ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1990, p. 196–201
0066-4804/90/020196-06$02.00/0

Vol. 34, No. 2

Effects of Antifungal Agents on the Function of Human Neutrophils In Vitro

EMMANUEL ROILIDES,[1] THOMAS J. WALSH,[1]* MARC RUBIN,[1]
DAVID VENZON,[2] AND PHILIP A. PIZZO[1]

Infectious Diseases Section, Pediatric Oncology Branch,[1] and Biostatistics and Data Management Section,[2] National Cancer Institute, Bethesda, Maryland 20892

Received 27 June 1989/Accepted 20 October 1989

Polymorphonuclear leukocytes (PMNs) are an important component of the host defense against fungi. We investigated the influence of five antifungal agents on PMN function and compared them with amphotericin B (AmB). The in vitro effects of AmB, flucytosine, ketoconazole, fluconazole, Sch-39304, and cilofungin (LY121019) on chemotaxis, phagocytosis, oxidative metabolism of PMN as reflected by superoxide anion (O_2^-) generation, and intracellular killing of *Candida albicans* blastoconidia were examined. With regard to chemotaxis in response to *N*-formylmethionyl-leucyl-phenylalanine, as measured by the multiwell chamber method, AmB induced a marked decrease (≥5 μg/ml), whereas ketoconazole at 5 μg/ml enhanced it. Phagocytosis was significantly decreased after pretreatment of PMNs with AmB and Sch-39304 (>5 and 1 to 10 μg/ml, respectively). O_2^- production after stimulation of PMNs with *N*-formylmethionyl-leucyl-phenylalanine was significantly decreased by AmB (>5 μg/ml) and enhanced by Sch-39304 (1 to 5 μg/ml). In contrast, intracellular killing, as tested by methylene blue staining, was enhanced by ketoconazole (5 μg/ml) and Sch-39304 (1 to 5 μg/ml). Flucytosine, fluconazole, and cilofungin did not affect PMN function at therapeutic concentrations. The results of this comprehensive study indicate that AmB, flucytosine, cilofungin, and the newer azoles, at safely achievable concentrations, generally do not suppress PMN function in vitro and may enhance selective functions.

Candida and *Aspergillus* species are the predominant fungal pathogens that cause life-threatening infections in neutropenic cancer patients. Since polymorphonuclear leukocytes (PMNs) are an important component of the host defense against opportunistic fungi, any suppression of their function could exacerbate further the ability of the host to control these infections. A number of commonly used drugs, including antineoplastic agents, analgesics, and antibiotics, have been shown to influence PMN function. Although a wide array of antibiotics has been evaluated (3), data regarding antifungal agents are more limited.

Unlike antibacterial antibiotics, only a small number of antifungal agents have been available. Amphotericin B (AmB), a polyene antibiotic, has been the cornerstone antifungal therapy for nearly 30 years. In addition, a few other antifungal agents have been developed, some of which may be used in patients who are immunocompromised. These include flucytosine (5-FC); miconazole; and the investigational agents itraconazole (22, 27), fluconazole (22, 27), cilofungin (LY121019) (14), and Sch-39304 (T. J. Walsh, J. W. Lee, J. Peter, R. Schaufele, M. Rubin, and P. A. Pizzo, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 169, 1988). The antifungal azoles are fungistatic rather than fungicidal at therapeutically achievable concentrations (13). Thus, it is important to ensure that the antifungal agent per se does not exert a negative effect on the host defense system.

Among the existing antifungal compounds, AmB is the only agent which has been tested extensively for interference with the PMN function. Moreover, the literature on the effects of antifungal agents shows conflicting data. Some investigators have reported that AmB inhibits chemotaxis (5, 19, 28) and phagocytosis but not intracellular killing of *Candida albicans* blastoconidia at 1 μg/ml (7, 15, 18). Others have found that phagocytosis and killing are significantly reduced only above 10 μg/ml (P. Van der Auvera, P. Joly, M. Bonnet, A. Leteux, C. Heymans, M. Husson, and F. Meunier, 28th ICAAC, abstr. no. 1520, 1988). Finally, AmB inhibits chemiluminescence of normal PMNs (2), although there are also reports of the opposite effect (24). 5-FC has been reported to suppress chemiluminescence (5) but to have no effect on chemotaxis (5, 10). Ketoconazole has been found to suppress (8) or not affect (19) chemotaxis of human phagocytic cells. Abruzzo et al. (1, 2) found that chemiluminescence is decreased by ketoconazole, fluconazole, and itraconazole, whereas 5-FC, fluconazole, and miconazole have no effect on mixed mouse spleen cell populations.

