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Isavuconazole Therapy Protects Immunosuppressed Mice from Mucormycosis

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We studied the *in vitro* and *in vivo* efficacies of the investigational drug isavuconazole against mucormycosis due to *Rhizopus delemar*. Isavuconazole was effective, with MIC and minimal fungicidal concentration (MFC) values ranging between 0.125 and 1.00 μg/ml. A high dose of isavuconazole prolonged the survival time and lowered the tissue fungal burden of cyclophosphamide/cortisone acetate-treated mice infected with *R. delemar* and was as effective as a high-dose liposomal amphotericin B treatment. These results support the further development of this azole against mucormycosis.

Mucormycoses are among the most common infections afflicting immunocompromised hosts and are occurring at an increasing frequency (1–3). The risk factors for mucormycosis include compromised immune status due to hematologic malignancies, neutropenia, steroid treatment, hyperglycemia, ketoacidosis and other forms of acidosis, deferoxamine therapy, and trauma (4–6). Despite disfiguring surgical debridement and adjunctive antifungal therapy, the overall mortality rate of mucormycosis remains approximately 50% and can approach 100% in hematogenously disseminated and central nervous system disease and in patients with prolonged neutropenia (2, 7–11). Clearly, new strategies for preventing and treating mucormycosis are urgently needed.

The new investigational drug isavuconazole (BAL4815) (ISA) is the active compound of the water-soluble prodrug isavuconazonium sulfate (BAL8557). Following administration, the prodrug isavuconazonium sulfate is rapidly converted by esterases in plasma to isavuconazole (isavuconazole is the term used here to describe the medicinal product). ISA shows broad-spectrum activity against fungi and is currently in late-stage clinical development for invasive fungal diseases, including invasive mucormycosis and invasive aspergillosis. For example, ISA has activity against *Aspergillus* spp. (12–15) (including those resistant to itraconazole, caspofungin, and amphotericin B [13]), *Candida* spp. (16, 17) (including isolates resistant to fluconazole [18]), and *Cryptococcus* spp. (19, 20). Recent studies demonstrated promising *in vitro* activity of ISA against *Mucorales* (12, 21, 22) fungi that cause mucormycosis. Therefore, the activity of ISA was compared to that of liposomal amphotericin B (LAmB) in a murine model of mucormycosis. Since *Rhizopus* species are the most common *Mucorales* isolates obtained from patients with mucormycosis (8, 23, 24), these studies focused on *Rhizopus delemar*.

The MIC_{100} (defined as the lowest concentration that causes 100% growth inhibition relative to the drug-free growth control) values of ISA (Astellas Pharma Global Development, Inc., Northbrook, IL) were determined against four clinical isolates of *R. delemar* (fumaric-malic acid producers) or four clinical isolates of *Rhizopus oryzae* (lactic acid producers) (25) using the Clinical Laboratory and Standards Institute (CLSI) M38-A2 method (26). The minimal fungicidal concentrations (MFCs) were also determined by spotting samples from all of the 96-well plates on potato dextrose agar (PDA) plates supplemented with 0.1% Triton X-100 and incubating them at 37°C for 2 days. The MFC was defined as the lowest concentration of the drug at which the organism failed to grow on the PDA plate. Against *R. delemar* isolates, ISA had median MIC_{100} and MFC values of 0.188 μg/ml (25th and 75th quartiles, 0.0625 and 0.0625 μg/ml). All tested *R. oryzae* isolates had ISA MIC_{100} and MFC values of 0.125 μg/ml. These studies showed that isavuconazole is fungicidal, since the MFC values were equivalent to the MIC values.

