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Antagonistic Interactions between Azoles and Amphotericin B with Yeasts Depend on Azole Lipophilia for Special Test Conditions In Vitro

M. SCHEVEN[1]* AND F. SCHWEGLER[2]

Laborpraxis Dr. Reinhöfer, D-07973 Greiz,[1] and Abteilung Medizin, Pfizer GmbH, D-76139 Karlsruhe,[2] Germany

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The interactions of the azole antifungal agents fluconazole, itraconazole, ketoconazole, or miconazole with amphotericin B (AmB) in their effect on *Candida albicans* were investigated. These four azoles antagonized the fungistatic activity of AmB at sub-MICs if both substances acted simultaneously. This coincubation test was primarily developed to observe the azole-mediated demethylase inhibition quantitatively by bioassay. Interestingly, the occurrence of azole-AmB antagonism depended on azole lipophilia if specially selected test conditions were applied. By a consecutive incubation regimen, preincubation at high azole concentrations (1 to 50 μg/ml) and then subsequent incubation with AmB (1 μg/ml), only preincubation with the three lipophilic azoles decreased the fungicidal activity of AmB but not that of FCZ. It was shown that yeasts absorb only lipophilic azoles to a remarkable extent. This fact might be responsible for the absence of antagonism of FCZ to AmB when yeasts were incubated consecutively. It can be concluded with caution that consecutive treatment of candidiasis with FCZ and AmB does not necessarily result in a clinically relevant antagonism.

Alterations of the effects of amphotericin B (AmB) on pathogenic fungi by azoles were observed repeatedly in vivo as well as in vitro. The majority of reports mention antagonisms (2–4, 6, 7, 11, 12, 16, 17), but indifference (1, 10, 18) and synergism (10, 14, 18) have also been described. The results were influenced by a variety of factors, especially experimental conditions as well as the particular fungal strains or species. The fact that the results depend on the properties of antifungal agents has been described sporadically (12). At first, a postexposure antagonism of azoles to AmB was assumed. In experiments dealing with this question, it was found that lipophilic azoles are able to influence the fungicidal activity of subsequently applied AmB on yeasts but not that of fluconazole (FCZ). To detect this behavior of lipophilic azoles, special test conditions (unusually high drug concentrations as well as a high density of organisms) had to be selected. Decreasing both inoculum size and antifungal concentration resulted in a failure to detect an influence of azoles on the fungicidal activity of AmB. For this aim of the investigation, the test conditions had to be very different from those that work with drug concentrations similar to those found in vivo, that is, sub-MICs, to observe postantifungal effects (9) with possible clinical significance.

Additional tests that were performed in order to explain the dependence of the long-lasting effects of azoles on their lipophilicities also required large quantities of test organisms.

The results presented in this report focus on a further aspect of the azole-AmB antagonism. The antagonism between azoles and AmB has been explained by the inhibition of ergosterol synthesis and then a disappearance of ergosterol from the cell membrane (12, 13, 17, 19). The affinity of AmB for ergosterol precursors is not as high as that for ergosterol. This mode of antagonism has been confirmed experimentally (12, 17). Therefore, it should be possible to detect indirectly the α-demethylase inhibition due to azoles by measuring this antagonism. For this reason, a bioassay (coincubation test) was developed. The test measured the interaction of FCZ, itraconazole (ICZ), ketoconazole (KCZ), or miconazole (MCZ) with AmB in an azole concentration-dependent manner.

Another hypothesis regarding azole-polyene antagonism postulates a competitive binding of both AmB and azoles to common ligands in the fungal cell membrane (6, 11, 14).

Because of the obvious relevance of such a hypothesis regarding the authenticity of the coincubation test, the results of experiments dealing with this question are reported here.

MATERIALS AND METHODS

FCZ was kindly provided by Pfizer Inc., Karlsruhe, Germany. ICZ (product no. 30.211.44) and KCZ (batch no. G 3051) were kind gifts from Janssen Inc., Neuss, Germany. MCZ (Daktar) and AmB (Fungizone) were used in the commercial form as products from Janssen Inc. and Bristol-Myers-Squibb Inc., Munich, Germany, respectively.

