Treatment of candidaemia in premature infants: comparison of three amphotericin B preparations

Nehama Linder1,2*, Gil Klinger1,2, Itamar Shalit2,3, Itzhak Levy2,3, Shai Ashkenazi2,3, Gali Haski4, Orli Levit1,2 and Lea Sirota1,2

1Department of Neonatology, 2Infectious Disease Unit and 4Pharmacy, Schneider Children’s Medical Center of Israel, Petah Tikva, Israel; 2Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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Objective: The objective of this study was to compare the effectiveness and tolerability of three antifungal preparations, amphotericin B, liposomal amphotericin B (LamB) and amphotericin B colloidal dispersion (ABCD), in the treatment of neonatal Candida bloodstream infection (CBSI).

Patients and methods: All patients hospitalized in the neonatal intensive care unit from 1996 to 2000 with CBSI were enrolled. Patients with a serum creatinine concentration of <1.2 mg/dL received amphotericin B, and those with serum creatinine ≥1.2 mg/dL received LamB or ABCD. Complete blood counts, and renal and hepatic function tests were obtained before, during and after treatment; blood cultures were performed daily until three consecutive cultures were negative. If cultures were positive for more than 10 days with clinical signs of fungal infection and/or persistent thrombocytopenia, a second antifungal drug was added.

Results: Fifty-six infants met the study criteria: four term and 52 preterm, including 36 extremely low birth weight infants. Amphotericin B was the initial treatment for 34, LamB for 6 and ABCD for 16 infants. No differences in mortality were found between the three groups. Sterilization of the blood was achieved with amphotericin B in 67.6% of patients, LamB in 83.3% and ABCD in 57.1%, when used as monotherapy; with the addition of a second antifungal agent, success rates were 100%, 83.3% and 92.8%, respectively. There were no differences between the groups in the time to resolution of fungaemia. No patients had immediate local or systemic adverse events and none showed deterioration in renal function.

Conclusion: ABCD and LamB appear to be effective, safe and well tolerated in premature infants with CBSI and renal dysfunction. Larger trials are needed before routine use can be recommended.

Keywords: liposomal amphotericin B, amphotericin B colloidal dispersion, Candida, premature infants

Introduction

The incidence of Candida bloodstream infections (CBSI) in the neonatal intensive care unit (NICU) has increased in recent years.1,2 In preterm infants after the third day of life, the most important risk factors for the development of late onset sepsicaemia are low birth weight (LBW) and low gestational age (GA).3 Treatment, which currently consists of long-term broad-spectrum antibiotics and parenteral nutrition, is a two-edged sword: while survival has improved, affected infants, especially those with very low birth weight (VLBW), are at greater risk of fungal infection mainly because of treatments used and their delivery by central venous lines.4-10 Renal function is often impaired during this period because of iatrogenic dehydration, administration of nephrotoxic agents and infections.

Amphotericin B is the most common antifungal agent for the treatment of CBSI.10 However, nephrotoxicity may limit its usefulness in infants with impaired renal function.11 Liposomal amphotericin B (LamB), amphotericin B colloidal dispersion (ABCD) and amphotericin B lipid complex (ABLC) may serve as more useful alternatives, with equal effectiveness to amphotericin B,12-14 but apparently less nephrotoxicity.15,16 The aim of the present study was to report our experience with three antifungal preparations in the treatment of neonates with CBSI and normal or abnormal renal function.
Patients and methods

Patients

The study sample included all infants admitted to the NICU of Schneider Children’s Medical Center of Israel between January 1, 1996 and December 31, 2000 with a culture-confirmed diagnosis of CBSI. The type of antifungal treatment administered was determined by the serum creatinine concentration at the start of therapy. In our experience, *Candida* sepsis appears at ≈2 weeks of age when the normal mean serum creatinine concentration for infants weighing <1500 g is 0.6 ± 0.005 mg/dL. An increase of serum creatinine to a concentration of <1.2 mg/dL, indicating a 50% decrease in the glomerular filtration rate, was considered acceptable for amphotericin B (Bristol-Myers Squibb, France) treatment. Infants with concentrations of ≥1.2 mg/dL were treated with LamB (AmBisome; Gilead Sciences Ltd, Ireland) if they presented before April 1, 1998 or with ABCD (Amphocil, Sequs, Gumed-Mediquip Ltd, Israel) if they presented later. Over the two study periods treatment policies and the processing techniques in the clinical microbiology laboratory were similar.

Empiric treatment was initiated if sepsis was suspected clinically prior to the receipt of the culture results. If cultures remained negative for *Candida* for the first 72 h, treatment was discontinued and the infant excluded from the analysis.

