Infections caused by opportunistic fungal pathogens remain an important clinical problem. *Candida albicans* is the major fungal pathogen. Deep-seated infections due to this organism are an important cause of nosocomial infections, and the morbidity and mortality associated with *C. albicans* infections remain significant. Although in recent years there has been an expansion in the number of antifungal drugs available, in many cases the treatment of fungal infections is unsatisfactory.

This situation has led to an ongoing search for new antifungal agents. The determination of ultimate outcome is a function of multiple variables, though much of it can be explained on the basis of intrinsic microbiological activity (in vitro) and the serum concentration-time profile (in vivo) (11). Correlations are available for *β*-lactam antibiotics, aminoglycosides, and quinolones (12–15, 19, 21, 24, 25, 27, 28). In the case of *β*-lactams, improved outcome is associated with time that serum drug concentrations remain above the MIC (*t > MIC*) for the pathogenic agent (22, 23, 28). In contrast, for aminoglycosides and quinolones, the maximum antimicrobial effect is associated with higher ratios of maximum concentration of drug in serum (*Cₘₐₓ*) or area under the concentration-time curve (AUC) to the MIC (*Cₘₐₓ/MIC* or AUC/MIC ratios, respectively) (13, 15, 26).

However, little is known about antifungal agents (7, 29, 30). Anaissie et al. (2) showed some correlation between in vitro parameters and in vivo efficacy, but no clear reference to pharmacokinetic (PK) parameters was described. Graybill et al. (16) reported results obtained with fluconazole-susceptible or -resistant isolates in an experimental murine candidiasis model. The correlation found in this study was not very high. In vitro susceptibility tests could predict in vivo response to fluconazole: susceptible *C. albicans* strains (MIC ≤ 0.25 µg/ml) required lower daily doses than did resistant *C. albicans* strains (MICS from 8 to 64 µg/ml). However, in most cases, therapeutic decisions are governed more by the clinical experience of the physician than by the preclinical results even when amphotericin B is considered (18).

Recently, Louie et al. (20) defined the pharmacodynamic (PD) parameter that optimizes outcome in deep-seated *C. albicans* infections treated with fluconazole intraperitoneally, based on a murine model of systemic candidiasis, and taking into account the fungal reduction in the kidneys. Dose fractionation studies showed that the AUC/MIC ratio (20) best predicted the outcome with fluconazole (3).

Fungal protein synthesis (FPS) inhibitors are a new family of antifungal drugs, with a novel mechanism of action (9, 10, 17) and no former related therapeutic experience. The aim of the present study was thus to define a possible correlation between the PK properties of FPS inhibitors and therapeutic efficacy,
using a murine model of invasive systemic candidiasis, and to provide a rationale for dose selection in the first study of efficacy in humans.


**MATERIALS AND METHODS**

*C. albicans* isolate. *C. albicans* 4711E (GlaxoWellcome culture collection, Greenwood, United Kingdom) was used throughout the study. The strain was maintained in Sabouraud dextrose (SAB) agar (Difco, Detroit, Mich.) with 15% glycerol at −70°C until required. For inoculum preparation, *C. albicans* was cultured on SAB agar (Difco) plates. The resulting growth was collected from the plates in sterile 0.9% NaCl, and infecting inocula were adjusted by the spectrophotometric method to 10⁶ CFU/ml. Then, the adjusted taining twofold dilutions of the drugs in 0.1 ml of medium. Starting inocula were Bonaduz, Bonaduz, Switzerland) was used to prepare microdilution panels con-

Serum protein binding of GM 237354. Binding was determined in mouse serum by equilibrium dialysis using [3H]GM 237354. Radiochemical purity was determined by HPLC as described elsewhere (17). Brieﬂy, a Microlab AT Plus robot (Hamilton Bonaduz, Switzerland) was used to prepare microdilution panels containing twofold dilutions of the drugs in 0.1 ml of medium. Starting inocula were adjusted by the spectrophotometric method to 10⁶ CFU/ml. Then, the adjusted y with an automatic plate reader (IEMS; Lab Systems, Helsinki, Finland) set at 620 nm. The MIC was deﬁned as the lowest concentration of antifungal agent which prevented any visible growth or which inhibited growth by 95% compared with that in drug-free control wells. The median MIC after the incubation period was log CFU per gram) in the absence and presence of treatment.

