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Relationship between In Vitro Activities of Amphotericin B and Flucytosine and pH for Clinical Yeast and Mold Isolates

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In this study, we investigated the pH dependency of the in vitro activities of amphotericin B (AMB) and flucytosine (5FC) against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Rhizopus* spp., and *Scedosporium prolificans* in RPMI 1640 buffered with citrate buffer (pH 4.0, 5.0, 5.4, and 6.0), citrate-phosphate buffer (pH 5.4, 6.0, 6.4, and 7.0), and 3-[*N*-morpholino]propanesulfonic acid (MOPS) (pH 6.4, 7.0, 7.4, and 7.9). For 5FC, no significant differences were found between MICs obtained with the different buffers, while for AMB, significant differences were found. The MICs obtained with citrate-phosphate buffer were approximately 1 twofold-dilution step higher than the MICs obtained with MOPS. We demonstrated that the in vitro activities of AMB and 5FC against yeast and mold isolates were pH dependent. The in vitro activity of AMB decreased when the pH was lowered, while the in vitro activity of 5FC increased. The effect of the pH on the in vitro activities was dependent not only on the antifungal agent tested but also on the microorganism. For AMB, there was a nonlinear relationship (median r^2, 0.864) for *Candida* spp., *C. neoformans*, *A. fumigatus*, and *Rhizopus* spp. over the pH range tested. The mean MICs ranged from 0.5 to 2.52 μg/ml at pH 7.0 and from 20.16 to 32 μg/ml at pH 5.0. For *S. prolificans*, there was no relationship. For 5FC, there was a linear relationship for *Candida* spp. (median r^2, 0.767) and a nonlinear relationship for *C. neoformans* and *A. fumigatus* (median r^2, 0.882) over the pH range tested. The mean MIC values ranged from 0.125 to 1,024 μg/ml at pH 7.0 and from 0.02 to 4 μg/ml at pH 5.0. For *Rhizopus* spp. and *S. prolificans*, the relationship could not be determined, since the MIC was >1,024 μg/ml over a pH range of 4.0 to 7.9.

In vitro susceptibility testing can be used to help predict outcomes in the treatment of invasive fungal infections. A good standard in vitro susceptibility test has to provide reproducible results, detect the resistance of fungi, and correlate with clinical outcome (10). There are many factors involved, which have made it difficult to develop and standardize in vitro susceptibility tests for fungi. Some of these factors are inherent to the antifungal agents, and others are related to the test conditions. Taking into account all these factors, the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) has published standard methods for the in vitro antifungal susceptibility testing of yeast (M27-A2) and of filamentous fungi (M38-A) (7, 8).

Although the standardized CLSI method has been found to give better inter- and intracenter reproducibility, in vitro antifungal susceptibility testing is still faced with several problems, such as the poor correlation of in vitro results with clinical outcomes for some organism-drug combinations. The poor correlation may be partly due to various factors related to the host, drugs, fungus, and their interactions (16), such as the recovery of granulocytes, which play roles in clinical outcome but are not taken into account in the in vitro susceptibility tests. It may also be partly due to various variables, such as pH, which may influence the activity of antifungal agents and which deviate significantly from the clinical situation for in vitro susceptibility tests (5).

Viviani et al. demonstrated that the MICs of flucytosine (5FC) against *Cryptococcus neoformans* obtained in yeast nitrogen base (YNB) buffered at pH 5.4 correlated well with clinical outcome, while the MICs obtained in YNB buffered at the standard pH value of 7.0 did not (15). We also demonstrated that the MICs of 5FC against *Aspergillus fumigatus* obtained in RPMI 1640 buffered at pH 5.0 correlated better with in vivo efficacy in a murine model of invasive aspergillosis than the MICs obtained in RPMI 1640 buffered at the standard pH value of 7.0 did (D. T. A. te Dorsthorst, J. W. Mouton, J. F. G. M. Meis, and P. E. Verweij, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-225, 2004).

In another study, we looked at the influence of pH on the in vitro activity of antifungal agents. We demonstrated that the in vitro antifungal activities of amphotericin B (AMB), itraconazole, and 5FC against *A. fumigatus*, *A. flavus*, and *A. terreus* changed at low pH (14). For both AMB and itraconazole, the in vitro activity decreased when the pH was lowered from 7.0 to 5.0, while for 5FC, the in vitro activity increased when the pH was lowered.

