

Efficacy of Posaconazole as Treatment and Prophylaxis against *Fusarium solani*[∇]

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Invasive fusariosis is a highly aggressive fungal infection associated with high mortality in heavily immunocompromised patients. Although posaconazole is efficacious as salvage therapy against infections caused by *Fusarium* species, concerns remain regarding this agent in the setting of reduced potency. To evaluate the efficacy of posaconazole as treatment or prophylaxis against invasive fusariosis caused by *Fusarium solani*, we utilized a neutropenic murine model of disseminated disease. ICR mice were administered escalating doses of posaconazole (6.25, 12.5, 25, or 50 mg/kg of body weight twice daily [BID]) by oral gavage beginning 2 days prior to inoculation in the prophylaxis studies or beginning 12 h after inoculation as treatment. Therapy was continued until day 9 postinoculation, and animals were monitored off therapy until day 15 for survival. Fungal burden was assessed as CFU in the kidneys. A clear dose-response relationship was observed, as the highest dose of posaconazole (50 mg/kg) was the most effective in prolonging survival and reducing tissue fungal burden both as prophylaxis and as treatment. This dose response was associated with high posaconazole serum concentrations as measured by bioassay. However, the extent of efficacy was also dependent on the infecting inoculum, as greater increases in survival and reductions in fungal burden were observed with the lower inocula tested. In this model high dosages of posaconazole were effective as treatment and prophylaxis against disseminated fusariosis caused by *F. solani*.

Fusarium species are among the leading causes of invasive mold infections among patients with hematologic malignancies and those undergoing hematopoietic stem cell transplantation (HSCT), with mortality exceeding 70% (1, 2, 15). Risk factors for disseminated infection include neutropenia, lymphopenia, graft-versus-host disease, corticosteroids, and immunosuppressive therapy (1, 2). *In vitro* studies have reported moderate to negligible activity of available antifungals against *Fusarium*, with amphotericin B and itraconazole having minimal activity (3, 8, 16). Reduced *in vitro* potency has also been reported for voriconazole and posaconazole when measured by standard microdilution methodology (3, 8, 16). However, both voriconazole and posaconazole have been shown to be effective in animal models of invasive fusariosis, including infections caused by isolates with reduced *in vitro* susceptibility (4, 10, 20).

Modifications to testing parameters used in determining the *in vitro* activity (e.g., inoculum size and incubation period) may greatly affect the MIC against *Fusarium* isolates (8, 16). This suggests that the *in vitro* activity as measured by standard microdilution methodology may not be predictive of *in vivo* efficacy. Our objective was to assess the *in vivo* efficacy of posaconazole against disseminated fusariosis caused by *Fusarium solani*, both as treatment and as prophylaxis, and to compare this activity to the *in vitro* potency measured at 24 and 48 h against different starting inocula. In addition, posaconazole concentrations were measured in animals that were ad-

ministered the same dosages used for treatment and prophylaxis.

MATERIALS AND METHODS

Isolate. *F. solani* species complex clinical isolate 95-2478 was obtained from the University of Texas Health Science Center at San Antonio. Conidia were inoculated onto potato dextrose agar and allowed to incubate at 37°C for 10 days. A mixture of micro- and macroconidia were harvested by flooding the plates with sterile physiologic saline and gentle scraping.

Antifungals. For *in vitro* testing, posaconazole (Schering-Plough, Kenilworth, PA) was dissolved in dimethyl sulfoxide (DMSO), and further dilutions were made in RPMI medium supplemented with 2% glucose and buffered with 0.165 M 4-morpholinepropanesulfonic acid (pH 7.0). For *in vivo* studies, the commercially available posaconazole oral suspension (Noxafil; Schering-Plough) was used.

***In vitro* susceptibility and pharmacodynamics.** The *in vitro* potency and pharmacodynamics of posaconazole were measured using the CLSI M38-A broth microdilution and the tetrazolium salt 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) colorimetric assays, respectively (8, 11, 13). Briefly, conidia were added to microtiter trays containing posaconazole (final concentration range, 0 to 8 µg/ml) in RPMI with 2% glucose to achieve a starting inoculum of either 1×10^4 or 1×10^5 conidia/ml. After 24 and 48 h of incubation, the MIC was read as the lowest concentration of posaconazole that resulted in complete visual inhibition of growth compared to the growth control. The XTT assay was performed in triplicate as previously reported (12). Absorbance was read at 492 nm using a microplate spectrophotometer, and readings were converted to percent absorbance (growth control wells set as 100%, medium control wells set as 0%).