Little is known about the effects of the investigational antifungal compounds 5-FC, itraconazole, Sch-39304, and cilofungin. Moreover, no single study has comprehensively examined the effects of established and investigational antifungal compounds on multiple PMN functions. We therefore studied the effects of AmB, 5-FC, ketoconazole, fluconazole, Sch-39304, and cilofungin on chemotaxis, phagocytosis, superoxide anion (O_2^-) generation, and intracellular killing of *C. albicans*.

MATERIALS AND METHODS

Preparation of PMNs. PMNs were prepared from heparinized whole blood of healthy adult donors (preservative-free heparin, 5 to 10 U/ml) by the method described by Boyum (6). Briefly, dextran sedimentation (1% [wt/vol]) of the whole blood was followed by Ficoll gradient centrifugation of the

* Corresponding author.

leukocyte-rich supernatants. Dextran 500 was purchased from Pharmacia Fine Chemicals (Piscataway, N.J.), and Ficoll (Lymphocyte Separation Medium) was purchased from Organon Teknika Corp. (Durham, N.C.). Contaminating erythrocytes were lysed by suspension of the PMN pellet in distilled H_2O for 15 to 20 s, followed by the addition of hypertonic saline to correct the tonicity of the solution. The PMNs were suspended finally in Hank balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+}. Viability of PMNs was tested by the trypan blue exclusion method by counting the stained (dead) versus unstained (alive) cells on a hemacytometer.

C. albicans. The strain of *C. albicans* used in all the experiments was clinical isolate 86-21, which was obtained from the Microbiology Service of the Clinical Center at the National Institutes of Health, and was extensively studied in vitro and in vivo in our laboratory. The isolate was preserved in fractions of skim milk at −40°C and was grown on Sabouraud agar plates for 24 h at 37°C before the experiments. Under these conditions the *Candida* cells grew only in the blastoconidial phase without any germ tubes or pseudohyphae. These cells were suspended in HBSS, and their concentration was adjusted to 10^7 CFU/ml with a hemacytometer.

Drugs. AmB-desoxycholate powder (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) was dissolved in distilled H_2O to 5 mg/ml and subsequently in 5% glucose solution to 1 mg/ml. Final dilutions in the range of 0.1 to 20 μg/ml were made in HBSS. Ketoconazole (kindly provided by Janssen Pharmaceutica, Inc., Piscataway, N.J.) was dissolved in 0.2 N HCl to a concentration of 50 mg/ml and subsequently in distilled H_2O to a concentration of 100 μg/ml. Final dilutions in the range of 0.01 to 10 μg/ml were made in HBSS. 5-FC (kindly provided by Hoffmann-La Roche Inc., Nutley, N.J.) as a solution of 1 mg/ml was further diluted in HBSS to the range of 5 to 500 μg/ml. Fluconazole (kindly provided by Pfizer Central Research, Groton, Conn.) was dissolved in normal saline at 1 mg/ml. Final dilutions were made in HBSS (1 to 50 μg/ml). Sch-39304 (kindly provided by Schering Corp., Orange, N.J.) was dissolved in lactated Emulphor EL-719L 9 (GAF Corp., Wayne, N.J.) to a concentration of 100 μg/ml, finally brought to the concentrations tested (1 to 25 μg/ml) in HBSS. Emulphor and lactic acid without Sch-39304 in equivalent concentrations were used as controls. Cilofungin (kindly provided by Eli Lilly & Co., Indianapolis, Ind.) was dissolved in phosphate-buffered polyethylene glycol to a concentration of 50 mg/ml. Subsequent dilution to the final concentrations (0.1 to 100 μg/ml) was made in HBSS. Polyethylene glycol in the maximal equivalent concentration was also used as a control. For all the drugs, the range of concentrations studied included and exceeded the achievable concentrations in plasma. The known upper limits of safely achievable concentrations in plasma are as follows: AmB, 4 μg/ml; 5-FC, 100 μg/ml; ketoconazole, 5 μg/ml; fluconazole, 25 μg/ml; Sch-39304, 2 μg/ml; and cilofungin, 40 μg/ml.

Drug pretreatment of cells. Prior to the assays, 10^6 PMNs in HBSS were incubated at 37°C for 30 min with or without the antifungal compounds. Cell counting after this incubation period with each of the drugs showed that there was no lysis of PMNs during this procedure, except with AmB at or higher than 20 μg/ml. The drugs remained in the test tubes throughout the assay period.