In this study, we investigated whether the same changes were found for the in vitro activity of AMB and 5FC against other molds (*Rhizopus* spp. and *Scedosporium prolificans*) and yeasts (*Candida* spp. and *C. neoformans*) when the pH was lowered from 7.0 to 5.0. In addition, we studied the changes in in vitro activity over a pH range of 4.0 to 7.9 to more precisely determine pH dependency.

(Results of this investigation were partly presented at the

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MATERIALS AND METHODS

Isolates. Four clinical yeast isolates (two *Candida krusei* and two *C. neoformans* isolates), six clinical mold isolates (two *A. fumigatus*, one *Rhizopus microsporus* var. *rhizopodiformis*, one *Rhizopus oryzae*, and two *S. prolificans* isolates), and two control strains (*Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258) were tested. The clinical isolates were obtained from the private collection of the Department of Medical Microbiology, Radboud University Nijmegen Medical Center, The Netherlands. Isolates had been frozen in glycerol broth at −80°C. Yeast isolates were revived by subculturing twice on Sabouraud dextrose agar plates supplemented with 0.5% chloramphenicol for 24 h and 48 h for the *Candida* spp. and *C. neoformans*, respectively, at 35°C. Mold isolates were revived by subculturing twice on Sabouraud dextrose agar tubes for 5 to 7 days at 35°C. All isolates were tested in triplicate on different days.

Medium. RPMI 1640 medium (with L-glutamine and without bicarbonate) (GIBCO BRL, Life Technologies, Woerden, The Netherlands) was buffered to pH 4.0, 5.0, 5.4, and 6.0 with 10 mM citrate buffer, to pH 5.4, 6.0, 6.4, and 7.0 with 10 mM citrate-phosphate buffer, and to pH 6.4, 7.0, 7.4, and 7.9 with 0.165 mol/liter 3-[*N*-morpholino]propanesulfonic acid (MOPS; Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Antifungal agents. AMB (Bristol-Myers Squibb, Woerden, The Netherlands) and 5FC (Valeant, Zoetermeer, The Netherlands) were obtained as powders. AMB was dissolved in dimethyl sulfoxide at a concentration of 3,200 μg/ml, and 5FC was dissolved in distilled water at a concentration of 6,400 μg/ml to prepare stock solutions. The stock solutions were held at −80°C until use.

Susceptibility testing. MICs were determined by a broth microdilution method according to CLSI guidelines (M27-A2 and M-38A) (7, 8), with the exception that other buffers were used where necessary (see "Medium" above). Briefly, serial twofold dilutions of the antifungal agents were prepared in RPMI 1640 medium. The final concentrations of the antifungal agents ranged from 0.001 to 256 μg/ml for AMB and from 0.001 to 1,024 μg/ml for 5FC. Aliquots of 100 μl of the drugs at concentrations of two times the targeted final concentrations were dispensed in the wells of flat-bottomed 96-well microtiter plates (Costar, Corning, NY).

Cell and spore suspensions were prepared spectrophotometrically and were further diluted in RPMI 1640 medium. In order to obtain final inoculum concentrations of 5.0×10^2 to 2.5×10^3 cells/ml for the yeast isolates and 0.4×10^4 to 5×10^4 conidia/ml for the mold isolates, 100-μl portions of the inocula were added to the wells. The microtiter plates were incubated at 35°C for 72 h.

After 24 h (*Rhizopus* spp.), 48 h (*Candida* spp. and *A. fumigatus*), and 72 h (*C. neoformans* and *S. prolificans*), growth was assessed spectrophotometrically at 405 nm with a microplate reader (Anthos htIII; Anthos Labtec Instruments, Salzburg, Austria). The optical densities (ODs) of the blanks, which consisted of uninoculated plates incubated together with the inoculated plates, were subtracted from the ODs of the inoculated plates. The percentage of growth for each well was calculated by comparing the OD of the well with that of the drug-free control based on the following equation: (OD of drug-containing well/OD of the drug-free well) × 100%. For AMB, the lowest concentration that showed no more than 10% of growth in comparison to the growth control (MIC-0) (6) and, for 5FC, the lowest concentration that showed no more than 50% of growth in comparison to the growth control (MIC-2) were taken as MIC endpoints (7, 8).