Bioassay. Serum concentrations of posaconazole were measured in uninfected and nonimmunosuppressed mice following 5 doses of posaconazole at 6.25, 12.5, 25, or 50 mg/kg of body weight administered twice daily (BID) 4 h following administration of the last dose. Posaconazole concentrations were measured by bioassay using previously described methods (9, 19). Briefly, 100 µl of serum was instilled in duplicate into wells cored into potato dextrose agar that had been inoculated with 1×10^4 cells/ml of *Candida kefyr* ATCC 66028. After 48 h of incubation, zones of inhibition were measured, and posaconazole concentrations were interpolated from a standard curve.

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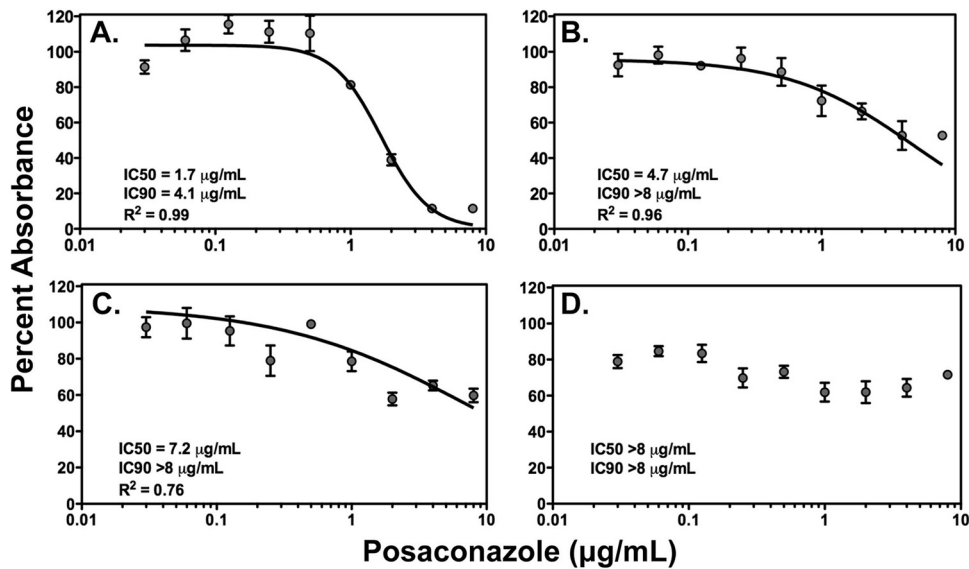


FIG. 1. *In vitro* dose-response curves for posaconazole against *Fusarium solani* clinical isolate 95-2478 at 1×10^4 conidia/ml (A and B) and 1×10^5 conidia/ml (C and D) after 24 h (A and C) and 48 h (B and D) of incubation. Curves were generated by fitting XTT absorbance data to a four-parameter inhibitory sigmoid model. The concentrations resulting in 50% and 90% inhibition of growth (IC_{50} and IC_{90}) and coefficients of determination (R^2) are reported.

Infection model. An established murine model of invasive fusariosis was used for all experiments (4). Outbred ICR mice were rendered temporarily neutropenic by a single dose of 5-fluorouracil (150 mg/kg intravenously [i.v.]) 1 day prior to inoculation. Mice were infected intravenously using an 0.2-ml volume of inoculum. Different starting inocula were tested and ranged from 7.5×10^4 to 2.9×10^6 conidia/mouse. This protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio, and animals were maintained in accordance with the standards of the American Association for Accreditation of Laboratory Animal Care (14).

Prophylaxis and treatment. For treatment and prophylaxis studies four posaconazole regimens (6.25, 12.5, 25, and 50 mg/kg administered by oral gavage BID) were compared to untreated controls. In prophylaxis studies ($n \geq 10$ animals per regimen within each regimen), posaconazole was begun 2 days prior to inoculation, while in treatment studies ($n \geq 20$ animals per regimen) therapy began 12 h after inoculation. Animals that appeared moribund prior to the end of the study were euthanized, the kidneys were harvested, and death was recorded as occurring the next day. Kidneys were homogenized in sterile saline by using a tissue homogenizer, and serial dilutions were prepared in sterile saline and plated onto potato dextrose agar. Following 48 h of incubation, the number of CFU per gram of kidney tissue for each animal was calculated. Treatment studies were repeated on separate occasions to ensure reproducibility.

