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Pharmacodynamics of a New Triazole, Posaconazole, in a Murine Model of Disseminated Candidiasis

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Previous in vivo studies have characterized the pharmacodynamic characteristics of two triazole compounds, fluconazole and ravuconazole. These investigations demonstrated that the 24-h area under the concentration-time curve (AUC)/MIC ratio is the critical pharmacokinetic-pharmacodynamic (PK-PD) parameter associated with treatment efficacy. Further analysis demonstrated that a free-drug triazole 24-h AUC/MIC ratio of 20 to 25 was predictive of treatment success in both experimental models and clinical trials. We used a neutropenic murine model of disseminated *Candida albicans* infection to similarly characterize the time course activity of the new triazole, posaconazole. The PK-PD parameters (percent time above MIC, AUC/MIC ratio, and peak serum drug level/MIC ratio) were correlated with in vivo efficacy, as measured by organism number in kidney cultures after 48 h of therapy. Kinetics and protein binding following oral posaconazole dosing were performed in neutropenic infected mice. Peak levels and AUC from 0 h to ∞ values were nonlinear over the 16-fold dose range studied. Serum drug elimination half-life ranged from 12.0 to 17.7 h. Protein binding was 99%. Single dose postantifungal effect studies demonstrated prolonged suppression of organism regrowth after serum posaconazole levels had fallen below the MIC. Treatment efficacy with the four dosing intervals studied was similar, supporting the AUC/MIC ratio as the PK-PD parameter predictive of efficacy. Nonlinear regression analysis also suggested that the AUC/MIC ratio was strongly predictive of treatment outcomes (AUC/MIC ratio $R^2 = 83\%$; peak serum drug/MIC ratio $R^2 = 85\%$; time that serum levels of posaconazole remained above the MIC $R^2 = 65\%$). Similar studies were conducted with 11 additional *C. albicans* isolates with various posaconazole susceptibilities (MIC, 0.015 to 0.12 μg/ml) to determine if a similar 24-h AUC/MIC ratio was associated with efficacy. The posaconazole free-drug AUC/MIC ratios were similar for all of the organisms studied (6.12 to 26.7, mean ± SD = 16.9 ± 7.8, *P* value, 0.42). These free-drug AUC/MIC ratios are similar to those observed for other triazoles in this model.

Antimicrobial pharmacodynamic characterizations have provided an understanding of the relationship between drug exposure and treatment efficacy. Therapeutic outcome predictions based upon these pharmacodynamic studies have correlated well in treatment against both susceptible and resistant pathogens (3). In addition, the strength of these in vivo predictions has been shown to be independent of animal species, infection site, and duration of treatment studied. These pharmacodynamic analyses in animal infection models have proven useful for the design of optimal dosing regimens and the validation of susceptibility breakpoint guidelines (8, 9, 18).

Prior in vivo studies have demonstrated that the pharmacokinetic-pharmacodynamic (PK-PD) parameter predictive of triazole efficacy against *Candida albicans* is the 24-h area under the concentration-time curve (AUC)/MIC ratio (1, 2, 16). Studies have also suggested that a 24-h AUC/MIC ratio target in the range of 20 to 25 is associated with treatment efficacy in experimental in vivo models when defined as the microbiologic endpoint of 50% effective dose (ED_{50}) or 80% survival in animals (1, 16, 23, 26; K. Sorenson, S. Corcoran, S. Chen, D. Clark, V. Tembe, O. Lomovskaya, and M. Dudley, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1271, 1999). The AUC/MIC ratio has also been shown predictive of outcomes in fluconazole clinical trials of both mucosal and deep-seated *Candida* infections (1, 14, 22; C. J. Clancy, C. A. Kauffman, A. Morris, M. L. Nguyen, D. C. Tanner, D. R. Snydman, V. L. Yu, and M. H. Nguyen, Progr. Abstr. Infect. Dis. Soc. Am. 36th Annu. Meet., abstr. 98, p. 93, 1998).

In the present study we have characterized the PK-PD parameter that is predictive of efficacy of a new triazole, posaconazole, in a neutropenic murine model of disseminated candidiasis. Furthermore, we have determined the magnitude of the PK-PD parameter required to achieve efficacy for numerous strains of *C. albicans* with various azole susceptibilities in order to provide a framework for the preliminary in vivo breakpoints for posaconazole.

