Combination Therapy in Experimental Invasive Aspergillosis

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Combination antifungal therapy was assessed in an immunosuppressed rabbit model of invasive aspergillosis. Treatment with fluconazole, amphotericin B, or a combination of both significantly prolonged survival of animals lethally challenged with *Aspergillus fumigatus*. High-dose amphotericin B was the most effective therapy for invasive aspergillosis. Although no antagonism was seen when fluconazole was given prophylactically or therapeutically in combination with amphotericin B, combination therapy did not augment the antifungal activity of amphotericin B. Animals given a sublethal challenge of *A. fumigatus* had lower mortality rates when given amphotericin B, fluconazole as treatment or prophylaxis, or various combination therapies. Only animals treated with flucytosine had mortality rates comparable to those of controls. No antagonism was observed with combinations of fluconazole and amphotericin B, flucytosine and amphotericin B, or fluconazole and flucytosine. These observations provide evidence that fluconazole, flucytosine, and amphotericin B used in various combinations are not antagonistic and may provide some insight into the treatment of invasive aspergillosis in humans.

Invasive aspergillosis is associated with significant morbidity and mortality despite therapy with amphotericin B [1–3]. Also, the management of invasive aspergillosis may be complicated by the toxicity associated with amphotericin B therapy [3, 4]. A number of new antifungal agents have been developed for use in patients with serious fungal infections, particularly the newer azoles, which may prove to be more effective and less toxic than amphotericin B [5]. Some of these newer agents have been or are currently being studied in patients with serious fungal infections, and many of these agents have also been studied in animal models of fungal disease [5]. Since invasive aspergillosis is often recalcitrant to current therapeutic regimens, combination therapy with an azole and amphotericin B or the prophylactic use of an azole followed by amphotericin B for treatment of invasive aspergillosis may improve the management of patients at risk for this disease [6]. However, in vivo antagonism between ketoconazole and amphotericin B has been demonstrated in an animal model [7]. This observation has led to some reluctance to combine an azole with amphotericin B in the treatment of fungal infections.

In previous studies, we observed that amphotericin B alone and fluconazole alone improved survival, lowered the tissue burden of infection, and reduced the level of *Aspergillus* antigenemia in our immunosuppressed rabbit model of infection [4, 8, 9]; also, prophylaxis with fluconazole before challenge reduced dissemination of infection [10]. However, oral fluconazole did not sterilize infected tissues [5]. Therefore, the present study was designed to evaluate the efficacy of combination antifungal therapy, primarily fluconazole plus amphotericin B, in our immunosuppressed leukopenic rabbit model of invasive aspergillosis, with particular attention to the possibility of antagonism. The efficacy of amphotericin B against *Aspergillus fumigatus* following fluconazole prophylaxis was also assessed. In addition, the effectiveness of flucytosine combined with either amphotericin B or fluconazole was evaluated.

Methods

The methods used have been described in detail previously [4, 5, 8–12]. Briefly, in our experimental model, New Zealand White rabbits were immunosuppressed with a single dose of cyclophosphamide (200 mg; Bristol-Myers Squibb, Evansville, IN) given intravenously on the first day and triamcinolone acetonide (10 mg; Westwood Pharmaceuticals, Buffalo, NY) given subcutanously each day throughout the duration of the experiments. With this immunosuppressive regimen, the rabbits have reduced total white blood cell counts through day 7, with a nadir on day 4 [4, 5, 10]. At 24 h after immunosuppression, groups of 8–10 rabbits were challenged intravenously with a lethal inoculum of 10^6 *A. fumigatus* conidia or a sublethal challenge of 10^5 conidia. Antifungal therapy was initiated 24 h after challenge or 48 h before challenge in the prophylaxis studies, as described below. The duration of therapy in surviving rabbits was 5–6 days. Each group contained 1 untreated control rabbit. Blood was obtained daily for determining total white blood cell counts

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and serum aspergillus antigen levels by our competitive ELISA as described previously [5, 8–12].

