Pharmacokinetics and Concentration-Dependent Efficacy of Isavuconazole for Treatment of Experimental Invasive Pulmonary Aspergillosis

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We studied the pharmacokinetics and efficacy of the broad-spectrum triazole isavuconazole for the treatment of experimental invasive pulmonary aspergillosis (IPA) in persistently neutropenic rabbits. Treatment started 24 h after endotracheal administration of Aspergillus fumigatus inoculum; study subjects included rabbits receiving orally administered prodrug isavuconazonium sulfate (BAL8557) equivalent to active moiety isavuconazole (ISA; BAL4815) at 20 (ISA20), 40 (ISA40), and 60 (ISA60) mg/kg (of body weight)/day, with an initial loading dose of 90 mg/kg (ISA90), and untreated rabbits (UC). There were significant concentration-dependent reductions of residual fungal burden (log CFU/gram) and of organism-mediated pulmonary injury, lung weights, and pulmonary infarct scores in ISA40- and ISA60-treated rabbits in comparison to those of UC (P < 0.001). ISA20-treated (P < 0.05), ISA40-treated, and ISA60-treated (P < 0.001) rabbits demonstrated significantly prolonged survival in comparison to that of UC. ISA40- and ISA60-treated animals demonstrated a significant decline of serum (1→3)-β-D-glucan levels (P < 0.05) and galactomannan indices (GMIs) during therapy following day 4 in comparison to progressive GMIs of UC (P < 0.01). There also were significantly lower concentration-dependent GMIs in bronchoalveolar lavage (BAL) fluid from ISA40- and ISA60-treated rabbits (P < 0.001). There was a direct correlation between isavuconazole plasma area under the concentration-time curve from 0 to 24 h (AUC0 –24) and residual fungal burdens in lung tissues, pulmonary infarct scores, and total lung weights. In summary, rabbits treated with isavuconazole at 40 and 60 mg/kg/day demonstrated significant dose-dependent reduction of residual fungal burden, decreased pulmonary injury, prolonged survival, lower GMIs in serum and BAL fluid, and lower serum (1→3)-β-D-glucan levels.

Isavuconazole is a new broad-spectrum triazole antifungal agent that has been recently approved by the FDA for primary treatment of invasive aspergillosis and mucormycosis (7–9). Isavuconazole in vitro demonstrates superior hyphal growth inhibition and MICs against Aspergillus fumigatus in comparison to those of voriconazole (10–14). However, there is a paucity of laboratory animal data for isavuconazole against Aspergillus spp. The pharmacodynamics of isavuconazole was explored in a murine neutropenic IPA model by Lepak and colleagues (15) and also in an invasive aspergillosis model of immunocompetent mice by Seyedmousavi et al. (16). We therefore studied the efficacy and pharmacokinetics of isavuconazole in treatment of experimental IPA in persistently neutropenic rabbits.

Invasive pulmonary aspergillosis (IPA) is a life-threatening infection in immunosuppressed patients, particularly in those with severe and prolonged neutropenia as a consequence of aplastic anemia, those undergoing myelo-ablative chemotherapy for the treatment of cancer, and those receiving immunosuppressive medication for rejection prophylaxis after organ transplantation or treatment of graft-versus-host disease in allogeneic bone marrow transplantation (1–5).

Invasive pulmonary aspergillosis is the most common cause of infectious pneumonic death in bone marrow transplant recipients, accounting for 45% of all such lethal cases. Mortality rates of IPA in cancer patients have varied between 13% and 100% depending on the recovery from neutropenia and status of underlying disease.

Current treatment of IPA in immunosuppressed hosts relies on the administration of voriconazole as primary therapy. Unfortunately, the overall rate of response of invasive aspergillosis to voriconazole remains at approximately 50% to 60%, with responses as low as nearly 30% in hematopoietic stem cell transplantation recipients (6). Although voriconazole is an important therapeutic advance against IPA, the problems of visual hallucinations, cutaneous solar hypersensitivity, hepatotoxicity, drug interactions, variable plasma pharmacokinetics (PK), and need for therapeutic drug monitoring warrant the development of new antifungal agents against Aspergillus spp. Clearly, new strategies are needed for the treatment of IPA.