Stock solutions. Ten grams of each of ICZ and KCZ was dissolved in 1 liter of dimethyl sulfoxide (DMSO; Vogler, Rotterdam, Holland). AmB (Fungizone; 50 mg) was dissolved in sterile deionized water, and FCZ was dissolved in sterile physiological saline. The final concentrations were 1,280 and 2,000 μg/ml, respectively. Stock solutions of ICZ and KCZ were stored at room temperature, and FCZ and MCZ were stored at 4°C. The AmB stock solution was stored in aliquots of 1 ml at −20°C.

Test medium. The test medium was a solution of RPMI 1640 medium (R 8755; Sigma, St. Louis, Mo.) in deionized water supplemented with 2% (wt/vol) 3-*N*-morpholino-*o*-propanesulfonic acid (MOPS; Sigma) and 1% (wt/vol) glucose and was adjusted to pH 7.0. Further preparation of the liquid medium was done according to the recommendations of the producer.

For diffusion testing, 2% (wt/vol) purified agar (A 11396; Serva, Heidelberg, Germany) was added. This mixture was applied to 90-mm petri dishes with a final thickness of 4.0 mm.

General methods. Serial broth dilutions were made in a microdilution version with final volumes of 400 μl (Cliniplate; EFLAB, Helsinki, Finland) or in a macrodilution assay with 5.0 ml as the final volume (glass tubes of 12 by 75 mm). After incubation, yeast growth was measured densitometrically as follows. Growth in the microdilution assay was measured with an enzyme-linked immunosorbent assay reader (SLT Instruments, Crailsheim, Germany) at a wavelength of 450 nm; growth in the macrodilution assay was measured with a photometer (SPEKOL 11; Carl-Zeiss Jena, Germany) at a wavelength of 620 nm. All measurements were made three times.

* Corresponding author. Mailing address: Laborpraxis Dr. Reinhöfer, Dr.-Wichmann-Str. 12, D-07973, Greiz, Germany. Phone: (011493661)4622420. Fax: (011493661)462424.

Azole-AmB coincubation. (i) Pretest. The MIC of AmB was determined as follows. A yeast inoculum (2.5 ml; grown on Sabouraud's agar overnight) with an optical density of 0.80 ± 0.04 (SPEKOL 11; wavelength, 620 nm, 5-cm cuvette) was added to each 2.5 ml of a serial dilution of AmB, and then the preparations were incubated for 4 h at 36°C. The MIC of AmB was defined as the lowest concentration of a serial AmB dilution which produced growth inhibition of ≥95% (see Fig. 1).

(ii) Main test. A yeast inoculum with an optical density equal to that used in the preliminary test was added to a serial dilution of an azole as described above. After a short incubation (1 to 2 h), AmB was added to reach final concentrations of 0.7 to 0.8 the MIC. This short azole preincubation resulted in a coincubation with both substances and should initialize the azole-mediated depletion of ergosterol reserves in the cell membrane. The short incubation was followed by incubation for 4 h at 36°C. At temperatures of 36°C, this short time of incubation produced curves with optical densities which varied with the azole dilutions in a sigmoidal manner, which is appropriate for determining reliable values of the azole concentrations which antagonize the activity of AmB by half (50% inhibitory concentration [IC_{50}]). Prolonged incubation resulted in inaccurate curves or required very low incubation temperatures. Finally, densitometry was performed.

Coincubation tests were also done as microdilution assays; 1% (vol/vol) pooled human serum was added to the test medium. To avoid agglutination problems, pools of sera were pretreated with killed yeast cells to remove antibodies against *Candida albicans.*

(iii) Double diffusion test. The decrease in the critical concentrations of AmB by FCZ (demonstrated by a reduction in the inhibition zone of AmB) was determined in a calibrated agar double-diffusion test. Further details will be discussed separately.

