Safety and efficacy of Intralipid emulsions of amphotericin B

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To evaluate the clinical role of amphotericin/20% Intralipid emulsions (ILA), we conducted a Medline search of the English literature to locate the relevant case reports and clinical studies involving the use of this formulation. Due to differences in study design and definitions, we applied a set of treatment outcome definitions to determine the clinical efficacy of this treatment modality. Only 37 patients received ILA for the treatment of documented fungal infections. Using our definitions, four were considered successfully treated, one improved, two failed, and 30 were unevaluable. While infusion-related adverse events and nephrotoxicity were reportedly reduced with ILA, use of adjunctive therapies and concomitant nephrotoxic agents, and comparisons with high infusion concentrations complicate evaluation. Furthermore, incomplete and conflicting data exist regarding the physiochemical stability of ILA. The currently available data do not support recommendations for the use of this formulation for the treatment of systemic fungal infections.

Introduction

Amphotericin B is a polyene macrolide that remains the antifungal agent of choice for a variety of systemic fungal infections (Gallis, Drew & Pickard, 1990; Lyman & Walsh, 1992). However, the use of this agent may be associated with infusion-related adverse events, such as chills, fever, rigors and hypotension, metabolic derangements, and dose-limiting nephrotoxicity (Maddux & Barriere 1980; Branch, 1988; Gallis et al., 1990; Lyman & Walsh, 1992). Lipid formulations of amphotericin B, including liposomes and lipid complexes, have been developed to minimize these effects (Janknegt et al. 1994; Meunier, 1994). Data from animal studies and preliminary clinical trials indicate the usefulness of these formulations in the treatment of fungal infections. Of note, however, the dosages used and the types of fungal infections studied were highly variable (de Marie, Janknegt & Bakker-Woudenberg, 1994).

While the exact mechanism for the reduced toxicity remains to be determined, the selective transfer of amphotericin B from the lipid formulation to the target fungal cell, with reduced mammalian cell uptake, is suggested (Janknegt et al. 1992). The disposition of liposomes following parenteral administration strongly depends upon
physiochemical characteristics such as particle size, bilayer rigidity, and surface electrical charge. Furthermore, the phospholipid to amphotericin B ratio and the type of phospholipid are important determinants of fungicidal activity and toxicity (Janknegt et al., 1992). Therefore, study results for each liposomal formulation must be interpreted independently with respect to efficacy and toxicities, as no direct comparison has been made of the new formulations. Several lipid formulations of amphotericin B (Abelcet, Liposome Company; Ambisome, Nexstar and Amphocil, Sequus Pharmaceuticals) are now licensed in several European countries, while only one (Abelcet) is commercially available in the USA.

Following the concept of a lipid-based delivery system for amphotericin B, European investigators evaluated the use of a parenteral fat emulsion (20% Intralipid, Pharmacia) as the delivery vehicle for amphotericin B. However, even though in-vitro and animal data appear encouraging (Kirsh et al., 1988; Lamb et al., 1991; Joly et al., 1994), only limited human data exist on the use of Intralipid/amphotericin B (ILA) in the treatment of fungal infections (Caillot et al., 1992, 1993, 1994; Chavanet et al., 1992; Moreau et al., 1992; Leake, Appleyard & Hartley, 1994; Macedo et al., 1994; Smith & Strozyk, 1994; Anderson & Clark, 1995). In addition, published data are incomplete and conflicting on the bioavailability and pharmaceutical and physiochemical stability of such formulations. Earlier reviews on the use of ILA suggested that further studies were needed to clearly define its clinical role (Vita & Schroeder, 1994; Anderson & Clark, 1995). Nonetheless, the ease of preparation, low expense and the limited availability of the commercially produced lipid formulations of amphotericin B make ILA an attractive option for the administration of amphotericin B.

To identify all available relevant information regarding the use of ILA, a Medline search was conducted using the Medical Subject Headings 'Amphotericin B' or 'Fat Emulsions (intravenous)', cross-referenced with key words 'Intralipid' and/or 'lipid-complex', and 'amphotericin B', respectively. These search parameters were used in the January 1985–May 1995 database and were restricted to English language publications. The purpose of this review is to examine the information from clinical trials and case reports with respect to efficacy and toxicities of ILA in the treatment of systemic fungal infections.

Pharmaceutics

Various methods of reconstitution of amphotericin B in Intralipid are described in the literature. Reconstitution methods include direct dissolution of amphotericin B in 20% Intralipid (Caillot et al., 1993, 1994) or dissolution of amphotericin B in 5% dextrose (Chavanet et al., 1992; Moreau et al., 1992), or sterile water for injection (Leake et al., 1994) before diluting into 20% Intralipid. Some reports failed to describe the preparation methods of the 20% Intralipid formulation of amphotericin B (Caillot et al., 1992; Macedo et al., 1994; Smith & Strozyk, 1994; Anderson & Clark, 1995). One study reported a final concentration of 2 mg/L, but the volume of ILA infusions required to achieve the daily doses reported in this study make this number unlikely (Caillot et al., 1994). While a final infusion concentration of 0.1 mg/mL is recommended for traditional amphotericin B/5% dextrose formulations (Trissel, 1994), the infusion concentrations utilized in the literature for ILA ranged from 0.02 to 2 mg/mL.

