Chemotherapeutic Evaluation of Clotrimazole [Bay b 5097, 1 (o-Chloro-α-α-Diphenylbenzyl) Imidazole]

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Clotrimazole has a broad spectrum of activity against yeast and filamentous fungi in vitro and also in vivo when given orally or parenterally to experimentally infected mice and when administered orally or topically to infected guinea pigs. In vitro a distinct inoculum effect has been observed with a number of strains of Candida and Torulopsis; minimal inhibitory concentrations have tended to increase with increased incubation time. With prolonged incubation times, resistance can be developed to clotrimazole in vitro, but this resistance is readily reversible upon passage in drug-free broth. The degree of in vivo activity of clotrimazole against Candida depends on the severity of infection used. Orally it appears to be more effective when administered by gavage than when given mixed in the diet. Pretreatment with the agent may decrease its efficacy because of drug inactivation. Against dermatophytes, clotrimazole has a degree of activity similar to griseofulvin when given orally, but it is less active than tolNAftate topically in cutaneous infection of Trichophyton mentagrophytes in guinea pigs. In vitro, but not in vivo, some gram-positive and gram-negative bacteria are inhibited by low concentrations of clotrimazole.

Clotrimazole [Bay b 5097, 1-(o-chloro-α-α-diphenylbenzyl) imidazole] is a new systemic antifungal agent first described by Plemple et al. (3, 4). These initial reports, as well as a more recent study of Shadomy (5), showed clotrimazole to have a high degree of activity in vitro against a wide variety of pathogenic yeasts and filamentous fungi. Oral activity in mice with experimental yeast and dermatophyte infections and topical activity against cutaneous Trichophyton infections in guinea pigs have also been described (3, 4), and initial clinical reports (1, 2) appeared promising. In vivo tests by Shadomy (6) against systemic fungal infections in mice were less promising, although this may partially be explained by the presence of a drug-induced mechanism for in vivo drug inactivation. In the studies reported below, clotrimazole has been compared with tolNAftate and griseofulvin in a number of test systems.

MATERIALS AND METHODS

The lot of clotrimazole used was no. 1506/69 (Delbay Pharmaceuticals); tolNAftate (lot M-3138) and griseofulvin (lot M1-21119) were from Schering Corp. Drug stock solutions were prepared in ethanol or dimethylformamide (DMF) for in vitro tests, since clotrimazole was insoluble in water at concentrations greater than 20 to 25 μg/ml. Tube dilution tests were done by conventional procedures in Sabouraud broth for yeasts and fungi, yeast beef broth for bacteria, and simplified Trypticase serum medium for Trichomonas vaginalis. Male CF-1 mice weighing approximately 20 g each and male albino guinea pigs weighing approximately 250 g each were used. Drug suspensions for oral or parenteral dosing were prepared in 0.5% aqueous carboxymethyl cellulose containing a drop of Tween 80. These suspensions were sonically treated to reduce particle size. Topical preparations were made in 1% polyethylene glycol (PEG) 400. Treatment schedules and infection procedures are detailed with the results but generally follow those described earlier for bacterial infections in mice (8) and Trichophyton mentagrophytes infections in guinea pigs (7, 9).

The microbiological assay for clotrimazole in serum is a modification of that developed by Holt (personal communication) with Candida pseudotropicalis Carshoton as the test organism. We use a paper disc-agar diffusion standard curve type of assay with seeded Brain Heart Infusion agar as the medium. The lower limit of sensitivity of the assay is 0.2 μg/ml in serum.

