Comparison of In Vivo Activity of Fluconazole with That of Amphotericin B against *Candida tropicalis*, *Candida glabrata*, and *Candida krusei*

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Fluconazole (UK-49,858) is a new oral bis-triazole antifungal agent with demonstrated activity against *Candida albicans*. Because of the increasing importance of infections due to other species of *Candida*, we studied the efficacy of fluconazole in a rat model of established systemic candidiasis, using clinical isolates of *C. tropicalis*, *C. glabrata*, and *C. krusei*. In normal rats, oral fluconazole at both 20 and 80 mg/kg per day for 7 days reduced both kidney and liver titers of *C. tropicalis* and *C. glabrata* compared with those in control animals and was only slightly inferior to amphotericin B. Both fluconazole and amphotericin B were ineffective in reducing kidney titers of *C. krusei*, but amphotericin B was more effective than fluconazole in reducing liver titers. Fluconazole showed no increased efficacy at the higher dose of 80 mg/kg per day compared with 20 mg/kg per day in any experiment. These results suggest that oral fluconazole may be useful in the treatment of established disseminated candidiasis caused by species other than *C. albicans*. Further in vivo studies are needed, however, to define minimum effective doses and length of therapy and to test additional *Candida* isolates.

Infections with *Candida* species are an important cause of morbidity and mortality in immunocompromised patients. Fungemia and disseminated candidiasis have been associated with malignancy, broad-spectrum antibiotics, cytotoxic drugs, and total parenteral nutrition (5, 6, 10). Although *Candida albicans* is the most common species found, there has been a marked increase in the number of serious infections in these patients caused by other *Candida* species, including *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and *C. glabrata* (1, 3, 5, 8, 9, 17). Amphotericin B is the standard therapy for serious infections due to *Candida* species, but may be associated with significant toxicity. More infections are occurring with species of *Candida* resistant to amphotericin B (2). Additional drugs, therefore, are needed for both prophylaxis and treatment of infections caused by *Candida* species.

Fluconazole (UK-49,858), an investigational bis-triazole antifungal agent, has been shown to have excellent activity against systemic candidiasis caused by *C. albicans* in normal and immunosuppressed animals (11–13, 16). Advantages of fluconazole include its high levels in serum after oral administration and minimal toxicity even at high doses. To our knowledge, however, the efficacy of fluconazole in the treatment of *Candida* infections caused by species other than *C. albicans* has not been studied. The purpose of this study was to compare the efficacy of oral fluconazole with that of amphotericin B in a model of disseminated candidiasis, using clinical isolates of *C. tropicalis*, *C. krusei*, and *C. glabrata*.


MATERIALS AND METHODS

Animals. Outbred male Sprague-Dawley rats, 225 to 250 g, were housed in groups of five to seven per cage with free access to water and rodent chow (Wayne Pet Food, Continental Grain Co., Chicago, Ill.). After 7 days of acclimatization, the rats were randomly assigned to experimental groups.

Compounds. Fluconazole (UK-49,858; lot R-9) was provided by Pfizer, Inc., Groton, Conn. This compound was preweighed at weekly intervals into sterile tubes and stored at −70°C until the day of use. Solubilization was performed by warming the powder to room temperature, adding sufficient sterile distilled water to make a 5-mg/ml solution, and vortexing until the powder dissolved. Amphotericin B (Fungizone; control 6C86692), provided in part by E. R. Squibb & Sons, Princeton, N.J., was reconstituted with 10 ml of sterile distilled water, and final suspensions for administration were prepared by dilution with a 5% glucose injection USP with a pH of 4.6, yielding a concentration of 0.3 mg/ml.

Inocula. Clinical blood isolates of *C. tropicalis*, *C. glabrata*, and *C. krusei* stored at −70°C in 1-ml aliquots were used for fresh subcultures on Sabouraud dextrose agar plates (Difco Laboratories, Detroit, Mich.), prepared 24 h prior to inoculation, and incubated at 37°C. The *C. tropicalis* isolate was provided by John E. Bennett, National Institutes of Health, Bethesda, Md. The *C. glabrata* and *C. krusei* isolates were blood isolates from patients at West Virginia University Hospitals, Inc., who had had disseminated candidiasis. A 10-ml portion of nonbacteriostatic normal saline was used to suspend subcultured organisms on the day of inoculation, using sufficient organisms to produce a turbid suspension when vortexed. A portion of suspension was then adjusted by serial dilutions in saline to correspond to a McFarland standard of 0.5 which consistently produced a concentration of 10⁶ CFU/ml. The following inocula were used: free injection: *C. tropicalis*, 10⁵ CFU/ml; *C. glabrata*, 10⁴ CFU/ml; *C. krusei*, 10⁴ CFU/ml. (These inocula were

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found in preliminary experiments to infect >90% of animals without early lethality, using the present methods.) Verification of the original concentration of each isolate was done with serial 10-fold dilutions of the original suspension cultured on Sabouraud dextrose agar by using the pour plate technique, incubating at 37°C for 48 h, and counting the CFU per milliliter. Each rat was injected in a lateral tail vein with 0.2 ml of prepared suspension of one of the Candida species.

