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Interaction between Posaconazole and Amphotericin B in Concomitant Treatment against *Candida albicans* In Vivo

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The interaction of posaconazole and amphotericin B was evaluated in concomitant treatment of *Candida albicans* systemic infections in immunocompetent mice by using four strains of *C. albicans* with different susceptibilities to fluconazole. Posaconazole and amphotericin B were each tested at four dose levels alone and in all possible combinations against each *C. albicans* strain. Survival curves of mice treated with combinations of posaconazole and amphotericin B were statistically compared with those of mice treated with the component monotherapies. Of the 64 total combinations evaluated against the *C. albicans* strains (16 combinations per strain), 20.3% were more effective in prolonging mouse survival than both of the monotherapies, 45.3% were more effective than one of the monotherapies, and 32.8% were similar to both monotherapies. No evidence of antagonism was observed between posaconazole and amphotericin B in this mouse model, consistent with in vitro results against the same strains.

The clinical use of azoles in combination with amphotericin B (AMB) is still controversial because of the potential for antagonism between the two drugs (9, 12, 18, 22). This potential comes from their mechanisms of action; azoles block ergosterol biosynthesis, while AMB causes membrane damage by binding to ergosterol. Various experimental fungal infection models have been used to address the issue of combinational dosing, but the results have been mixed. In systemic candidiasis models in mice with *Candida albicans*, Louie et al. (11) observed antagonism between the triazole fluconazole (FLC) and AMB, while Sugar and Liu (23) reported antagonism between another triazole, itraconazole, and AMB. Louie et al. (10, 12) also found that FLC was antagonistic to AMB therapy against experimental *C. albicans* endocarditis, endophathalmitis, and pyelonephritis in rabbits. However, Sanati et al. (17) did not observe antagonism when FLC and AMB were used in combination against *C. albicans* in invasive candidiasis in neutropenic mice or in endocarditis in rabbits. Sugar et al. (21) reported no antagonism between FLC and AMB in invasive candidiasis with *C. albicans* in immunocompetent or immunocompromised mice.

Posaconazole (POS) is a broad-spectrum antifungal triazole which recently completed phase III clinical trials (7). The experiments described in this report were performed to determine the interaction between POS and AMB in concomitant combination therapy against systemic *C. albicans* infection in mice.

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MATERIALS AND METHODS

Antifungal agents. POS clinical oral suspension was used in these experiments, and dilutions were made in sterile water for injection. AMB (Fungizone) was obtained from Apothecon, Bristol-Myers Squibb, Princeton, N.J., and prepared according to the manufacturer's directions.

***C. albicans* strains and in vitro activity testing.** All *C. albicans* strains were from the Schering-Plough Research Institute fungal culture collection and included one FLC-susceptible (FLC-S) strain C43, one FLC-susceptible, dose-dependent (FLC S-DD) strain C210, and two FLC-resistant (FLC-R) strains C284 and C335. MICs were determined by the standard NCCLS method M27-A (14). MICs of FLC for strains C43, C210, C284, and C335 were 0.125, 16, 64, and 64 μg/ml, respectively. Drug interactions between POS and AMB were determined by a checkerboard microdilution method. The endpoints for POS alone and for the POS-AMB combinations were read at 80% inhibition, while that for AMB alone was read at 100% inhibition. The fractional inhibitory concentration (FIC) index (5) was defined as synergistic if the FIC was ≤0.5, indifferent if it was >0.5 but ≤4, and antagonistic if it was >4.

Mice. Charles River Laboratories (Wilmington, Mass.) CF1 mice (white, male) were used in these studies. At the time of infection, the mice weighed 18 to 20 g. These studies were carried out in accordance with the *Guide to the Care and Use of Laboratory Animals* of the National Institutes of Health (15) and the Animal Welfare Act in an Association for Assessment Accreditation of Laboratory Animal Care-accredited program.

Systemic infection model and drug therapy. *C. albicans* strains were grown for 48 h on Sabouraud dextrose agar, and inocula were prepared as saline suspensions as described previously (6). Initiation of systemic infection occurred on day 0 by intravenous injection (tail vein). Inocula ranged from ca. 5×10^6 (strains C43 and C210) to ca. 1×10^7 (strains C284 and C335) CFU/mouse. Drug therapy to groups of 10 mice began at 4 h postinfection on day 0 and continued once daily through day 3. POS and AMB were each tested at 4 dose levels alone and in all possible combinations in a checkerboard fashion against each *C. albicans* strain (see Table 2). The 4 dose levels for each drug were selected from preliminary dose-response experiments (data not shown) to include the full range of survival efficacy, from maximum to intermediate to minimal, in the survival curves. This was done to ensure that POS and AMB would be tested in combinations involving high-, intermediate-, and low-dose levels of each drug (similar to doing a checkerboard in vitro MIC test). For each strain, there were 16 drug combination groups and 8 monotherapy groups of mice. Concomitant combination therapy was achieved by administering POS orally followed immediately by AMB intraperitoneally. Control animals were administered sterile water for injection. Sur-

