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Activity of Fluconazole (UK 49,858) and Ketoconazole against *Candida albicans* In Vitro and In Vivo

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Fluconazole (UK 49,858), a new orally administered bis-triazole, was compared with ketoconazole for activity in synthetic broth dilution susceptibility tests against *Candida albicans* and also in treatment of experimental systemic candidal infections in rats. In vitro studies indicated that fluconazole activity is less sensitive to acidic medium than is that of ketoconazole. At physiologic pH, fluconazole was approximately 16-fold less active than ketoconazole against 35 representative isolates of *C. albicans*. Two additional isolates (K-1 and K-3) recovered from patients who had failed ketoconazole therapy were 32- to 64-fold more resistant than the median of each drug for other isolates. In animal studies, fluconazole was very effective in prolonging survival of rats infected with a representative candidal strain. With an inoculum sufficient to kill 29 of 38 sham-treated animals, only 1 of 18 animals treated with 0.5 mg of fluconazole per kg per day died compared with 13 of 20 animals treated with 10.0 mg of ketoconazole per kg per day. However, when similar fluconazole treatment was administered to rats infected with the more resistant strain, K-1, no prolongation of survival was found. Thus, in vivo and in vitro results between strains correlated well for fluconazole. However, in comparing results between drugs, ketoconazole was 16-fold more active in vitro and fluconazole was 20-fold more active in vivo. This discrepancy may be due to drug distribution, modes of drug metabolism, or other pharmacologic differences between the two agents.

Systemic candidiasis is a significant complication of immunosuppressive diseases, surgical procedures, and immunosuppressive medical therapies. Standard treatment of systemic candidiasis involves intravenous amphotericin B, which commonly engenders a variety of untoward effects. Other agents have been proposed as also of value in treating or preventing systemic candidiasis (6, 15, 31). However, their use is more controversial.

Fluconazole (UK 49,858) is a new bis-triazole antifungal agent. Because its absorption after oral administration is good (14), fluconazole may be effective as an orally administered therapy. Furthermore, its pharmacologic characteristics differ markedly from those of the related commercially available drug ketoconazole in that fluconazole is renally excreted, readily penetrates cerebrospinal fluid, and has a serum half-life that is sufficiently long to result in high drug levels in plasma with a single daily dose. These differences make fluconazole an interesting possible alternative to currently available therapies.

In this report, we present our findings of the activity of fluconazole compared with that of ketoconazole against *Candida albicans* both in broth dilution susceptibility tests and in experimental systemic infection. Because in vitro test conditions have been found to influence results with other imidazole agents significantly (2, 7, 8, 11, 12, 19, 21, 27), we considered it of importance to examine some of the likely variables in our in vitro comparisons.

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MATERIALS AND METHODS

Antifungal agents and media. Fluconazole (UK 49,858) and ketoconazole were supplied as powders by the respective drug manufacturers (Pfizer Inc., Groton, Conn.; Janssen Pharmaceutica, Inc., New Brunswick, N.J.). Synthetic amino acid medium-fungal (SAAMF) was prepared as described previously (3). For most studies, SAAMF was constituted with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer adjusted to a pH of 7.4. In selected studies, morpholinepropanesulfonic acid (MOPS), Tris, and sodium phosphate buffers (Sigma Chemical Co., St. Louis, Mo.) were substituted for HEPES and, the pH was varied between 3.0 and 7.4. In some studies, the medium was supplemented with 1 or 10% bovine serum albumin or fresh rat serum.