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MATERIALS AND METHODS

Animals. Healthy female New Zealand White rabbits (Covance Research Products, Inc., Denver, PA) weighing 2.6 to 3.5 kg at the time of endotracheal inoculation were used in our experiments. All rabbits were monitored under humane care and use of standards in facilities, accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and according to the guidelines of the National Research Council (17) for the care and use of laboratory animals and under the approval of the Animal Care and Use Committee of the Weill Cornell Medical Center, New York, NY. Rabbits were individually housed and maintained with water and standard rabbit feed ad libitum. Atraumatic vascular access was established by modified surgical placement of a Silastic tunneled central venous catheter as previously described (18). The Silastic catheter permitted nontraumatic venous access for administration of parenteral agents and for repeated blood sampling for study of plasma pharmacokinetics, serum galactomannan and (1→3)-β-D-glucan levels, and biochemical and hematological parameters.

Organism, inoculum, and inoculation. A well-characterized clinical isolate of Aspergillus fumigatus, NIH 4215 (ATCC MYA-1163), initially obtained from a neutropenic patient with autopsy-proven pulmonary aspergillosis, was used for preparation of the inoculum, as previously described (19). All rabbits received predetermined inocula of 1 × 10⁶ to 1.25 × 10⁷ conidia of A. fumigatus in a volume of 250 μl to 350 μl administered endotracheally under general anesthesia on day 2 of the experiments, as previously described (19). The MIC of isavuconazole against A. fumigatus, determined according to Clinical and Laboratory Standards Institute (CLSI) standard M38-A microdilution method (20), was 1 μg/ml.

Immunosuppression and maintenance of neutropenia. Immunosuppression and profound persistent neutropenia (a neutrophil concentration of <100 neutrophils/μl) were established and maintained with intravenous (i.v.) administration of cytarabine (ara-C) (Zydis Hospira Oncology Private Ltd., Gujarat, India for Hospira, Inc., Lake Forest, IL) at an initial dosage of 525 mg/m² for five consecutive days (days 1 to 5), and methylprednisolone (Solu-Medrol, Pfizer for Pharmacia & Upjohn Co., New York, NY) at a dosage of 5 mg/kg/day on days 1 and 2, as described elsewhere (19). Maintenance doses of ara-C at 484 mg²/m² were administered on days 8, 9, 13, and 14 of the study. Cefazidime (75 mg/kg i.v. twice daily; Glaxo Pharmaceuticals, Research Triangle Park, NC), gentamicin (5 mg/kg i.v. every other day; Elkins-Sinn, Inc., Cherry Hill, NJ), and vancomycin (15 mg/kg i.v. daily; Abbott Laboratories, North Chicago, IL) were administered from day 4 of immunosuppression for the prevention of opportunistic bacterial infections during neutropenia. For prevention of antibiotic-associated diarrhea due to Clostridium spironforme, all rabbits received 50 mg of vancomycin per liter of drinking water.

Antifungal compounds and treatment regimens. Prodrug isavuconazonium sulfate (BAL8557) powder, provided by Astellas Pharma Global Development, Inc., was dissolved in sterile water, and the same concentration of BAL8557 was dissolved in 5% dextrose injection solution (D5W; Baxter Healthcare Global Development, Inc., Deerfield, IL) and 100 μl of a dilution (10⁻¹) were cultured on Sabouraud dextrose agar (DIFCO Laboratories, Detroit, MI) for an initial period of 21 days at 35°C. Each rabbit was sampled and cultured for quantitative counts, and the CFU of A. fumigatus was considered negative.