TABLE 1. In vitro interaction between POS and AMB against four *C. albicans* strains with different susceptibilities to FLC

Strain	MIC[a] (μg/ml) of:			POS-AMB FIC	Interpretation
	POS	AMB	POS-AMB		
C43 (FLC-S)	0.03	2	0.008, 0.5	0.50	Synergy
C43 (FLC-S)	0.03	2	0.016, 0.5	0.78	Indifferent
C210 (FLC S-DD)	1	2	0.5, 0.5	0.75	Indifferent
C284 (FLC-R)	0.25	1	0.125, 0.125	0.62	Indifferent
C284 (FLC-R)	0.25	4	0.06, 1	0.49	Synergy
C335 (FLC-R)	0.125	2	0.016, 1	0.63	Indifferent

[a] MICs were determined using NCCLS method M27-A (14).

vival was monitored for 10 days. Mice were not cultured for organ fungal burdens.

Statistical analysis. Each *C. albicans* strain was tested twice, and the results were combined for statistical analysis. Wilcoxon tests were performed to compare the survival curves (Kaplan Meier). A P value of <0.05 indicated that the overall survival curves were statistically significantly different. No multiplicity adjustments were made.

RESULTS

In vitro interaction between POS and AMB. POS and AMB were tested in vitro alone and in combination against the four *C. albicans* strains which exhibited a range of susceptibilities to FLC (Table 1). The MICs of POS ranged from 0.03 to 1, while the MICs of AMB ranged from 1 to 4. The drug interaction results were mixed but were primarily indifferent. The POS-AMB interactions with strains C43 and C284 were synergistic and indifferent in different experiments, while those with strains C284 and C335 were indifferent. No antagonism was observed.

Effect of combination dosing against systemic candidiasis in mice. The four *C. albicans* strains were tested in a systemic infection model with immunocompetent mice. Table 2 shows the 16 POS and AMB combinations used for each strain and the averaged percent survival data from two experiments per strain on day 4 (the day after the last dose) and day 10 (end of experiment) for the combinations and their component monotherapies. Survival curves from day 0 to day 10 for each concomitant combination of POS and AMB were statistically compared to those for the POS and AMB monotherapies, and the resulting P values are also shown in Table 2. Survival curves are shown as examples of the data in Fig. 1A (strain C43) and 1B (strain C284). Both graphs show the combination of the highest dose level of POS and the second-highest dose level of AMB used against that strain, compared to the component monotherapies and controls. The emphasis in the graphs on the second-highest dose level of AMB is to show the drug interaction with a lower, but still efficacious, level of AMB, where potential antagonism could still be observed, as opposed to the highest level of AMB, where antagonism may be masked by the maximum efficacy of AMB. In Fig. 1A, for the FLC-S strain C43, mice treated with the POS and AMB combination survived significantly longer than those treated with the component monotherapies ($P < 0.05$) or the controls. In Fig. 1B, for the FLC-R strain C284, mice treated with the POS and AMB combination survived similarly to those treated with the component monotherapies ($P > 0.05$) but longer than the controls.

Table 3 shows a summary of all of the interactions between POS and AMB listed in Table 2. In these studies, an antagonistic combination was defined as providing less survival efficacy than one or both of the component drugs alone. Against all four *C. albicans* strains, 20.3% of the POS and AMB combinations tested were more effective than either POS or

