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Antifungal Activity of Tioconazole (UK-20,349), a New Imidazole Derivative

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Tioconazole (UK-20,349), a new antifungal imidazole derivative, was compared with miconazole for activity in vitro against *Candida* spp., *Torulopsis glabrata, Cryptococcus neoformans, Aspergillus* spp., and dermatophyte fungi (*Trichophyton* spp. and *Microsporum* spp.). Tioconazole was more active than miconazole against all the fungal species examined except *Aspergillus,* against which both agents showed similar activity. Both tioconazole and miconazole inhibited the growth of all fungi examined at concentrations well below their quoted minimum inhibitory concentrations. Their activity against fungi in vivo was investigated in mice infected systemically with *Candida albicans.* Both agents significantly reduced the numbers of viable *Candida* cells recoverable from the kidneys of infected animals, with tioconazole producing a generally more marked reduction. After administration of a single oral dose (25 mg/kg) to beagle dogs or white mice, higher and more sustained circulating levels of bioactive drug were detectable of tioconazole than of miconazole. These observations suggest that tioconazole may have potential in the treatment of both superficial and systemic mycoses in humans.

Superficial fungal infections caused by dermatophytes and yeasts are common clinical problems. Some infections of the vagina and glabrous skin are treated satisfactorily by topical therapy with a variety of agents, and some dermatophyte infections are treated by oral therapy with griseofulvin (2, 10). The newer imidazole antifungal drugs, clotrimazole and miconazole, possess a very broad spectrum of antifungal activity (13, 14, 17, 19, 22), and resistance to them is rare (13). Their proven efficacy has led to their widespread acceptance for topical use (5, 8, 9, 23).

Systemic fungal infections, e. g., coccidioidomycosis, blastomycosis, and histoplasmosis, are much less common and tend to be restricted to specific parts of the world. However, systemic mycoses in the compromised host, especially in patients undergoing immunosuppressive or cancer therapy, are an increasing problem (13). The opportunistic fungus is most commonly a species of *Candida* or *Aspergillus,* and the infection is frequently life threatening. The choice of therapy is limited, with amphotericin B, introduced in 1955, still the drug of choice in spite of the frequency and severity of side effects resulting from its use (1, 4). The only other agent of significance is 5-fluorocytosine which has a limited spectrum of antifungal activity, and its use in therapy frequently leads to the emergence of resistant strains (13, 18). Clotrimazole and miconazole have been evaluated in the treatment of systemic mycoses, each with limited success (6, 7, 11, 13, 15, 20, 24). Orally administered clotrimazole is frequently associated with gastrointestinal intolerance (13, 14, 24) and, since parenteral formulations are not available, its value in systemic therapy is severely limited. Miconazole appears to be better tolerated (3, 13) and is showing some promise in the therapy of systemic mycoses when administered either orally or parenterally (6, 11, 13, 16, 20, 21).

The purpose of this paper is to describe the in vitro and in vivo antifungal properties of tioconazole, a new 1-substituted imidazole derivative (Fig. 1), in comparison with those of the now widely used agent miconazole.

MATERIALS AND METHODS

Compounds. Tioconazole was synthesized at Pfizer Research Laboratories, Sandwich, Kent, U.K. The compound was isolated as the hydrochloride salt. Miconazole nitrate was a laboratory sample (batch L97/1) generously supplied by Janssen Pharmaceutica, Beerse, Belgium. Concentrations of both compounds are expressed in terms of base.

Fungi. Organisms used in this study were clinical isolates, the majority of which were collected since the beginning of 1976.

Assessment of the effect of different media on antifungal activity. The effect of four different agar media on the antifungal activity of tioconazole and miconazole was assessed before selecting the one most

