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Pharmacokinetics and Pharmacodynamics of Fluconazole for Cryptococcal Meningoencephalitis: Implications for Antifungal Therapy and *In Vitro* Susceptibility Breakpoints

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Fluconazole is frequently the only antifungal agent that is available for induction therapy for cryptococcal meningitis. There is relatively little understanding of the pharmacokinetics and pharmacodynamics (PK-PD) of fluconazole in this setting. PK-PD relationships were estimated with 4 clinical isolates of *Cryptococcus neoformans*. MICs were determined using Clinical and Laboratory Standards Institute (CLSI) methodology. A nonimmunosuppressed murine model of cryptococcal meningitis was used. Mice received two different doses of fluconazole (125 mg/kg of body weight/day and 250 mg/kg of body weight/day) orally for 9 days; a control group of mice was not given fluconazole. Fluconazole concentrations in plasma and in the cerebrum were determined using high-performance liquid chromatography (HPLC). The cryptococcal density in the brain was estimated using quantitative cultures. A mathematical model was fitted to the PK-PD data. The experimental results were extrapolated to humans (bridging study). The PK were linear. A dose-dependent decline in fungal burden was observed, with near-maximal activity evident with dosages of 250 mg/kg/day. The MIC was important for understanding the exposure-response relationships. The mean AUC/MIC ratio associated with stasis was 389. The results of the bridging study suggested that only 66.7% of patients receiving 1,200 mg/kg would achieve or exceed an AUC/MIC ratio of 389. The potential breakpoints for fluconazole against *Cryptococcus neoformans* follow: susceptible, ≤2 mg/liter; resistant, >2 mg/liter. Fluconazole may be an inferior agent for induction therapy because many patients cannot achieve the pharmacodynamic target. Clinical breakpoints are likely to be significantly lower than epidemiological cutoff values. The MIC may guide the appropriate use of fluconazole. If fluconazole is the only option for induction therapy, then the highest possible dose should be used.

Cryptococcal meningoencephalitis is a leading cause of global infectious morbidity and mortality (1). There are approximately one million cases per year in the world and 600,000 deaths (1). The majority of the global disease burden occurs in sub-Saharan Africa, where cryptococcal meningoencephalitis is intricately linked with the extensive and persistent AIDS epidemic. There are relatively few therapeutic options, and little information on the pharmacokinetics and pharmacodynamics (PK-PD) of currently available antifungal agents for the treatment of this neglected fungal disease.

In many parts of the world, fluconazole is the only antifungal agent that is available for treatment of cryptococcal meningoencephalitis. While there is extensive information on the use of fluconazole for consolidation and suppressive therapy, there is less information on optimal induction regimens. Recent clinical trials suggest that higher dosages (e.g., 1,200 to 2,000 mg/day) result in improved antifungal activity (2, 3), although further antifungal activity is evident with the addition of flucytosine, suggesting that this dosage may still be submaximal (3). A further understanding of the pharmacokinetic and pharmacodynamic relationships for fluconazole would provide additional insight into optimal induction regimens and aid in establishing *in vitro* susceptibility breakpoints.

Here, we estimated the pharmacokinetics and pharmacodynamics of fluconazole for cryptococcal meningoencephalitis. We used a well-validated model of murine cryptococcal meningoencephalitis and a number of clinical strains with a range of MICs. We investigated the predictive value of the MIC for fluconazole exposure-response relationships. We bridged the results to humans to reflect on appropriate *in vitro* susceptibility breakpoints for fluconazole for cryptococcal meningoencephalitis.

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MATERIALS AND METHODS

Isolates and MICs. A well-characterized clinical strain of *Cryptococcus neoformans*, H99 (ATCC 208821), was purchased from LGC Standards and used to define the initial pharmacokinetic and pharmacodynamic relationships for fluconazole. The original intention in this study was to use strains from a previous publication documenting clinical failure resulting from increased fluconazole MICs (4). Repeat MIC testing of these

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strains demonstrated that the MICs were much lower than originally reported, suggesting that the mechanism of resistance was unstable. Therefore, three clinical strains of *Cryptococcus neoformans* (identified to the species level using standard microbiological techniques) were studied (F/13186, F/6137, and F/20886). These isolates were obtained from the Mycology Reference Laboratory, University Hospital of South Manchester, and were chosen on the basis of the recorded susceptibility testing result. Fluconazole MICs were subsequently repeated a total of 5 times using the methodology outlined in the Clinical and Laboratory Standards Institute (CLSI) M27-A3 document (5). Briefly, susceptibility testing was performed in RPMI 1640 broth with 0.2% glucose, using an inoculum of 0.5×10^3 to 2.5×10^3 CFU/ml with incubation for 72 h at 35°C. The endpoint for determination of the MIC was defined as ≥50% reduction in growth relative to the growth of control organisms.

Murine model of cryptococcal meningoencephalitis. All laboratory animal experiments reported in this study were performed under United Kingdom Home Office project license PPL40/3101 and had also received ethical approval from The University of Manchester. A previously described murine model of cryptococcal meningoencephalitis was used (6). Male CD1 mice (Charles River Ltd.) weighing 23 to 28 g were used for all experiments. No immunosuppression was used. The mice were housed in vented HEPA-filtered cages and had free access to food and water. Inoculation with 3.8×10^5 organisms intravenously (i.v.) via the lateral tail vein in 0.25 ml phosphate-buffered saline (PBS) (Invitrogen, Paisley, United Kingdom) resulted in a persistent nonlethal infection in the brain for at least 10 days. The target inoculum was verified using quantitative cultures.