Data analysis. The XTT reduction assay data were fitted to a four-parameter inhibitory sigmoid model (modified Hill equation) using computer curve-fitting software (Prism 5; GraphPad Software, Inc.) to derive concentrations resulting in 50% and 90% inhibition (IC_{50} and IC_{90} , respectively). Goodness of fit was assessed by R^2 and standard error of the IC_{50} value. Survival was plotted by Kaplan-Meier analysis, and differences in median survival time and percent survival were analyzed by the log rank test and Fisher's exact test, respectively. Differences in fungal burden endpoints between control and each antifungal regimen were assessed for significance by the Mann-Whitney test. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

***In vitro* susceptibility and pharmacodynamics.** Similarly to previous studies we found discordant *in vitro* susceptibility and pharmacodynamic results for posaconazole between the two incubation periods and starting inocula (16). At the lower inoculum, a 4-fold increase in the MIC from 2 to 8 $\mu\text{g/ml}$ was observed between the 24- and 48-h time points. Similarly, when

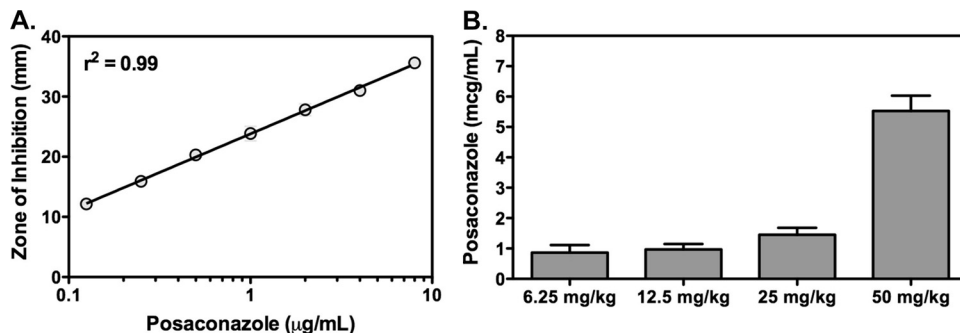


FIG. 2. Posaconazole bioassay standard curve (A) and serum concentrations (B) measured in uninfected and nonimmunosuppressed mice. Blood was collected 4 h after the fifth dose in each regimen (posaconazole at 6.25, 12.5, 25, and 50 mg/kg by oral gavage BID) used in the treatment and prophylactic studies. *Candida kefyr* ATCC 66028 served as the indicator organism for the bioassay, and zones of inhibition were measured after 48 h of incubation.

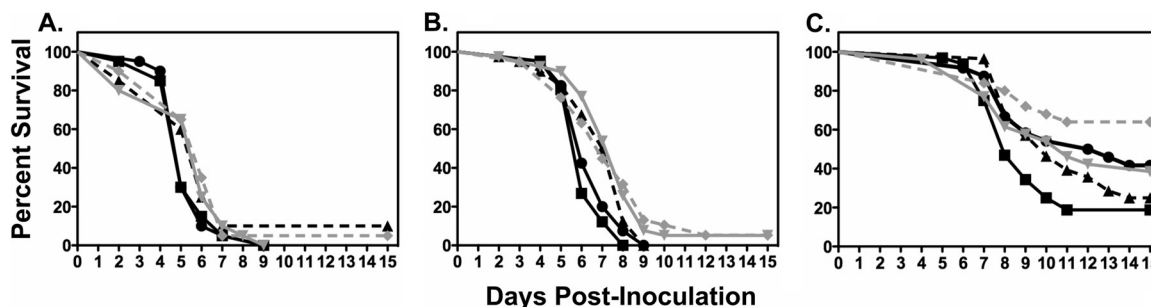


FIG. 3. Kaplan-Meier survival curves for the different posaconazole treatment dosage groups at the high inoculum (2.9×10^6 conidia/animal) (A), mid-inoculum range (6.5×10^5 to 6.8×10^5 conidia/animal) (B), and low inoculum range (7.7×10^4 to 7.8×10^4 conidia/animal) (C). Twice-daily dosing with oral posaconazole (black circles, 6.25 mg/kg; black triangles, 12.5 mg/kg; gray inverted triangles, 25 mg/kg; gray diamonds, 50 mg/kg) was initiated 12 h following intravenous inoculation in neutropenic mice, and values were compared to those for untreated controls (black squares).

