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Original article

Combined action of micafungin, a new echinocandin, and human phagocytes for antifungal activity against *Aspergillus fumigatus*

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Abstract

Micafungin, a new echinocandin, inhibits fungal cell wall beta-glucan synthesis. We postulated micafungin and host phagocytic cells could act together in damaging fungi. Using the metabolic XTT assay, micafungin alone (0.01 and 0.10 μg/ml) inhibited *Aspergillus fumigatus* germlings by 48% and 61%, respectively. Polymorphonuclear neutrophils (PMNs) inhibited germlings by 53%. Micafungin at 0.01 or 0.10 μg/ml and PMNs resulted in additive inhibition, 82% and 99%, respectively. Monocyte-derived macrophage (MDM) monolayers inhibited germling growth by 66%; micafungin (0.01 or 0.10 μg/ml) alone inhibited by 32% and 42%, respectively. MDMs and micafungin (0.01 or 0.10 μg/ml) caused an additive inhibition of growth, 85% and 95%, respectively. Hyphae were generated by incubation of conidia for 24 h with or without micafungin. PMNs alone, added to hyphae, inhibited growth by 19% in the subsequent 20 h. Hyphae generated in the presence of micafungin (0.10 μg/ml) and subsequently cultured with micafungin for 24 h inhibited growth by 64%. PMNs plus micafungin resulted in 82% inhibition. Monocytes alone inhibited hyphal growth by only 5%. Hyphae produced in the presence of micafungin (0.01 μg/ml) and incubated again with micafungin for 24 h inhibited growth by 47%; combination with monocytes resulted in 62% inhibition. These data indicate that micafungin inhibits growth of tissue forms of *A. fumigatus*, and phagocytes and micafungin together have an additive effect. These findings support the thesis that the greater efficacy of micafungin in vivo compared with in vitro could be due to combined effect of phagocytic cells and micafungin.

Keywords: Micafungin; Human phagocytes; *Aspergillus fumigatus*

1. Introduction

Invasive fungal infections, especially invasive aspergillosis, have become an important cause of mortality and morbidity among immunocompromised patients in recent years. Despite progress in diagnosis by using molecular techniques, antifungal strategies, and the development of new antifungal drugs, the results are still disappointing [1]. Immunomodulation and the collaborative effect of antifungal agents with host cells are currently under study in fungal infections [2–7].

Micafungin (FK463, Fujisawa) is of the echinocandin class of antifungals that inhibits beta-1,3-D-glucan synthesis in the fungus cell wall [8]. Micafungin causes incomplete growth inhibition of *Aspergillus* species in vitro, but micafungin has excellent activities in several mouse infection models of *Aspergillus fumigatus* [9–11]. Antifungal efficacy in animal models is considered to be the combined result of the host defense system in addition to the direct antifungal effect of the drug itself [8,12].

The aim of the present study was to examine the efficacy and collaboration of micafugin with human polymorphonuclear neutrophils (PMNs), monocytes, or monocyte-derived macrophages (MDMs) against tissue forms of *A. fumigatus*.

2. Materials and methods

2.1. A. fumigatus

A patient isolate of *A. fumigatus*, AF-10, stored at the California Institute for Medical Research, San Jose, CA was

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used for all experiments. AF-10 was incubated on Saboraud's dextrose agar plates at 37 °C to form conidia. The conidia were collected in 0.05% Tween-80 in saline and filtered through sterile gauze. The conidia were washed, diluted in saline, and counted. To make germlings (single filament without septa), conidia were incubated in RPMI-1640 at 37 °C for 4 h and then for 24 h at room temperature. To make hyphae, conidia were incubated in RPMI-1640 at 37 °C in 5% CO_2 + 95% air for 24 h.

2.2. Micafungin (FK 463)

Micafungin was supplied by Fujisawa Pharmaceutical Company, Osaka, Japan and stored at 4 °C. Micafungin was diluted in RPMI-1640 or complete tissue culture medium (CTCM, RPMI-1640 plus 10% autologous human serum) and added to cultures to obtain the desired final concentration.

2.3. PMN assay

PMNs were isolated by 6% dextran-70 sedimentation of heparinized human blood, followed by density gradient centrifugation of the buffy coat diluted 1:1 with RPMI-1640 on Histopaque 1077 (Sigma Chemical Co., St Louis, Mo.). The pelleted cells were collected in 0.85% NH_4Cl to lyse contaminating red blood cells. PMNs were washed, counted, and suspended in CTCM. PMNs were added to wells of 96-well microtest plates (Becton Dickinson, Franklin Lakes, NJ, #353072) containing *A. fumigatus* germlings or hyphae and incubated with and without micafungin at 37 °C in 5% CO_2 + 95% air for 24 h. In experiments with hyphae of *A. fumigatus*, hyphae were produced in the presence or absence of micafungin, and micafungin was present during the time of challenge with PMNs. The XTT assay was performed to measure antifungal activity of micafungin, PMNs, and the combination of micafungin plus PMNs.