Fluconazole. The results of preliminary pharmacokinetic experiments suggested that the use of the clinical formulation of i.v. fluconazole was too dilute to enable sufficient systemic drug exposure to fully characterize the pharmacodynamic relationships. The necessary dosages would have required the use of volumes that exceeded those that are safe and acceptable for mice. To circumvent this problem, fluconazole capsules (Pfizer, Sandwich, Kent, United Kingdom) were broken, and fluconazole powder was dissolved in sterile saline with 0.03% Noble agar (Oxoid, Basingstoke, United Kingdom) as previously described (7). The mixture was administered to mice orally and was well tolerated. Groups of mice received daily dosing of fluconazole beginning 24 h postinoculation. These dosages were chosen to encompass the systemic drug exposures that are achievable and clinically relevant for humans. These exposures were identified from preliminary dose-finding experiments and a previously published pharmacokinetic study (8).

Pharmacokinetic studies. The concentration-time profile of fluconazole in plasma and in the cerebrum following oral administration was defined in 72 mice. One cohort of mice received 125 mg of fluconazole/kg of body weight/day, while the other cohort received 250 mg/kg/day. Fluconazole therapy was initiated 24 h postinfection. Groups of 3 mice were serially sacrificed at 24.5, 25, 26, 28, 30, 48, 72.5, 73, 74, 76, 78, and 96 h postinfection. Serum samples were obtained by cardiac puncture after terminal anesthesia with 5% isoflurane (Baxter UK, Newberry, United Kingdom). Subsequently, murine brains were removed intact and sectioned. One half of each brain was stored in a sterile plastic bag at −80°C prior to high-performance liquid chromatography (HPLC) analysis, while the other half was used to estimate the cerebral fungal density (see below).

Pharmacodynamic studies. The pharmacodynamics of fluconazole were determined using three cohorts of mice; one cohort received no fluconazole, one cohort received 125 mg/kg/day, and one cohort received 250 mg/kg/day. Fluconazole was administered only once per day. All experiments were performed twice. Groups of three mice were serially sacrificed at 1, 24, 72, 144, 168, 192, and 240 h postinfection. Half of each brain from a mouse was weighed, placed in 1 ml PBS, and homogenized. Serial 10-fold dilutions were then plated onto Sabouraud dextrose agar (Oxoid, Basingstoke, United Kingdom) and incubated at 30°C for 48 h before determining the cerebral fungal density.

Measurement of fluconazole concentrations using HPLC. Fluconazole concentrations in mouse plasma and cerebrum samples were measured by high-performance liquid chromatography (Shimadzu Prominence), using a modified version of a previously described method (9). Briefly, an extraction step involved the use of dichloromethane (Fisher Scientific, Loughborough, United Kingdom). A Hypersil BDS 5-μm column (250 by 4.6 mm) (Fisher Scientific, Loughborough, United Kingdom) was used throughout. The injection volume for both plasma and cerebrum was 50 μl. A standard curve encompassing 0.4 to 25 mg/liter was constructed in the respective matrix, from stock solutions of fluconazole (1,000 mg/liter) in methanol (Fisher Scientific, Loughborough, United Kingdom).

The internal standard was phenacetin at 5 mg/liter (Sigma-Aldrich, Dorset, United Kingdom). The starting mobile phase consisted of 1% of solution A and 99% of solution B. Solution A consisted of 0.05 M ammonium acetate (Fisher Scientific, Loughborough, United Kingdom) with 0.1% triethylamine (Fisher Scientific, Loughborough, United Kingdom) and 20% acetonitrile (Fisher Scientific, Loughborough, United Kingdom) (vol/vol), and solution B consisted of 0.1% formic acid (Fisher Scientific, Loughborough, United Kingdom) in acetonitrile (vol/vol). A gradient over 9 min was used that progressed to 20% solution A and 80% solution B. A flow rate of 1.2 ml/min was used. Fluconazole and the internal standard were detected using UV at 262 nm, and they eluted after 5.6 and 8.7 min, respectively.

Data analysis and mathematical modeling. The pharmacokinetics and pharmacodynamics of fluconazole for each of the four strains were described using a 5-compartment mathematical model consisting of the following five ordinary differential equations:

$$dX_1/dt = B(1) - K_a X_1 \quad (1)$$

$$dX_2/dt = -\left[k_{cp} + k_{cb} + (\mathrm{SCL}/V_c)\right](X_2) + k_{bc}X_3 + k_{pc}X_4 + K_a X_1 \quad (2)$$

$$dX_3/dt = k_{cb}X_2 - k_{bc}X_3 \quad (3)$$

$$dX_4/dt = k_{cp}X_2 - k_{pc}X_4 \quad (4)$$

$$dN/dt = \mathrm{Kgmax}\left[1 - (N/\mathrm{POPMAX})\right](N) \quad (5a)$$

$$*\left(1 - \left\{(X_3/V_{\mathrm{brain}})^{\mathrm{Hg}}/\left[(X_3/V_{\mathrm{brain}})^{\mathrm{Hg}} + C_{50}g^{\mathrm{Hg}}\right]\right\}\right) \quad (5b)$$

$$-\mathrm{Kkmax}\left\{(X_3/V_{\mathrm{brain}})^{\mathrm{Hk}}/\left[(X_3/V_{\mathrm{brain}})^{\mathrm{Hk}} + C_{50}k^{\mathrm{Hk}}\right]\right\}(N) \quad (5c)$$