Identification and management of patients with CBSI

CBSI was suspected in the presence of any combination of apathy, apnoea, bradycardia, tachycardia, hypotension and thrombocytopenia. Prior to therapy, blood, cerebrospinal fluid (CSF) and suprapubic urine cultures were performed on all infants suspected of sepsis. On positive identification of a fungal infection, all indwelling catheters were removed and cultured, and ophthalmological examination, cerebral, hepatic and renal ultrasound, and echocardiography were performed. Blood cultures were repeated daily until three consecutive cultures were negative. One to two millilitres of blood were taken using sterile technique and cultured, and suprapubic aspiration were repeated only if the initial cultures were positive or if pathological findings were noted on the cerebral or renal ultrasound scan. The ophthalmological examination, cerebral and renal ultrasound, and echocardiography were repeated routinely on the eighth day after diagnosis and thereafter according to the clinical criteria. All extremely low birth weight (ELBW) infants are routinely screened for intraventricular haemorrhage (IVH) and retinopathy of prematurity. IVH is graded according to severity, with grade 4 indicating the worst prognosis.

Concentrations of creatinine, blood urea nitrogen and electrolytes, liver function tests and complete blood counts with a differential were determined before onset of treatment, 24 and 48 h after onset of treatment, and 24 h after the last dose. The tests were repeated during treatment as necessary. If renal function had not returned to normal by the end of treatment, renal function tests were performed monthly for six months. Routine brainstem-evoked potentials were tested in all infants on release from the NICU.

Treatment protocol

All drugs were administered via a peripheral vein in the following doses: amphotericin B, 1 mg/kg/day; LamB, 5 mg/kg/day; ABCD, 3 mg/kg/day on the first day of treatment and 5 mg/kg/day thereafter. In cases of positive urine culture or fungus ball, a second antifungal agent was added immediately. If the *Candida* infection persisted for more than 10 days and there were clinical signs of fungal infection and/or persistent thrombocytopenia, a second antifungal agent was introduced. In uncomplicated cases, treatment was continued for 14 days after the last positive blood culture. In complicated cases with a persistent focus of infection, treatment was continued until resolution of the lesion. This research was performed according to the guidelines of the local ethics board.

Statistical analysis

Statistical analysis was performed using the Biomedical Statistical Software Package (BMDP Statistical Software 1992, University of California, Chief Editor W. J. Dixon). For comparison between the groups, we used the Student’s t-test for continuous variables and Pearson’s χ² test for discrete variables. As the distribution of certain variables was not normal, we applied a square root transformation. A P value of <0.05 was considered significant.

Results

Between January 1, 1996 and December 31, 2000, 4201 patients were admitted to the NICU of Schneider Children’s Medical Center of Israel. Of these, 456 were VLBW infants (1000–1500 g) and 397 were ELBW infants (<1000 g). Antifungal medication was administered initially to 67 infants with a presumptive diagnosis of CBSI. It was discontinued after 72 h in 11 of them in whom the diagnosis was not confirmed; nine were found to have bacterial sepsicaemia.

The remaining 56 infants (mean ± s.d. weight 1075 ± 578 g, GA 28.0 ± 4.3 weeks) with culture-proven CBSI accounted for 1.3% of all the NICU patients. Four of the 56 (7.1%) were term infants and 52 (92.9%) were preterm, including four (7.1%) larger preterm infants (>1500–2000 g), 12 (21.4%) VLBW infants, and 36 (64.3%) ELBW infants.

Table 1. Clinical characteristics of premature infants with CBSI

<table>
<thead>
<tr>
<th></th>
<th>Amphotericin B (n = 34)</th>
<th>LamB (n = 6)</th>
<th>ABCD (n = 14)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>29.3 ± 4.2</td>
<td>26.2 ± 2.1</td>
<td>25.2 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1197 ± 623</td>
<td>901 ± 273</td>
<td>779 ± 170</td>
<td>&lt;0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Onset of infection (days)</td>
<td>24.4 ± 24.6</td>
<td>14.0 ± 3.9</td>
<td>15.8 ± 8.7</td>
<td>NS</td>
</tr>
<tr>
<td>Ventilation (days)</td>
<td>19.9 ± 23.8</td>
<td>18.0 ± 11.0</td>
<td>31.4 ± 24.7</td>
<td>&lt;0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prenatal steroids (%)</td>
<td>57.6</td>
<td>0</td>
<td>18</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup>Comparison of amphotericin B group with LamB and ABCD groups.

<sup>b</sup>Difference significant between amphotericin B group and ABCD group only.