A modification of the semiquantitative culture technique of Graybill and Kaster [13] was used to evaluate and compare the aspergillus tissue burden. Samples of liver, kidney, lung, and brain were manually chopped, weighed, diluted 1:10 (wt/vol) with sterile saline, and homogenized for 25 s with an electric tissue homogenizer (Tri-R Instruments, Rockville Center, NY). Then 1.0- and 0.1-mL volumes of each organ homogenate were plated in duplicate on Sabouraud dextrose and blood agar. The plates were incubated for 24–36 h at 37°C, and colonies were counted as described previously [9]. Also, liver, spleen, kidney, lungs, and brain were examined histologically at the time of autopsy or sacrifice (48–72 h after completion of therapy for the treated rabbits) as described previously [4, 5, 8–12]. Organs were considered positive when more than one colony of *A. fumigatus* was present on ~1 g of minced organ tissue placed directly on Sabouraud dextrose and blood agar plates or when semiquantitative cultures of tissue homogenates contained ≥20 cfu/g of tissue [4]. Tissue burden of *Aspergillus* was evaluated with semiquantitative cultures that could detect 20–7500 cfu/g of tissue.

Criteria used in evaluating efficacy. Three criteria are used for evaluating the efficacy of antifungal therapy in our rabbit model of invasive aspergillosis, as described previously [4, 5, 8–12]: mortality rate, reduction in the number of *Aspergillus* organisms in target organs in each animal studied, and reduction in the level of circulating *Aspergillus* carbohydrate antigen as measured by our competitive ELISA technique [4, 11].

Therapy in rabbits challenged with a lethal inoculum. Immunosuppressed rabbits were challenged with a lethal inoculum of 10^6 *A. fumigatus* conidia, divided into groups, and treated with amphotericin B (fungizone; E. R. Squibb & Sons, Princeton, NJ), fluconazole (Pfizer, Groton, CT), or a combination of amphotericin B plus fluconazole. Amphotericin B was diluted with 5% dextrose in sterile water at a ratio of 1 mg of drug to 10 mL of diluent and was given intravenously over 30–60 min through a lateral ear vein at 1.5 or 0.5 mg/kg/day for 5 days. Fluconazole was dissolved at 4 mg/mL in sterile water and was administered orally via a gastric gavage tube (American Pharmaseal, Valencia, CA) at 60 or 120 mg/kg/day for 6 days. Another group of rabbits was treated with the combination of low-dose amphotericin B (0.5 mg/kg/day) for 5 days and low-dose fluconazole (60 mg/kg/day) for 6 days. Antifungal therapy was initiated 24 h after challenge in all treatment studies.

In other experiments, groups of rabbits received fluconazole prophylactically before intravenous challenge with a lethal (10^6 conidia) inoculum. Fluconazole at 60 mg/kg/day was begun 48 h before challenge and was continued daily for 9 days as single-drug therapy or, in some experiments, as combination therapy with amphotericin B at 0.5 mg/kg/day begun 24 h after challenge and given for 5 days.

Therapy in rabbits challenged with a sublethal inoculum. Immunosuppressed rabbits were challenged with a sublethal inoculum of 10^5 *A. fumigatus* conidia, subdivided into groups, and treated with amphotericin B alone at 0.25 or 0.5 mg/kg/day for 5 days, fluconazole alone at 60 or 120 mg/kg/day for 6 days, or flucytosine alone at 100 mg/kg/day for 6 days. Flucytosine (Roche, Nutley, NJ) was dissolved in sterile water at a concentration of 10 mg of flucytosine/mL of water and administered orally via a gastric gavage tube. Other groups of rabbits were treated with flucytosine at 100 mg/kg/day for 6 days in combination with either amphotericin B at 0.5 mg/kg/day for 5 days or fluconazole at 120 mg/kg/day for 6 days. Antifungal therapy was initiated 24 h after challenge.

Sublethally challenged rabbits received prophylactic fluconazole at 60 or 120 mg/kg/day initiated 48 h before challenge. Prophylactic fluconazole at 60 mg/kg/day was also given in combination with treatment regimens, begun 24 h after challenge, of 0.25 and 0.5 mg/kg/day of amphotericin B.

Statistical analysis. The two-tailed Wilcoxon rank sum test and χ^2 analysis were used when appropriate. Statistical significance was defined as $P < .05$.