where X_1, X_2, X_3, and X_4 are the amounts of fluconazole (in milligrams) in the gut, central compartment (i.e., plasma), brain, and peripheral compartment, respectively. $B(1)$ is the total amount of drug administered by oral gavage (in milligrams), and K_a is the first-order rate constant that links the gut with the central compartment. SCL (liter/h) is the clearance from the central compartment, and V_c and V_{brain} are the volumes of the central compartment and brain, respectively. k_{cp}, k_{pc}, k_{cb}, and k_{bc} are the first-order rate constants that connect the central (c), peripheral (p), and brain (b) compartments. N is the density (organisms/gram brain) of *Cryptococcus neoformans*. Kgmax is the maximum rate of fungal growth in the brain ($\log_{10}$ CFU/g/h), and POPMAX is the theoretical maximum density within the brain. Hg is the slope function for the suppression of growth, and $C_{50}g$ is the concentration of drug in the brain that produces half-maximal suppression of growth. Kkmax is the maximum rate of fluconazole-induced kill, and Hk is the slope function for the fungal kill. $C_{50}k$ is the concentration of fluconazole in the brain where the rate of fungal killing is half-maximal.

Equation 1 describes the rate of change of fluconazole concentrations in the gut.

Equation 2 describes the rate of change of fluconazole in the central compartment (plasma).

Equation 3 describes the rate of change of fluconazole in the brain.

Equation 4 describes the rate of change of fluconazole in the peripheral compartment (i.e., everything other than the blood and the brain).

Equation 5 describes the rate of change of fungal burden in the brain that contains terms describing the capacity-limited growth of *Cryptococ-*

cus (equation 5a), the drug-associated suppression of growth (equation 5b), and the drug-associated fungal kill (equation 5c).

The weighting functions for the PK-PD data were obtained by initially fitting the same structural mathematical model to the pharmacokinetic and pharmacodynamic data from each strain, using the maximum likelihood estimator within the program ADAPT 5 (10). The model was fitted to the data using the population pharmacokinetic program Nonparametric Adaptive Grid (Big NPAG) (11). Both the mean and median parameter estimates were explored and discriminated on the basis of a linear regression of the observed values versus the predicted values (i.e., intercept, slope, and coefficient of determination [r^2]) for both the pharmacokinetic and pharmacodynamic data.

The area under the concentration-time curve from 0 to 24 h at steady state (AUC_{0-24}) for the regimens used in this study was estimated using integration in ADAPT 5 (10). The area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC ratio) values for each strain was then calculated using the mean estimate for the MIC from each of the 5 experiments (see above). The decline in fungal burden was calculated using the model-predicted values for the fungal density in control mice. The relationship between the AUC versus decline in fungal density and the relationship between the AUC/MIC ratio versus decline in fungal density was modeled using the following sigmoid Emax model:

$$\text{Decline in } \log_{10} \text{CFU/g} = \frac{\text{Emax} \times \text{drug exposure}^H}{E_{50}{}^H + \text{drug exposure}^H}$$

where Emax is the asymptotic decline in fungal burden induced by fluconazole therapy, H is the Hill coefficient (slope function), drug exposure is the AUC value or AUC/MIC ratio value, and E_{50} is the magnitude of drug exposure at which the decline in fungal burden is half-maximal.

To determine the potential impact of the MIC on the exposure-response relationships, the fit of the sigmoid Emax model (assessed using the coefficient of determination) to the AUC versus antifungal effect relationship was compared with that obtained using the AUC/MIC ratio versus antifungal effect relationship.

Determination of drug exposure targets, *in vitro* susceptibility breakpoints, and extrapolating to humans. The clinical consequences of the experimental data and the mathematical models were explored using a previously published population pharmacokinetic model fitted to 113 HIV-infected patients receiving fluconazole (12). The parameter values and their associated variances were inserted into the PRIOR subroutine of ADAPT 5 (10) and a 5,000-patient Monte Carlo simulation was performed. The AUC_{0-24} at steady state for each simulated patient receiving 1,200 mg of fluconazole per day was determined by integration. The MICs for 5,733 isolates of *Cryptococcus neoformans* using Clinical and Laboratory Standards Institute (CLSI) methodology were obtained from the study of Espinel-Ingroff et al. (13).

The most appropriate endpoint(s) for further pharmacodynamic analyses in this model of cryptococcal meningoencephalitis is/are not known. Ultimately, we used a stasis endpoint (i.e., the magnitude of drug exposure that prevents progressive fungal growth—or the drug exposure for which the estimated fungal density in the brain at the end of therapy ($t = 240$ h postinoculation) was the same as that at the time of initiation of therapy ($t = 24$ h postinoculation). The AUC value and the AUC/MIC ratio for each strain that produced stasis were determined from the respective mathematical models. The mean value for each of the four strains was then calculated.