Organisms. Thirty-seven isolates of *C. albicans* were used in these experiments. Thirty-two isolates (kindly furnished by John E. Bennett, Bethesda, Md.) had been selected from a panel collected from several sources within the United States for their different susceptibilities to an unrelated antifungal agent, flucytosine (28, 29). Each of these isolates was stored at −70°C in yeast nitrogen base broth with 10% glycerol prior to these studies. Two other isolates were obtained from the Veterans Administration Medical Center, Tucson, Ariz. Strain B113, which had been used previously in animal studies (9, 32), was provided by Edward Balish of Madison, Wis. Two strains, also used by others, were isolated by Charles Kirkpatrick of Denver, Colo., from patients with chronic mucocutaneous candidiasis whose candidal lesions had relapsed during prolonged therapy with ketoconazole (13, 26). The day before they were to be tested, isolates were inoculated into SAAMF broth for in vitro studies or yeast nitrogen base broth for in vivo studies.

Broth dilution testing. MICs and 50% inhibitory concentrations ($IC_{1/2}$s) were determined as previously described (3,

TABLE 1. Effect of pH and medium on results of broth dilution tests of the susceptibility of *C. albicans* to fluconazole or ketoconazole

Drug	Buffer	Mean MIC/$IC_{1/2}$ (μg/ml)[a] with an initial medium pH of:			
		3.0	5.0	6.0	7.4
Fluconazole	MOPS-Tris	1.0/0.5	1.0/0.5	1.0/0.25	0.25/0.25
	HEPES	2.0/0.5	1.0/0.5	1.0/0.25	0.5/0.125
Ketoconazole	MOPS-Tris	4.0/2.0	0.125/0.031	0.031/0.016	0.031/0.031

[a] Geometric mean from two strains.

7) with test conditions modified for some specific experiments as detailed in the text. Briefly, twofold dilutions of the antifungal agents were prepared in media to final concentrations of 0.004 to 32.0 μg/ml. Yeast inocula were adjusted to 2×10^3 yeast cells per ml by hemacytometer counting. Equal volumes of the inocula and diluted drug were mixed (final volume, 2.0 ml) in plastic tubes and incubated at 37°C without agitation. Both MIC and $IC_{1/2}$ readings were determined by a single observer after 22 h while growth in a drug-free control was still active. The $IC_{1/2}$ was calculated, by using turbidimetric data, as the lowest drug concentration satisfying the criterion $\%T \geq [\%T_{control} + 0.5(100 - \%T_{control})]$, in which $\%T$ equals percent transmission and control equals the turbidity of the drug-free tube.

In vivo studies. Systemic candidiasis was produced as previously described (9) by using anesthetized, outbred, cesarean-delivered, adult (180 to 200 g) male Sprague-Dawley rats. After centrifuging and suspending the yeast three times in pyrogen-free sterile saline, we diluted overnight growths of the infecting inocula in sterile nonbacteriostatic saline so that each animal received 1.5×10^6 to 3.0×10^6 CFU in 0.5 ml by a single intravenous injection. Six hours after injection of the candida suspension and then daily thereafter, the rats received various doses of fluconazole, ketoconazole, or diluent alone (sham treatment) in 1.0-ml amounts by oral gavage. The rats were examined and deaths were recorded twice daily for 21 days. Results were analyzed by censored-survival data statistics (23, 24).

In separate studies, serum was obtained from rats 0, 1, 2, 3, 6, and 22 h after a single dose of 2.0 mg of fluconazole or 10 mg of ketoconazole per kg. Sera were assayed for drug levels by a plate assay (18) with SAAMF and *Candida pseudotropicalis* ATCC 28838 as the indicator organism. The sensitivity of these studies was 0.3 μg/ml. The results were used to estimate peak levels and half-lives of the drugs in serum.

RESULTS

Effects of inoculum size, pH, buffers, and protein supplementation on broth dilution susceptibility results. Two strains (C-17 and B113) were used to explore the effects of various test conditions on susceptibility results with fluconazole and, in some studies, ketoconazole for comparison. Strain C-17 has been used for several years in our laboratory for in vitro testing with other antifungal agents, and strain B113 has been used by several other investigators for animal studies of systemic candidiasis.

