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Clotrimazole (Bay b 5097): In Vitro and Clinical Pharmacological Studies

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Clotrimazole (Bay b 5097) is a new synthetic antifungal drug with in vitro activity against *Candida* spp., *Torulopsis glabrata*, and *Saccharomyces* spp. Pharmacological studies in man after the oral administration of 1.5 and 3 g of clotrimazole produced mean peak concentrations in the serum of 1.16 and 1.29 μg/ml, respectively, 2 hr after administration. In six patients taking 1.5 g of clotrimazole every 6 hr, there was a progressive decline in the serum concentrations after administration of a dose on days 1, 4, and 8. Nine other patients begun on a similar schedule manifested gastrointestinal symptoms attributed to the clotrimazole and were unable to complete the study. Concentrations of active drug in the urine were less than 1% of the administered dose.

Systemic fungal infections contribute significantly to the morbidity and mortality of patients with hematological malignancies (1, 7) and of transplant patients receiving immunosuppressive therapy (13, 17, 20). Currently, the only antifungal drugs available for the treatment of these infections are amphotericin B and 5-fluorocytosine. The efficacy of amphotericin B is, however, limited by its inherent toxicity (21). The clinical evaluation of 5-fluorocytosine, though still in progress, appears promising (18, 22).

Clotrimazole (Bay b 5097, 1[O,chloro-α-α-diphenylbenzyl]imidazole) is a new synthetic antifungal drug developed in Germany (Fig. 1). Several reports indicate that this agent has broad in vitro antifungal activity against both pathogenic yeasts and filamentous fungi (10, 12, 16, 23). The in vivo activity of clotrimazole in experimental fungal infections in animals is variable (12, 15, 23), and, although clinical activity has been reported (4, 9, 11), little information on its pharmacological action in humans is available. The present study further defines the in vitro activity of clotrimazole and describes clinical pharmacological studies in man.

MATERIALS AND METHODS

In vitro studies. Susceptibility tests on 156 clinical isolates of *Candida* spp., *Torulopsis glabrata*, and *Saccharomyces* spp. were performed by a broth dilution method (6). The organisms were inoculated into Sabouraud broth and incubated at 37 C for 24 hr. The drug was dissolved in 95% ethanol and then added to Sabouraud broth to give a concentration of 50 μg/ml. Serial twofold dilutions with broth were than made to 0.1 μg/ml. Tubes containing the drug were inoculated with 50 μliters of a 10^{-3} dilution of overnight growth of the organisms, and the results were read after 24, 48, and 72 hr of incubation at 37 C.

Of the 112 strains of *Candida* spp. tested, 80% were *C. albicans*; 26 strains of *T. glabrata* and 18 strains of *Saccharomyces* spp. were also tested. The *Candida* spp. were isolated from throat and sputum cultures (49%), from stool (21%), from blood (10%), and from skin, urine, and autopsy tissue cultures (20%). Of the *T. glabrata* strains, 70% were isolated from sputum and stool cultures, and 61% of the *Saccharomyces* were from stool specimens.

In vivo studies. Clinical pharmacological studies were performed in 7 normal volunteers and 16 ambulatory cancer patients. Clotrimazole was supplied as 500-mg capsules by Delbay Pharmaceuticals Inc., Bloomfield, N.J. All subjects were adults with normal renal and hepatic function as measured by blood urea nitrogen, serum creatinine, serum bilirubin, serum glutamic oxalacetic transaminase, and serum alkaline phosphatase. There was an interval of at least 48 hr between studies in the same individuals, and all studies were done after an overnight fast. Single-dose studies were performed in seven normal volunteers and one cancer patient with doses of 1.5 and 3 g of clotrimazole. Serum specimens were collected immediately prior to each study and at 1, 2, 3, 4, and 6 hr after administration of the drug. Urine specimens were collected immediately prior to each study and for 6 hr after drug administration.