Consecutive azole-AmB incubation. In order to obtain significant effects of azole preincubation on the fungicidal activity of subsequently applied AmB, the following test conditions were selected. About 2×10^7 to 1×10^8 CFU of *C. albicans* per ml (which is necessary to obtain a sufficient number of survivors) were incubated in 5.0 ml of RPMI 1640 broth containing 50 μg of each azole per ml (the high concentration was necessary to produce a significant decrease in the fungicidal activity of AmB) with an additional azole control well. Two further azole free controls contained 0.5% (wt/vol) DMSO and 1% (vol/vol) Tween 80. Separate tests were performed with preincubation with serial dilutions of ICZ ($n = 2$) and KCZ ($n = 1$).

The incubation times with the azoles were 24 to 48 h at room temperature (this long incubation period ensured an effect on the fungicidal activity of AmB because a minimum of 6 h was required to produce this effect). Then, the yeast aliquots were washed twice by centrifugation and resuspension steps. Aliquots were drawn from resuspended final pellets to inoculate microdilution plates with a final concentration of 1 μg of AmB per ml and about 2×10^7 to 4×10^7 CFU/ml. AmB-free wells were also incubated. These blank wells were used to detect the postantibiotic effects of azoles as well as those of growth controls (determined from azole-free wells). This incubation took place at room temperature for 24 h (both yeast growth and the time-dependent loss of AmB activity increase with an increase in the incubation temperature; therefore, a low incubation temperature allowed a prolonged overnight incubation). After agitation, the contents of each well were diluted 10- and 100-fold. A total of 50 μl of these dilutions was applied to the surfaces of Sabouraud agar plates by using Drzigalski spatulas. After incubation for 24 to 48 h at 36°C, the colonies were counted.

Additional tests. As reported above, yeasts were also preincubated with the following lipophilic agents: *o*-, *m*-, and *p*-dichlorobenzene, 2,2′,4,4′-tetrachlorodiphenylene, 2-aminobenzyl-imidazole (high-pressure liquid chromatography [HPLC] standards, Amchro Inc., Sulzbach, Germany), and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (Medac Inc., Hamburg, Germany).

Azole-AmB competition assay. About 2×10^7 to 1×10^8 CFU of *C. albicans* per ml was incubated with 10 μg of AmB per ml for 15 min or with 50 μg of KCZ for 24 h. After washing the yeast aliquots three times, the final pellets were resuspended in 5.0 ml of a solution of one of the antifungal agents. In addition, yeast aliquots were incubated in solutions without antifungal agents. All specimens were stored for 6 h at room temperature. After final pelleting, the concentrations of the antifungal agents in the supernatants were determined. AmB was measured photometrically at a wavelength of 334.5 nm in 5-cm cuvettes. KCZ was determined by bioassay after boiling for 2 h in the supernatant.

Azole release from preloaded yeast cells. About 2×10^7 to 1×10^8 CFU of *C. albicans* per ml (density was determined in a Neubauer chamber) was preincubated with azoles as mentioned above and was then washed three times. The final pellets were incubated in 2 ml of broth for 24 h. Then, the amounts of the released azoles were determined by bioassay.

For this purpose, the antifungal activity in the supernatant determined by serial dilution in a respective microtiter broth assay was compared with that of a standard dilution of one of the azoles on the same plate and with the same inoculum of the test strain (*C. albicans* Leuschke) by the following method. The antifungal activity of the azole standard was determined by calculation of the IC_{50}; the antifungal activity in the supernatant was defined by the half maximal inhibiting dilution (ID_{50}). The IC_{50}s or ID_{50}s were obtained from a plot of the optical turbidities versus the serially diluted azole concentrations or versus the dilution factors, respectively. The half-maximal optical densities and, subsequently, the corresponding abscissa values $x(IC_{50}, c_0)$ and $x(ID_{50}, d_0)$ (where c_0 is one-half of the standard azole concentration and d_0 equals 1/2) were determined from the sigmoidal plots. The term α is a proportionality factor between dilution step i and the abscissa values x_i (c_i, c_0) and x_i (d_i, d_0) for IC_{50} and ID_{50}, respectively. IC_{50}s and ID_{50}s are then given by the following respective equations: $IC_{50} = c_0\, e^{-x(IC_{50},\, c0)\ln 2/\alpha}$ (equation 1), and $ID_{50} = d_0\, e^{-x(ID_{50},\, d0)}\ln 2/\alpha$ (equation 2). By definition, the ID_{50} fold dilution of the supernatant reduced its azole concentration (c) to the IC_{50} by the following equation: $c = IC_{50}/ID_{50}$ (equation 3).