MATERIALS AND METHODS

Organisms. Twelve clinical isolates of *C. albicans* designated K-1, 412, 580, 98-17, 98-234, 98-210, 5810, 1490, 2-76, W-2, 2438, and 2183 were chosen to include both fluconazole-susceptible and -resistant strains. Isolates were also chosen based upon a relatively similar degree of virulence in this animal model, as determined by the amount of growth in the kidneys of untreated animals over 48 h (mean ± standard deviation [SD] = 3.29 ± 0.53 $\log_{10}$ CFU/kidney). *C. albicans* K-1 and 580 were isolated from patients with systemic candidiasis. The other isolates were from mucosal infections. Five of the isolates had reduced susceptibility to fluconazole in vitro (four susceptible, dose dependent and one resistant). Isolates 412, 2307, 2512, and 2183 were kindly provided by J. Lopez-Ribot (15). Isolate 2-76 was kindly provided by T. White (21). The organisms were maintained, grown, subcultured, and quantified on Sabouraud dextrose

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agar (SDA) plates (Difco Laboratories, Detroit, Mich.). Twenty-four hours prior to study, organisms were subcultured at 35°C.

Antifungal agent. Posaconazole was obtained as a powder from Schering-Plough Pharmaceuticals for in vitro susceptibility testing. The powder was stored desiccated at ambient temperature. Drug solutions for in vitro studies were prepared on the day of study by dissolving the powder in dimethyl sulfoxide. Drug for oral administration to animals was obtained as a 40-mg/ml clinical suspension from Schering-Plough and was stored at ambient temperature. Lower dose levels were prepared using sterile water as the diluent.

In vitro susceptibility testing. MICs were determined using the NCCLS M27-A method (17). Determinations were performed in duplicate on three separate occasions. Final results are expressed as the mean of these results.

Animals. Six-week-old ICR/Swiss specific-pathogen-free female mice weighing 23 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, Ind.). Animals were housed in groups of five and allowed access to food and water ad libitum. Animals were maintained in accordance with the American Association for Accreditation of Laboratory Care criteria (19). Animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial VA Hospital.

Infection model. Mice were rendered neutropenic (polymorphonuclear cells $< 100/mm^3$) by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, Ind.) subcutaneously for 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before infection. Absolute white blood cell and neutrophil counts were monitored every 24 h throughout the period of study with a Coulter Counter and peripheral blood smears, respectively. Neutrophil counts remained at or below $100/mm^3$ throughout the study.

Organisms were subcultured on SDA 24 h prior to infection. The inoculum was prepared by placing three to five colonies into 5 ml of sterile pyrogen-free 0.9% saline warmed to 35°C. The final inoculum was adjusted to a 0.6 transmittance at 530 nm. Fungal counts of the inoculum determined by viable counts on SDA were 5.22 ± 0.14 $\log_{10}$ CFU/ml.

Disseminated infection with the *Candida* organisms was achieved by injection of 0.1 ml of inoculum via lateral tail vein 2 h prior to start of drug therapy. At the end of the study period, animals were sacrificed by CO_2 asphyxiation. After sacrifice the kidneys of each mouse were immediately removed and placed in sterile 0.9% saline at 4°C. The homogenate was then serially diluted 1:10, and aliquots were plated on SDA for viable fungal colony counts after incubation for 24 h at 35°C. The lower limit of detection was 100 CFU/ml. Results were expressed as the mean CFU/kidney for two mice (four kidneys).