the concentration-response curve was obtained using the XTT colorimetric assay, a 2.75-fold increase in the posaconazole IC_{50} value was also measured between the two incubation periods, and the IC_{90} value was also increased ($4.1 \mu\text{g/ml}$ versus $>8 \mu\text{g/ml}$, respectively) (Fig. 1A and B). When the starting inoculum was increased to 1×10^5 conidia/ml, the potency of posaconazole against this isolate was markedly reduced at both 24 and 48 h as measured by elevated MICs ($>8 \mu\text{g/ml}$ at both time points) and further increases in the calculated IC_{50} and IC_{90} values (Fig. 1C and D).

Serum concentrations. Posaconazole serum concentrations were measured by bioassay at 4 h following the fifth dose in uninfected and nonimmunosuppressed mice. This time point was chosen as it has been reported that a posaconazole concentration of $\geq 0.7 \mu\text{g/ml}$ drawn 3 to 5 h postdose is associated with improved prophylactic efficacy (7). In addition, average serum concentrations of $>1.2 \mu\text{g/ml}$ were associated with improved efficacy when posaconazole was used as salvage therapy for invasive aspergillosis (22). As shown in Fig. 2, the highest posaconazole dosage group (50 mg/kg BID) achieved a median posaconazole serum concentration of $5.3 \mu\text{g/ml}$ (range, 4.9 to $6.2 \mu\text{g/ml}$), and decreasing concentrations were observed as the dosages were lowered.

Treatment. In treatment studies an inoculum effect and a small dose-response relationship were observed for posaconazole. In the initial study, no improvement in survival (Fig. 3) or reduction in tissue fungal burden within the kidneys (Table 1) was observed following inoculation with 2.9×10^6 conidia/animal. Based on these results this inoculum was used only once ($n = 10$ animals per group) and lower infecting inocula were evaluated for the remainder of the study. In the mid-inoculum studies (6.5×10^5

to 6.8×10^5 conidia/animal), median survival increased in animals that received BID posaconazole doses at 12.5 mg/kg (7.5 days), 25 mg/kg (8 days), and 50 mg/kg (7 days) compared to untreated controls (6 days; $P < 0.05$), although the percentage of animals in these groups surviving to the end of the study period was not improved (range, 0 to 5%). These results were consistent with the modest reductions in kidney tissue fungal burden within the 25- and 50-mg/kg dosage groups compared to untreated controls (Table 1). No differences in tissue fungal burden were observed in untreated controls that succumbed early to infection (≤ 5 days postinoculation) compared to those that survived longer (≥ 7 days).

When the infecting inocula were further lowered (7.7×10^4 to 7.8×10^4 conidia/animal), improvements in median survival (>15 days) and percent survival (64%) were observed for those animals in the posaconazole 50-mg/kg BID dosage group compared to untreated controls (8 days and 18%, respectively; $P \leq 0.005$). In addition, a significant reduction in kidney fungal burden was also observed in mice that received this regimen. However, it should also be noted that in the control group, the median survival was increased and tissue fungal burden was lower than in the corresponding untreated control group in the mid-inoculum level. Although the median survival times in the posaconazole 6.25-, 12.5-, and 25-mg/kg BID groups were longer than those of untreated controls (12.5, 10, and 11 days, respectively), these differences were not significant, nor were the percentages of animals that survived to the end of the study period (42, 25, and 38%, respectively). However, posaconazole at 25 mg/kg BID did significantly reduce fungal burden within the kidneys.

