CONCISE COMMUNICATION

Prophylactic Administration of Liposomal Amphotericin B Is Superior to Treatment in a Murine Model of Invasive Aspergillosis after Hematopoietic Cell Transplantation

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With use of a novel model of invasive Aspergillus fumigatus, the efficacy of prophylactic versus therapeutic administration of liposomal amphotericin B (L-AmB) was tested in C57BL/6 mice. After lethal irradiation and transplantation of whole bone marrow (d 0), animals were challenged with conidia either intravenously or via nasal instillation on d +3 and divided into 3 groups: group I received 5% dextrose in water throughout the study period; group II received L-AmB, 5 mg/kg, beginning on d +4; and group III received L-AmB, 5 mg/kg on d −4, d −2, d 0, and d +2, then daily starting d +4. Groups I and II did not survive intravenous challenge, whereas group III had a 40% survival rate. After nasal instillation of conidia, the survival was 25%, 35%, and 85% for mice in groups I, II, and III, respectively. These results demonstrate that prophylactic administration of L-AmB increased early survival against lethal challenge with A. fumigatus, compared with therapy instituted after infection.

Since the advent of prophylactic, preemptive, and treatment strategies for the treatment of cytomegalovirus, invasive aspergillosis has emerged as the most lethal infectious complication after allogeneic hematopoietic cell transplantation (HCT). Modifications in the chemotherapeutic preparative regimens and the transplanted grafts have resulted in a significant shortening of the period of neutropenia. However, the incidence of invasive aspergillosis remains between 8% and 15%. Furthermore, mortality after diagnosis of either proved or probable invasive aspergillosis in the setting of allogeneic HCT is 92%–100%, despite aggressive treatment with a growing roster of appropriate antifungal agents [1]. Therefore, the lethality of this infection underscores the necessity of preventing this infection.

Empirc institution of broad-spectrum antifungal therapy for fever unresponsive to 3–4 days of antibacterial administration is a widely accepted practice, but the nephrotoxicity of amphotericin B deoxycholate (D-AmB) precludes full-dose therapy in patients who have undergone HCT. The reduced potential for nephrotoxicity of the lipid formulations of amphotericin B (L-AmB) permits administration of higher doses even after HCT, and L-AmB has been shown to have the least potential for nephrotoxicity [2]. These lipid formulations reach significant concentrations in the lung, liver, spleen, brain, and kidneys, especially after administration of higher doses. However, in a recent multicenter trial [3], empiric administration of either D-AmB or L-AmB still resulted in a significant number of proved or probable breakthrough fungal infections during the brief 7-day follow-up period, of which the most common breakthrough pathogen was Aspergillus fumigatus.

As a result of such breakthrough infections, a limited number of prophylactic strategies have been tested clinically. Fluconazole has been shown to reduce the incidence and mortality caused by systemic Candida albicans infections after HCT, but, as would be predicted, the incidence of mold infections was unchanged. Although prophylactic administration of oral itraconazole solution has been reported to reduce death caused by proven fungal infections, these studies included only a limited number of HCT recipients, and breakthrough aspergillus infections still occurred [4].

The protracted postdosing and broad-spectrum antifungal activity of L-AmB suggests a favorable profile for its use in a prophylactic strategy [5]. In the 2 published studies of prophylactic administration of 1–2 mg/kg of L-AmB after HCT, fungal colonization was reduced, but decreases in reported mortality could not be attributed to a decrease in fungal-related deaths.
[6, 7]. These equivocal results may be explained by pharmaco-
kinetische studien in animals that demonstrate the need for doses
>1–2 mg/kg to achieve significant tissue levels in all organs. On
the basis of these findings, a single high dose of L-AmB given
up to 1 week prior to challenge was shown to reduce the growth
of C. albicans and Histoplasma capsulatum in mice during che-
motherapy-induced neutropenia [8].

Demonstrating the efficacy of antifungal agents with activity
against aspergillus has been more problematic. Published mu-
rine models of invasive aspergillosis have relied on chemother-
apy-induced neutropenia or corticosteroids to render the mice
susceptible to infection. No prophylactic studies have been pub-
lished that have used animal models that replicate the immu-
nodeficiency that results from HCT. Therefore, a murine model
was developed to study the efficacy of prophylactic versus early
treatment in the face of lethal challenge. The results of the
present study demonstrate that prophylactic administration of
L-AmB was more effective in preventing death than adminis-
tration of L-AmB started within 24 h after fungal challenge.

Materials and Methods

**Mouse strains.** Congenic strains of C57BL/6 mice were used
as donors and recipients. Donor mice were used at age 6–8 weeks
and recipients at age 12–16 weeks.

**Irradiation.** Host mice received a total dose of 950 cGy ad-
ministered at 3-h intervals (200-kV machine; Philips RT250) and
were provided with water that contained 1.1 g/L neomycin sulfate
and 10° U/L polymyxin B sulfate for the duration of the study.