Chemotaxis. Chemotaxis of PMNs was measured by the 48-multiwell chamber method developed by Falk et al. (11). Suspensions of 45 μl containing 10^6 PMNs per ml in HBSS previously treated with various concentrations of drugs were added to the upper wells of a 48-multiwell chemotaxis chamber (Neuroprobe, Inc., Cabin John, Md.). Polycarbonate filters (25 by 80 mm), polyvinylpyrrolidone coating free, with 3-μm holes were used. In the lower wells, either 10^{-8} M *N*-formylmethionyl-leucyl-phenylalanine (FMLP; Sigma Chemical Co., St. Louis, Mo.), which served as a chemoattractant, or buffer (HBSS) was added; and the chamber was incubated at 37°C in a humidified atmosphere with 5% CO_2 for 60 min. After that, the filters were removed, air dried, fixed, stained with Diff-Quick (Diagnostic Systems Inc., Gibbstown, N.J.), and mounted onto glass slides. The cells on the lower surface of the filters were counted by using a Zeiss microscope and a chemotaxis counter (Optomax System IV; ITI, Burlington Mass.). The percentage of the number of the chemoattracted pretreated compared with the number of nontreated cells was estimated for each experiment and each chemotaxis filter.

O_2^- release assay. Following treatment with antifungal compound, 10^6 PMNs were mixed with cytochrome *c* (5×10^{-5} M; Sigma Chemical Co.) and the stimulus. Three different stimuli were used in these experiments: FMLP (5×10^{-7} M), phorbol myristate acetate (PMA; 0.5 μg/ml; Sigma Chemical Co.), and opsonized zymosan A particles (approximately 2 mg/ml). FMLP and PMA were stored in concentrated form at −40°C. Zymosan A was opsonized by incubation at 37°C for 30 min in the presence of 50% human AB serum (GIBCO Laboratories, Grand Island, N.Y.) and thoroughly washed with HBSS (20). The reduction of ferric to ferrous cytochrome *c* was measured as a change in absorption at a wavelength of 550 nm on a spectrophotometer (Gilford 260; Ciba Corning Diagnostics Corp., Oberlin, Ohio). The nanomoles of O_2^- produced by 10^6 PMNs in 5 min were calculated by using the extinction coefficient 29.5.

Phagocytosis and intracellular killing of *C. albicans* by PMNs. Equal numbers (10^6) of drug-pretreated or buffer-treated PMNs and *C. albicans* blastoconidia were mixed in a 1-ml final volume (HBSS) together with 10% human AB serum and incubated for 15 min at 37°C on a shaker. At the end of this incubation, 200 μl of the reaction volume was removed; and cytospins were prepared, fixed, and stained by Diff-Quick. The proportion of PMNs containing blastoconidia after 100 PMNs were counted gave the percent phagocytosis, whereas the average number of blastoconidia in each phagocytosing cell gave the phagocytic index (PI). The incubation at 37°C continued for another 45 min (total of 1 h). The intracellular killing of the blastoconidia was tested by the methylene blue staining by method described by Lehrer and Cline (16). Methylene blue was added at a concentration of 0.01%, and the cells were left at 37°C for an additional 3 min. Wet mounts were prepared, and the number of methylene blue-stained (dead) versus unstained (alive) intracellular blastoconidia was counted (a total of 300 blastoconidia). The intracellular killing (IK) was estimated by the following formula: IK (%) = (stained [dead] blastoconidia/stained + unstained [alive] blastoconidia) × 100.

Statistics. Trends in the observed effects with increasing concentrations of drugs were analyzed by using the method of Tukey et al. (25).

RESULTS

Four different healthy volunteers served as PMN donors for testing the effect of each antifungal drug on the PMN function, and the results are expressed as the mean ± standard error (SE).

TABLE 1. Effects of the antifungal drugs on chemotaxis of human PMNs to FMLP

Drug concn (μg/ml)	% Migrated PMNs (mean ± SE) with[a]:				
	AmB	5-FC	Ketoconazole	Fluconazole	Cilofungin
0.01			106 ± 14		
0.1	103 ± 11		105 ± 4		112 ± 15
1.0	74 ± 6		101 ± 3	92 ± 5	142 ± 31
2.0	71 ± 11				
5.0	49 ± 7[b]	106 ± 11	134 ± 6[b]	83 ± 13	
10.0	25 ± 5[b]	91 ± 7	130 ± 11[b]	79 ± 7	143 ± 47
20.0	—[c]			121 ± 19	
50.0		109 ± 6		110 ± 17	
100.0		92 ± 16			152 ± 53
500.0		104 ± 5			

[a] The results are expressed as percentages of migrated PMNs treated with various concentrations of the drugs compared with untreated control cells. Shown are results of three experiments.
[b] $P < 0.001$.
[c] At 20 μg/ml, AmB was lethal to PMNs and thus prevented functional assays at this concentration.

Viability of cells. The viability of the PMNs tested by trypan blue exclusion was >95% for all the drugs in the range of concentrations used. AmB was toxic for the PMNs at concentrations higher than 10 μg/ml. Because of this toxicity, it could not be used for functional assays at concentrations higher than 10 μg/ml.

