Efficacy of a new formulation of amphotericin B in a murine model of disseminated infection by Candida glabrata

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Objectives: Amphotericin B poly-aggregates are a new formulation of amphotericin B, which can be obtained cheaply. In this study, we tested the efficacy of this new formulation for treating a disseminated infection by Candida glabrata in a murine model.

Methods: Mice were rendered neutropenic by intraperitoneal cyclophosphamide and intravenous 5-fluorouracil administration and infected intravenously with $2 \times 10^8$ cfu of C. glabrata. The efficacy of the new formulation of amphotericin B was evaluated by survival and tissue burden studies. The experiments were repeated using three different clinical strains of C. glabrata.

Results and conclusions: Amphotericin B poly-aggregates showed an efficacy similar to that of amphotericin B deoxycholate and liposomal amphotericin B in the treatment of a disseminated murine infection by C. glabrata.

Keywords: candidiasis, animal models, poly-aggregates

Introduction

Amphotericin B is a polycene with a broad range of activity, available since the 1950s and still extensively used for treating a wide spectrum of fungal infections. The classical and most frequently used formulation of this drug is amphotericin B deoxycholate. However, its important adverse effects, mainly nephrotoxicity, make it inappropriate in many cases.1,2 Recently, newer lipid formulations of amphotericin B with less adverse effects and activities similar to that of amphotericin B deoxycholate have become available, but their high costs limit enormously their clinical use, especially in developing countries.

Due to the low aqueous amphotericin B solubility, amphotericin B molecules are usually in an aggregated disposition. In the conventional deoxycholate preparation (Fungizone), amphotericin B is mainly in a dimer form. Different types of aggregation of dimers or poly-aggregates can be obtained by heating Fungizone3 or depending on certain interactions with excipients.4,5 Another way to control the aggregation state of amphotericin B is related to the pH conditions during the formulation preparation procedure.6 This latest way to obtain amphotericin B in a poly-aggregated state is cheap and this new amphotericin B formulation has previously demonstrated efficacy in the treatment of experimental leishmaniasis.6 Moreover, this new formulation has been proven experimentally to be less toxic than the conventional amphotericin B deoxycholate, which allows its administration at high doses in a way similar to the most expensive lipid formulations.6

We have tested the efficacy of this new formulation in a murine model of Candida glabrata infection using three different clinical strains, comparing it with the traditional deoxycholate and liposomal formulations.

Materials and methods

Three clinical isolates, FMR 8487, FMR 8489 and FMR 8497, the first two isolated from urine and the third one from an exudate, at our university hospital, were used in the study. Following the CLSI broth microdilution reference method, all three strains showed an identical in vitro susceptibility to amphotericin B, i.e. MICs of 1 mg/L. They were subcultured on Sabouraud dextrose agar (SDA) plates and incubated at 35°C for 24 h.

Male OF1 mice were immunosuppressed by a single intraperitoneal injection of 200 mg/kg cyclophosphamide plus a single intravenous injection of 150 mg/kg 5-fluorouracil on the day of infection.7

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Amphotericin B poly-aggregates against C. glabrata

The procedure followed the standards approved by the Animal Welfare Committee of the Rovira i Virgili University.

Amphotericin B was purchased as Fungizone (Squibb Industria Farmacéutica S.A., Barcelona, Spain). Liposomal amphotericin B as AmBisome and amphotericin B raw material were provided by Gilead Sciences S.A. (Madrid, Spain) and by Bristol-Myers Squibb, respectively. Amphotericin B poly-aggregates were prepared as follows: amphotericin B as raw material (50 mg) was dispersed in 5 mL of a water solution formed by sodium deoxycholate (41 mg), dibasic sodium phosphate (10 mg) and monobasic sodium phosphate (0.9 mg). The resulting homogeneous suspension was also diluted in water to a final volume of 10 mL and the amphotericin B molecular organization was analysed by spectrophotometry showing the same absorption spectra reported previously.6

The efficacy of the different drugs was evaluated through prolongation of mice survival and reduction in fungal tissue burden of mice challenged by the three strains of C. glabrata. For the survival and tissue burden studies, groups of 10 mice were randomly established for each strain, one for each treatment and one as control. Mice were challenged with 2 × 10⁸ cfu in 0.2 mL into the lateral tail vein. The different groups were treated as follows: liposomal amphotericin B (10 mg/kg of body weight/day),8 free poly-aggregates of amphotericin B (5 or 10 mg/kg/day), both given intravenously, and amphotericin B deoxycholate (1.5 mg/kg/day), given intraperitoneally.7 All treatments began 24 h after challenge and the therapy lasted for 5 days.

