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Fluconazole (UK-49,858) Treatment of Candidiasis in Normal and Diabetic Rats

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Fluconazole (UK-49,858), a new oral bistriazole antifungal agent, was compared with amphotericin B in the treatment of established systemic infection with *Candida albicans* in normal and diabetic rats. In normal rats, oral fluconazole at 10 mg/kg per day for 7 days reduced *Candida* colony counts in the kidneys and livers as well as amphotericin B did and was nearly as effective as amphotericin B in a 21-day treatment trial. There was no further reduction in *Candida* colony counts when normal rats were treated with fluconazole at 40 mg/kg twice a day for 7 days. In streptozotocin-induced diabetic rats, fluconazole at 20 mg/kg per day for either 7 or 21 days compared favorably with amphotericin B in efficacy. Results of our study suggest that oral fluconazole may be useful in the treatment of established disseminated candidiasis in normal as well as diabetic hosts.

Disseminated candidiasis is associated with malignancies, broad-spectrum antibiotics, cytotoxic chemotherapy, intra-abdominal surgery, intravascular catheters, and hyperalimentation (2, 8, 10). Diabetes mellitus and urinary tract abnormalities predispose patients to urinary tract infections with *Candida albicans* (3). Amphotericin B is the standard drug used to treat both disseminated and renal candidiasis, but its use may be limited by toxicity. Ketoconazole has been used for mucocutaneous *Candida* infections (1), but it is unreliable for disseminated infections. Ketoconazole can cause a decrease in serum testosterone (12), producing gynecomastia, and it can interfere with adrenal corticosteroid synthesis (20).

Fluconazole (UK-49,858) is an investigational bistriazole antifungal agent (Pfizer Inc., Groton, Conn.) which has demonstrated activity against *C. albicans* as well as other fungi that are pathogenic in humans (7, 11, 17, 19). Oral fluconazole is well absorbed and nontoxic and is eliminated by the kidneys in a biologically active form, a feature which may be an advantage in treating renal candidiasis (6). In contrast to ketoconazole, in vivo and in vitro studies to date have shown that fluconazole appears to have little effect on the synthesis of testosterone in mammalian cells (4).

Fluconazole has been shown to be effective in systemic infections with *C. albicans* in normal mice when 2.5 to 10.0 mg/kg per day is used (19). Other studies in animals have suggested a superiority of fluconazole over ketoconazole in disseminated *Candida* infections (14, 16, 19). To our knowledge, the efficacy of oral fluconazole in treating established systemic candidiasis in diabetic animals has not been examined. In addition, oral fluconazole has appeared promising in the aforementioned animal studies, but it has not been compared with amphotericin B in animals with chronic established candidiasis, in which a very high dose of fluconazole might be expected to be advantageous.

The purposes of the present study were (i) to compare the efficacy of oral fluconazole with that of amphotericin B in the treatment of established systemic *Candida* infections in normal and diabetic rats, and (ii) to compare the efficacy of twice-daily, high-dose oral fluconazole with that of amphotericin B in normal rats with candidiasis.

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MATERIALS AND METHODS

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

Compounds. Fluconazole (lot R-9) was provided by Pfizer Inc. 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 it until it was dissolved. Amphotericin B (Fungizone; control 6C86692) was provided in part by E. R. Squibb & Sons, Princeton, N.J., and was reconstituted with 10 ml of sterile distilled water; and final suspensions for administration were prepared by diluting them with a 5% glucose injection USP with a pH of 4.6, yielding a concentration of 0.3 mg/ml. Streptozotocin (Sigma Chemical Co., St. Louis, Mo.) was prepared fresh as a 3% solution in 0.1 M citrate buffer at pH 4.5 and was stored on ice during the injection procedures. Protamine zinc insulin (protamine, zinc, and Iletin I; U-40; Eli Lilly & Co., Indianapolis, Ind.) was also diluted daily with sterile normal saline to a final concentration of 0.001 U/ml.