Treatment regimens. Drug therapy was begun 3 days after inoculation. Groups of rats inoculated with the various Candida species were randomly assigned to one of our four treatment groups: amphotericin B, 1.0 mg/kg per day intraperitoneally; fluconazole, 20 mg/kg per day by gavage given once a day with a blunt metal cannula; fluconazole, 40 mg/kg given twice daily by gavage; or control group, 3.33 ml of distilled water-glucose solution per kg given intraperitoneally (a volume equal to that of the amphotericin preparation).

For the C. tropicalis and C. krusei experiments, each treatment group consisted initially of 10 rats. For the C. glabrata trial, five rats were present in each group at the beginning of therapy.

All rats were treated for 7 days and then sacrificed by pentobarbital injection 2 days after the last treatment.

Culture techniques and statistics. Both kidneys were sterile and excised and decapsulated. Approximately half of each kidney was weighed and homogenized in 1 ml of sterile saline. A segment of liver from each rat was treated similarly. Aliquots of 0.1 ml of homogenate were then cultured on Sabouraud dextrose agar plates in duplicate, two 100-fold dilutions of each homogenate, inverted, and incubated for 48 h at 37°C.

The CFU per gram of tissue for each kidney and liver from each rat was converted to log_{10} for statistical manipulation. This culture technique does not detect <10 CFU/g reliably, so all samples with 0 CFU/g were assigned a log_{10} = 0 corresponding to 1 CFU/g. Analysis of variance was used to compare mean log colony counts of kidneys of various treatment groups; the liver counts were analyzed separately. Fisher’s exact test was used to compare the numbers of animals in each treatment group in an experiment which had negative kidney or liver cultures. A P value of <0.05 was considered to be statistically significant.

In vitro susceptibility testing. In vivo susceptibility studies were done by S. White and S. Shadomy, using a modification of broth macrodilution methods described previously and used for other antifungal agents (9, 15, 18). Amphotericin B was tested in antibiotic medium 3 FDA (Penassay broth 0243; Difco). Fluconazole testing was performed in synthetic amino acid medium-fungal (provided by M. G. Rinaldi, University of Texas Health Science Center at San Antonio, and available commercially from American Biorganic, Inc., North Tonawanda, N.Y.). Amphotericin B solution was prepared by dissolving the stabilized deoxycholate suspension in 100% dimethyl sulfoxide to yield a stock solution concentration of 5,000 μg/ml. Further twofold serial dilutions were accomplished in antibiotic medium 3 FDA to prepare final test concentrations ranging from 100 to 0.05 μg/ml. The test concentrations were placed in 1-ml disposable tubes (Falcon 2054 plastic tubes) in 1.0-ml volumes and were prepared fresh and used the same day. A 5-ml amount of fluconazole was dissolved in 5 ml of water, resulting in a stock concentration of 5,000 μg/ml. The antymycotic was further serially diluted to achieve test concentrations ranging from 100 to 0.05 μg/ml. The test concentrations were dispensed in 1-ml volumes in plastic tubes and used as described above.

A control organism, Saccharomyces cerevisiae (ATCC 9763), as well as the isolates of C. tropicalis, C. glabrata, and C. krusei used for the infection studies, were prepared for susceptibility testing by growing each isolate on Sabouraud dextrose agar slants at 30°C for 48 h. Testing of each Candida isolate was done as follows. A loopful of each organism was removed from the overnight slant and suspended in 5 ml of sterile saline. The saline suspension was adjusted to provide a reading of 95% transmittance when measured in a spectrophotometer (Bausch & Lomb) set at 530 nm. This reading corresponded to approximately 10^6 CFU/ml and was verified by plate counts. The test medium was inoculated (95% suspension) into 12 test tubes at concentrations ranging from 100 to 0.05 μg/ml in duplicates, using 1-ml pipettes. MICs were determined visually at the time growth became turbid in the growth control tubes, usually at 48 h. The MIC was defined as the lowest concentration of antifungal agent which prevented clearly visible growth.

### RESULTS

**C. tropicalis.** Fluconazole at 20 mg/kg per day reduced kidney C. tropicalis titers compared with the control (P < 0.05), but amphotericin B was more effective (P < 0.05) (Table 1). There was no further reduction in kidney yeast titers with fluconazole at 80 mg/kg per day orally (P = 0.69). The results were similar in the liver. With amphotericin B, five of nine rats showed negative liver cultures; with fluconazole at 80 mg/kg per day, 4 of 9 rats did so; and with fluconazole at 20 mg/kg per day, 4 of 10 rats did so. None of 10 control rats had negative liver cultures (P > 0.05). No deaths occurred in any group until sacrifice. The MIC of amphotericin B for the C. tropicalis isolate was 0.39 μg/ml, and that of fluconazole was 100 μg/ml.