RESULTS

In vitro activity. The in vitro antifungal activity of clotrimazole, tolNAftate, and griseofulvin against a variety of pathogenic yeasts and filamentous fungi is shown in Table 1. The minimal
| Organism                          | No. of strains | Clotrimazole (μg/ml) | Tolnaftate (μg/ml) | Griseofulvin (μg/ml) |
|----------------------------------|----------------|----------------------|--------------------|----------------------|
| <i>Cladosporium carrionii</i>    |                | >25                  | >25                | >25                  |
| <i>Epidermophyton floccosum</i>  | 2              | 0.75                 | 0.03               | 3.0                  |
| <i>Keratinomyces ajelloi</i>     |                | 0.03                 | 0.003              | 17.5                 |
| <i>Microsporum audouinii</i>     |                | 0.03                 | 0.003              | 0.3                  |
| <i>M. canis</i>                  | 3              | 0.03-0.8             | 0.003-0.008        | 0.75                 |
| <i>M. cookei</i>                 |                | 0.75                 | 0.003              | 0.75                 |
| <i>M. distortum</i>              |                | 0.03                 | 0.008              | 0.3                  |
| <i>M. gypseum</i>                | 2              | 0.1-0.75             | 0.03               | 3.0                  |
| <i>M. nanum</i>                  |                | 0.75                 | 0.03               | 0.75                 |
| <i>Phialophora jeaneselmei</i>   |                | >25                  | >25                | >25                  |
| <i>P. pedrosoi</i>               |                | 0.3                  | >25                | >25                  |
| <i>Trichophyton concentricum</i> |                | 0.08                 | 0.008              | 7.5                  |
| <i>T. discoides</i>              |                | 0.03                 | 0.008              | 0.75                 |
| <i>T. ferrugineum</i>            |                | 0.75                 | 0.03               | 7.5                  |
| <i>T. gllainae</i>               |                | 0.03                 | 0.008              | 7.5                  |
| <i>T. gournii</i>                |                | 0.08                 | 0.008              | >25                  |
| <i>T. megnini</i>                | 2              | 0.03                 | 0.003-0.008        | 0.75-17.5            |
| <i>T. rubrum</i>                 |                | 0.3                  | 0.008              | 3.0                  |
| <i>T. schoeleinii</i>            |                | 0.03                 | 0.003              | 0.75                 |
| <i>T. soudanense</i>             | 2              | 0.03-0.8             | 0.003-0.03         | 0.3-0.8              |
| <i>T. terrestrae</i>             |                | 0.75                 | 0.003              | 7.5                  |
| <i>T. tonsurans</i>              |                | 0.03                 | 0.008              | 3.0                  |
| <i>T. verrucosum</i>             |                | 0.3                  | 0.03               | >25                  |
| <i>T. violaceum</i>              |                | 0.03                 | 0.008              | 0.75                 |
| <i>T. yaoundei</i>               |                | 0.8-3.0              | 0.8-3.0            | 3.0                  |
| <i>Candida albicans</i>          | 18             | 0.01-0.8             | 0.003-0.03         |                     |
| <i>Nocardia asteroides</i>       | 4              | 0.3-3.0              |                    |                     |
| <i>N. brasiliensis</i>           | 4              | 0.03-0.8             |                    |                     |
| <i>N. madurae</i>                | 1              | 0.8                  |                    |                     |
| <i>Saccharomyces cerevisiae</i>   | 1              | 7.5                  |                    |                     |
| <i>Torulopsis glabrata</i>       | 11             | 0.8-7.5              |                    |                     |

The inhibitory concentrations (MIC) are given in micrograms per milliliter. Most of the dermatophytes, <i>Torulopsis</i>, and <i>Candida</i> cultures were fairly recent clinical isolates. The dermatophytes were obtained primarily from the Skin and Cancer Hospital in Philadelphia and the College of Physicians and Surgeons of Columbia University. Eighteen of the <i>T. mentagrophytes</i> were isolated from Vietnam. The <i>Torulopsis</i> cultures were from T. Eickhoff, University of Colorado. The results show that clotrimazole indeed had a broad spectrum of activity against yeasts and filamentous fungi. Its activity against filamentous fungi in vitro was less than that of tolnaftate but in many cases greater than that of griseofulvin. It had anti-<i>Candida</i> activity which both tolnaftate and griseofulvin lacked.