Induction of diabetes. In the diabetic rat trial, rats were fasted overnight and then given intraperitoneal streptozotocin at 65 mg/kg of body weight 4 days prior to the *C. albicans* inoculations. Urine glucose and ketone concentrations were monitored daily by using Keto-Diastix (Ames Co., Elkhart, Ind.) reagent strips. Glucose levels in serum were determined at the time of sacrifice by using a glucose oxidase assay and were read spectrophotometrically at 600 nm. During the course of the diabetic trial, the severity of the

diabetes was controlled by using regular insulin and protamine zinc insulin. Regular insulin (0.2 to 0.4 U) was given daily to each rat that appeared lethargic or moribund or had lost >15 g of weight per day, concurrent with a glucose level in urine of 1,000 mg/dl or ketone levels in urine of 80 mg/dl. Only rats with a glucose level in serum of >250 mg/dl at the time of sacrifice were included in the experiment.

Inoculum. A clinical isolate of *C. albicans* (passaged twice after isolation) stored at −70°C in 1-ml fractions was used for fresh subcultures on Sabouraud dextrose agar plates (Difco Laboratories, Detroit, Mich.) that were prepared 24 h prior to inoculation and incubated at 37°C. The *C. albicans* isolate used was a blood isolate from a patient who died of disseminated candidiasis at West Virginia University Hospital. Ten milliliters of nonbacteriostatic normal saline was used to suspend subcultured organisms on the day of inoculation by using a sufficient number of organisms to produce a turbid suspension when the suspension was vortexed. A portion of the suspension was then adjusted by serial dilutions in saline to correspond to a McFarland standard of 0.5, which consistently produced a concentration of 10^6 CFU/ml, and was then diluted in saline to 10^5 CFU/ml (an inoculum that consistently infected >90% of normal rats in this model). Verification of the concentration was done by using serial Sabouraud dextrose pour plates of 10-fold dilutions of the original suspension, incubating it at 37°C for 48 h, and counting the CFU per milliliter. Inocula were prepared by serially diluting the original suspensions with sterile normal saline.

Treatment regimens. Drug therapy was begun 4 days after inoculation with *C. albicans*. Amphotericin B was given by daily intraperitoneal injection of 1.0 mg/kg of body weight. Fluconazole was given once a day by gavage with a blunt metal cannula at 10 mg/kg per day in the nondiabetic rat trial and at 20 mg/kg per day in the diabetic rat trial. Control rats received 3.33 ml of distilled water-glucose solution per kg intraperitoneally (a volume equal to that of the amphotericin B preparation). Courses of therapy of 1 and 3 weeks were used for both normal and diabetic rats. One group of animals receiving each drug regimen was sacrificed 28 days after they finished a 21-day course of therapy, to study relapse after treatment. In the high-dose fluconazole experiment, normal rats received fluconazole orally at 40 mg/kg twice a day for 7 days.

Culture techniques and statistical analysis. All rats except those in the 28-day relapse study were sacrificed 4 days after their last treatment. Both kidneys were sterilely 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. Fractions of 0.1 ml of homogenate were then cultured on Sabouraud dextrose agar plates in duplicate with 200-fold dilutions of each homogenate, inverted, and incubated for 48 h at 37°C.

The CFU per gram of tissue for each kidney and the liver from each rat was converted to $\log_{10}$ values for statistical manipulation. This culture technique did not detect less than 10 CFU/g reliably, so all samples with zero colonies on the pour plates were assigned 1 CFU/g, which corresponded to a $\log_{10}$ of 1 = 0. Analysis of variance was used to compare mean log colony counts in kidneys of rats in the various treatment groups, and the liver counts were compared separately. The Fisher exact test was used to compare the numbers of animals in each treatment group in an experiment which had completely negative kidney and liver cultures. A P value of <0.05 was considered to be statistically significant.