Survival. The survival time, in days postinoculation, was recorded for each rabbit in each group. Following humane endpoints, rabbits were euthanized by i.v. administration of pentobarbital (65 mg of pentobarbital sodium/kg of body weight; Beuthanasia-D Special [euthanasia solution]; Schering-Plough Animal Health Corp., Union, NJ) on day 13 postinoculation, 24 h after the last dose of study drug.

Galactomannan detection. Serum samples from each rabbit were collected every other day and stored at −80°C before analysis. Galactomannan antigen levels were determined in serial serum samples and postmortem-obtained BAL fluid by a one-stage immunoenzymatic sandwich microplate assay method (23) (Plateia Aspergillus enzyme immunoassay [EIA]; Bio-Rad, Marnes la Coquette, France) according to the manufacturer’s instructions and described elsewhere (22). Enzyme immunoassay data were expressed as a serum galactomannan index (GMI) plotted over time. The GMI for each test serum or BAL fluid sample was equal to the absorbance of a standard sample divided by the absorbance of a threshold serum provided by the manufacturer. A GMI of less than 0.5 was considered negative.

Detection of (1→3)-β-D-glucan levels. Serum from each rabbit was collected every other day for determination of (1→3)-β-D-glucan levels by using a colorimetric assay (Fungitell; Associates of Cape Cod, Inc.) read at 405 nm (with 490-nm background subtraction), based upon para-nitroanilide absorption at that wavelength, performed according to the manufacturer’s instructions and described in detail elsewhere (24). The (1→3)-β-D-glucan levels were determined by taking the mean optical density of the duplicate readings and comparing with the standard curve of predetermined concentrations. Interpretation of (1→3)-β-D-glucan values, according to the manufacturer’s instructions, was as follows: <60 pg/ml, negative; 60 to 79 pg/ml, indeterminate; and ≥80 pg/ml, positive. The median correlation coefficient (r) of the standard curves performed in these studies was 0.9992 (range, 0.9982 to 0.9998).

Pharmacokinetic studies. (i) Single-dose pharmacokinetics of isavuconazole. The plasma pharmacokinetics of isavuconazole in rabbits was investigated for each dosage group of four noninfected healthy rabbits. Isavu-
conazole was administered as a single dose orally at dosages of 20, 40, 60, and 90 (ISA90) mg/kg/day. Following withdrawal of a baseline predose, blood samples were collected into heparinized syringes from each rabbit at the following time points from the beginning of isavuconazole administration: 1, 2, 4, 8, 12, 18, 24, and 48 h. Plasma was immediately separated by centrifugation at 4,000 × g, and samples were stored in 2-ml Sarstedt microtubes at −80°C prior to analysis.

(ii) Optimal sampling pharmacokinetics of isavuconazole. The plasma pharmacokinetics of isavuconazole was studied in five to eight infected animals each per dosage cohort. Time points for sampling were determined by inspection of full plasma concentration profiles obtained in normal rabbits following administration of similar dosages based upon previous plasma pharmacokinetic studies. Plasma sampling was performed on day 6 of antifungal therapy. Blood samples were drawn predose and at 1, 4, 8, and 24 h postdosing. Plasma was immediately separated by centrifugation and stored at −80°C until assayed.

LC-MS/MS assay. (i) Plasma samples. Paraoxon at 0.1 M (10 μl per 1 ml of plasma) was added to the blank EDTA/K3 and blank Li-heparin rabbit plasma to inhibit esterases. Spiking solutions of BAL0004815 and BAL0008728 were prepared by serial dilution in dimethyl sulfoxide (DMSO) and acetonitrile (ACN)–0.05% trifluoroacetate (TFA) out of a 2-mg/ml stock solution with a range of 0.5 μg/ml to 500 μg/ml. Calibration of samples was performed by spiking 1 μl of each DMSO (or ACN–0.05% TFA) solution in 99 μl of blank EDTA/K3 plasma followed by 300 μl of ACN–0.05% TFA containing 1 μg/ml of BAL0004815-d4 and pyridooxazinone as internal standards. After centrifugation, 10 μl of the supernatant was injected into the liquid chromatography-tandem mass spectrometry (LC-MS/MS) instrument (QTRAP; Applied Biosystems) (25). The quality control (QC) samples were prepared like the calibrators but using blank Li-heparin plasma instead of EDTA/K3 plasma. For the samples, 50 μl of plasma was mixed with 150 μl of ACN–0.05% TFA containing 1 μg/ml of BAL0004815-d4 and pyridooxazinone as internal standards and then treated like the calibrators.