Results

Lethal challenge. Untreated immunosuppressed rabbits challenged with a lethal inoculum of *A. fumigatus* conidia develop progressive invasive aspergillosis, which is fatal in 100% of animals by day 9 after challenge as described previously [5, 9, 10] and as shown in figure 1 for the present studies. The survival of rabbits treated with fluconazole, amphotericin B, or a combination of the two begun 24 h after challenge is also shown in figure 1. Survival was significantly prolonged with fluconazole therapy at 60 and 120 mg/kg/day ($P < .005$ for both dosages vs. controls) and amphotericin B at 0.5 and 1.5 mg/kg/day ($P < .001$ for both dosages vs. controls), as well as with the combination of fluconazole at 60 mg/kg/day plus amphotericin B at 0.5 mg/kg/day ($P < .005$ vs. controls). There was no significant difference in mortality among the treated groups ($P > .1$). Of importance, there was no evidence of antagonism between fluconazole and amphotericin B when used in combination in these doses.

When fluconazole was given prophylactically (48 h before challenge) at 60 mg/kg/day alone or in combination with amphotericin B at 0.5 mg/kg/day, survival was also significantly prolonged ($P < .001$ for both regimens vs. controls). Although the difference in survival between prophylactic and therapeutic fluconazole (60 mg/kg/day) was not statistically significant, mortality was lower (40% vs. 17%) in rabbits given fluconazole prophylactically. The cumulative mortality observed in immunosuppressed lethally challenged rabbits was 100% (18/18) in untreated controls, 40% (4/10) in each group of animals treated with fluconazole at either 60 or 120 mg/kg/day, 17% (2/12) in those receiving fluconazole at 60 mg/kg/day prophylactically, 9% (1/11) in animals given fluconazole at 60 mg/kg/day plus amphotericin B 0.5 mg/kg/day, 8% (1/12) in those given prophylactic fluconazole at 60 mg/kg/day plus amphotericin B at 0.5 mg/kg/day, and 0 in those treated with amphotericin B at 0.5 (0/11) or 1.5 mg/kg/day (0/8). Since all animals treated with amphotericin B survived their infection, the possibility of combina-

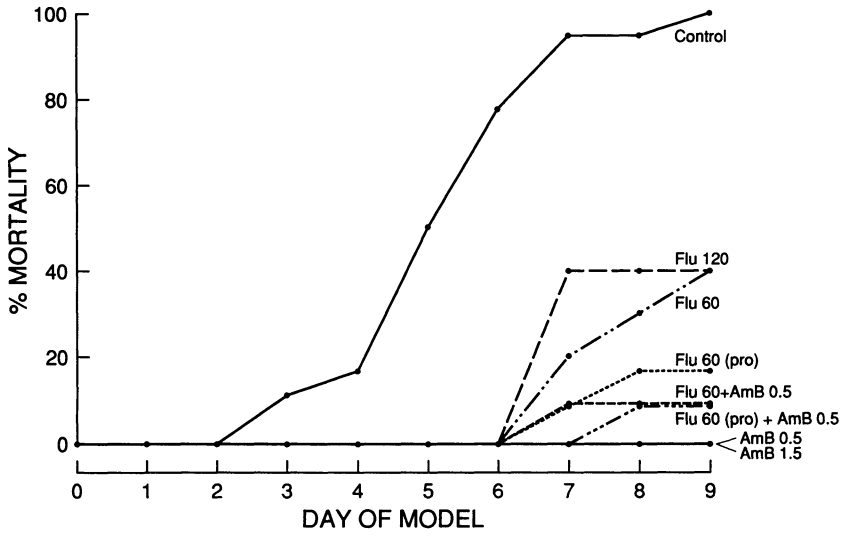

Figure 1. Cumulative mortality of rabbits lethally challenged with *Aspergillus fumigatus*. Control, infected untreated animals; Flu 120, 60, fluconazole at 120 or 60 mg/kg/day; pro, prophylaxis; AmB 0.5, 1.5, amphotericin B at 0.5 or 1.5 mg/kg/day.

tion therapy improving survival could not be assessed, but no evidence of antagonism was seen when therapeutic or prophylactic fluconazole was given in combination with amphotericin B.

