ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1982, p. 355–357
0066-4804/82/020355-03$02.00/0

Vol. 21, No. 2

Combined Activity of Ketoconazole and 5-Fluorocytosine on Potentially Pathogenic Yeasts

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Received 8 September 1981/Accepted 6 November 1981

In vitro studies based on periodic viable count determinations indicated that the two antifungal drugs ketoconazole and 5-fluorocytosine generally were at least additive in their combined effect on various opportunistic yeast pathogens, including some resistant to 5-fluorocytosine.

Suitability for oral administration is a major advantage of the two antifungal drugs ketoconazole (KCZ) and 5-fluorocytosine (5-FC). Unfortunately, 5-FC has several well-known disadvantages that restrict its use clinically (2). Originally designated as compound R 41400, KCZ is a highly promising, new imidazole-containing drug that was synthesized and initially evaluated in the laboratories of Janssen Pharmaceutica, Beerse, Belgium (8, 11). In this report, we have assessed the in vitro activity of KCZ–5-FC combinations against various potentially pathogenic or opportunistic species of yeasts. Opportunistic yeast infection is a problem of increasing importance in clinical medicine, particularly in immunosuppressed patients (4). More effective and totally oral drug regimens would be a decided advantage in these situations. At present, information regarding the chemotherapeutic potential of imidazole–5-FC combinations is quite limited (1, 3, 6, 7).

Gifts of 5-FC and KCZ were supplied by Hoffmann-La Roche Inc., Nutley, N.J. and by Janssen Pharmaceutica, New Brunswick, N.J., respectively. KCZ was dissolved in 0.004 N HCl, and 5-FC was dissolved in deionized water. After filter sterilization, the solutions were further diluted in sterile water.

The two primary yeast strains used were *Candida albicans* ATCC 11651 and *Candida parapsilosis* ATCC 14054. Single clinical isolates of *C. albicans*, *Candida tropicalis*, and *Torulopsis glabrata* were obtained from D. Serstock, Laboratory Service, VA Medical Center, Minneapolis, Minn. Inoculum cells were grown for 16 to 18 h in 20 ml of a synthetic liquid medium (1) at 37°C with rotary shaking (150 rpm). Several 5-FC–resistant mutants were derived from *C. albicans* 11651 in the following manner. Flasks containing 100 ml of synthetic medium each were inoculated with approximately 10^8 colony-forming units of *C. albicans* and incubated as described above for 2 h. 5-FC was then added at a concentration of 5.0 μg/ml, and incubation was continued until heavy turbidities were obtained (6 to 7 days). Culture samples were diluted 1:200 in saline, and 0.01-ml volumes were spread on plates of synthetic medium solidified with agar and containing 5.0 μg of 5-FC per ml. After incubation, colonies were picked and streaked on new plates for final isolation.

In the drug combination experiments, KCZ and 5-FC were added to 20-ml volumes of medium in 50-ml flasks. Each was inoculated to a density of 1.5×10^5 to 2.0×10^5 colony-forming units per ml. Viable yeast counts were monitored at intervals during shake incubation at 37°C by standard plate count methods (1). Plates were usually incubated for 2 days at 37°C before counting.

The data in Fig. 1 and 2 show a comparison of the effects of KCZ and 5-FC alone and in combination on the two reference organisms and three clinical isolates. Concentrations of KCZ approximated serum levels achievable in vivo (5). Note that a concentration of 5.0 μg/ml was only partially inhibitory, except for *C. parapsilosis*, where 0.50 μg/ml was moderately fungicidal. Concentrations of 5-FC were well below serum levels achievable in vivo (9), but this condition was necessary to detect any antagonistic or positive drug interaction. Note that 5-FC alone at ≤0.40 μg/ml provided periods of fungistatic or fungicidal activity followed by rapid growth. The population of *C. albicans* 11651 present after 120 h of treatment was tested and found susceptible to 5-FC (Fig. 1). Therefore, transient 5-FC activity apparently reflects escape of susceptible organisms from the effects of low-level drug. In general, antifungal activities of KCZ–5-FC combinations greatly exceeded those seen with either drug alone, and the role of KCZ appeared to be more than simply growth suppression of 5-FC–resistant mutants. The single unexplained deviation from this cooperative

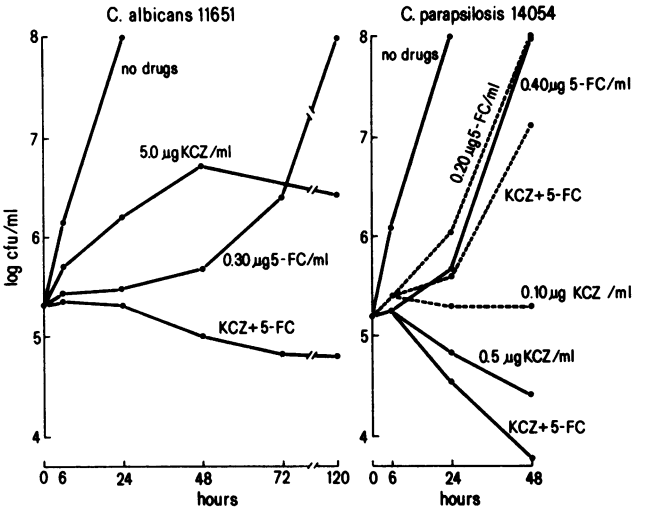

FIG. 1. Inhibitory activity of KCZ and 5-FC both singly and in combination against ATCC strains of *C. albicans* and *C. parapsilosis*. The population of *C. albicans* cells that emerged by 120 h in the culture treated with 0.30 μg of 5-FC per ml alone retained 5-FC susceptibility.