**Antifungal treatment.** Therapy was initiated 1 h after inoculation and was continued for 7 days. Deaths were recorded daily up to 28 days postinoculation (1). GM 237354 was administered at 25, 50, 100, and 200 mg/kg every 4, 8, and 12 h for total daily doses of 5 to 240 mg/kg. The AUC over 24 h (AUC) and Cₘₐₓ were calculated after a multiple-dose regimen with the above doses administered every 4, 8, and 12 h. Simulations were performed using the WinNonlin software package.

Fungal burden of kidneys. Twelve hours after the end of treatment, three randomly selected animals from groups dosed every 8 h were sacrificed. The kidneys were removed, weighed, and homogenized with 5 ml of cold sterile saline in a blender (Stomacher 400; Seward Medical, London, United Kingdom). Samples from each specimen were diluted and spread onto SAB plates. After 24 h of incubation at 35°C, the log CFU per gram of kidney were calculated with WinNonlin software (Scientific Consulting, Inc., Apex, N.C.).

**Efficacy parameters.** The efficacy parameters used to assess treatment success were the following: (i) percentage of survivors and median survival day obtained for each set of dosage regimens that are based on different dosing intervals (8). The relationship between PK parameters (total serum concentration-time data on the basis of a one-compartment open model with first-order absorption-elimination kinetics. AUC and Cₘₐₓ were calculated with WinNonlin software (Scientific Consulting, Inc., Apex, N.C.). PK simulations. PK profiles for 40, 20, 10, 5, and 2.5 mg/kg were simulated considering the experimental plasma data obtained after a single s.c. dose (25). The AUC over 24 h (AUC) and Cₘₐₓ were calculated after a multiple-dose regimen with the above doses administered every 4, 8, and 12 h.

**Correlation between survival and PK parameters (AUC and Cₘₐₓ).** Most of the interdependence among PK-pharmacodynamic parameters can be reduced by comparing the results of dosage regimens that are based on different dosing intervals (8). The relationship between PK parameters (total serum concentra-

where AUCₘₐₓ is the maximum AUC, Eₘₐₓ is the maximum effect, and AUCₜ is the AUC at which 50% of the maximum efficacy is obtained, and γ is the Hill factor determining the slope of the curve. After a preliminary analysis which showed a large uncertainty for the 12-h-dosing-interval PK-PD parameter estimates, which 50% of the maximum efficacy is obtained, and γ is the Hill factor determining the slope of the curve. After a preliminary analysis which showed a large uncertainty for the 12-h-dosing-interval PK-PD parameter estimates, Eₘₐₓ was fixed at 100% (i.e., the theoretical maximum effect which would be observed in noninfected untreated control animals, (i.e., 100% survival during the experimental period 2,800% day 28 days 100%), the effect Kₑ measured in control, infected-nontreated animals (975% 95% day 3), and the effect Kᵢ measured during the effect will be examined through the model with the Hill equation using WinNonlin software and the Nelder-Mead algorithm. No weighting was used (21). The general equation used is given as

\[ E = \frac{E_{\text{max}} \cdot \text{AUC}^\gamma}{\text{AUC}_0 + \text{AUC}^\gamma} \]

where AUC is the steady-state 24-h AUC obtained after PK simulations. Eₛ is the net effect observed, Eₘₐₓ is the maximum net effect, AUCₗ is the AUC at which 50% of the maximum efficacy is obtained, and γ is the Hill factor determining the slope of the curve. After a preliminary analysis which showed a large uncertainty for the 12-h-dosing-interval PK-PD parameter estimates, Eₘₐₓ was fixed at 100% (i.e., the theoretical maximum effect which would be observed in noninfected untreated control animals, (i.e., 100% survival during the experimental period 2,800% day 28 days 100%), the effect Kₑ measured in control, infected-nontreated animals (975% 95% day 3), and the effect Kᵢ measured during the effect will be examined through the model with the Hill equation using WinNonlin software and the Nelder-Mead algorithm. No weighting was used (21). The general equation used is given as

