Antifungal activity of amphotericin B–lipid admixtures in experimental systemic candidosis in naive mice

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We have shown previously that admixtures of amphotericin B (AMB) and Intralipid (AMB–IL) obtained by vigorous and prolonged agitation are stable and can be standardized. These preparations exhibited in-vitro activity against various Candida spp., and had significantly lower toxicity. The present study was undertaken to evaluate the activity of AMB–IL admixtures in vivo in comparison with the conventional formulation of AMB (Fungizone), using a murine model of experimental systemic candidosis. ICR female mice (4–6 weeks old) were injected iv with $5 \times 10^4$ Candida albicans CBS 562. The animals developed a lethal infection (100%) within 10 days. Systemic candidosis was demonstrated by the presence of fungal elements in kidneys and spleen tissue, and by enumeration of cfu of Candida in the tissue homogenates. AMB–IL or AMB was administered iv 48 h post-Candida inoculation for 5 consecutive days. Four experiments with 108 mice treated with AMB $5 \times 0.4$ mg/kg and followed up for 6 weeks, showed that the mean survival percentages at the end of the experiment were 0, 24.9 and 52.5% for the untreated group, conventional AMB-treated and AMB–IL-treated groups, respectively. The mean survival time (MST) was 7.4, 25 and 30 days for the untreated, conventional AMB-treated and AMB–IL-treated groups, respectively. Use of increased doses of AMB showed that conventional AMB at doses greater than $5 \times 1$ mg/kg caused immediate animal death. AMB–IL was used at doses of AMB up to $5 \times 2$ mg/kg. Experiments with 104 mice revealed that the mean survival percentage at the end of the experiment was 0, 34.5, 58.6 and 97% for the untreated, conventional AMB-treated ($5 \times 1$ mg/kg), AMB–IL-1-treated ($5 \times 1$ mg/kg) and AMB–IL-2-treated ($5 \times 2$ mg/kg) groups, respectively. The MST was 7, 27.8, 34.8 and 41.4 days for the untreated, conventional AMB-treated, AMB–IL-1-treated and AMB–IL-2-treated groups, respectively. The results of this study reveal that AMB–IL is significantly more effective in treating systemic murine candidosis than conventional AMB.

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Organisms/Mouse

C. albicans with either conventional AMB (as a control) or A M B–IL at different doses (range 0.4-2 mg/kg). Treatment began 48 h after inoculation of Candida and consisted of five consecutive daily injections of A M B (conventional A M B or A M B–IL). A control group with infected and sham-treated mice (injected with buffer–PBS) was included. Survival was followed up for 42 days. A assessment of the activity of A M B–IL was based on the parameters described above for characterization of the systemic candidosis model and was evaluated by mortality and/or morbidity of untreated animals in comparison with animals treated with conventional A M B [expressed as survival rate and mean survival time (MST)].

Evaluation of in-vivo toxicity of A M B–IL

A cute toxicity was determined for A M B–IL in comparison with conventional A M B, in Candida-infected and non-infected animals. Mice were injected into the tail vein for 5 consecutive days with various doses of A M B (0.4-2 mg/kg) either as conventional A M B or A M B–IL, and surveyed for mortality.

Results

Experimental murine candidosis

Inoculation of 4-6 week old female ICR mice (20 mice) with $5 \times 10^4$ C. albicans organisms/mouse resulted in 100% mortality within 5-10 days. Organs examined macroscopically for pathological signs before further processing, showed microabscesses. Microscopic observation of organ homogenates stained with calcofluor demonstrated the presence of hyphae and budding yeasts. Sections of the kidneys stained by PAS revealed large fungal lesions, which contained yeast cells and hyphal elements.

To characterize the extent of fungal colonization of the kidney, a target organ in systemic candidosis, we enumerated cfu in kidneys of 20 mice on the day of death. We found that their kidneys were infected with greater than $10^6$ C. albicans organisms (range $1.6 \times 10^5$-3 $\times 10^7$; mean 8.6 $\pm 0.86 \times 10^6$).