To determine the effect of multiple dosing on serum levels, 15 cancer patients being treated for their disease in protected-environment units (2) were begun on clotrimazole, 1.5 g every 6 hr for 8 days. All of these patients were receiving prophylactic oral nonabsorbable antibacterial antibiotics (3) but no other antifungal agents during the period of study. The studies

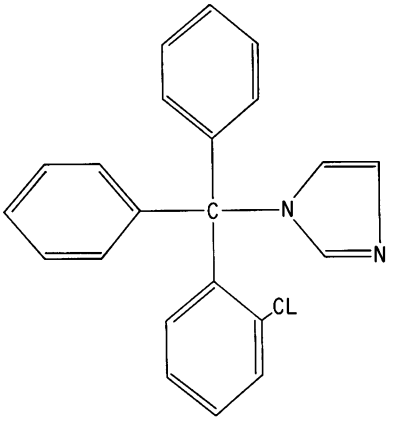

FIG. 1. *Chemical structure of clotrimazole.*

were not conducted during courses of cancer chemotherapy. Drug concentrations in serum were determined as described above after the first, the thirteenth (after 72 hr of administration), and the twenty-ninth (after 7 days of administration) doses. Urine specimens were collected for 24 hr after the first dose.

Concentrations of clotrimazole in serum and urine were measured by an agar well method with *C. pseudotropicalis* var. *carshalton* as the test organism. The organism was inoculated into Sabouraud broth from a fresh agar slant and was incubated for 18 to 24 hr at 37 C. A 50-μliter sample of the broth culture was inoculated into 50 ml of brain heart infusion agar, which was then distributed in plates. Wells (0.75 mm in diameter by 0.75 mm deep) were cut into the agar and filled with 0.1 ml of the specimens to be measured. The plates were then incubated at 37 C for 18 hr, and the zones of inhibition were measured and compared with a standard curve. The standard curve was prepared by use of a 100 μg/ml solution of clotrimazole in 95% ethanol. Dilutions of this solution were made with normal human serum to contain 0.5, 1.0, 2.0, and 4.0 μg/ml. Zones of inhibition were measured after 18 hr of incubation at 37 C. All determinations were performed in triplicate.

The standard error of the mean was calculated by the method of Mantel (8). The 95% confidence limits were determined as twice the standard error of the mean.

RESULTS

In vitro studies. The antifungal activity of clotrimazole against *Candida* spp., *T. glabrata*, and *Saccharomyces* spp. is shown in Fig. 2. The minimal inhibitory concentration (MIC) for 112 strains of *Candida* was 3.12 μg or less/ml. In particular, 70% of the isolates were susceptible to 1.56 μg or less/ml. The MIC for 18 strains of *Saccharomyces* was 6.25 μg or less/ml, and for 26 strains of *T. glabrata* the MIC was 12.5 μg or less/ml. However, only 25% of the latter were susceptible to 1.56 μg or less of clotrimazole per ml. A significant difference was observed during the study between the MIC at 24 and 72 hr for each group of organisms tested. An example of this difference is shown in Fig. 2 for the *Candida* spp. There was, however, no significant difference for the values at 48 and 72 hr.

In vivo studies. Figure 3 shows the mean concentrations in the serum of subjects after the oral administration of 1.5 and 3 g of clotrimazole. The mean peak concentration in serum after the 1.5-g dose was 1.16 μg/ml at 2 hr. The mean concentration in serum at 6 hr was 0.24 μg/ml. The range of peak concentrations was from 0.15 to 2.5 μg/ml and occurred from 1 to 4 hr after the 1.5-g dose.