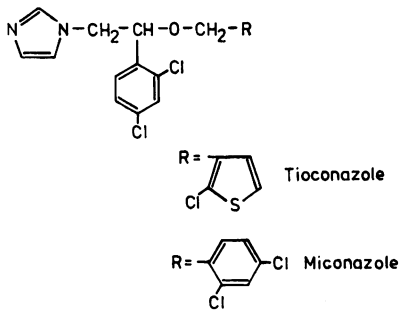

FIG. 1. *Structures of tioconazole and miconazole.*

suitable for use in minimum inhibitory concentration (MIC) determinations. Commercially available Sabourand dextrose agar (SDA; London Analytical and Bacteriological Media Ltd.), diagnostic sensitivity test agar (Oxoid Ltd.), and yeast morphology agar (Difco Laboratories) were made up according to the manufacturers' directions, and yeast nitrogen base medium was prepared according to the formula of Shadomy (18). A method similar to that described by Hoeprich and Huston (12) was used for measuring antifungal activity. The liquid agar media (125 ml) were seeded with *Candida pseudotropicalis* (C84) and poured into separate flat glass assay plates (30 by 30 cm). Standard solutions of tioconazole and miconazole were prepared in 0.1 M phosphate buffer (pH 7) and placed in stainless-steel cups (6-mm internal diameter, 0.3-ml total volume), which were positioned on the surface of the seeded agar. The plates were incubated for 24 h at 37°C, and the zones of inhibition of growth were measured and compared for the different media.

Determination of MIC. MICs were determined using a standard agar plate technique. The compounds were dissolved in dimethyl formamide, and water was added to give a final compound concentration of 1 mg/ml in 10% dimethyl formamide–water. From this solution, serial twofold dilutions of the compounds in water were incorporated into diagnostic sensitivity test agar over an appropriate concentration range (normally 100 μg/ml down to 0.01 μg/ml). Organisms were inoculated onto the surface of the agar, using a multipoint inoculator (Denley Instruments) which delivers approximately 1 μl of the fungal inoculum. The temperature and duration of incubation varied with the different fungi, as did the preparation and standardization of inocula.

Candida spp., *Torulopsis glabrata*, and *Cryptococcus neoformans* were grown overnight at 37°C in brain heart infusion broth (Difco Laboratories). The concentration of cells in the culture was determined in a hemocytometer and then appropriately diluted in broth to 10^6 cells per ml. MICs were read after incubation at 37°C for 48 h.

Inocula of dermatophyte fungi were prepared from a 5- to 10-day growth of the organism on SDA. The dermatophyte mycelium was scraped from the agar and macerated in brain heart infusion broth using an overhead-drive homogenizer. The inoculum was standardized by turbidimetry, diluting as appropriate with brain heart infusion broth (final light transmission reading on an absorptiometer [Evans Electroselenium Ltd.] was 65%). MICs were read after incubation at 28°C for 6 days.

For *Aspergillus* spp., a spore suspension was prepared by washing off the spores from a 2- to 5-day growth of the organism on SDA with saline. The concentration of spores was determined with a hemocytometer and diluted to 10^6 spores per ml with brain heart infusion broth. MICs were read after incubation at 37°C for 48 h.

In all cases the MIC was that concentration of compound which totally suppressed visible growth of the organism.

Determination of the effect on *Candida* spp. of concentrations below the MIC. Agar plates containing different concentrations of tioconazole were inoculated as described in the procedure for determining MICs and incubated at 37°C for 48 h, and the MIC end point was recorded. The areas of fungal growth and the inoculated areas from plates where no growth was visible were excised from the plates and homogenized in brain heart infusion broth (0.9 ml). The number of viable cells present was determined by colony counting after plating out serial 10-fold dilutions of the broth onto diagnostic sensitivity test agar plates and incubating at 37°C for 48 h.

Experimental *Candida* infections in mice. Specific-pathogen-free female mice of weight range 18 to 22 g (A. Tuck and Son Ltd., Essex, England) were used in these studies. The inoculum was prepared from saline washings of an overnight growth of *C. albicans* (strain 44) on an SDA plate. These washings were standardized by turbidimetry to approximately 2.5×10^6 cells per ml. Mice were inoculated via the tail vein with 0.2 ml of this suspension. Tioconazole and miconazole, suspended by sonication in 0.1 M phosphate buffer (pH 7) containing 10% Cremophor EL (BASF, Ludwigshaven, Germany), were administered orally, subcutaneously, or intravenously 1 and 4 h postinfection. Groups of five mice from each treatment group, and five infected control mice, were sacrificed 24 and 48 h postinfection. Kidneys were removed, pooled in the appropriate groups of five, weighed, and homogenized in saline (5 ml). Serial 10-fold dilutions of the homogenate were plated out on SDA plates and incubated at 37°C for 48 h. The colonies of *Candida* were counted, and the number of viable *Candida* cells per gram of kidney tissue was calculated.