By definition, there was a linearity between the azole concentrations in supernatants and reciprocals of the ID_{50}s. For quantitative determination of test sensitivity, unknown azole concentrations had to be at least twofold the IC_{50}s found in the respective standard bioassay. Repeated testing of standard preparations of the four azoles had the following IC_{50} ranges: FCZ, 0.15 to 0.20 μg/ml; ICZ, 20 to 40 ng/ml; and KCZ and MCZ 2 to 5 ng/ml. Thus, yeasts were incubated with FCZ concentrations of 1,000 μg/ml instead of the 50 μg/ml used for the lipophilic azoles to compensate for the lower sensitivity of the FCZ bioassay. The coefficients of variation with two different concentrations of FCZ in serum and urine were 10.3% (13.2 μg of FCZ per ml) and 10.2% (97 μg of FCZ per ml), respectively. HPLC determinations (done by A. Wildfeuer, Illertissen, Germany) showed good agreement with the serum FCZ levels found in this test. The azole release from cells broken by boiling in a water bath was also investigated. However, this test was limited by the thermal instability of MCZ.

TABLE 1. IC_{50}s for FCZ-dependent AmB antagonism (coincubation macrodilution assay) of the strains tested

Species	FCZ IC_{50} (μg/ml)
Candida albicans Leuschke	0.98 ± 0.07[a]
Candida albicans 1631	0.37
Candida albicans Hohendorf	0.26
Candida albicans H29	0.68
Candida albicans 7859	1.2
Candida albicans 305	0.80
Candida albicans H12	1.1
Candida albicans 7114	0.57
Candida parapsilosis 1384	1.7
Candida parapsilosis 1385	2.15
Candida parapsilosis 1569	5.9
Candida krusei 1264	~10
Candida krusei 1265	2.8
Candida krusei G	2.7
Candida krusei	4.0
Candida tropicalis G	0.80
Candida tropicalis 944	1.5
Candida melinii G	1.8
Cryptococcus neoformans I	2.8
Candida glabrata 1565	5.1

[a] Mean of four tests.

RESULTS

In coincubation tests, the effect of AmB on *C. albicans* was reduced in a concentration-dependent manner by the azoles FCZ, ICZ, KCZ, and MCZ. To quantify this antagonism, the azole concentrations which reduced the activity of about of 0.8 the MIC of AmB by 50% (IC_{50}s) were determined. For FCZ, IC_{50}s were also determined for several *Candida* species. These results are given in Table 1. Figure 1 provides the result of a preliminary experiment, and Fig. 2 provides the results of the four main AmB-FCZ coincubation tests. The antagonism disappeared at AmB concentrations exceeding 85 ng/ml, which was the approximate MIC. This behavior shows the necessity of pretests to determine the MIC of AmB. All four azoles had the same effect on *C. albicans*, but the lipophilic azoles were characterized by remarkably lower IC_{50}s (Table 2). By the quantitative agar double-diffusion assay, the critical concentration of AmB was nearly doubled by FCZ (*C. albicans* Leuschke).

By preincubation with 50 μg of FCZ per ml, the viable counts after the action of AmB, which was used at a fungicidal