Analysis of results. In order to approximate normal distributions, the drug concentrations were transformed by logarithmic transformation to $\log_2$ values, and these values were used for the analysis of the results. The high and low off-scale MICs were included by converting to the next-higher and -lower twofold drug concentrations, respectively.

(i) Effect of pH on the MICs. In order to determine whether there were changes in the in vitro activities of AMB and 5FC when the pH was lowered from 7.0 to 5.0, the difference in in vitro activities was calculated. For each isolate, the mean $\log_2$ MIC at pH 7.0 was subtracted from the mean $\log_2$ MIC at pH 5.0. The differences in in vitro activities were given in twofold-dilution steps.

In order to determine pH dependency, we studied the changes in the in vitro activities over a pH range of 4.0 to 7.9. For each isolate, the relationship between the in vitro activities of AMB and 5FC and the pH was determined by linear or nonlinear regression analysis. Nonlinear regression was done by using a four-parameter logistic model (sigmoid curve with variable slope) known as the E_{max} model. The 50% effective concentration (EC_{50}) [pH value that gives the MIC equaling the maximal MIC − 0.5 × (the maximal MIC − the minimal MIC)], the EC_{20} [pH value that gives the MIC equaling the maximal MIC − 0.8 × (the maximal MIC − the minimal MIC)], and the EC_{80} [pH value that gives the MIC equaling the maximal MIC − 0.2 × (the maximal MIC − the minimal MIC)] were calculated. For both linear and nonlinear regressions, the goodness of fit was checked by the r^2 values and residuals. Analysis was carried out using the GraphPad Prism Software (San Diego, Calif.).

(ii) Effect of buffer on the MIC. In order to determine whether there were statistically significant differences between MICs obtained with the different buffers, the $\log_2$ MICs of AMB and 5FC obtained with citrate buffer at pH 5.4 and 6.0 were compared with the $\log_2$ MICs obtained with citrate-phosphate buffer at pH 5.4 and 6.0. Also, the $\log_2$ MICs obtained with citrate-phosphate buffer at pH 6.4 and 7.0 were compared with the $\log_2$ MICs obtained with MOPS at pH 6.4 and 7.0. Results were analyzed by the Mann-Whitney U test (AMB) or the unpaired *t* test (5FC). Statistical significance was defined as $P < 0.01$.

RESULTS

Almost all isolates grew well in RPMI 1640 buffered to pHs ranging from 4.0 to 7.9 after 24 to 72 h of incubation at 35°C. With the exception of the two *C. neoformans* isolates, the OD values of the growth controls of the other isolates (after subtraction of the OD values of the blanks) were higher than 0.1.

The MICs of AMB and 5FC found at pH 7.0 for the two control strains were within the limits recommended by the CLSI (7, 8).

Effect of pH on the MICs. In general, the MIC of AMB increased when the pH was lowered, while the MIC of 5FC decreased when the pH was lowered.

Table 1 shows the susceptibilities of the 12 isolates to AMB and 5FC determined at pH 7.0 (buffered with MOPS) and at pH 5.0 (buffered with citrate buffer). For the *Candida* sp., *C. neoformans*, *A. fumigatus*, and *Rhizopus* sp. isolates, the mean MICs of AMB increased by 3 to 6 twofold-dilution steps when the pH was lowered from 7.0 to 5.0, while for the *S. prolificans* isolates, the mean MIC decreased with 2 twofold-dilution steps when the pH was lowered. The relationships between the in vitro activities of AMB and the pH over a range of 4.0 to 7.9 for the 12 isolates were determined by regression analysis (see Table 2). For the *Candida* sp. isolates, *C. neoformans* AZN 467, the *A. fumigatus* isolates, and *R. microsporus* var. *rhizopodiformis* AZN 1185, nonlinear relationships were found. The E_{max} model fitted the data well, since the r^2 values ranged from 0.745 to 0.918 (median, 0.864). For *C. neoformans* AZN 9019 and *R. oryzae* AZN 6373, there were also relationships between the in vitro activities and the pH, but this could not be confirmed by regression analysis. For the *S. prolificans* isolates, no relationship was found. Figure 1 shows the relationships between the in vitro activities of AMB and the pH over a range of 4.0 to 7.9 for *C. neoformans* AZN 467, *A. fumigatus* AZN 8196, and *S. prolificans* AZN 7901.