In vitro susceptibility testing. In vitro susceptibility studies were done by S. White and S. Shadomy by a modification of the broth macrodilution methods described previously and as 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 Biorganics, Inc., North Tonawanda, N.Y.). Amphotericin B solution was prepared by dissolving the stabilized Desoxycholate (BBL Microbiology Systems, Cockeysville, Md.) 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 to prepare final test concentrations ranging from 100 to 0.05 μg/ml. The test concentrations were placed in 1-ml disposable tubes (2054 plastic tubes; Becton Dickinson Labware, Oxnard, Calif.) in 1.0-ml volumes and were prepared fresh and used the same day. A total of 5 mg of fluconazole was dissolved in 5 ml of water, resulting in a stock concentration of 5,000 μg/ml. The antimycotic was further serially diluted to achieve test concentrations ranging from 100 to 0.05 μg/ml. These test concentrations were dispensed in 1-ml volumes in plastic tubes and used as described above.

A control organism, *Saccharomyces cerevisiae* ATCC 9763, and the isolate of *C. albicans* used for the infection studies were prepared for susceptibility testing by growing them on Sabouraud dextrose agar slants at 30°C for 48 h. A loopful of the organism to be tested 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 it was measured in a spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.) 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 duplicate by using 1-ml pipettes. MICs were determined visually at the time that growth became turbid in the growth control tubes, usually at 48 h. The MIC was defined as the lowest concentration of antifungal agent which inhibited clearly visible growth.

RESULTS

Systemic infection with *C. albicans* in normal rats. In the 7-day treatment trial, fluconazole at 10 mg/kg per day reduced *Candida* titers in both the livers and kidneys compared with the titers in the controls ($P < 0.05$) and was as effective as amphotericin B (Table 1). Negative liver and kidney cultures in the same animals occurred in 3 of 9 amphotericin B-treated rats, 2 of 9 fluconazole-treated rats, and 2 of 10 control rats ($P > 0.10$). In the 21-day treatment trial, fluconazole reduced *Candida* titers in the livers and kidneys compared with the titers in the controls ($P < 0.01$), but was less effective than amphotericin B in the kidneys ($P < 0.01$). Negative liver and kidney cultures occurred in five of eight amphotericin B-treated rats, one of eight fluconazole-treated rats, and zero of eight controls ($P > 0.05$). In a 21-day treatment trial in which rats convalesced for 28 days after their last treatment, fluconazole reduced *Candida* titers better than amphotericin B did in the liver ($P < 0.05$), while the drugs showed similar efficacies in the kidneys ($P > 0.10$). Negative liver and kidney cultures resulted in two of five

TABLE 1. Fluconazole (10 mg/kg per day) in candidiasis in normal rats

Trial	Drug treatment[a] (no. of rats)	Mean $\log_{10}$ CFU/g of tissue ± SE	
		Liver	Kidney[b]
7-Day Rx[c]	AMB (9)	0.34 ± 0.23	0.90 ± 0.31
	FLU (9)	0.77 ± 0.40	1.28 ± 0.35
	CONT (10)	1.68 ± 0.53	3.11 ± 0.73
21-Day Rx (sacrificed at 4 days post-Rx)	AMB (8)	0.20 ± 0.20	0.20 ± 0.13
	FLU (8)	0.21 ± 0.21	2.02 ± 0.43
	CONT (8)	3.13 ± 0.31	5.27 ± 0.66
21-Day Rx (sacrificed at 28 days post-Rx)	AMB (5)	0.92 ± 0.39	0.52 ± 0.32
	FLU (5)	0.00 ± 0.00	0.81 ± 0.37
	CONT (4)	3.38 ± 0.53	4.45 ± 0.73

[a] Abbreviations: AMB, Amphotericin B; FLU, fluconazole; CONT, control.
[b] Mean for right and left kidneys.
[c] Rx, Treatment.

amphotericin-treated rats, two of five fluconazole-treated rats, and one of four controls ($P > 0.10$).