NS, not significant.
In infants. Over the five year period the distribution of the Candida species was as follows: Candida albicans (n = 36, 64.3%); Candida parapsilosis (n = 12, 21.4%); mixed C. albicans and C. parapsilosis (n = 4, 7.1%); Candida glabrata (n = 3, 5.4%); and Candida tropicalis (n = 1, 1.8%). No clusters of cases were identified. In five infants, the same Candida species grew in the suprapubic urine culture and their blood cultures. No infant had a positive CSF culture.

Steroid treatment significantly more often than the other two groups. Over the five year period the distribution of the infants treated with amphotericin B were of significantly older GA than the infants given LamB or ABCD, and had significantly higher birth weight than the ABCD group. They also received than the LamB or ABCD, and had a significantly higher birth weight than the ABCD group. They also received more and had significantly fewer days of intermittent mandatory ventilation than the ABCD group. Ophthalmological examination and cerebral, hepatic and renal ultrasound did not show signs of localized spread of the fungal infection. Echocardiography was abnormal in five infants. The findings were a thrombus in the ductus venosus, an echogenic mass in the foramen ovale (diameter = 2.4 cm), and a thrombus in the right atrium in three infants who survived. Two infants died: one was found to have a right atrial fungal ball and the other had a right atrial thrombus (Table 2, infants 8 and 4, respectively).

Crude mortality was 14.8% (eight infants) for the whole sample, with no significant differences among the treatment groups. Five were treated with amphotericin B, two with ABCD and one with LamB. The clinical and laboratory characteristics of these eight infants appear in Table 2. Two infants had positive blood cultures for Candida in the 72 h period prior to their deaths. No autopsies were performed.

The persistence of CBSI was similar among the three groups (Table 3). Treatment with one or two agents yielded sterile blood cultures for Candida in 52 patients (96.3%). Sterilization was achieved with a single agent in 36 of 54 patients of the whole group (66.7%). Use of monotherapy or polytherapy achieved the following sterilization rates: 67.6% and 100% for the amphotericin B group; 83.3% and 83.3% for the LamB group, and 57.1% and 92.8% for the ABCD group (non-significant for all comparisons). The supplementary antifungal medications were fluconazole (9 patients), 5-fluorocytosine (5FC) (8 patients) and combined 5FC+fluconazole (1 patient) (non-significant for all comparisons).

The duration of CBSI was 6.9 ± 5.5 days for the whole cohort, with no significant difference among the treatment groups. Resolution occurred in 4.9 ± 3.2 days in the 36 infants treated with a single agent.
and 11.6 ± 6.9 days in those who required additional antifungal medi-
cation (Table 3).

No immediate local or systemic adverse effects, such as skin rash,
hypotension or tachycardia, were noted during treatment. Initial renal
function values were significantly closer to normal in the ampho-
etericin B group than in the LamB and ABCD groups. These levels did
not change significantly in the first 48 h of treatment. During treat-
ment renal function improved in all treatment groups (Table 4). In no
infant did renal function deteriorate during treatment. Function
returned to normal range by the end of treatment in 98% of the sam-
ple, with no significant differences between the groups. No infant
required dialysis. Potassium supplementation during treatment was
required by 16 infants (47%) in the amphotericin B group and in none
of the infants in the other groups.

Liver function tests, including albumin, aspartate aminotransferase,
gamma glutamate-pyruvate transaminase and alkaline phosphatase,
increased marginally and non-significantly in infants receiving
amphotericin B, and to a lesser extent after receiving LamB. This
slight elevation is commonly seen in septic infants on prolonged
hyperalimentation. Platelet levels increased during treatment in all
treatment groups. No differences in white blood cell and platelet
counts were noted before, during or after treatment among the three
groups. By the end of treatment, all infants had platelet concen-
trations of >100 000 platelets/mm³, and by age 6 months, all had
normal platelet counts of >150 000 platelets/mm³.

Brainstem-evoked potentials were abnormal in only one infant
from the amphotericin B group.

Discussion

The reported incidence of CBSI ranges from 4%–12%.1–5 In this
study, we detected CBSI in 2.6% of VLBW infants and 9.1% of ELBW
infants admitted to the NICU over a 5 year period.