For the *Candida* sp. isolates and *C. neoformans* AZN 467, the mean MICs of 5FC decreased with 3 to 5 twofold-dilution steps, and for the *A. fumigatus* isolates, the mean MIC decreased with 11 twofold-dilution steps when the pH was lowered from 7.0 to 5.0, while for *C. neoformans* AZN 9019, the mean MICs were almost identical at pH 7.0 and at pH 5.0. For the *Rhizopus* sp. and *S. prolificans* isolates, the difference could not be determined, because high off-scale MICs (mean MIC > 1,024 μg/ml) were found at both pH 7.0 and pH 5.0. The relationships between the in vitro activities of 5FC and the pH over a range of 4.0 to 7.9 for the 12 isolates were also determined by regression analysis (see Table 2). For the *Candida* sp. isolates, a linear relationship was found. The model fitted the data well, since the r^2 values ranged from 0.681 to 0.906 (median, 0.767). The slopes ranged from 1.08 to 2.30 (median, 1.91) and significantly deviated from zero ($P < 0.01$). For *C.*

TABLE 1. Susceptibilities of six yeast and six mold isolates to AMB and 5FC determined in RPMI 1640 buffered at pH 7.0 and 5.0[a]

Isolate	Mean (range) MIC (μg/ml)				Difference in in vitro activity found when pH was lowered from 7.0 to 5.0 (twofold dilutions)[b]	
	AMB		5FC		AMB	5FC[c]
	pH 7.0	pH 5.0	pH 7.0	pH 5.0		
C. parapsilosis ATCC 22019	0.79 (0.5–1)	32	0.125	0.02 (0.016–0.031)	5.33	−2.65
C. krusei ATCC 6258	1.26 (1–2)	25.4 (16–32)	6.35 (4–8)	0.5	4.33	−3.67
C. krusei AZN 1_31	2.52 (2–4)	25.4 (16–32)	8 (2–64)	0.79 (0.5–1)	3.33	−3.33
C. krusei AZN 2_12	2	32	4 (2–16)	0.2 (0.031–0.5)	4	−4.34
C. neoformans AZN 467	0.79 (0.5–1)	25.4 (16–32)	2	0.05 (0.031–0.125)	5	−5.34
C. neoformans AZN 9019	0.63 (0.5–1)	25.4 (16–32)	5.04 (1–32)	4	5.33	−0.33
A. fumigatus AZN 58	0.79 (0.5–1)	32	1.024	0.5 (0.25–1)	5.33	−11
A. fumigatus AZN 8196	1	32	80.53 (32–128)	0.031	5	−11.34
R. microsporus var. *rhizopodiformis* AZN 1185	0.5	20.16 (16–32)	>1,024	>1,024	5.33	ND[c]
R. oryzae AZN 6373	0.5	25.4 (16–32)	>1,024	>1,024	5.67	ND
S. prolificans AZN 7989	322.54 (256–>256)	101.59 (64–256)	>1,024	>1,024	−1.67	ND
S. prolificans AZN 7901	256 (128–>256)	64 (32–128)	>1,024	>1,024	−2	ND

[a] RPMI 1640 medium was buffered to pH 7.0 with MOPS and to pH 5.0 with 10 mM citrate buffer.
[b] The differences were calculated by subtracting the mean $\log_2$ MICs found at pH 7.0 from the mean $\log_2$ MICs found at pH 5.0.
[c] ND, not done because high off-scale MIC values (MIC >1,024 μg/ml) were found at pH 7.0 and at pH 5.0.

neoformans AZN 467 and the *A. fumigatus* isolates, nonlinear relationships were found. The E_{max} model fitted the data well, since the r^2 values ranged from 0.822 to 0.927 (median, 0.882). For *C. neoformans* AZN 9019, there was also a relationship between the in vitro activity and the pH, but this could not be confirmed by regression analysis. For the *Rhizopus* spp. and *S. prolificans* isolates, the relationships could not be determined because high off-scale MICs (MIC > 1,024 μg/ml) were found at all pH values. Figure 2 shows the relationships between the in vitro activity of 5FC and the pH over a range of 4.0 to 7.9 for *C. krusei* AZN 1_31, *C. neoformans* AZN 467, *A. fumigatus* AZN 8196, and *S. prolificans* AZN 7901.