Compatibility and stability data are limited for ILA. In a study of murine candidiasis, Kirsh et al. (1988) reported a lipid emulsion of amphotericin B, initially solubilized in
Safety and efficacy of Intralipid/amphotericin B emulsions

a deoxycholate/dimethylacetamide solution and then diluted in 20% Intralipid (dd-ILA) to a final amphotericin B concentration of 1 mg/mL, to be stable for up to 1 year by high performance liquid chromatography (HPLC). Currently, there are no data regarding the bioactivity of this or other amphotericin B emulsions over time. However, two reports raised questions regarding the ability of amphotericin B to partition into the lipid phase of Intralipid fat emulsions. Washington, Lance & Davis (1993) suggested that the type of emulsions used in the clinical studies then reported were not stable, and that at least 95% of the amphotericin B had not distributed to the oil phase, resulting in precipitation. These observations have recently been confirmed (Trissel, 1995). In a set of comparative stability tests, Trissel examined a 0.6 mg/mL concentration of amphotericin B in dextrose and ILA (using both 10% and 20% Intralipid; amphotericin B reconstituted with sterile water). Samples of each formulation were thoroughly mixed, stored for 1 h at 23°C, and then centrifuged at 5000 rpm for 1 h. No changes were visualized in the amphotericin B in dextrose, but both ILA samples produced yellow precipitates. Analysis of the particle sizes in the three formulations after undisturbed storage at 23°C for 1 h showed that the amphotericin B in dextrose solution had a substantially lower number of particles and considerably fewer particles ≥ 10μm in size than the two Intralipid samples. Nearly 50% of the total particle counts in the 20% samples were particles ≥ 10μm. Measurements of amphotericin B concentrations in the resultant supernatant and particle sizes associated with either 10% or 20% Intralipid alone (without amphotericin B) were not reported. This preliminary evidence suggests that when mixed with fat emulsions, amphotericin B does not partition into the lipid phase, but instead exists as particles. This raises concerns regarding ILA formulations, as in-line filters may be needed to prevent lodgement of precipitant particles in a patient’s microvasculature. However, these concerns remain speculative as no embolic events have been reported with the administration of the ILA formulation. Whether filtration of these preparations results in removal of the active drug remains to be seen. Additional data are available regarding the biophysical properties of ILA via the use of circular dichroism spectroscopy (Swenson et al., 1995), although the data from this analysis of ILA are not consistent with results from earlier investigations (Joly et al., 1994; Chavanet & Caillot, 1995). Further data regarding the pharmaceutical and physiochemical stability and bioavailability of these formulations are required to ensure safe preparation, storage and administration of such emulsions.

Pharmacokinetics

The pharmacokinetic characteristics of ILA infusions in human subjects are shown in Table I. Only three studies and one case report measured serum amphotericin B levels following parenteral administration of ILA (Caillot et al., 1992, 1994, Chavanet et al., 1992; Leake et al., 1994); three of these compared levels achieved by ILA and traditional amphotericin B formulations. Two studies utilized bioassays to determine amphotericin B concentrations while one used HPLC; in the case report by Leake et al. (1994), the assay methodology was not stated. The mean daily doses used in these studies were similar (~1 mg/kg), although the times of serum level sampling were not uniform. In two of the studies, amphotericin B concentrations were graphically determined as the actual values were not reported (Caillot et al., 1992; Chavanet et al., 1992). The study by Chavanet et al. (1992) reported blood concentrations that differ a thousand-fold from other studies. When compared with serum concentrations
<table>
<thead>
<tr>
<th>Reference</th>
<th>Method of concentration determination</th>
<th>Treatment group</th>
<th>Mean daily dose (mg/kg)</th>
<th>$C_{\text{max}}$ (mg/L)</th>
<th>$C_{\text{min}}$ (mg/L)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caillot et al. (1994)</td>
<td>HPLC</td>
<td>AmB in dextrose</td>
<td>0.99 ± 0.12</td>
<td>2.75 ± 1.46</td>
<td>1.28 ± 0.74*</td>
<td>$C_{\text{max}}$ 5min post infusion on day 5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILA</td>
<td>1.08 ± 0.15</td>
<td>1.87 ± 0.83</td>
<td>0.74 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td>Chavanet et al. (1992)</td>
<td>Bioassay</td>
<td>AmB in dextrose</td>
<td>1</td>
<td>1800$^*$</td>
<td>700$^*$</td>
<td>$C_{\text{min}}$ 12h post infusion on day 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILA</td>
<td>1</td>
<td>1050$^*$</td>
<td>450$^*$</td>
<td></td>
</tr>
<tr>
<td>Caillot et al. (1992)</td>
<td>Bioassay</td>
<td>AmB in dextrose</td>
<td>0.92 ± 0.1</td>
<td>2.6$^r$</td>
<td>1.7$^r$</td>
<td>$C_{\text{max}}$ 8h post infusion on day 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILA</td>
<td>1 ± 0.2</td>
<td>2.7$^r$</td>
<td>1.8$^r$</td>
<td></td>
</tr>
<tr>
<td>Leake et al. (1994)</td>
<td>Not stated</td>
<td>ILA</td>
<td>1</td>
<td>1.12 serum</td>
<td>NS</td>
<td>results from one patient</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12 CSF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^aP < 0.05; ^bP = 0.01; ^c$exact values not given, concentrations were graphically estimated; $^dP < 0.005. AmB. Amphotericin B; ILA, amphotericin B in 20% Intralipid; HPLC, high performance liquid chromatography; NS, not stated.
Safety and efficacy of Intralipid/amphotericin B emulsions