The results of tube dilution tests with clotrimazole against a number of bacterial strains in yeast-beef broth at pH 7.4 are shown in Table 2. The data are the results of several tests with both ethanol and DMF as solvents for the stock drug solutions. Minimal bactericidal concentrations (MBC) were determined by streaking the tubes used for MIC determinations on Mueller Hinton agar. These data indicate that clotrimazole had a high degree of in vitro activity against strains of <i>Staphylococcus</i> and <i>Streptococcus</i>. Occasional strains of <i>Proteus</i> and <i>Salmonella</i> were also sensitive, but the other gram-negative bacteria were not. The action of clotrimazole was primarily bacteriostatic since MBC levels were substantially higher than MIC levels.

The effect of inoculum size and incubation time on the in vitro activity of clotrimazole was tested against seven laboratory strains of <i>C. albicans</i> with 3 ml of Sabouraud dextrose broth used per tube. The results for several of the strains showed a distinct inoculum effect. Increased MIC values were observed with increased inoculum size and also with increased incubation time.

This was examined further (Table 3) against a small group of recent clinical isolates of <i>C.
Salmonella and Pseudomonas were included. The inoculum effect with clotrimazole appeared to be greater than that seen with nystatin. Nystatin showed increasing MIC values with increased incubation time which has been attributed to the instability of nystatin in solution.

A similar study was conducted with 11 isolates of T. glabrata. With these strains, a distinct inoculum effect was also seen, with many of the strains also showing an increase in MIC with increased incubation time.

The studies on the effect of inoculum size and incubation time suggested that we might be able to develop resistance to clotrimazole with Candida. Table 4 shows the results of such a study. Four strains of C. albicans were allowed to incubate in broth containing various concentrations of clotrimazole. In the first passage, after 4 days of incubation, growth was observed at 1 μg/ml with three of the strains and at 0.5 μg/ml for the fourth strain. No growth was observed at 5 μg/ml. The footnoted cultures were used as a source of inoculum for the second passage, which again after 4 days of incubation showed one of the strains growing at 25 μg/ml, two at 10 μg/ml, and one at 1 μg/ml. The footnoted tubes were used as a source of inoculum for the next passage with 4 days of incubation. At this time, three of the four strains were growing at a concentration of 50 μg/ml, whereas the fourth was growing at a concentration of 10 μg/ml.

An inoculum effect and incubation time experiment with these four strains after the third passage (at which time three were growing at 50 μg/ml and one at 10 μg/ml) is shown in Table 5. A distinct inoculum effect was also seen with these cultures, although the last one probably cannot be considered resistant. Increased incubation time also resulted in increased MIC values. The resistance was lost after several passages of these cultures in broth not containing clotrimazole.

The antitrichomonal activity of clotrimazole was tested in vitro against a recent T. vaginalis isolate in STS medium. Clotrimazole at 100 μg/ml inhibited growth by 78% in 24 hr and was cidal after 48 hr. No activity was detected at 25 μg/ml or less.

In vivo activity. Clotrimazole was tested for protective activity orally and subcutaneously (Table 6) in mice infected intraperitoneally with bacteria or intravenously with C. albicans. Inoculum sizes were chosen in this experiment to kill all of the infected mice 24 hr after infection. Mice were treated twice, shortly before and 5 hr after infection. Survivors were determined 48 hr after infection. Clotrimazole was active against gram-positive bacterial infections in mice when given at very high doses subcutaneously. It was essentially inactive orally. The Candida infection