Pharmacokinetic studies in dogs and mice. Male beagle dogs, from an in-house breeding colony, and specific-pathogen-free female mice (A. Tuck and Son Ltd.) were used in these studies. The dogs were of average weight 15 kg (range, 14.0 to 16.5 kg), and the mice were of average weight 20 g (range, 18 to 22 g). All animals were fasted for 18 h before, and for the duration of, an experiment. Water was freely available at all times. Tioconazole or miconazole was administered by gavage as a suspension (10 ml per dog and 0.4 ml per mouse) in 10% Cremophor EL–0.1 M phosphate buffer, pH 7.0, at a dose level of 25 mg/kg of body weight. After dosing of dogs, blood samples (5 ml) were withdrawn at intervals from the saphenous vein of a rear leg via a cannula. Serum was separated by conventional procedures. Bladder urine was obtained

by catheter except for final samples (6 to 24 h postdose), which were free-voided while the dogs were housed overnight in metabolism cages.

Serial blood samples (20 μl), collected in heparinized capillary tubes, were obtained from each mouse by orbital sinus puncture at intervals up to 6 h after dosing. Urine samples were collected from separate groups of similarly dosed mice, housed in metabolism cages.

Samples of dog serum and urine and mouse blood and urine were analyzed for tioconazole or miconazole concentrations by bioassay.

Bioassay procedure. A standard agar plate bioassay technique was used for measuring the concentrations of tioconazole and miconazole in serum, blood, and urine. The indicator organism *C. pseudotropicalis* (C84) was grown on yeast morphology agar (125 ml) in flat, glass dishes measuring 30 by 30 cm. Standard solutions of tioconazole and miconazole, covering a range of concentrations from 0.39 to 12.5 μg/ml, prepared in normal dog serum, in heparinized mouse blood, or in 0.1 M phosphate buffer (pH 7.0) in lieu of urine, were used for references purposes. For estimations of tioconazole and miconazole concentrations in dog serum or urine, 0.3 ml of test samples or standards was placed in 6-mm-internal diameter stainless-steel cups on the surface of the seeded agar. For estimations in mouse blood or urine, 20-μl test samples of both unknowns and standards were placed in 6-mm-diameter wells cut in the seeded agar. Plates were incubated overnight at 28°C, and the zones of inhibition of growth were measured. A calibration curve of mean inhibition zone diameter against log concentration was constructed from results obtained with standard solutions. The concentration of antifungal agent in each test sample was obtained by referring the mean inhibition zone diameter to this calibration curve.

RESULTS

Influence of the growth medium on antifungal activity. The largest zones of inhibition of *C. pseudotropicalis* (C84) for both tioconazole and miconazole were obtained in diagnostic sensitivity test agar; the smallest were obtained in SDA. Hoeprich and Huston (12) reported similar observations on the activity of miconazole in SDA and yeast nitrogen base agar. Diagnostic sensitivity test agar was selected for testing the susceptibility of fungi to tioconazole and miconazole, because, of the media studied, it was least antagonistic to these agents and was nutritionally adequate for a wide variety of fungi.

Antifungal activity in vitro. The data in Table 1 show that tioconazole was more active than miconazole against *Candida* spp., *Cryptococcus neoformans, T. glabrata, Trichophyton* spp., and *Microsporum* spp. The two imidazoles were essentially equally active against *Aspergillus* spp. Both compounds effected significant levels of growth inhibition at concentrations well below the MIC. For example, the growth of *C. albicans* (C66) was inhibited by 70% at a concentration of 0.2 μg of tioconazole per ml. The MIC against this strain was 6.2 μg/ml, at which more than 99.9% inhibition of growth occurred. Similar effects were observed with both tioconazole and miconazole for all the species of fungi examined.

***C. albicans* infection in mice.** After intravenous administration of a *C. albicans* suspension to mice, the predominant site from which viable *Candida* cells can be isolated from the infected animals at 24 and 48 h postinfection is the kidney (unpublished data). Substantially more viable cells can normally be recovered after 48 h than after 24 h, indicating that the organism multiplies at this site.

In the experiments summarized in Table 2, 8.6 $\times 10^5$ to 1.4 $\times 10^6$ *Candida* cells per g of kidney tissue were recovered from untreated control mice at 24 h postinfection, and 7.2 $\times 10^6$ to 9.4 $\times 10^6$ *Candida* cells per g of kidney tissue were recovered at 48 h. Tioconazole or miconazole, when administered orally, subcutaneously, or intravenously to infected mice, significantly re-

TABLE 1. *Activity in vitro of tioconazole and miconazole against yeasts, Aspergillus spp., and dermatophyte fungi*

