Fungicidal Activity of Tioconazole in Relation to Growth Phase of
Candida albicans and Candida parapsilosis

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It was shown that tioconazole possesses an important property not shared by ketoconazole and micronazole, its well-known relatives in the imidazole group of antifungal drugs. At a concentration of $3.8 \times 10^{-5} \text{ M}$, tioconazole, like miconazole, caused rapid 2- to 3-log reductions in CFU per milliliter when added to late-lag- or logarithmic-phase Candida albicans or Candida parapsilosis cells. Only tioconazole, however, exerted similar reductions when added to diluted stationary-phase cultures. This growth-phase-independent lethal action has important clinical implications and may explain the superior performance of tioconazole, which was observed in earlier comparative drug studies.

Tioconazole (TCZ), a synthetic imidazole-containing antifungal drug, was originally prepared and developed at the Pfizer Research Laboratories in England. From the first comparative study published by the Pfizer group in 1979 (7), it appeared that this agent possessed antifungal properties and other biological characteristics that in certain respects made it superior to the primary imidazole available at the time, namely miconazole (MCZ). TCZ was more active in vitro against a number of opportunistic yeast pathogens and several dermatophytes, and it appeared to be superior in the treatment of mice experimentally infected with Candida albicans. Furthermore, results indicated that TCZ, unlike MCZ, might be administered effectively via the oral route. Surprisingly, little has been published on the action and chemotherapeutic potential of TCZ during the past 5 years. In 1980, Odds reported that TCZ was about equal to MCZ and superior to the promising new imidazole ketoconazole (KCZ) in its activity against various dermatophytes, opportunistic yeasts, and filamentous fungal opportunists in vitro (12). A very recent paper from this same laboratory, however, indicated that TCZ might be better than MCZ against yeasts (13). The Pfizer group published a second study in 1983, describing more fully the advantages of TCZ over MCZ as a topical agent, but no information was presented on the possibilities of systemic use (11). Johnson et al. showed that low levels of TCZ inhibited germ tube elongation in strains of C. albicans more effectively than did MCZ but not as effectively as did KCZ (8). From the standpoint of metabolic interactions, workers at Pfizer demonstrated that low-level TCZ, like other imidazoles, is a potent inhibitor of sterol biosynthesis in yeasts (10, 14). Recent studies in our laboratory indicated that on a mole-for-mole basis, the potential of TCZ for direct, lethal cell damage significantly exceeds that of MCZ or any one of a number of other imidazoles (3). This observation served as the primary stimulus for the investigation presented here. The results complement a very recent report published in this journal by Leffer and Stevens concerning the inhibitory and, more importantly, the fungicidal activities of imidazoles against C. albicans (9). In their experiments with 11 strains of C. albicans, Leffer and Stevens found that both the fungistatic and fungicidal properties of TCZ surpassed those of four other imidazoles studied, including KCZ and MCZ. The findings presented here confirm and extend those of Leffer and Stevens, providing a partial explanation for the marked fungicidal action of TCZ that they observed, and raise new possibilities with respect to the chemotherapeutic potential of TCZ.

MATERIALS AND METHODS

Sources of C. albicans 11651 and Candida parapsilosis 14054, the various culture media employed, and conditions of yeast cultivation were described previously (2, 4). Briefly, for experimental work, organisms were grown at 37°C with rotary shaking in 20-ml volumes of a synthetic liquid medium (pH 7) containing yeast nitrogen base, L-asparagine, and glucose. Inocula were obtained from cultures grown in this manner to late logarithmic or early stationary phase (i.e., 17 to 18 h). MCZ (miconazole nitrate) and KCZ (ketoconazole base), both of which were gifts from Janssen Pharmaceutica, New Brunswick, N.J., and TCZ (tioconazole base), which was a gift from Pfizer, Sandwich, Kent, United Kingdom, were dissolved in Me2SO at $3.8 \times 10^{-3} \text{ M}$ and added to yeast cultures. Appropriate controls with drug-free Me2SO were included in the experiments. Viability estimations were done by standard dilution and plate count methods, as described earlier (4).