Amphotericin B is the drug of choice for the treatment of CBSI in
premature infants.10 However, concern about potential kidney and
liver damage10,15,16 and the need to treat premature infants who have
impaired renal function has led to the use of alternative antifungal
agents. ABCD and LamB have been found useful for the treatment of
invasive fungal infections in adults and children when ampho-
etericin B is contraindicated because of impaired renal function or
concomitant use of other nephrotoxic agents, and in cases of treat-
ment failure.11–16

CBSI is associated with a high morbidity and mortality.20,21 Con-
trary to our expectations, in the present study outcome in the LamB

Table 3. Time to resolution of fungaemia and need for additional antifungal preparation in the three treatment groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Amphotericin B</th>
<th>LamB</th>
<th>ABCD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (days)</td>
<td>19.3 ± 13.4</td>
<td>19.0 ± 7.6</td>
<td>17.4 ± 8.2</td>
</tr>
<tr>
<td>Fungaemia (days)</td>
<td>8.4 ± 5.8</td>
<td>5.2 ± 2.1</td>
<td>6.5 ± 5.8</td>
</tr>
<tr>
<td>Positive blood cultures (number)</td>
<td>7.1 ± 4.2</td>
<td>5.1 ± 3.8</td>
<td>5.6 ± 4.6</td>
</tr>
<tr>
<td>Eradication: single antifungal medication (%)</td>
<td>67.6</td>
<td>83.3</td>
<td>57.1</td>
</tr>
<tr>
<td>Eradication: two or more antifungal medications (%)</td>
<td>100</td>
<td>83.3</td>
<td>92.8</td>
</tr>
<tr>
<td>Hospitalization (days)</td>
<td>71.7 ± 40.7</td>
<td>85.5 ± 39.0</td>
<td>92.0 ± 57.2</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>14.7</td>
<td>16.7</td>
<td>14.2</td>
</tr>
</tbody>
</table>

No data expressed as mean ± S.D.

Table 4. Hepatic and renal function at the start of treatment, 24 h after beginning treatment, and at the end of therapy

<table>
<thead>
<tr>
<th>Test</th>
<th>Time</th>
<th>Amphotericin B</th>
<th>LamB</th>
<th>ABCD</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dL)</td>
<td>Start</td>
<td>22.0 ± 19.6</td>
<td>42.8 ± 30.7</td>
<td>64.3 ± 26.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>21.7 ± 19.5</td>
<td>44.5 ± 28.1</td>
<td>62.2 ± 25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>14.9 ± 23.5</td>
<td>23.2 ± 15.0</td>
<td>42.0 ± 44.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>Start</td>
<td>0.8 ± 0.4</td>
<td>1.44 ± 0.4</td>
<td>1.52 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>0.8 ± 0.4</td>
<td>1.0 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>0.6 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>SGOT (U/L)</td>
<td>Start</td>
<td>29.0 ± 9.9</td>
<td>46.8 ± 30.8</td>
<td>31.0 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>33.5 ± 15.0</td>
<td>68.4 ± 78.4</td>
<td>38.0 ± 33</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>End</td>
<td>62.4 ± 82.7</td>
<td>63.0 ± 69.5</td>
<td>39.0 ± 42</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Difference significant between amphotericin B group and ABCD group only.

BUN, blood urea nitrogen; SGOT, aspartate aminotransferase; NS, not significant.
Candidaemia in NICU—three amphotericin B preparations

and ABCD groups, which included younger and smaller infants who had received less steroid preparation to enhance lung maturity, was similar to that of the amphotericin B group. Cure rates were 67.6%, 83.3% and 57.1% for amphotericin B, LamB and ABCD, respectively, when used as single agents (non-significant). A previous review of NICU patients reported cure rates of 27%–100% for amphotericin B.2,3 Two studies have been conducted using LamB to treat premature infants with sample sizes of 33 and 14; the respective cure rates were 63.6% and 86%.22,23 In 11 neonates treated with ABLC, the cure rate was 72.7%.14 All these studies found LamB and ABLC to be as effective as conventional amphotericin B for the eradication of CBSI.

We did not find signs of nephrotoxicity or hepatotoxicity with any of the antifungal preparations used. In fact, renal function normalized in 94.2% of the surviving infants given LamB or ABCD. Some researchers have found transient nephrotoxic and hepatotoxic effects in adults and children using lipid-based forms of amphotericin B, although significantly fewer than with conventional amphotericin B.13 Studies of premature infants given LamB showed no cases of nephrotoxicity,14,15 and only one case of transient hepatotoxicity.15 We also detected no signs of haematological toxicity. The increase in mean number of platelets during the course of treatment indicates that the Candida infection, not the antifungal medication, was the cause of the thrombocytopenia.

Indications that amphotericin B may have little to no toxicity in LBW infants may influence the appraisal of the theoretical benefits of the new antifungal medications such as LamB, ABCD and ABLC.24

In summary, ABCD and LamB appear to be effective and well tolerated in preterm infants with invasive Candida infections and renal dysfunction. However, larger trials of these antifungal preparations are needed in this patient population to confirm our findings.

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References