Chemotaxis. We examined the effects of the compounds on PMN chemotaxis by using 10^{-8} M FMLP as a chemoattractant. The results of these experiments are shown in Table 1. AmB had an adverse effect at the higher concentrations (≥5 μg/ml; $P < 0.001$). By contrast, in all the donors ketoconazole significantly enhanced chemotaxis at concentrations of ≥5 μg/ml ($P < 0.001$). 5-FC, fluconazole, and cilofungin had no consistent effect on chemotaxis. Because of the adverse effect of the solvent Emulphor on the adherence of the cells on the filter surface, chemotaxis experiments with Sch-39304 were not performed.

Phagocytosis of *C. albicans* blastoconidia. The phagocytic activity of PMNs was tested after pretreatment with various concentrations of the antifungal drugs and was compared with that after control buffer pretreatment. The results of the percent phagocytosis and the PI are shown in Table 2. AmB decreased the percent phagocytosis ($P < 0.001$) but not the PI at concentrations of >5 μg/ml. Ketoconazole did not affect either percent phagocytosis or PI at concentrations of <10 μg/ml. 5-FC decreased only the percent phagocytosis at the higher concentrations (≥100 μg/ml) and in the PMNs of only two of four donors. However, the trend was not statistically significant. The same was true for fluconazole; the decrease of both the percent phagocytosis and the PI at higher concentrations was not statistically significant (P value for PI was 0.056). Sch-39304 decreased percent phagocytosis and PI within the range of 1 to 10 μg/ml (both statistically significant at $P < 0.05$); however, the same effect was induced by the solvent of the drug (lactated Emulphor). Cilofungin did not have any adverse effect on either percent phagocytosis or PI.

O_2^- generation. The tripeptide FMLP, PMA, and opsonized zymosan A were used to stimulate the PMNs for O_2^- production in order to study three different mechanisms of cellular activation. Table 3 shows the results of experiments when FMLP, at a concentration of 5×10^{-7} M, was used as a stimulus of the PMNs. AmB showed a decrease of O_2^- production at concentrations of >5 μg/ml ($P < 0.001$) but not at concentrations in the lower therapeutic range. Ketoconazole, 5-FC, fluconazole, and cilofungin did not show significant effects on O_2^- production, although there were trends of enhancement in some of them.

Both Sch-39304 and the solvent itself (lactated Emulphor) consistently showed marked (almost fivefold) enhancement of the O_2^- production at the lower concentrations ($P < 0.05$). In more detailed experiments, the peak of the effect was found at 0.5 μg/ml, and it was due to Emulphor only. The effects of the drugs on the PMA-stimulated or opsonized zymosan A-stimulated PMNs were similar to those of FMLP. However, Sch-39304 did not show enhancement of O_2^- production with PMA or opsonized zymosan A.

Intracellular killing of *C. albicans* blastoconidia. Table 4 shows the effects of the compounds on the fungicidal function of PMNs, as tested by incubation of equal numbers of PMNs and blastoconidia at 37°C for 1 h together with 10% human AB serum. With the exception of AmB and cilofungin, none of the antifungal agents had a fungicidal effect on the *C. albicans* strain used in these experiments, in the

TABLE 2. Effects of the antifungal drugs on phagocytosis of *C. albicans* blastoconidia by human PMNs[a]

Drug concn (μg/ml)	AmB		5-FC		Ketoconazole		Fluconazole		Sch-39304		Cilofungin	
	%[b]	PI[c]	%	PI	%	PI	%	PI	%	PI	%	PI
0	75 ± 2	2.1 ± 0.2	74 ± 6	2.0 ± 0.2	58 ± 6.4	1.8 ± 0.1	67 ± 8	2.1 ± 0.2	69 ± 4	1.9 ± 0.1	63 ± 7	1.7 ± 0.1
0.01					55 ± 11.6	1.7 ± 0.01						
0.1	70 ± 7	2.1 ± 3			60.3 ± 9.5	1.6 ± 0.1					67 ± 5	1.7 ± 0.1
1.0	71 ± 5	2.1 ± 0.2			56.3 ± 10.1	1.6 ± 0.1	58 ± 8	2.0 ± 0.2	55 ± 4	1.5 ± 0.1	61 ± 6	1.7 ± 0.1
2.0	69 ± 9	2.1 ± 0.3										
5.0	64 ± 12	2.2 ± 0.4	73 ± 9	1.7 ± 0.3	58 ± 10	1.7 ± 0.1	61 ± 6	1.9 ± 0.5	46 ± 5[d]	1.5 ± 0.1[d]		
10.0	48 ± 9[e]	1.8 ± 0.2	66 ± 9	1.8 ± 0.4	45.3 ± 7.9	1.6 ± 0.2	59 ± 8	2.0 ± 0.3	48 ± 8[d]	1.5 ± 0.1[d]	68 ± 8	1.7 ± 0.1
20.0	—[f]						53 ± 11	1.7 ± 0.2				
25.0									59 ± 6	1.6 ± 0.1		
50.0			65 ± 9	1.9 ± 0.3			52 ± 12	1.5 ± 0.2				
100.0			60 ± 11	1.7 ± 0.3							66 ± 4	1.6 ± 0.04
500.0			60 ± 16	1.8 ± 0.5								