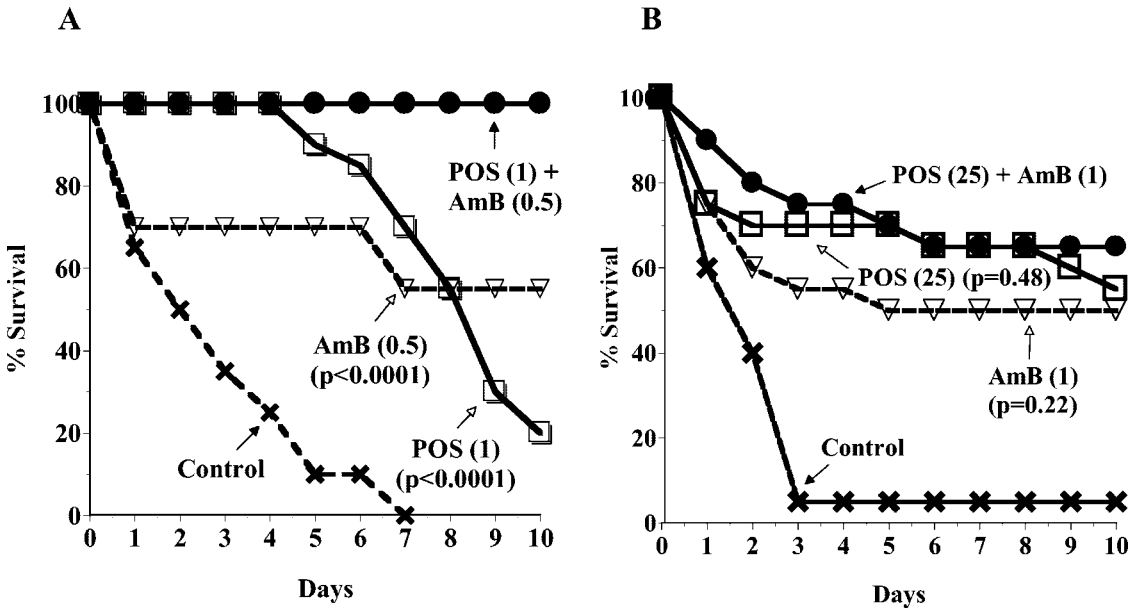

FIG. 1. Concomitant combination treatment with POS and AMB of systemic infection in mice with FLC-S *C. albicans* C43 (A) or FLC-R *C. albicans* C284 (B). The combination of POS and AMB, POS alone, or AMB alone was administered once daily for 4 days starting 4 h postinfection on day 0. Dose levels (in milligrams/kilogram of body weight) are indicated in parentheses. Controls were administered sterile water for injection. The survival data were averaged from two experiments (10 mice per group in each experiment). The P values were determined by comparing the survival curve for the combination to each of those for the drugs alone.

TABLE 2. Effect of POS-AMB combinations on mouse survival compared to POS or AMB alone against *C. albicans*