\[ \text{log}(\text{AUC}) = \text{log}(\text{AUC}_0) + \frac{\text{log}(E_{\text{max}}) - \text{log}(E_{\text{max}})}{\gamma} \]

and phosphate-octane sulfonic acid (Reactivos Scharlau S.L., Barcelona, Spain) solution buffered at pH 5. The flow rate was 1 ml/min, and UV detection of the compound was performed at 215 nm. Chromatography was isocratic at 78% acetonitrile. Standard curves (linearity from 100 to 2000 ng/ml) were generated by adding known amounts of GM 237354 to pooled mouse serum (Charles River France Inc.). Before HPLC analysis, standard and unknown samples were depop-

Twelve hours after the end of treatment, three randomly selected animals from groups dosed every 8 h were sacrificed. The kidneys were removed, weighed, and homogenized with 5 ml of cold sterile saline in a blender (Stomacher 400; Seward Medical, London, United Kingdom). Samples from each specimen were diluted and spread onto SAB plates. After 24 h of incubation at 35°C, the log CFU per gram of kidney were calculated with WinNonlin software (Scientific Consulting, Inc., Apex, N.C.). PK simulations. PK profiles for 40, 20, 10, 5, and 2.5 mg/kg were simulated considering the experimental plasma data obtained after a single s.c. dose (25). The AUC over 24 h (AUC) and Cₘₐₓ were calculated after a multiple-dose regimen with the above doses administered every 4, 8, and 12 h. Simulations were performed using the WinNonlin software package.

Systemic infection. A pilot experiment to determine the inoculum sizes of *C. albicans* that would result in a survival time for infected-nontreated control animals of at least 7 days was performed (data not shown). Mice were challenged intravenously with 200 µl of the appropriate inoculum (10⁷ CFU) into the lateral tail vein. Thirteen infected animals were left untreated, and the rest were randomly assigned to treatment groups of 10 to 13 animals each.

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Thus, for 90% efficacy,

\[ E_{\text{ther}} = 0 \cdot 9 \cdot E_{\text{max}} \]

**Correlation between kidney burden and PK parameters (AUC and \( C_{\text{max}} \)).** The relationship between i.e. AUC (total serum concentrations) and kidney burden was evaluated with an inhibitory sigmoid \( E_{\text{max}} \) model, including the baseline \( E_0 \), which is the kidney burden in infected-nontreated animals at the end of the 7-day treatment period. The equation used is given as:

\[ E = E_0 - \frac{E_{\text{max}} \cdot \text{AUC}}{AUC_{\text{th}} + \text{AUC}} \]

where AUC is the steady-state 24-h AUC obtained after PK simulations, \( E \) is the effect measured, \( E_{\text{max}} \) is the maximum net effect, \( AUC_{\text{th}} \) is the AUC at which 50% of the maximum therapeutic efficacy is obtained, and \( \gamma \) is the Hill coefficient. Individual data (approximately three animals per dose) were used for the analysis. The same modeling conditions as described above were used.

The AUC which would provide a desired therapeutic effect, \( E_{\text{ther}} \) (i.e., 90% of the maximum therapeutic efficacy), can be calculated as follows:

\[ \log(\text{AUC}) = \log(AUC_{\text{th}}) + \left( \frac{\log(E_0 - E_{\text{max}}) - \left( \frac{E_{\text{max}}}{AUC_{\text{th}} + \text{AUC}} \right) }{\gamma} \right) \]

Thus, for 90% of maximal efficacy,

\[ E_{\text{ther}} = E_0 - 0.9 \cdot E_{\text{max}} \]

**Correlation between efficacy and PK-PD parameters (AUC/MIC and \( t > \text{MIC} \)).** AUC/MIC, defined as the ratio between AUC and the corresponding MIC (27), would be another parameter to be assessed in terms of free serum concentrations for different compounds of the same class. \( AUC_{\text{th}}/\text{MIC} \) values could be calculated in terms of free concentrations by correcting for the binding of a specific compound (unbound fraction = 0.05 for GM 237354 in mice), the \( AUC_{\text{th}}/\text{MIC} \) values being estimated for total concentrations as described in the previous section. However, PK-PD data are available only for one FPS inhibitor, GM 237354, and consequently AUC/MIC values cannot be compared for different compounds of the same class and will not be presented in Results.