Semiquantitative results of organ cultures from rabbits given a lethal challenge are shown in table 1. Extensive infection occurred in the liver, lung, and kidney tissues of all untreated control rabbits. Fluconazole at 60 mg/kg/day, given either prophylactically or therapeutically, reduced the tissue burden of *A. fumigatus* in liver tissues >10-fold but did not reduce the burden of infection in other organs, while fluconazole at 120 mg/kg/day reduced the tissue burden of aspergillus in liver and kidney by 100-fold compared with controls. Amphotericin B at 1.5 mg/kg/day was the most effective therapeutic regimen and was significantly better than amphotericin B at 0.5 mg/kg/day in reducing the tissue burden in liver and lung. Furthermore, the effectiveness of the lower dose of amphotericin B (0.5 mg/kg/day) was similar to those of all other treatment groups studied. Also, the effectiveness of the combination of fluconazole at 60 mg/kg/day plus amphotericin B at 0.5 mg/kg/day was not significantly different from those of all other treatment groups. Although neither of the combination treatment groups demonstrated antagonism, amphotericin B at 1.5 mg/kg/day was the only regimen that sterilized the liver and kidney and significantly reduced the tissue burden of *Aspergillus* organisms in the lung. These data suggest that high-dose amphotericin B therapy may be effective against invasive aspergillosis. Furthermore, we observed a significant difference (by χ^2 analysis) between amphotericin B at 0.5 and 1.5 mg/kg/day in the *Aspergillus* burden of the liver ($P < .001$) and lung ($P < .04$) that was not seen in any of the combination treatment groups when they were compared with amphotericin B at 0.5 mg/kg/day.

The number of positive organ cultures in the treated animals and controls is also shown in table 1. Amphotericin B at

Table 1. Cultures of tissue from temporarily immunosuppressed lethally challenged rabbits.

	Colony counts (mean $\log_{10}$ cfu/g of tissue ± SE)				No. positive cultures/ no. rabbits cultured			
Treatment group (*n*)	Liver	Lung	Kidney	Brain	Liver	Lung	Kidney	Brain
Control (18)	3.5 ± 0.1	2.8 ± 0.2	3.2 ± 0.2	0.8 ± 0.3	18/18	18/18	18/18	18/18
Flu 60 (10)	2.3 ± 0.5*	3.3 ± 0.2	2.9 ± 0.4*	0.9 ± 0.4	7/10	10/10	9/10	3/10†
Flu 120 (10)	1.4 ± 0.4*	2.1 ± 0.4	1.0 ± 0.4*	0.2 ± 0.2	6/10‡	8/10	4/10‡	1/10†
Flu 60 pro (12)	2.8 ± 0.3	3.0 ± 0.4	3.3 ± 0.2	1.0 ± 0.4	12/12	11/12	11/12	4/12†
AmB 0.5 (11)	2.6 ± 0.3*	2.7 ± 0.3	0.5 ± 0.3*	1.1 ± 0.5	10/11	10/11	3/11†	5/11‡
AmB 1.5 (8)	0*	1.2 ± 0.5*	0*	0.3 ± 0.3	0/8†	3/8‡	0/8†	1/8†
Flu 60 + AmB 0.5 (11)	2.7 ± 0.3	2.0 ± 0.5	1.1 ± 0.4*	1.2 ± 0.4	10/11	8/11	5/11‡	5/11‡
Flu 60 pro + AmB 0.5 (12)	3.3 ± 0.2	2.4 ± 0.3	0.8 ± 0.4*	1.5 ± 0.5	12/12	11/12	4/12†	6/12‡

NOTE. Flu 60, 120, fluconazole 60 or 120 mg/kg/day; AmB 0.5, 1.5, amphotericin B, 0.5 or 1.5 mg/kg/day; pro, prophylaxis.

* $P < .01$ vs. controls (Wilcoxon rank sum).

† $P < .001$ vs. controls (χ^2).

‡ $P < .005$ vs. controls (χ^2).

Table 2. Serum *Aspergillus* antigen values in temporarily immunosuppressed lethally challenged rabbits.