Next, the efficacy of the prodrug isavuconazonium sulfate was evaluated in a neutropenic mouse model of intratracheal infection (27) caused by *R. delemar* 99-880 (a brain isolate with ISA MIC_{100} and MFC values of 0.25 μg/ml). Male ICR mice (23 to 25 g; Taconic Farms, Germantown, NY) were used in this study. They were given irradiated feed and sterile water containing 50 μg/ml Baytril (enrofloxacin; Bayer) *ad libitum* (to control for bacterial infection). Neutropenia was induced by cyclophosphamide (200 mg/kg of body weight intraperitoneally [i.p.]) and cortisone acetate (500 mg/kg subcutaneously) on days −2 and 3 relative to infection. This treatment regimen results in ~10 days of leukopenia with a total white blood cell count dropping from ~13,0000/cm^3 to almost no detectable leukocytes as determined by the Unopette system (Becton-Dickinson and Co., Franklin Lakes, NJ). After sedation with ketamine and xylazine, the mice were intratracheally infected with 2.5×10^5 spores of *R. delemar* 99-880 (27). Treatment with the prodrug isavuconazonium sulfate (80, 110, and 215 mg/kg, prepared fresh daily in irrigation water and given orally three times daily [t.i.d.]) started 8 h postinfection and continued through day 4. The higher dose of 215 mg/kg t.i.d. of isavuconazonium sulfate demonstrated enhanced efficacy over that of placebo treatment of mice (70% survival in the isavuconazo-

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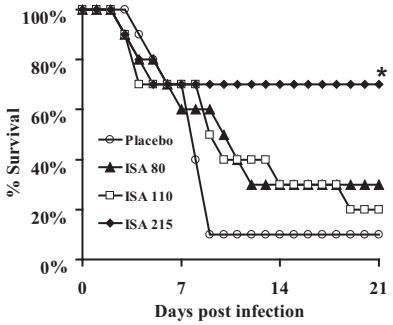

FIG 1 Isavuconazole enhanced survival of neutropenic mice with mucormycosis pneumonia. Mice ($n = 10$ per arm) were infected intratracheally with 2.5×10^5 spores of *R. delemar* 99-880 (inhaled inoculum was 4.1×10^3 spores). Isavuconazonium sulfate treatment started 8 h postinfection and continued three times daily at 80, 110, or 215 mg/kg by oral gavage through day 4 postinfection. Placebo mice were infected and treated with sterile irrigation water. *, $P < 0.05$ compared to placebo-treated mice by log rank test.

nium sulfate-treated mice versus 10% survival for placebo mice [treated orally with irrigation water] after 21 days) (Fig. 1).

Since a high dose of ISA (215 mg/kg t.i.d.) demonstrated efficacy against *R. delemar* infection, the efficacy of this dose was compared against that of a high dose of LAmB (AmBisome; Gilead Sciences Inc., Forest City, CA) in treating mucormycosis, which is considered the standard therapy for mucormycosis in this model (28). LAmB was dissolved initially in sterile irrigation water and diluted in 5% dextrose water (D5W) according to the manufacturer's instructions. Neutropenic mice were infected intratracheally as described above. Eight hours later, treatment with isavuconazonium sulfate (215 mg/kg t.i.d., given orally) or LAmB (15 mg/kg, given once daily through tail vein injection) started and continued through day 4. Neutropenic mice infected intratracheally and administered a comparable volume of vehicle (i.e., D5W) served as placebo controls. The primary endpoint for efficacy was the time to moribundity of infected mice. ISA was as effective as LAmB in treating neutropenic mice for mucormycosis. The twenty-one-day survival rates for the prodrug isavuconazonium sulfate-, LAmB-, and placebo-treated mice were 65%, 40%, and 15%, respectively (Fig. 2A).

Because ISA increased the survival rate of neutropenic mice infected with *R. delemar*, the effect of drug treatment on the tissue fungal burden in target organs was determined. The mice were infected as described above and treated until day 3 relative to infection when the mice were sacrificed and their lungs and brains harvested and tested for tissue fungal burden (representing primary and secondary target organs [27]) by quantitative PCR (qPCR) (29). Treatment of the mice with the prodrug resulted in an approximately 1-log decrease in lung and brain fungal burdens compared to those of placebo-treated controls. This reduction in tissue fungal burden was comparable to that elicited by LAmB treatment (Fig. 2B).