On the other hand, \( t > \text{MIC} \) has been successfully used to describe PK-PD relationships with different antibacterials (14, 27, 28). \( t > \text{MIC} \) was determined for each dosing regimen based on free compound concentration. Its relationship with effect was first evaluated graphically and could be described by a sigmoid \( E_{\text{max}} \) model for the 8- and 12-h dosing intervals (data for the 4-h dosing interval could not be analyzed, as \( t > \text{MIC} \) was 100% whatever the dose and the corresponding ES value [see Fig. 6]) as

\[ E = \frac{E_{\text{max}}^* (t > \text{MIC})^*}{(t > \text{MIC})} \]

**RESULTS**

**PKs.** Figure 1 displays the GM 237354 serum concentration-time curve observed after s.c. administration of 50 mg/kg (total concentration) or simulated after i.e. a 40-mg/kg s.c. dose (free and total concentrations). Table 1 displays the main PK parameters, including those obtained in a pilot study performed to explore dose proportionality.

PK simulations for AUC and \( C_{\text{max}} \) were based on total serum concentrations and were carried out for repeated dosing using the PK parameters estimated above for the 50-mg/kg dose. Single-dose PK parameters were used to predict repeated-dosing PKs (Table 2), as GM 237354 did not show accumulation, even when using the shortest dosing interval (every 4 h) and the highest dose simulated (40 mg/kg). \( t > \text{MIC} \) values were derived from free concentration profiles, derived from simulated total serum concentrations corrected for binding.

**Efficacy parameters.** All untreated animals died within 8 and 11 days postinfection. Survival accumulative distributions are displayed in Fig. 2, with derived AUSTC and ES values shown in Table 3. Kaplan-Meier survival analysis showed statistical differences between the treated animals and the untreated controls, even at the lowest dose, i.e., 2.5 mg/kg every 12 h (\( P \leq 0.0001 \)). As shown in Fig. 3, a good consistency was observed between short-term (\( C. \text{ albicans in kidneys 7 days postinfection} \)) and long-term (survival rate at day 28 or AUSTC) measures (data available for all endpoints only for the 8-h dosing interval).

**Correlation between survival and PK parameters (AUC and \( C_{\text{max}} \)).** Using total plasma concentrations, a graphic visual evaluation showed efficacy to be related independently of the dosing interval only to AUC, not to \( C_{\text{max}} \) (Fig. 4 and 5). Results from Table 3 were modeled to obtain the parameters shown in Table 4. Several models were tested, as summarized in Table 4 and explained as follows.

Using a sigmoid \( E_{\text{max}} \) model for each dosing interval separately, the data could be well fitted for the 8- and 4-h dosing intervals; however, the fit was reasonably good only for the

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>s.c. single dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{\text{th}} (\mu g \cdot h/ml)</td>
<td>46.04</td>
</tr>
<tr>
<td>( C_{\text{max}} ) (\mu g/ml)</td>
<td>23.04</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>0.52</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (\mu g/ml)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* \( AUC_{\text{th}} \) AUC from 0 h to infinity; \( t_{1/2} \), half-life; \( T_{\text{max}} \), time after administration at which \( C_{\text{max}} \) is reached.

**TABLE 2. PK parameters (AUC and \( C_{\text{max}} \)) predicted after s.c. repeated dosing**

<table>
<thead>
<tr>
<th>Simulated dose (mg/kg)</th>
<th>AUC over 24 h (\mu g \cdot h/ml) for dosing interval:</th>
<th>Predicted value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>8 h</td>
</tr>
<tr>
<td>40</td>
<td>220.8</td>
<td>110.4</td>
</tr>
<tr>
<td>20</td>
<td>110.4</td>
<td>55.3</td>
</tr>
<tr>
<td>10</td>
<td>55.3</td>
<td>27.6</td>
</tr>
<tr>
<td>5</td>
<td>27.6</td>
<td>13.8</td>
</tr>
<tr>
<td>2.5</td>
<td>13.8</td>
<td>6.9</td>
</tr>
</tbody>
</table>

