Posaconazole Mono- or Combination Therapy for Treatment of Murine Zygomycosis

Ashraf S. Ibrahim,1,2* Teclegiorgis Gebremariam,1 Julie A. Schwartz,3 John E. Edwards Jr.,1,2 and Brad Spellberg1,2

Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor—University of California Los Angeles Medical Center, Torrance, California; David Geffen School of Medicine at UCLA, Los Angeles, California; and Charles River Laboratories, Davis, California

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We compared the efficacy of combination posaconazole-liposomal amphotericin B (LAmB) therapy to monotherapy with either drug in diabetic ketoacidotic or neutropenic mice with disseminated zygomycosis caused by Rhizopus oryzae. Combination therapy was no better than LAmB alone, and posaconazole monotherapy did not improve survival or reduce fungal burden versus placebo.

Posaconazole has in vitro activity against the agents of zygomycosis and has been used as salvage therapy for patients refractory to or intolerant of polyenes (2, 15). To determine if up-front posaconazole-polyene combination therapy would improve the outcome of zygomycosis, we tested the efficacy of liposomal amphotericin B (LAmB), posaconazole, or the combination LAmB plus posaconazole therapy in our standard diabetic ketoacidotic (DKA) and neutropenic mouse models of disseminated zygomycosis.

For the DKA model, BALB/c male mice were rendered diabetic with a single intraperitoneal injection of 210 mg/kg streptozotocin 10 days prior to fungal challenge, as we have previously described (3–5). Glycosuria and ketonuria were confirmed in all mice prior to infection. For the neutropenic model, mice were made neutropenic by a single intraperitoneal dose of 200 mg of cyclophosphamide/kg of body weight on day −2 relative to infection, resulting in approximately 7 days of neutropenia (6, 7, 12, 13). Mice were infected via the tail vein with Rhizopus oryzae 99-880, a clinical brain isolate known to be virulent in the murine model (4, 5, 11). LAmB (Gilead Sciences), posaconazole oral suspension (Western Medical Supplies), a combination of both, or placebo (5% dextrose solution) was administered once daily for 4 days starting at 24 h postinfection.

For the initial experiment in the DKA model, a dose-ranging study was conducted to determine the optimal dose of posaconazole. DKA mice were infected via the tail vein with R. oryzae 99-880 and treated with posaconazole at 10, 30, or 60 mg/kg/day administered via oral gavage. No dose of posaconazole improved survival versus placebo (Fig. 1A). Both lower doses of posaconazole resulted in a trend to worse survival (P = 0.1 for 10 and 30 mg/kg/day versus placebo).

For the subsequent combination study, DKA mice were infected with R. oryzae 99-880 and treated with 15 mg/kg/day LAmB intravenously (i.v.), 60 mg/kg/day posaconazole by oral gavage, a combination of both, or placebo. LAmB monotherapy and the combination of LAmB plus posaconazole significantly improved time to death compared to placebo (P < 0.05 for both comparisons by log rank test) (Fig. 1B). In contrast, posaconazole monotherapy mediated a nonsignificant trend to improve time to death compared to placebo (P = 0.2). There was no significant difference in time to death between mice treated with combination therapy and LAmB monotherapy (P = 0.3).

To define the impact of antifungal therapy on tissue fungal burden in the DKA model, mice were infected as described above and treated with LAmB, posaconazole, combination therapy, or placebo. Treatment was initiated 8 h after infection and continued once daily until the morning of day 2 postinfection (three doses total), prior to the onset of deaths in the placebo group. On the afternoon of day 2 postinfection, mice were euthanized and kidneys and brains were harvested, homogenized by gentle rolling in Whirl-Pak bags, and plated on potato-dextrose agar plus 0.1% Triton for quantification of tissue fungal burden. Additionally, tissue samples were fixed in zinc formalin and processed for histopathology. Posaconazole did not reduce tissue fungal burden compared to placebo, whereas LAmB and combination therapy mediated ~10-fold reductions in kidney and ~30-fold reductions in brain fungal burdens (Fig. 1C). There was no significant difference between organ fungal burdens in mice treated with combination therapy versus LAmB alone. Histopathology was concordant in showing that posaconazole was not effective at reducing tissue fungal burden (Fig. 2).

