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Treatment of Disseminated *Torulopsis glabrata* Infection with DO870 and Amphotericin B

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***Torulopsis glabrata*, an opportunist pathogen in immunosuppressed patients, is resistant to many antifungal agents, and there are no established treatment regimens for this organism. The mouse model was used to evaluate treatment with DO870, amphotericin B, fluconazole, and their combination. Mice were immunosuppressed with 5 mg of gold sodium thiomalate given intraperitoneally 1 day prior to intravenous infection with 10^8 *T. glabrata* cells. Treatment with a new antifungal triazole, DO870, at doses ranging from 1 to 50 mg/kg of body weight administered per os either daily or on alternate days; fluconazole at 100 mg/kg twice a day per os; or amphotericin B at 3 mg/kg/day intraperitoneally was begun 1 day after infection. Treatment for 5 days was followed by sacrifice 2 days later for determining CFU counts in spleen and kidney tissue. For a fluconazole-sensitive isolate (MIC of DO870, < 1.25 μg/ml), DO870 at 5 mg/kg/day significantly reduced counts in kidney and spleen tissue ($P < 0.05$), amphotericin B was modestly effective, and the combination of DO870 (25 mg/kg) and amphotericin B (3 mg/kg) was markedly more effective than either drug alone ($P < 0.01$). Three additional isolates were resistant in vitro to DO870 (MIC, 4 μg/ml). No reduction in CFU in kidney or spleen tissue was observed with DO870 when compared with counts in control tissue. DO870 is effective in vivo against at least some isolates of *T. glabrata* and when combined with amphotericin B can exert additive effects.**

Torulopsis glabrata (*Candida glabrata*), an opportunistic pathogen, has been seen increasingly in immunosuppressed AIDS and transplant patients, where it has been reported in up to 25% of patients with fungemia (1, 6, 11). To date, there is no standard therapy for infection with this organism, which has shown variable in vitro susceptibility to present antifungal agents including amphotericin B, fluconazole, and flucytosine (7). DO870, a new triazole antifungal agent, has been shown to have excellent activity in vivo and in vitro against *Candida albicans* and *Cryptococcus neoformans*, including fluconazole-resistant isolates in normal and immunocompromised mice (2, 3, 5, 13). Using an immunosuppressed mouse model of *T. glabrata* infection, we evaluated the effect of amphotericin B, fluconazole, and DO870 against disseminated *T. glabrata* infection.

MATERIALS AND METHODS

Animals. Outbred ICR Harlan Sprague-Dawley male mice, 20 to 25 g, were housed five per cage with free access to water and food.

Immunosuppression. Mice were immunosuppressed with either 5 mg of gold sodium thiomalate (Myochrysine; Merck Sharp & Dohme, West Point, Pa.) given intraperitoneally 1 day prior to infection (8, 17) or 5-fluorouracil (5FU; Hoffman-LaRoche, Nutley, N.J.) administered intravenously at 150 mg/kg of body weight. Immunosuppression was verified by leukocyte counts performed the day after administration of the gold sodium thiomalate or 5FU as well as at 5, 10, and 15 days posttreatment. With both agents, the peripheral blood leukocyte counts were reduced to less than 100/mm^3 for 15 days.

Organisms. Four clinical isolates of *T. glabrata* were obtained from the Fungal Research Laboratory of M. G. Rinaldi, University of Texas Health Science Center at San Antonio. One day prior to challenge, the organisms were subcultured to 50 ml of brain heart infusion (BHI) broth (BBL, Cockeysville, Md.). The fungi were incubated on a shaker at 37°C overnight, washed twice with 0.9% NaCl, quantified with a hemacytometer, and adjusted to the desired concentration in normal saline. The counts were verified by determining CFU counts with 10-fold serial dilutions in 0.9% NaCl. Each mouse was infected with 1.1×10^8 to 7×10^8 CFU/ml in the lateral tail vein given in a 0.2-ml volume. The *T. glabrata* cells were agitated between each injection to achieve a uniform suspension.