[a] The results are expressed as the means of four experiments ± SE.
[b] Percent phagocytosis [(number of PMNs with blastoconidia/total number of PMNs counted) × 100].
[c] PI is total number of blastoconidia phagocytosed/number of phagocytosing PMNs.
[d] $P < 0.05$.
[e] $P < 0.001$.
[f] At 20 μg/ml, AmB was lethal to PMNs and thus prevented functional assays at this concentration.

TABLE 3. Effects of the antifungal drugs on O_2^- generation by human PMNs on stimulation with FMLP[a]

Drug concn (μg/ml)	nmol of O_2^- produced (mean ± SE) with[a]:					
	AmB	5-FC	Ketoconazole	Fluconazole	Sch-39304	Cilofungin
0	2.27 ± 0.6	1.7 ± 0.1	2.5 ± 0.4	2.1 ± 0.4	1.7 ± 0.3	1.6 ± 0.4
0.01			2.5 ± 0.5			
0.1	2.4 ± 0.7		2.3 ± 0.6			1.6 ± 0.4
1.0	2.7 ± 0.7		2.5 ± 0.5	1.9 ± 0.4	7.8 ± 2.7[b]	1.4 ± 0.4
5.0	2.4 ± 1.2	1.8 ± 0.3	3.6 ± 1.0	2.6 ± 0.8	6.5 ± 2.6[b]	
10.0	0.3 ± 0.2[c]	2.4 ± 0.7	4.3 ± 1.2	2.5 ± 0.6	3.5 ± 1.3	0.9 ± 0.2
20.0	—[d]			1.3 ± 0.6		
25.0					0.00[c]	
50.0		1.9 ± 0.4		1.8 ± 1.1		
100.00		1.9 ± 0.4				0.7 ± 0.5
500.00		1.4 ± 0.5				

[a] FMLP was used at 5×10^{-7} M. The results are expressed as nanomoles of O_2^- produced by 10^6 PMNs in 5 min. Shown are results of four experiments.
[b] $P < 0.05$, because of the solvent Emulphor.
[c] $P < 0.001$.
[d] At 20 μg/ml, AmB was lethal to PMNs and thus prevented functional assays at this concentration.

absence of PMNs, as evidenced by resistance to methylene blue staining and by growth of *Candida* colonies on Sabouraud agar plates.

AmB enhanced the intracellular killing of the blastoconidia above the base-line activity of 18.7 ± 2.3%. However, this was not statistically significant because of large variation among the donors. By comparison, ketoconazole had a positive effect on intracellular killing of the blastoconidia at the concentrations tested ($P < 0.05$). 5-FC, fluconazole, and cilofungin did not show any statistically significant effect. The compound Sch-39304 enhanced intracellular killing at relatively low therapeutic concentrations (from 1 to 5 μg/ml; $P < 0.001$). However, Sch-39304 and cilofungin, when they reached very high concentrations (25 and 100 μg/ml, respectively), had an adverse effect on intracellular killing of *C. albicans* without reducing PMN viability.

DISCUSSION

Our findings in the present study and those of previous investigators studying the effects of antifungal agents on PMN function are summarized in Table 5. Although a number of investigators have previously reported on the effects of selected antifungal agents, especially AmB, on PMN function, the present study is the most comprehensive investigation of the effects of established and investigational antifungal compounds on four principal PMN functions.

Among the systemically acting antifungal compounds, AmB has been studied most extensively. Most investigators have shown that AmB inhibits chemotaxis (5, 19; F. Bernaudin, J. Hathorn, R. Schaufele, and P. A. Pizzo, 26th ICAAC, abstr. no. 829, 1986) and phagocytosis (>10 μg/ml) but not killing of *C. albicans* (7, 15), whereas others have found that both phagocytosis and intracellular killing are reduced at >10 μg/ml (Table 5). The depressive effect on phagocytosis may be due to the binding of AmB to sterols of the PMN and concomitant cell membrane injury. The same action could be responsible for the depressed O_2^- generation and chemiluminescence found in this and other studies (2, 5, 19).

