Activity of Ketoconazole and Its Deacyl Derivative Against *Plasmodium falciparum* and *Candida* Isolates

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Deacylketoconazole was 15- to 50-fold more active against *Plasmodium falciparum* than was ketoconazole, based on [³H]hypoxanthine uptake and quantitative parasite counts. In contrast, there were no significant differences between these drugs in their activity against clinical isolates of *Candida* spp.

Imidazoles such as ketoconazole (R 41,400) and miconazole (R 14,889) have a wide spectrum of antimicrobial activity that includes some fungi (2, 8) and gram-positive bacteria (17), *Leishmania tropica* (1), and chloroquine-resistant *Plasmodium falciparum* (10). Because the deacyl derivative of ketoconazole (R39,519) is 10- to 15-fold more active than ketoconazole is against *L. tropica* (1), and because deacylketoconazole may be produced from ketoconazole by acid hydrolysis (Analysis Report No. 575, Janssen Pharmaceutica, 1979) with the HCl necessary for its oral absorption (18), we undertook these studies to determine whether deacylketoconazole was also more active than ketoconazole against *P. falciparum* and *Candida* isolates and whether it might account for the antifungal activity observed in vivo in patients receiving oral ketoconazole (2).

We examined the activity of ketoconazole and deacylketoconazole in vitro against two isolates of *P. falciparum* and 26 isolates of *Candida* spp. Honduras I/CDC is a chloroquine-susceptible isolate of *P. falciparum* inhibited by ≥ 20 ng of chloroquine base per ml in vitro; Indochina I/CDC is a chloroquine-resistant isolate of *P. falciparum* inhibited by ≥ 150 ng of chloroquine base per ml (10). The 26 isolates of *Candida* (12 *Candida albicans* and 14 *Candida tropicalis*), were obtained from the blood cultures of 26 different patients in the Barnes Hospital Microbiology Laboratory and identified by standard criteria (13).

Stock solutions (50,000 $\mu\text{g/ml}$) of ketoconazole and deacylketoconazole were prepared in 0.2 N HCl and diluted within 30 min in either the tissue culture medium (*P. falciparum*) or sterile distilled water (*Candida* spp.). Examination of this material by high-pressure liquid chromatog-

raphy revealed no evidence of degradation (data not shown). Each experiment also included controls with appropriate amounts of drug-free HCl.

P. falciparum parasites were grown in the RPMI 1640 tissue culture medium developed by Trager and Jensen (15) in a modular incubation chamber (Billups-Rothenberg, Inc., Del Mar, Calif.) with 3% O₂, 3% CO₂, and 94% N₂ (11). [³H]hypoxanthine uptake was measured as described by Desjardins et al. (5).

Quantitative parasite counts were determined as described previously (10, 16). The antiparasitodal activity of the imidazoles was expressed as the average percent inhibition of [³H]hypoxanthine uptake (based on a minimum of three determinations) or as the percent inhibition of parasite replication (based on quantitative parasite counts).

The imidazole concentrations necessary to reduce [³H]hypoxanthine uptake or quantitative parasite counts by 50% (50% effective dose) were determined with the method of Litchfield and Wilcoxon (9) by linear regression (the least-squares technique [4]).

Candida isolates were grown in yeast nitrogen base broth (Difco Laboratories, Detroit, Mich.) and tested for their susceptibility to imidazoles by the agar dilution technique with an inoculum of $\sim 10^3$ colony-forming units (12) delivered with a Steers replicator (14). The testing medium was yeast nitrogen base (Difco) to which both 2% agar (Difco) and aqueous solutions of ketoconazole or deacylketoconazole had been added. The yeast nitrogen base was sterilized by filtration through a 0.45- μm filter (Nalgene Labware Division, Rochester, N.Y.); the agar was autoclaved at 121°C and 15 lb/in² for 15 min. Controls included plates containing appropriate dilutions of a standard antifungal agent (amphotericin B) and a *Saccharomyces cerevisiae* isolate susceptible to amphotericin B (≤ 2 $\mu\text{g/ml}$) obtained from Hoffman-LaRoche, Inc. (Nutley, N.J.).