Activity in vitro. The in vitro susceptibility studies were performed by the broth macrodilution technique proposed by the National Committee for Clinical Laboratory Standards (7). The medium was RPMI 1640 with glutamine, buffered by MOPS (morpholinepropanesulfonic acid) at pH 7.0 (American Bioganics, Niagara Falls, N.Y.). The inoculum was prepared from 24-h Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) subcultures of *T. glabrata* incubated at 35°C. Colonies of *T. glabrata* were suspended in 5 ml of sterile distilled water, and the cell density was adjusted with a spectrophotometer to a 0.5 McFarland standard at a 530-nm wavelength. The working suspension was a 1:100 dilution of the organisms followed by a 1:20 dilution of the suspension with RPMI broth medium. A volume of 0.9 ml of the adjusted solution was dispensed into tubes containing 0.1 ml of 10 times the concentrations of the drugs to be tested. The test tubes were incubated at 35°C for 48 h. The MIC of amphotericin B was the lowest concentration that permitted no visible growth. For the azoles, the MIC was the lowest concentration that

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TABLE 1. MICs for four isolates of *T. glabrata*[a]

Isolate no.	MIC (μg/ml) of:		
	DO8790	AmB	FLU
1	<0.125	0.5	2
2	4	0.5	16
3	4	0.5	64
4	4	1.0	>64

[a] Abbreviations: AmB, amphotericin B; FLU, fluconazole.

produced at least 80% inhibition of growth in comparison with that of the control. Inconsistent results were rechecked by duplicate testing of the isolates. Quality control strains of *T. glabrata* (ATCC 90029 and ATCC 90030) were included on each day of repeated testing. As shown in Table 1, the MIC of DO870 for isolate 1 was <0.125 μg/ml and that for isolates 2, 3, and 4 was 4 μg/ml. The latter isolates were considered resistant, since the MIC was the highest observed in our study and agreed with the data of Peng and Galgiani (16), who reported a MIC range of 0.125 to 4.0 μg/ml for *T. glabrata*.

Antifungal agents. DO870 was obtained from Zeneca Pharmaceuticals (Macclesfield, Cheshire, England). Fluconazole was provided by Pfizer-Roerig Pharmaceuticals (New York, N.Y.), and amphotericin B was obtained from Lyphomed (Deerfield, Ill.). The DO870 was prepared daily in 0.5% polysorbate (Tween 80), sonicated, and administered per os by an esophageal cannula (gavage) (p.o.) in a volume of 0.2 ml per dose. Amphotericin B was diluted to the desired concentration in 5% dextrose in water and administered at a volume of 0.2 ml intraperitoneally. Fluconazole was suspended in 0.3% Noble agar (Difco Laboratories) and administered at 0.2 ml p.o. via gavage.

Treatment regimens. After infection, the mice were randomized into treatment groups of 10 mice each. Drug therapy was begun the day following the infecting challenge. The mice were treated for 5 days with either amphotericin B (3 mg/kg/day) intraperitoneally, fluconazole (100 mg/kg twice a day [BID]) p.o., or DO870 (25, 10, 5, or 1 mg/kg/day or 10 or 5 mg/kg on alternate days [q.o.d.]) p.o. Other groups of mice were treated with a high-dose combination of amphotericin B (3 mg/kg/day) plus DO870 (25 mg/kg/day) or a low-dose combination of amphotericin B (2 mg/kg/day) plus DO870 (1 mg/kg/day). Control mice received 0.2 ml of 0.3% Noble agar p.o. Forty-eight hours after the last dose of antifungal agent, the mice were anesthetized with methoxyflurane (Metophane; Pitman-Moore, Washington Crossing, N.J.) and sacrificed by cervical dislocation. Both kidneys and the spleen were excised by a sterile technique, weighed, and homogenized in 2 ml of sterile 0.9% saline with 60 μg of amikacin per ml and 60 μg of piperacillin per ml. The homogenates were diluted by serial 10-fold dilution in saline, and 0.1 ml of each dilution and the undiluted homogenate were cultured in duplicate on Sabouraud dextrose agar for 48 h. CFU per gram of tissue was calculated. The minimal count was 18 CFU/g of tissue.

Statistical analysis. One-way analysis of variance was used. Multiple comparisons were made by Tukey's Studentized Range test using the logs of the CFU counts and were analyzed. A P value of <0.05 was considered statistically significant.

RESULTS

Table 1 presents in vitro susceptibilities. Isolate 1 was susceptible to both DO870 and fluconazole. Isolates 2 to 4 were resistant to DO870 and fluconazole.

