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Efficacy of UK-49,858 (Fluconazole) against *Candida albicans* Experimental Infections in Mice

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UK-49,858 (fluconazole), a new, orally absorbed bis-triazole derivative, has been evaluated against systemic infections with *Candida albicans* in normal and immunosuppressed mice and against an intestinal infection with *C. albicans* in immunosuppressed mice. Orally administered ketoconazole was used as a comparison agent throughout, and orally administered amphotericin B was included for comparative in the experimental intestinal infection. In a 10-day dosage regimen, UK-49,858 was far more active than ketoconazole against systemic infections with *C. albicans* in normal and immunosuppressed mice. In normal mice, extension of UK-49,858 dosing to 30 days resulted in prolongation of survival to over 90 days, and up to 60% of treated animals had no detectable *C. albicans* in their kidneys. In addition, over 90% of mice with intestinal candidiasis had culture-negative feces after a 3-day treatment with UK-49,858, but only 62 and 23% of mice gave this response after amphotericin B and ketoconazole therapy, respectively. These data suggest that UK-49,858 may be of value in the treatment of systemic and gastrointestinal infections due to *C. albicans* in humans.

The high morbidity and mortality caused by opportunistic systemic fungal infections (10, 12), their increasing incidence (2, 3), and the lack of safe, effective therapy (4, 11, 18) has prompted a search for safer and more effective drugs. UK-49,858 (fluconazole) is a new bis-triazole derivative, developed at Pfizer Ltd., which has been reported (15) to exhibit superior efficacy to that of ketoconazole in fulminating systemic candidiasis (10^7 CFU per animal) of normal and immunosuppressed animals as well as in superficial infections (15). *Candida albicans* is the most common opportunistic fungal pathogen (9, 12, 22), and so we have examined UK-49,858 in comparison with ketoconazole in less rapidly progressing but still lethal systemic infections (10^4 to 10^5 CFU per animal) and with ketoconazole and amphotericin B in a gastrointestinal infection in mice.

MATERIALS AND METHODS

Fungi. *C. albicans* Y0102 from the Pfizer culture collection (Pfizer Central Research, Sandwich, Kent, England) was stored freeze-dried or under liquid nitrogen, and when needed fresh cultures were grown on Sabouraud dextrose agar for 24 h at 28°C. This isolate is a standard test strain used in our laboratory and responds to azoles in a manner typical of other isolates (9, 15). Inocula for the in vivo models were prepared from washed blastospores in saline, standardized by turbidimetry with an absorptiometer (Evans Electroselenium Ltd., Halstead, England), and checked by hemocytometer counts.

Compounds. UK-49,858 was synthesized at Pfizer Central Research Laboratories (U.S. patent 4,404,216 Sept./1983). Ketoconazole (batch no. B15/1) was a laboratory sample generously supplied by Janssen Pharmaceutica, Beerse, Belgium. Amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) was a commercial sample. Concentrations of all compounds are expressed in terms of base. In view of the poor aqueous solubility of ketoconazole and amphotericin B at physiological pH, all compounds were dispersed in 10% Cremophor EL (BASF, Ludwigshaven, Federal Republic of Germany) in 50 mM sodium phosphate buffer (pH 7.0) (15).

Laboratory animals. Outbred female TFI albino mice (A. Tuck and Son Ltd., Southend, England), weighing 18 to 20 g each, were used for all experiments, including the pharmacokinetics. They were housed and maintained as described previously (15). Nine or ten animals per group were used in all the systemic infections, and eight animals per group were used for the intestinal infection. Each experiment was carried out at least in triplicate. Results reported for the systemic infections are from single representative experiments, whereas the intestinal data are a compilation of data from all experiments.