For both strains, increasing the inocula from 10^2 to 10^5 yeast cells per ml increased the MIC of fluconazole from 0.25 to 2.0 μg/ml. $IC_{1/2}$ results varied no more than twofold (from 0.125 to 0.25 μg/ml) and agreed most closely with MICs at inocula of 10^2 and 10^3 yeast cells per ml. Subsequent studies were performed with inocula adjusted to 10^3 yeast cells per ml.

Certain buffers have previously been shown to antagonize other antifungal agents (3). For this reason, we compared susceptibility results obtained with SAAMF constituted with HEPES, MOPS-Tris, or sodium phosphate buffer, each at the same molarity and adjusted to an initial pH of 7.4, for two strains of candida. Drug activity was unaffected by the choice of buffer system.

Reducing the pH in either HEPES- or MOPS-Tris-buffered SAAMF from 7.4 to 3.0 resulted in two- to fourfold increases in MIC or $IC_{1/2}$ results with fluconazole (Table 1). In marked contrast, ketoconazole results were 64- to 128-fold higher at a pH of 3.0 than at 7.4, findings that agree with those of previous reports (19). Up to 10% supplementation with either bovine serum albumin or fresh rat serum resulted in no change in MIC or $IC_{1/2}$ results for either drug against strain C-17. Because of these findings and those of our previously published study, we chose to perform subsequent in vitro tests at a pH of 7.4 and to use HEPES as the buffer.

MIC and $IC_{1/2}$ results for the 37 *C. albicans* isolates used in these studies are summarized in Table 2. For 32 of the 35 representative isolates (not selected for clinical failure of ketoconazole therapy), MICs for fluconazole ranged between 0.125 and 0.5 μg/ml. An equally narrow modal distribution was obtained in $IC_{1/2}$ results. For ketoconazole, MICs for the 32 strains were between 0.008 and 0.031 μg/ml, approximately 16-fold lower than those for fluconazole. For both drugs, few isolates were more resistant than the others. Indeed, depending on the specific drug examined and the endpoint used, one to three of the isolates were only fourfold more sensitive than strains K-1 and K-3, the strains collected from patients who had relapsed while receiving ketoconazole therapy. Strains K-1 and K-3 had MICs of 16.0 μg/ml with fluconazole and 0.5 to 0.25 μg/ml with ketoconazole.

In vivo studies. *C. albicans* B113 was selected for these studies because it had been used previously and because its in vitro susceptibility was found to equal the median for fluconazole and ketoconazole. On the other hand, strain K-1 was selected because previous studies (26) and our own in vitro results indicated this strain to be more resistant to imidazoles. Results of our in vivo efficacy studies are summarized in Table 3.

TABLE 2. Susceptibility to fluconazole and ketoconazole of 37 strains of *C. albicans*, including 2 strains (K-1 and K-3) from patients who had failed ketoconazole therapy

Drug	Strain(s)	MIC (μg/ml)		
		Range	For 50% of strains	For 90% of strains
Fluconazole	35 Strains	0.063–4.0	0.25	0.25
	Strain K-1	16.0		
	Strain K-3	16.0		
Ketoconazole	35 Strains	0.008–0.125	0.016	0.031
	Strain K-1	0.5		
	Strain K-3	0.25		

TABLE 3. Survival of rats infected intravenously with either strain B113 (susceptible) or strain K-1 (resistant) of *C. albicans* in relation to treatment with fluconazole or ketoconazole

Infecting strain	Agent and dose (mg/kg per day)	Days of treatment	No. of 21-day survivors/ no. treated	Median survival (days) of rats that died (range)
B311	Sham	3–6	9/38	5.4 (3–13)
	Fluconazole			
	0.1	3	2/10	5.0 (3–9)
	0.3	3	4/10	5.0 (4–7)
	0.3	6	2/10	5.0 (4–8)
	0.5	3	17/18[a]	11.0 (11)
	Ketoconazole			
	2.0	3	5/20	5.5 (3–9)
	10.0	3	5/10	5.5 (4–11)
	10.0	6	2/10	5.0 (4–11)
K-1	Sham	3	1/8	6.0 (5–7)
	Fluconazole			
	0.5	3	0/8	6.0 (6–7)

[a] Significantly different from sham treatment ($P < 0.01$).