The potential validity of this pharmacodynamic target value was further assessed by placing it in a clinical context. We determined the predicted decline in fungal burden in the brains of mice exposed to fluconazole AUCs that are the same as those that are expected in humans receiving 1,200 mg of fluconazole per day. This dosage was chosen on the basis of a recent clinical trial and current recommendations from Infectious Diseases Society of America (IDSA) (14, 15). A regimen of 1,200 mg of fluconazole per day results in a higher rate of decline in fungal burden in the cerebrospinal fluid (CSF) of patients with cryptococcal meningitis compared with the use of 800 mg/day and is therefore generally recommended for induction therapy (2). We readily acknowledge it may be possible to use higher dosages of fluconazole than 1,200 mg/day, although at the current time there is a relative paucity of clinical, safety, and pharmacokinetic data with such regimens. An expectation for the predicted decline in fungal burden across the MIC distribution of *Cryptococcus neoformans* was calculated. In this process, AUCs were determined for each of 5,000 simulated patients receiving 1,200 mg of fluconazole/day. These AUC values were then divided by the MIC value (i.e., 0.125, 0.25, etc., up to 64 mg/liter) to generate a set of AUC/MIC ratio values for each simulated patient who is theoretically infected with cryptococcal strains with MICs ranging from 0.125 to 64 mg/liter. The predicted decline in $\log_{10}$ CFU/g induced by each AUC/MIC ratio was then calculated using the sigmoid Emax model describing the AUC/MIC-versus-effect relationship that was originally estimated in mice (see Fig. 4). A mean value of the estimated decline was calculated from each of the 5,000 simulated patients at a fixed MIC value. This value was then multiplied by the fraction of the total cryptococcal population for each MIC value obtained from a recent publication (13). Each of those products summed to obtain an expectation for the predicted decline in fungal density across the entire distribution of *Cryptococcus neoformans*.

TABLE 1 MICs for the *Cryptococcus neoformans* strains used in this study

Isolate	MIC (mg/liter)	
	Mode (range)	Mean
H99	4 (2–4)	3.67
F/13186	8 (4–8)	7.33
F/6317	2 (2–8)	3.33
F/20886	2 (2–4)	3

RESULTS

Isolates and MICs. The mode, range, and mean values of the MICs for each of the strains used in this study are summarized in Table 1.

Pharmacokinetic studies. The pharmacokinetics of fluconazole were linear. Following oral gavage, there was a rapid increase in serum and brain fluconazole concentrations, followed by biexponential decay in both compartments. The predicted concentration-time profile of fluconazole in plasma and in the brain for mice receiving 125 and 250 mg/kg/day is shown in Fig. 1. The ratio of the area under the concentration-time curve in the cerebrum ($AUC_{cerebrum}$) to the area under the concentration-time curve in plasma (AUC_{plasma}) was 46.9%. There was no evidence of hysteresis: the shapes of the concentration-time profiles in plasma and brain were similar (Fig. 1).

Murine model of cryptococcal meningoencephalitis. Each of the strains resulted in a sustained nonlethal infection for at least 10 days postinoculation. The i.v. inoculation of *Cryptococcus neoformans* resulted in logarithmic growth in the brain in the initial 4 to 7 days (Fig. 2). With the exception of *Cryptococcus neoformans* F/6317, the rate of growth in the brain subsequently slowed and plateaued with a final fungal density of $\log_{10}$ CFU/g cerebrum of approximately 5 to 7 (see Fig. 2 and estimates for POPMAX in Table 2). The maximum rates of growth (Kgmaxs) were similar in three of the strains, but the rate was lower in strain F/6317 (see Fig. 2). These differences were also apparent in the estimates for Kgmax where the value for F/6317 (0.04 $\log_{10}$ CFU/g/h) was approximately half of the estimates for the other three strains.

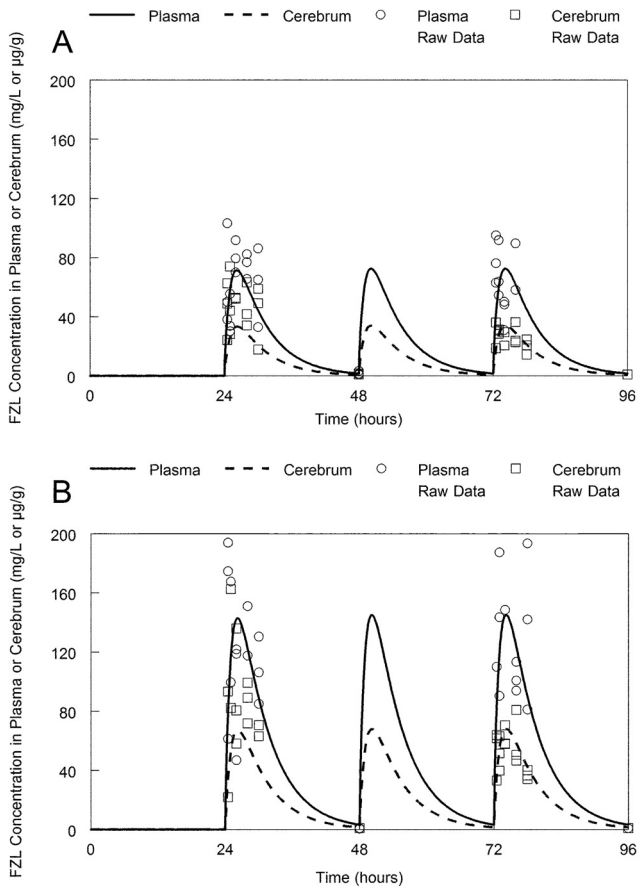

FIG 1 Model predictions for the pharmacokinetics of fluconazole (FZL) in plasma (solid line) and in the cerebrum (broken line) in mice receiving 125 mg/kg/day (A) and 250 mg/kg/day (B). Raw data from individual mice are shown by the open circles (plasma) and open squares (cerebrum).

Pharmacodynamic studies. The oral administration of fluconazole caused a dose-dependent decline in fungal burden in the brain for all strains (Fig. 2). While there were some clear differences in the exposure-response relationships, the administration of 125 mg/kg and 250 mg/kg/day was generally associated with submaximal and static antifungal activity, respectively. At least within the confines of the experimental conditions used in this study, fluconazole therapy prevented progressive fungal growth but did not appear to cause a decline in the density of organisms in the brain (Fig. 2).

