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Pharmacokinetic Studies of Fluconazole in Rabbits Characterizing Doses Which Achieve Peak Levels in Serum and Area under the Concentration-Time Curve Values Which Mimic Those of High-Dose Fluconazole in Humans

ARNOLD LOUIE,[1] QING-FENG LIU,[1] GEORGE L. DRUSANO,[1,2] WEIGUO LIU,[1] MARTIN MAYERS,[3] ELIAS ANAISSIE,[4]† AND MICHAEL H. MILLER[1]*

Divisions of Infectious Diseases[1] and Clinical Pharmacology,[2] Albany Medical College, Albany, New York 12208; Department of Ophthalmology, Albert Einstein College of Medicine, Bronx, New York 10017[3]; and Section of Infectious Diseases, University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030[4]

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We conducted steady-state pharmacokinetic studies with high-dose fluconazole with rabbits and human volunteers. We then derived mathematical equations that predict the doses of fluconazole that should be given to rabbits to produce 24-h area under the concentration-time curve values and maximum concentrations in serum that are similar to those measured for humans given 800 to 2,000 mg of fluconazole per day. These equations provide a rational basis for designing future efficacy studies with rabbits and in evaluating the strength with which results of previously conducted studies using rabbit infection models can be extrapolated to the clinic.

Rabbit models of systemic candidiasis and deep-seated fungal infections are used to compare the relative efficacies of various antifungal regimens (4, 9, 10, 13–15). Often these models demonstrate that the best outcomes are associated with the administration of higher doses of amphotericin B or fluconazole (9, 10, 13, 14). However, for fluconazole, the higher doses of fluconazole studied may result in concentrations in serum in rabbits that are above those achieved with the maximum safely tolerated dose in humans of 1,600 mg/day (2). In humans, doses of 2,000 mg/day are associated with central nervous system toxicities in 30% of subjects studied (2). Thus, the findings derived from animal models of systemic candidiasis and deep-seated fungal infections may have limited clinical relevance.

In the current study, we defined steady-state maximum concentrations of drug in serum (C_{max}s) and 24-h areas under the concentration-time curves (AUCs) of incremental doses of fluconazole in the serum of rabbits and correlated these pharmacokinetic parameters with those associated with the highest dose of fluconazole that has been shown to be safe in humans (e.g., 1,600 mg/day). From our data, we derived equations to predict the doses of fluconazole that should be used in rabbits to attain serum pharmacokinetic parameters that are equivalent to those achieved with doses of 800 to 2,000 mg/day in humans. These equations provide a rational basis for designing future efficacy studies with rabbits and in evaluating the strength with which results of previously conducted efficacy studies using rabbits can be extrapolated to the clinic.

The pharmacokinetics of fluconazole were determined in 2- to 3-kg male New Zealand White rabbits (Hare Marland, Nutley, N.J.) by a previously described protocol (8). Fluconazole was infused intravenously over 10 min at doses of 15, 20, 30, or 50 mg/kg of body weight into four to five rabbits per dose at 12-h intervals for four doses. Blood was collected at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h following the last fluconazole administration.

In humans, the pharmacokinetics of fluconazole were determined at steady state in six patients with neoplasms and documented or presumptive mold infections (reference 2 and unpublished data). Four hundred milligrams of fluconazole was given intravenously every 12, 8, 6, or approximately 4.8 h to achieve total daily doses of 800, 1,200, 1,600, and 2,000 mg/day, in one, one, three, and one patient, respectively. Fluconazole was administered intravenously as 2-h infusions with a control pump for a minimum of 96 h. Once steady state was reached, plasma samples were collected at the start of a 2-h infusion (0 h) and 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 h after the start of that 2-h infusion.