Systemic infections with *C. albicans* in normal mice. Infections in normal mice were produced by injection (via the lateral tail vein) of 2×10^5 saline-washed blastospores of *C. albicans* Y0102 (9), a dose generally lethal for placebo-treated animals within 15 days. Groups of animals received either UK-49,858 (dose range, 0.1 to 2 mg/kg) or ketoconazole (dose range, 20 to 100 mg/kg). Doses, in 0.4 ml of diluent, were administered orally by gavage once daily for 10 or 30 consecutive days, starting 3 days postinfection. The numbers of *C. albicans* in the kidney were determined by sacrificing surviving animals at the end of the experimental period, homogenizing the kidneys in sterile saline in a Silverson mixer (Silverson Machines Ltd., Chesham, England), and plating dilutions of the homogenate onto Sabouraud dextrose agar. Plates were incubated at 37°C, and the number of yeast colonies was counted (50 CFU/g of kidney was the minimum detectable number).

Statistical analysis was carried out by using the two-tailed Mann Whitney test, which examines differences in the distribution of times to death. For comparisons in which both groups of mice had survivors at the end of the experiment, the chi-square test was used to examine differences in the proportion of survivors in each group.

Systemic infection with *C. albicans* in immunosuppressed mice. Animals were immunosuppressed with daily oral doses of dexamethasone acetate (1 mg/kg), starting 4 days before infection. The infection was established by intravenous

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TABLE 1. Efficacy of UK-49,858 and ketoconazole against systemic candidiasis in normal or immunocompromised mice

Experiment no. (animal status)	Treatment regimen			No. of survivors[a]/ total no. inoculated	Fatalities			
					During therapy		After therapy	
	Days of drug dosage	Drug	Daily oral dose (mg/kg)		No.	Mean day of death (range)	No.	Mean day of death (range)
1. Normal	3–13	No drug		0/9	9	7.3 (6–9)		
		UK-49,858	0.1	0/10	6	10.2 (9–13)	4	20.3 (15–28)
			1.0	1/10	0		9	24.4 (18–45)
		Ketoconazole	20	0/10	10	8.6 (1–11)		
2. Normal	3–33	No drug		0/10	10	12.2 (8–27)		
		UK-49,858	0.1	4/10	4	26.7 (20–32)	2	36 (35–37)
			2.0	9/10	0		1	43
		Ketoconazole	25	0/10	8	9 (5–30)	2	34.5 (34–35)
			100	5/10	4	22 (14–32)	1	64
3. Compromised	2–12	No drug		0/9	9	6.6 (4–10)		
		UK-49,858	2.5	0/10	3	5.3 (4–7)	7	13.4 (13–15)
			10	0/10	3	11.6 (11–12)	7	16.3 (14–19)
		Ketoconazole	50	0/10	10	7.6 (3–11)		

[a] Experiment 1 was terminated on day 33 postinfection; experiment 2 was terminated on day 91 postinfection; experiment 3 was terminated on day 20 postinfection.

injection of 10^4 blastospores of *C. albicans*, and placebo-treated control animals died within 10 days. Starting 48 h postinfection, groups of animals received either UK-49,858 in doses ranging from 2.5 to 10 mg/kg or ketoconazole in a dose of 50 mg/kg orally, once daily for 10 consecutive days. The results were analyzed by using the two-tailed Mann Whitney test.

Intestinal infection with *C. albicans*. A chronic infection of the lower part of the intestinal tract can be established in suitably compromised mice (7, 19, 20). Animals received drinking water containing neomycin sulfate (1 mg/ml) continuously starting 4 days before infection; 2 days before infection they were immunosuppressed with a single subcutaneous dose of cortisone acetate (80 mg/kg). Placebo-treated animals inoculated intragastrically with 5×10^6 blastospores had counts of $>10^3$ *C. albicans* cells per g (fresh weight) of feces for at least 2 weeks postinoculation. Oral therapy was started 1 h after inoculation and continued twice daily (8.0 a.m. and 4.0 p.m.) for 3 days. Efficacy was assessed by measuring the numbers of viable *C. albicans* cells in fecal samples at 1, 7, and 14 days after inoculation. Fecal homogenates (in saline) were plated onto Sabouraud dextrose agar containing doxycycline at 100 μg/ml and incubated at 37°C for 48 h. The results were expressed as the numbers of animals with no *C. albicans* recovered from the feces (minimum detection level, 100 CFU/g) and analyzed by using the chi-square test. The effect of drug carry-over into the feces was minimized by dilution.