Treatment group	No. antigen-positive/ no. animals tested	Median antigen level in ng/mL (range)*
Control	9/9	1050 (235–5000)
Flu 60	9/10	45 (<10–330)
Flu 120	8/10	40 (<10–125)
Flu 60 pro	12/12	107 (20–1580)
AmB 0.5	11/11	37 (16–58)
AmB 1.5	7/8	21 (<10–28)
Flu 60 + AmB 0.5	11/11	32 (<10–320)
Flu 60 pro + AmB 0.5	12/12	76 (25–440)

NOTE. Flu 60, 120, fluconazole 60 or 120 mg/kg/day; pro, prophylaxis; AmB 0.5, 1.5, amphotericin B 0.5 or 1.5 mg/kg/day.

* Calculated from maximum antigen level for each rabbit in respective treatment group.

1.5 mg/kg/day sterilized liver and kidney ($P < .001$ vs. controls), but some infection remained in the lung of 3 and in the brain of 1 of 8 rabbits. Amphotericin B at 0.5 mg/kg/day was less effective than at 1.5 mg/kg/day in sterilizing all organs studied (table 1). Infection remained in the liver and lung of 10, in the brain of 5, and in the kidney of 3 of 11 rabbits treated with amphotericin B at the lower dose. Although fluconazole at 120 mg/kg/day reduced the tissue burden of infection, the liver, lung, kidney, and brain of some animals remained infected, as did organs in those receiving fluconazole as treatment or prophylaxis at 60 mg/kg/day alone or in combination with amphotericin B at 0.5 mg/kg/day (table 1). No evidence of antagonism was seen with the combination of fluconazole and amphotericin B.

These doses of fluconazole and amphotericin B alone and in combination dramatically reduced or eliminated antigenemia compared with untreated controls (table 2). Low levels of circulating antigen, remaining in most of the treated animals, correlated with the persistence of infection, as was observed in our earlier studies [4, 5, 8–12]. However, the lowest median antigen level was observed with high-dose amphotericin B (1.5 mg/kg/day) therapy.

Sublethal challenge. Untreated immunosuppressed rabbits challenged with a sublethal inoculum of *A. fumigatus* conidia developed invasive aspergillosis at a mortality rate of 35% (7/20). Only rabbits treated with flucytosine at 100 mg/kg/day had a mortality rate (33% [3/9]) comparable to that of untreated animals, even though the MIC of flucytosine was 1.6 μg/mL by agar dilution against our strain of *A. fumigatus*. In comparison, animals receiving fluconazole at 60 or 120 mg/kg/day as treatment (1/9 [11%] and 2/13 [15%], respectively) or prophylaxis (2/14 [14%] and 0/7, respectively) had lower mortality rates, as did animals treated with fluconazole at 120 mg/kg/day plus flucytosine at 100 mg/kg/day (2/15 [13%]). However, survival was 100% in groups treated with amphotericin B at 0.25 or 0.5 mg/kg/day, prophylactic fluconazole alone at 120 mg/kg/day, prophylactic fluconazole at 60 mg/kg/day plus amphotericin B at 0.25 or 0.5 (0/10) mg/kg/day, or amphotericin B at 0.5 plus flucytosine at 100 mg/kg/day. Thus, lower mortality rates were seen in all groups treated with combination therapy. Even though there was no significant difference in mortality rates among any of the treated groups of animals, those receiving fluconazole or amphotericin B either alone or in combination with another agent had lower death rates. No antagonism was seen with combination therapy. However, in the doses used in these studies, combination therapy did not improve survival significantly above that found with amphotericin B in sublethally challenged rabbits.

Semiquantitative results of organ cultures from rabbits given a sublethal challenge are shown in table 3. Extensive infection occurred in liver, lung, and kidney tissues of untreated control animals. Fluconazole at 60 or 120 mg/kg/day, either prophylactically or therapeutically, reduced the tissue burden of *A. fumigatus* in almost all organs (except the brain) by 10- to 100-fold compared with untreated controls. Similarly, the tissue burden of infection was reduced in liver, lung, and kidney of animals treated with amphotericin B at 0.25 or 0.5 mg/kg/day or flucytosine at 100 mg/kg/day, except for the livers of rabbits treated with amphotericin B at 0.25 mg/kg/day (table 3). Combination therapy with fluconazole plus amphotericin B at both doses studied, fluconazole plus flucytosine, and amphotericin B plus flucytosine also significantly reduced the tissue burden of infection compared with untreated controls, except for the livers of animals treated with fluconazole at 120 mg/kg/day plus flucytosine at 100 mg/kg/day. There was no evidence of antagonism in the animals treated with combination therapy.