### Table 2. In vitro antibacterial activity of clotrimazole in yeast-beef broth (pH 7.4)

| Organism                     | Avg MIC (μg/ml) | MBCb |
|------------------------------|-----------------|------|
|                              | 24 hr | 48 hr |     |
| Staphylococcus aureus        |       |       |     |
| Gray                         | 0.75  | 0.75  | 5.0  |
| Smith                        | 0.75  | 3.0   | 10.0 |
| W                            | 0.75  | 0.75  | 5.0  |
| 11631                        | 0.75  | 3.0   | 50.0 |
| 209P                         | 0.75  | 0.75  | 10.0 |
| Streptococcus pyogenes       |       |       |     |
| C                            | 0.75  | 0.75  | 10.0 |
| C 203                        | 0.75  | 3.0   | 10.0 |
| 4                            | 0.75  | 3.0   | 10.0 |
| 3                            | 0.75  | 0.75  | 10.0 |
| 5                            | 0.75  | 0.75  | 10.0 |
| 6                            | 0.75  | 0.75  | 10.0 |
| Enterobacter aerogenes       |       |       |     |
| 4                            | 0.75  | 0.75  | 50.0 |
| 3                            | >50   | >50   | >50  |
| 824                          | 0.75  | 0.75  | 25.0 |
| Escherichia coli             |       |       |     |
| McFadden                     | >50   | >50   | >50  |
| 5                            | >50   | >50   | >50  |
| 19                           | >50   | >50   | >50  |
| Screen                       | >50   | >50   | >50  |
| Klebsiella pneumoniae        |       |       |     |
| 432                          | >50   | >50   | >50  |
| 845                          | >50   | >50   | >50  |
| Proteus mirabilis            |       |       |     |
| McFadden                     | >50   | >50   | >50  |
| P. rettiger                  | >50   | >50   | >50  |
| 525                          | >50   | >50   | >50  |
| 928                          | >50   | >50   | >50  |
| P. vulgaris                  |       |       |     |
| eye                          | 0.75  | 3.0   | >50  |
| 1                            | 0.75  | 0.75  | >50  |
| 2                            | 0.75  | 3.0   | >50  |
| Pseudomonas aeruginosa       |       |       |     |
| 621                          | >50   | >50   | >50  |
| 650                          | >50   | >50   | >50  |
| Salmonella sp.               |       |       |     |
| 2A                           | >50   | >50   | >50  |
| 830                          | 0.75  | 3.0   | 10.0 |
| Sc                           | >50   | >50   | >50  |
| 827                          | 0.75  | 0.75  | 10.0 |
| 1                            | 0.3   | 0.3   | 50.0 |

a Average value for two to three tests for each strain.

b Tubes streaked on Mueller Hinton agar after 24 hr of incubation.
Table 3. In vitro activity of clotrimazole and nystatin against clinical isolates of Candida albicans

| Drug     | Culture | MIC (μg/ml) at various inoculum levels | ~10^6 cells | ~10^6 cells | ~10^6 cells | ~10^6 cells |
|----------|---------|----------------------------------------|-------------|-------------|-------------|-------------|
|          |         |                                        | 24 hr       | 48 hr       | 24 hr       | 48 hr       | 24 hr       | 48 hr       |
| Clotrimazole | 1298   | >25 >25                                | 0.8         | 0.8         | 0.3         | 0.3         | <0.01       | 0.3         |
|          | 989     | >25 >25                                | >25         | >25         | 7.5         | >25         | 0.3         | 0.8         |
|          | 669     | >25 >25                                | 0.3         | 7.5         | 0.3         | 0.8         | 0.3         | 0.3         |
|          | 1058    | >25 >25                                | 0.03        | 0.3         | 0.03        | 0.03        | <0.01       | 0.3         |
|          | 992     | >25 >25                                | 0.03        | 0.3         | <0.01       | 0.3         | <0.01       | 0.3         |
| Nystatin | 1298    | 3.0 7.5                                | 3.0         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |
|          | 989     | >25 >25                                | 7.5         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |
|          | 669     | 7.5 7.5                                | 3.0         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |
|          | 1058    | 7.5 7.5                                | 3.0         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |
|          | 992     | 3.0 7.5                                | 3.0         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |
|          | 429     | 3.0 7.5                                | 7.5         | 7.5         | 3.0         | 7.5         | 3.0         | 7.5         |

* Tested in Sabouraud dextrose broth in a volume of 3 ml/tube. Incubation was at 37 C.