**Isolation of whole bone marrow.** Whole bone marrow cells
were harvested by use of Hanks’ balanced salt solution with 2% fetal
calf serum, penicillin, and streptomycin (Gibco BRL). Erythrocytes
were depleted via ammonium-chloride lysis, washed twice with me-
dia to remove residual lysing solution, and kept on ice until
transplantation.

**Transplantation of whole bone marrow cells.** Mice were anes-
ethetized in a chamber saturated with a mixture of Isoflurane (Ab-
rott Laboratories) and oxygen. One million whole bone marrow
cells were transplanted into the retro-orbital vein.

**Administration of L-AmB.** L-AmB (AmBisome; Fujisawa
Healthcare) was reconstituted according to the manufacturer’s
specifications. Appropriate dilutions were made with use of sterile
5% dextrose in water (D,W) for injection via the tail vein each day.
Mice were matched for age, sex, and weight for each experiment
and divided into groups that received the following: group I, in-
travenous (iv) administration of D,W from d –4 prior to lethal
irradiation and transplantation; group II, iv D,W from d –4
through d +3, followed by 5 mg/kg daily of iv L-AmB starting on
d +4; group III, 5 mg/kg of iv L-AmB every other day from d –4
through d +2, then daily starting on d +4; and group IV, 1 mg/
kg daily of iv L-AmB, then 5 mg/kg daily starting on d +4. Group
III received D,W on all days that L-AmB was not administered.
Mice were challenged with either 100 conidia of iv A. fumigatus
or 1–2 × 10° conidia via nasal instillation on d +3, 36 h prior to
the d +4 dose of L-AmB or D,W.

**Preparation of Aspergillus conidia.** An isolate of A. fumigatus
known to cause fatal sinusitis in a patient after allogeneic HCT
was plated onto Sabouraud dextrose agar (SDA; Becton Dickin-
son) and incubated for 48 h at 37°C. Sterile saline was poured onto
the surface of the fungal lawn, which was scraped gently with an
inoculating loop. Conidia were resuspended in normal saline and
filtered through sterile gauze. The suspension was examined for
hyphal elements, and the concentration of conidia was determined
by both counts and colony growth. Suspensions were stored at 4°C.

**Culture of target organs.** Mice were killed if they exhibited
clinical evidence of disease or 20% loss of baseline body weight;
all remaining surviving mice were killed at the end of the 24-day
study period. Spleen, liver, kidneys, lungs, and brains were har-
vested, weighed, and then homogenized with 2 mL of Dulbecco’s
modified Eagle medium (Gibco BRL). Tissue homogenate (0.3 mL)
was plated on freshly made SDA plates and incubated at room
temperature. Colonies were counted at 5, 7, and 10 days after
plating. Fragments of organs were also fixed for histologic ex-
amination.

**Statistics.** The log-rank test was used for paired data analyses
of the Kaplan-Meier survival curves.

**Results**

After transplantation, mice remained neutropenic for a mini-
um of 14 days. A control group of mice that received D,W
daily after transplantation but were not infected remained
healthy throughout the study period. Mice that served as con-
trols for the lethal irradiation dose died between d +12 and d
+14.

**iv Challenge with A. fumigatus.** After iv challenge with
conidia, all mice that received only D,W (group I) throughout
the study period or that received L-AmB only after challenge
(group II) died within 1 week. In contrast, 40% of the mice in
group III survived (P < .05) for 24 days after transplantation
and were killed for culture and histologic examination of their
organs (figure 1A).

**Intranasal (inl) challenge with A. fumigatus.** After nasal
instillation of conidia, only 25% and 35% of the mice in groups
I and II, respectively, survived. In contrast, 85% of mice that
received L-AmB prior to and after challenge (group III; P < .05)
survived for the remainder of the 24-day study period. The
administration of 1 mg/kg/d of L-AmB (group IV) prior to
challenge did not result in a significantly improved survival,
compared with group I (figure 1B).

**Tissue fungus load.** Four days after inl administration of
conidia, a high fungus load in the lungs of moribund mice in
groups I, II, and IV was confirmed by culture. Acutely branch-
ning hyphal elements consistent with A. fumigatus could be read-
ily seen in histologic samples of the lung. Of interest, fungal
 tissue load remained significant even in the surviving mice in
group II. In contrast, no fungus was isolated from the tissue
of mice that had received prophylactic administration of L-
AmB. Mice in group III did not display evidence of illness
throughout the study period (table 1). After iv challenge, A.
Figure 1. Survival of mice after infection with *Aspergillus fumigatus* on day 3 posttransplantation (d+3) with either 100 cfu intravenously (A) or cfu by nasal instillation (B). By either route of fungal challenge, prophylactic administration of 5 mg/kg every other day of liposomal amphotericin B (L-AmB) resulted in a significantly higher survival rate ( ), compared with administration of L-AmB 36 h after infection (L-AmB treatment) or with control mice (5% dextrose in water [D5W]). Prophylactic administration of 1 mg/kg/d L-AmB for 4 days prior to intranasal challenge with *A. fumigatus* did not significantly improve survival. Arrow indicates day of infection. The log-rank test was used on paired data analysis for statistical significance. The results from 2 separate experiments are shown.