Fungi	No. of isolates	MIC (μg/ml)			
		Tioconazole [GM[a] (range)]		Miconazole [GM[a] (range)]	
Candida albicans	11	4.7	(1.6–12.5)	15.5	(6.3–>25)
Candida spp.	7	0.7	(<0.01–6.3)	3.0	(<0.01–25)
Cryptococcus neoformans	3	0.1	(0.01–0.4)	0.7	(0.5–0.8)
Torulopsis glabrata	3	0.06	(0.05–0.07)	1.0	(0.8–1.1)
Aspergillus fumigatus	3	5.7	(3.1–9.4)	6.2	(3.1–12.5)
Aspergillus spp.	3	4.9	(3.1–6.2)	4.9	(3.1–6.2)
Trichophyton mentagrophytes	2	0.1	(0.08–0.18)	0.7	(0.6–0.9)
Trichophyton rubrum	2	0.5	(0.5)	1.7	(1.6–1.9)
Microsporum canis	2	0.4	(0.3–0.5)	1.7	(1.6–1.8)
Microsporum gypseum	1	0.5		1.6	

[a] GM, Geometric mean of MICs.

TABLE 2. *Effect of tioconazole and miconazole on the recovery of viable Candida cells from the kidneys of infected mice*

Dose[a] (mg/kg)	Route	Recovery of *Candida* (% of control)[b]			
		Tioconazole		Miconazole	
		24 h	48 h	24 h	48 h
100	Oral	<0.5 ± 0.1	3.0 ± 1.7	<1.0 ± 0.4	7.9 ± 3.3
50	Oral	4.5 ± 2.9	11.1 ± 5.0	14.7 ± 8.8	5.0 ± 3.2
25	Oral	7.3 ± 1.6	35.8 ± 20.9	69.0 ± 25.4	26.6 ± 9.8
100	Subcutaneous	0.8 ± 0.3	2.4 ± 0.5	2.8 ± 0.6	5.5 ± 0.6
50	Subcutaneous	1.1 ± 0.6	6.0 ± 2.1	3.3 ± 1.7	16.6 ± 7.9
25	Subcutaneous	1.4 ± 0.8	41.5 ± 26.8	7.8 ± 5.4	52.0 ± 34.1
50	Intravenous	3.3 ± 1.9	1.9 ± 0.9	4.0 ± 1.5	9.6 ± 0.8
25	Intravenous	10.7 ± 0.2	7.5 ± 2.9	34.9 ± 26.6	90.3 ± 6.9
12.5	Intravenous	72.1 ± 22.8	6.0 ± 2.4	55.2 ± 12.1	52.9 ± 33.5

[a] Mice were given the doses stated at 1 and 4 h postinfection.

[b] Values are the means ± standard error of three experiments at 24 h and two at 48 h. The number of viable *Candida* cells in the kidneys was determined at 24 and 48 h postinfection and expressed as a percentage of the number recovered from untreated control mice.

duced the number of viable cells recoverable from the kidneys. The magnitude of the reduction was generally greater at 24 h than at 48 h postinfection and was dose related in the majority of cases. Overall, effects of tioconazole were more pronounced than those of miconazole by all three routes of administration.

Pharmacokinetics in dogs and mice. As the data presented in Fig. 2 show, there were striking differences in the concentrations of tioconazole and miconazole in the sera of dogs after oral administration of doses of 25 mg/kg. Serum concentrations of both compounds peaked 2 h after dosing, but at 4.8 μg/ml for tioconazole and 0.6 μg/ml for miconazole. At 6 h after dosing the concentrations of these agents were 2.2 and 0.3 μg/ml, respectively. Less than 0.1% of the administered dose of either tioconazole or miconazole was excreted in the urine over a period of 24 h after dosing.

The concentrations of tioconazole in blood of mice receiving 25 mg/kg orally were significantly higher than those of miconazole, but the difference in both peak and sustained levels was less striking than that observed in dogs (Fig. 3). Peak concentrations were 2.1 μg/ml for tioconazole and 1.4 μg/ml for miconazole. As with dogs, less than 0.1% of the administered doses of either compound was excreted in the urine over a period of 24 h after dosing.