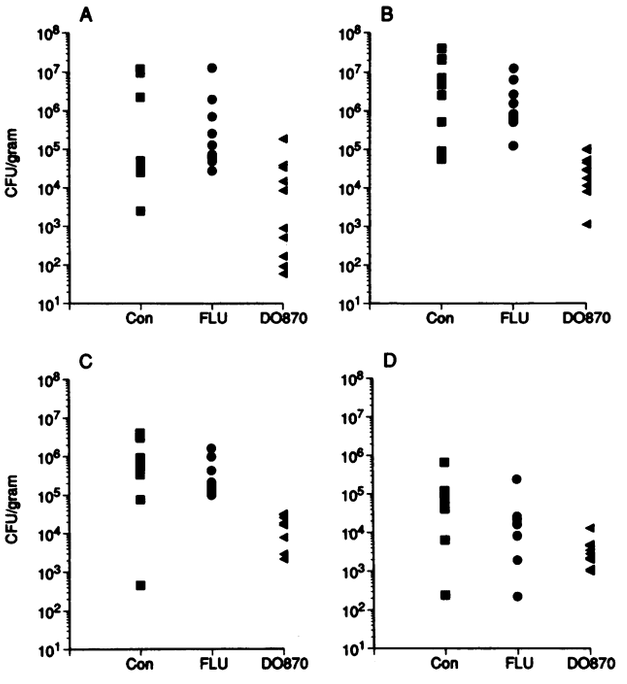

FIG. 1. Comparison of CFU per gram of tissue in control (Con) mice and mice treated with fluconazole (FLU) (100 mg/kg BID) or DO870 (50 mg/kg/day) after immunosuppression with 5FU (A and B) or gold sodium thiomalate (C and D). Panels A and C show results for kidney tissue, and panels B and D show results for spleen tissue (n = 10).

Immunosuppression. 5-FU or gold sodium thiomalate reduced the peripheral leukocyte counts to <100/mm^3 in the mice 1 day following treatment. Previous experiments with 5FU showed that 150 mg/kg similarly reduced the leukocyte counts with the least toxicity for the period of treatment (unpublished data). Because we wished to evaluate *T. glabrata* in mice with impaired macrophage and neutrophil function, we used gold sodium thiomalate for immunosuppression (13) in the subsequent studies. A comparison of mice immunosuppressed with gold sodium thiomalate versus 5FU and treated with fluconazole or DO870 is shown in Fig. 1. Overall, DO870 (50 mg/kg/day) was more active than fluconazole (100 mg/kg/day) in reducing the CFU burden in both kidney and spleen tissue, regardless of the method used for immunosuppression ($P < 0.05$). There was a modest but significant reduction in the CFU per gram of kidney tissue when the mice immunosuppressed with gold sodium thiomalate were treated with fluconazole ($P < 0.05$), but there was not a significant reduction in the CFU counts in kidneys of mice immunosuppressed with 5FU. In both groups, there was a significant reduction in the CFU burden in the spleen when mice were treated with DO870 instead of fluconazole. In all of the subsequent studies, the mice were immunosuppressed with gold sodium thiomalate.

Dose range studies. Results for infections with isolate 1 treated with DO870 at various concentrations ranging from 1 to 25 mg/kg/day are shown in Fig. 2. Daily dosing at 25 mg/kg/day suppressed the spleen tissue CFU burden the most. At 5 mg/kg/day (kidney) and 1 mg/kg/day (spleen), the tissue CFU burden was also reduced. However, q.o.d. dosing with DO870 at 10 mg/kg (a dose equivalent to 5 mg/kg/day for 2 days) did not significantly reduce CFU in either the kidneys or

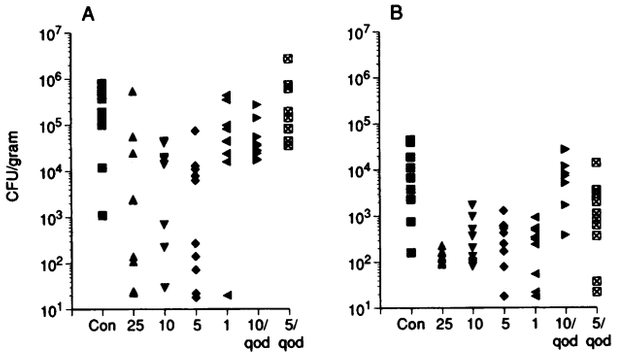

FIG. 2. Dose range study comparing effects of daily dosing with DO870 at 25, 10, 5, and 1 mg/kg/day with DO870 administered q.o.d. at 10 and 5 mg/kg. Panels: A, kidney; B, spleen. Con, control; $n = 10$.

