage of fat in the admixture that was present as particles measuring 0.79–5.04 μm and 1.59–25 μm in diameter. The percentage fat concentration of the admixture existing as enlarged lipid globules (> 5.09 μm diameter) was twice that present in Intralipid without Fungizone.

In study B, the admixture prepared with 500 mL Intralipid was not homogeneous with a clear precipitate occurring in the lower part of the bottle. Creaming or liberation of free oil were not observed. For the solution in 50 mL Intralipid pH was not modified, but osmolality was lower. The particle size in the admixture of Fungizone in 50 mL Intralipid was completely different (Table II) compared with 20% Intralipid alone.

**Discussion**

Amphotericin B remains the treatment of choice for many fungal infections, but its clinical usefulness is limited by its toxicity. Chills and fever are experienced by 30–60% of patients treated with Fungizone. Specific premedication, such as corticosteroids or anti-histamines, are supposed to decrease these side effects but the results are poor. Impairment of renal function is an important dose limiting factor. Recent studies have shown that amphotericin B encapsulated in liposomes is less toxic than standard amphotericin B, and liposomal amphotericin B has been effective in the treatment of severe mycoses, even in patients who failed to respond to the conventional formulation and in those who had developed nephrotoxicity (Mills et al., 1994). Potential problems of formulating amphotericin B in liposomes, such as stability and homogeneity, led some investigators (Kirsh et al., 1988) to study a solution made with amphotericin B diluted with sodium deoxycholate dimethylacetamide and added to Intralipid. The results on cells and mice of this experimental solution were interesting, with reductions in amphotericin B toxicity. Following this report different studies were performed in patients with two types of solution. In some studies, Fungizone was mixed directly with Intralipid (Caillot et al., 1994). In one study, Fungizone was first mixed with 5% dextrose and then with Intralipid (Moreau et al., 1992). There have been no published results for the stability and particle size of these mixtures. Our results show that these two types of dilution led to an unstable solution with a precipitation of Fungizone, and hence a risk of discontinuous administration of the drug. We observed an increased number of particles with diameter > 1 μm compared with the initial Intralipid emulsion.

**Table I.** Study A: percentage of fat in the admixture existing as particles of diameter 0.79–5.04 μm (10^{-3} dilution) and 1.59–25 μm (10^{-3} dilution)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Intralipid 0.79–5.04 μm</th>
<th>1.59–25 μm</th>
<th>Intralipid + Fungizone 0.79–5.04 μm</th>
<th>1.59–25 μm</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>9.04</td>
<td>0.42</td>
<td>10.27</td>
<td>1.89</td>
</tr>
<tr>
<td>1</td>
<td>9.24</td>
<td>0.41</td>
<td>10.85</td>
<td>2.18</td>
</tr>
<tr>
<td>2</td>
<td>9.53</td>
<td>0.43</td>
<td>10.92</td>
<td>1.82</td>
</tr>
<tr>
<td>3</td>
<td>9.63</td>
<td>0.44</td>
<td>10.36</td>
<td>1.61</td>
</tr>
<tr>
<td>4</td>
<td>9.41</td>
<td>0.41</td>
<td>9.81</td>
<td>1.66</td>
</tr>
<tr>
<td>5*</td>
<td>9.29</td>
<td>0.43</td>
<td>10.56</td>
<td>3.41</td>
</tr>
<tr>
<td>24*</td>
<td>9.45</td>
<td>0.43</td>
<td>10.86</td>
<td>3.35</td>
</tr>
</tbody>
</table>

*Measurements made after homogenization of admixture.
Table II. Study B. particle size, pH and osmolality of various Fungizone admixtures measured after 3 and 24 h

<table>
<thead>
<tr>
<th></th>
<th>IL 50 mL</th>
<th>IL 50 mL</th>
<th>IL</th>
<th>IL 500 mL</th>
<th>IL 500 mL</th>
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</thead>
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<tr>
<td></td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>pH</td>
<td>7.84</td>
<td>7.95</td>
<td>7.88</td>
<td>7.88</td>
<td>8.08</td>
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<tr>
<td>Osmolality (mosm/kg)</td>
<td>330</td>
<td>263</td>
<td>216</td>
<td>216</td>
<td>330</td>
</tr>
<tr>
<td>Mean diameter (nm)</td>
<td>356</td>
<td>367</td>
<td>378</td>
<td>405</td>
<td>372</td>
</tr>
<tr>
<td>% particles ≥ 1 μm</td>
<td>0.23</td>
<td>2.68</td>
<td>2.04</td>
<td>3.62</td>
<td>14.89</td>
</tr>
</tbody>
</table>

+ 75 mg amB
+ 150 mg amB

*IL, Intralipid, *amB, amphotericin B.
The number of particles ≥1 μm per mL of solution is 1.5–4-fold higher than in Intralipid without Fungizone. The number of particles ≥2 μm per mL of solution is 10–25-fold higher than the original emulsion. Microscopic examination indicated that these bigger particles were Fungizone poorly suspended in Intralipid. The average diameter of each unilamellar vesicle in liposomal amphotericin B (Ambisome, Vestar, Cambridge, UK) is less than 100 nm. The practical guidelines for preparing and administering Fungizone recommend dilution in 5% dextrose. This solution was shown to be a chemically and physically stable colloidal suspension (Kintzel & Smith, 1992).

Liposomal formulations of amphotericin B are expensive and this is one reason why alternative formulations, such as Fungizone in Intralipid, have been explored. It remains uncertain whether treatment with liposomal amphotericin B will generate savings in the overall cost of treatment (Persson et al., 1992). However, such economic considerations should not lead to home-made lipid formulations of amphotericin B which have not been fully evaluated (Washington, Lance & Davis, 1993). Further investigations are needed, but the physical modifications which occur in Fungizone Intralipid indicate that this formulation should not be administered to patients.

References


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