Efficacy of high doses of liposomal amphotericin B in the treatment of experimental aspergillosis

María Teresa Martín, Joan Gavaldà*, Pedro López, Xavier Gomis, José Luís Ramírez, Dolores Rodríguez, Oscar Len, Queralt Jordano, Isabel Ruiz, Marta Rosal, Benito Almirante and Albert Pahissa

Infectious Diseases Research Laboratory, Infectious Diseases Division, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

17 March 2003; returned 25 May 2003; revised 26 September 2003; accepted 3 October 2003

Objective: Differences in efficacy between deoxycholate amphotericin B (d-AmB) and escalating doses of liposomal amphotericin B (L-AmB) were evaluated in a model of invasive pulmonary aspergillosis in persistently steroid-immunosuppressed rats.

Methods: Animals were infected intratracheally with a conidial suspension of a clinical isolate of Aspergillus fumigatus and randomized to receive intravenously 5% dextrose, 1 mg/kg/day of d-AmB or 3, 5 or 10 mg/kg/day of L-AmB.

Results: All the antifungal treatments improved survival, although no differences were found among the groups, perhaps because of treatment-related toxicity. In animals surviving long enough to receive at least five doses of antifungal treatment, there were significant reductions in paired lung weight in the 5 and 10 mg/kg/day L-AmB groups as compared with the controls (P = 0.004 and 0.001, respectively) and with the 3 mg/kg/day L-AmB group (P = 0.007 and 0.002, respectively). Significant decreases in fungal biomass, measured indirectly by chitin quantification, were found only in the 10 mg/kg/day L-AmB group as compared with controls (P = 0.003), d-AmB (P = 0.007) and 3 mg/kg/day L-AmB (P = 0.001).

Conclusion: Infusion of L-AmB doses as high as 10 mg/kg/day may be a good therapeutic option for the management of invasive pulmonary aspergillosis developing in the context of steroid immunosuppression, although further studies are needed to assess this approach.

Keywords: antifungal treatment, efficacy, invasive pulmonary aspergillosis

Introduction

Current strategies for the treatment of invasive pulmonary aspergillosis (IPA) yield suboptimal overall response rates of around 35%–50%, underlining an urgent need for new therapeutic approaches to this infection. In recent years, lipid formulations of amphotericin B, such as liposomal amphotericin B (L-AmB) have become available, with rates of adverse effects lower than the conventional formulation, thus allowing the use of large doses.1

Animal pharmacokinetic models have shown that the larger the dose of L-AmB, the higher the tissue concentration achieved, even in the lungs,2 where aspergillus infection used primarily to be located.

Infusion of large L-AmB doses, which have been proven safe for humans,1 could therefore provide drug concentrations of sufficient strength to be fungicidal in the lungs; this is considered a promising strategy for the treatment of IPA.

A dose-escalating study was carried out, in an experimental model of IPA induced in persistently steroid-immunosuppressed rats, comparing the efficacy of 3, 5 and 10 mg/kg/day L-AmB with that of 1 mg/kg/day deoxycholate amphotericin B (d-AmB). Among other parameters, the chitin content of the lungs was used to assess the therapeutic response, since it permits quantitative estimation of the fungal load in tissues of infected animals.3

*Correspondence address. Servei de Malalties Infeccioses, Hospital General Vall d’Hebron, Passeig Vall d’Hebron, 119–129, 08035 Barcelona, Spain. Tel: +34-93-4894033; Fax: +34-93-2746057; E-mail: jgavalda@vhebron.net
Efficacy of high doses of liposomal amphotericin B in the treatment of experimental aspergillosis