With strain B113, a sharp dose response was observed between 0.3 and 0.5 mg of fluconazole per kg per day. In the combined results of two separate studies, death occurred in only 1 of 18 animals receiving 0.5 mg/kg on each of three consecutive days. Animals receiving 0.3 mg/kg for 3 or 6 days appeared to obtain no benefit. In fact, results with 6 days of treatment were slightly worse than with 3 days of treatment. Whether this pattern is due to drug toxicity could not be determined from our studies since treatment of uninfected animals was not included as a control. However, results from other studies indicate that fluconazole is very well tolerated (16).

With ketoconazole, considerably higher doses were required to produce any evidence of a therapeutic effect. With the highest drug dose studied (10 mg/kg) for 3 to 6 days of treatment, 7 of 20 animals survived compared with 3 of 20 sham-treated animals (the paired subset of the 38 sham-treated controls shown in Table 3). However, this trend did not constitute a statistically significant difference. In contrast to these studies, substituting strain K-1 for B113 and treating rats with fluconazole at a dosage of 0.5 mg/kg per day for 3 consecutive days resulted in no indication of protection.

Measurements of drug levels in serum at 1.0, 2.0, 3.0, 6.1, and 22.0 h after 10 mg of ketoconazole per kg demonstrated 1.52, 1.23, 0.73, ≤0.3, and ≤0.3 μg/ml. These measurements suggest the serum half-life to be approximately 3 h. Similar studies 1.1, 2.3, 3.8, 6.7, and 22.7 h after 2.0 mg of fluconazole per kg demonstrated 1.14, 1.11, 1.46, 0.89, and ≤0.3 μg/ml. Fluconazole half-life in serum appears to be at least 6 h. However, this estimate is less precise because of the prolonged peak which may be due to somewhat delayed absorption. The areas under the curves derived from these measurements were 2.9 and 8.9 μg · h for ketoconazole and fluconazole, respectively. All fluconazole levels measured in serum after 0.5 mg/kg were below the sensitivity of our assay. However, if fluconazole levels after 2.0 or 0.5 mg/kg are proportional to the dose, extrapolations from the measured results suggest that mean drug concentrations during treatment with 0.5 mg of fluconazole or 10 mg of ketoconazole per kg would be similar.

DISCUSSION

A striking feature of our in vitro studies with fluconazole and ketoconazole was the uniform activity of each drug against the representative strains of *C. albicans*. MIC results for each drug were within a fourfold range for at least 32 of the 35 representative strains. This uniformity differs from reports of studies with ketoconazole in which unbuffered medium was used (22; S. Shadomy, H. P. Yu, S. C. White, W. E. Dismukes, and the CMCS Group, Program Abstr. 21st Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 275, 1981) but are in excellent agreement with the results of Minagawa et al. (19). In the latter studies, in which acidic media antagonized ketoconazole activity, the range of MIC results was very broad, conveying the impression that various isolates of *C. albicans* differed markedly in in vitro sensitivity. However, when the pH was nearly physiologic, MIC results for the same isolates were very low and nearly identical. These findings suggest that the diverse MICs for ketoconazole seen in unbuffered medium may be due to differences in acid production and those seen in acid medium may be due to differences among isolates in their growth rates at low pH. Neither of these characteristics are directly related to the actual ability of the drug tested to inhibit yeast growth. Fluconazole was not appreciably influenced by differences in medium pH, and therefore the pH of media may not be as important a variable in testing this drug.

