Efficacy of Single-Dose Liposomal Amphotericin B or Micafungin Prophylaxis in a Neutropenic Murine Model of Invasive Pulmonary Aspergillosis[▽]

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In a neutropenic murine model of invasive pulmonary aspergillosis, prophylaxis with single doses of liposomal amphotericin B or micafungin at ≥ 5 mg/kg of body weight improved animal survival and suppressed the lung fungal burden for up to 7 days after infection, demonstrating the potential utility of infrequent dosing with these antifungals.

Lipid formulations of the amphotericin B (LFAB) and echinocandin antifungals are important options for the treatment of invasive aspergillosis, especially in patients who are poor candidates for oral triazoles due to absorption problems, drug interactions, or hepatic toxicity (18). The necessity for the administration of LFAB or echinocandins by daily intravenous infusions, however, somewhat limits their utility for long-term use as primary or secondary prophylaxis in the outpatient setting. Recently, a strategy of intermittent dosing (i.e., one to three times weekly) of liposomal amphotericin B (L-AMB) or micafungin (MCFG) has been suggested as a means for improving the practicality of intravenous therapy in ambulatory patients (5, 6, 8, 9, 13). In vivo, pharmacokinetic-pharmacodynamic studies of amphotericin B (3, 10, 15, 20) and the echinocandins (2, 9, 12, 16, 17, 19) have demonstrated that total drug exposure, defined by the ratio of the area under the concentration-time curve to the MIC or the ratio of the peak drug concentration to the MIC, is the critical variable for the determination of dosing efficacy in experimental models of candidiasis and aspergillosis. Therefore, administration of the same cumulative dose of L-AMB or MCFG over an extended dosing interval should not, in theory, compromise the effectiveness of an antifungal regimen for prophylaxis against aspergillosis.

Single high-dose regimens of L-AMB (5 to 20 mg/kg of body weight) have been shown to protect both immunocompetent and immunocompromised mice challenged with lethal inocula of *Candida albicans* or *Histoplasma capsulatum* (7). Adler-Moore and colleagues also found that regimens of intermittent dosing with L-AMB (8 to 20 mg/kg) were as effective as daily dosing regimens in neutropenic mice with invasive candidiasis (1). Furthermore, Gumbo and colleagues reported that once-weekly therapy with MCFG at doses up to 100 mg/kg was as effective as daily MCFG therapy for disseminated *Candida glabrata* infection in persistently neutropenic mice (9). The efficacies of intermittent L-AMB and MCFG dosing have not

been compared, however, in neutropenic animal models of invasive pulmonary aspergillosis (IPA). In the present study, we examined whether single doses of L-AMB or MCFG could protect persistently neutropenic mice challenged with a sino-pulmonary inoculum of *Aspergillus fumigatus*.

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Eight-week old female BALB/c mice (weight, 18 to 22 g; Charles River Laboratories, Boston, MA) were immunosuppressed with intraperitoneal (i.p.) injections of cyclophosphamide (150 mg/kg) at 4 days and 1 day prior to inoculation, with an additional injection given 3 days after inoculation to maintain neutropenia for at least 6 days after infection (11). A single 250-mg/kg i.p. dose of cortisone acetate was also administered to animals 1 day prior to infection to suppresses the activity of alveolar macrophages that clear inhaled *Aspergillus* conidia (11). The mice were then anesthetized 24 h later with 6% nebulized isoflurane in oxygen, and a 50- μ l droplet containing 2.5×10^6 *A. fumigatus* 293 conidia was slowly instilled in the nares by use of a micropipette (11). The test isolate was confirmed to be susceptible to both AMB (MIC, 0.25 μ g/ml) and MCFG (minimum effective concentration, 0.25 μ g/ml) by Etest and broth microdilution (CLSI [formerly NCCLS] approved standard M38-A) methods (14). After inoculation, the animals were carefully monitored for 7 days for two or more signs of morbidity (matted fur, weight loss, limited ambulation, respiratory difficulty). Any animal determined to be in a morbid state was euthanized by CO₂ narcosis, and death was recorded as occurring 8 h later.

We tested the prophylactic efficacies of L-AMB and MCFG regimens by administering a single i.p. dose of either antifungal (5, 10, or 20 mg/kg) or 5% dextrose water (D5W) alone (control) to groups of 20 mice each 24 h prior to infection. All doses were prepared from clinical powder formulations (Astellas Inc. Deerfield, IL), according to the manufacturer's instructions, and were diluted with D5W before administration to the mice.