Treatment of systemic murine candidosis with low doses of A M B

The experiments involved naive ICR mice inoculated iv with $5 \times 10^4$ C. albicans organisms and treated 48 h later with either A M B–IL, or conventional A M B at a concentration of 0.4 mg/kg, or buffer. Treatment was administered for 5 consecutive days (total A M B dose 2 mg/kg). A nalms were surveyed for up to 42 days. Data obtained from four experiments with 108 mice are summarized in Figures 1 and 2. Both formulations (conventional A M B and A M B–IL) significantly increased the survival of the mice as compared with the sham-treated controls, with over 50% of mice surviving when treated with A M B–IL. Specifically, the mean percentages of surviving mice at day 42 were 0, 24.85 $\pm 1.84$ and 52.48 $\pm 3.38$% for the untreated, conventional A M B-treated and A M B–IL-treated groups, respectively. The follow-up of the course of infection indicated that conventional A M B and A M B–IL increased the survival time of the treated mice. T hus, the MSTs in those mice that succumbed to infection were 7.38 $\pm 0.57$, 25 $\pm 1.77$ and 30 $\pm 2.09$ days for the untreated control, conventional A M B-treated and A M B–IL-treated groups, respectively (Figure 2).
To attempt to improve the efficacy of the AMB–IL preparations we planned experiments with higher doses of AMB. Towards this aim we first had to establish the maximum doses of AMB to which the mice would be tolerant. We injected 84 mice with either conventional AMB or AMB–IL in doses of 0.4–2 mg/kg for 5 consecutive days. We found that the maximum tolerated dose was 1 mg/kg × 5 for conventional AMB, with higher doses causing immediate death. AMB–IL at a concentration of 2 mg/kg (total 10 mg/kg) did not cause death during an observation period of 6 weeks.

Based on these results we performed four additional treatment experiments with 104 mice, using higher doses of AMB in the AMB–IL preparations. We thus had four groups in each experiment: sham (buffer-treated) controls, conventional AMB-treated group (1 mg/kg × 5) and two groups (1 and 2) treated with AMB–IL (1 mg/kg × 5 and 2 mg/kg × 5). These experiments revealed that the mean survival percentages at day 42 were 0, 34.5, 58.6 and 97% for the untreated, conventional AMB-treated, AMB–IL-1-treated and AMB–IL-2-treated groups, respectively (Figure 3). The MSTs were 7, 27.8, 34.8 and 41.4 days for the untreated, conventional AMB-treated, AMB–IL-1-treated and AMB–IL-2-treated groups, respectively (Figure 4). These experiments indicated that AMB–IL is more effective in treating systemic murine candidosis than conventional AMB, especially at the higher concentration of 10 mg/kg.

Discussion
The aim of the present study was to evaluate in vivo the efficacy of amphotericin B–Intralipid admixtures in comparison with conventional AMB. Towards this aim we used...
The evaluation was based on accepted criteria, including mortality and morbidity, the latter being assessed primarily by quantitative determination of fungal infection of the kidneys. We chose an experimental model in which the development of infection was not too rapid—mortality began at day 5, which enabled a follow-up of the course of infection. The fungal inoculum adopted—5 × 10^4 organisms/mouse—produced 100% mortality within 10 days. This in turn permitted reliable evaluation of the treatment.

The initial A M B treatment, which consisted of five consecutive injections of 0.4 mg/kg, was based on methods described in the literature. The experiments revealed that both formulations of A M B (conventional A M B and A M B–IL) significantly increased the survival of animals and affected the course of infection in comparison with untreated controls. The data also showed that A M B–IL is more effective than conventional A M B in treatment of murine candidiasis, demonstrating a significant difference between the survival rate of conventional A M B-treated versus A M B–IL-treated animals. However, the A M B–IL treatment did not save almost half of the animals from succumbing to the experimental candidiasis.

In order to attempt to increase the efficacy of the treatment, an increase in the dose of A M B was needed. An increase in the dose of the A M B beyond the level of 5 × 1 mg/kg was possible only for A M B–IL admixtures, since conventional A M B at such doses was lethal. The higher dose of A M B (5 × 2 mg/kg) in the A M B–IL admixtures was very effective, leading to almost 100% survival. To follow up these promising results we plan to evaluate this treatment regimen in immunocompromised animals.

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