Table 1. Results of the therapeutic efficacy in the treatment groups

<table>
<thead>
<tr>
<th>Rats surviving ≥ 5 days of treatment</th>
<th>Total no. of rats</th>
<th>Days of survivala</th>
<th>no. of animals (% of total)</th>
<th>mean log_{10} of cfu/g of lungb</th>
<th>weight of paired lungs in gc</th>
<th>log_{10} μg of glucosamine/paired lungsd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>4.44 ± 2.79</td>
<td>11 (32.4)</td>
<td>2.85 (1.08–4.61)</td>
<td>2.59 (2.22–2.96)</td>
<td>2.18 (1.95–2.42)</td>
</tr>
<tr>
<td>d-AmB 1 mg/kg/day</td>
<td>26</td>
<td>7.35 ± 2.51*</td>
<td>20 (76.9)</td>
<td>2.75 (2.37–3.14)</td>
<td>2.04 (1.67–2.41)</td>
<td>2.06 (1.85–2.26)</td>
</tr>
<tr>
<td>L-AmB 3 mg/kg/day</td>
<td>26</td>
<td>7.69 ± 2.80*</td>
<td>19 (73.1)</td>
<td>3.82 (3.33–4.31)</td>
<td>2.40 (1.95–2.86)</td>
<td>2.14 (1.92–2.36)</td>
</tr>
<tr>
<td>L-AmB 5 mg/kg/day</td>
<td>24</td>
<td>8.33 ± 2.55*</td>
<td>20 (83.3)</td>
<td>3.07 (2.38–3.75)</td>
<td>1.64 (1.38–1.90)*</td>
<td>1.88 (1.74–2.01)</td>
</tr>
<tr>
<td>L-AmB 10 mg/kg/day</td>
<td>27</td>
<td>7.33 ± 2.69*</td>
<td>19 (70.4)</td>
<td>2.51 (2.05–2.97)‡</td>
<td>1.52 (1.28–1.77)*‡</td>
<td>1.59 (1.35–1.83)*‡‡</td>
</tr>
</tbody>
</table>

aData shown as mean ± S.D.
bData expressed as mean ± S.D.
cData expressed as mean ± 95% confidence interval.
dData expressed as mean ± 95% confidence interval.

Methods

A previously described animal model of IPA was used. SPF female Wistar rats weighing 180–200 g were obtained from Harland Iberica (Barcelona, Spain). Rats were fed an 8% protein diet and sterile water ad libitum. The experimental protocol was approved by the Ethics Committee of Vall d’Hebron Hospitals and the Generalitat de Catalunya.

Animals were immunosuppressed with 125 mg/kg of subcutaneous cortisone acetate (Sigma Chemical Co, St. Louis, MO, USA) three times per week—starting 14 days before the fungal challenge—until the end of the experiment. Four days before the infection, a sterile silastic catheter was inserted surgically through the right jugular vein into the inferior vena cava to administer antifungal treatment. The line was tunneled subcutaneously, brought to the interscapular region and connected to a subcutaneous port. On day 14, rats were infected intratracheally with 0.3 mL of a conidial suspension, adjusted in sterile saline to 8 × 106 cells/mL. A clinical isolate of Aspergillus fumigatus (MIC of amphotericin B = 1 mg/L, in accordance with NCCLS procedures) was used.

Antifungal therapy was administered intravenously starting 24 h after infection and lasted 10 days. Animals were assigned randomly to receive 5% dextrose (control, n = 34), 1 mg/kg/day of d-AmB (Fungizone, Bristol-Myers Squibb Group, Spain) (n = 26), or 3 (n = 26), 5 (n = 24) or 10 (n = 27) mg/kg/day of L-AmB (AmBisome, Gilead Sciences, Spain). They were checked periodically every day until day 12 after fungal infection. Mortality was recorded throughout this period. Surviving rats were sacrificed 24 h after the last dose of antifungal treatment. Dead and euthanized animals were dissected. Lungs were removed aseptically, weighed and homogenized in 10 mL of sterile distilled water for chitin assay, which was used to quantify the fungal burden in the lungs, as described elsewhere. A small aliquot of lung homogenate was plated onto Sabouraud dextrose agar and trypticase soy blood agar plates to assess fungal growth qualitatively and to rule out bacterial superinfection.