Strain	Combination			POS alone[a]			AMB alone[a]		
	Dose levels (mg/kg)[b]	% Survival on day[c]:		% Survival on day[d]:		*P* value[e]	% Survival on day[d]:		*P* value[e]
		4	10	4	10		4	10	
C43	1, 1	100	100	100	20	<0.0001	85	70	0.0088
	1, 0.5	100	100	100	20	<0.0001	70	55	<0.0001
	1, 0.1	95	60	100	20	0.0037	50	45	0.0370
	1, 0.02	100	40	100	20	0.0410	35	0	<0.0001
	0.5, 1	80	60	55	5	0.0065	85	70	0.5500
	0.5, 0.5	100	70	55	5	<0.0001	70	55	0.1900
	0.5, 0.1	95	40	55	5	0.0067	50	45	0.1900
	0.5, 0.02	95	15	55	5	0.0054	35	0	<0.0001
	0.2, 1	60	60	15	0	0.0150	85	70	0.3500
	0.2, 0.5	75	65	15	0	0.0006	70	55	0.4800
	0.2, 0.1	60	55	15	0	0.0230	50	45	0.6300
	0.2, 0.02	45	10	15	0	0.2900	35	0	0.1300
	0.05, 1	90	85	20	0	<0.0001	85	70	0.2500
	0.05, 0.5	70	50	20	0	0.0010	70	55	0.9500
	0.05, 0.1	55	55	20	0	0.0280	50	45	0.6700
	0.05, 0.02	25	10	20	0	0.4200	35	0	0.3000
C210	25, 5	100	80	75	40	0.0150	75	65	0.2100
	25, 1	100	70	75	40	0.0820	85	70	0.7300
	25, 0.1	90	45	75	40	0.6500	50	50	0.2900
	25, 0.02	65	5	75	40	0.0280	35	15	0.3300
	10, 5	75	70	35	0	0.0002	75	65	0.6700
	10, 1	90	55	35	0	0.0004	85	70	0.4300
	10, 0.1	55	50	35	0	0.1300	50	50	0.9000
	10, 0.02	55	20	35	0	0.0720	35	15	0.2100
	5, 5	100	95	50	0	<0.0001	75	65	0.1700
	5, 1	85	70	50	0	<0.0001	85	70	0.9700
	5, 0.1	60	50	50	0	0.0790	50	50	0.8600
	5, 0.02	85	10	50	0	0.0021	35	15	0.0210
	1, 5	100	95	15	0	<0.0001	75	65	0.0170
	1, 1	85	70	15	0	<0.0001	85	70	0.9300
	1, 0.1	50	35	15	0	0.1300	50	50	0.4600
	1, 0.02	20	5	15	0	0.4200	35	15	0.4800
C284	25, 5	90	90	70	55	0.0160	75	60	0.0420
	25, 1	75	65	70	55	0.4800	55	50	0.2200
	25, 0.2	80	55	70	55	0.8900	30	15	0.0042
	25, 0.05	80	55	70	55	0.6900	15	10	0.0005
	10, 5	75	70	80	55	0.5200	75	60	0.8100
	10, 1	75	70	80	55	0.5600	55	50	0.2700
	10, 0.2	70	70	80	55	0.6600	30	15	0.0210
	10, 0.05	85	80	80	55	0.1400	15	10	0.0001
	5, 5	80	65	55	35	0.0370	75	60	0.7800
	5, 1	55	55	55	35	0.3000	55	50	0.9500
	5, 0.2	60	60	55	35	0.2000	30	15	0.1300
	5, 0.05	50	40	55	35	0.9800	15	10	0.5100
	1, 5	60	55	20	10	0.0380	75	60	0.3200
	1, 1	65	65	20	10	0.0110	55	50	0.6200
	1, 0.2	55	35	20	10	0.2000	30	15	0.6000
	1, 0.05	45	15	20	10	0.5700	15	10	0.8300
C335	100, 5	100	100	100	65	0.0040	75	70	0.0088
	100, 1	95	95	100	65	0.0280	70	70	0.0300
	100, 0.25	95	90	100	65	0.1100	45	40	0.0005
	100, 0.1	95	55	100	65	0.3500	20	10	<0.0001
	25, 5	95	95	100	30	0.0001	75	70	0.0370
	25, 1	100	100	100	30	<0.0001	70	70	0.0087
	25, 0.25	100	75	100	30	0.0130	45	40	0.0020
	25, 0.1	90	35	100	30	0.9700	20	10	<0.0001
	5, 5	90	90	70	40	0.0030	75	70	0.1200
	5, 1	85	65	70	40	0.1300	70	70	0.9000
	5, 0.25	75	55	70	40	0.7500	45	40	0.1900
	5, 0.1	70	40	70	40	0.8900	20	10	0.0014
	1, 5	80	75	30	5	<0.0001	75	70	0.6400
	1, 1	55	55	30	5	0.0490	70	70	0.5500

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TABLE 2—*Continued*

Strain	Combination			POS alone[a]			AMB alone[a]		
	Dose levels (mg/kg)[b]	% Survival on day[c]:		% Survival on day[d]:		*P* value[e]	% Survival on day[d]:		*P* value[e]
		4	10	4	10		4	10	
	1, 0.25	45	40	30	5	0.3200	45	40	0.9400
	1, 0.1	35	15	30	5	0.8500	20	10	0.7300

[a] POS or AMB alone at dose levels used in the combination.
[b] Doses of POS are given first, followed by doses of AMB.
[c] Percent survival of combination-treated mice on day 4 (day after final dose) or day 10 (end of experiment) postinfection (average of the results from two experiments per strain, 10 mice per group per experiment).
[d] Percent survival of POS alone- or AMB alone-treated mice on day 4 (day after final dose) or day 10 (end of experiment) postinfection (average of the results from two experiments per strain, 10 mice per group per experiment).
[e] *P* values from Wilcoxon tests comparing survival curves (from day 0 to day 10) of combination versus POS or AMB alone at the dose level used in the combination. A significant difference between survival curves was observed if the *P* value was <0.05.

AMB alone ($P < 0.05$) in prolonging the survival of mice. In addition, 45.3% of the combinations were more effective than one of the drugs alone ($P < 0.05$) and similar to the other drug alone ($P > 0.05$), while 32.8% of the combinations were similar to both drugs alone ($P > 0.05$). Only one combination of POS and AMB was less effective than one of the drugs alone (POS in this case), and no combinations were less effective than both POS and AMB alone. Overall, 98.4% of the 64 combinations tested against the four *C. albicans* strains showed no evidence of antagonism between POS and AMB in this model of systemic candidiasis in immunocompetent mice.