achieved with amphotericin B in dextrose, one study found significantly lower peak concentrations ($C_{\text{max}}$) (Chavanet et al., 1992), while two found significantly lower trough concentrations ($C_{\text{min}}$) of amphotericin B in the ILA group (Chavanet et al., 1992; Caillot et al., 1994). The variation in reported amphotericin B levels may be attributed to the differences in assay methodology, sampling times, preparation methods of the ILA formulation and/or patient specific parameters. It is unclear whether whole blood or serum concentrations were measured in the studies as centrifugation of blood samples may cause sedimentation of lipid-complexed amphotericin B particles. This may have accounted for the observed variation in measured levels (Janknegt et al., 1992). Of note, there does not appear to be currently any clinical indication for the measurement of serum amphotericin B concentrations. A comparison of the therapeutic index between ILA and traditional amphotericin B formulations may provide more clinically relevant information. Nonetheless, the above studies suggested that administration of ILA may result in lower amphotericin B levels as compared with traditional formulations.

Animal studies

The in-vivo antifungal activity of ILA has been studied in murine models of candidiasis and cryptococcosis. Using DBA/2 mice intravenously infected with *Cryptococcus neoformans*, Joly et al. (1994) compared the efficacy of amphotericin B in dextrose and ILA at various dosages. An infusion concentration of 5 mg/mL was used for both formulations; treatment was initiated nine days after infection. The authors reported that for amphotericin B in dextrose and ILA the maximum tolerated doses, defined as the dose responsible for less than 15% lethality, were 0.8–1.2 and 2 mg/kg respectively. While survival time after treatment with single doses of 0.8 mg/kg of amphotericin B in dextrose and ILA were significantly different from controls, no significant difference between these two treatment groups was observed. Comparison of single treatments with 1.2 mg/kg of traditional amphotericin B and 2 mg/kg of ILA revealed a statistically significant increase in survival ($P < 0.05$) with the latter. Comparisons of organism counts in brain, kidney, lung and spleen three days after treatment revealed no significant differences between traditional amphotericin B and ILA at 1.2 mg/kg. However, significant differences were observed between 1.2 mg/kg of amphotericin B in dextrose and 2 mg/kg of ILA in brain and spleen organism counts ($P < 0.05$), but not in kidney and lung counts. The authors concluded that the superior therapeutic activity of ILA was due to the ability to administer higher doses, thereby achieving higher tissue concentrations.

Kirsh et al. (1988) used Webster-derived, CD-1 mice intravenously infected with *Candida albicans* to compare the efficacy of amphotericin B in dextrose, ILA, and dd-ILA in various single dosages 48 h after infection. The authors reported that the maximum tolerated dose, defined as the dose at which no deaths due to amphotericin B occurred within 4 days of injection in normal mice, was 1 mg/kg for amphotericin B in dextrose and 9 mg/kg for amphotericin B emulsion. However, the authors do not differentiate between the maximum tolerated doses of ILA and dd-ILA. Comparisons revealed that treatment with 1 mg/kg of traditional amphotericin B or 9 mg/kg AmB emulsion improved survival compared with controls, with 20% of mice given traditional amphotericin B mice and 100% of amphotericin B emulsion-treated mice alive 34 days after treatment. The authors concluded that the emulsion formulation of amphotericin B is preferable to the
Table II. Outcome definitions

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cure</td>
<td>No clinical signs of infection, documented negative fungal cultures from previously positive sites and patient not receiving antifungal therapy after completion of AmB therapy.</td>
</tr>
<tr>
<td>Improvement</td>
<td>Decreased clinical signs of infection with or without positive fungal cultures after completion of AmB therapy.</td>
</tr>
<tr>
<td>Failure</td>
<td>Little or no improvement in clinical status with persistent positive fungal cultures or death due to fungal infection or after completion of AmB therapy.</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>Lack of documented fungal infection, lack of documented negative follow up fungal cultures after treatment, concurrent treatment with other antifungal agent(s), treatment for nonsystemic infections, or death during treatment where death due to fungal infection could not be ruled out</td>
</tr>
</tbody>
</table>

traditional formulation. Of note, cultures of kidneys taken 35 days after treatment of mice with 9 mg/kg of amphotericin B emulsion produced viable *C. albicans*.

These experimental studies indicate that ILA may be as effective as the traditional AmB formulation but the applicability of these results to humans remains in question due to the differences in duration of therapy, infusion concentrations, and the modes of acquisition of the fungal infection.