The fit of the mathematical model to the data was highly acceptable for most of the strains (see solid lines in Fig. 2). The final parameter values from each of the mathematical models are summarized in Table 2. For each strain, the median parameter values provided a better fit than the means of the mathematical model to the data. A linear regression of the observed values versus predicted values for plasma, brain, and $\log_{10}$ CFU/g values prior to the Bayesian step was statistically significant and in each case had an intercept and slope that approximated 0 and 1, respectively (data not shown). The r^2 values for the pharmacodynamics of strains H99, F/13186, F/6317, and F/20886 were 0.92, 0.66, 0.87, and 0.93, respectively.

A progressively higher AUC_{0-24} resulted in progressively more antifungal activity (Fig. 3). The fit of the sigmoid Emax model to the AUC-versus-effect data was satisfactory, but the r^2 value was only 0.53. In contrast, the fit of the same model to the AUC/MIC ratio-versus-effect data was better with an r^2 value of 0.80 (Fig. 3), suggesting that the MIC accounted for a considerable amount of the original unexplained (or residual) variance.

Definition of the pharmacodynamic target. The AUC and AUC/MIC ratio values associated with stasis (i.e., the drug exposure required to prevent progressive fungal growth) in mice were estimated using each of the mathematical models fitted to data from the respective strains (Fig. 3 and 4). The AUC/MIC values for isolates H99, F/13186, F/6317, and F/20886 that were required for stasis were 340.6, 454.3, 453.5, and 308.7, respectively. Therefore, the mean AUC/MIC ratio target in mice was 389.3, and this was associated with a decline in $\log_{10}$ CFU/g of 3.67 in this murine model (Fig. 4).

An AUC/MIC ratio target of 389.3 with a corresponding decline in murine cerebral burden of $\log_{10}$ CFU/g of 3.67 was closely approximated by an estimated (or expected) decline in fungal density of $\log_{10}$ CFU/g of 3.72 for patients receiving 1,200 mg of fluconazole/day and infected with a population of *Cryptococcus neoformans* isolates with a distribution of MICs described in a recent study (13). Therefore, the use of a stasis endpoint as a pharmacodynamic target appeared justifiable in subsequent determination of breakpoints for fluconazole against *Cryptococcus neoformans*.

***In vitro* susceptibility breakpoints.** A population pharmacokinetic model of McLachlan and Tett (12) fitted to pharmacokinetic data obtained from 113 HIV-infected patients receiving fluconazole was used to determine the proportion of simulated patients that achieved an AUC/MIC ratio of ≥389.3 across the expected distribution of MICs for *Cryptococcus neoformans*. The parameter values from this publication were used to calculate the AUC values predicted from each fluconazole regimen. The AUCs were calculated using integration with the ADAPT 5 program (10). The proportion of simulated patients that exceeded this drug exposure target was 100% for patients infected with strains possessing MICs of ≤1 mg/liter. For strains with higher MICs, the proportion of patients achieving the breakpoint rapidly declined. The percentage success for patients infected with isolates with an MIC of 2 and 4 mg/liter were 96.8 and 52, respectively. For isolates with MICs of 8 mg/liter, only 0.64% of patients achieved the pharmacodynamic target (Fig. 5). It was expected that the proportion of all patients receiving 1,200 mg/day who were predicted to achieve or exceed the pharmacodynamic target was 66.7%.

DISCUSSION

Fluconazole monotherapy is the only therapeutic option for induction therapy of cryptococcal meningoencephalitis in many resource-poor health care settings. The experimental basis for the use of fluconazole for this indication was established over 25 years ago (16) and subsequently confirmed in multiple laboratory animal and clinical studies (see, for example, references 17 to 20). Early clinical studies used relatively low dosages for induction therapy (e.g., 200 to 400 mg/day) (4, 19, 21). While clinical efficacy can be demonstrated with these dosages, the microbiological and clinical outcomes are often inferior to amphotericin B-based regimens, thus prompting studies examining higher dosages of fluconazole (2, 3, 22). The use of ≥1,200 mg of fluconazole/day as monotherapy is recommended in guidelines from the Infectious