Effect of buffer on the MIC. For AMB, there was no significant difference between MICs obtained with citrate buffer and with citrate-phosphate buffer ($P > 0.01$). The mean MICs obtained with citrate buffer and citrate-phosphate buffer were 33.82 and 32.67 μg/ml, respectively, at pH 5.4 and 22.16 and 19.43 μg/ml, respectively, at pH 6.0. There was a significant difference between the MICs obtained with citrate-phosphate buffer and those obtained with MOPS ($P < 0.01$). The MICs obtained with citrate-phosphate buffer were approximately 1 twofold-dilution step higher than the MICs found with MOPS. The mean MICs obtained with citrate-phosphate and MOPS were 7.84 and 3.41 μg/ml, respectively, at pH 6.4 and 6.68 and 2.43 μg/ml, respectively, at pH 7.0.

For 5FC, the results of the *Rhizopus* sp. and *S. prolificans* isolates were not used, because high off-scale MICs were found (MIC > 1,024 μg/ml). For the *Candida* sp., *C. neoformans*, and *A. fumigatus* isolates, there were no significant differences between MICs obtained with citrate buffer and those obtained with citrate-phosphate buffer or with citrate-phosphate buffer and with MOPS ($P > 0.01$). The mean MICs obtained with

TABLE 2. Results of regression analysis of the relationships between the MICs of AMB and 5FC and the pH over a range of 4.0 to 7.9

Isolate	Relationship between $\log_2$ MIC and pH[a]							
	AMB				5FC			
	r^2	EC_{50}	EC_{20}	EC_{80}	r^2	EC_{50}	EC_{20}	EC_{80}
C. parapsilosis ATCC 22019	0.898	6.35	6.84	5.87	0.708	L	L	L
C. krusei ATCC 6258	0.838	6.62	7.30	5.94	0.906	L	L	L
C. krusei AZN 1_31	0.745	6.33	7.28	5.38	0.681	L	L	L
C. krusei AZN 2_12	0.918	6.77	7.58	5.95	0.826	L	L	L
C. neoformans AZN 467	0.873	6.27	7.00	5.56	0.822	6.57	5.46	7.69
C. neoformans AZN 9019	NF	NF	NF	NF	NF	NF	NF	NF
A. fumigatus AZN 58	0.802	6.48	6.97	6.00	0.882	5.20	5.03	5.37
A. fumigatus AZN 8196	0.902	6.71	7.25	6.17	0.927	6.31	5.78	6.84
R. microsporus var. *rhizopodiformis* AZN 1185	0.855	6.89	7.86	5.91	NF	NF	NF	NF
R. oryzae AZN 6373	NF	NF	NF	NF	NF	NF	NF	NF
S. prolificans AZN 7989	NF	NF	NF	NF	NF	NF	NF	NF
S. prolificans AZN 7901	NF	NF	NF	NF	NF	NF	NF	NF

[a] For AMB against *Candida* spp., *C. neoformans*, and *A. fumigatus* and for 5FC against *C. neoformans* and *A. fumigatus*, data were obtained by nonlinear regression analysis using the E_{max} model with variable slopes. For 5FC against *Candida* spp., data were obtained by linear regression (L). NF, no fit.