The number of positive organ cultures in the treated animals and controls is also shown in table 3. There was a significant difference in the number of organs sterilized, for most tissues studied, by each treatment compared with controls, except for animals treated with fluconazole at 60 mg/kg/day or flucytosine at 100 mg/kg/day. Results with the combination of fluconazole plus flucytosine were significantly different from those in untreated controls only for lung tissue (table 3). No antagonism was observed in any of the combination treatment groups.

All treatment regimens reduced antigenemia substantially compared with untreated controls (table 4). Serum antigen levels were reduced to <100 ng/mL in animals treated with amphotericin B at 0.5 mg/kg/day, amphotericin B at 0.5 mg/kg/day plus fluconazole at 60 mg/kg/day, and amphotericin B at 0.5 mg/kg/day plus flucytosine at 100 mg/kg/day. Amphotericin B at 0.25 mg/kg/day and fluconazole at either 60 or 120 mg/kg/day resulted in median serum antigen levels >100 ng/mL. Animals treated with the combination of fluconazole at 60 mg/kg/day plus amphotericin B at 0.25 mg/

Table 3. Cultures of tissue from temporarily immunosuppressed sublethally challenged rabbits.

Treatment group (*n*)	Colony counts (mean $\log_{10}$ cfu/g of tissue ± SE)				No. positive cultures/no. rabbits cultured			
	Liver	Lung	Kidney	Brain	Liver	Lung	Kidney	Brain
Control (20)	3.5 ± 0.1	3.1 ± 0.0*	2.9 ± 0.3*	0.4 ± 0.0	20/20	19/19	17/19	5/20
Flu 60 (9)	2.0 ± 0.5†	3.0 ± 0.2	2.1 ± 0.3	0.4 ± 0.2	7/9	9/9	9/9	2/9
Flu 120 (13)	2.3 ± 0.3†	1.4 ± 0.4†,‡	1.5 ± 0.5	0.2 ± 0.1	13/13	7/11	6/13	1/13
Flu 60 pro (14)	1.4 ± 0.4†	2.2 ± 0.8†	1.2 ± 0.1	0.2 ± 0.2	7/14§	8/14	5/14§	2/14
Flu 120 pro (7)	0.3 ± 0.3†	1.8 ± 0.5†	0†	0	1/17‖	5/7	0/7‖	0/7
AmB 0.25 (8)	2.8 ± 0.4	0.8 ± 0.4†	0.7 ± 0.4†	0	7/8	3/8§	3/8	0/8
AmB 0.5 (11)	1.6 ± 0.4†	1.3 ± 0.4†	0†	0.4 ± 0.3	7/11	6/11§	0/11‖	1/11
Flucytosine 100 (9)	3.1 ± 0.1	2.3 ± 0.1†	2.3 ± 0.5†	0.5 ± 0.4	9/9	9/9	7/9	2/9
Flu 60 pro + AmB 0.25 (9)	2.1 ± 0.6†	1.4 ± 0.4†	0†	0	6/9	6/9	1/9‖	0/9
Flu 60 pro + AmB 0.5 (10)	1.5 ± 0.4†	0.7 ± 0.4†	0.5 ± 0.3†	0	7/10	3/10‖	2/10‖	0/10
Flu 120 + 5-FC 100 (14)	2.9 ± 0.2	1.6 ± 0.4†	1.8 ± 0.4	0¶	15/15	9/15	9/15	0/14
AmB 0.5 + 5-FC 100 (5)	0.4 ± 0.4†,**	0†,**	0†	0	1/5‖	0/5‖	0/5‖	0/5

NOTE. Flu 60, 120, fluconazole 60 or 120 mg/kg/day; AmB 0.25, 0.5, amphotericin B 0.25 or 0.5 mg/kg/day; 5-FC 100, flucytosine 100 mg/kg/day; pro, prophylaxis.