Pharmacokinetics. The single-dose pharmacokinetics of posaconazole were determined in individual neutropenic infected ICR/Swiss mice following oral gavage administration of 320, 80, and 20 mg/kg administered in 0.2-ml volumes. Samples were analyzed by a microbiologic assay. Protein binding studies utilized previously described ultrafiltration methods (7). For the microbiologic assay, *Candida kefyr* ATCC 46764 was used as the assay organism in yeast nitrogen base agar supplemented with glucose and trisodium citrate (28). Groups of three halothane-anesthetized mice were sampled three times by retrooribital puncture, and blood was collected in heparinized capillary tubes (Fisher Scientific, Pittsburgh, Pa.). The volume collected with each sample ranged from 30 to 50 μl. Less than 5% of the total mouse blood volume was collected from any individual animal. The samples were collected at 3- to 12-h intervals over 24 h. Capillary tubes were immediately centrifuged (model MB centrifuge; International Equipment Co.) at $10{,}000 \times g$ for 5 min. The serum samples (10 μl each) were then placed in the agar wells. Assays of serum and standard curves were performed on the same day. Intraday variation was less than 5%. The lower limit of detection for this assay was 0.50 μg/ml.

A noncompartmental model was used in the kinetic analysis. Pharmacokinetic constants including elimination half-life and concentration at time zero (C_0) were calculated via nonlinear least-squares techniques. The AUC was calculated by the trapezoidal rule. For treatment doses for which no kinetics were determined, pharmacokinetic parameters were estimated by linear extrapolation from the highest and lowest dose levels used in the above kinetic studies.

In vivo PAFE. Infection in neutropenic mice was produced as described above. Two hours after infection with *C. albicans* K-1, mice were treated with one of three single oral doses of posaconazole (10, 5, or 2.5 mg/kg). Groups of two treated and two control mice were sacrificed at sampling intervals ranging from 3 to 12 h. Control growth was determined with five sampling points over 24 h. Posaconazole-treated animals were sampled six times over 48 h. Kidneys were removed at each time point and processed immediately for CFU determination as outlined above. The time that serum levels of posaconazole remained above the MIC ($T>$MIC) for the organism following the three doses was calculated from the pharmacokinetic data. Free drug concentrations were utilized for kinetic calculations. Total drug concentrations remained above the MIC for the entire period of study. The postantifungal effect (PAFE) was calculated by determining the time it took for controls to increase 1 $\log_{10}$ CFU/kidney (c) and subtracting this from the amount of time it took organisms from the treated animals to grow 1 $\log_{10}$ CFU/kidney (t) after serum drug levels fell below the MIC for the organism [$\text{PAFE} = t - c$] (6).

Pharmacodynamic parameter determinations. Neutropenic mice were infected with *C. albicans* K-1 2 h prior to the start of therapy. Twenty-four dosing regimens were chosen to determine the impact of dose level and dosing interval on posaconazole efficacy. These 24 regimens were comprised of six total dose levels (0.625, 2.5, 10, 40, 160, and 640 mg/kg/24 h), four dosing intervals (every 6, 12, 24, and 48 h), and a single treatment duration (48 h). This wide variety of regimens was used to minimize the interdependence among the three pharmacodynamic parameters studied and also to describe the complete dose-response relationship. Groups of two mice were treated with each dosing regimen. Drug was administered by oral gavage in 0.2-ml volumes. Mice were sacrificed at the end of therapy, and kidneys were removed for CFU determinations as described above. Untreated control mice were sacrificed just before treatment and at the end of the experiment. Efficacy was defined as the change in $\log_{10}$ CFU/kidney over the study period and was calculated by subtracting the mean $\log_{10}$ CFU/kidney in treated mice from the mean number of CFU from kidneys of two mice at the end of therapy in untreated animals.

Pharmacodynamic parameter magnitude determinations. Studies similar to those described above were performed with 11 additional strains of *C. albicans* (98-17, 98-234, 98-210, 412, 580, 1490, 2-76, W-2, 2438, 5810, and 2183). Attempts were made to choose organisms with different susceptibilities to posaconazole. However, the range of posaconazole MICs in our stock of organisms varied only eightfold. This group of organisms includes both fluconazole-susceptible, -susceptible dose-dependent, and -resistant strains. Dosing studies were designed to vary the magnitude of the pharmacodynamic parameters. The five total dose levels varied from 0.08 to 20 mg/kg/24 h. Doses were administered twice (every 24 h) for the 2-day study period. Groups of two mice were again used for each dosing regimen. At the end of the study, mice were euthanized and kidneys were immediately processed for CFU determinations.