After the 3-g dose of clotrimazole, the mean peak concentration in serum was 1.29 μg/ml and occurred at 2 hr. The mean concentration at 6 hr

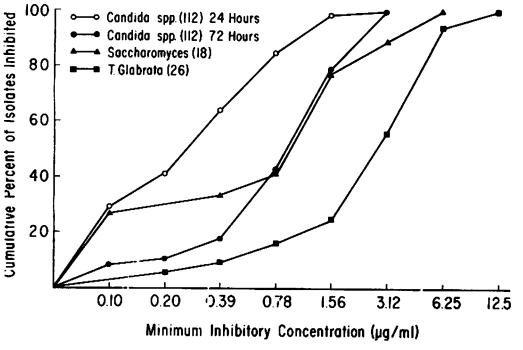

FIG 2. *Susceptibility to clotrimazole of clinical isolates of Candida spp., T. glabrata, and Saccharomyces spp. The numbers in parentheses represent the number of organisms tested. The MIC for the T. glabrata and Saccharomyces isolates represents the value at 72 hr.*

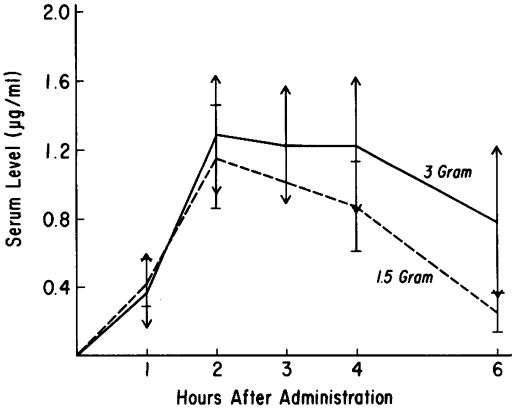

FIG. 3. *Mean drug concentrations in serum after the administration of 1.5 and 3 g of clotrimazole in eight subjects. The bars represent the 95% confidence limits for each determination.*

was 0.78 μg/ml. At this dosage, the range of peak concentrations was 0.49 to 3.5 μg/ml and occurred from 2 to 6 hr after administration.

Only 6 of the 15 patients who were begun on a dosage regimen of 1.5 g of clotrimazole every 6 hr could tolerate the drug for the 8 days of study. The other nine patients were unable to continue owing to various degrees of nausea, vomiting, abdominal pain, and diarrhea. Figure 4 shows the mean serum concentrations obtained in the six patients who completed the study. Studies were conducted after the first dose on days 1, 4, and 8. After the initial dose, a mean peak concentration of 1.55 μg/ml was observed at 3 hr; this fell to 1.25 μg/ml at 6 hr. On day 4, immediately prior to the first dose, there was a mean serum concentration of 0.43 μg/ml, which rose to a mean peak at 4 hr of 1.03 μg/ml. The mean concentration in serum 6 hr after this dose was 0.83 μg/ml. After 7 days of administration, the mean peak concentration was only 0.65 μg/ml, again at 4 hr after the dose. At 6 hr, the mean serum concentration was 0.27 μg/ml.

Figure 5 illustrates the difference between the serum concentrations obtained after the first dose in the six patients who tolerated the continuous administration and the nine patients who were unable to continue taking the drug after a variable number of doses. In the former group, a mean peak concentration of 1.55 μg/ml was observed at 3 hr, compared with a mean peak concentration of 1.08 μg/ml at 4 hr in the latter group. Two of the patients who subsequently became intolerant of the drug had no demonstrable serum activity after the initial 1.5-g dose of clotrimazole.

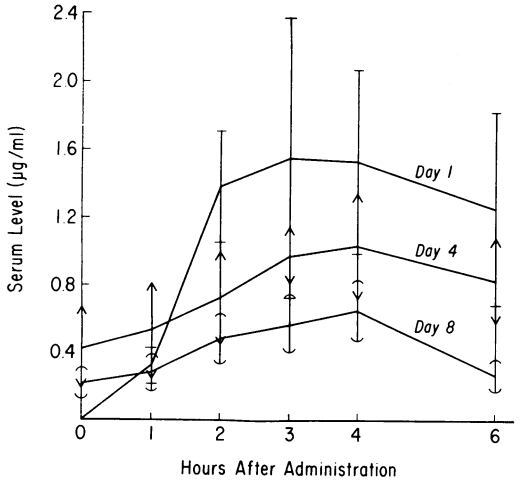

FIG. 4. *Mean drug concentrations in serum after the first dose on days 1, 4, and 8 in six patients taking 1.5 g of clotrimazole every 6 hr.*