Table 4. Induction of resistance to clotrimazole in Candida albicans

| Conditions | Strain | Growth (+) at various conc (μg/ml) |
|------------|--------|-----------------------------------|
|            |        | 0.1 | 0.5 | 1.0 | 5.0 | 10.0 | 25.0 | 50.0 |
| Passage 1, 4 days of incubation | 400 | + | + | + | + | + | + | + |
|            | 401 | + | + | + | + | + | + | + |
|            | 404 | + | + | + | + | + | + | + |
|            | 406 | + | + | + | + | + | + | + |
| Passage 2, 4 days of incubation | 400 | + | + | + | + | + | + | + |
|            | 401 | + | + | + | + | + | + | + |
|            | 404 | + | + | + | + | + | + | + |
|            | 406 | + | + | + | + | + | + | + |

Table 5. Effect of inoculum size and incubation time on in vitro activity of clotrimazole

| Candida strain | Inoculum size (no. of organisms) | MIC (μg/ml) at various times of incubation |
|---------------|----------------------------------|-------------------------------------------|
|               | 24 hr | 48 hr | 72 hr | 96 hr |
| 400R          | >25   | >25   | >25   | >25   |
| 401R          | >25   | >25   | >25   | >25   |
| 404R          | >25   | >25   | >25   | >25   |
| 406R          | >25   | >25   | >25   | >25   |

* Inoculum of ~10^6 cells taken from this tube for next passage.

Table shows the per cent in the diet and the drug intake in milligrams per kilogram per day that this represented based on the weight of the mice and diet consumption. Survivors were determined at various days after intravenous infection with Candida. In the groups treated starting the day of infection, 50% of the control mice had died 2 days after infection, two-thirds had died 3 days after infection, and 8 of the 10 responded more readily to oral treatment than to parenteral treatment in contrast to the bacterial infections.

In another study (Fig. 1), we used milder challenges with Candida which resulted in death after 3 days. Much lower doses of clotrimazole were effective in delaying death as shown in the survival curves.

Table 7 shows the result of treatment of Candida infections by administering the drug in the diet. Groups of 7 mice each with 10 mice in control groups were given diets containing various levels of clotrimazole for 5 days, either starting the day of infection or 2 days before infection. The
mice were dead 4 days after infection. In the second test with treatment starting 2 days before infection, similar survival patterns were noted in the control group. In the second test, the larger drug intakes reflect the fact that the mice were eating normally for 2 days before being infected. Increased survival time was noted with both experiments; the number of mice surviving in each of the groups appeared to be lower than that observed when the dose was administered orally by gavage. Complete protection was not obtained with doses as high as 600 mg per kg per day starting 2 days before infection or 240 mg per kg per day starting on the day of infection. Some dose-related response was noted, with doses below 20 or 30 mg per kg per day being ineffective.

A brief study of the effect of pretreatment on the efficacy of clotrimazole against C. albicans infections in mice was performed. One group of mice was treated with 50 mg per kg per day orally for 5 days, rested for 2 days, and then infected with Candida. A second group was treated with vehicle for the same period of time before infection. A group of controls was not pretreated at all. Mice in all groups were then treated orally shortly before and 3 hr after infection. The results of this probing study seemed to indicate that, in mice pretreated with clotrimazole, the drug was less efficacious than in those pretreated with the vehicle. This study was then expanded to include groups of 30 mice each at several different dose levels (Table 8). One group of 120 mice was pretreated orally with 50 mg per kg per day for 5 days and rested for 2 days before being tested. The second

| Infection organism       | PDs (mg/kg)* |
|--------------------------|--------------|
|                          | Oral         | Subcutaneous |
| Staphylococcus aureus    | >250         | 200          |
|albicans                 |              |              |
| S. aureus W.             | >250         | 180          |
| Streptococcus pyogenes C.| ~250         | 180          |
| Salmonella paratyphi B.  | ~250         | >250         |
| Candida albicans PC.     | ~100         | 250          |

*Total dose divided in two and given shortly before and 3 hr after intraperitoneal infection. *Candida was given intravenously. PDs, 50% protective dose.