(ii) Lung tissue samples. Two samples were prepared from each lung tissue. One equivalent of rabbit lung piece was mixed with two equivalents of water by using the Ultra-Turrax (IKA) dispenser. Fifty microliters of this lung-water solution was quenched with 150 μl of ACN–0.05% TFA containing 1 μg/ml of BAL0004815-d4 and pyridooxazinone as internal standards. After centrifugation, 10 μl of the supernatant was injected into the LC-MS/MS. For quantification, standard curves of BAL0004815 and BAL0008728 were prepared with a range of 2.5 to 5,000 ng/ml in lung-water solution. This “lung-water matrix” was

FIG 1 Response of primary pulmonary aspergillosis in persistently neutropenic rabbits to antifungal therapy measured by mean pulmonary tissue residual fungal burden (log CFU/gram), mean lung weight, mean pulmonary infarct score, and survival in untreated controls (UC) and rabbits receiving oral isavuconazole (BAL4815). An initial loading dose of 90 mg/kg of isavuconazole was administered orally, and thereafter the drug was administered daily at 20 mg/kg (ISA20), 40 mg/kg (ISA40), and 60 mg/kg (ISA60). Values are means ± SEMs. For the measure of survival, the values on the y axis are probability of survival. Survival was plotted by Kaplan-Meier analysis. Differences in survival of treatment groups and untreated controls were analyzed by log rank test. P values are indicated as follows: *, P < 0.05, decreased infarct score in ISA20-treated rabbits in comparison to that of UC; †, P < 0.001, decreased residual fungal burden, lung weight, and infarct score in ISA40- and ISA60-treated rabbits in comparison to that of UC; ‡, P < 0.001, prolonged survival of rabbits treated with ISA40 and ISA60 in comparison to that of UC; §, P < 0.05, prolonged survival of rabbits treated with ISA20 in comparison to that of UC.
made by mixing one equivalent of rabbit lung with two equivalents of water. The lung-water solution was spiked (1 µl of spiking solutions in 99 µl of lung-water matrix) and treated like the samples.

(iii) BAL fluid samples. BAL fluid and BAL fluid spiking solutions of BAL0004815 and BAL0008728 were prepared by serial dilution, respectively, in DMSO and ACN–0.05% TFA out of a 2-mg/ml stock solution with a range of 0.5 µg/ml to 500 µg/ml. Calibration of samples was performed by spiking 1 µl of each DMSO (or ACN–0.05% TFA) solution in 99 µl of 0.9% NaCl solution followed by 300 µl of ACN–0.05% TFA containing 1 µg/ml of BAL0004815-d4 and pyridoxazinone as internal standards. After centrifugation, 10 µl of the supernatant was injected into the LC-MS/MS. The QC samples were prepared like the calibrators but using normal rabbit BAL fluid supernatant instead of 0.9% NaCl solution. For the samples, 50 µl of rabbit BAL fluid or BAL fluid supernatant was