* Equal value for all dosing intervals as there is no evidence of compound accumulation upon repeated dosing.
12-h dosing data (larger coefficients of variation [CVs] for the estimated parameters). AUC$_{50}$ was estimated at 21.7 and 34.7 mg·h/ml and $\gamma$ was estimated at 1.95 and 2.34 for dosing intervals of 8 and 4 h, respectively. For an efficacy target of 90% of maximum effect, AUC$_{90}$ was predicted as 67 mg·h/ml (8-h dosing interval).

A sigmoid $E_{\text{max}}$ model with a common $\gamma$ value but a different AUC$_{50}$ value for each dosing interval did not provide any substantial improvement in the fit. No real change in AUC$_{50}$ estimates was observed.

A sigmoid $E_{\text{max}}$ model pooling data from all three dosing intervals provided a reasonable fit, with AUC$_{50}$ estimated at 29.3 mg·h/ml and $\gamma$ estimated at 1.5 (Table 4). The simple $E_{\text{max}}$ model pooling data from all three dosing intervals gave a poorer fit.

Correlation between survival and $t > \text{MIC}$. The PK-PD relationship with $t > \text{MIC}$ at steady state was derived for ES (net effect on AUSTC) using $t > \text{MIC}$ calculated for unbound concentrations (5% of the corresponding total concentrations) as depicted in Fig. 1. Figure 6 shows the very steep relationship between ES and $t > \text{MIC}$, with 50% of $E_{\text{max}}$ being achieved with a $t > \text{MIC}$ of 77.1 and 59.2% for the 8- and 12-h dosing intervals, respectively. $\gamma$ was estimated as 15.9 and 6.2 for the 8- and 12-h intervals, respectively (Table 5). For the 4-h dosing interval, $t > \text{MIC}$ was 100% for all doses, although efficacy was poor for certain doses (Fig. 2 and 6). This shows that $t > \text{MIC}$ is not a valid PK-PD predictor.

Correlation between kidney burden and PK parameters (AUC and C$_{\text{max}}$). Figure 7 shows an excellent correlation between AUC (total serum concentrations) and kidney burden (log CFU per gram) (observed and fitted data; data available only for the 8-h dosing interval). PK-PD parameters were estimated at 37.1 mg·h/ml for AUC$_{50}$, 4.40 log CFU/g for $E_{\text{max}}$, and 1.51 for $\gamma$. The baseline $E_{\text{max}}$ was estimated at 6.03 log CFU/g. For an efficacy target of 90% of maximum effect, AUC$_{90}$ was predicted as 159 mg·h/ml.

A satisfying consistency in the PK-PD parameter estimates was observed between the two different efficacy endpoints, i.e., AUSTC and kidney burden (Table 4 and Fig. 3; 8-h interval). No modeling was performed with $C_{\text{max}}$, as (i) $C_{\text{max}}$ was not selected as a satisfying PK-PD predictor for the PK-PD analysis of AUSTC (see above) and (ii) the kidney burden and AUSTC endpoints are consistent regarding the dose relationship (Fig. 3, similar dose providing 50% of net effect).

---

**TABLE 3.** Long-term efficacy obtained with different dosing intervals throughout the treatment period

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Value (% · day) for dosing interval$^a$</th>
<th>AUSTC</th>
<th>$E_{\text{max}} = K_t - K_k$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>8 h</td>
<td>12 h</td>
</tr>
<tr>
<td>40</td>
<td>2,800</td>
<td>2,755</td>
<td>2,330</td>
</tr>
<tr>
<td>20</td>
<td>2,775</td>
<td>2,620</td>
<td>1,510</td>
</tr>
<tr>
<td>10</td>
<td>2,350</td>
<td>2,065</td>
<td>1,665</td>
</tr>
<tr>
<td>5</td>
<td>1,535</td>
<td>1,390</td>
<td>1,345</td>
</tr>
<tr>
<td>2.5</td>
<td>1,330</td>
<td>1,349</td>
<td>1,318</td>
</tr>
<tr>
<td>Untreated</td>
<td>975</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ AUSTC values are obtained from plots of cumulative survival versus days (Fig. 2).
DISCUSSION