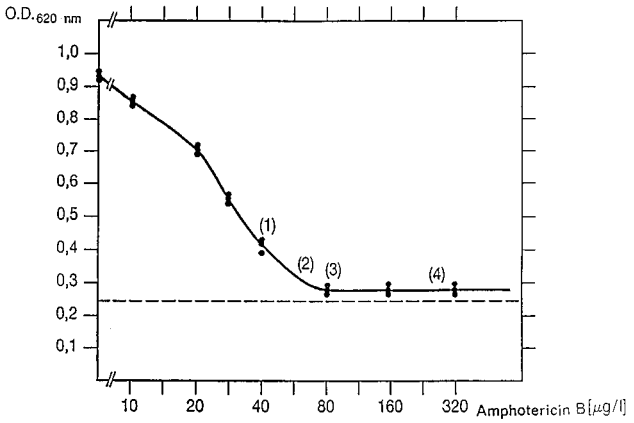

FIG. 1. Preliminary determination of the AmB MIC (macrodilution assay) in a FCZ-AmB coincubation test (with 2 h of preincubation with FCZ) with *C. albicans* Leuschke. The MIC was determined with 80 ng/ml. OD_{620}, optical density at 620 nm.

concentration of 1 μg/ml, was not remarkably altered. On average, the survival rate was enhanced about twice by FCZ (Table 3). Since it was defined for this antagonism that the survival rate should be enhanced by more than 10 times by azole preincubation, FCZ did not produce an antagonism as defined. In contrast, preincubation with the lipophilic azoles enhanced the viable counts up to 10,000 times (Table 4).

TABLE 2. MIC_{50}s and IC_{50}s of the antagonism to AmB in coincubation tests of the azoles FCZ, ICZ, KCZ, and MCZ with *C. albicans* Leuschke[a]

Compound	MIC_{50} (μg/ml)	IC_{50} (μg/ml)
FCZ	0.45	1.0
ICZ	0.125	0.3
MCZ	0.00004	0.004
KCZ	0.00125	0.00165

[a] The concentrations inhibiting 50% of strains tested (MIC_{50}) were determined by a microdilution assay with an inoculum of about 10^6 CFU/ml. Coincubation tests were done by macrodilution assay.

The effect was closely related to the azole concentration (Fig. 3). Azole concentrations producing a remarkable antagonism to the fungicidal activity of subsequently applied AmB were comparatively much higher than therapeutically relevant levels in body fluids. Additionally, the inocula necessary to produce this effect were also found to be very high (range of 2×10^7 to 2×10^8 CFU/ml). Preincubation with other lipophilic substances did not produce any comparable antagonism. This is also true for DMSO and Tween 80. Furthermore, it was shown that AmB was released from yeast cells in the presence of 50 μg of KCZ per ml at amounts clearly less than those