TABLE 1. *Fusarium solani* kidney tissue fungal burden in mice that received posaconazole as treatment beginning 12 h after inoculation^a

Inoculum (no. of conidia/animal; no. of animals/group)	Median log ₁₀ CFU/g in kidneys (range) for group:				
	Control	PSC at mg/kg:			
		6.25	12.5	25	50
High (2.9×10^6 ; 10)	5.8 (4.9–6.1)	5.6 (5.1–6.2)	5.3 (4.7–5.5)	5.3 (4.8–5.9)	5.2 (4.4–5.9)
Middle (6.5×10^5 – 6.8×10^5 ; >20)	6.1 (3.4–6.4)	6.0 (4.4–6.5)	5.8 (4.8–6.5)	5.6 (2.4–6.4)*	5.4 (3.7–6.4)*
Low (7.7×10^4 – 7.8×10^4 ; >20)	5.3 (3.1–6.3)	5.0 (2.7–5.9)	5.1 (1.2–6.2)	5.0 (1.5–5.9)*	4.6 (1.2–6.0)*

^a Median log₁₀ CFU/g values and ranges are reported for each dosage group. *P* values are reported for posaconazole (PSC) regimens with significant reductions in log₁₀ CFU/g compared to untreated controls (*, $P < 0.05$).

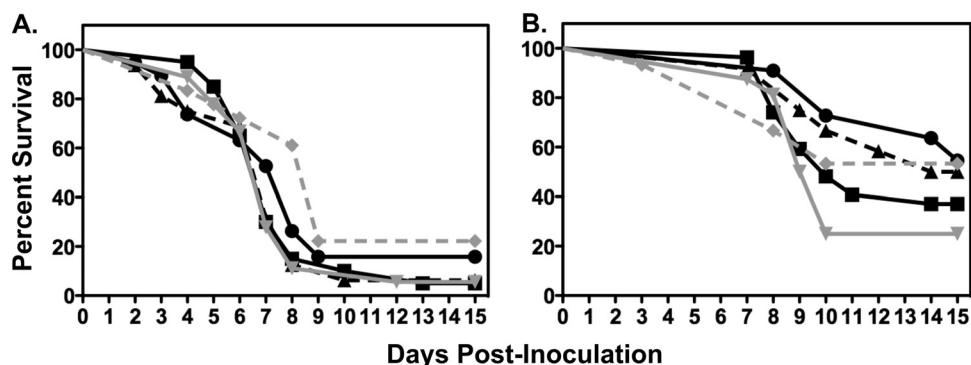


FIG. 4. Kaplan-Meier survival curves for the different posaconazole prophylaxis dosage groups at the mid-inoculum (6.5×10^5 conidia/animal) (A) and low inoculum (7.5×10^4 conidia/animal) (B). Twice-daily oral dosing with posaconazole (black circles, 6.25 mg/kg; black triangles, 12.5 mg/kg; gray inverted triangles, 25 mg/kg; gray diamonds, 50 mg/kg) was initiated 2 days prior to intravenous inoculation in neutropenic mice, and values were compared to those for untreated controls (black squares).

Prophylaxis. Due to the poor overall survival among the treatment groups and untreated controls at the highest inoculum studied, only the mid- and low inoculum ranges were evaluated in the prophylaxis studies. In the mid-inoculum study (6.5×10^5 conidia/animal), the median survival (9 days) was significantly improved in animals that received posaconazole at 50 mg/kg BID compared to untreated controls (7 days; $P \leq 0.002$; Fig. 4). In addition, tissue fungal burden was significantly reduced within the kidneys at this dosage level compared to untreated controls (Table 2). However, no improvements in survival or reductions in tissue fungal burden were observed with the other posaconazole regimens. As with the treatment studies, no differences in tissue fungal burden were observed between untreated controls that succumbed early to infection and those that survived longer.

Similar to that observed in the treatment studies, an inoculum effect was observed for survival in animals administered posaconazole prophylaxis. As shown in Fig. 4, the median survival in all prophylaxis groups and untreated controls at the low inoculum was higher than that observed at the mid-inoculum. However, only animals that received posaconazole at 50 mg/kg BID had a median survival (>15 days) that was significantly greater than that of untreated controls (10 days; $P = 0.02$), and only this dosage resulted in significant reductions in tissue burden within the kidneys (Table 2).

DISCUSSION

Invasive infections caused by *Fusarium* species are difficult to treat and are often associated with poor clinical outcomes.