Other authors have already published some correlations between in vitro and in vivo antifungal activities in animal models (L. Appenzeller, E. Lim, P. Wong, M. Fadem, P. Motchkin, M. Bakalinsky, and R. Little, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F187, 1996; 16, 20, 29). However, much less experience exists with a rational approach for the design of the first clinical trial involving antifungals. One difficulty is the extrapolation of the experimental animal model results to humans, due to PK dissimilarities between humans and laboratory animals (4, 5).

The present study was designed to establish correlations between PK parameters and efficacy. The ultimate aim is to identify a PK parameter (e.g., AUC or $C_{\text{max}}$) or a PK-PD parameter (e.g., $t > \text{MIC}$ or AUC/MIC ratio) which could be used as a common predictor of in vivo antifungal activity. The PK characteristics are different in each species and depend more critically on specific metabolic paths (6). However, the same PK or PK-PD parameter values (based on free concentrations) predictive of i.e. 90% efficacy in an animal species are likely to provide a similar efficacy and thus could be used to more accurately extrapolate results within different species.

In order to identify the predictor of efficacy, it is critical to explore several daily doses fractionated using different dosing intervals. Most of the interdependence among PK-PD parameters can be reduced by comparing the results of dosage regimens that are based on different dosing intervals (8).

The $E_{\text{max}}$ model has been successfully used to describe PK-PD relationships (12, 19, 21, 25) in the antibacterial field. In these reports, the most frequently used efficacy parameter was the difference between the CFU in the absence and that in the presence of the antibacterial compound. In our case, we have also used survival-related parameters such as AUSTC. This approach has been validated because we found a good
PK-PD relationship for both survival-related parameters and the presence of *C. albicans* in kidneys.

Regarding survival measures, AUSTC was selected for PK-PD modeling as being more sensitive than the percentage of survivors at the end of the study or the mean survival day. The relationship between the net effect on AUSTC and the MIC was 100% for all doses. This implies that, when near-complete efficacy was observed, unbound concentrations were essentially above the MIC over the whole dosing interval. However, *t > MIC* was 100% in the 4-h dosing interval group though good efficacy was not observed at low doses. This demonstrated that *t > MIC* is not a PK-PD predictor of efficacy.

Regarding tissue burden, the AUC$_{50}$ value needed to diminish the *C. albicans* burden in its target organ (kidney in this infection model) was found to be at 37.1 µg h/ml and the Hill coefficient γ was found to be at 1.5 following repeated dosing every 8 h (no data for the other dosing intervals). These values

### TABLE 4. PK-PD parameter estimates for the relationship between the net effect ES on survival time curve (AUSTC) and kidney burden and GM 237354 AUC (total serum concentrations)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>Dosing interval (h)</th>
<th>γ (CV %)</th>
<th>$E_{\text{max}}$ (CV %)</th>
<th>Baseline $E_{\text{0}}$ (CV %)</th>
<th>AUC$_{50}$ (CV %)</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival time</td>
<td>Sigmoid $E_{\text{max}}$ model for each dosing interval</td>
<td>12</td>
<td>0.9 (40.7)</td>
<td>NA</td>
<td>41.2 (37.5)</td>
<td>0.862</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.9 (20.3)</td>
<td>NA</td>
<td>21.7 (11.4)</td>
<td>0.985</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2.3 (19.7)</td>
<td>NA</td>
<td>34.7 (9.4)</td>
<td>0.989</td>
<td></td>
</tr>
<tr>
<td>Survival time</td>
<td>One sigmoid $E_{\text{max}}$ model for each dosing interval, common γ</td>
<td>12</td>
<td>1.7 (17.7)</td>
<td>NA</td>
<td>39.8 (19)</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3.5 (18.6)</td>
<td>NA</td>
<td>21.4 (18.4)</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>Survival time</td>
<td>One sigmoid $E_{\text{max}}$ model for all dosing intervals (pooled data)</td>
<td>12, 8, 4</td>
<td>1.5 (19.4)</td>
<td>NA</td>
<td>29.3 (12.7)</td>
<td>0.927</td>
<td></td>
</tr>
<tr>
<td>Survival time</td>
<td>One simple $E_{\text{max}}$ model for all dosing intervals (pooled data)</td>
<td>12, 8, 4</td>
<td>NA</td>
<td>27.8 (18.7)</td>
<td>0.920</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney burden</td>
<td>Inhibitory sigmoid $E_{\text{max}}$ model</td>
<td>8</td>
<td>1.5 (29.7)</td>
<td>4.4 (21.5)</td>
<td>6.03 (3.3)</td>
<td>37.1 (31.9)</td>
<td>0.970</td>
</tr>
</tbody>
</table>