Case reports and clinical studies

To date, four case reports and five clinical studies regarding the use of ILA in humans have been described in the English literature (Caillot *et al.*, 1992, 1993, 1994; Chavanet *et al.*, 1992; Moreau *et al.*, 1992; Leake, Appleyard & Hartley, 1994; Macedo *et al.*, 1994; Smith & Strozyk, 1994; Anderson & Clark, 1995). Variations in the outcome definitions in these studies complicate evaluation of the clinical efficacy of ILA. For this reason, we applied our own set of definitions of treatment outcomes to facilitate a uniform assessment of the studies (Table II). A summary of the design and treatment outcomes of each study is listed in Table III. A summary of the toxicities reported in these studies is shown in Table IV.

Case reports

Case reports have described 14 patients treated with ILA. Of these, seven had documented systemic fungal infections due to either candidaemia (*n* = 6, Macedo *et al.*, 1994; Smith & Strozyk, 1994) or cryptococcal meningitis (*n* = 1, Leake *et al.*, 1994), while the remaining five were treated empirically (Smith & Strozyk, 1994; Anderson & Clark, 1995). Two patients had oral candidiasis (Smith & Strozyk, 1994). The daily ILA doses these patients received ranged from 0.02-1 mg/kg, with infusion concentrations ranging from 0.02-1 mg/mL. Adjunctive prophylaxis for infusion-related adverse events (IRAE) was given to six patients, but was ineffective in three. Concurrent treatment with either an aminoglycoside or vancomycin was reported in six patients. Electrolyte abnormalities were found in five patients (Leake *et al.*, 1994; Smith & Strozyk, 1994), but were not stated in the remaining two case reports. None of the reports associated nephrotoxicity with the use of ILA, with the highest cumulative dose received being 1820 mg (Smith & Strozyk, 1994). However, Smith & Strozyk (1994) reported that a
Table III. Study design and treatment outcome

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Treatment groups</th>
<th>No. of patients</th>
<th>Treatment indication (number of patients)</th>
<th>Mean daily dose* (mg/kg/d)</th>
<th>Cumulative dose* (mg/kg)</th>
<th>Outcome*</th>
<th>I</th>
<th>Fail</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson &amp; Clark (1995)</td>
<td>case report</td>
<td>ILA</td>
<td>1</td>
<td>documented infection 0 1</td>
<td>(0.5–1)</td>
<td>330mg</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Macedo et al. (1994)</td>
<td>case reports</td>
<td>ILA</td>
<td>3</td>
<td>candidaemia 0 3 6–12</td>
<td>0.6</td>
<td>6–12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Smith &amp; Strozyk (1994)</td>
<td>case reports</td>
<td>ILA</td>
<td>9</td>
<td>candidiasis 4 NS</td>
<td>NS</td>
<td>732.2mg (125–1820mg)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Leake et al. (1994)</td>
<td>case report</td>
<td>ILA</td>
<td>1</td>
<td>cryptococcal meningitis 0 (0.02–1)</td>
<td>NS</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Caillot et al. (1992)</td>
<td>retrospective</td>
<td>AmB in dextrose</td>
<td>12</td>
<td>NS 14 candidaemia 12.0 24.8</td>
<td>0.92 ± 0.1 NS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Caillot et al. (1993)</td>
<td>prospective</td>
<td>ILA</td>
<td>10</td>
<td>NS 14 candidaemia 12.0 24.8</td>
<td>1 ± 0.2 NS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Moreau et al. (1992)</td>
<td>randomized</td>
<td>AmB in dextrose</td>
<td>16</td>
<td>pulmonary aspergillosis 1 candidaemia 16</td>
<td>0.8 (0.7–1.0) 8.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Chavanet et al. (1992)</td>
<td>prospective</td>
<td>AmB in dextrose</td>
<td>11</td>
<td>oral candidiasis 0 1 4 14.5</td>
<td>0.8 (0.7–1.0) 14.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Caillot et al. (1994)</td>
<td>randomized</td>
<td>AmB in dextrose</td>
<td>21</td>
<td>candidaemia 21 1 UTI 19 14.3 ± 8.5</td>
<td>0.99 ± 0.12 8.3 ± 4.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Patient totals</td>
<td>AmB in dextrose</td>
<td>ILA</td>
<td>86</td>
<td>14 46</td>
<td>1.08 ± 0.15 14.3 ± 8.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
</tbody>
</table>

*Ranges provided in parenthesis if standard deviation not stated.

*See Table II for outcome definitions.

*Cultures were obtained from sputum, urine, blood, catheter tips and oropharynx.

*Plus 5-fluctyosine 99–200mg/kg/d for 3–32 days in 12 of these patients.

*One patient also received 5-FC, dose not stated.

*Causative organism not specified.

*Patients with documented fungal infections.

AmB, Amphotericin B; ILA, amphotericin B in 20% Intralipid; I, improvement; NE, not evaluable; NS, not stated; UTI, urinary tract infection.
Table IV. Adverse effects