Systemic infections with *C. albicans* in diabetic rats. In a 7-day treatment trial with fluconazole at 20 mg/kg per day, both amphotericin B- and fluconazole-treated rats had reduced *Candida* colony counts in the livers compared with those in the controls ($P < 0.001$) (Table 2). Both amphotericin B and fluconazole also reduced the *Candida* titers in the kidneys compared with the titers in the controls ($P < 0.05$). There was no significant difference in colony counts in the kidneys ($P = 0.65$) or in the livers ($P = 0.18$) for rats treated with either drug. No rats in any group had negative cultures.

In the diabetic rat experiment with 21 days of fluconazole therapy given at 20 mg/kg per day, both amphotericin B- and fluconazole-treated rats had reduced *Candida* titers in the liver compared with the titers in the controls ($P < 0.001$). There was no difference between rats in the antifungal treatment groups in this experiment. Although both drugs also significantly reduced *Candida* titers in the kidneys, amphotericin B was more effective than fluconazole ($P < 0.001$). With amphotericin B therapy 2 of 15 rats had negative kidney and liver cultures, while both fluconazole and control groups had 0 of 15 rats each with completely negative cultures ($P > 0.05$). Glucose concentrations measured in serum at the time of sacrifice were similar for all groups of rats in the diabetic rat trials (amphotericin B group, 602 ± 103 mg/dl [standard deviation]; fluconazole group 565 ± 182 mg/dl; control group, 591 ± 146 mg/dl; $P > 0.05$).

TABLE 2. Experiment with diabetic rats treated with fluconazole at 20 mg/kg per day

Treatment	No. of rats	Mean $\log_{10}$ CFU/g of tissue ± SE	
		Liver	Kidney[a]
7 Days			
Amphotericin B	12	1.74 ± 0.27	3.30 ± 0.39
Fluconazole	15	2.16 ± 0.29	3.57 ± 0.42
Control	14	3.18 ± 0.25	4.64 ± 0.44
21 Days			
Amphotericin B	15	1.25 ± 0.24	0.88 ± 0.81
Fluconazole	14	2.66 ± 0.13	3.45 ± 0.49
Control	15	5.90 ± 0.20	5.50 ± 0.28

[a] Mean for right and left kidneys.

TABLE 3. High-dose fluconazole treatment results in normal rats

Treatment[a]	Mean $\log_{10}$ CFU/g of tissue ± SE	
	Liver	Kidney[b]
Amphotericin B	0.53 ± 0.26	0.52 ± 0.17
Fluconazole	0.87 ± 0.24	1.77 ± 0.42
Control	3.08 ± 0.31	4.94 ± 0.47

[a] Fluconazole was administered at 40 mg/kg twice a day for 7 days. There were 10 rats in each group.
[b] Mean for right and left kidneys.

Systemic infection in normal rats treated with high-dose fluconazole twice daily. Treatment with either fluconazole at 40 mg/kg twice a day (total, 80 mg/kg per day) for 7 days or amphotericin B reduced *Candida* titers in livers compared with the titers in the controls ($P < 0.001$) (Table 3). There was no difference between the efficacy of amphotericin B and fluconazole in this experiment ($P > 0.05$). Both drugs also reduced titers of *Candida* in kidneys significantly compared with the titers in the controls ($P < 0.05$), but amphotericin B was more effective than fluconazole ($P < 0.05$). With amphotericin B treatment 3 of 10 rats had negative kidney and liver cultures; with fluconazole treatment it was 2 of 10 rats, and for controls it was 0 of 10 rats ($P > 0.05$).

In vitro susceptibility results. The MICs for the *C. albicans* isolate used were 0.20 μg/ml for amphotericin B and 100 μg/ml for fluconazole.