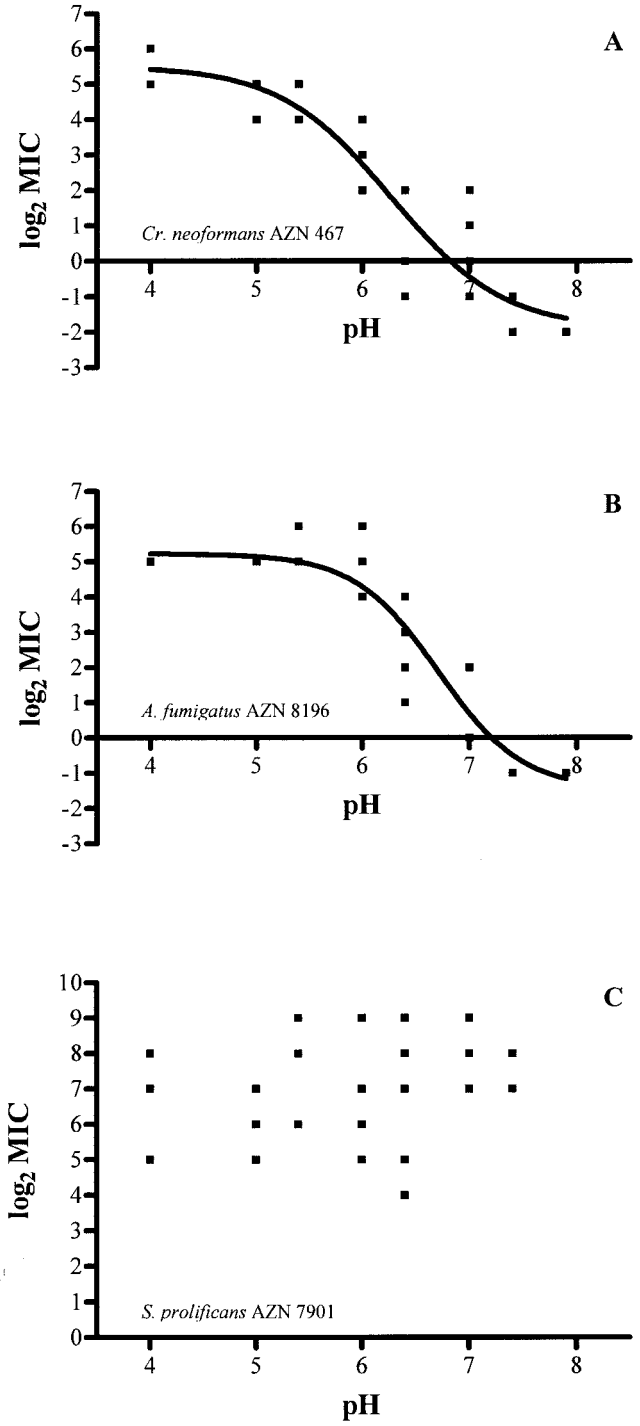

FIG. 1. Graphic representation of the relationships between the in vitro activities of amphotericin B and pH values ranging from 4.0 to 7.9 for *C. neoformans* AZN 467 (A), *A. fumigatus* AZN 8196 (B), and *S. prolificans* AZN 7901 (C).

citrate buffer and citrate-phosphate buffer were 0.67 and 0.53 μg/ml, respectively, at pH 5.4 and 1.45 and 1.53 μg/ml, respectively, at pH 6.0. The mean MICs obtained with citrate-phosphate buffer and MOPS were 4.35 and 2.25 μg/ml, respectively, at pH 6.4 and 8 and 8.22 μg/ml, respectively, at pH 7.0.

DISCUSSION

In this study, we demonstrated that the in vitro activities of AMB and 5FC against yeast and mold isolates were pH dependent. The in vitro activity of AMB decreased when the pH was lowered, while the in vitro activity of 5FC increased when the pH was lowered. The effect of the pH on the in vitro activity was dependent not only on the antifungal agent tested but also on the microorganism tested. Lowering the pH affected the MICs of AMB for all microorganisms in relatively similar fashions. At acidic pH, all microorganisms appear resistant to AMB. Although this could be due to the lower activity of AMB at lower pH, another explanation could be the chemical inactivation of the drug over time. The decreases in the in vitro activities of AMB against *Candida* spp., one *C. neoformans* isolate, *A. fumigatus*, and *R. microsporus* var. *rhizopodiformis* could be described by sigmoid curves. The sigmoid curves showed that the decreases in in vitro activities were primarily found over pH ranges of 7.9 to 5.4 (see the EC_{20} and EC_{80} values in Table 2). For the other *C. neoformans* isolate and the *R. oryzae* isolate, the decreases in in vitro activities could not be confirmed by regression analysis. For *S. prolificans* isolates, no relationship was found between the pH and the in vitro activity of AMB. Lowering the pH affected the MICs of 5FC for some microorganisms more than for others. The increase in in vitro activity of 5FC against *Candida* spp. could be described by a linear curve, while for one *C. neoformans* isolate and the *A. fumigatus* isolates, the increases could be described by sigmoid curves. The linear curve showed that the increase in in vitro activity for *Candida* spp. was found over a pH range of 7.9 to 4.0, while the sigmoid curves showed that the increases in in vitro activities for one *C. neoformans* isolate and the *A. fumigatus* isolates were primarily found over a pH range of 7.7 to 5.0 (see the EC_{20} and EC_{80} values in Table 2). For the other *C. neoformans* isolate, the increase in in vitro activity could not be confirmed by regression analysis. For the *Rhizopus* sp. and *S. prolificans* isolates, the relationships between the pH and the in vitro activities of 5FC could not be determined because of high off-scale MICs. An explanation for the increase in the in vitro activity of 5FC could be that protonation of the NH_2 group at low pH leads to a better uptake of 5FC by the fungal cell.