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment groups</th>
<th>No. of patients</th>
<th>Infusion concentration (mg/mL)</th>
<th>Mean cumulative dose* (mg/kg)</th>
<th>Mean serum creatinine* (µmol/L) pre</th>
<th>Mean serum creatinine* (µmol/L) post</th>
<th>Concurrent AG or VM?</th>
<th>IRAEs</th>
<th>Reports or use of adjunctive therapy</th>
<th>Amphotericin B discontinued (no. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson &amp; Clark (1995)</td>
<td>ILA</td>
<td>1</td>
<td>1</td>
<td>330mg</td>
<td>141</td>
<td>106</td>
<td>no</td>
<td>no</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td>Macedo et al. (1994)</td>
<td>ILA</td>
<td>3</td>
<td>NS</td>
<td>6–12</td>
<td>NS</td>
<td>NS</td>
<td>yes</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Smith &amp; Strozyk (1994)</td>
<td>ILA</td>
<td>9</td>
<td>0.5</td>
<td>732.2mg (125–1820)</td>
<td>NS</td>
<td>17.7*</td>
<td>yes</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Leake et al (1994)</td>
<td>ILA</td>
<td>1</td>
<td>0.02–0.7</td>
<td>42</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0</td>
</tr>
<tr>
<td>Caillot et al. (1992)</td>
<td>AmB in dextrose</td>
<td>12</td>
<td>1</td>
<td>NS</td>
<td>81.4 ± 21.2</td>
<td>109.7 ± 43.4</td>
<td>NS</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Caillot et al. (1992)</td>
<td>ILA</td>
<td>10</td>
<td>1</td>
<td>NS</td>
<td>83.2 ± 14.2</td>
<td>90.3 ± 15</td>
<td>NS</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Caillot et al. (1993)</td>
<td>ILa</td>
<td>14</td>
<td>1–2</td>
<td>24.8</td>
<td>82.3 ± 25.7</td>
<td>96.5 ± 25.7*</td>
<td>yes</td>
<td>1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Moreau et al. (1992)</td>
<td>AmB in dextrose</td>
<td>16</td>
<td>0.16–0.32</td>
<td>8.8</td>
<td>61 (34–87)</td>
<td>120 (74–302)</td>
<td>yes</td>
<td>12</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Chavanet et al. (1992)</td>
<td>ILa</td>
<td>16</td>
<td>0.16–0.32</td>
<td>14.5</td>
<td>63 (36–85)</td>
<td>90 (72–135)</td>
<td>yes</td>
<td>5</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Caillot et al. (1994)</td>
<td>AmB in dextrose</td>
<td>11</td>
<td>1.6</td>
<td>4</td>
<td>79.6 ± 13.3*</td>
<td>121 ± 31.8*</td>
<td>NS</td>
<td>9</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Caillot et al. (1994)</td>
<td>ILa</td>
<td>21</td>
<td>0.002</td>
<td>8.3 ± 4.3</td>
<td>74.3 ± 14.2*</td>
<td>117.7 ± 25.7*</td>
<td>yes</td>
<td>16</td>
<td>15</td>
<td>5</td>
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*Ranges provided in parenthesis if standard deviation not stated.

*Reported as the mean change (range) after treatment.

*Twelve patients also received 5-flucytosine 99–200mg/kg for 3–30 days.

*Significant changes in creatinine clearance found post treatment ($P = 0.01$)

$P = 0.04$.

*Serum creatinine levels were measured on day 10 of treatment.

$P = 0.01$.

ILA, amphotericin B in 20% Intralipid; AG, aminoglycoside; AmB, amphotericin B; NS, not stated; IRAEs, infusion related adverse events; VM, vancomycin.
Safety and efficacy of Intralipid/amphotericin B emulsions

post-ILA treatment increase in serum creatinine of 70.7 μmol/L occurred. Whether this increase was seen in the patient who received the highest cumulative dose is unclear. Post-treatment serum creatinine levels were not mentioned in two reports (Leake et al., 1994; Macedo et al., 1994). Applying our outcome definitions, two patients were successfully treated (Macedo et al., 1994), one improved (Leake et al., 1994), one failed (Smith & Strozyk, 1994), and ten were not evaluable due to either empirical treatment (n = 1, Anderson & Clark, 1995), or lack of follow-up cultures (n = 1, Macedo et al., 1994; n = 6, Smith & Strozyk, 1994), or non-systemic fungal infection (n = 2, Smith & Strozyk, 1994).

Clinical studies

Retrospective studies. One retrospective study evaluated 22 patients receiving amphotericin B in dextrose versus ILA treatment following myeloablative therapy (Caillot et al., 1992). Daily doses ranged from 0.75 to 1.05 mg/kg and 0.75 to 1.5 mg/kg for amphotericin B in dextrose and ILA groups, respectively. The infusion concentration of both formulations was 1 mg/mL. With regard to toxicity, the authors reported that significantly more patients treated with amphotericin in dextrose experienced renal insufficiency than the ILA patients (9 vs 4, P = 0.02) after 6 days of treatment. Interestingly, no significant differences were found when comparison was made at 4, 8, 10 and 12 days. Comparison of pre- and post-treatment serum creatinine levels revealed that no significant increases in serum creatinine levels occurred within the ILA group, while a significant difference was seen in the amphotericin B in dextrose group (P = 0.006). The duration of treatment ranged from 2 to 29 days in the amphotericin B in dextrose group and 5 to 21 days in the ILA group. However, the total dose received is not stated for any of the patients included in the study. IRAE were reported in terms of courses of treatment and were not patient specific. It was reported that dexamethasone was required in six of the amphotericin B treatment courses. Four courses of traditional amphotericin B treatment were discontinued due to adverse effects compared to none in the ILA group. There is no specific reason stated for the discontinuation of amphotericin B therapy. The authors concluded that the ILA formulation was clinically better tolerated and less toxic than the conventional formulation. Efficacy of ILA was not addressed in this study as patient outcomes were not reported. The toxicity data are difficult to evaluate due to the wide variation in treatment duration, lack of information on total amphotericin B dose received, relatively high infusion concentrations of the traditional preparation (Trissel, 1994), and comparisons of IRAE in terms of infusion courses rather than in the number of patients.