Fluconazole concentrations in rabbit serum were determined by microbiological (6, 8) and high-pressure liquid chromatography (2) assays previously described. Pharmacokinetic analysis was performed with a nonlinear least-square regression program, RSTRIP (Micromath Scientific Software, Salt Lake City, Utah). The most appropriate pharmacokinetic models were determined by using model selection criteria based upon a modified form of Akaike's information criterion (1). To determine the AUC, the trapezoidal method was used from time zero to the last time point. Since fluconazole was administered at different intervals in rabbits and humans, the AUCs were standardized to 24 h. Regression lines and their 95% confidence bounds were constructed for the dose of fluconazole versus both the 24-h AUC and the C_{max} of fluconazole measured in plasma with the statistical program SYSTAT for Windows, version 6.0 (SPSS, Inc., Evanston, Ill.).

Dose-response C_{max} and 24-h AUC data were characterized for rabbits and humans at steady state. Concentration-time curves for rabbits given 15, 20, 30, or 50 mg/kg every 12 h and for humans receiving fluconazole at 1,600 mg/day are shown in Fig. 1. The relationships between incremental increases in flu-

* Corresponding author. Mailing address: Division of Infectious Diseases, Mail Code A-49, Albany Medical College, Albany, NY 12208. Phone: (518) 262-5343. Fax: (518) 262-6727. E-mail: michael_miller@ccgateway.amc.edu.

† Present address: University of Arkansas Medical Science Center, Little Rock, AR 72205.

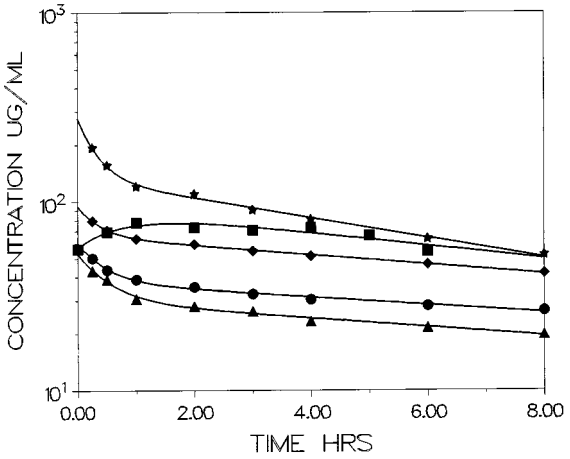

FIG. 1. Concentration-time curves at steady state for rabbits receiving 15 (triangles), 20 (circles), 30 (diamonds), and 50 (stars) mg/kg every 12 h and patients receiving a total dose of 1,600 mg/day (squares).

conazole dose and C_{max} and 24-h AUC are shown in Fig. 2. In rabbits, administration of fluconazole at 15, 10, 30, and 50 mg/kg every 12 h resulted in C_{max}s of 42.1, 50.2, 79.0, and 192.3 μg/ml, respectively, at steady state (Fig. 2, right panel). The corresponding 24-h AUCs were 552.66, 710.96, 1,154.80, and 1,789.50 mg · h/liter, respectively (Fig. 2, left panel). In rabbits, the relationship between fluconazole dose and C_{max} is described by the linear equation $C_{max} = 4.4063D - 35.780$, $r^2 = 0.97$. The relationship between fluconazole dose and 24-h AUC is described by the linear equation $AUC_{24} = 35.6880D + 25.964$, $r^2 = 0.99$. For both equations, D represents the milligram-per-kilogram dose of fluconazole administered intravenously every 12 h. The terminal elimination half-life of fluconazole in rabbits was 11.55 ± 3.4 h.

In humans, 400 mg of fluconazole was administered intravenously every 12, 8, 6, or approximately 4.8 h to achieve total daily doses of 800 ($n = 1$ patient), 1,200 ($n = 1$), 1,600 ($n = 3$), and 2,000 ($n = 1$) mg/day, respectively; each dose was administered as a 2-h infusion. Steady-state C_{max}s measured at the end of the 2-h infusion were 34.0, 51.8, 77.2, and 91.8 μg/ml, respectively (Fig. 2, right panel). The steady-state 24-h AUCs for 800, 1,200, 1,600, and 2,000 mg of fluconazole per day were 813.27, 1,110.30, 1,661.68, and 1,939.04 mg · h/liter, respectively (Fig. 2, left panel). The terminal elimination half-life for the 1,600-mg/day dose was 11.8 h.