Data analysis. A sigmoid dose-effect model was used to measure the in vivo potency of posaconazole. The model is derived from the Hill equation: $E = (E_{max} \times D^N)/(ED_{50}{}^N + D^N)$, where E is the observed effect (change in $\log_{10}$ CFU/kidney compared with untreated controls at the end of the treatment period), D is the total dose, E_{max} is the maximum effect, ED_{50} is the dose required to achieve 50% of the E_{max}, and N is the slope of the dose-effect relationship. The correlation between efficacy and each of the three parameters studied was determined by nonlinear least-squares multivariate regression analysis (Sigma Stat; Jandell Scientific Software, San Rafael, Calif.). The coefficient of determination (R^2) was used to estimate the percent variance in the change of $\log_{10}$ CFU/kidney over the treatment period for the different dosing regimens that could be attributed to each of the pharmacodynamic parameters. Calculations were performed using both total and free drug concentrations.

We also calculated the dose required to produce 25, 50, and 75% of the maximal effect (ED_{25}, ED_{50}, and ED_{75}) over the treatment period for each of the dosing intervals. The calculated values for each dosing interval were compared using analysis of variance (ANOVA; Sigma Stat; Jandell Scientific Software). If the AUC/MIC parameter was more predictive of posaconazole in vivo activity, then these calculated doses would be similar for each dosing interval. If $T>$MIC was the predictive parameter, the calculated doses would be lower with shorter dosing intervals. And lastly, if the peak serum drug level/MIC ratio was the predictive parameter, the calculated doses would be smaller with longer dosing intervals.

The ED_{50} was determined for the 24-h dosing regimen for each of the 12 strains. The magnitude of the pharmacodynamic parameter predictive of the efficacy of posaconazole was compared for each of the 12 isolates at the respective ED_{50} dosing level. Again, both total and free drug concentrations were considered. The significance of differences among these values was determined by ANOVA (Sigma Stat; Jandell Scientific Software). A two-tailed P value of <0.05 was considered statistically significant.

RESULTS

In vitro susceptibility testing. The 48-h MICs for the 12 *C. albicans* organisms studied varied eightfold (range, 0.015 to 0.12 μg/ml). The fluconazole MICs for this group of organisms varied more than 500-fold (range, 0.25 to >128 μg/ml).

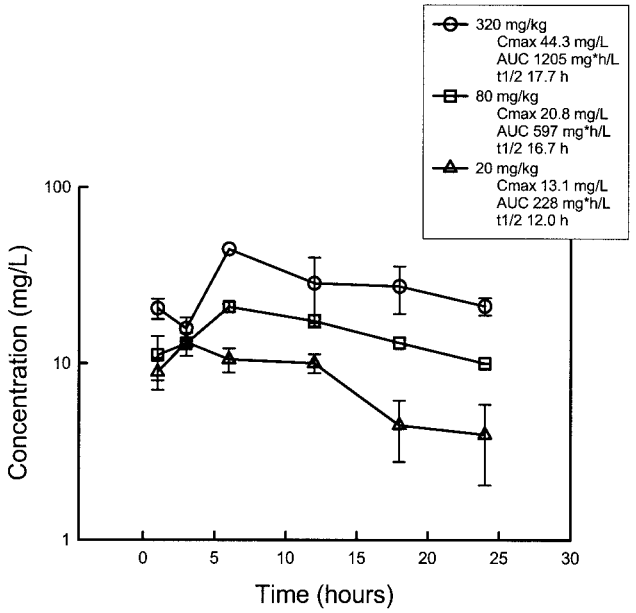

FIG. 1. Serum posaconazole concentrations after administration of oral doses of 20, 80, and 320 mg/kg in neutropenic infected mice. Each symbol represents the geometric mean ± SD of the levels in the sera of three mice.