**C. glabrata.** Fluconazole at both 20 and 80 mg/kg per day reduced kidney C. glabrata titers compared with the control (P < 0.05) and was as effective as amphotericin B (P > 0.05) (Table 2). In the liver, both fluconazole and amphotericin B reduced C. glabrata colony counts compared with the control (P < 0.05). In this case, the fluconazole was less effective than amphotericin B and only marginally more effective than the control. There were no rats in any group with negative cultures. Two rats in the amphotericin B group died on day 1 of treatment. The MIC of amphotericin B for C. glabrata was 0.2 μg/ml, and the fluconazole MIC was 100 μg/ml.

**C. krusei.** Achieving a high rate of infection in our rat model was possible only by using a very high (10^8 CFU/ml) inoculum of C. krusei. Once infection was established, however, it was difficult to reduce colony counts with a
The amphotericin effective than increased C. therapy 0.05). In have been due to the agents work krusei as drugs human blood isolates of C. CFU/ml. of development 10 Lg/ml, mg/kg mg/kg 20 Fluconazole a Mean for right and left kidneys. All therapy groups lower than control, P < 0.05. b Amphotericin B lower than any other regimen, P < 0.05.

7-day course of any regimen studied. There were no rats in any group that had negative kidney or liver cultures, and there were no deaths before sacrifice. Both fluconazole and amphotericin B were ineffective in reducing kidney titers of C. krusei (P > 0.05) (Table 3). Amphotericin B was more effective than fluconazole in reducing liver C. krusei titers (P < 0.05). As in the previous experiments, fluconazole showed no increased efficacy at the higher dose of 80 mg/kg per day. The MIC of amphotericin B for the C. krusei isolate was 0.78 μg/ml, and that of fluconazole was 100 μg/ml.

**DISCUSSION**

Antifungal agents used for either prophylaxis or empiric therapy in immunocompromised patients should be active optimally against C. albicans as well as other Candida species. In our rat model, we found that fluconazole was effective in reducing tissue titers of one isolate of C. tropicalis and one isolate of C. glabrata. In contrast, fluconazole was ineffective in reducing titers of C. tropicalis, while amphotericin B reduced liver titers by only 1 log. This relatively poor response to amphotericin B by the C. krusei isolate may have been due to the intermediate susceptibility to amphotericin as measured by MIC testing (MIC, 0.78 μg/ml). The observation that fluconazole was ineffective against the C. krusei isolate may be important because the two antifungal agents work via different mechanisms (7, 14). This possible cross-resistance should be sought in other amphotericin B-insusceptible isolates with longer treatment courses before any conclusions should be drawn. We also tested two human blood isolates of C. parapsilosis, but the efficacy of drugs could not be assessed because neither strain used was pathogenic in our rat model even with an inoculum of 10⁶ CFU/ml.

The Candida concentrations required to produce infection reliably in our rat model were high, ranging from 10⁶ to 10⁹ CFU/ml. This situation may not be analogous to the development of disseminated candidiasis in humans. The final Candida tissue titers achieved in control animals, however, were similar to those produced by lower inocula of C. albicans in our experiments. It is likely that lower inocula of C. tropicalis, C. glabrata, and C. krusei would produce a similar degree of infection in immunosuppressed animals.

Although fluconazole at both 20 and 80 mg/kg per day was effective in reducing both liver and kidney titers of C. tropicalis as well as C. glabrata, fluconazole was generally less effective than amphotericin B in our model. Two amphotericin B-treated rats in the C. glabrata experiment died after receiving only one dose. It is possible that the apparent advantage of amphotericin B may have been less had these animals survived and if Candida colony counts could have been measured. A larger sample size of animals would be needed to resolve this issue.

No improvement in efficacy was seen with the higher dose of fluconazole. This observation may result from the fact that the primary mechanism of action of fluconazole is inhibition of ergosterol synthesis by targeting of a single enzyme in the pathway. Its activity may be limited by the kinetics of the drug-enzyme interaction or the degree of enzyme inhibition produced by drug binding, since absorption is nearly complete and distribution to the kidney is particularly efficient (13). Thus, although the higher dose given twice a day overcomes the problem of a short half-life (4 h) in the rat (4), enzyme saturation at low drug concentration or incomplete enzyme inhibition by the drug may preclude realization of greater benefit from the high levels in serum achievable without apparent side effects.

The high MICs of fluconazole when tested in synthetic amino acid medium-fungal did not correlate with the efficacy of the drug in our model against the isolates of C. tropicalis or C. glabrata used. When complex media are used for in vitro testing of fluconazole, the results may be falsely high and may not correlate with clinical efficacy (13). In our fluconazole testing, even with the essential medium synthetic amino acid medium-fungal, the MICs were still high, emphasizing the problem of discrepancies between in vitro and in vivo susceptibilities of Candida isolates to fluconazole (14).

Fluconazole has been shown to be effective in treating C. albicans infections and superior to ketoconazole in several animal models (13, 16). Although in our study fluconazole showed less efficacy against Candida species than amphotericin B, there are potential advantages to this antifungal agent, including a low toxicity profile and oral administration.

Fluconazole showed promise in its activity against C. tropicalis and C. glabrata in the rat model. Further trials are needed in both humans and animals to test this agent against more isolates of Candida species, using prolonged therapy, determining optimal dosages, and assessing efficacy in neutropenic models.

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**LITERATURE CITED**