FIG 2 (Top) Expression of galactomannan antigenemia in persistently neutropenic rabbits with pulmonary aspergillosis in untreated controls and rabbits receiving oral dosing of isavuconazole (BAL4815). An initial loading dose of 90 mg/kg of isavuconazole was administered orally, and thereafter the drug was administered daily at 20, 40, and 60 mg/kg. Values are means ± SEMs. (Bottom) BAL fluid galactomannan antigen levels in persistently neutropenic rabbits with pulmonary aspergillosis in untreated controls and rabbits treated with isavuconazole at 20, 40, and 60 mg/kg/day p.o. Values are means ± SEMs. P values are indicated as follows: †, P < 0.01, lower GMI in ISA40- and ISA60-treated rabbits than in UC; †, P < 0.001, lower GMI in BAL from rabbits treated with ISA40 and ISA60 than in that of UC (P values were obtained by comparison to UC by ANOVA with Bonferroni’s correction for multiple comparisons).
mixed with 150 μl of ACN–0.05% TFA containing 1 μg/ml of BAL0004815-d4 and pyridooxazinone as internal standards and then treated like the calibrators.

**Determination of pharmacokinetic parameters.** Pharmacokinetic parameters for isavuconazole were determined from plasma concentration data using noncompartmental methods (WinNonlin Professional version 4.1; Pharsight Corp., Mountain View, CA). Pharmacokinetic measures were maximum observed plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC) through 24 h after the first dose (AUC_{0–24}) or at steady state (AUC_{ss}) calculated by the linear trapezoidal rule, clearance (CL) calculated by dividing dose by AUC, volume of distribution at steady state (V_{ss}) calculated by multiplying the dose by the ratio of the area under the first moment curve to the square of AUC, and terminal elimination half-life (t_{1/2}) calculated from a linear regression of the log-linear portion of the log concentration-time curve.

**Statistical analysis.** Comparisons between the groups were performed by analysis of variance (ANOVA) with Bonferroni’s correction for multiple comparisons or the Mann-Whitney U test, as appropriate. The central hypothesis of this analysis was based upon the response of isavuconazole in comparison to that of untreated controls. A two-tailed P value of ≤0.05 was considered to be statistically significant. Survival was plotted by Kaplan-Meier analysis. Differences in survival of treatment groups and untreated controls were analyzed by log rank test. Values are expressed as means ± standard errors of the means (SEMs). Pharmacokinetic parameters were compared using ANOVA or Student’s t test, as appropriate. Correlation of AUC_{0–24} and outcome variables was performed using Pearson’s correlation method.

**RESULTS**

Rabbits treated with ISA20 (1.32 ± 0.21 log CFU/g; mean ± SEM), ISA40 (0.06 ± 0.06 log CFU/g), and ISA60 (0.12 ± 0.06 log CFU/g) showed significant decreases (P ≤ 0.001) in residual fungal burden in comparison to that of UC (2.44 ± 0.12 log CFU/g) (Fig. 1). There also was a significant reduction in organism-mediated pulmonary injury as measured by total lung weight and infarct score. Rabbits treated with ISA40 and ISA60 showed significantly decreased (P ≤ 0.001) total lung weights in comparison to that of UC (23.5 ± 2.4 and 24.8 ± 3.6 g, respectively, versus 48.9 ± 2.2 g). Rabbits treated with ISA40 and ISA60 also demonstrated significant decreases (P ≤ 0.001) in pulmonary infarct score in comparison to that of UC (0.56 ± 0.34 and 1.22 ± 0.40, respectively, versus 5.71 ± 0.18). ISA20-treated rabbits had significantly lower infarct scores (P ≤ 0.01) (4.00 ± 0.27 in comparison to that of UC (Fig. 1). There also was significantly prolonged (P ≤ 0.001) survival of rabbits treated with ISA40 (6 of 9 [66.7%] surviving) and ISA60 (4 of 9 [44.4%]) in comparison to that of UC (0 of 8 [0%]) (Fig. 1).

Rabbits treated with ISA40 and ISA60 had lower GMIs during the study. ISA40- and ISA60-treated rabbits showed significantly lower (P < 0.01) GMIs than that of UC rabbits (Fig. 2, top). Consistent with the lower serum GMI, there were significantly lower (P < 0.001) GMIs in BAL fluid from rabbits treated with ISA40 and ISA60 than that of UC rabbits (Fig. 2, bottom). There were significantly lower (P ≤ 0.05) plasma (1→3)-β-D-glucan levels during experiments in ISA20-, ISA40-, and ISA60-treated rabbits (P ≤ 0.01) than for UC (Fig. 3).