DISCUSSION

Our studies demonstrate that concomitant combination treatment of systemic candidiasis in immunocompetent mice with POS and AMB was not antagonistic, as determined by using survival as the efficacy endpoint. Instead, 20.3, 45.3, and 32.8% of the total combinations tested against four *C. albicans* strains resulted in survival of mice, which was more effective than both monotherapies, more effective than one of the monotherapies, and similar to both monotherapies, respectively. In addition, the combination of POS and AMB was effective and not antagonistic even against strains with reduced susceptibility to FLC, where much higher doses of POS were used in the combinations. The finding of no antagonism in the in vivo experiments was consistent with the in vitro results against the same strains.

In these studies we did not investigate the effect of POS and AMB on fungal burdens in organs or the effect of sequential dosing of POS relative to AMB. However, Najvar et al. (13) reported that concomitant POS and AMB or sequential dosing (initial dosing with POS followed a day later by AMB) in a pulmonary *Aspergillus flavus* infection model in immunocompromised mice were not antagonistic, as determined by both survival and lung burden results.

Although pharmacokinetic (PK) data and PK-pharmacodynamic (PD) analyses were not obtained or performed in our combination dosing studies, the PK data for POS and the PK-PD data for POS monotherapy and AMB monotherapy were reported previously. Following oral administration of POS to mice, Nomeir et al. (16) observed a dose-related increase in the maximum concentration in serum (up to 80 mg/kg) and area under the concentration-time curve (AUC, up to 120 mg/kg). Andes et al. (4) studied PK-PD data for POS against *C. albicans* in neutropenic mice and reported that the 24-h AUC/MIC ratio was the PK-PD parameter associated with POS efficacy in this model. The mean free drug AUC/MIC ratio of 16.9 for POS was similar to the ratio of

TABLE 3. Summary of interactions of POS-AMB combinations compared to one or both of POS or AMB alone against the four *C. albicans* strains[a]

Strain	No. of combinations tested	No. of combinations:				
		More effective[b] than both alone	More effective[b] than one alone	Similar[b] to both alone	Less effective[b] than one alone	Less effective[b] than both alone
C43 (FLC-S)	16	5	9[c]	2	0	0
C210 (FLC S-DD)	16	2	6[c]	7	1[c]	0
C284 (FLC-R)	16	1	7[c]	8	0	0
C335 (FLC-R)	16	5	7[c]	4	0	0
Total (%)	64	13 (20.3)	29 (45.3)	21 (32.8)	1 (1.6)	0

[a] Based on statistical analysis of survival curves through day 10 postinfection with 4 dose levels of each drug alone and in all possible combinations (data from 2 experiments per strain were pooled).
[b] More effective or less effective means the survival curves for the combinations were significantly different from those for the drug(s) alone ($P < 0.05$). Similar means the survival curves for the combinations and the drug(s) alone were not significantly different ($P > 0.05$).
[c] These combinations were also similar to the other drug alone.

25 observed with other triazoles. In addition, these authors also indicated that POS exhibited prolonged (20 to 30 h) post-antifungal effect (PAFE) for free drug, potentially due to sub-MIC effects. Andes et al. (3) also reported that the PK-PD parameter predictive of efficacy for AMB was the peak serum level/MIC ratio and that AMB also had a prolonged (23 to 30 h) PAFE. The prolonged PAFEs of POS and AMB may have contributed to the antifungal efficacy of combination treatment with these drugs observed in our studies.

Antagonism between azoles and AMB has been observed in some, but not other, literature reports involving animal models of fungal infections. Louie et al. (10, 11) showed antagonism between FLC and AMB, and Sugar and Liu (23) showed the same result with itraconazole and AMB with *C. albicans* infection models. Lewis et al. (9) also reported that preexposure to itraconazole reduced the efficacy of subsequent treatment with AMB in murine pulmonary aspergillosis. The absence of FLC-AMB antagonism was observed in murine candidiasis studies (17, 21) and by George et al. (8) in an immunosuppressed rabbit model of aspergillosis, by Anaissie et al. (2) against *Trichosporon beigelii* infection in mice, and by Barchiesi et al. (5) against murine systemic cryptococcosis. Other triazole-AMB combinations were also not antagonistic, including saperconazole against murine systemic candidiasis (20) and SCH 39304 against murine systemic candidiasis (19) and cryptococcal meningitis (1).

We anticipate that POS will be used in combination with other antifungals, potentially including AMB, for treatment of serious fungal infections in patients. The efficacy observed in mice with the combination of POS and AMB suggests this combination could be effective in clinical fungal infections. However, the lack of antagonism in our studies indicates that the combination could potentially be tried clinically with less concern for antagonism.

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