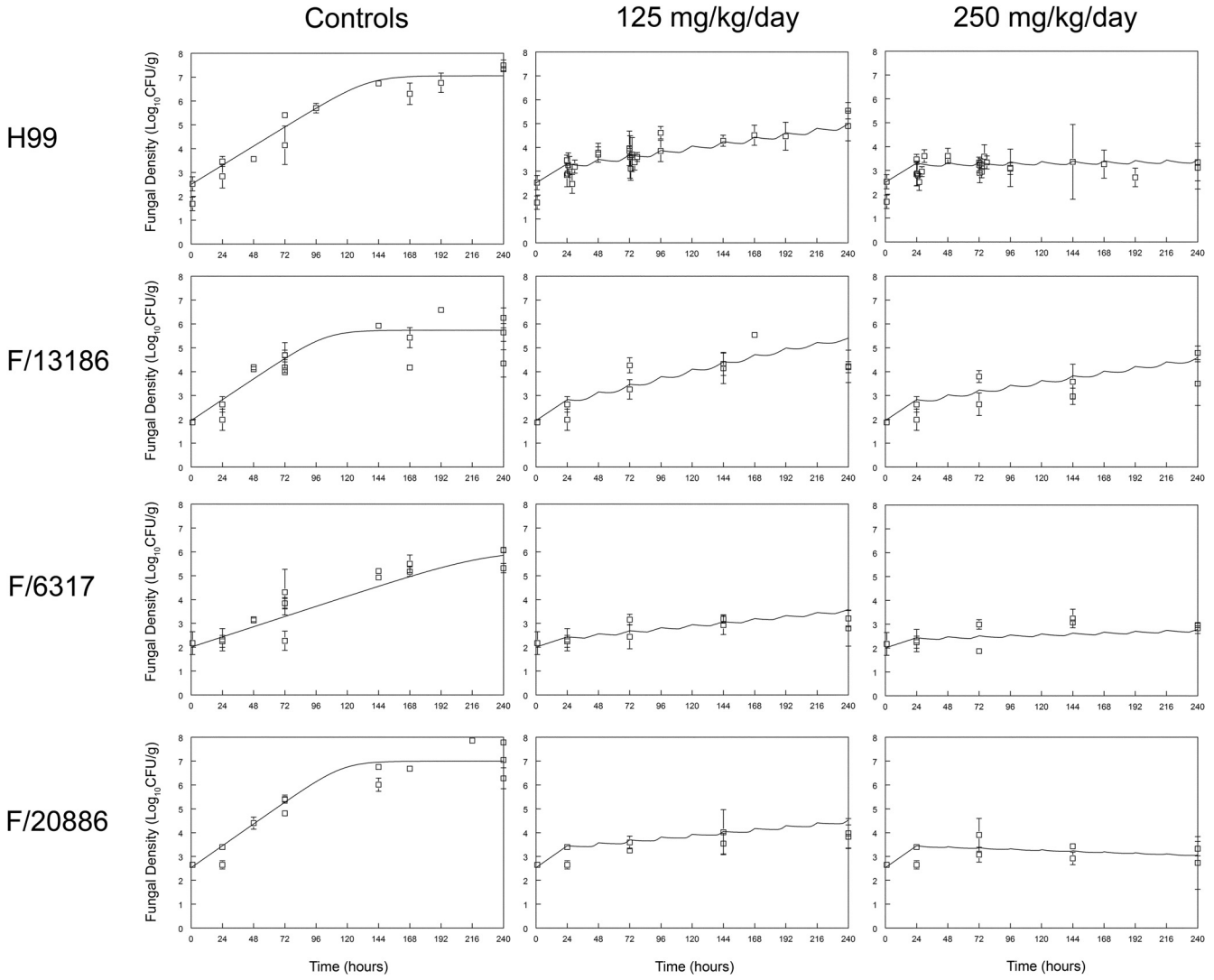

FIG 2 Pharmacodynamics of fluconazole against four strains of *Cryptococcus neoformans*. Mice received no fluconazole (controls) or received 125 mg/kg and 250 mg/kg/day of fluconazole orally. In all cases, the horizontal and vertical axes represent time and the cerebral fungal burden, respectively. The solid line is the fit of the respective mathematical models to the data from each strain. Raw data (open circles) are means ± standard deviations (error bars) for 3 mice. With the exception of strain F/13186, approximately 250 mg/kg/day is required to achieve stasis (i.e., the fungal burden at the start of therapy is the same as the end of therapy).

Diseases Society of America (15). While improved clinical responses are observed with higher fluconazole dosages (circa 1,200 to 2,000 mg/day) (2, 3), the additional antifungal activity that is observed with the addition of flucytosine (3, 14, 22) suggests that these higher dosages of fluconazole (as monotherapy) do not produce maximal antifungal activity. Thus, further studies are required to investigate ways in which fluconazole-containing antifungal regimens can be further optimized.

Our study provides a pharmacodynamic rationale and understanding for the submaximal effect associated with 800 and 1,200 mg of fluconazole/day. A regimen of 1,200 mg/day is predicted to result in approximately one-third of patients failing therapy simply because they are unable to generate sufficient drug exposure (AUC) with respect to the MIC of the invading pathogen. The PK-PD models suggest that clinical outcomes would be even worse with the use of 800 mg/day, which is consistent with clinical observations where the rate of decline of fungal density in the CSF with this regimen is lower than that observed with 1,200 mg/day (2). Suboptimal drug exposure may manifest as clinical failure or the emergence of drug-resistant organisms, as has been described in at least some clinical studies (4). While the molecular basis of this reduced susceptibility following fluconazole exposure is increasingly well understood (see, for example, reference 23), the extent to which this occurs in routine clinical practice is unclear, principally because of difficulties in studying this in a systematic way in resource-poor settings where induction therapy with fluconazole is mostly used.

Experimental studies in mice and retrospective clinical studies suggest that the MIC (or other *in vitro* measures of antifungal potency—see, for example, Bauer et al. [24]) is an important prognostic factor for determining the ultimate clinical outcome (22, 25–28). For example, the probability of clinical failure in pa-

TABLE 2 Parameter values from the mathematical model for each isolate[a]