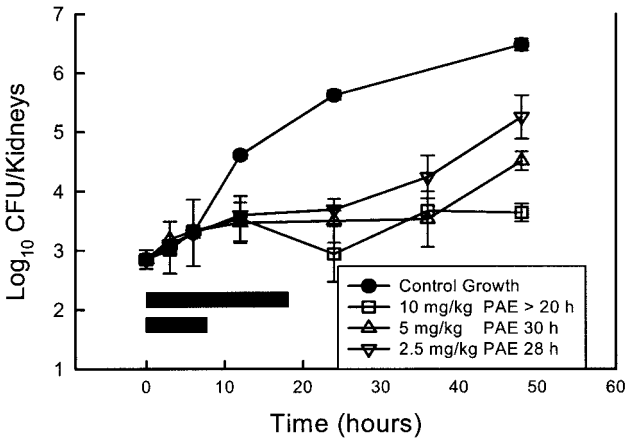

FIG. 2. In vivo PAFE following posaconazole doses of 10, 5, and 2.5 mg/kg against *C. albicans* K-1 in neutropenic infected mice. Each symbol represents the mean ± SD for two mice. The width of a horizontal bar represents the time that serum free drug levels exceeded the MIC.

Pharmacokinetics. The serum time course of posaconazole in infected neutropenic mice following oral doses of 320, 80, and 20 mg/kg is shown in Fig. 1. Peak serum drug levels and the AUC increased in a nonlinear fashion with dose escalation. Peak levels were achieved within the 6 h for each of the doses and ranged from 13.1 ± 0.25 to 44.3 ± 0.07 μg/ml. The elimination half-life ranged from 12.0 to 17.7 h. The AUC from 0 h to ∞ ($AUC_{0-\infty}$), as determined by the trapezoidal rule, ranged from 228 to 1,205 mg · h/liter with the lowest and highest doses, respectively. Protein binding in mouse serum was 99% at concentrations of 100 and 400 μg/ml. The lower limit of detection for the bioassay precluded study of lower dose levels.

In vivo PAE. Following tail vein inoculation of 10^6 CFU/ml, the *C. albicans* burden in the kidneys of untreated mice increased 3.63 ± 0.16 $\log_{10}$ CFU/kidney over 24 h. Control growth of 1 $\log_{10}$ CFU/kidney in untreated mice was achieved in 8.8 h. No drug carryover was observed in treatment groups. Based upon the above pharmacokinetics, the three doses of posaconazole studied (2.5, 5, and 10 mg/kg) would produce serum (free drug) levels above the MIC for the *Candida* organism (0.03 μg/ml) for 0, 7, and 19 h, respectively. None of the doses produced a net reduction in organisms compared to the numbers at the start of therapy. Growth curves for both the control group as well as those following the single doses of posaconazole are shown in Fig. 2. Posaconazole suppressed regrowth of organisms at each of the doses studied. Considering free drug levels, organism regrowth was suppressed from >20 to 30 h. Despite the fact that free drug levels with the lowest dose studied would never achieve levels above the MIC, growth was similarly suppressed for a prolonged period due to sub-MIC effects.

Pharmacodynamic parameter determinations. At the start of therapy, kidneys had 4.68 ± 0.15 $\log_{10}$ CFU/kidney. After 48 h the organisms grew to 2.92 ± 0.13 $\log_{10}$ CFU/kidney in untreated mice. Drug carryover was not observed in any of the samples. Over the range of posaconazole doses studied, compared to burden at the end of the study period in untreated animals, maximal organism reduction with the various dosing intervals ranged from 3.57 ± 0.35 to 4.35 ± 0.54 $\log_{10}$ CFU/kidney. The dose-response curves for each of the four dosing intervals are shown in Fig. 3. As the dosing interval was shortened from an every-48-h to every-6-h administration, the dose-response curves retained a similar shape, indicating similar efficacies. There was not a significant difference among the doses necessary to produce the ED_{50} and the ED_{75} (Table 1). The dose necessary to achieve an ED_{25} was significantly less for the 48-h regimen than for the 6-h regimen. There was not a significant difference among the other ED_{25} values. The relationships between microbiologic effect and each of the