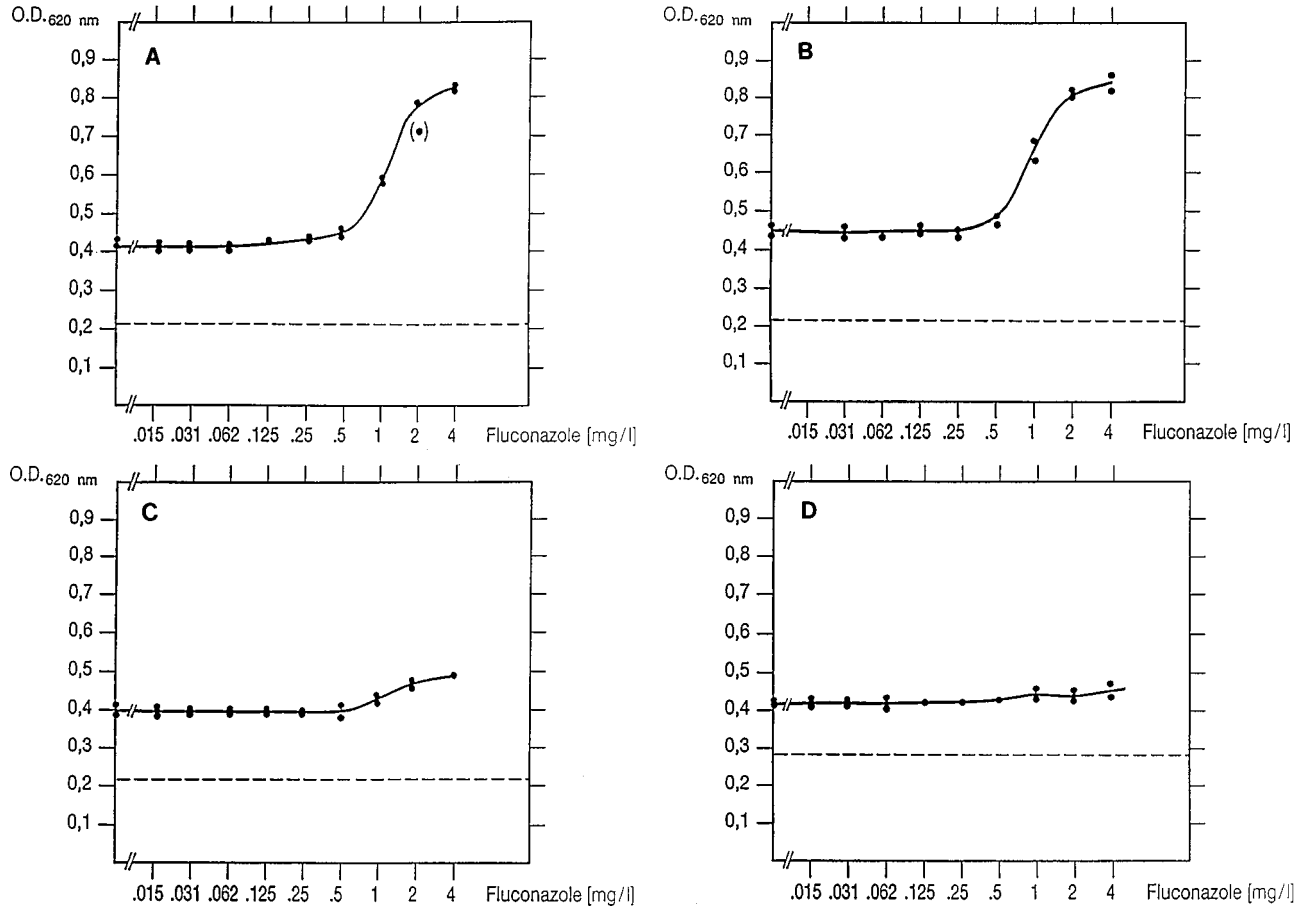

FIG. 2. Results of four main tests of FCZ-AmB coincubation (*C. albicans* Leuschke). Each test was performed with a different concentration of AMB: 42 ng/ml (A), 63 ng/ml (B), 85 ng/ml (C), and 250 ng/ml (D). With 250 ng of AMB per ml, no antagonism was seen. OD_{620}, optical density at 620 nm.

TABLE 3. Rates of survival of yeast cells after incubation with AmB and previously treated with 50 μg of four azoles per ml[a]

Compound	CFU of survivors per ml
Control	600 ± 280
FCZ	1.100 ± 280
ITZ	42.000 ± 10.600
KCZ	420.000 ± 113.000
MCZ	625.000 ± 71.00

[a] These rates are an example of the antagonizing effects of high concentrations of the lipophilic azoles against the fungicidal activity of AmB (1 μg/ml). In the incubation with AmB, the yeast strain *C. albicans* Leuschke was used at an inoculum into AmB of about 2×10^7 CFU/ml. Values are medians ± standard deviations.

released into wells containing saline. Analogous results were obtained if KCZ was released in the presence of AmB. Therefore, competition between AmB and KCZ can be excluded. More AmB was released in wells containing ICZ or MCZ than in blank wells, but the same results were found with blank wells containing 1% Tween 80. Therefore, such tests requiring non-

TABLE 4. Semiquantitative azole-AmB antagonism against 31 strains of *C. albicans*[a]