Our finding of a moderate, although not statistically significant, enhancement of intracellular killing by AmB may be due to its immunoadjuvant properties at therapeutic concentrations. AmB administered as a single 10-mg/kg dose to mice was protective against subsequent challenge with either *C. albicans* or *Staphylococcus aureus*. Effector cells from treated mice showed enhanced in vitro fungicidal activity in a ^{51}Cr-release assay (4). Macrophages from intact mice

TABLE 4. Effects of the antifungal drugs on intracellular killing of *C. albicans* blastoconidia by human PMNs

Drug concn (μg/ml)	% of killing (mean ± SE) with[a]:					
	AmB	5-FC	Ketoconazole	Fluconazole	Sch-39304	Cilofungin
0	18.7 ± 2.3	15.5 ± 1.5	14.3 ± 2.5	15.3 ± 0.9	13.0 ± 1	19.0 ± 4.0
0.01			13.8 ± 1.8			
0.1	24.5 ± 1.5		13.8 ± 2.4			14.5 ± 0.5
1.0	21.0 ± 7.5		18.3 ± 1.5	16.3 ± 2.4	27.5 ± 5.5[b]	17.0 ± 4.0
2.0	19.7 ± 5.5					
5.0	22.7 ± 7.5	15.8 ± 1.8	20.8 ± 2.3[c]	18.5 ± 2.5	21.0 ± 2.0[b]	
10.0	29.7 ± 9.9	15.0 ± 1.6	17.0 ± 1.1	19.7 ± 3.2	16.0 ± 4.0	18.0 ± 2.0
20.0	—[d]					
25.0					3.0 ± 0.01[b]	
50.0		18.5 ± 2.0		17.7 ± 3.9		
100.0		16.0 ± 2.0				8.0 ± 2.0[b]
500.0		11.0 ± 1.5				

[a] Expressed as the (number of methylene blue-stained blastoconidia divided by the total number of counted blastoconidia [stained + unstained]) × 100. Shown are results of four experiments.
[b] $P < 0.001$.
[c] $P < 0.05$.
[d] At 20 μg/ml, AmB was lethal to PMNs and thus prevented functional assays at this concentration.

TABLE 5. Effects of antifungal drugs on PMN function summarized from results of the present study and previous studies[a]

Drug	Reference	Chemotaxis	O_2^- release	Chemiluminescence	Phagocytosis	Fungicidal or bactericidal activity	Comment
Fungizone	This study	D (≥5)[b]	D (>5)		D (>5)[b]	N or I	
	2			D (>1.25)			Mouse spleen cells
	5	D (2)		D (5–20)	D (≥20); *C. albicans*	D (≥20); *C. albicans*	
	19	D (1–10)		D	D; *S. aureus*	D or N; *S. aureus*	
	24			I (5–100)			
	Bernaudin et al.[c]	D (>1)					
	Van der Auvera et al.[d]	D (2.1–3.8)	D (0.5–2)		D (>10); *S. aureus*, *C. albicans*	D (>10); *S. aureus*, *C. albicans*	
Pure AmB	7, 15				D (1); *C. albicans*	N; *C. albicans*	
	28	D	D (≥4.65)				
5-FC	This study; 5	N	N		N	N	
	2			N (<20)			
	10	N					Chemoattractant: suspension of *C. albicans*
Ketoconazole	This study	I (≥5)	I (≥5)		N (<10)	I (1–10)[e]	
	2			D (>2.5)			Mouse spleen cells
	12, 15				N (1–6); *C. albicans*	N (1–6); *C. albicans*	
	17	N			N (1); *C. albicans*		
	19	N (1–5)	N	N; *S. aureus*	N; *S. aureus*		
	21				D (1–20)		
	23					D (5–20); *C. albicans*	
	29	I or N					Chemoattractant: mycetoma agent
Fluconazole	This study	N	N		N	N	
	1			N (<20)			
	23					N (5–20); *C. albicans*	
Sch-39304	This study		I (1–5)[f]	D (1–10)[e]	I (1–5)[b]		
Cilofungin	This study; Van der Auvera et al.[d]	N	N		N	N	

[a] The numbers in parentheses denote concentrations (in micrograms per milliliter) at which the effect was observed. Abbreviations: I, increase; D, decrease; N, not affected.
[b] $P < 0.001$.
[c] Bernaudin et al., 26th ICAAC.
[d] Van der Auvera et al., 28th ICAAC.
[e] $P < 0.005$.
[f] $P < 0.05$, because of the solvent Emulphor.

treated with AmB in vitro also showed enhanced fungicidal activity (4). Although we studied the intracellular killing of the phagocytosed blastoconidia by PMNs, a direct effect of the drug on the yeast extracellularly or even intracellularly cannot be excluded.

5-FC is commonly used in combination with AmB. Our findings indicated that 5-FC does not impair normal PMN function at therapeutic levels of up to 100 μg/ml in blood. These findings differ somewhat from those of Abruzzo et al. (2), who found that chemiluminescence is depressed at >20 μg/ml.