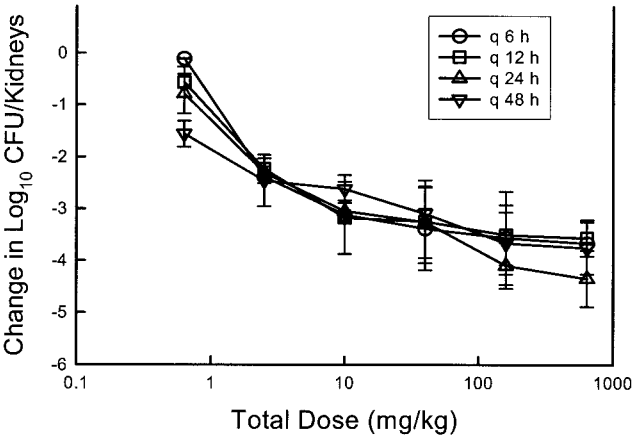

FIG. 3. Relationship between the 24-h total dose and the change in $\log_{10}$ CFU per kidney over the treatment period for posaconazole administered at different dosing intervals in a neutropenic murine model of disseminated candidiasis. Each symbol represents data for two mice.

TABLE 1. Relationship between posaconazole dosing interval and outcome in a disseminated candidiasis model

Endpoint	Dose (95% CI) for interval of:				*P* value[a]
	q6h	q12h	q24h	q48h	
ED_{25}	1.23 (0.7–1.8)	0.84 (0.6–1.1)	0.60 (0.1–1.1)	0.20 (0.2–0.6)	0.04
ED_{50}	1.88 (1.4–2.4)	1.73 (1.2–2.1)	3.20 (0.3–6.1)	1.90 (0.2–3.6)	0.59*
ED_{75}	2.86 (2.3–3.4)	3.58 (3.3–3.8)	14.0 (1.4–26.0)	26 (1.7–49)	0.07

[a] *, only significant difference in pairwise multiple comparison (Tukey test) was for dosing every 6 h (q6h) and q48h. *P* values are based on ANOVA.

pharmacodynamic parameters, percent time above MIC, AUC/MIC, and peak drug level/MIC, are shown in Fig. 4 (free drug). The data regressed with the AUC and the peak level in relation to the MIC had the strongest relationships, with an R^2 of 83 and 85%, respectively. T>MIC had the weakest correlation with treatment efficacy, whether total or free drug levels were considered, with R^2 values of 33 and 65%, respectively. That the peak drug level/MIC ratio was also important in this model is a reflection of the strong interrelationship between these concentration-associated parameters.

Magnitude of the pharmacodynamic parameter associated with efficacy. At the start of therapy the kidneys had 3.57 ± 0.37 (range, 3.02 to 4.68) $\log_{10}$ CFU/kidney. Each of the 12 *C. albicans* strains grew similarly in the animals. The range of organism growth in control animals was 3.29 ± 0.53 (range, 2.66 to 4.68 $\log_{10}$ CFU/kidney). The relationship between the posaconazole free-drug 24-h AUC/MIC ratio and efficacy with the 12 strains is displayed in Fig. 5. The relationship among the treatment groups was strong (R^2 = 70%). The posaconazole dose necessary to achieve 50% of the maximal effect varied 53-fold (range, 0.25 to 13.3 mg/kg). However, the 24-h AUC/MIC ratios representative of these doses varied only fourfold (free-drug AUC/MIC range, 6.12 to 26.7) (Table 2). There was not a significant difference among these AUC/MIC ratios (P = 0.41).

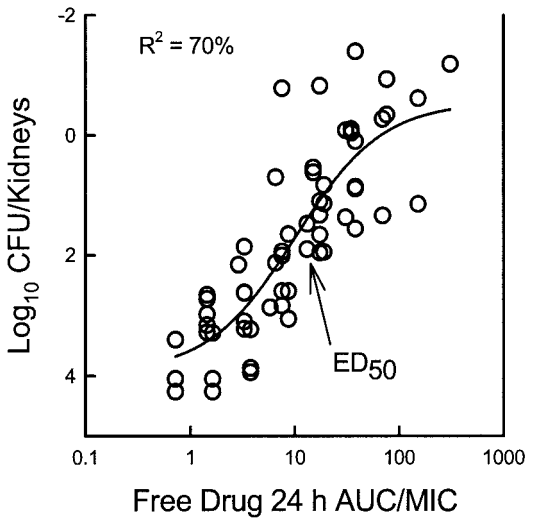

FIG. 5. Relationship between the free-drug 24-h AUC/MIC ratio and the $\log_{10}$ CFU per kidney after 2 days of treatment for posaconazole against 12 *C. albicans* organisms. Each symbol represents data for two mice. R^2 is the coefficient of determination.