Parameter[b]	H99		F/13186		F/6317		F/20886	
	Median	SD	Median	SD	Median	SD	Median	SD
K_a (h^{-1})	0.87	1.01	1.32	1.59	1.07	1.03	1.3	1.88
SCL/*F* (liter/h)	0.005	0.0026	0.007	0.003	0.005	0.003	0.005	0.003
V_c/F (liter)	0.007	0.0007	0.01	0.002	0.01	0.01	0.01	0.003
k_{cp} (h^{-1})	14.47	6.12	10.85	5.03	3.47	2.36	14.22	4.61
k_{pc} (h^{-1})	12.44	8.02	18.91	2.94	27.42	1.66	19.77	8.49
k_{cb} (h^{-1})	24.63	5.35	15.59	5.82	23.63	4.83	21.66	5.5
k_{bc} (h^{-1})	13.29	4.77	14.71	3.28	14.27	5.49	16.55	6.05
V_{brain}/F (liter)	0.028	0.009	0.02	0.016	0.036	0.005	0.03	0.009
Kgmax ($\log_{10}$ CFU/g/h)	0.077	0.018	0.084	0.007	0.041	0.0002	0.088	1.83
Hg	8.43	0.33	1.57	2.57	19.26	0.52	6.655	0.92
$C_{50}g$ (mg/liter)	3.63	1.77	2.49	5.33	4.76	1.22	1.62	0.01
POPMAX (CFU/g of cerebrum)	11,370,000	6,811,080	542,108	27,605,800	1,147,144	406,218	9,888,772	3,562,500
Kkmax ($\log_{10}$ CFU/g/h)	0.032	0.01	0.019	0.03	0.017	0.003	0.025	0.013
Hk	1.129	0.059	1.18	0.051	1.07	0.33	1.29	3.39
$C_{50}k$ (mg/liter)	21.06	6.39	16.44	10.28	15.55	1.37	38.42	13.81
Initial condition (CFU/g of cerebrum)	317.87	139.24	89.07	46.39	101.29	1.2	339.98	131.34

[a] The estimated parameter values and standard deviations from the mathematical models fitted to each of the strains.
[b] See text for a definition of the parameters. *F*, bioavailability.

tients induced with fluconazole progressively increases with higher MICs when measured using a microdilution technique (27). Nevertheless, *in vitro* susceptibility testing is generally not advocated as a component of routine clinical care in any clinical setting, nor is it a prerequisite for enrollment in therapeutic clinical trials (15). The results of our study do suggest that the MIC is an important determinant of exposure-response relationships and may help predict the therapeutic response to fluconazole. *In vitro* susceptibility testing may help identify a subset of patients that could be optimally treated with fluconazole. Unfortunately, however, there are considerable obstacles for the routine use of microdilution testing in resource-poor settings where induction therapy with fluconazole is mostly used. A simpler *in vitro* method would be required to make this feasible in that particular context.

This study is the first to provide a pharmacodynamic rationale to aid in the establishment of *in vitro* susceptibility breakpoints for fluconazole against *Cryptococcus neoformans*. The results of a recent study suggest that the epidemiological cutoff values (ECOFFs) for fluconazole against *Cryptococcus neoformans* are 8 to 16 mg/liter. An ECOFF is simply the highest MIC of an isolate

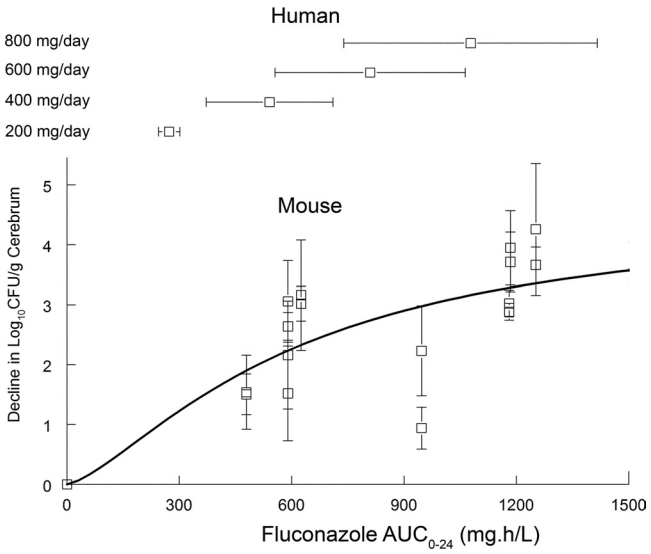

FIG 3 Relationship between the fluconazole area under the concentration-time curve at steady state (AUC_{0-24}) and decline in fungal burden in the brain relative to controls. Data are means ± standard deviations (error bars). The solid line is the fit of the sigmoid Emax model. The experimental data are placed in a clinical context by showing the means ± standard deviations of AUC values for 5,000 simulated patients receiving 200, 400, 600, and 800 mg of fluconazole/day.

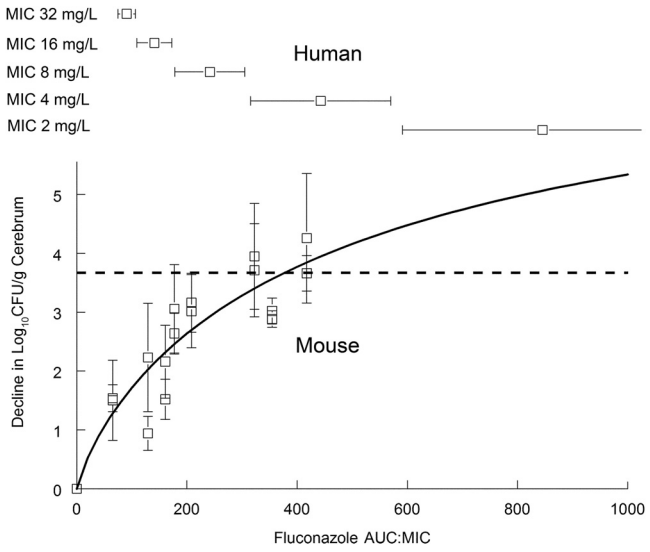

FIG 4 Relationship between the ratio of the fluconazole area under the concentration-time curve (AUC) to MIC (AUC/MIC ratio) and the decline in fungal density in the brain. Data are means ± standard deviations (error bars). The solid line is the fit of the sigmoid Emax model. The experimental data are placed in a clinical context by showing the means ± standard deviations of the AUC/MIC ratios for 5,000 simulated patients receiving 1,200 mg of fluconazole/day and infected with strains with various MIC values given at the top of the figure. As the MIC increases from 2 to 32 mg/liter, there is a progressive decline in the AUC/MIC ratio and a smaller corresponding antifungal effect.