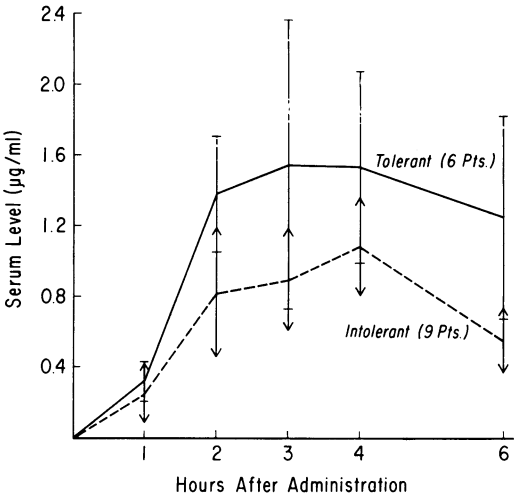

FIG. 5. *Mean drug concentrations in serum after the first 1.5-g dose of clotrimazole in the 15 patients begun on continuous administration of the drug for 8 days. Nine patients subsequently became intolerant of the clotrimazole.*

The urinary excretion of clotrimazole after the 1.5- and 3-g doses was extremely low. Less than 1% of the administered dose could be detected as active drug in urine collections for 6 hr after the dose. Similarly, 24-hr collections during the first day of continuous dosing failed to reveal more than 1% of the total dose administered during that time.

DISCUSSION

In the present study, the in vitro activity of clotrimazole against the three groups of fungi tested was similar to that reported previously. The MIC for the majority of *Candida* spp. was 1.56 μg or less/ml, a level that can be achieved in the serum by oral administration of the drug. Somewhat less activity was observed for clotrimazole against *T. glabrata* and *Saccharomyces* spp. Although serious infections may be caused by these latter two organisms (14, 19), disseminated candidiasis constitutes the major threat to patients who are susceptible to systemic fungal infections (1).

The results of the pharmacology studies are of interest in several respects. In the single-dose studies, the peak concentration in serum occurred from 1 to 6 hr after administration, and there was considerable variability in these peak concentrations, a result which suggests that the absorption of clotrimazole is erratic. In addition, two patients failed to show any serum activity of clotrimazole after a 1.5-g dose.

Clotrimazole was poorly tolerated when given

in multiple daily doses for several days. Gastrointestinal symptoms including nausea, vomiting, abdominal pain, and diarrhea led to cessation of the clotrimazole in 9 of the 15 patients. These symptoms were clearly due to the clotrimazole because they subsided promptly in all patients when the drug was discontinued. Goldstein and Hoeprich (5) reported a similar experience with clotrimazole and suggested the need for a better-tolerated oral preparation or a parenteral form of the drug if therapeutic trials are not to be compromised. The difference in the mean serum concentrations after the first 1.5-g dose between the patients who tolerated subsequent administration and those who were intolerant (Fig. 5) can probably be explained by the nausea produced by the clotrimazole; thus, the intolerance appears to be due to a local effect of the drug.

The other finding of interest was the progressive decline in the serum concentrations of clotrimazole after continuous dosing, as shown in Fig. 4. One possible explanation for this finding is the induction of microsomal enzymes by the clotrimazole. There is evidence in animals that enzyme induction occurs after multiple dosing with this drug (15; M. G. Rinaldi and P. D. Hoeprich, Bacterial. Proc., p. 123, 1971). Also, this mechanism of increased drug metabolism could explain, in part at least, the treatment failures in some animal infections (15). An alternative explanation for the decreasing serum concentrations after continuous administration is the nausea, attributed to the clotrimazole, which occurred in the majority of patients by the end of the study. The concomitant administration of nonabsorbable antibiotics could also be a contributory factor.

The development of a better-tolerated oral preparation or a suitable parenteral form of clotrimazole will facilitate the therapeutic evaluation of this agent, which clearly possesses significant in vitro activity against a wide range of pathogenic fungi.

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