Table 1. Fungus load in the lungs of mice cultured at 7 days (d+7) and 24 days (d+24) posttransplantation after intranasal administration of *Aspergillus fumigatus* (1–2 × 10⁶ cfu) 3 days posttransplantation.

<table>
<thead>
<tr>
<th>Group, characteristic</th>
<th>d+7</th>
<th>d+24</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, D,W</td>
<td>1940 ± 647</td>
<td>ND</td>
</tr>
<tr>
<td>II, L-AmB treatment, 5 mg/kg</td>
<td>2508 ± 1871</td>
<td>18,014 ± 8533</td>
</tr>
<tr>
<td>III, L-AmB prophylaxis, 5 mg/kg</td>
<td>ND²</td>
<td>0</td>
</tr>
<tr>
<td>IV, L-AmB prophylaxis, 1 mg/kg</td>
<td>2028 ± 853</td>
<td>ND²</td>
</tr>
</tbody>
</table>

NOTE. Data are mean cfu/g of lung tissue ± SE. n = 3 for each time point. D,W, 5% dextrose in water; L-AmB, liposomal amphotericin B; ND, not done.

² No evidence of illness.

Discussion

Invasive aspergillosis after HCT has an inordinately high fatality rate, despite early administration of appropriate antifungal agents. Clinical studies have invariably confirmed the occurrence of breakthrough mold infections after administration of even broad-spectrum antifungal agents when they are administered empirically [2, 9, 10]. In a well-designed clinical trial that compared empiric D-AmB therapy with L-AmB therapy, the total incidence of proved and probable fungal infections did not appear to be significantly different between the groups [3]. Thus, until there are significant improvements in the immunosuppressive regimens used after transplantation or proven immunotherapy, the prevention of aspergillosis remains the most effective strategy in decreasing death due to this infection.

The pharmacokinetic and toxicity profile of L-AmB is more favorable than that of D-AmB, making full-dose therapy possible even after HCT and a reasonable choice for a prophylactic drug. L-AmB has also been shown to reduce dissemination of infection compared with D-AmB in a rat model of pulmonary aspergillosis [11] and to prevent murine systemic candidiasis and histoplasmosis [8]. However, prophylactic administration of low doses of L-AmB [6, 7] has been shown to significantly decrease only fungal colonization, not the incidence of invasive fungal infections or mortality in neutropenic patients or after HCT.

Other animal models of invasive aspergillosis have relied on chemotherapy-induced neutropenia with or without corticosteroids to render the mice susceptible to infection. In those models, the number of conidia required to induce lethal disease has been reported to be between 1 × 10⁴ and 1 × 10⁵ with iv injection and between 1 × 10⁴ and 1 × 10⁵ with inl administration [12]. In our model of lethal irradiation and HCT, although both the lethal iv and inl doses are decreased, there was a less dramatic impact on the lethal inl dose, which suggests that the alveolar macrophage population is not as radiosensitive as the hematopoietic populations. The profound immunopathology demonstrated by this preclinical model parallels the increased mortality in patients after HCT, compared with patients who have other risk factors for invasive aspergillosis.

The data presented show that prophylactic administration of L-AmB is superior to treatment started even very early postinfection after HCT in the protection against lethal invasive aspergillosis. In addition, prophylactic administration of L-AmB
resulted in greater protection against death after inl administration of conidia compared with iv inoculation. This may be due to differences in effective drug concentrations relative to the delivery of the conidia or differences in the degree of compromise of the local immune effect cells. The trend toward an increased latency to death in the mice that received prophylactic administration of L-AmB may also suggest that the presence of drug in tissues at the time of infection may slow fungal proliferation. The absence of a protective effect of 1 mg/kg/d of L-AmB is also consistent with limited clinical studies. The ability to isolate fungus from the lungs of the survivors that received L-AmB daily beginning 36 h after infection suggests that, once established, it is difficult to eradicate the fungus, which further underscores the need for prevention.

The prevention of death due to invasive aspergillosis in this preclinical model of HCT suggests that the results in clinical trials of antifungal prophylaxis with L-AmB were equivocal because of an inadequate dose of L-AmB. Because tissue accumulation of L-AmB follows nonlinear and progressive pharmacokinetics (clearance and volume decreased and half-life increased with dose and time), 5 mg/kg has been shown to result in disproportionately higher tissue levels in the liver, spleen, lung, kidneys, and plasma than 1–2 mg/kg [5, 13–15].

The laboratory and clinical studies cited have demonstrated that chemoprophylaxis of invasive candidal infections is possible. The results reported herein are the first demonstration that invasive aspergillosis also can be prevented even in the setting of severe immune compromise.

References