AUC_{0–24} values after a single oral dose in normal animals for ISA20, ISA40, ISA60, and ISA90 were 3.3 × 10^{3} ± 0.4 × 10^{3}, 5.9 × 10^{3} ± 0.8 × 10^{3}, 32.8 × 10^{3} ± 6.7 × 10^{3}, and 58.2 × 10^{3} ± 7.4 × 10^{3} ng · h/ml, respectively, and for clearance were 15.7 ± 3.1, 17.8 ± 2.3, 3.4 ± 0.9, and 2.0 ± 0.6 ml/h (Fig. 4; Table 1). On the other hand, AUC_{0–24} values after per os (p.o.) administration of BAL8557 for 6 days to the infected animals for ISA20, ISA40, and ISA60 were 59.7 × 10^{3} ± 13.8 × 10^{3}, 141 × 10^{3} ± 32.9 × 10^{3}, and 197 × 10^{3} ± 27.8 × 10^{3} ng · h/ml, respectively, consistent with linear kinetics (Fig. 4; Table 1).
FIG 4 (Top) Mean plasma profiles of BAL4815 after single oral-dose administration of BAL8557 prodrug equivalent to active compound of 20, 40, 60, and 90 mg/kg/day. (Bottom) Mean plasma profiles of isavuconazole after oral dose administration of prodrug isavuconazonium sulfate for 6 days to the infected animals. A loading oral dose of 90 mg/kg of isavuconazole was administered, followed by once-daily maintenance doses of 20, 40, and 60 mg/kg.
and lung weight (activity similar to those of liposomal amphotericin B (26, 27) and rabbits with invasive pulmonary aspergillosis reveals antifungalies in the same animal model system of persistently neutropenic mental rabbit model showed dose proportionality in near steady state. This is similar to that which is observed in human volunteers (25, 28, 29) receiving as much as 600 mg. The near steady-state plasma exposure of isavuconazole in the rabbit model where maximal antifungal activity was achieved ranged from $59.7 \times 10^3$ to $141 \times 10^3$ ng · h/ml, which also correlates with the plasma exposure achieved in the dose regimen used in the phase III clinical trial of invasive aspergillosis (30). Following a loading regimen of 372 mg of isavuconazonium sulfate (equivalent to 200 mg isavuconazole, active moiety) given every 8 h over 48 h and a maintenance dose of 372 mg/day, Desai and colleagues reported a mean plasma AUC in patients of 97.9 μg · h/ml for this dosing regimen (31), which falls just under the AUC of the 40-mg/kg/day isavuconazole maintenance dosage achieved in the rabbit model. Consistent with human plasma pharmacokinetic data, clearance of isavuconazole is relatively low in single- and multiple-dosing studies of the rabbits and is compatible with an extended plasma half-life.

Our data indicate that dosages above 20 mg/kg/day in rabbits

Figure 5 depicts the pulmonary distribution of isavuconazole. There was a significant pharmacodynamic correlation between AUC and key outcome variables of residual fungal burden ($r = 0.61; P = 0.045$), pulmonary infarct score ($r = 0.66; P = 0.017$), and lung weight ($r = 0.44; P = 0.052$) (Fig. 6). The maximum effect on residual fungal burden and on pulmonary infarct score appears to coincide with an AUC of approximately 20,000 ng · h/ml.

**DISCUSSION**

Isavuconazole in this study was highly effective in treatment of experimental invasive pulmonary aspergillosis in persistently neutropenic rabbits as measured by a panel of outcome variables: residual fungal burden, survival, markers of pulmonary injury (lung weights and infarct scores), serum GMI, BAL fluid GMI, and serum (1→3)-β-d-glucan. The dosages of 40 mg/kg/day and 60 mg/kg/day yielded AUCs of $141 \times 10^3$ ng · h/ml and were comparable in antifungal activity by all parameters. Isavuconazole at 20 mg/kg ($59.7 \times 10^3 \pm 14 \times 10^3$ ng · h/ml) was less active than at the higher dosages. These findings might suggest that administration of higher dosages of isavuconazole for some isolates of *A. fumigatus* with elevated MICs may be beneficial.