A limited number of other studies also demonstrated that the in vitro activities of AMB and 5FC against yeast (*Candida* spp. and *C. neoformans*) and mold isolates (*Aspergillus* spp.) were pH dependent (9, 14, 15).

We also demonstrated that the in vitro activity of AMB was affected by the buffer used, while the in vitro activity of 5FC was not. For AMB, the MICs found in RPMI 1640 medium buffered with citrate-phosphate buffer were significantly higher than the MICs found in RPMI 1640 medium buffered with MOPS. The difference in MICs was approximately 1 twofold-dilution step. An explanation for this difference may be that phosphate buffers are buffers that readily traverse the cell membrane and can theoretically produce unexpected interactions with antifungal agents (7, 8).

We assume that these differences in MIC do not affect the pH dependency, since we found that in both RPMI 1640 medium buffered with citrate-phosphate buffer and RPMI 1640 medium buffered with MOPS the in vitro activities of AMB

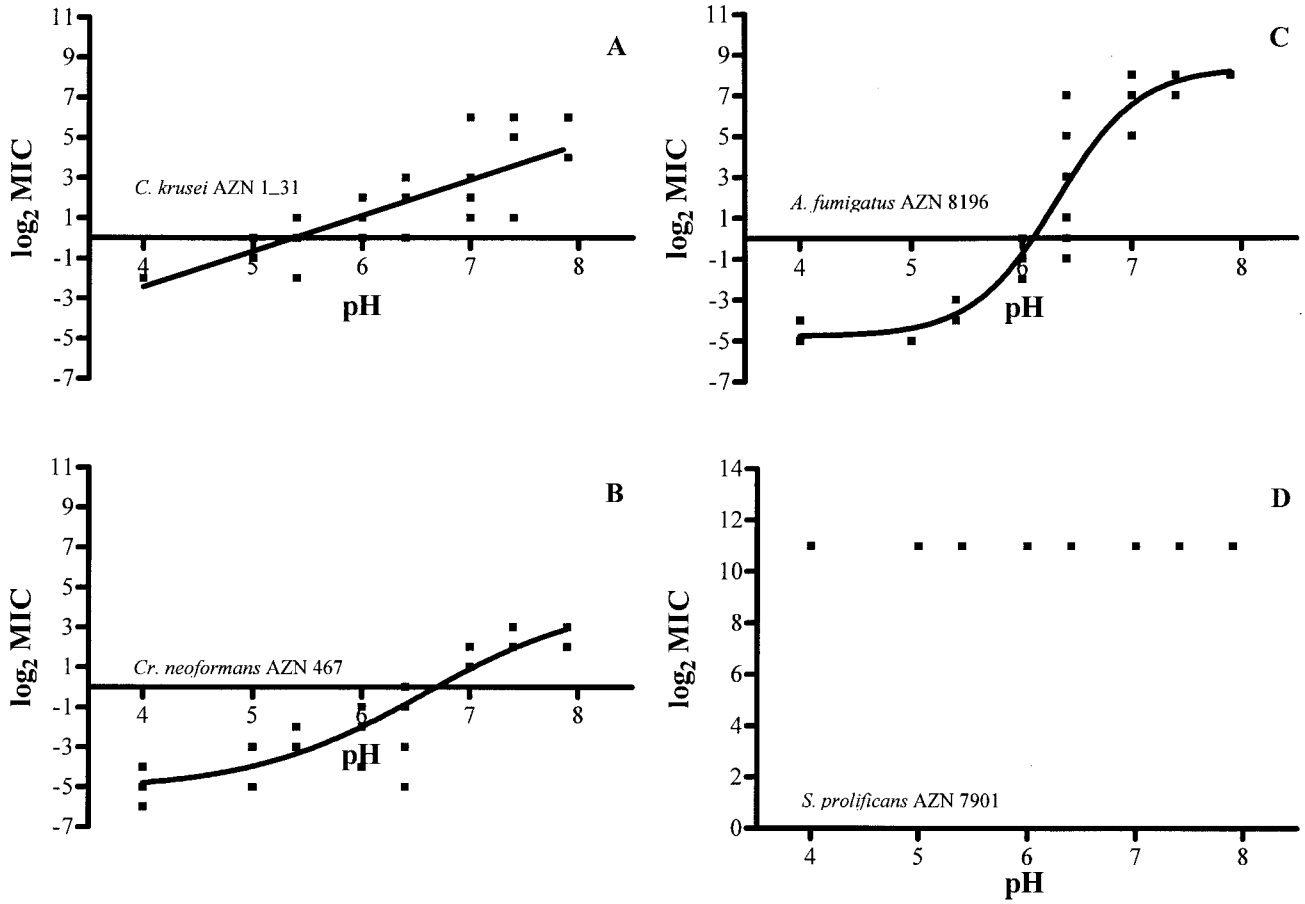

FIG. 2. Graphic representation of the relationships between the in vitro activities of flucytosine and pH values ranging from 4.0 to 7.9 for *C. krusei* AZN 1_31 (A), *C. neoformans* AZN 467 (B), *A. fumigatus* AZN 8196 (C), and *S. prolificans* AZN 7901 (D).

against *Candida* spp., *C. neoformans*, *A. fumigatus*, and *Rhizopus* spp. decreased when the pH was lowered.

The choice of an in vitro susceptibility test and its variables is based on inter- and intracenter reproducibility, on the ability to provide a wide range of MICs that allow discrimination among resistant and sensitive strains, and on the correlation of in vitro results with clinical outcomes. Buffered medium at a pH value of 7.0 has been accepted as the standard for the in vitro susceptibility testing of microorganisms (7, 8). However, the in vitro results obtained with this standard pH do not always correlate well with clinical outcome. Viviani et al. showed that MICs of 5FC against *C. neoformans* isolates obtained with YNB at pH 5.4 correlated well with clinical outcome, while MICs obtained with YNB at the standard pH value of 7.0 did not (15). We also demonstrated that the MICs of 5FC against *A. fumigatus* obtained in RPMI 1640 buffered at a pH value of 5.0 correlated better with in vivo efficacy in a murine model of invasive aspergillosis than did the MICs obtained in RPMI 1640 buffered at the standard pH value of 7.0 (D. T. A. te Dorsthorst, et al., 44th ICAAC). Thus, there is some evidence that the predictive value of MICs of 5FC determined at a low pH is greater than those determined at the standard pH. Further studies will need to confirm this finding for 5FC. In contrast, the effect of a low pH on the MICs of AMB was similar for all microorganisms. A discriminatory effect of pH does not seem to be present.