Pharmacokinetic studies with ketoconazole in mice. Ketoconazole (40 mg/kg) was administered in 10% Cremophor EL as a single oral dose to a group of five mice. Serial blood samples (10 μl), collected in heparinized capillary tubes, were obtained from each mouse by orbital sinus puncture at intervals up to 6 h. Levels in blood were measured by agar plate bioassay with yeast morphology agar (Difco Laboratories, Detroit, Mich.) and an azole-sensitive strain of *Candida pseudotropicalis* (9).

RESULTS

Systemic infections with *C. albicans* in normal mice. Treatment with 10 daily doses of UK-49,858 at 0.1 or 1.0 mg/kg significantly ($P < 0.01$) prolonged survival compared with that of the control animals and those receiving 20 mg of ketoconazole per kg (Table 1). Ketoconazole was not effective. In a subsequent study, treatment was continued for 30 days: 4 of 10 mice receiving 0.1 mg of UK-49,858 per kg and 9 of 10 mice receiving 2.0 mg of UK-49,858 per kg survived until day 91 after infection (Table 1). In contrast, only 5 of the 10 mice receiving 100 mg of ketoconazole per kg survived. UK-49,858 (0.1 and 2.0 mg/kg) produced a significant ($P < 0.01$) increase in survival over that of control animals and those receiving 25 mg of ketoconazole per kg. The difference between UK-49,858 (2.0 mg/kg) and ketoconazole (100 mg/kg) was also significant ($P = 0.05$). Animals surviving to the end of the experiment were sacrificed, and 6 of 9 of these receiving 2.0 mg of UK-49,858 per kg and 1 of 5 receiving 100 mg of ketoconazole per kg had no detectable *C. albicans* in their kidneys.

Systemic infections with *C. albicans* in immunosuppressed mice. Treatment with UK-49,858 at 10 mg/kg for 10 days starting 48 h after infection significantly ($P < 0.01$) prolonged survival compared with that of placebo-treated animals and those treated with ketoconazole at doses up to 50 mg/kg, which was ineffective (Table 1). Treatment with UK-49,858 at 2.5 mg/kg also produced a significant ($P < 0.05$) improvement in survival over that of the ketoconazole-treated group. At death all animals had high levels of *C. albicans* in their kidneys.

Pharmacokinetic studies with ketoconazole in mice. The

TABLE 2. Levels of ketoconazole in blood after single-dose oral administration to mice at 40 mg/kg

Time (h)	Mean level ± SD (μg/ml)
0.25	9.8 ± 3.7
0.5	12.3 ± 3.0
1.0	11.3 ± 4.7
2.0	10.9 ± 6.1
4.0	3.2 ± 2.2
6.0	<3.1

TABLE 3. Efficacy of UK-49,858, ketoconazole, and amphotericin B against intestinal candidiasis in mice[a]

Compound	Oral dose (mg/kg)	No. of mice with negative fecal culture/total no. (%) at days postinfection:					
		1		7		14	
Control	0	0/24	0	2/23[b]	9	2/23	9
UK-49,858	2.5	0/24	0	15/21[b,d]	71	20/22[c]	91
Ketoconazole	10	1/24	4	4/22[b,c]	18	5/21[d]	23
Amphotericin B	20	15/24	62	12/22[b,c]	55	14/23	61

[a] Therapy twice daily for 3 days starting 1 h after infection. Negative culture defined as no *C. albicans* isolated from the feces (limit of detection, 100 CFU/g). Results of three experiments.
[b] One death.
[c] No sample from one animal during a 3-h sampling period.
[d] No sample from two animals during a 3-h sampling period.

peak level in blood for ketoconazole-treated mice, 11.3 to 12.3 μg/ml, was achieved between 30 and 60 min after dosing (Table 2). The pharmacokinetics of UK-49,858 are described elsewhere (8).

