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In Vitro Activity and *In Vivo* Efficacy of Anidulafungin in Murine Infections by *Aspergillus flavus*▽

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Anidulafungin (AFG) showed high activity against 27 strains of *Aspergillus flavus* by use of broth microdilution and disk diffusion methods. This drug was effective *in vivo* in a murine model of disseminated infection with five isolates tested. AFG was able to prolong survival and reduce tissue burden of infected mice but not able to reduce galactomannan serum concentrations. The AFG serum levels were above the corresponding minimum effective concentrations (MEC) for all of the strains tested.

Aspergillus flavus is the second most important agent of invasive aspergillosis (IA) after *Aspergillus fumigatus* (9) and is considered more virulent and resistant to antifungal drugs than other *Aspergillus* species (6). Voriconazole is the recommended drug for primary treatment of IA, with lipid formulations of amphotericin B and echinocandins being alternative therapies when voriconazole fails (8, 18). Anidulafungin (AFG) shows *in vitro* and *in vivo* activities against *Aspergillus* similar to those of the other echinocandins (16) but seems to have a wider range of action, a lower toxicity, and fewer drug interactions (2). Nevertheless, AFG has been evaluated *in vivo* only against *A. fumigatus* experimental infections (11, 12, 17), and little is known about its *in vivo* efficacy against other *Aspergillus* species.

We have tested the *in vitro* activities of AFG against 27 *A. flavus* strains from different origins by using two different methods, microdilution (3) and disk diffusion methods, determining the minimum effective concentrations (MEC) (5) and the inhibition zone diameters (IZD), respectively, in order to determine if there were any significant differences among both techniques.

Disk diffusion was carried out using nonsupplemented Mueller-Hinton agar and 6-mm-diameter paper disks containing 5 μg of AFG (4).

For the *in vivo* studies, we chose five isolates. Male OF1 mice were immunosuppressed (13) and used following the procedural standards approved by the Animal Welfare and Ethics Committee of Rovira i Virgili University. Mice were challenged intravenously with a conidial suspension of 1×10^4 CFU in 0.2 ml of saline. AFG was administered at 1, 5, or 10 mg/kg of body weight intraperitoneally once a day. These doses were selected based on previous experimental studies of aspergillosis by *A. fumigatus* (11, 12, 16). All treatments began 24 h after challenge and lasted for 7 days. Groups of 10 animals were established. Control animals received no treatment. For survival studies, mice were checked daily for 30 days. For tissue burden studies, animals were sacrificed on day 5 after infection. Kidneys and spleens were aseptically removed and homogenized in 1 ml sterile saline. Serial dilutions were plated on potato dextrose agar and incubated for 24 h at 35°C.

Mean survival times were estimated by the Kaplan-Meier method and compared among groups using the log rank test. Colony counts in tissue burden studies were analyzed by the Mann-Whitney U test. A P value of ≤ 0.05 was considered statistically significant.

In addition, groups of 5 immunosuppressed mice were challenged with the strain FMR 10084 to determine galactomannan serum levels by enzyme immunoassay (Platelia *Aspergillus*) on the last day of therapy, as a marker of the treatment response.

Additional groups of 5 mice were similarly infected with strain FMR 10084 and treated with the same doses described above to determine levels of AFG in serum by bioassay. Drug standards were prepared in methyl alcohol and placed in wells of 4 mm in diameter in yeast nitrogen base agar. *Candida albicans* ATCC 90028 was the test organism. To calculate serum drug concentrations, diameters of growth inhibition for serum from treated mice, 4 h after the last dosing on day 5 of therapy, were compared to the standard straight line.

Both MEC (≤ 0.06 μg/ml) and IZD (22 to 35 mm) values suggested a good *in vitro* activity of AFG against *A. flavus*.

All of the AFG doses significantly prolonged survival for all strains tested, with the exception of AFG at 1 mg/kg for two strains. For two strains, AFG at 10 mg/kg significantly improved the results obtained with AFG at 1 mg/kg. In any case, there were no significant differences between the doses of AFG at 5 and 10 mg/kg (Fig. 1).

In spleen and kidney, all of the AFG doses significantly reduced the fungal load with respect to that for the control group, with the exception of the lowest dose against one strain in each organ. In both organs and with a few exceptions, AFG showed a dose-dependent response (Table 1).