* Data from 19 rabbits.

† $P < .01$ vs. controls (Wilcoxon rank sum).

‡ Data from 11 rabbits.

§ $P < .005$ vs. controls (χ^2).

‖ $P < .001$ vs. controls (χ^2).

¶ Data from 13 rabbits.

** $P < .05$ vs. AmB 0.5 (Wilcoxon rank sum).

kg/day had lower median antigen levels than those treated with amphotericin B at 0.25 mg/kg/day only.

Discussion

The outcome of invasive aspergillosis in immunosuppressed patients remains poor even with the use of amphotericin B [2, 3, 6]. Recovery of neutropenia appears to be of signal importance in determining prognosis [6]. Therefore, agents with improved efficacy and decreased toxicity are needed to improve the management of aspergillus infections in these patients. Also, antifungal prophylaxis or early empiric antifungal therapy may reduce the morbidity and mortality associated with invasive aspergillosis in high-risk patients [14–17]. Newer azoles, used alone or in combination with other antifungal agents, may offer several advantages in the treatment of invasive fungal infection. Some of these newer azoles (e.g., fluconazole, itraconazole, and saperconazole) demonstrate several of these advantageous characteristics, including broad-spectrum antifungal activity, excellent absorption after oral administration, and minimal acute toxicity [12]. Fluconazole also has solubility characteristics that permit intravenous administration, extensive central nervous system penetration, and a prolonged half-life in humans [10, 18, 19]. In earlier studies, fluconazole was shown to have significantly more activity against *A. fumigatus* and *Aspergillus flavus* than ketoconazole in a murine model [20]. This protective effect of fluconazole against aspergillosis, however, was not confirmed in other studies with a murine model but these results may be due to the relatively rapid clearance of fluconazole in mice [19].

Combination therapy with an azole plus amphotericin B or the use of an azole prophylactically followed by amphotericin B therapeutically for invasive aspergillosis may improve the management of patients at risk for this infection [6]. However, the oral imidazole ketoconazole, which has

Table 4. Serum *Aspergillus* antigen values in temporarily immunosuppressed sublethally challenged rabbits.

Treatment group	No. antigen-positive/ no. animals tested	Median antigen level in ng/mL (range)*
Control	18/18	2000 (35–5000)
Flu 60	9/9	195 (<10–500)
Flu 60 pro	14/14	143 (21–1000)
Flu 120	10/11	102 (<10–5000)
Flu 120 pro	6/7	265 (<10–500)
AmB 0.25	6/8	640 (<10–2000)
AmB 0.5	10/11	13 (<10–30)
Flucytosine 100	8/9	155 (<10–550)
Flu 60 pro + AmB 0.25	8/9	335 (<10–500)
Flu 60 pro + AmB 0.5	7/10	14 (<10–28)
Flu 120 + 5-FC 100	13/14	152 (<10–5000)
AmB 0.5 + 5-FC 100	5/5	32 (19–120)

NOTE. Flu 60, 120, fluconazole 60 or 120 mg/kg/day; pro, prophylaxis; AmB 0.25, 0.5, amphotericin B 0.25 or 0.5 mg/kg/day; 5-FC 100, flucytosine 100 mg/kg/day.

* Calculated from maximum antigen level from each rabbit in respective treatment group.

limited in vitro activity against *Aspergillus* organisms, is not useful for treating patients with invasive aspergillosis and has been shown to be antagonistic to amphotericin B in an experimental model of disseminated aspergillosis [7]. These data have caused some concern about combining an azole with amphotericin B in the treatment of fungal infections. Therefore, the present study was designed to evaluate the efficacy of combination antifungal therapy in our experimental model of invasive aspergillosis, with particular attention to the possibility of antagonism.