One of the explanations for the predictive value of MICs of 5FC determined at low pH could be that the pH at the site of infection is lower than 7.4 (in the human body, the pH is carefully regulated at pH 7.4), due to the production of organic acids by fungi (in particular aspergilli) (11). Kauffman et al. described three different phases during the growth of *Aspergillus* species (4). These phases could be characterized by changes in medium pH. During the first phase (phase I), the fungus starts to grow and uses glucose as a carbon source. The use of glucose is associated with the production of organic acids and a resulting drop in medium pH. When glucose becomes limited, the organic acids are used as a secondary carbon source, and the medium pH rises again (phase II). After reaching a maximum, the pH stabilizes or drops slightly (phase III). The duration of phase I depends on the glucose concentration. Increasing concentrations of glucose resulted in prolongations of phase I followed by increases in pH or no increase at all. Extrapolating these in vitro growth characteristics to the clinical setting, it seems likely that, depending on the concentration of glucose at the site of infection, the fungus will produce organic acids and the pH at the site of infection will drop below 7.4. Not only the production of organic acids by fungi but also

the presence of necrosis (12) and the lysozyme activities of granulocytes and macrophages (3) may lower the pH at the site of infection.

Unfortunately, the pH values at the site of infection caused by yeasts or molds are unknown. However, there are some studies in which the pHs of bacterial abscesses were determined. The pHs of bacterial abscesses varied, depending on the location and bacterial agent present, but overall they were found to be lower than 7.4. One clinical study showed that the pHs ranged from 5.7 (empyema) to 7.2 (liver abscess) (2), and another clinical study showed that the pHs of intra-abdominal and anorectal abscesses ranged between <6 and 6.87 and between <6 and 6.98, respectively (13). In intra-abdominal abscesses, pH values ranged form 6.71 to 6.85 (1).

We conclude that further studies are necessary to determine the influence of pH on the distribution of the MICs of several antifungal agents for various yeasts and molds and on the correlation between in vitro results with clinical outcome.

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