Non-randomized prospective studies.

A non-randomized prospective study was performed by Caillot et al. (1993) to examine the tolerance and efficacy of ILA for the treatment of candidaemia in fourteen neutropenic patients with haematological malignancies. All patients received aminoglycoside and β-lactam therapy empirically for febrile neutropenia. Vancomycin, followed by amphotericin B, treatment was initiated if fever persisted. Twelve patients received ILA plus 5-flucytosine (5-FC) and two received ILA alone. Doses for ILA and 5-FC ranged from 0.73–1.895 mg/kg/day and 99–200 mg/kg/day, respectively. ILA
was infused at a concentration of either 1 or 2 mg/mL. The total dose of ILA received ranged from 5.4 to 55.7 mg/kg with duration of treatment ranging from 6 to 62 days. The authors reported a significant decrease in the mean serum creatinine clearance post-treatment ($P = 0.01$), but not in mean serum creatinine levels. Only one patient experienced IRAE and whether this patient received adjunctive therapy is unknown. Electrolyte supplementation was not reported. With regard to efficacy, the two patients who received ILA alone were successfully treated, while the remaining patients are not evaluable due to combination treatment with 5-FC. The authors concluded that ILA was clinically well tolerated and beneficial in the treatment of candidaemia in these patients. However, the noncomparative nature of the study precludes an evaluation of the potential for decreased risk of nephrotoxicity with ILA treatment.

Randomized prospective studies.

A prospective study was performed by Moreau et al. (1992). Sixteen patients in each group were randomized to receive ILA or amphotericin B in dextrose for empirical treatment of fever persisting despite antibacterial treatment (vancomycin, an aminoglycoside and a β-lactam). Patients were excluded from the study if the duration of amphotericin B therapy was expected to be shorter than 7 days, if their baseline serum creatinine was greater than 106.1 μmol/L (1.2 mg/dL) or if they had received cyclosporine A. Infusion concentrations ranged from 0.16 to 0.32 mg/mL and daily doses of amphotericin B ranged from 0.7 to 1 mg/kg in both groups. Duration of treatment ranged from 4 to 25 days in the amphotericin B in dextrose group and 7 to 40 days in the ILA group. All patients received 10 mg of iv diphenhydramine before infusion and 25 mg of hydrocortisone was added if fever and rigors occurred. The incidence of IRAE was statistically different between the two groups; 12 AmB in dextrose patients and 5 ILA patients experienced clinical side effects related to amphotericin B infusions ($P < 0.05$) and all responded to adjunctive therapy. However, the authors did not compare the daily dose or infusion concentrations that were administered to these patients. Sodium intake was reported to be similar in both groups, but potassium or magnesium supplementation were not stated. A significantly higher number of patients in the traditional preparation group experienced renal toxicity ($n = 9$ vs 2, $P < 0.05$) as measured after 11 and 18 days of therapy. Three patients were found to have fungal infections after initiation of treatment, two cases of pulmonary aspergillosis (one in each group) and one case of candidaemia in the AmB in dextrose group. Results of follow up cultures after treatment in these patients were not stated, but successful outcomes were reported; one patient received a combination of amphotericin B with 5-FC. The authors concluded that efficacy could not be evaluated but that the ILA formulation resulted in less acute nephrotoxicity than traditional amphotericin B formulations. Our analysis of the efficacy data in this study is in agreement with that of the authors. None of the three patients with documented fungal infections had cultures following treatment. The toxicity data presented here are also difficult to evaluate for the following reasons. Comparison of the two groups did not include treatment duration or the cumulative doses of vancomycin or aminoglycoside received. It is also unclear how the renal and clinical toxicities observed relate to the infusion concentrations used in the study. Furthermore, the infusion concentrations of the AmB in dextrose formulation are higher than those generally recommended (Trissel,
Comparison of renal toxicity was not evaluated after the full course of treatment in all patients complicating evaluation of the observed serum creatinine levels. Specifically, these comparisons were based on the number of patients experiencing renal toxicity as defined by the authors and not by serum creatinine levels.