Control animals administered D5W succumbed to infection within 5 to 7 days, with only 10% surviving at day +7 (Fig. 1A). In contrast, all of the single-dose L-AMB or MCFG regimens improved the rate of animal survival compared to the rate of survival for the controls (55 to 90% and 10%, respectively; $P <$

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FIG. 1. Single-dose MCFG or L-AMB prolongs survival and reduces the lung fungal burden in persistently neutropenic mice challenged with *Aspergillus fumigatus*. (A) Each datum point represents the proportion of surviving animals ($n = 20$ per dosing group) at the respective time point following infection (day 0). All curves were significantly different from control by the log-rank (Mantel-Cox) test ($P < 0.0001$). (B) The lung fungal burden was determined at day +7 or prior to day +7 for animals euthanized because of a moribund condition. Each datum point represents the quantity (in CE) of *A. fumigatus* DNA in the lungs of animals determined by quantitative real-time PCR. Solid bars represent the median fungal burden. The dotted line represents the median lung fungal burden threshold associated with animal mortality. P values were determined by analysis of variance with Dunn's correction for multiple comparisons with the results for the controls.

0.02, Mantel-Cox log rank test). L-AMB regimens dosed at 5 or 10 mg/kg resulted in 90% animal survival at day +7 ($P < 0.001$ compared with the results for the controls). Marginally lower survival rates were observed in animals treated with 20 mg/kg L-AMB or 10 mg/kg MCFG ($P < 0.001$ compared with the results for the controls). Single doses of MCFG at 5 mg/kg and 20 mg/kg were less effective, with only 55% of the animals being alive at day +7 ($P = 0.02$ compared with the results for the controls).

As a secondary end point, the lung fungal burden in 10 to 20 mice from each treatment group was determined at the time of euthanization by a previously described quantitative real-time PCR assay (Fig. 1B) (4, 10). The median *A. fumigatus* conidial equivalent (CE) lung fungal burden in control animals was 3.2×10^6 (range, 5.4×10^5 to 1.5×10^7). Pretreatment with L-AMB at the 5-mg/kg dose was associated with a 0.75- $\log_{10}$ reduction in the median fungal burden ($P > 0.05$ compared with the results for the controls), and pretreatment with L-AMB at the 10- and 20-mg/kg doses was associated with a 1.3- $\log_{10}$ reduction ($P < 0.01$). Pretreatment with MCFG at 5 and 10 mg/kg reduced the fungal burden by 1.3 $\log_{10}$ ($P < 0.01$); however, pretreatment with MCFG at the 20-mg/kg dose reduced the median fungal burden by only 0.7 $\log_{10}$ ($P > 0.05$).

To further evaluate the activity of MCFG in the model, we compared the patterns of hyphal invasion on day +7 by Gemori methenamine staining of lung tissue sections in repeat experiments with five animals per dose (Fig. 2). The hyphae in the control animals demonstrated elongated branching patterns in a classic starburst pattern (Fig. 2a and b). However, the fungi in tissue from the MCFG-treated animals exhibited degenerative reductions in hyphal length and swelling compared to the fungi in tissue from the untreated controls (Fig. 2c to f). Animals treated with MCFG at 20 mg/kg exhibited swollen dysmorphic hyphal clusters, but these were present at higher densities per lung field compared to the densities in animals pretreated with MCFG at 10 mg/kg (Fig. 2g and h).

In conclusion, we found that single doses of L-AMB or MCFG could protect neutropenic mice following a sinopulmonary challenge with *A. fumigatus*. Although these results should

FIG. 2. Histopathological evidence of dose-dependent suppression of fungal growth in persistently neutropenic mice following single-dose MCFG prophylaxis. Lungs from five mice per treatment group were fixed in 10% (vol/vol) formaldehyde, stained with Grocott methamine silver nitrate (GMS), and viewed by light microscopy. Representative images are presented. (a, c, e, and g) Magnification, $\times 100$; (b, d, f, and h) magnification, $\times 400$. A dose-dependent degeneration in the hyphal mass was observed throughout the lung fields. Few hyphae were observed in areas of consolidated lung in animals that received MCFG at 10 mg/kg. However, dysmorphic-swollen hyphae were present in the infiltrates of some animals treated with MCFG at 20 mg/kg.

be confirmed in studies with other isolates and other models of IPA, our results offer encouraging evidence that intermittent dosing of L-AMB or MCFG could be an effective prophylactic strategy that deserves further evaluation in studies with patients.

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