Ketoconazole did not exert an adverse effect on PMN functions in our study and in most of the previous studies (15, 17, 19, 26). Our finding of an enhancement of intracellular killing was similar to that of De Brabander et al. (9) and may have been due to the inhibition of germination by ketoconazole, with subsequent easier killing of the *Candida* cells by PMNs.

Fluconazole, a new bis-triazole, is a very promising antifungal agent with a very long half-life and the ability to penetrate well into cerebrospinal fluid (22, 27). Our findings suggest that it does not interfere with the PMN function at the therapeutically achievable levels (5 to 20 μg/ml), which concurs with the results of Senior and Shaw (23).

Sch-39304 is a new investigational triazole with potent in vivo antifungal activity (14; Walsh et al. 28th ICAAC). Sch-39304 markedly enhanced O_2^- production after stimulation of pretreated cells with FMLP at therapeutic concentrations of the compound (0.5 to 1 μg/ml). We found, however, that this enhancement of O_2^- production was due to the solvent Emulphor that was used for solubilization of the drug. O_2^- production after stimulation with PMA or

opsonized zymosan A was not enhanced. The synthetic tripeptide FMLP, a bacterial product analog, induces O_2^- generation by binding to specific surface receptors on PMNs. Phorbol esters act directly on the protein kinase C system of the cell and represent a nonspecific stimulus of oxidative metabolism of PMNs. Zymosan A consists of killed yeast cells that activates O_2^- production through attachment and ingestion. The effect of Emulphor may be due to activating FMLP receptors on the surface of the cells. Whether this activation represents an increase of the number or the affinity of the FMLP receptors needs further investigation.

Cilofungin is a novel echinocandin compound which is fungicidal against *Candida* spp. This compound specifically inhibits glucan synthetase of *Candida* spp. and does not interfere with mammalian cellular metabolism at safely achievable concentrations. That cilofungin specifically binds to glucan synthetase as its specific mechanism of action may explain its lack of adverse effects on human PMNs, which do not possess this enzyme.

In summary, these findings indicate that AmB at ≥5 μg/ml (concentrations in serum considered to have systemic toxicity) may exert deleterious effects on PMN chemotaxis, phagocytosis, and O_2^- production. However, AmB, 5-FC, ketoconazole, fluconazole, cilofungin, and Sch-39304, at concentrations that are safely achievable in serum, generally do not exert an adverse affect on PMN chemotaxis, phagocytosis, O_2^- generation, or fungicidal activity. Additional studies are required to characterize the effect of antifungal compounds on PMNs of patients receiving antifungal agents.

ACKNOWLEDGMENT

This study was supported in part by a grant from Eli Lilly & Co., Indianapolis, Ind.