Intestinal infection with *C. albicans* in immunosuppressed mice. Antibiotic therapy combined with cortisone immunosuppression predisposed mice to chronic intestinal candidiasis. Thus, feces from control animals contained between 5×10^3 and 1×10^6 CFU/g for at least 14 days after infection. Oral therapy with 2.5 mg of UK-49,858 per kg resulted in a negative culture in 71 and 91% of animals by day 7 and 14 after infection, respectively (Table 3). The numbers of culture-negative animals at day 7 and 14 were significantly different ($P < 0.001$ by the chi-square test) from the numbers of culture-negative control animals and those given 10 mg of ketoconazole per kg. A total of 62% of mice given 20 mg of amphotericin B per kg were culture negative by day 1 after infection, a significant ($P < 0.001$) difference from all other groups. However, subsequently the number of culture-negative animals remained fairly constant, and by day 7 there was no significant difference between the amphotericin B- and the UK-49,858-treated group ($P > 0.10$). After 14 days, UK-49,858 was significantly better ($P < 0.05$) than amphotericin B.

DISCUSSION

Opportunistic pathogens, in particular *C. albicans*, represent an increasing threat to patients with impaired defense mechanisms (10, 12, 17, 22). We have previously shown that UK-49,858 is more active than ketoconazole and provides significant protection against fulminating *C. albicans* infections in normal or cyclophosphamide-treated mice (15). UK-49,858 also exhibited greater efficacy than ketoconazole against the less rapidly progressive infections reported on here. Thus, the majority of normal animals receiving UK-49,858 at 1 mg/kg for 10 days survived for 20 days. Treatment with 2 mg/kg for 30 days not only extended survival to at least 90 days but resulted in five of nine animals with culture-negative results. Ketoconazole was less effective, and only one of the five surviving animals receiving 100 mg/kg for 30 days was cured. These results were not due to the poor oral bioavailability of ketoconazole in our mice, as the peak levels obtained in blood were closely similar to those already reported for mice (1). In addition, UK-49,858 was more than 20 times as effective as ketoconazole in mice immunocompromised by daily administration of dexamethasone. Published data confirm that high doses of ketoconazole (20 to 160 mg/kg) are required for efficacy against lethal systemic *C. albicans* infections in rodents (5, 14, 16). Despite these animal data, ketoconazole has been used successfully to treat candidiasis in humans (6), which suggests that UK-49,858 may also be effective in the therapy of human candidiasis.

The gut has been shown to act as a reservoir for *Candida* spp. in humans (12), and systemic spread may occur by persorption through the gut wall in immunosuppressed patients (10, 12). Oral treatment with a polyene or ketoconazole is used to combat this problem. In the mouse model of intestinal infection presented here, amphotericin B rapidly reduced the number of *C. albicans* in the feces to levels below the minimum detectable, whereas UK-49,858 produced a more gradual decline. However, when assessed at 14 days, UK-49,858 (2.5 mg/kg) produced a 91% cure with no detectable *C. albicans* in the feces of 20 of the 22 mice, while amphotericin B (20 mg/kg) and ketoconazole (10 mg/kg) cleared only 62 and 23% of animals, respectively. The one published study with ketoconazole in a similar animal infection showed that doses of 20 to 40 mg/kg were required to produce clearance in 75 to 80% of animals (21). The excellent activity of UK-49,858 in this model and in a rat model of palatal candidiasis (M. Martin, personal communication) suggests that it may be of value for the treatment of candidiasis of the digestive tract.

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