There was an excellent linear correlation between the total dose of fluconazole given to humans over 24 h and both C_{max} values ($C_{max} = 0.0542K - 13.680$, $r^2 = 0.99$) and 24-h AUC values ($AUC_{24} = 0.9822K + 6.031$, $r^2 = 0.98$); K represents the total dose administered over 24 h (Fig. 2). For the highest dose of fluconazole that is not associated with central nervous system side effects in humans (1,600 mg/day), the measured C_{max} was 77.2 μg/ml; the calculated 24-h standardized AUC was 1,576.86 mg · h/liter.

Others reported that 400 mg of fluconazole per day resulted in a C_{max} of 18.9 μg/ml and a 24-h AUC of 350 mg · h/liter in healthy human volunteers (5). When these points are displayed relative to the regression lines for C_{max} and 24-h AUC, they are well within the 95% confidence intervals surrounding these lines (Fig. 2), indicating the linear nature of fluconazole pharmacokinetics over the high dose range examined and extending down to the most commonly used fluconazole dose.

The equations that were derived solely from our data were combined to determine the total daily dose of fluconazole that should be administered to rabbits to result in C_{max} and 24-h AUC values that mimick those seen with high-dose fluconazole in humans. The resultant formulas were $D_{C_{max}} = (0.0542K + 22.10)/4.4063$ and $D_{AUC_{24}} = (0.9822K - 19.933)/35.6880$, where D represents the dosage given to rabbits every 12 h and K represents the total daily dose of fluconazole when it is given to humans at 400 mg per infusion. These equations indicate that rabbits would need to receive 24.70 and 43.48 mg of flucon-