* AUC$_{50}$ values are shown as microgram-hours per milliliter; $E_{\text{0}}$ and $E_{\text{max}}$ values are shown as log CFU per gram. The net effect was defined as $ES = (K_p - K_p')$ (see Materials and Methods). $E_{\text{max}}$ values for survival were fixed at 1.825% survival day. Correlation is that between observed and predicted values provided by WinNonlin software. NA, not applicable.

### TABLE 5. PK-PD parameter estimates for the relationship between the net effect ES on survival time curve (AUSTC) and kidney burden and $t > \text{MIC}$ (free serum concentrations)

<table>
<thead>
<tr>
<th>Dosing interval</th>
<th>$t &gt; \text{MIC}$ (CV %)</th>
<th>γ (CV %)</th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>8 h</td>
<td>77.1 (2.1)</td>
<td>15.9 (35.6)</td>
<td>0.979</td>
</tr>
<tr>
<td>12 h</td>
<td>59.2 (5.9)</td>
<td>6.2 (46.5)</td>
<td>0.824</td>
</tr>
</tbody>
</table>

* NA, not applicable, as $t > \text{MIC}$ values are 100% for all doses $t > \text{MIC}$ values are shown as percentages of the dosing intervals. Correlation is that between observed and predicted values provided by WinNonlin software.
are similar to the estimates for the net effect on AUSTC (AUC50 = 21.7 μg · h/ml; γ = 1.9) for the same dosing interval.

We can conclude the following. (i) A good agreement was found between the PK and therapeutic efficacy of GM 237354 at different dosing regimens using an experimental systemic C. albicans infection model in mice. The 7-day kidney colonisation represents infection of the target organ, and a good consistency was found between Candida burden and mortality (survival time and percentage of survivors). (ii) PK-PD relationships between efficacy measures (survival time curve and kidney burden) and AUC at steady state were evaluated using PK parameters obtained with total plasma concentrations. PK-PD relationships using t > MIC at steady state were assessed using unbound serum concentrations. (iii) The effect was well predicted independently of the dosing interval only by AUC. The various efficacy endpoints used for modeling (net effect on survival time curve, percentage of survivors, and kidney burden) provided similar PK-PD trends. (iv) For 50% efficacy, AUC50 was estimated at 21.7 and 37.1 μg · h/ml for the survival time curve and the reduction in kidney burden (8-h dosing interval data), respectively. These values corresponded to a daily dose of ~ 60 mg/kg in mice. (v) At near-maximal efficacy for the effect on survival, t > MIC90 was close to 90% in the 8- and 12-h dosing interval groups. However, t > MIC is not predictive of efficacy; in the 4-h dosing interval group, efficacy was dose dependent, although t > MIC was 100% at all doses. (vi) PK-PD relationships have to be validated using new FPS inhibitor compounds and Candida spp. with different susceptibility patterns. However, these relationships could be already useful for the more accurate design of studies involving large animal species and humans, after correction for any plasma protein binding between species. Future work destined to increase knowledge in this field is warranted.

ACKNOWLEDGMENTS

We thank members of the Organic Chemistry group for providing GM 237354 and E. Herreros and her team for performing susceptibility testing (MIC). We also thank Centro de Investigacion Farmacologica (C.I.F.) for technical assistance. We thank the reviewers for their in-depth review of the manuscript, pertinent comments, and excellent suggestions.

REFERENCES