2.4. Monocyte assay

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized human blood by 6% dextran-70 sedimentation, followed by density gradient centrifugation of buffy coat diluted 1:1 with RPMI-1640 on Histopaque 1077 (Sigma). PBMC layers were harvested, washed, and counted. Three milliliters of PBMCs (2.5×10^6/ml CTCM) was plated in human serum-coated 120-mm plastic Petri dishes and incubated for 2 h at 37 °C. Non-adherent cells were aspirated, and plates were washed twice with warm RPMI-1640. Adherent cells were collected after incubation of plates with 0.01 M EDTA mixed with an equal volume of RPMI-1640 for 15 min at room temperature. Non-adherent cells were washed, counted, and suspended in CTCM. Fifteen to 20% of plated PBMCs were obtained as adherent PBMCs and termed monocytes. Monocytes were added to microtest plate wells containing *A. fumigatus* germlings. In studies of hyphae, conidia were incubated first with or without micafungin at 37 °C for 24 h; then monocytes ± micafungin were added to hyphae. The XTT assay was performed to measure antifungal activity of micafungin, monocytes, and the combination of micafungin plus monocytes.

2.5. MDM assay

PBMCs were collected from heparinized blood, as described above. Then, 0.2 ml of 5×10^6 PBMCs/ml CTCM was dispensed per well of a microtest plate. After incubation for 2 h at 37 °C in 5% CO_2 incubator, non-adherent cells were aspirated, and wells were rinsed once with RPMI-1640. Approximately 10–15% of plated PBMCs were adherent, 5×10^5/well. Adherent cells were incubated in CTCM for 5 days with a change of medium every 48 h. MDMs were challenged with germlings in RPMI-1640 (2×10^6/ml). Germlings were added to MDM monolayer ± micafungin and cultured for 24 h at 37 °C in 5% CO_2 + 95% air. Cultures were washed several times with distilled water to lyse MDMs, and the XTT assay was performed.

2.6. XTT assay

Cultures in microtest plate wells were centrifuged (2000 rpm, 10 min), and supernatants were carefully aspirated. Wells were washed twice with 0.2 ml of distilled water. Human cells were lysed or killed, as shown by debris and deformed cells observed by microscopy. Fungal growth was measured by the colorimetric XTT-coenzyme Q method. (2,3)-*Bis*-(2-methoxy-4-nitro-5-sulphenyl-(2H)-tetrazolium-5-carboxanilide) sodium salt (XTT, Sigma) at 0.5 mg/ml plus 2.3-dimethoxy-5-methyl-1,4-benzoquinone (coenzyme Q, Sigma) at 0.04 mg/ml in phosphate-buffered saline (pH 7.4) constituted the test solution. XTT test solution (0.2 ml) was added to each well, and incubation at 37 °C for 1 h was performed. Then, 0.1 ml of aliquot was transferred to corresponding wells of another plate, and the absorbance at 450 nm was recorded with a microtest reader (OpsysMR™, Dynex Technologies INC., Chantilly, VA) as previously described [2,3].

To determine the change in absorbance (ΔAb) in experimental cultures, absorbance by XTT alone was subtracted from absorbance by experimental cultures. The percentage of inhibition was calculated by the formula [1-(ΔAb experimental/ΔAb control)] × 100. The rationale of this method has already been described [2,3].

2.7. Statistics

Student's *t*-test was used for statistical analysis of the data, and significance was set at $P < 0.05$. The GB-STAT program (Microsoft, Richmond, VA) was used for Bonferroni's adjustment to the *t*-test, where appropriate.

3. Results

3.1. Effect of micafungin on the growth and germination of A. fumigatus conidia

A. fumigatus conidia were incubated with 1, 20, 100 μg/ml micafungin in RPMI-1640 for 24 h at 37 °C in 5% CO_2 + 95% air. As shown in Table 1, growth from conidia was inhibited in a concentration-dependent manner. Hyphae produced by conidia in the presence of micafungin in this experiment showed morphological changes, i.e. antler-like growth compared with normal hyphal form.

In a second experiment, lower concentrations of micafungin (0.01, 0.10, and 1.00 μg/ml) were tested against 10^2, 10^3, and 10^4 conidia/well. Micafungin at 0.01 μg/ml inhibited growth from 10^2 conidia (94%), 10^3 conidia (61%), and 10^4 conidia (50%), indicating that antifungal activity of micafungin was conidial inoculum size dependent. Similar results were obtained with micafungin at 0.1 and 1.0 μg/ml under these conditions.