DISCUSSION

The model of candidiasis in rats used in our experiments is one of a subacute systemic infection that is usually well tolerated by the animals for several weeks but is not cleared spontaneously. This model, with treatment delayed for several days, mimics human infection. This is unlike mouse lethality studies, in which therapy is begun concomitant with injection of an otherwise lethal inoculum. We chose to study fluconazole in the oral form for the advantage of ease of administration compared with that of amphotericin B. At 10 mg/kg per day, fluconazole appeared to be as effective as amphotericin B. In a 21-day treatment trial at the same dose, however, fluconazole was as effective as amphotericin B for the liver infection but was less effective for the kidney infection. In the 21-day treatment trial designed to study relapse and cure, in which a 28-day convalescence was incorporated after the last dose, the efficacy of the two drugs was similar. In these animals, which had well-established visceral infections, both fluconazole and amphotericin B treatments produced two of five rats with microbiological cures, which was not significant with this sample size. These data suggest that in this model fluconazole may be as effective as amphotericin B and that higher total doses and longer treatment may be required for a cure with either drug.

In the diabetic rat experiments, we used 20 mg of fluconazole per kg per day because of the severe infection in these animals, even at the lower inoculum of *C. albicans* (10^4 CFU/ml). At 7 days of therapy, amphotericin B and fluconazole were similar in efficacy. However, at 21 days of therapy amphotericin was more effective in the kidneys. The severity of diabetes plus the progressive nature of the candidiasis in this model may explain the fact that few rats had negative cultures even after 21 days of therapy with either drug.

The half-life of fluconazole in humans is about 22 h, but in rats it is only 4 h (6). We postulated that twice-a-day dosing in the rat might be advantageous. Because fluconazole appears to be a safe, well-tolerated drug and because of the previously described results, we used a higher dose administered twice daily to adequately compare it with amphotericin B in the last experiment. At a dose of 40 mg/kg twice daily, fluconazole again had efficacy similar to that of amphotericin B in the liver, but amphotericin B was still better in the kidney. Treatment with fluconazole at 80 mg/kg per day orally versus that at 10 mg/kg per day resulted in similar titers in the kidneys and livers after 7 days. It is possible that with a susceptible isolate of *C. albicans*, the lower dose of fluconazole would be sufficient for efficacy in the rat, with higher doses conferring no advantage. Larger trials and a dose-response curve in this model might demonstrate an optimal dose.

In most of our experiments, amphotericin B was superior to fluconazole in the treatment of infection in the kidneys. This may have been due to the severity of the *Candida* infection in the rat kidney and the propensity of this organ to involvement with *C. albicans* when inoculated hematogenously (13). Also, like the imidazoles, fluconazole may demonstrate fungistatic activity even at high doses (5), while amphotericin B is fungicidal.

The high MIC of fluconazole when tested in synthetic amino acid medium-fungal did not correlate with the efficacy of the drug in our model against the isolate of *C. albicans* that we used. It has been shown that when complex media are used for in vitro testing of fluconazole, the MIC results may be falsely high and may not correlate with clinical efficacy (16). Complex media appear to contain substances which may inhibit fluconazole in vitro (7). Furthermore, in vitro susceptibility testing of fluconazole is troublesome and difficult to reproduce among different laboratories even when the same growth medium is used (17). In our fluconazole testing with a defined medium, the MIC was still high, emphasizing the continued problem of discrepancies between the in vitro and the in vivo susceptibilities of *C. albicans* to fluconazole. At present, in vivo animal models of fungal infections appear to be the best method of assessing the relative antifungal effectiveness of the drug before use in humans (17).

Fluconazole has excellent activity against *C. albicans* in this animal model that is only slightly inferior to that of amphotericin B. The major advantages of the use of fluconazole in humans include oral administration and the low toxicity profile found in the studies that have been done to date. A longer duration of therapy or combination therapy with other antimicrobial agents may be required to achieve mycological cure of established renal or hepatic candidiasis.

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