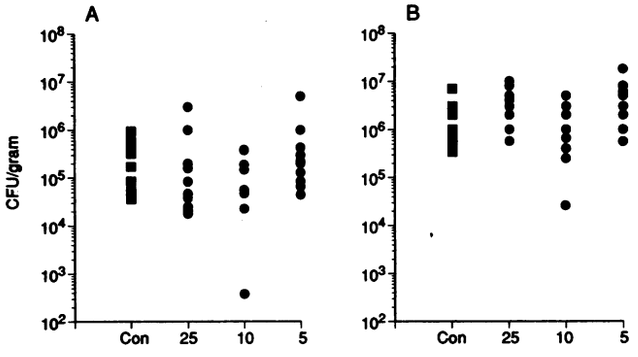

FIG. 4. Dose range study comparing efficacies of DO870 at 25, 10, and 5 mg/kg/day for treatment of infection with isolate 2. Panels: A, kidney; B, spleen. Con, control; $n = 10$.

the spleen. Therefore, despite a slow clearance, a DO870 elimination half-life of 30 h (2a), daily dosing is required.

Combination therapy. The high-dose combination of DO870 (25 mg/kg/day) and amphotericin B (3 mg/kg/day) decreased the CFU per gram of kidney tissue ($P < 0.05$) more than either antifungal agent alone for isolate 1, the fluconazole-susceptible isolate (Fig. 3A). An additive effect was undetectable for the spleen CFU counts at these antibiotic concentrations (Fig. 3B). Low-dose combination treatment with amphotericin B (2 mg/kg/day) and DO870 (1 mg/kg/day) showed no additive effect (Fig. 3C and D).

Resistant strains. When mice infected with *T. glabrata* isolates 2, 3, and 4 (DO870 MIC, 4 μg/ml) were treated with 5, 10, or 25 mg of DO870 per kg per day, no reduction in CFU per gram of kidney or spleen tissue was detected (Fig. 4, isolate 2). At 100 mg/kg/day, DO870 had no effect on either the kidney or the spleen tissue CFU burden (result not shown).

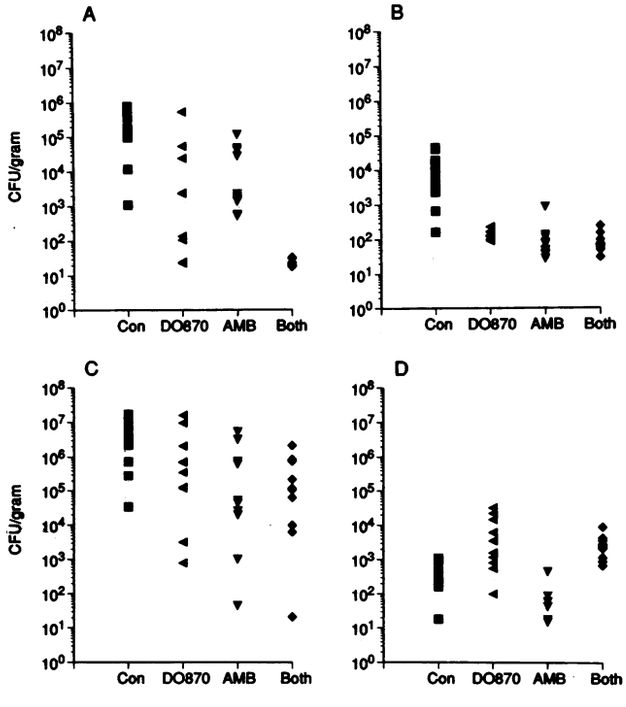

FIG. 3. Comparison of treatment with DO870 at 25 mg/kg/day, amphotericin B (AMB) at 3 mg/kg/day, and the combination (Both) (panels A [kidney] B [spleen]) and DO870 at 1 mg/kg/day, amphotericin B at 2 mg/kg/day, and the combination (panels C [kidney] and D [spleen]). Con, control; $n = 10$.

DISCUSSION

Disseminated *T. glabrata* infection was achieved in mice immunosuppressed with either gold sodium thiomalate or 5FU with an inoculum of 10^8 CFU/ml. Organs infected in preliminary studies included the spleen, kidneys, liver, and lungs. Cell-mediated immunity is believed to play an important role in immunity to fungal infections, and macrophages may contribute to the release of polypeptide cytokines which may alter the antimicrobial activity of effector cells such as neutrophils. Kowanko et al. (10) showed that granulocyte macrophage colony-stimulating factor enhanced the in vitro killing of *T. glabrata*. We therefore examined models with suppression of neutrophils (5FU) as well as inhibition of macrophage function (gold sodium thiomalate). Since the target organ for *T. glabrata* is the kidney and since DO870 concentrates in the reticuloendothelial system, the kidneys and spleen were chosen for these studies.