We next tested the efficacy of antifungal therapies in our neutropenic mouse model. Similar to the DKA model, in neutropenic mice with zygomycosis, posaconazole did not improve time to death compared to placebo (P = 0.9), whereas LAmB monotherapy did improve time to death compared to placebo (P = 0.006). Combination therapy mediated a nonsignificant trend to improved time to death versus placebo (P = 0.1) and was not superior to LAmB monotherapy. Both LAmB monotherapy and combination therapy improved time to death compared to posaconazole monotherapy (P = 0.02 for LAmB; P = 0.04 for combination therapy versus posaconazole) (Fig. 3A).
To define the impact of antifungal therapy on tissue fungal burden in neutropenic mice, the experiment was repeated with treatment initiated 8 h after infection and continued once daily for three doses prior to harvesting kidneys and brains. None of the treatments reduced kidney fungal burden compared to placebo in neutropenic mice (Fig. 3B). However, both LAmB and combination therapy reduced kidney fungal burden compared to posaconazole monotherapy ($P < 0.03$ for both com-
As in the kidney, posaconazole did not reduce brain fungal burden relative to placebo. However, both LAmB and combination therapy reduced brain fungal burden compared to both placebo and posaconazole (P < 0.03 for all comparisons). There was no significant difference in efficacy between LAmB and combination therapy.

Because posaconazole has in vitro activity against Mucorales, it has been used as a salvage therapy for patients with zygomycosis refractory to or intolerant of polyenes. Furthermore, due to the poor outcomes of zygomycoses treated with polyene monotherapy, the possibility of up-front combination therapy with polyenes and posaconazole is intriguing. We found no evidence of an additive benefit of posaconazole when combined with LAmB, either in terms of time to death or tissue fungal burden in mice with disseminated zygomycosis caused by R. oryzae. Furthermore, concordant with prior murine studies (1, 10, 14), monotherapy with posaconazole had little activity in mice infected with R. oryzae. A recent study found that posaconazole enhanced the activity of a subtherapeutic dose of amphotericin B deoxycholate (AmB) but was of no additional benefit when a full dose of AmB was used to treat R. oryzae infection in neutropenic mice (10).

We cannot exclude a benefit of higher posaconazole doses. However, 100 mg/kg/day of posaconazole was no more effective than lower doses in a previous study of murine zygomycosis (1). We cannot exclude the possibility of a benefit of combination therapy during intranasal or inhalational zygomycosis, or against other strains or species of Mucorales. Nevertheless, as R. oryzae is the most common cause of zygomycosis, accounting for >70% of cases (8, 9), our data do not support the use of combination polyene-posaconazole therapy for disseminated zygomycosis.

**FIG. 3.** Efficacy of combination LAmB plus posaconazole versus monotherapy with either drug in the neutropenic mouse model of zygomycosis. A) Survival of uninfected control neutropenic mice (n = 10) or of neutropenic mice with zygomycosis (the R. oryzae 99-880 average inoculum was 9.2 × 10^3 spores i.v.) treated with LAmB (15 mg/kg/day; n = 9), posaconazole (60 mg/kg/day; n = 19), combination therapy (n = 10), or placebo (n = 19), *P < 0.04 for LAmB or combination therapy versus posaconazole by log rank test. B) Kidney or brain fungal burdens in neutropenic mice (n = 10 per group) infected i.v. with 3 × 10^3 R. oryzae 99-880 cells. Data are displayed as medians ± interquartile ranges. The y axes reflect lower limits of detection of the assay. *, P < 0.05 versus placebo and posaconazole; †, P < 0.03 versus posaconazole by the nonparametric Mann-Whitney test.
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REFERENCES