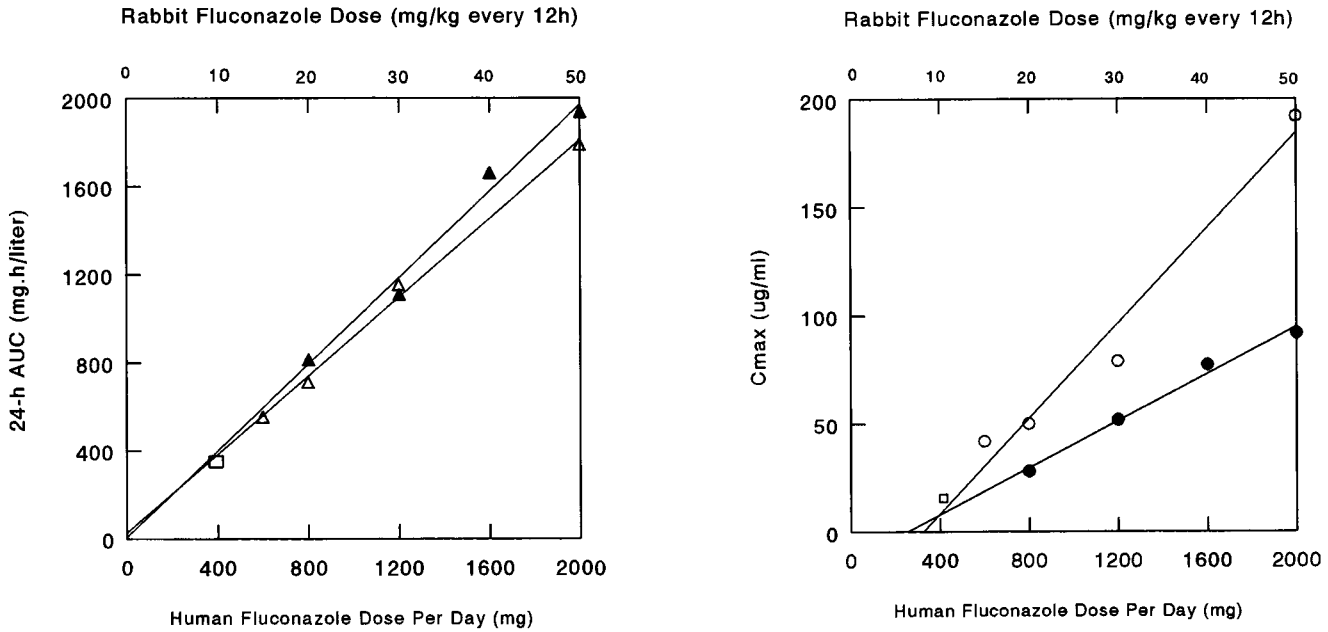

FIG. 2. Relationship between AUC (left) (triangles) and C_{max} (right) (circles) for fluconazole in the serum of rabbits (open symbols) receiving 15, 20, 30, and 50 mg/kg every 12 h and patients (closed symbols) receiving total daily dosages of fluconazole of 800, 1,200, 1,600, and 2,000 mg. Data for separate group of patients who received 400 mg/day are also shown (5) (squares).

azole per kg every 12 h to mimic the C_{max} and 24-h AUC of 77.2 μg/ml and 1,576.86 mg · h/liter, respectively, that are measured for patients given fluconazole at 1,600 mg/day.

For many systemic fungal diseases, it is difficult, if not impossible, to recruit sufficient numbers of patients to conduct meaningful antifungal drug efficacy studies. Thus, drug efficacy is often defined in experimental rabbit models in which the doses of drug evaluated are arbitrarily chosen (4, 9, 10, 13). The results of experimental infection models would have questionable clinical significance if the doses studied resulted in serum pharmacokinetics that were toxic to humans, and the models would not evaluate the full potential of the drug if the doses studied resulted in serum pharmacokinetic values that were lower than those seen with clinically prescribed doses.

Previously, we showed that the pharmacodynamic variable most closely linked with outcome for fluconazole is the AUC/MIC ratio (7). The pharmacodynamic variable associated with toxicity is unknown. Thus, in the current study we defined both the serum C_{max} and 24-h AUCs for dosages of fluconazole in humans and rabbits that are higher than those previously described (3, 5, 12). We found that linear equations expressed the relationship between the dose of fluconazole administered and each of these pharmacokinetic parameters in both species. Then we derived equations that predicted the doses of fluconazole that should be given to rabbits to result in serum C_{max}s and 24-h AUCs that are comparable to the C_{max}s and 24-h AUCs that are measured in humans who receive 800 to 2,000 mg of this azole per day. Doses higher than 2,000 mg/day were not examined in humans because this dose was associated with central nervous system side effects in 30% of recipients (2).

The equations that provide the dose equivalence of fluconazole between humans and rabbits have a number of potential applications. First, they can be used to determine the strength with which outcomes associated with fluconazole therapy in rabbit models of deep-seated fungal infections can be extrapolated to the clinic. Second, they may serve as a tool with which one can reassess the discordance between results seen with humans and in rabbit models of fungal infection, such as the failure of 400 mg of fluconazole per day to prevent the development of aspergillosis in patients (11) while 60 mg/kg/day offered effective prophylaxis in rabbit infection models (9). Our equations demonstrate that a dose of >2,000 mg per day needs to be given to patients to result in the same 24-h AUC that was associated with the dose used for rabbits. Third, knowledge of the doses of fluconazole for rabbits that result in pharmacokinetic parameters that are toxic to humans can be used to define the highest doses of drug that should be used for rabbits to identify the maximum benefit of fluconazole for the treatment of new and emerging fungal infections, including those caused by *Candida parapsilosis*, *Torulopsis glabrata*, and *Fusarium* species.

Finally, it should be recognized that the methods described in this study may serve as a paradigm for defining the dose equivalence for C_{max}s and 24-h AUCs between humans and any other animal species for almost any drug. Using these equations to select doses for animals that result in clinically relevant C_{max}s and 24-h AUCs will facilitate the evaluation of the full potential of established and investigational drugs.

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