Strain	Antagonism of[b]:			
	MCZ	KCZ	ICZ	FCZ
B305	++	++	+	0
R	++	++	++	0
L	++	+++	+	0
541/93	+	++	0	0
376/93	++	++	++	0
541(w)/93	++	++	++	−
1157/93	++	++	++	0
1168/93	++	++	++	0
1167/93	+	0	0	0
1314/93	++	++	0	−
1320/93	0	+	0	−
1342/93	+++	++	++	0
1318(g)/93	+++	++	++	0
M1	++	++	+	0
M2	+++	++	++	0
M3	++	++	0	0
1295/93	++++	+++	++++	+
1294/93	+	+	+	0
1316/93	+	+++	+++	0
1476/93	+++	++	+++	0
1532/93	+	++	+	−
1536/93	+	+	+	0
1560/93	++	++	++	0
1569/93	0	0	+	0
1544/93	++	++	++	+
1554/93	+	+	++	0
1661/93	+	++++	+++	0
1658/93	0	+++	++	0
1668/93	+++	++	++	0
1669/93	+	++	0	0
1649/93	++++	0	+++	−

[a] Data were obtained from consecutive incubations. +, antagonism; 0, indifference; −, synergism. The interactions are summarized as follows. Antagonism was found for 28, 28, 25, and 2 of 31 strains tested with MCZ, KCZ, ICZ, and FCZ, respectively. Indifference was found for 3, 3, 6, and 24 of 31 strains tested with MCZ, KCZ, ICZ, and FCZ, respectively. Synergism was found for 0, 0, 0, and 5 of 31 strains tested with MCZ, KCZ, ICZ, and FCZ, respectively.

[b] The number of symbols represents the order of magnitude of the alteration of survival rates compared with the for the controls.

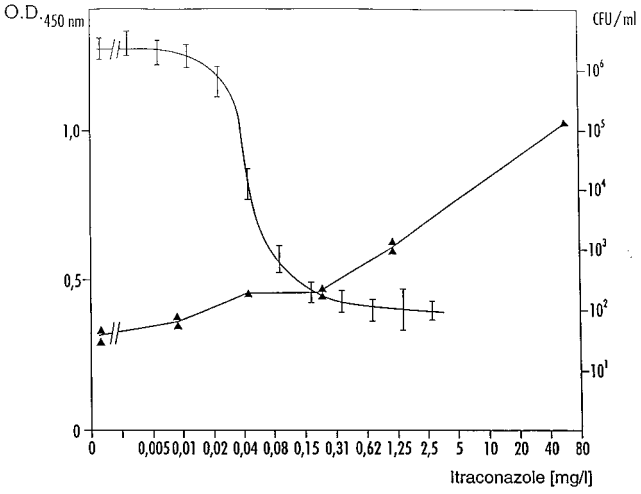

FIG. 3. Concentration-dependent antagonism of preincubation of *C. albicans* Leuschke with various concentrations of ICZ on the fungicidal action of subsequently applied AmB (1 μg/liter). The triangles represent the respective viable counts (two determinations). In parallel, the results of a microtiter susceptibility test with the same *Candida* strain is shown (inoculum density, about 10^6 CFU/ml). The vertical lines indicate the range of optical densities from three determinations for each ICZ dilution. A remarkable degree of antagonism of ICZ on the subsequent fungicidal activity of AmB did not occur at ICZ concentrations near its MIC. OD_{450}, optical density at 450 nm.

ionic detergents should be considered nonvalid with regard to the aim of this investigation.

After preincubation of three strains of *C. albicans* with the four azoles, washing and subsequent incubation in 2 ml of broth (whole or broken cells), the release of ICZ, KCZ, and MCZ (MCZ release was at room temperature) was demonstrated by bioassay, but release was not demonstrated for FCZ.

This was also true with enhancement of the FCZ concentration up to 1,000 μg/ml. Removal of the cell membranes with methanol solutions buffered with phosphate-buffered saline resulted in a strong increase in the cellular storage capacities of the lipophilic azoles, but FCZ again was not bound at detectable amounts. The total amounts of lipophilic azoles released after loading the yeast cells with a solution containing 50 μg of each azole per ml were about 100 to 400 ng of KCZ per 10^8 CFU, 20 to 50 ng of MCZ per 10^8 CFU, and 10 to 30 ng of ICZ per 10^8 CFU.