DISCUSSION

There are several new triazole compounds under development (20, 25). Animal infection models have demonstrated the potency of these new triazole compounds against a variety of *Candida* spp. (4, 10, 25). However, these investigations have not determined the in vivo PK-PD parameter and parameter magnitude associated with treatment outcome with these drugs. The present studies were designed to see if the PK-PD characteristics of the new triazole, posaconazole, were similar to those observed with fluconazole and ravuconazole (1, 2, 16).

The time course of antifungal activity of these triazole compounds against *C. albicans* has been well described (1, 11, 16). Studies have demonstrated concentration-independent organism killing, but prolonged inhibitory effects after drug levels have fallen below the MIC (PAFE) (1). The single-dose in vivo studies with fluconazole observed PAFEs in the range of 4 h to more than 20 h. The present posaconazole studies also found prolonged PAFE durations when free drug levels were considered (20 to 30 h). These in vivo determinations could not

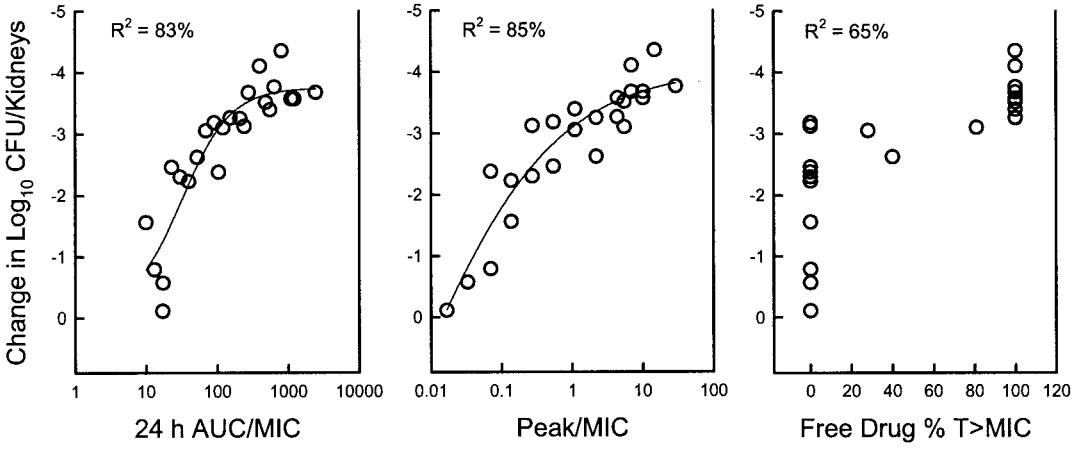

FIG. 4. Relationship between free-drug T>MIC, AUC/MIC, peak/MIC, and the change in $\log_{10}$ CFU per kidney. Each symbol represents data for two mice. R^2 is the coefficient of determination.

TABLE 2. Posaconazole efficacy against 12 *C. albicans* isolates in a disseminated candidiasis model

C. albicans strain	MIC (mg/liter)	ED_{50} (mg/kg/24 h)	24-h AUC/MIC	
			Total drug	Free drug
2-76	0.015	0.25	1,140	11.4
W2	0.015	0.93	2,513	25.1
K1	0.03	3.2	2,640	26.4
412	0.03	3.25	2,667	26.7
5810	0.06	0.89	612	6.12
1490	0.06	1.01	660	6.60
2438	0.06	9.36	2,517	25.2
2183	0.06	3.74	1,450	14.5
580	0.06	7.33	2,172	21.8
98-17	0.12	5.51	917	9.17
98-234	0.12	12.3	1,483	14.8
98-210	0.12	13.3	1,488	15.0
Mean ± SD				16.9 ± 7.8

differentiate between persistent growth suppression due to initial supra-MIC serum drug concentrations and those potentially due to sub-MIC effects. We feel it is likely that the majority of the in vivo PAFEs observed with the triazoles are due to the latter.

Multiple dosing interval studies with other triazole drugs have shown that treatment outcome is dependent upon the total amount of drug (AUC) and not the dosing interval. The present analysis measured outcomes following posaconazole therapy with a total dose range of more than 1,000-fold and four dosing intervals. These investigations also found that treatment efficacy was dependent most upon the total amount of drug (AUC).