3.2. Effect of low concentrations of micafungin on the growth of A. fumigatus germlings

A. fumigatus germlings were made using the method described above (0.1 ml/well) and treated with 0.1 ml of CTCM ± lower concentration (0.01, 0.05, and 0.1 μg/ml) of micafungin. Two sources of serum (commercial human serum and fresh frozen human serum) were used to compare any potential difference according to the type of CTCM. Micafungin showed significant concentration-dependent growth inhibition of *A. fumigatus* germlings, even at a concentration of 0.01 μg/ml (Table 2). In contrast to the conidial experiment, low concentration of micafungin in CTCM did not induce morphological changes in germlings as they grew into hyphae.

3.3. Activity of PMNs and micafungin against A. fumigatus germlings

As shown in Table 3, PMNs alone significantly inhibited *A. fumigatus* germlings' growth, by more than 50%. Micafungin, even at a concentration of 0.01 μg/ml, inhibited growth of germlings. There was a collaborating effect between PMNs and micafungin. Inhibition by PMNs plus micafungin against *A. fumigatus* germlings was significantly higher than either PMNs or micafungin alone. Importantly, PMNs plus >0.05 μg/ml concentration of micafungin completely inhibited growth of germlings as measured by this assay.

In another experiment, where the conidia inoculum was 10^3/well and PMNs 8×10^5/well, inhibition by PMNs alone was 78%, micafungin (0.01 μg/ml) 29%, and the combination 85%. This indicates that the optimal conditions for showing additive collaboration of PMNs and micafungin against germlings are those shown in Table 3.

3.4. Activity of MDMs and micafungin against A. fumigatus germlings

Compared with PMNs, MDMs showed similar growth inhibitory activity against *A. fumigatus* germlings, when they were added to MDM monolayers. MDMs plus micafungin produced significantly more inhibition of *A. fumigatus* ger-

Table 1
Effect of micafungin (FK, μg/ml) on the germination and growth of *A. fumigatus* conidia

Treatment	ΔA_{450} [a]	% Inhibition	$P<$ [b]		
RPMI-1640	239 ± 22	0	Ref		
+FK 1.0	137 ± 20	43	0.01	Ref	
+FK 20.0	92 ± 32	62	0.01	NS	Ref
+FK 100.0	58 ± 12	76	0.01	0.01	NS

[a] Conidia, 10^3/well in RPMI-1640, were incubated overnight at 37 °C with or without 1, 20, or 100 μg/ml micafungin. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; NS, not significant ($P > 0.05$).

Table 2
Effect of lower dose of micafungin (FK, μg/ml) on the growth of *A. fumigatus* germlings

Treatment	ΔA_{450} [a]	% Inhibition	$P<$ [b]		
RPMI + 10% CHS [c]	825 ± 167	0	Ref		
+FK 0.01	530 ± 63	36	0.05	Ref	
+FK 0.05	375 ± 86	55	0.01	0.05	Ref
+FK 0.1	292 ± 79	65	0.01	0.01	NS
RPMI + 10% FFHS [c]	911 ± 59	0	Ref		
+FK 0.01	604 ± 59	34	0.01	Ref	
+FK 0.05	368 ± 67	60	0.01	0.01	Ref
+FK 0.1	264 ± 12	70	0.01	0.01	0.05

[a] Conidia, 10^3/well in RPMI-1640, were incubated 4 h at 37 °C, then 18 h at room temperature to produce germlings. Serum and 0.01, 0.05, or 0.1 μg/ml of micafungin were added and incubated 18 h more, and an XTT test was performed. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; NS, not significant ($P > 0.05$).
[c] CHS, commercial human serum; FFHS, fresh frozen human serum.

Table 3
Activity of PMNs and micafungin (FK, μg/ml) against *A. fumigatus* germlings

Treatment	ΔA_{450} [a]	% Inhibition	*P*< [b]				
			I	II [c]	III [d]	IV [e]	V
CTCM	619 ± 75	0	Ref				
+FK 0.01	324 ± 35	48	0.01	Ref			
+FK 0.05	260 ± 48	58	0.01		Ref		
+FK 0.1	245 ± 54	61	0.01			Ref	
PMNs (2×10^5/well)	291 ± 37	53	0.01				Ref
+FK 0.01	117 ± 38	82	0.01	0.05			0.05
+FK 0.05	13 ± 9	98	0.01		0.01		0.01
+FK 0.1	6 ± 7	99	0.01			0.01	0.01
PMNs (1×10^5/well)	277 ± 16	56	0.01				Ref
+FK 0.01	178 ± 20	72	0.01	0.01			0.01
+FK 0.05	6 ± 9	99	0.01		0.01		0.01
+FK 0.1	1 ± 1	100	0.01			0.01	0.01