A recent study demonstrated that oral-gastric doses of the prodrug isavuconazonium sulfate at 10, 40, 160, and 640 mg/kg produced serum peak levels of 0.51 to 25.4 μg/ml ISA and an elimination half-life of 1 to 5 h (30). The short half-life of this drug is in contrast to the long half-life of >50 h seen in humans following a single-dose administration (31). Therefore, the prodrug was administered three times daily over a series of doses that would provide a range of exposures, some of which should result in serum peak levels of ISA above the registered MIC and MFC values of 0.125 to 1.0 μg/ml. Indeed, the higher dose of 215 mg/kg (prodrug isavuconazonium sulfate) given three times daily, which would have resulted in serum peak levels of >12.5 μg/ml ISA and a half-life of >3.1 h (30), demonstrated enhanced protection of mice from mucormycosis. This protection was equivalent to the efficacy demonstrated by a high dose of LAmB.

In addition to the activity in the cyclophosphamide/cortisone acetate-treated mice, ISA activity has been demonstrated in animal models of other fungal infections, including invasive aspergillosis and disseminated candidiasis (14, 32, 33). Finally, the availability of ISA in oral and intravenous formulations provides a clear advantage for this azole for use in different medical scenarios. However, given the frequency of this infection in patients with diabetes, future studies of ISA efficacy should also be carried out in a diabetic murine mucormycosis model (8, 9). In summary, given

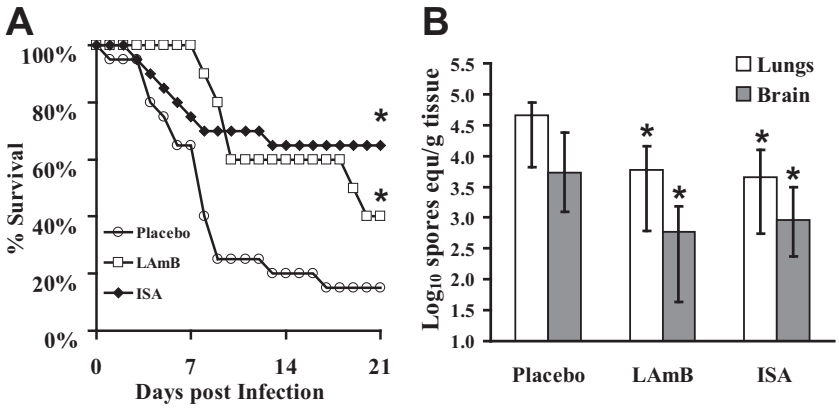

FIG 2 Isavuconazole is as effective as high-dose LAmB in improving survival (A) and reducing fungal burden (B) of neutropenic mice from mucormycosis. Mice ($n = 10$ per arms for both A and B) were infected intratracheally with 2.5×10^5 spores of *R. delemar* 99-880 (average inhaled inoculum was 1.3×10^3 cells). Isavuconazonium sulfate (215 mg/kg t.i.d., by oral gavage) or LAmB (15 mg/kg every day by i.v. injection) treatment started 8 h postinfection and continued through day 4 postinfection (A) and day 3 postinfection (B). Placebo mice were infected and treated with 5% dextrose water. (A) Survival of mice through day 21. *, $P = 0.025$ or 0.004 for LAmB or ISA compared to placebo by log rank test, respectively. (B) Fungal burden was measured by qPCR with a conidial standard curve (29, 34). All qPCR results are expressed as $\log_{10}$ spore equivalents per gram of tissue. *, $P < 0.05$ compared to placebo by Wilcoxon rank sum test.

the *in vitro* and *in vivo* evidence of the activity of ISA against *Rhizopus*, these results warrant further development of this azole for the treatment of mucormycosis.

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