Bioassay results are shown in Table 2. At day 5 of treatment, antifungal levels in serum were above the corresponding MEC values for all of the strains tested, increasing significantly with dose escalation. None of the doses of AFG was able to significantly reduce the galactomannan serum concentrations in comparison with that for the control group at the end of therapy (Table 2).

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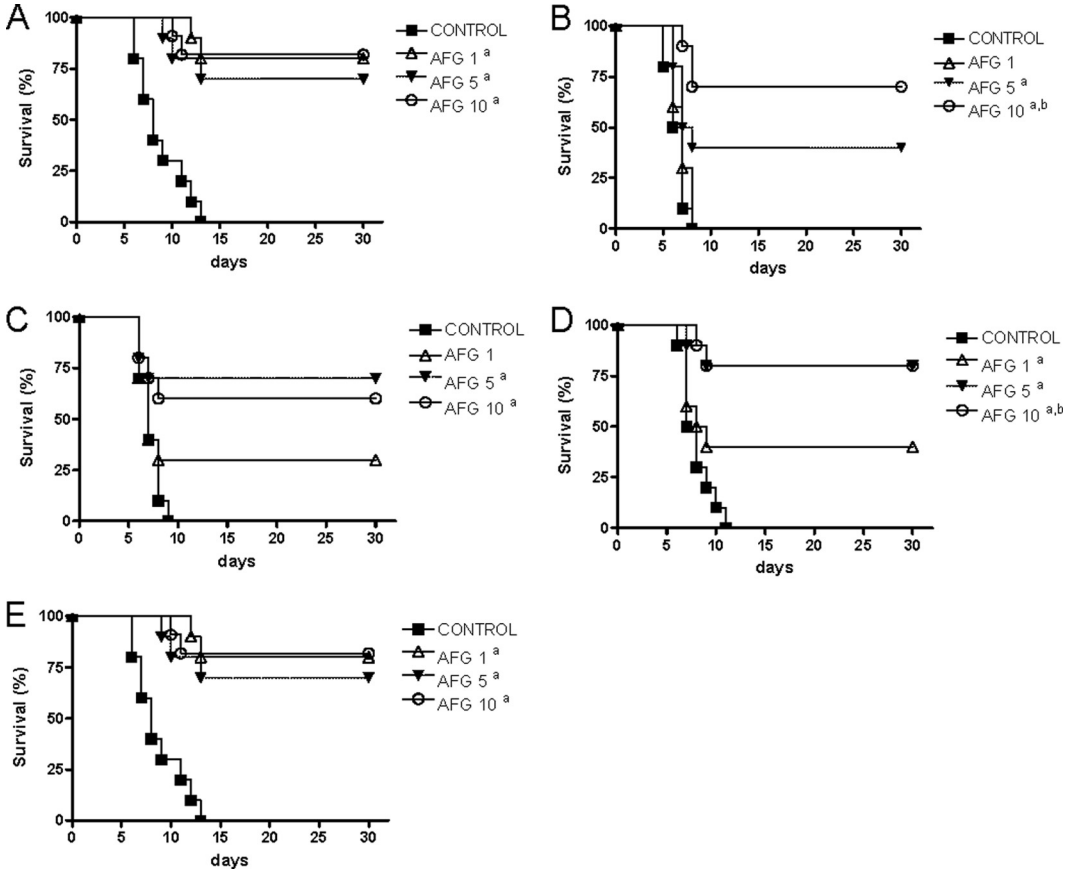

FIG. 1. Cumulative mortality of mice infected with 1×10^4 CFU of strain FMR 9965 (A), FMR 8756 (B), FMR 9960 (C), FMR 10084 (D), or FMR 9966 (E) of *A. flavus*. AFG 1, anidulafungin at 1 mg/kg; AFG 5, anidulafungin at 5 mg/kg; AFG 10, anidulafungin at 10 mg/kg. a, $P < 0.05$ versus control; b, $P < 0.05$ versus AFG at 1 mg/kg.