DO870 was more effective than fluconazole for treatment of infection with a fluconazole- and DO870-susceptible isolate of *T. glabrata*, regardless of the method of immunosuppression. It was not, however, active against isolates of *T. glabrata* which are resistant in vitro to fluconazole and DO879. Ross et al. (18) suggested the in vitro breakpoint for DO870 to be a MIC of ≤2.5 μg/ml. Peng and Galgiani (16) showed similar MICs for *T. glabrata* ranging from 0.125 to 4.0 μg/ml, which were 32 to 16 times higher than those typical for *C. albicans* (median, 0.0037 μg/ml) or *C. neoformans* (median, 0.0075 μg/ml). No standards for susceptibility or resistance have been established for *T. glabrata*. Although more isolates will need to be examined, it appears from our in vivo studies that a DO870 MIC of 4 μg/ml may correspond to resistance of *T. glabrata*.

Our results showed that although DO870 has a long half-life of 30 h, q.o.d. dosing was not effective in vivo with DO870 administered at 5 or 10 mg/kg q.o.d. compared with 5 or 10 mg/kg/day. This is in contrast to reports by Edwards et al. (4, 5), which showed that five oral doses of DO870 at 5 mg/kg at 48-h intervals were protective for treatment of *C. albicans* infection. For treatment of murine cryptococcal meningitis, DO870 at 10 mg/kg q.o.d. was superior to fluconazole (2). Our results are in agreement with the latter study, which also

showed DO870 at 50 mg/kg to be superior to fluconazole. Combination therapy with amphotericin B (3 mg/kg/day) and DO870 (25 mg/kg/day) was significantly better than either antifungal agent alone in reducing the isolate 1 CFU burden in kidney tissue.

DO870, a new triazole, has exhibited excellent antifungal activity against systemic murine infections with *C. neoformans* and *C. albicans* in both kidney and brain tissue of normal and immunocompromised mice (4). DO870 is also active in vitro against fluconazole-resistant *C. albicans* (4). In vivo, DO870 is reported to be 5- to 25-fold more potent than fluconazole for treatment of murine vaginal and systemic infections (3) and 2- to 7-fold superior to fluconazole in normal mice versus 3- to 90-fold superior in mice immunosuppressed with either cortisone or cyclophosphamide (14).

The kidney is the target organ of systemic *Candida* infection in mice. The treatment of systemic *Candida* infection in immunocompromised patients continues to be a major problem. Infections respond poorly to treatment with antifungal drugs, and cure is dependent on reestablishment of host resistance (11). *T. glabrata* is often found as a urinary tract pathogen causing cystitis and pyelonephritis and is reported to account for 25 to 33% of positive urine cultures for immunocompromised hosts (15). *T. glabrata* is often more resistant than *C. albicans* and is therefore more difficult to treat. Experimental data indicate that neither amphotericin B nor the triazoles (fluconazole and itraconazole) are able to decrease the number of CFU in the kidneys of persistently neutropenic mice with systemic *C. albicans* infection (19). DO870 showed no in vivo effect with DO870-resistant isolates which had variable in vitro susceptibility to fluconazole. In contrast, DO870 gave protection with a fluconazole-DO870-susceptible isolate for which the DO870 MIC was <0.125 μg/ml, and DO870 was superior to fluconazole for this strain. This is in contrast to results reported with *Candida krusei*, another more resistant yeast species, with which survival was increased but DO870 failed to reduce the yeast burden in the kidneys of immunosuppressed mice (9).

DO870 was effective in vivo against at least some strains of *T. glabrata* and combined with amphotericin B exerted some additive effects. The maximal dose of DO870 has not been determined for humans, and it is uncertain whether the higher doses which may be required for effective treatment of *T. glabrata* infection will be clinically effective or tolerated. Further studies with neutropenic models, both humans and animals, are needed, not only to test this agent against more isolates of *T. glabrata* but also to evaluate the use of prolonged therapy and to determine the optimal dosages.