DISCUSSION

The azole-AmB antagonism is assumed to be caused by the inhibition of ergosterol synthesis due to azoles. Some preliminary reports dealing with this mode of action used IC_{50}s of the incorporation of [^{14}C]acetate into whole cells (or microsomes) as a measure of the extent of demethylase inhibition. A median IC_{50} of 0.28 ± 0.1 μg of FCZ per ml for [^{14}C]acetate incorporation into six *C. albicans* strains (whole cells) was reported (14). As for coincubation (with short azole preincubation), IC_{50}s of 0.2 to 0.5 μg of FCZ per ml were found (microdilution assay). Except for ICZ, the IC_{50}s of the lipophilic azoles for antagonism of AmB were at least two decade orders of magnitude lower than those of FCZ.

These findings correspond to those from reports that lipophilic azoles produce comparably lower IC_{50}s for [^{14}C]acetate incorporation (14). This parallelism is not accidental but suggests the dependence of the antagonism on ergosterol synthesis inhibition.

Enhancement of the critical AmB concentration found by

quantitative agar double-diffusion assay agrees with other reports. Duplication of the AmB MIC by clotrimazole (6) and enhancement of the fractional inhibition coefficients of AmB by FCZ by two to four by the checkerboard agar dilution method have been reported (11).

Furthermore, it was demonstrated that relatively high concentrations of the lipophilic azoles ICZ, KCZ, and MCZ were able to reduce the fungicidal activity of subsequently applied AmB. In the case of FCZ, no significant antagonism (subsequent AmB fungicidal activity) occurred. This effect was only observed if the azole preincubation period was at least 6 h (data not shown). However, by using a preincubation period of 2 h, additive effects were seen. It was found that the azole-induced depletion of the ergosterol reserves in the membrane requires at least 1 h and that the complete exchange of ergosterol by its methylated precursors under the influence of azoles occur after about 6 h of exposure (6a). The latency of azole antagonism to the subsequent fungicidal action of AmB might relate to the latencies of these major sterol changes. In coincubation tests, the formation of these changes also occurred since they worked with sub-MICs of AmB with an azole exposure period of 5 to 6 h.

There is a parallel effect of azole antagonism to the subsequent fungicidal activity of AmB. KCZ, MCZ, and, to a lesser extent, ICZ produced a long-lasting postantifungal effect (9) which was never seen for FCZ (60 strains were examined [data not shown]). A short-lasting postantifungal effect of FCZ of 0.5 to 2 h has been described (9). In contrast, we observed post-antibiotic inhibition effects of up to 100 h because of the influence of MCZ or KCZ (both at 5 to 50 μg/ml; the extent was concentration dependent). Intercalculations of KCZ and MCZ into the cell membrane have been reported (19). Furthermore, involvement of these effects on antifungal activity was suggested (13, 19). FCZ cannot exert such effects. Therefore, long-lasting postantifungal effects and antagonism to subsequently acting AmB could depend on such a competitive (4, 6, 11) membrane interaction. However, the finding that KCZ and AmB impair each other's release from yeasts contradicts this hypothesis. Because of the lack of antagonizing effects of lipophilic agents other than azoles on the fungicidal activity of subsequently applied AmB, the dependence of the antagonism on azole moieties is evident, and the hypothesis involving the membrane is again contradicted. Therefore, the parallelism between a postantifungal effect and the antagonism of lipophilic azoles to subsequently acting AmB should have another cause. In azole release tests, removal of azoles from previously loaded yeast cells was detected only when lipophilic azoles were used. Additionally, the barrier function of the cell membrane found in experiments with FCZ (8, 14, 15) was not responsible for the nondetectable binding of FCZ to yeast cells, as shown in experiments with methanol-treated cells. It can therefore be assumed that this effect is due to an affinity of FCZ to cellular ligands that is comparably weaker than the affinities of the lipophilic azoles. These bonds suggest nonspecific noncovalent Van der Waals types of bonds.

The lack of an influence of FCZ on the fungicidal action of AmB, which was determined in consecutive tests, may be of clinical importance. According to the data presented here, consecutive treatment with AmB following initial FCZ therapy without a loss of activity should be possible. This assumption has been confirmed in a recent clinical report (5). Whether this is also true for the other substances tested must be clarified in further studies.

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