On average, the isolates that we studied were inhibited by 16-fold less ketoconazole than fluconazole. This difference appeared to exist over a 3-log range of starting inocula and with each of three different buffer systems. Since ketoconazole, but not fluconazole, is antagonized by acidity, the difference between the two drugs was lost with low pH. SAAMF is a defined medium the constituents of which are relatively simple compounds. Nonetheless, our studies do not exclude the possibility that one or more of its constituents antagonize fluconazole more than ketoconazole as a possible reason for the systematic difference between the in vitro activities of these two drugs.

In comparing fluconazole and ketoconazole treatments of systemic candidal infection in rats, the relative efficacy of the two drugs was found to be the reverse of their ranking by in vitro tests. Whereas fluconazole at doses of 0.5 mg/kg per day for 3 days of treatment resulted in survival of nearly all animals, ketoconazole at a dose of 10 mg/kg per day given for 3 to 6 days was not significantly effective. Others, using larger doses in similar studies, have been able to demonstrate therapeutic efficacy of ketoconazole (17, 22, 25, 30). In our studies, we neither examined histology nor performed quantitative cultures, procedures which might have been more sensitive at detecting antifungal effects of ketoconazole. Nonetheless, our results in animals indicate at least 20-fold greater efficacy of fluconazole in vivo despite the finding of 16-fold lesser activity in vitro.

Several pharmacologic differences between the two drugs may account for the discrepancies between in vitro activity and in vivo efficacy. First, absorption of the dose could vary between the two drugs. Of fluconazole orally administered to rats, 82% has been recovered in the urine, indicating that absorption is nearly complete (14). Approximately 5% of ketoconazole is recovered from rat feces as unchanged drug, suggesting that this drug may also be well absorbed (10). However, a more precise estimate of ketoconazole absorption would require comparative studies with intravenously administered drug. Second, the modes of clearance of ketoconazole and fluconazole are different. Very little ketocon-

azole is recovered from the urine and presumably is not concentrated in the kidney (10). In contrast, fluconazole is predominantly eliminated by the kidneys. Since the kidney is the primary target organ in this experimental model (32), the differences in route of excretion between the two drugs could be important. A third difference may be the lower percentage of serum protein binding of fluconazole compared with that of ketoconazole (5, 14). We were unable to demonstrate an effect of protein supplements on ketoconazole or fluconazole results, and previous studies reported that the addition of horse serum resulted in either no difference or greater ketoconazole activity (1, 21). Nonetheless, in vivo effects of serum protein binding have not been evaluated for either antifungal agent. A fourth difference between the drugs is their rates of clearance from serum. Since fluconazole has a longer half-life, the effective dose may be greater compared with an equivalent milligram-per-kilogram dose of ketoconazole. Our studies do not exclude this difference as a contributing factor since our assay was not sufficiently sensitive to measure serum levels after the fluconazole dose that was effective in vivo. However, extrapolating from serum measurements obtained after a fourfold higher fluconazole dose, our results suggest that the areas under the curves after 0.5 mg of fluconazole or 10.0 mg of ketoconazole per kg were similar.

Despite the discrepancies discussed above, for each drug viewed separately the in vitro resistance of strains K-1 and K-3 appears to correlate with the previously reported clinical failure (13). Ryley et al. (26) reported that murine candidiasis produced by these same isolates was resistant to treatment by ketoconazole and another imidazole. Our studies demonstrated significantly less fluconazole efficacy also against strain K-1 compared with our standard strain, B113. Our studies, along with those of Ryley et al., indicate that resistance which develops to one imidazole antifungal agent may extend to other members of this class of drugs.

Very few strains of *C. albicans* have been identified as resistant to ketoconazole or other imidazoles. Our studies suggest that most strains collected prior to the wide use of imidazoles in general practice appear more sensitive than strains K-1 and K-3. However, the lack of standardized methods of antifungal susceptibility testing hinders reliable detection of imidazole resistance (4, 20). It is of interest that our relatively small study identified one to three strains whose susceptibilities are only fourfold greater than those of isolates from patients with clinical relapse. This finding raises some concern that primary imidazole resistance in *C. albicans* may not be rare.