For toxicity studies, groups of steroid- and non-steroid-treated uninfected rats were given a 10 day course of antifungal treatment, using a scheme similar to that of the therapeutic efficacy study; mortality and biochemical parameters (liver and kidney function) were determined. Survival was analysed using Kaplan–Meier plots. Lung weight (in grams) and pulmonary chitin content (in log_{10} μg of glucosamine per paired lungs) were expressed as the mean and 95% confidence interval of the mean, and were compared using one-way ANOVA analysis and the Tukey’s pairwise test as statistics, at an overall error rate of 5%. P values less than or equal to 0.015 were considered significant.

Results

The results of the therapeutic efficacy for the different regimens are summarized in Table 1.

As shown in Figure 1, mean survival time was increased significantly with all amphotericin B treatments with respect to the control group (P ≤ 0.0015). No significant differences in survival were found among the four treatment regimens studied; however, survival data were not analysed further since treatment-related mortality was found in uninfected immunosuppressed rats, as explained later.

Inter-group differences in lung weight and chitin content were found in animals that survived long enough to receive at least five doses of treatment. Paired lung weight, indicative of organism-mediated pulmonary injury, was significantly lower in animals given 5 or 10 mg/kg/day of L-AmB as compared with the control (P = 0.004 and 0.001, respectively) or 3 mg/kg/day L-AmB groups (P = 0.007 and 0.002, respectively). A dose-dependent effect was also found for the chitin content of lungs, which was significantly lower in rats receiving 10 mg/kg/day of L-AmB than in controls (P = 0.003), and the d-AmB (P = 0.007) and 3 mg/kg/day L-AmB (P = 0.001) groups.

No signs of toxicity were observed in uninfected non-immunosuppressed animals, whereas deaths were recorded in non-infected steroid-treated rats given d-AmB (three out of eight), 5 mg/kg/day of L-AmB (two out of seven) or 10 mg/kg/day of L-AmB (four out of eight) for 4 days or more. Analytical parameters determined in blood samples at the end of the experiment were insufficiently definitive to establish the origin of the drug-related toxicity (data not shown).

Discussion

In this study, the efficacy of d-AmB and escalating doses of L-AmB were compared using an experimental model of pulmonary aspergillosis. The aim was to determine whether increases in L-AmB dosage up to 10 mg/kg/day could lead to an improvement in antifungal efficacy.

There were no differences in survival among the groups receiving antifungal treatment. However, this was probably related to the fact that chronic administration of steroids and amphotericin B (particularly at high doses) in uninfected rats caused the death of animals. The
different experimental models and endpoints. Leenders et al. found that both d-AmB and 10 mg/kg of L-AmB delayed mortality and significantly increased survival when compared with a control group. Nevertheless, only 10 mg/kg of L-AmB reduced dissemination to the right lung and completely prevented dissemination to extra-pulmonary organs in neutropenic rats. Dosages equal to or larger than 5 mg/kg/day have also resulted in both prolonged survival and decreased pulmonary injury in neutropenic rabbits. The dose-dependent efficacy of L-AmB may be of major importance in the management of pulmonary aspergillosis in immunosuppressed individuals. In our experimental model of IPA, the chronic infusion of L-AmB at doses as high as 10 mg/kg/day was more effective than lower doses of L-AmB or 1 mg/kg/day of d-AmB in reducing pulmonary chitin content as a measure of fungal burden. Increases in L-AmB doses up to 10 mg/kg/day could be useful for the treatment of pulmonary aspergillosis. Further clinical studies are needed to assess this approach.

Acknowledgements

We thank Celine Cavallo for assistance with the English.

This work has been supported by a grant from the Fondo de Investigaciones Sanitarias (FIS exp no. 99/0894)

References


Figure 1. Effect of d-AmB versus different doses of L-AmB or no treatment on the cumulative survival of rats with IPA. Solid square, control group; solid triangle, 1 mg/kg/day of d-AmB; open square, 3 mg/kg/day of L-AmB; open triangle, 5 mg/kg/day of L-AmB; open circle, 10 mg/kg/day of L-AmB.