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TABLE 1. Activity of deacylketokonazole and ketoconazole against *P. falciparum* in vitro^a

Strain (testing method)	50% Effective dose ($\mu\text{g/ml}$) (mean $\pm$ SEM)	
	Deacylketokonazole	Ketoconazole
Indochina I/CDC ([³ H]hypoxanthine uptake)	0.14 $\pm$ 0.01	2.83 $\pm$ 0.27
Indochina I/CDC (quantitative parasite counts)	0.40 $\pm$ 0.02	6.17 $\pm$ 0.63
Honduras I/CDC ([³ H]hypoxanthine uptake)	0.09 $\pm$ 0.01	5.10 $\pm$ 0.73

^a Parasitemias were determined by counting a minimum of 1,000 erythrocytes on Giemsa-stained smears after the parasites had been exposed to constant concentrations of the imidazoles for two developmental cycles (96 h) with daily medium changes. Deacylketokonazole was significantly more active than ketoconazole ($P < 0.02$, Student *t* test [4]).

After inoculation, the agar plates were incubated in the dark at 30°C in room air and read after 48 h when controls without drug had achieved confluent macroscopic growth. The lowest concentration of each drug that inhibited visible growth was defined as the minimum inhibitory concentration (MIC). Each test was performed in duplicate and recorded as the average of the two determinations.

Based on [³H]hypoxanthine uptake, deacylketokonazole was 20- to 50-fold more active than ketoconazole against either chloroquine-susceptible or chloroquine-resistant *P. falciparum* isolates (Table 1 and Fig. 1). Although both drugs were slightly less active when tested with quantitative parasite counts, deacylketokonazole was 15-fold more active than ketoconazole with this method.

Based on stage-specific parasite counts, both drugs reduced the number of mature erythrocytic forms (schizonts), from 16 to 20% in untreated controls to <1% at the highest imidazole concentrations tested. However, the most striking change was the increase in abnormal (vacuolated and pyknotic) forms, from <1% in untreated controls to $\geq 85\%$ of parasitized erythrocytes at the higher imidazole concentrations (data not shown).

In contrast, deacylketokonazole was not more active than ketoconazole against isolates of *Candida* (Table 2). The *C. albicans* isolates were more susceptible to the imidazoles tested than were *C. tropicalis* isolates, and both were 1 to 2 dilutions more susceptible to ketoconazole than to deacylketokonazole. These patterns were also observed with individual isolates; 22 of the 26 isolates tested were more susceptible to ketoconazole than to deacylketokonazole.

The results of the in vitro susceptibility studies reported here establish that deacylketokonazole has greater antiparasmodial activity than ketoconazole does against both chloroquine-susceptible and chloroquine-resistant strains of *P. falciparum* in vitro (Table 1 and Fig. 1). The differences in activity between the two drugs were similar as determined by the radiometric method (which is a measure of nucleic acid synthesis) and quantitative parasite counts (which do not discriminate between resting and metabolically active cells). The reason for the greater antiparasmodial activity of deacylketokonazole is unknown. There were no differences between these drugs in their ability to hemolyze unparasitized erythrocytes (data not shown).

Although there were no significant differences between ketoconazole and deacylketokonazole in their activity against clinical isolates of *Candida* spp., there was substantial variability among the isolates tested (Table 2). This variability has also been observed by other investigators, and seems to be characteristic of the *Candida*-imidazole interaction in vitro (3, 6, 7).

Because there are considerable differences in the activity of the imidazoles against fungi other than *Candida* (2, 6, 7), there may be significant differences between ketoconazole and deacylketokonazole in their activity against other (non-candidal) fungal pathogens. However, we have found insignificant levels of deacylketokonazole in high-performance liquid chromatographic studies of serum specimens from patients receiving oral ketoconazole (D. J. Krogstad and G. G. Granich, unpublished data). Therefore, we have concluded that deacylketokonazole does not

FIG. 1. Activity of ketoconazole and deacylketokonazole against the chloroquine-resistant (Indochina I/CDC) strain of *P. falciparum*, as measured by the inhibition of [³H]hypoxanthine uptake.

TABLE 2. Comparative activity of deacyl ketoconazole and ketoconazole against 26 isolates of *Candida* spp.

Drug	MICs ^a (μg/ml)					
	<i>C. albicans</i>			<i>C. tropicalis</i>		
	GM MIC (±SEM)	MIC ₅₀	MIC ₉₀	GM MIC (±SEM)	MIC ₅₀	MIC ₉₀
Deacyl ketoconazole	9.8 ± 1.8	31	31	58.4 ± 9.3	62	125
Ketoconazole	3.4 ± 1.9	2	31	19.1 ± 4.8	31	62

^a Abbreviations: GM MIC, geometric mean MIC; MIC₅₀ and MIC₉₀, concentration required to inhibit 50 and 90% of strains, respectively. The difference between the geometric mean MICs for deacyl ketoconazole and ketoconazole was not significant ($P > 0.05$, Student *t* test [4]).

contribute significantly to the anticandidal activity observed *in vivo* in patients receiving oral ketoconazole.

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