For survival studies, mice were checked daily for 15 days. For tissue burden studies, 1 day after the treatment finished, five of the surviving mice, randomly chosen, were sacrificed. Spleen and kidneys were aseptically removed, and the entire organs were homogenized in 1 mL of sterile saline. Serial 10-fold dilutions of the homogenates were plated on SDA, incubated at 35°C and examined daily for 3 days. The numbers of cfu/g of tissue were calculated.

Mean survival time was estimated by the Kaplan–Meier method and compared among groups using the log-rank test. Colony counts in tissue burden studies were analysed using the Mann–Whitney U-test. Calculations were made using SPSS 13.0 and Graph pad 4.0 for Windows.

Results

Figure 1 shows the results of survival studies obtained with the strains tested. Whereas all treatments prolonged the survival of mice infected with strain FMR 8489, none of them significantly prolonged the survival of mice infected with the strains 8497 and 8487 with the exception of the poly-aggregates at 5 mg/kg for the latter strain.

Tissue burden results are shown in Figure 2. Liposomal amphotericin B and amphotericin B deoxycholate significantly reduced the fungal load in kidneys and spleen for all the strains (P < 0.05) with the exception of the latter formulation in spleen for the strain 8487. The high dose of the amphotericin B poly-aggregate formulation significantly reduced the fungal load for all the strains and all the organs (P < 0.05) with the exception of spleen for the strain 8487. The low dose of the formulation was clearly less effective than the other formulations and doses at reducing the fungal burden in kidneys.

We observed no statistical differences between the deoxycholate formulation and liposomal amphotericin B in the survival and tissue burden studies. There were significant differences only in the reduction of fungal load between mice treated with the deoxycholate formulation and the amphotericin B poly-aggregated formulation at 10 mg/kg for the strain 8489 in spleen. Liposomal amphotericin B was more effective in reducing the tissue burden with respect to the amphotericin B poly-aggregated formulation for the 8487 and 8497 strains but not for the 8489 strain in kidneys.

Discussion

C. glabrata is one of the most common Candida non-albicans species causing severe human infections. Despite the newer alternative drugs, amphotericin B remains as one of the main options for the treatment of such infections.5 In a previous study using a murine model of disseminated infection by C. glabrata, we demonstrated that amphotericin B showed a higher efficacy than representatives of other antifungal classes such as echinocandins and azoles.7
Currently, many formulations of amphotericin are available with noticeable lesser adverse effects, but the use of these new drugs, as stated above, is greatly limited by their high cost.\textsuperscript{10,11} In our study, we observed a similar efficacy between the amphotericin B poly-aggregated formulation and the marketed amphotericin B formulations we tested.

The comparative effectiveness of the different amphotericin B formulations on the market is controversial because of the high degree of heterogeneity inherent in the experimental studies. This high variability, which can be due to the pathogenic agent or to the patient, makes it difficult to demonstrate significant differences in efficacy in clinical practice among the

Figure 2. Effects of the antifungal treatments on tissue burden of \textit{C. glabrata} FMR 8489 (a), FMR 8497 (b) and FMR 8487 (c) in kidneys and spleen of mice. AMB 1.5, amphotericin B deoxycholate at 1.5 mg/kg/day; LAMB 10, liposomal amphotericin B at 10 mg/kg/day; PAG 5 and PAG 10, amphotericin B poly-aggregates at 5 and 10 mg/kg/day, respectively. \textsuperscript{a}P value of <0.05 versus control. \textsuperscript{b}P value of <0.01 versus control and <0.05 versus the poly-aggregates. Horizontal lines of scatter plots indicate the mean values.
different formulations.\(^\text{12,13}\) Both sources of variability can be partially reduced in the animal models. In this work, different results were observed depending on the strain tested. Based on the data of the survival studies, the new formulation is not statistically different from the tested commercially available formulations. Furthermore, it is important to point out that the lower toxicity of the new formulation in relation to the conventional deoxycholate formulation allows the administration of doses of amphotericin B as high as 10 mg/kg.

Based on the tissue burden reduction, for the amphotericin B poly-aggregated formulation, better results were found, in general, in spleen than in the kidneys, since in this organ even the lower dose of such a formulation was able to reduce the fungal load for two of the three strains. This effect may be due to the fact that the disposition of drug molecules as large aggregates could enhance amphotericin B capture by the mononuclear phagocyte system cells located in liver, spleen and bone marrow.\(^\text{14}\) The targeting of the new amphotericin B poly-aggregated formulation to these organs may constitute the basis of new applications for this compound. For instance, in the treatment of visceral leishmaniasis, this formulation has proven to be clearly superior to the conventional deoxycholate formulations.\(^\text{6}\)

The new amphotericin B poly-aggregated formulation is an interesting alternative to the commercially available amphotericin B formulations we tested and warrants future studies with other fungal species and different infection types.

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Amphotericin B poly-aggregates against \textit{C. glabrata}

Transparency declarations

None to declare.

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