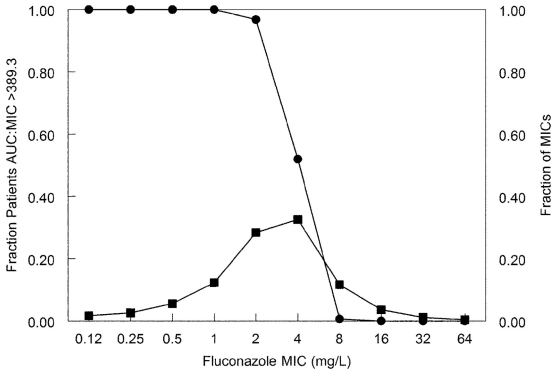

FIG 5 Attainment of the pharmacodynamic target (AUC/MIC ratio of ≥389) as a function of MIC. The solid squares delineate the MIC distribution of *Cryptococcus neoformans* estimated using CLSI methodology. The solid circles show the proportion of 5,000 simulated patients receiving 1,200 mg of fluconazole/day that have an AUC/MIC ratio of ≥389.3.

belonging to the wild-type population, and thereby identifies isolates with potential underlying resistance mechanisms. The results of our study suggest that these epidemiological cutoff values should not be used as clinical breakpoints. The pharmacodynamic analyses suggest the breakpoint for patients receiving 1,200 mg/day is much lower and bisects the wild-type population (i.e., susceptible, ≤2 mg/liter). A greater proportion of the wild-type population could be potentially treated with dosage escalation, but a dosage of 2,400 mg/day would be required before the modal value could be adequately covered (i.e., susceptible, ≤4 mg/liter). While dosages as high as 2,000 mg have been studied in clinical settings (3), the safety and pharmacokinetics of these higher dosages are not well defined. Thus, even the use of high fluconazole dosages does not enable the entire wild-type population to be adequately covered. Clinical evidence to support the establishment of breakpoints is limited. Witt et al. demonstrated a progressive increase in the probability of treatment failure with increasing MIC (27), and patients with AIDS receiving 800 to 1,000 mg of fluconazole/day had a longer median time to CSF culture negativity for isolates with MICs of 4 mg/liter compared with <4 mg/liter (56 versus 16 days, respectively) although this was not statistically significant (22). Until more comprehensive clinical data are available, the results from this study can guide the interpretation of susceptibility testing results.

There are a number of limitations of the current study that should be acknowledged: first, these analyses pertain only to the use of fluconazole monotherapy when used as primary induction therapy for cryptococcal meningoencephalitis caused by *Cryptococcus neoformans*. The predictive value of the MIC for patients receiving fluconazole for consolidation or long-term suppressive therapy following a period of induction therapy with other antifungal agents is not known. This is potentially because the fungal burden may be lower following induction therapy, or because of the development of drug resistance. Second, the breakpoints identified in this study are contingent on patients receiving 1,200 mg of fluconazole/day and are applicable only to meningoencephalitis. The appropriate breakpoints for patients with non-central nervous system (non-CNS) disease (e.g., cryptococcal pneumonia) are not known but may reasonably be different. Third, the most appropriate endpoint for experimental models of cryptococcal meningoencephalitis that are extrapolated to humans is not known. The endpoint used in this study (AUC/MIC ratio of 389) is at least achievable with the drug exposure expected with the use of a clinically relevant dosage of fluconazole (1,200 mg/day). Fourth, while the study extrapolating to humans (bridging study) used a population pharmacokinetic model fitted to HIV-infected individuals (12), the applicability of this model to patients in sub-Saharan Africa is not clear. Unfortunately, there is a rather surprising paucity of robust population PK data for fluconazole, especially at higher dosages, and further pharmacokinetic studies are urgently required. Fifth, the validity of these findings is predicated, as always, on an assumption that the rate and extent of trafficking of fluconazole from plasma into the CNS in humans and mice are comparable. In this regard, there are no robust estimates for the penetration of fluconazole into the human brain, although it is well-known that fluconazole does achieve exposures in the CSF that are 70 to 80% of those observed in plasma (29). Finally, there is an assumption that the fungal density in the cerebrum is a relevant endpoint for a deeper understanding of the therapeutics of human disease. Clearly, humans have both a meningitis and encephalitis, although the former is amenable for routine diagnosis and management via sampling of CSF. Whether there are important differences in the pharmacodynamics of antifungal agents in the cerebrum versus CSF remains poorly defined and deserves further study.

Collectively, therefore, this study provides further evidence that fluconazole monotherapy is an inferior strategy for induction therapy of cryptococcal meningoencephalitis. This study provides a pharmacodynamic rationale for this conclusion, rather than the frequently used explanation that fluconazole is merely a "fungistatic" antifungal agent. For patients for whom fluconazole is the only available antifungal agent for treatment of cryptococcal meningoencephalitis, the highest possible (or tolerated) dose should be used, and if at all possible in combination with flucytosine. There is, therefore, an ongoing requirement to improve global access to flucytosine, and for drug discovery programs to develop other orally bioavailable alternatives to fluconazole for treatment of cryptococcal meningoencephalitis.

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