Other investigators prospectively compared ILA with traditional amphotericin B in the treatment of oral candidiasis in HIV positive patients (Chavanet et al., 1992). Eleven patients were randomized to receive either amphotericin B in dextrose or ILA for 4 days. Patients were excluded if they had oesophagitis, oral Kaposi's sarcoma, known intolerance to amphotericin B, pancreatitis, hyperlipidaemia, or a serum creatinine greater than 114.9 μmol/L (1.3 mg/dL). ILA infusion concentrations were 2 mg/mL and amphotericin B in dextrose infusion concentrations were 1.6 mg/mL. A daily dose of 1 mg/kg was used in both treatment groups. No premedication was given unless severe chills or fever occurred during or after infusions. Hydration and electrolyte supplementation, except for magnesium, was given as needed. With regard to toxicity, the authors reported that the mean serum creatinine after treatment in the AmB in dextrose group was significantly greater than the ILA group \((P = 0.04)\). Therapy was discontinued in four patients treated with amphotericin B in dextrose, three for serum creatinine increases and one due to clinical side-effects. No significant changes in potassium or sodium levels were observed, but a significant difference was found in the mean change in serum magnesium levels after treatment (amphotericin B in dextrose, \(1.22 \pm 1.7 \text{ mmol/L vs ILA, } 0.22 \pm 1.2 \text{ mmol/L, } P = 0.05\)). There were no data regarding the amount of electrolyte supplementation required in any of the patients. Nine patients in the AmB in dextrose group experienced IRAE compared to three ILA patients \((P = 0.03)\). The authors stated that the IRAE in five of the former group of patients were severe enough to warrant treatment with dexamethasone and paracetamol. The authors concluded that the clinical and renal toxicities were reduced with the ILA formulation and that it had comparable efficacy to the formulation. Overall, the toxicity data presented is difficult to evaluate. The higher incidence of IRAE seen with the AmB in dextrose formulation may be a result of the infusion concentration employed, which was much higher than is generally recommended (Trissel, 1994). Furthermore, the duration of treatment was shorter than is usually required to resolve systemic infections. Since nephrotoxicity relates to the cumulative dose, a 4-day treatment course may not be long enough for a true comparison. Lastly, due to the non-systemic nature of the fungal infections in the study population, we find the efficacy data to be unevaluable.

In a randomized, prospective study, Caillot et al. (1994) compared ILA to AmB in dextrose in 42 patients with haematological malignancies. Twenty-one patients were assigned to each treatment group. Patients were excluded from the trial if they had received allogeneic bone marrow transplantation, had serum creatinine levels greater than 132.6 μmol/L (1.5 mg/dL) or a creatinine clearance less than 40 mL/min, had received cyclosporine A, or if they had received AmB treatment within the last 12 months. Amphotericin B treatment was initiated empirically if patients experienced fevers despite antibiotic treatment with vancomycin, an aminoglycoside and a β-lactam. Initial daily doses of amphotericin B were 1.0–1.1 mg/kg and the infusion concentration was 2 μg/mL in both groups. AmB infusions were discontinued if IRAE were not controlled with dexamethasone on two successive infusions or if severe renal impairment was observed. The mean daily dose was 0.99 ± 0.12 mg/kg in the amphotericin B in dextrose group and 1.08 ± 0.15 mg/kg in the ILA group and the mean total doses received were 8.3 ± 4.3 and 14.3 ± 8.5 mg/kg, respectively. The toxicity of the two
preparations was compared by requirements for adjunctive therapy for IRAE, electrolyte requirements, and by changes in renal function as measured by serum creatinine levels and creatinine clearance estimations. Statistical comparisons were made after 8 days of therapy only, even though the mean duration of treatment for the amphotericin B in dextrose and ILA groups was 8.4 and 12.8 days, respectively. Comparison of creatinine clearance between groups revealed that fourteen amphotericin B in dextrose patients and seven ILA patients experienced a >50% decrease in creatinine clearance ($P = 0.025$). Ten patients given amphotericin B in dextrose and two given ILA had a >75% increase in serum creatinine levels ($P = 0.007$). Of note, a significant increase in the serum creatinine ($P = 0.007$) and a significant decrease in creatinine clearance ($P = 0.03$) were seen during the initial twelve days of treatment within the ILA group. Treatment was discontinued in four patients given traditional amphotericin B infusions (due to renal toxicity) and none in the ILA group. The initial daily dose as well as the duration of concurrent treatment with amikacin and vancomycin was comparable between the two groups. Seven AmB in dextrose recipients versus two ILA patients required amikacin dose reductions ($P = 0.045$), while 12 and five, respectively, required vancomycin dose reductions ($P = 0.03$). Electrolyte supplementation was the same for both groups in terms of the quantity and number of patients requiring potassium, while significantly more patients in the amphotericin B in dextrose group required magnesium supplementation ($8 \text{ vs } 2$, $P = 0.02$). The incidence of IRAE was also compared. Fifteen patients in the amphotericin B in dextrose group required dexamethasone treatment compared to five ILA patients ($P = 0.002$). With respect to efficacy, only three out of the 42 patients treated had documented fungal infections. One patient in each of the groups had a catheter-related candidaemia, while one patient in the ILA group had a fungal urinary tract infection with an unreported organism. The authors stated that the three patients with documented fungal infections were successfully treated; however no documentation of negative cultures following amphotericin B treatment were included from either group. By our definitions of efficacy, all 42 patients in this study are unevaluable.