In earlier studies in our immunocompromised rabbit model of invasive aspergillosis, amphotericin B alone and fluconazole alone significantly reduced mortality as well as the level of aspergillus antigen in the serum, which correlated with the reduced tissue burden of *A. fumigatus* compared with untreated control animals [5, 9, 10]. Oral fluconazole reduced the tissue burden of *Aspergillus* organisms by 10- to 100-fold, but only amphotericin B at 1.5 mg/kg/day sterilized tissues [9, 10]. In the present studies, combination therapy and prophylaxis followed by combination therapy were evaluated in our lethal and sublethal model of invasive aspergillosis in an attempt to determine the efficacy of treatment and prophylaxis in both severe infection and moderate disease.

This rabbit model of invasive aspergillosis provides a rigorous test of efficacy when animals receive a lethal challenge of *A. fumigatus.* Mortality in untreated animals approaches 100%. In the present study, survival was significantly prolonged with fluconazole or amphotericin B at all doses studied and with combination therapy, even when lower doses of fluconazole and amphotericin B were used. Antagonism was not observed when amphotericin B and fluconazole were used in combination in these doses as judged by mortality, tissue burden of aspergillus organisms, and serum levels of aspergillus antigen. We also observed a lower mortality in animals given fluconazole alone as prophylaxis compared with the drug as therapy, though these differences were not statistically significant. Also, antagonism was not observed when fluconazole was given prophylactically followed by amphotericin B when evaluated by these same criteria. However, high-dose amphotericin B was the most effective therapy observed in this model, which suggests that higher dosages of amphotericin B may be beneficial in the treatment of invasive aspergillosis.

Similar results were observed in the present studies when animals were given a sublethal challenge. Only rabbits treated with flucytosine had a mortality rate comparable to that of untreated animals. In contrast, lower mortality rates were observed in all other treatment groups, including animals receiving fluconazole alone as treatment or prophylaxis, amphotericin B alone at low doses, or combination therapy with fluconazole plus amphotericin B, fluconazole plus flucytosine, or amphotericin B plus flucytosine. Again, no evidence of antagonism was observed in animals given a combination of two antifungal agents, whether the combination included fluconazole and amphotericin B, flucytosine and amphotericin B, or fluconazole and flucytosine. However, higher-dose amphotericin B was the most effective treatment in animals receiving a sublethal challenge of aspergillus organisms.

Combination antifungal therapy has also been studied in a neutropenic rabbit model of candidiasis, and amphotericin B combined with flucytosine was observed to be the most effective therapy [21]. In these studies, amphotericin B combined with ketoconazole was also superior to amphotericin B alone against infection in the kidney but was not as effective as amphotericin B combined with flucytosine. Importantly, no antagonism was observed between amphotericin B and ketoconazole in that model of candidal infection [21]. Also, recent studies have evaluated the postantifungal effect of flucytosine and fluconazole separately and in combination on *Candida albicans* in vitro [22]. A synergistic interaction of the two drugs at concentrations well below their individual MICs was observed, and the combination of these two antifungal agents induced a postantifungal effect that persisted 2½ h longer than those observed with each antifungal agent separately [22].

Few clinical studies have evaluated the efficacy of combination antifungal therapy or prophylaxis for invasive aspergillosis, and these studies were noncomparative [15, 23]. Nevertheless, the use of amphotericin B combined with flucytosine resulted in survival of 13 of 14 patients with invasive aspergillosis when this therapy was begun very early after the development of symptoms [24]. However, granulocytopenia resolved in all 13 patients who survived the infection. Similarly, the use of high-dose amphotericin B plus flucytosine has been evaluated as prophylaxis in patients who have had a previous episode of invasive aspergillosis and who have required another course of chemotherapy [15]. Antifungal therapy was started 48 h before chemotherapy and continued until resolution of granulocytopenia, and no patient died from invasive aspergillosis [15]. In this context, the present studies demonstrated significant improvement in the lung and liver of rabbits given a sublethal challenge of aspergillus organisms and treated with amphotericin B plus flucytosine compared with amphotericin B alone. Antagonism between flucytosine and amphotericin B was not observed. Similarly, antagonism was not observed between fluconazole and amphotericin B or fluconazole and flucytosine in this rabbit model of invasive aspergillosis. Thus, the antagonism reported previously between ketoconazole and amphotericin B [7] does not appear to be present when ketoconazole is replaced by fluconazole in that combination.

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