LITERATURE CITED

1. **Abruzzo, G. K., R. A. Fromtling, T. A. Turnbull, and D. M. Giltinan.** 1987. Effects of bifonazole, fluconazole, itraconazole, and terbinafine on the chemiluminescence response of immune cells. J. Antimicrob. Chemother. **20**:61–68.
2. **Abruzzo, G. K., D. M. Giltinan, T. P. Capizzi, and R. A. Fromtling.** 1986. Influence of six antifungal agents on the chemiluminescence response of mouse spleen cells. Antimicrob. Agents Chemother. **29**:602–607.
3. **Anderson, R.** 1985. The effect of antibiotics and of drug associations including antibiotics on the immunodefense system. *In* M. Neuman (ed.), Useful and harmful interactions of antibiotics. CRC Press Inc., Boca Raton, Fla.
4. **Bistoni, F., A. Vecchiarelli, R. Mazzola, P. Pucceti, P. Marconi, and E. Garaci.** 1985. Immunoadjuvant activity of amphotericin B as displayed in mice infected with *Candida albicans*. Antimicrob. Agents Chemother. **27**:625–631.
5. **Bjorksten, B., C. Ray, and P. G. Quie.** 1976. Inhibition of human neutrophil chemotaxis and chemiluminescence by amphotericin B. Infect. Immun. **14**:315–317.
6. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. Suppl. **97**:77–89.
7. **Chan, C. K., and E. Balish.** 1978. Inhibition of granulocyte phagocytosis of *Candida albicans* by amphotericin B. Can. J. Microbiol. **24**:363–364.
8. **Davies, R. R., and F. Zaini.** 1985. Antifungal drugs affecting the chemotaxis of polymorphonuclear neutrophils. Sabouraudia J. Med. Vet. Mycol. **23**:119–123.
9. **De Brabander, M., F. Aerts, J. Van Cutsem, H. Van den Bossche, and M. Borgers.** 1980. The activity of ketoconazole in mixed cultures of leukocytes and *Candida albicans*. Sabouraudia **18**:197–210.
10. **Denning, T. J. V., and R. R. Davies.** 1973. *Candida albicans* and the chemotaxis of polymorphonuclear neutrophils. Sabouraudia **11**:210–221.
11. **Falk, W., R. H. Goodwin, and E. J. Leonard.** 1980. A 48-well micro-chemotaxis assembly for rapid and accurate measurement of leukocyte migration. J. Immunol. Methods **33**:239–247.
12. **Farkas, B., and A. Dobozy.** 1983. The effect of ketoconazole on the phagocytosis and intracellular killing of Candida albicans by polymorphonuclear granulocytes. Mykosen **26**:22–26.
13. **Fromtling, R. A.** 1984. Imidazoles as medically important antifungal agents: an overview. Drugs Today **20**:325–349.
14. **Gordee, R. S., D. J. Zeckner, L. F. Ellis, A. L. Thakkar, and L. C. Howard.** 1984. *In vitro* and *in vivo* anti-candida activity and toxicology of LY121019. J. Antibiot. **37**:1054–1065.
15. **Johnson, E. M., D. W. Warnock, M. D. Richardson, and C. J. Douglas.** 1986. *In vitro* effect of itraconazole, ketoconazole and amphotericin B on the phagocytic and candidacidal function of human neutrophils. J. Antimicrob. Chemother. **18**:83–91.
16. **Lehrer, R. I., and M. J. Cline.** 1969. Interaction of *Candida albicans* with human leucocytes and serum. J. Bacteriol. **98**: 996–1009.
17. **Le Moli, S., R. Seminara, R. D'Amelio, O. Pontesilli, and R. Nisini.** 1983. *In vitro* effect of ketoconazole on human neutrophil chemotaxis and *Candida albicans* killing. Boll. Ist. Sieroter. Milan **62**:531–534.
18. **Lingaas, E., and T. Mldtvedt.** 1985. Dissociated effect of amphotericin B and desoxycholate on phagocytosis of *Escherichia coli* by human polymorphonuclear neutrophils. Drugs Exp. Clin. Res. **11**:747–754.
19. **Marmer, D. J., B. T. Fields, Jr., G. L. France, and R. W. Steele.** 1981. Ketoconazole, amphotericin B, and amphotericin B methyl ester: comparative in vitro and in vivo toxicological effects on neutrophil function. Antimicrob. Agents Chemother. **20**:660–665.
20. **Metcalf, J. A., J. I. Gallin, W. M. Nauseef, and R. K. Root.** 1987. Superoxide production, p. 109–114. *In* Laboratory manual of neutrophil function. Raven Press, New York.
21. **Rowan-Kelly, B., A. Ferrante, and Y. H. Thong.** 1984. Modification of polymorphonuclear leukocyte function by imidazoles. Int. J. Immunopharmacol. **6**:389–393.
22. **Saag, M. S., and W. E. Dismukes.** 1988. Azole antifungal agents: emphasis on new triazoles. Antimicrob. Agents Chemother. **32**:1–8.
23. **Senior, D. S., and J. T. B. Shaw.** 1988. *In vitro* effects of fluconazole (UK-49,858) and ketoconazole on mouse lymphocyte proliferation and on *Candida* blastospore destruction by human polymorphonuclear leukocytes. Int. J. Immunopharmacol. **10**:169–173.
24. **Supapidhayakul, S. R., L. R. Kizlaitis, and B. R. Andersen.** 1981. Stimulation of human and canine neutrophil metabolism by amphotericin B. Antimicrob. Agents Chemother. **19**:284–289.
25. **Tukey, J. W., J. L. Ciminera, and J. F. Heyse.** 1985. Testing the statistical certainty of a response to increasing doses of a drug. Biometrics **41**:295–301.
26. **Van Rensburg, C. E. J., R. Anderson, G. Joone, M. F. Van der Merve, and H. A. Eftychis.** 1983. The effects of ketoconazole on cellular and humoral immune functions. J. Antimicrob. Chemother. **11**:49–55.
27. **Walsh, T. J., and P. A. Pizzo.** 1988. Treatment of systemic fungal infections: recent progress and current problems. Eur. J. Clin. Microbiol. **7**:460–475.
28. **Yasui, K., M. Masuda, T. Matsuoka, M. Yamazaki, A. Komiyama, T. Akabane, and K. Murata.** 1988. Miconazole and amphotericin B alter polymorphonuclear leukocyte functions and membrane fluidity in similar fashions. Antimicrob. Agents Chemother. **32**:1864–1868.
29. **Yousif, M. A., and R. J. Hay.** 1987. Leucocyte chemotaxis to mycetoma agents—the effect of the antifungal drugs griseofulvin and ketoconazole. Trans. R. Soc. Trop. Med. Hyg. **81**:319–321.