The availability of advanced expanded-spectrum triazoles (i.e., voriconazole and posaconazole) has resulted in a modest improvement in treatment response. In a multicenter, open-label study of voriconazole as salvage therapy, a satisfactory global response was reported in 5 of 11 patients (45%) with fusariosis (17). However, it is important to remember that in this study stable disease was considered part of the global response. This is in contrast to a recent report of 21 patients who received posaconazole salvage therapy for proven or probable invasive fusariosis (18). In this retrospective analysis of three independent, multicenter open-label trials, which defined a successful outcome as complete or partial response and classified those with stable disease as failures, the overall response rate was 48%.

One potential reason for the poor response rates in clinical studies of invasive fusariosis is the limited activity of antifungal agents against *Fusarium* species (8, 10, 16). However, modifications to testing parameters used in determining the *in vitro* activity can affect these results. Paphitou et al. noted that an increased inoculum size and lengthened duration of incubation resulted in higher azole MICs (16). Interestingly, one of the *F. solani* isolates reported by these authors (24-h MIC, 2 $\mu\text{g/ml}$) was previously shown to be responsive *in vivo* to posaconazole at 100 mg/kg despite minimal *in vitro* activity of this azole (48-h MIC, >16 $\mu\text{g/ml}$) (10, 16). A recent study also reported improved survival in mice dosed with posaconazole at 100 mg/kg following infection with a *Fusarium oxysporum* isolate with an MIC of >16 $\mu\text{g/ml}$ (20).

The results of our study are consistent with these previous reports. Survival was improved and tissue fungal burden within

TABLE 2. *Fusarium solani* kidney tissue fungal burden in mice that received posaconazole as prophylaxis beginning 2 days prior to intravenous inoculation^a

Inoculum (no. of conidia/animal; no. of animals/group)	Median log ₁₀ CFU/g of kidneys (range) for group:				
	Control	PSC at mg/kg:			
		6.25	12.5	25	50
Middle (6.5×10^5 ; >10)	5.8 (1.3–6.5)	5.6 (4.0–6.2)	5.5 (3.2–6.2)	5.5 (3.2–6.3)	4.7 (1.3–6.2)*
Low (7.5×10^4 ; >10)	4.4 (3.3–6.1)	2.8 (1.6–6.0)	4.4 (1.3–5.9)	4.5 (1.8–6.1)	2.9 (1.3–5.1)**

^a Median log₁₀ CFU/g values and ranges are reported for each dosage group. *P* values are reported for posaconazole (PSC) regimens with significant reductions in log₁₀ CFU/g compared to untreated controls (*, $P = 0.02$; **, $P = 0.03$).

the kidneys was significantly reduced in mice that received escalating dosages of posaconazole. This was consistently found with the highest-dose regimen of posaconazole evaluated (50 mg/kg BID). We also found discrepancies between the *in vitro* activities of this azole when measured after 24 and 48 h and observed that the survival and tissue burden results were also related to the infecting inoculum sizes. This may be relevant as the lowest inoculum size used *in vitro* was similar to the lowest *in vivo* inoculum range where the greatest improvements in survival and reductions in tissue burden were observed. We also measured serum concentrations and found an association between levels achieved 4 h after administration of posaconazole and efficacy. Animals in the 50-mg/kg posaconazole BID group had the highest serum concentrations as well as the greatest improvements in survival and reductions in tissue burden. The median plasma concentration achieved in this group (5.3 µg/ml) was similar to the *in vitro* IC₉₀ value after 24 h of incubation at the lower starting inoculum (4.1 µg/ml) but was less than that for the higher inoculum (7.2 µg/ml). These data suggest that the *in vivo* efficacy was also related to the starting inoculum, which is in agreement with the results of Paphitou et al. (16), as well as clinical data demonstrating improved outcomes with higher posaconazole concentrations (6, 7, 22).

Although our results are consistent with those reported by others, several differences must be considered. The types of immunosuppression used, if any, were different among the studies, as were the species of *Fusarium* tested (*F. oxysporum* and *F. solani*), and the inoculum sizes used to establish infection. In addition, we did not evaluate the efficacy of combination therapy, which was recently shown to be effective (20). Finally, the serum concentrations measured at the highest-dose posaconazole regimen used in our study are much higher than can currently be achieved clinically (5–7, 21). A more detailed analysis comparing a larger number of *Fusarium* isolates along with a more rigorous pharmacokinetic/pharmacodynamic analysis is needed to clarify the relationships between *in vitro* potency, *in vivo* efficacy, and bloodstream concentrations.

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