![Fig. 1. Effect of Bay b 5097 therapy on survival time of mice infected intravenously with Candida albicans. Mice were treated shortly before and 5 hr after infection.](image)

Table 7. Activity of clotrimazole against Candida albicans infections when given in the diet

| Treatment                         | Diet (%) | Amt (mg per kg per day) | Per cent survivors at various times after infection |
|-----------------------------------|----------|-------------------------|---------------------------------------------------|
|                                   |          |                         | 1 day     | 2 days   | 3 days   | 4 days   | 5 days   | 6 days   | 7 days   |
| For 5 days starting day of infection | 0.50     | 240                      | 86        | 86       | 72       | 57       | 57       | 43       | 43       |
|                                    | 0.25     | 131                      | 86        | 86       | 57       | 43       | 43       | 43       | 43       |
|                                    | 0.125    | 72                       | 86        | 86       | 57       | 43       | 43       | 43       | 43       |
|                                    | 0.06     | 29                       | 86        | 86       | 57       | 29       | 29       | 29       | 29       |
|                                    | 0.03     | 15                       | 86        | 86       | 43       | 29       | 29       | 14       | 14       |
|                                    | 0.015    | 21                       | 86        | 57       | 29       | 29       | 29       | 29       | 29       |
|                                    | 0        |                          | 90        | 50       | 30       | 20       | 20       | 20       | 20       |
| For 5 days starting 2 days preinfection | 0.5      | 643                      | 86        | 72       | 57       | 57       | 57       | 57       | 57       |
|                                    | 0.25     | 221                      | 86        | 86       | 57       | 29       | 29       | 29       | 29       |
|                                    | 0.125    | 183                      | 100       | 86       | 72       | 57       | 57       | 57       | 57       |
|                                    | 0.06     | 78                       | 100       | 72       | 72       | 72       | 72       | 72       | 72       |
|                                    | 0.03     | 37                       | 86        | 72       | 72       | 57       | 57       | 57       | 57       |
|                                    | 0.015    | 23                       | 43        | 14       | 14       | 14       | 14       | 14       | 14       |
|                                    | 0        |                          | 80        | 50       | 20       | 20       | 20       | 20       | 20       |
group of 120 mice was treated with vehicle for 5 days and rested for 2 days, and an additional 10 control mice were not pretreated at all. All mice were then infected with *Candida* and were treated shortly before and 3 hr after intravenous infection. Survivors were determined over a period of 7 days. Treatment in both groups resulted in increased survival time. The dramatic effect of pretreatment as seen in the earlier experiment was not as obvious in this experiment with larger numbers of animals. After correction for the difference in survival time in the two groups of controls, no significant difference in efficacy was observed in those mice pretreated with drug for 5 days. These studies do not rule out, however, the possibility that pretreatment may result in increased ability of the mouse to handle the drug through more rapid metabolism, resulting in decreased efficacy of the agent.

Table 9 shows the in vivo activity of clotrimazole against topical *T. mentagrophytes* infections in guinea pigs in two tests. The growth from Sabouraud agar slants of *T. mentagrophytes* DA 480 incubated for 10 days at 27 °C was homogenized in Sabouraud broth to serve as inoculum. Inoculum was rubbed on the scarified shaved skin of each guinea pig with sandpaper. Lesions were graded from negative to plus 5 depending upon severity and cultured on Mycosel agar daily. Groups of five animals each were used, with treatment beginning on the 3rd day after infection and continuing for 10 days regardless of culture or lesion results. Cultures were taken and lesions were graded daily through 21 days. In the first test, clotrimazole and griseofulvin were given orally once a day as ultrasonically treated suspensions in aqueous 0.5% carboxymethyl cellulose. Clotrimazole and tolnaftate were used topically as 1% preparations in PEG 400 with treatments twice a day. Vehicle-treated and untreated controls were included. Vehicle-treated and untreated controls (groups 7 and 8) remained positive for culture through 18 days after the start of treatment, and this was reflected in the lesion scores for these groups. Tolnaftate treatment produced negative cultures and lesions in an average of 4.5 and 6 days, respectively, which was also reflected in the lack of an increase in the 10-day lesion scores over the 5-day lesion scores. Griseofulvin given orally at a level of 15 mg per kg per day produced negative cultures in 5.5 days and negative lesions 1 day later. It also produced a dramatic reduction in lesion scores. The data for tolnaftate and griseofulvin are quite similar to those obtained in previous experiments. Clotrimazole orally produced a dose-related response. It appears to have a potency similar to griseofulvin in this test with regard to the length of time required to produce negative cultures. However, lesion scores were not reduced as rapidly as with griseofulvin and the lesions persisted longer. When used topically, clotrimazole was effective but was less active than tolnaftate at the same treatment level. This was confirmed in a second test with higher levels. The deaths observed in the groups treated topically were not drug-related but appeared to result from chilling.