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REFERENCES

1. **Bodey, G. P.** 1984. Candidiasis in cancer patients. Am. J. Med. **77**(Suppl. 40):13–19.
2. **Correa, A., M. Albert, and J. Graybill.** 1992. Treatment of murine cryptococcal meningitis (CM) with ICI DO870 (ICI) versus Fluconazole (F). Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1048.

2a. **Edwards, J. R.** Personal communication.

3. **Edwards, J. R., and S. McGregor.** 1992. DO870, a triazole antifungal agent: activity against *Candida albicans* in experimental vaginal and systemic infections in mice. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1047.
4. **Edwards, J. R., K. Nairn, and S. McGregor.** Personal communication.
5. **Edwards, J. R., K. Nairn, G. M. Megson, G. L. Shepherd, and G. D. Hughes.** 1993. Activity of DO870 against *Candida albicans* and *Cryptococcus neoformans* showing reduced susceptibility to fluconazole. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 375.
6. **Fraser, V. J., M. Jones, J. Dunkel, S. Storfer, G. Medoff, and W. C. Dunagan.** 1992. Candidemia in a tertiary care hospital; epidemiology, risk factors, and predictors of mortality. Clin. Infect. Dis. **15**:414–421.
7. **Galgiani, J. N., M. S. Bartlett, A. Espinel-Ingroff, R. A. Fromtling, M. A. Pfaller, and M. G. Rinaldi.** 1992. Standard reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
8. **Jessop, J. D., B. Vernon-Roberts, and J. Harris.** 1973. Effects of gold salts and prednisolone on inflammatory cells. Ann. Rheum. Dis. **32**:294–300.
9. **Karyotakis, N. C., E. J. Anaissie, R. Hachem, M. C. Dignani, and G. Samonis.** 1993. Comparison of the efficacy of polyenes and triazoles against hematogenous *Candida krusei* infection in neutropenic mice. J. Infect. Dis. **168**:1311–1318.
10. **Kowanko, I. C., A. Ferrante, D. P. Harvey, and K. L. Carman.** 1991. Granulocyte-macrophage colony-stimulating factor augments neutrophil killing of *Torulopsis glabrata* and stimulates neutrophil respiratory burst and degranulation. Clin. Exp. Immunol. **83**:225–230.
11. **Kullberg, B., J. W. Van't Wout, R. J. M. Poell, and R. van Furth.** 1992. Combined effect of fluconazole and recombinant human interleukin-1 on systemic candidiasis in neutropenic mice. Antimicrob. Agents Chemother. **36**:1225–1229.
12. **Meuniser-Carpenter, F., T. E. Kiehn, and D. Armstrong.** 1981. Fungemia in the immunocompromised host: changing patterns, antigenemia, high mortality. Am. J. Med. **71**:363–370.
13. **Mochizuki, H., H. Yamada, T. Tsuda, T. Watanabe, and M. Mizota.** 1992. In vitro and in vivo antifungal activities of DO870, a novel triazole. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1046.
14. **Mochizuki, H., H. Yamada, T. Watanabe, T. Tsuda, and M. Mizota.** 1993. Excellent therapeutic efficacy of DO870, a triazole antifungal agent, against local infections and its mechanism of action. Program Abstr. 33rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 377.
15. **Neal, D. E., and G. Rodriguez.** 1993. Fungal prostatitis, p. 1–2. *In* V. T. Andriole and P. H. Wiernik (ed.), Cliniguide to fungal infections, vol. 4, issue 5. DellaCorte Publications, Inc., New York.
16. **Peng, T., and J. N. Galgiani.** 1993. In vitro studies of a new antifungal triazole, DO870, against *Candida albicans*, *Cryptococcus neoformans*, and other pathogenic yeasts. Antimicrob. Agents Chemother. **37**:2126–2131.
17. **Persillin, R. H., and M. Ziff.** 1966. The effect of gold salts on lysosomal enzymes to peritoneal macrophage. Arthritis Rheum. **9**:57–63.
18. **Ross, S., D. A. McGough, A. W. Fothergill, and M. G. Rinaldi.** 1992. In vitro susceptibility testing of DO870, an investigational antifungal triazole. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1045.
19. **Van't Wout, J. W., I. Linde, P. C. J. Leigh, and R. Van Furth.** 1988. Effect of irradiation, cyclophosphamide, and etoposide (VP-16) on number of peripheral blood and peritoneal leukocytes in mice under normal conditions and during acute inflammatory reaction. Inflammation **13**:1–14.