Comparison of these effects of isavuconazole to previous studies in the same animal model system of persistently neutropenic rabbits with invasive pulmonary aspergillosis reveals antifungal activity similar to those of liposomal amphotericin B (26, 27) and posaconazole (22). The antifungal activity of isavuconazole was similar across all major outcome variables that were used to study these antifungal agents. These dosages demonstrated a robust response to isavuconazole in significantly increased survival, resolution of circulating serum biomarkers [galactomannan and (1→3)-β-d-glucan], and reduction of markers of organism-mediated pulmonary injury (lung weight and pulmonary infarct score).

The plasma pharmacokinetics of isavuconazole in the experimental rabbit model showed dose proportionality in near steady state.

### TABLE 1 Pharmacokinetic parameters of isavuconazole (BAL4815) after oral administration of isavuconazonium (BAL8557) prodrug equivalent to active compound of 20, 40, 60, and 90 mg/kg/day

<table>
<thead>
<tr>
<th>Dose (mg/kg p.o.)</th>
<th>Single dose</th>
<th>6 doses to infected rabbits</th>
<th>CL (ml/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>$3.3 \times 10^3 \pm 0.4 \times 10^3$</td>
<td>$59.7 \times 10^3 \pm 13.8 \times 10^3$</td>
<td>$15.7 \pm 3.1$</td>
</tr>
<tr>
<td>40</td>
<td>$5.9 \times 10^3 \pm 0.8 \times 10^3$</td>
<td>$141 \times 10^3 \pm 32.9 \times 10^3$</td>
<td>$17.8 \pm 2.3$</td>
</tr>
<tr>
<td>60</td>
<td>$32.8 \times 10^3 \pm 6.7 \times 10^3$</td>
<td>$197 \times 10^3 \pm 27.8 \times 10^3$</td>
<td>$3.4 \pm 0.9$</td>
</tr>
<tr>
<td>90</td>
<td>$58.2 \times 10^3 \pm 7.4 \times 10^3$</td>
<td>$-a$</td>
<td>$2.0 \pm 0.6$</td>
</tr>
</tbody>
</table>

The loading dose of 90 mg/kg/day was administered only on day 1 to measure the exposure of a single loading dose.

<table>
<thead>
<tr>
<th>mg/lung (BAL4815)</th>
<th>ISA 20</th>
<th>ISA 40</th>
<th>ISA 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>ng/ml</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
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**FIG 5 (Top)** Concentrations of isavuconazole (BAL4815) in the lung tissue after oral administration of BAL8557 for 12 days to the infected animals. An initial loading dose of 90 mg/kg of isavuconazole was administered orally, and thereafter the drug was administered daily at 20, 40, and 60. **(Bottom)** Concentrations of isavuconazole in the BAL fluid and BAL fluid supernatant after oral administration of prodrug isavuconazonium sulfate for 12 days to the infected animals. An initial loading dose of 90 mg/kg of isavuconazole was administered orally, and thereafter the drug was administered daily at 20, 40, and 60 mg/kg.
REFERENCES

Pfizer, Methylgene, SigmaTau, and Trius. W.W.H. has acted as a consultant to research grants for experimental and clinical antimicrobial pharmacotherapeutics and receives support from the Save our Sick Kids Foundation, as well as as a Scholar of Pediatric Infectious Diseases of the Sharp Family Foundation. T.J.W. is a Scholar of the Henry Schueler Foundation and a research assistant in this work. We are grateful to Brigitte T. Huertas and Egle Petraityte for their laboratory technical assistance in this work.

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REFERENCES