The results of serum level determinations in mice after a single oral dose of clotrimazole with the modified Holt assay procedure are shown in Table 10. The drug appears to be absorbed rapidly from the intestinal tract and produces serum levels which persist for a period of 6 hr and are essentially negative at 24 hr after doses of 62.5 or 125 mg/kg. The effect of pretreatment on serum levels was determined in an additional test (Table 10). Pretreatment for 5 days, either

### Table 8. Effect of pretreatment on the efficacy of clotrimazole against Candida albicans in vivo

| Treatment | Oral dose* (mg/kg) | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days |
|-----------|-------------------|-------|--------|--------|--------|--------|--------|--------|
| Pretreated orally with 50 mg per kg per day for 5 days and then rested for 2 days (30 mice/treatment group) | 50 | 87 | 70 | 67 | 57 | 50 | 50 | 50 |
| | 25 | 50 | 44 | 44 | 37 | 33 | 33 | 33 |
| | 5 | 50 | 44 | 44 | 40 | 40 | 40 | 40 |
| | 0 | 40 | 23 | 23 | 13 | 13 | 13 | 13 |
| Pretreated with vehicle for 5 days and then rested 2 days (30 mice/treatment group) | 50 | 83 | 73 | 67 | 60 | 60 | 60 | 60 |
| | 25 | 67 | 53 | 53 | 50 | 50 | 50 | 50 |
| | 5 | 73 | 50 | 50 | 50 | 50 | 50 | 50 |
| | 0 | 50 | 47 | 43 | 40 | 33 | 33 | 33 |
| Pooled controls (90 mice) | 0 | 41 | 31 | 27 | 21 | 19 | 19 | 19 |

* Mice treated orally with total dose divided in two and given shortly before and 3 hr after intravenous infection.
EVALUATION OF CLOTRIMAZOLE

Activity of clotrimazole against cutaneous infections of Trichophyton mentagrophytes in guinea pigs

Table 9. Activity of clotrimazole against cutaneous infections of Trichophyton mentagrophytes in guinea pigs

| Test | Prepn | Dose and route | Average sum of lesion scores | Average day to become negative |
|------|-------|---------------|------------------------------|-------------------------------|
|      |       |               | 5 days | 10 days | Lesions | Cultures |
| A    | Clotrimazole, oral | 10 mg per kg per day, 10 days | 15.0 | 22.4 | 15.3<sup>a</sup> | 13.0<sup>a</sup> |
|      | Clotrimazole, oral | 50 mg per kg per day, 10 days | 13.0 | 16.4 | 12.8 | 5.4 |
|      | Clotrimazole, oral | 125 mg per kg per day | 11.3 | 12.3 | 7.3<sup>b</sup> | 4.5<sup>b</sup> |
|      | Clotrimazole, topical | 1% in PEG 400, 2X/day, 10 days | 15.8 | 23.7 | 13.3<sup>c</sup> | 8.7<sup>c</sup> |
|      | Griseofulvin, oral | 50 mg per kg per day, 10 days | 13.6 | 14.0 | 6.6 | 5.4 |
|      | Tolnaftate, topical | 1% in PEG 400, 2X/day, 10 days | 11.0 | 11.0 | 6.0 | 4.4 |
|      | Vehicle controls, topical | | | | >18<sup>d</sup> | >18<sup>d</sup> |
|      | Untreated controls | | | | >18 | >18 |
| B    | Clotrimazole, topical | 2% in PEG 400, 2X/day, 10 days | 14.2 | 25.0 | 13.6 | 8.0 |
|      | Clotrimazole, topical | 4% in PEG 400, 2X/day, 10 days | 15.6 | 26.0 | 12.0 | 9.0 |
|      | Tolnaftate, topical | 1% in PEG 400, 2X/day, 10 days | 10.6 | 11.0 | 6.6 | 4.5 |
|      | Control | | | | 19.5 | 44.5 |

<sup>a</sup> Two animals died in this group on days 9 and 10, respectively; one was positive by culture and both had positive lesions at death.
<sup>b</sup> One animal died on day 5 and had positive cultures and lesions.
<sup>c</sup> Two animals died on days 5 and 8, respectively, and both had positive cultures and lesions at death.
<sup>d</sup> Two animals died on day 10 and had positive cultures and lesions at death.