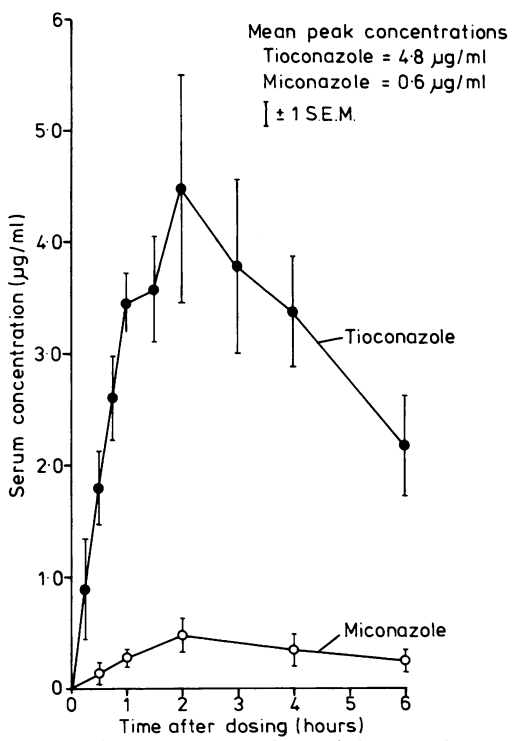

FIG. 2. *Serum concentrations of tioconazole and miconazole in dogs after oral dosing at 25 mg/kg. SEM, Standard error of the mean. Values are the means of three dogs for tioconazole and six dogs for miconazole.*

DISCUSSION

Tioconazole (Fig. 1), a new 1-substituted imidazole derivative, has exhibited significant inhibitory activity in vitro against yeasts, *Aspergillus* spp., and dermatophytes. Against *Candida* spp. it is approximately fourfold more active than miconazole. Tioconazole is also more potent than miconazole against *Cryptococcus neoformans, T. glabrata,* and dermatophytes. Against *Aspergillus* spp., tioconazole and miconazole exhibit similar activity.

Both agents partially inhibit the growth of all

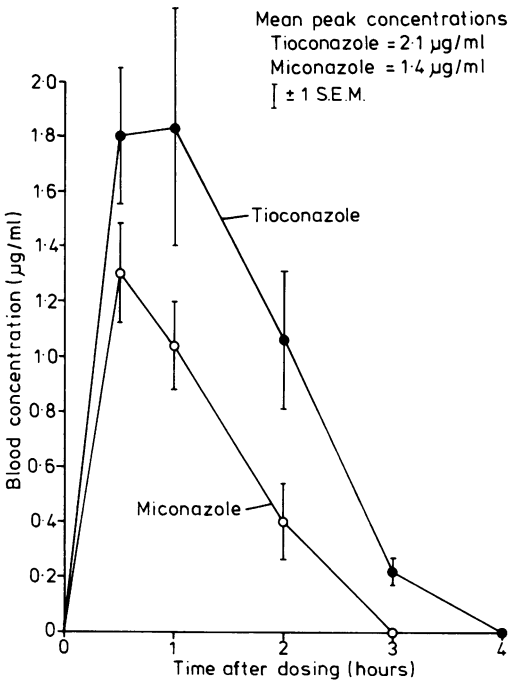

FIG. 3. *Blood concentrations of tioconazole and miconazole in mice after oral dosing at 25 mg/kg. SEM, Standard error of the mean. All values are the means obtained from 18 mice.*

the fungi tested at concentrations below their recorded MICs. From the quantitation of this effect against *Candida* isolates it is evident that inhibition is considerable and remains significant at concentrations approximately 30-fold less than the MIC. Inhibition of fungi by miconazole at concentrations below the MIC has also been reported by other workers (22). In view of its activity in vitro, appropriate formulations of tioconazole should prove to be clinically effective when applied topically in the treatment of superficial infections caused by dermatophytes and yeasts. Early clinical data on a topical formulation of tioconazole supports this conclusion (T. Fredriksson, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 18th, Atlanta, Ga., Abstr. 100, 1978).

Evidence of systemic antifungal activity has been obtained with tioconazole in mice infected intravenously with *C. albicans*. In this model, tioconazole significantly reduces the number of viable *Candida* cells recoverable from the kidneys in a dose-dependent manner. Miconazole exhibits a similar, but less pronounced, effect. In humans, miconazole has been used with some success, principally by the intravenous route, in the treatment of systemic mycoses caused by *Candida* spp. and other fungal pathogens (6, 11, 13, 16, 20, 21). The generally greater potency of tioconazole against fungi in vitro and its activity against *Candida* in the mouse model suggest that it may be more effective than miconazole in the treatment of human systemic mycoses.

The pharmacokinetic properties of tioconazole after oral administration, as judged by concentrations in the blood or serum, are encouraging, particularly in dogs. If this important property translates to humans, it offers the potential of antifungal therapy using the oral route of administration. As a first step in evaluating this possibility, oral pharmacokinetic and tolerance studies in human volunteers are planned.

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