RESULTS

In our system, $3.8 \times 10^{-3} \text{ M}$ MCZ (16 μg/ml) can exert direct, lethal, physicochemical cell damage against Candida species, but KCZ at the same molarity (20 μg/ml) or even at twice this concentration (40 μg/ml) cannot (1-4). However, under the conditions of the C. albicans experiment in which the drug was added to cells in stationary phase immediately after inoculation into fresh medium (Fig. 1) the lethal direct effect of MCZ apparently is not expressed (4). The high fungistatic activities of MCZ and KCZ at $3.8 \times 10^{-3} \text{ M}$ were nearly identical and probably reflect a common mechanism of action on a specific metabolic function (15). Fungistatic activity of the same magnitude as that shown in Fig. 1 resulted when the concentrations of KCZ and MCZ were doubled to $7.6 \times 10^{-3} \text{ M}$, the highest concentrations tested (data not shown). In contrast, when TCZ was tested at the $3.8 \times 10^{-3} \text{ M}$ level (15 μg/ml), there was marked fungicidal activity as evidenced by a 2-log reduction in CFU per milliliter within 6 h (Fig. 1). On a mole-for-mole basis, therefore, TCZ was markedly superior to MCZ and KCZ with respect to activity against stationary-phase cells. This finding is consistent with earlier results from our laboratory.
(3), and it prompted a more thorough investigation of TCZ activity in relation to the phase of growth in which C. albicans cells are treated. When \(3.8 \times 10^{-3}\) M TCZ was added at time zero to early-stationary-phase cells, viable counts remained constant for the first hour, followed by over a 2-log reduction in CFU per milliliter between 1 and 4 h (Fig. 2). Susceptibility to this fungicidal action increased significantly as cultures reached the end of lag phase and proceeded into the period of exponential growth as evidenced by the 3- to 4-log reductions in CFU per milliliter that occurred within 1 h after addition of the drug at these stages of the growth cycle. At \(1.9 \times 10^{-3}\) M, TCZ was not lethal when added at time zero to stationary-phase cells. The effect was only fungistatic in this case, and there was only a modest increase in susceptibility as cultures approached the end of lag phase. Further experiments showed that the antifungal properties of TCZ revealed in Fig. 2 do not simply reflect a unique characteristic of the C. albicans strain studied. A strain of C. parapsilosis behaved in a very similar manner toward TCZ (Fig. 3).

**DISCUSSION**

Earlier studies showed that as yeast cells pass through the various phases of growth, they undergo phenotypic changes that coincidentally confer resistance or susceptibility to the lethal, direct, physicochemical, cell-damaging effect of MCZ (4, 5). Inoculation of stationary-phase organisms into medium containing \(3.8 \times 10^{-3}\) M MCZ resulted in fungistasis upon subsequent incubation, but no fungicidal activity. However, if cells were incubated for 3 to 6 h without the drug, addition of MCZ then caused a rapid and extensive decrease in CFU per milliliter (4). It was demonstrated in this study that susceptibility to the lethal action of \(3.8 \times 10^{-3}\) M TCZ also increased as cultures of C. albicans and C. parapsilosis passed from stationary- to late-lag- to logarithmic-phase growth. However, the fungicidal nature of TCZ activity appeared to be relatively growth phase independent when compared with that of MCZ. At 15 \(\mu\)g/ml (i.e., \(3.8 \times 10^{-3}\) M), TCZ caused rapid 2- to 3-log reductions in CFU per milliliter during the early hours of drug exposure regardless of growth phase or physiological state of either organism. These results provide an explanation for the superiority of TCZ noted by Leffler and Stevens (9) and probably reflect greater capacity and versatility in the direct cell damage potential of TCZ than that of MCZ and other imidazoles (3), but this possibility has not been proven.

Apparently nothing has been published with regard to the pharmacokinetics of TCZ in humans, but whether serum levels of 15 \(\mu\)g/ml could be approached with oral or parenteral administration is questionable (6). Nevertheless, the fact that this moderately elevated concentration of TCZ was clearly fungicidal when tested against representative strains of two important opportunistic yeast pathogens regardless of growth phase or physiological state seems sufficient justification for further evaluation of TCZ as a systemic antifungal agent. Because of its apparently unique fungicidal properties, TCZ could eventually prove superior to those imidazoles now used widely in topical preparations for the treatment of superficial or cutaneous mycotic infections. Finally, the possibility of developing new azole compounds with pharmacological and antifungal properties superior to any now in existence is reinforced by these TCZ studies. In view of the virtually unlimited opportunities for molecular modifications within theazole group, it seems more likely that
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