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

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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

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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 survival</th>
<th>no. of animals (% of total)</th>
<th>mean log₁₀ of cfu/g of lung</th>
<th>weight of paired lungs in g</th>
<th>log₁₀μg of glucosamine/paired lungs</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 are shown as mean ± S.D.
*bData expressed as mean 95% confidence interval.
†P < 0.015 compared with control, †P < 0.015 compared with d-AmB 1 mg/kg/day, ††P < 0.015 compared with 3 mg/kg/day of L-AmB.

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 tunnelled 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 x 10⁶ conidia/mL, in accordance with NCCLS procedures.

An 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.

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 log₁₀ micrograms 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
nature of this possible cumulative cross-toxicity could not be determined, although it has been shown that the combination of d-AmB and steroids has deleterious effects in mice. To our knowledge, no interaction between steroids and L-AmB has been described in humans.

Since survival rates could not be used as a reliable parameter, other therapeutic endpoints were evaluated. When mean log$_{10}$ cfu was compared, a significant difference was found only between the 3 and 10 mg/kg/day L-AmB groups ($P = 0.012$). This absence of differences may be related to the fact that single conidia, clusters of spores, or hyphal fragments—having a great variety of lengths—can all be quantified as unique colonies in culture plates. Since a cfu has not yet been defined for filamentous moulds, the number of cfu probably does not accurately reflect the viable fungal biomass. Therefore, quantitative or semi-quantitative cultures would not be a reliable index to estimate reductions in tissue fungal loads, as previously indicated by other authors. To avoid this problem, it has been suggested that a more reliable method of measuring fungal burden in tissues would be by quantification of fungal cell elements. Thus, quantification of chitin, a $\beta$-1,4 glycan found in the fungal cell wall that increases with hyphal growth, was used to compare the decrease in the aspergillar load in the lungs of the animals of the different treatment groups. By means of chitin quantification, significant differences in efficacy were found between the largest dose of L-AmB and the controls, d-AmB and 3 mg/kg/day of L-AmB. Additionally, a non-significant trend towards a reduced pulmonary fungal load was found with 5 mg/kg/day of L-AmB. Similar results were found using the lung weight as an endpoint for therapeutic efficacy. Our results are in the line with those reported by other groups in neutropenic animals, although it is difficult to establish comparisons between 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 extrapulmonary 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.

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**References**