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LITERATURE CITED

1. **Aerts, F., M. DeBrander, H. van den Bossche, J. van Cutsem, and M. Borgers.** 1980. The activity of ketoconazole in mixed cultures of fungi and human fibroblasts. Mykosen **3**:53–67.
2. **Brass, C., J. Z. Shainhouse, and D. A. Stevens.** 1979. Variability of agar dilution-replicator method of yeast susceptibility testing. Antimicrob. Agents Chemother. **15**:763–768.
3. **Calhoun, D. L., and J. N. Galgiani.** 1984. Analysis of pH and buffer effects on flucytosine activity in broth dilution susceptibility testing of *Candida albicans* in two synthetic media. Antimicrob. Agents Chemother. **26**:364–367.
4. **Calhoun, D. L., G. D. Roberts, J. N. Galgiani, J. E. Bennett, D. S. Feingold, J. Jorgensen, G. S. Kobayashi, and S. Shadomy.** 1986. Results of a survey of antifungal susceptibility tests in the United States and interlaboratory comparison of broth dilution testing of flucytosine and amphotericin B. J. Clin. Microbiol. **23**:298–301.
5. **Daneshmend, T. K., and D. W. Warnock.** 1983. Clinical pharmacokinetics of systemic antifungal drugs. Clin. Pharmacokinet. **8**:17–42.
6. **Fazio, R. A., P. C. Wickremesinghe, and E. L. Arsura.** 1983. Ketoconazole treatment of *Candida esophagitis*—a prospective study of 12 cases. Am. J. Gastroenterol. **78**:261–264.
7. **Galgiani, J. N., and D. A. Stevens.** 1976. Antimicrobial susceptibility testing of yeasts: a turbidimetric technique independent of inoculum size. Antimicrob. Agents Chemother. **10**:721–726.
8. **Galgiani, J. N., and D. A. Stevens.** 1978. Turbidimetric studies of growth inhibition of yeasts with three drugs: Inquiry into inoculum-dependent susceptibility testing, time of onset of drug effect, and implications for current and newer methods. Antimicrob. Agents Chemother. **13**:249–254.
9. **Galgiani, J. N., and D. B. VanWyck.** 1984. Ornithyl amphotericin methyl ester treatment of experimental candidiasis in rats. Antimicrob. Agents Chemother. **26**:108–109.
10. **Heel, R. C.** 1981. Pharmacokinetic properties, p. 67–73. *In* H. B. Levine (ed.), Ketoconazole in the management of systemic fungal disease. ADIS Press, New York.
11. **Hoeprich, P. D., and P. D. Finn.** 1972. Obfuscation of the activity of antifungal antimicrobials by culture media. J. Infect. Dis. **126**:353–361.
12. **Hoeprich, P. D., M. A. Saubolle, and A. C. Houston.** 1977. Susceptibility testing of fungi, p. 101–106. *In* A. Bondi, J. T. Bartola, and J. E. Prier (ed.), The clinical laboratory as an aid in chemotherapy of infectious diseases. University Park Press, Baltimore.
13. **Horsburgh, C. R., Jr., and C. H. Kirkpatrick.** 1983. Long-term therapy of chronic mucocutaneous candidiasis with ketoconazole: experience with twenty-one patients. Am. J. Med. **74**:23–29.
14. **Humphrey, M. J., S. Jevons, and M. H. Tarbit.** 1985. Pharmacokinetics of UK-49,858, a metabolically stable triazole antifungal drug, in animals and humans. Antimicrob. Agents Chemother. **28**:648–653.
15. **Jordan, W. M., G. P. Bodey, V. Rodriguez, S. J. Ketchel, and J. Henney.** 1979. Miconazole therapy for treatment of fungal infections in cancer patients. Antimicrob. Agents Chemother. **16**:792–797.
16. **Kobayashi, G. S., S. Travis, and G. Medoff.** 1986. Comparison of the in vitro and in vivo activity of the bis-triazole derivative UK 49,858 with that of amphotericin B against *Histoplasma capsulatum*. Antimicrob. Agents Chemother. **29**:660–662.
17. **Lefler, E., and D. A. Stevens.** 1985. New azole compounds: vibunazole (Bay n7133) and Bay *l*9139, compared with ketoconazole in the therapy of systemic candidosis and in pharmacokinetic studies, in mice. J. Antimicrob. Chemother. **15**:69–75.
18. **Lund, M. E., D. J. Blazevic, and J. M. Matsen.** 1973. Rapid gentamicin bioassay using a multiple-antibiotic-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **4**:569–573.
19. **Minagawa, H., K. Kitaura, and N. Nakamizo.** 1983. Effects of pH on the activity of ketoconazole against *Candida albicans*. Antimicrob. Agents Chemother. **23**:105–107.
20. **National Committee for Clinical Laboratory Standards.** 1985. Antifungal susceptibility testing; committee report, vol. 5, no. 17. National Committee for Clinical Laboratory Standards, Villanova, Pa.
21. **Odds, F. C.** 1982. Interactions among amphotericin B, 5-fluorocytosine, ketoconazole, and miconazole against pathogenic fungi *in vitro*. Antimicrob. Agents Chemother. **22**: 763–770.
22. **Odds, F. C., L. J. R. Milne, J. C. Gentles, and E. H. Ball.** 1980. The activity *in vitro* and *in vivo* of a new imidazole antifungal, ketoconazole. J. Antimicrob. Chemother. **6**:97–104.
23. **Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G.**