TABLE 1. Effects of antifungal treatment on colony counts of *A. flavus* FMR 9965, FMR 8756, FMR 9960, FMR 10084, and FMR 9966 in spleen and kidney tissues of mice

A. flavus isolate	AFG dose (mg/kg/day)	Mean $\log_{10}$ CFU/g (95% CI)[a]	
		Spleen	Kidney
FMR 9965	None	2.54 (2.31–2.76)	3.28 (3.15–3.41)
	1	2.07 (1.94–2.20)a	1.80 (1.27–2.33)a
	5	1.92 (1.71–2.12)a	1.76 (1.43–2.09)a
	10	1.77 (1.63–1.91)a,b	0.81 (0.47–1.14)a,b,c
FMR 8756	None	2.67 (2.51–2.82)	2.65 (2.51–2.79)
	1	2.43 (2.30–2.56)a	2.20 (1.94–2.45)a
	5	2.26 (2.12–2.41)a	1.67 (1.29–2.05)a,b
	10	2.23 (2.06–2.41)a	1.55 (1.15–1.94)a,b
FMR 9960	None	2.58 (2.42–2.74)	2.36 (2.18–2.54)
	1	2.34 (2.17–2.51)a	2.27 (2.07–2.46)
	5	2.34 (2.20–2.48)a	1.71 (1.40–2.02)a,b
	10	2.23 (2.09–2.37)a	1.57 (1.25–1.90)a,b
FMR 10084	None	2.51 (2.34–2.68)	3.15 (2.95–3.36)
	1	2.14 (1.98–2.30)a	2.00 (1.16–2.83)a
	5	2.08 (1.95–2.22)a	1.36 (0.98–1.74)a
	10	1.82 (1.70–1.96)a,b,c	1.08 (0.61–1.55)a,b
FMR 9966	None	2.90 (2.80–3.00)	2.93 (2.83–3.04)
	1	2.73 (2.57–2.89)	2.36 (2.10–2.62)a
	5	2.68 (2.52–2.83)a	1.23 (0.95–1.52)a,b
	10	2.64 (2.53–2.76)a	1.40 (1.12–1.69)a,b

[a] 95% CI, 95% confidence interval. a, $P < 0.05$ versus control; b, $P < 0.05$ versus AFG at 1 mg/kg; c, $P < 0.05$ versus AFG at 5 mg/kg.

Histological studies of control mice and, to a lesser degree, those treated with AFG at 1 mg/kg showed kidney invasion by fungal cells, with signs of necrosis, like parenchyma destruction and karyolysis, caused by angioinvasion and consequent ischemia, but an inflammatory response was not observed. Mice treated with 5 and 10 mg/kg showed renal congestion, but there was no sign of necrosis, inflammatory response, or invasion by fungal elements.

Several experimental and clinical studies have demonstrated good *in vitro* and *in vivo* activities of echinocandins against aspergillosis (18). Our *in vitro* results showed a remarkable

TABLE 2. Determination of AFG and galactomannan levels in serum[a]

AFG dose (mg/kg)	Serum level (μg/ml), mean ± SD	GMI[b], mean ± SD
None		6.48 ± 0.34
1	2.36 ± 1.21	6.28 ± 0.12
5	10.14 ± 1.04	6.30 ± 0.17
10	13.19 ± 1.65	6.22 ± 0.22

[a] AFG levels in serum were determined on day 5 of therapy for mice infected with 1×10^4 CFU of *A. flavus* strain FMR 10084, 4 h after the last dosing; galactomannan levels in serum were determined for mice infected with 1.4×10^2 CFU of the same strain and treated for 7 days with AFG.

[b] GMI, galactomannan index.

correlation between the results obtained with disk diffusion and broth microdilution methods. In our *in vivo* study, AFG demonstrated high efficacy against *A. flavus* infections. Other authors had already demonstrated the efficacy of AFG in experimental infections but against *A. fumigatus* (11, 12, 17). In the current study, although the three doses of AFG showed serum concentrations above the MECs of the isolates tested and were effective against the fungal infection, the two higher doses performed better than 1 mg/kg, suggesting a concentration-dependent efficacy, as other authors previously observed with caspofungin (19).

In contrast, the good results obtained with the therapy were not accompanied by a decrease in galactomannan serum levels. This fact was also observed in disseminated and pulmonary murine infections by *A. fumigatus* (1, 10, 14, 15). It has been suggested that due to their fungistatic activity, the echinocandins are not able to reduce galactomannan levels in serum, since this phenomenon was not observed in the treatment of aspergillosis with fungicidal drugs, like azoles (1). Similarly, in a case of invasive aspergillosis treated with caspofungin (CSP), an unexpected rise in galactomannan levels was observed (7). In this case, the authors suggested that the reduction in 1,3-β-D-glucan caused by the exposure to echinocandins and the consequent degradation of the cell wall could lead to a release of galactomannan, thus increasing its circulating levels.

Our results suggest that AFG may be considered a therapeutic option in the management of IA.

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