Table 10. Serum levels of clotrimazole in mice after a single oral dose

| Pretreatment | Dose (mg/kg) | Effect of pretreatment |
|--------------|-------------|-----------------------|
|              | Serum levels (μg/ml) at various times (mean and range) |
|              | 1 hr | 3 hr | 6 hr | 24 hr |
| None | 125 | 3 | 1.8 | 2.0 | 2.5 | 0.2 | (0.8–2.4) | (1.2–2.9) | (1.9–3.4) | (0–0.3) |
| With carboxymethyl cellulose for 5 days<sup>a</sup> | | | | | | | | | | |
| 62.5 | 1 | 1.7 | 1.6 | 1.4 | 0 | (0.9–2.0) | (0.8–2.2) | (1.4–3.2) | 0 |
| Orally with clotrimazole (50 mg per kg per day) for 5 days<sup>a</sup> | | | | | | | | | | |
| 62.5 | 1 | 0.5 | 0.5 | 0.6 | 0 | (0–0.5) | (1.2–2.0) | (1.9–3.4) | (0.2–0.8) |
| With clotrimazole (0.1%)<sup>b</sup> in diet for 5 days<sup>a</sup> | | | | | | | | | | |
| 62.5 | 1 | 0.2 | 0.2 | 0.3 | 0 | (0.2–0.8) | (1.2–2.0) | (1.9–3.4) | (0.2–0.8) |

<sup>a</sup> Treated mice were rested (not treated) for the 2 days before dosing for serum level determinations.
<sup>b</sup> Concentration of 0.1% in the diet resulted in an average drug intake of 180 mg per kg per day.

by oral gavage treatment or in the diet, with a rest of 2 days resulted in markedly reduced serum levels. This suggests that treatment induces enzymes which degrade clotrimazole.

DISCUSSION

The in vitro antifungal spectrum of clotrimazole described above agrees favorably with that reported in previous studies (3–5). Our studies...
have, in addition, demonstrated antibacterial activity in vitro specifically against Staphylococcus and Streptococcus. The striking inoculum effect seen with Candida and Torulopsis should be considered in explaining differences in MIC values obtained against similar organisms by different laboratories. It also anticipates differences to come with further study on this agent. The development of resistance to clotrimazole in vitro has not been reported by others. Our results in this regard may result from the prolonged incubation times used in contrast to the usual rapid passage at increasing concentration of drug. However, the resistance we have observed rapidly reverts to normal sensitivity when the culture is passed in drug-free media.

In vivo antibacterial tests were not encouraging despite the rather strong in vitro antibacterial activity of clotrimazole. Previous tests of Plempel et al. (3, 4) and Shadomy (6) have differed as to the efficacy of oral clotrimazole treatment in systemic Candida infections in mice. Shadomy (6) explained these differences on the basis of differences in length of infection and on the length of the treatment period. Our test model was more like that of Plempel in that the observation period was short-term and involved shorter treatment periods. Also, as pointed out by Shadomy (6), treatment-stimulated drug inactivation occurs; this finding is supported by the lower serum level values we have observed.

The variable results in therapy of systemic fungal infections point to the need for careful evaluation of treatment schedules in further tests.

The oral treatment of T. mentagrophytes infections in guinea pigs is of considerable interest. The activity of clotrimazole approximated that of griseofulvin. Topically, in our model, clotrimazole was not as effective as tolnaftate. The data reported here suggest that the therapeutic evaluation of clotrimazole in man may require definition of optimal treatment regimens to best utilize the potential of this new broad-spectrum agent.

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