The authors concluded that the ILA formulation of amphotericin B is better tolerated and less nephrotoxic than the traditional formulation. They also stated that the efficacy of ILA was not evaluable due to the low number of documented fungal infections, which is consistent with our assessment. In addition to the lack of efficacy data, our analysis of the toxicity data reveal several limitations. The stated infusion concentration used, 2 $\mu$g/mL, would result in excessively large infusion volumes of 20% Intralipid or 5% dextrose. Ten patients given amphotericin B in dextrose were reported to have had a greater than 150% increase in serum creatinine and a greater than 75% decrease in creatinine clearance (the authors’ definitions of renal toxicity), but only four patients had treatment discontinued. Furthermore, the serum creatinine or creatinine clearance values are not reported past day 10 of treatment. This may produce inaccurate comparisons due to the concurrent administration of nephrotoxic drugs along with considerable variation in treatment duration. In addition, higher mean total doses were administered to the ILA group (14.3 vs 8.3 mg/kg). Specifically, the authors noted significant changes from baseline serum creatinine and creatinine clearance in the ILA patients, suggesting that duration of ILA and concomitant therapies should be considered when evaluating the nephrotoxicity of ILA.
Conclusions

Based upon our review of the English literature on the use of ILA, the human studies performed to date do not provide conclusive data on the efficacy of ILA formulations for the treatment of systemic fungal infections. Notably, a majority of the studies utilized empirical treatment strategies which complicate evaluation; only one prospective, randomized study specifically examined the use of ILA in patients with documented fungal infections (oral candidiasis—Chavanet et al., 1992), where the use of amphotericin B is generally not considered. Furthermore, evaluation of ILA efficacy is complicated by the fact that definitions of treatment outcomes were either not stated or were not uniform in the studies and, some studies allowed for either prior or concurrent use of other antifungal agents (Chavanet et al., 1992; Caillot et al., 1993). Of the 146 human subjects included in these studies and case reports, 86 patients received amphotericin B therapy as ILA and only 37 of these patients actually had documented fungal infections (Table III). Only patients with systemic fungal infections were included in our efficacy analysis; patients with oral (13) or urinary (1) infections were deemed unevaluable. Using our definitions of treatment outcomes, of the 23 patients with systemic fungal infections, only four were successfully treated, one improved and two failed treatment with ILA. Although 30 of the 37 patients with documented infections were not evaluable (Table II). The four patients that were successfully treated for candidaemia received mean daily ILA doses of 0.6 to 1.435 mg/kg. The patient who improved was treated for cryptococcal meningitis using daily ILA doses of 0.02 to 1 mg/kg. The treatment duration for both of these patient groups was not stated. The two patients who failed treatment had candidaemia and received mean daily ILA doses of 1.2 mg/kg (the daily dose for one patient was not stated) with mean treatment duration ranging from 21.9 to 24 days. One of these candidaemias was reported to be catheter related but whether the catheter was removed was not stated (Caillot et al., 1993).

Although results from animal studies indicate that the ILA formulation may allow infusion of larger daily doses resulting in greater efficacy (Kirsh et al., 1988; Joly et al., 1994), these findings have proven to be variable in human subjects. In comparison to traditional amphotericin B formulations, the average daily doses of ILA used in the studies and case reports are higher (0.8 mg/kg vs 1.1 mg/kg, respectively). The highest daily dose of ILA reported is 1.895 mg/kg (Caillot et al., 1993). It remains speculative whether yet higher doses of ILA are needed to overcome a possible decreased bioavailability of amphotericin B in lipid emulsions.

Some protection from the toxicity (both IRAE and nephrotoxicity) associated with traditional AmB infusions may be afforded by the ILA formulation at conventional doses, but comparisons have been made with amphotericin B in dextrose infusion concentrations greater than those recommended, making the validity of these comparisons uncertain. In addition, the different preparation methods of ILA described could conceivably result in varying degrees of IRAE and nephrotoxicity. The specific infusion concentrations of ILA associated with IRAE requiring adjunctive treatment vary widely (0.002–2 mg/mL), suggesting that these side effects may not be concentration-dependent. With respect to nephrotoxicity, comparative studies have found post-treatment serum creatinine levels increase less with ILA than with traditional amphotericin B formulations even though the mean cumulative doses administered were higher. However, these findings need to be interpreted with caution.
since patients in both groups received concurrent nephrotoxic agents. Nonetheless, IRAE and renal toxicity were still reported in patients despite the use of ILA formulations. Caillot et al. (1994) reported that a significant increase in mean serum creatinine and a significant decrease in mean creatinine clearance were found following average daily ILA doses of 1.08 mg/kg ($P = 0.007$ and 0.03 respectively). Furthermore, the study using high dose ILA (mean cumulative dose 24.8 mg/kg) found a significant decrease in creatinine clearance post-treatment ($P = 0.01$, Caillot et al., 1993). Overall, these data suggest that the renal toxicity may be delayed, but unavoidable, with the use of ILA.

Compatibility and stability are other important aspects in the evaluation of the ILA formulation. The findings of Washington et al. (1993) and Trissel (1995) raise questions about the stability and safety of the ILA preparations. Although embolic episodes associated with the use of ILA have not been reported, this possibility cannot be overlooked. Whether the use of in-line filters can help prevent possible precipitant-particle associated complications without affecting efficacy remains to be determined.

The results of the clinical studies published to date do not indicate that ILA is useful in the treatment of systemic fungal infections and many questions regarding this formulation of amphotericin B remain. These include compatibility and stability, the degree of nephrotoxicity at high doses, and the possible requirements for in-line filters. Due to the lack of efficacy data presented in the available clinical studies, recommendations regarding the use of Intralipid/amphotericin B emulsions for the treatment of systemic fungal infections cannot be substantiated.

References


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