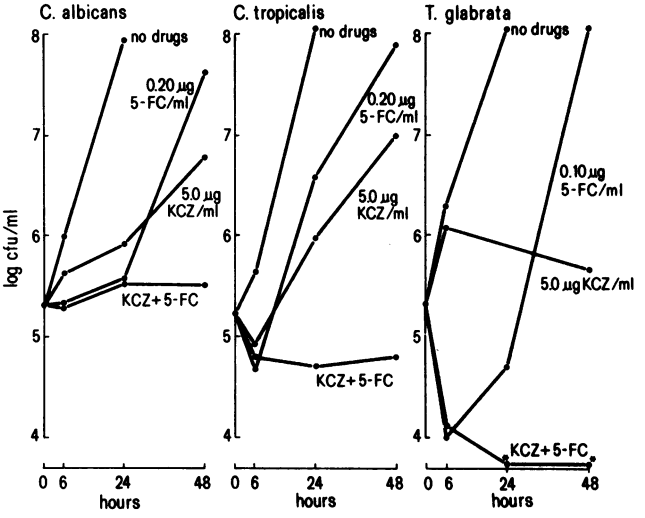

FIG. 2. Inhibitory activity of KCZ and 5-FC both singly and in combination against three clinical yeast isolates. *, $\leq 1 \times 10^3$ colony-forming units per ml.

drug interaction occurred with *C. parapsilosis*. Although concentrations of 0.50 μg of KCZ plus 0.40 μg of 5-FC per ml were at least additive, 5-FC at 0.20 μg/ml antagonized the activity of KCZ at 0.10 μg/ml.

We assessed combined drug activity against three 5-FC–resistant derivatives of *C. albicans* 11651 (Fig. 3). Mutant 5FCR-1 was unaffected by 50.0 μg of 5-FC per ml but retained the typical pattern of susceptibility to KCZ. With both drugs, growth was identical to that seen in the culture containing KCZ alone. Different results were obtained with 5FCR-2 and 5FCR-3, which were nearly identical to each other. A strong but very brief period of fungistasis occurred in the presence of 50.0 μg of 5-FC per ml alone. KCZ patterns again were typical. After 24 h of incubation, combined drug activity exceeded that of the imidazole alone in both cases.

Measurement of viability as a function of exposure time is one of the more rigorous methods to assess antimicrobial drug action, but it is

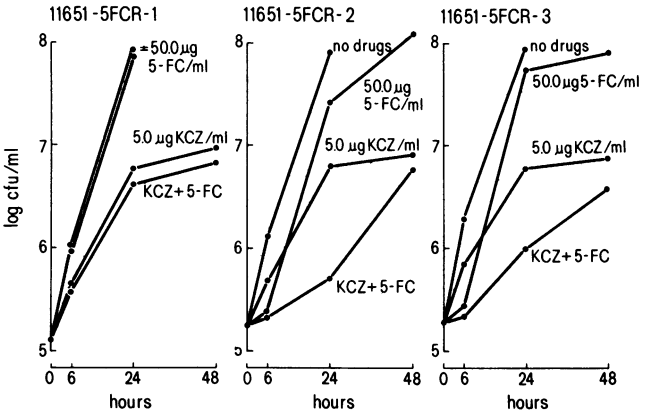

FIG. 3. Inhibitory activity of KCZ and 5-FC both singly and in combination against three 5-FC–resistant mutants of *C. albicans* ATCC 11651.

impractical for surveys of large numbers of different organisms. In this study, we sacrificed exhaustive survey for rigor. Results were generally indicative of an additive or synergistic KCZ–5-FC interaction against various opportunistic yeasts, including some showing 5-FC resistance. Furthermore, the data were consistent with previous studies from this laboratory on clotrimazole–5-FC combinations (1) and with the recent work of Dupont and Drouhet (3), who studied effects of 5-FC–econazole and 5-FC–miconazole combinations against strains of *C. albicans* and *Cryptococcus neoformans*. In apparent contrast, Graybill et al. (6) and Harvey et al. (7) failed to observe any positive interaction between KCZ and 5-FC. However, an assessment of this drug combination was not the primary thrust of these reports, and the negative findings were based on experiments with single strains of *C. neoformans* (6) and *Blastomyces dermatitidis* (7). Moreover, strains of *B. dermatitidis* generally are not susceptible to 5-FC (10).

Based on these studies, it appears that combined activity of KCZ and 5-FC against opportunistic yeast pathogens is usually at least additive. We conclude that this drug combination is sufficiently promising to justify evaluation in an in vivo system.

This work was supported by the Veterans Administration.

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