Smith. 1976. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. Br. J. Cancer **34**:585–612.

24. **Peto, R., M. C. Pike, P. Armitage, N. E. Breslow, D. R. Cox, S. V. Howard, N. Mantel, K. McPherson, J. Peto, and P. G. Smith.** 1977. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br. J. Cancer **35**:1–39.
25. **Plempel, M.** 1984. Antimycotic activity of AY N 7133 in animal experiments. J. Antimicrob. Chemother. **13**:447–463.
26. **Ryley, J. F., R. G. Wilson, and K. J. Barrett-Bee.** 1984. Azole resistance in *Candida albicans*. Sabouraudia **22**:53–63.
27. **Shadomy, S.** 1971. *In vitro* antifungal activity of clotrimazole (Bay b 5097). Infect. Immun. **4**:143–148.
28. **Stiller, R. L., J. E. Bennett, H. J. Scholer, M. Wall, A. Polak, and D. A. Stevens.** 1982. Susceptibility to 5-fluorocytosine and prevalence of serotype in 402 *Candida albicans* isolates from the United States. Antimicrob. Agents Chemother. **22**:482–487.
29. **Stiller, R. L., J. E. Bennett, H. J. Scholer, M. Wall, A. Polak, and D. A. Stevens.** 1983. Correlation of *in vitro* susceptibility test results with *in vivo* response: flucytosine therapy in a systemic candidiasis model. J. Infect. Dis. **147**:1070–1077.
30. **Van Cutsem, J., J. Fransen, F. Van Gerven, and P. A. Janssen.** 1985. Oral treatment with ketoconazole in systemic candidosis of guinea-pigs: microbiology, hematology, and histopathology. Sabouraudia **23**:189–198.
31. **Vandevelde, A., A. A. Mauceri, and J. E. Johnson III.** 1972. 5-Fluorocytosine in the treatment of mycotic infections. Ann. Intern. Med. **77**:43–51.
32. **Wong, B., E. M. Bernard, J. W. M. Gold, D. Fong, A. Silber, and D. Armstrong.** 1982. Increased arabinitol levels in experimental candidiasis in rats: arabinitol appearance rates, arabinitol/creatinine ratios, and severity of infection. J. Infect. Dis. **146**:346–352.