The concordance of PK-PD parameter magnitudes among animal species and in humans has been demonstrated for a variety of antibacterials (5). This should not be surprising, given that PK-PD parameters can correct for differences in pharmacokinetics among animal species. Furthermore, the drug receptors for antimicrobials are in the pathogen and therefore are similar in all animals. Studies with numerous antimicrobials have also shown that the magnitude of the PK-PD parameter required for efficacy is similar for drugs within the same class, provided free drug concentrations are considered, and it is similar in the treatment of organisms with reduced susceptibility (3, 5). Thus, the results of studies from these experimental models have been shown to be useful for the design of dosing regimens in humans and for the more-rational development of in vivo susceptibility breakpoints (9, 18).

In vivo observations with other triazoles have found that an AUC/MIC ratio in the range of 20 to 25 produces 50% of the maximal microbiologic effect (ED_{50}) against both triazole-susceptible and -resistant strains. Similar AUC/MIC ratios were also found to be predictive of fluconazole clinical trial outcomes (14, 22; Clancy et al., Infect. Dis. Soc. Am., 1998). In these studies we also chose to utilize the dose necessary to produce a 50% maximal effect to allow comparison of these data with the prior triazole data.

One major difference between the newer triazoles and fluconazole is the degree of protein binding (25). Fluconazole has a low degree of protein binding in all species studied (10%). Because of this low degree of protein binding across animal species, total drug levels were utilized for PK-PD parameter calculations in the fluconazole publication (1). Each of the newer triazole compounds has a much higher degree of protein binding. Because of this discrepancy, the present studies attempted to determine the impact of protein binding on treatment outcome. In general, it is accepted that only free drug is pharmacologically active. This is related to the limited ability of protein-bound drug to diffuse across cellular membranes to reach the drug target. The impact of protein binding upon antimicrobial agents has been most clearly shown for antibacterials (7, 12, 13). Previous in vivo studies have not commonly considered the impact of azole protein binding. A recent PK-PD evaluation of the new triazole ravuconazole demonstrated that in vivo-in vitro correlations were strongest when free drug levels were utilized for the in vivo drug concentration time course (2). In the present study with posaconazole, protein binding determinations were performed in mouse serum collected from neutropenic, infected animals, attempting to closely mimic the binding that would occur in treatment studies. These studies with organisms with nearly eightfold MIC variations suggested that when free drug posaconazole concentrations are considered, treatment efficacy is similar to that observed with other triazoles.

One important weakness of the present study is the difference between the very low MICs (0.015 to 0.12 μg/ml) of this potent drug and the lower limit of detection of the pharmacokinetic assay used (0.5 μg/ml). Under this circumstance, examination of the relationship between serum drug concentrations at these lower concentrations and effects are based upon estimation or extrapolation. This is an increasingly common issue in PK-PD analysis of drugs with enhanced in vitro potencies. Although the lower limit of assay detection in the kinetic study is up to an order of magnitude higher than the lowest MIC with the microbiologic assay used, even a more sensitive high-pressure liquid chromatography assay would not allow study of drug concentrations at or below the MIC. We are, however, encouraged that our extrapolation of drug concentrations below those we were able to measure resulted in a strong PK-PD relationship (Fig. 4 and 5). Thus, it is not likely that the estimates were terribly inaccurate. Furthermore, the PK-PD magnitude relationships for posaconazole in this study are extremely similar to those reported with other drugs from the class using this infection model.

The present studies with the new triazole, posaconazole, identified the 24-h AUC/MIC parameter as predictive of efficacy. Furthermore, the magnitude of the 24-h AUC/MIC parameter required for posaconazole efficacy against a large number of *C. albicans* isolates was similar, including fluconazole-resistant organisms. In addition, the mean free drug 24-h AUC/MIC of 16.9 is similar to the value of 25 observed for other triazoles in this infection model. This investigation supports the pharmacodynamic theory that the PK-PD parameter and the parameter magnitude predictive of in vivo efficacy are similar for antiinfective compounds with similar mechanisms of action, including antifungals.

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