[a] Conidia, 10^3/well in RPMI-1640, were incubated 4 h at 37 °C, then 18 h at room temperature to produce germlings. PMNs (2×10^5/well or 1×10^5/well) and 0.01, 0.05, or 0.1 μg/ml of micafungin were added and incubated 18 h more, and an XTT test was performed. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; blank, statistics not done.
[c] Metabolism of XTT by *A. fumigatus* germlings from FK (0.01 μg/ml) cultures compared with that of PMNs plus same concentration of FK culture.
[d] Metabolism of XTT by *A. fumigatus* germlings from FK (0.05 μg/ml) cultures compared with that of PMNs plus same concentration of FK culture.
[e] Metabolism of XTT by *A. fumigatus* germlings from FK (0.1 μg/ml) cultures compared with that of PMNs plus same concentration of FK culture.

mling growth (75–95%) than either MDMs alone (66%) or micafungin alone (32–42%), when a low inoculum (200 germlings/well) was used (Table 4). When MDMs were challenged with a higher inoculum of germlings (1000 germlings/well), only MDMs plus 0.1 μg/ml micafugin showed significant collaborative activity (61%) compared with either MDMs (44%) or micafungin alone (38%) (Table 5).

3.5. Activity of PMNs and micafungin against A. fumigatus *hyphae*

In preliminary experiments, hyphae were produced in the presence or absence of micafungin. Growth of hyphae generated in the presence of micafungin was inhibited 16% by PMNs alone. This inhibition was not significantly different from inhibition by PMNs alone against hyphae formed in the absence of micafungin (18%). However, it was found in this experiment that if micafungin was present during generation of hyphae, and also during challenge with PMNs, there was an additive effect. Subsequently, in studies of PMNs and micafungin against hyphae, micafungin was present (as described in Section 2) (Table 6). PMNs alone significantly inhibited *A. fumigatus* hyphal growth, and the combination of PMNs and micafungin showed an additive inhibitory effect. This was true when the starting inoculum was either 500 or 250 conidia per well.

3.6. Activity of monocytes and micafungin against A. fumigatus *hyphae*

As shown in Table 7, monocytes alone did not show significant growth inhibitory effect against *A. fumigatus* hyphae. However, monocytes + micafungin significantly inhibited the growth (66–72%) of hyphae previously exposed to micafungin. The combination of monocytes and micafungin resulted in an additive inhibition of hyphal growth (Table 7).

Table 4
Activity of MDMs and micafungin (FK, μg/ml) against *A. fumigatus* germlings (200/well)

Treatment	ΔA_{450} [a]	% Inhibition	*P*< [b]						
CTCM	464 ± 29	0	Ref						
+FK 0.01	318 ± 36	32	0.05	Ref					
+FK 0.05	281 ± 11	40	0.01		Ref				
+FK 0.1	273 ± 37	42	0.01			Ref			
MDMs	152 ± 11	66	0.01	0.01			Ref		
+FK 0.01	120 ± 32	75	0.01	0.01			NS	Ref	
+FK 0.05	74 ± 17	85	0.01		0.01		0.01	0.05	Ref
+FK 0.1	27 ± 7	95	0.01			0.01	0.01	0.01	0.01

[a] MDM monolayers were produced by 5 days incubation of adherent PBMCs. MDM monolayers were challenged with germlings with or without micafungin for 24 h at 37 °C, and the XTT test was performed. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; NS, not significant ($P > 0.05$); blank, statistics not done.

Table 5
Activity of MDMs and micafungin (FK, μg/ml) against *A. fumigatus* germlings (1000/well)

Treatment	ΔA_{450} [a]	% Inhibition	$P<$ [b]						
CTCM	601 ± 93	0	Ref						
+FK 0.01	373 ± 60	38	0.01	Ref					
+FK 0.05	329 ± 43	46	0.01		Ref				
+FK 0.1	377 ± 27	38	0.01			Ref			
MDMs	342 ± 49	44	0.01	NS	NS	NS	Ref		
+FK 0.01	308 ± 31	49	0.01	NS			NS	Ref	
+FK 0.05	262 ± 49	57	0.01		NS		NS	NS	Ref
+FK 0.1	236 ± 40	61	0.01			0.01	0.05	0.05	NS

[a] MDM monolayers were produced by 5 days incubation of adherent PBMCs. MDM monolayers were challenged with germlings with or without micafungin for 24 h at 37 °C, and the XTT test was performed. Values are means ± standard deviations for quadruplicate measurements.
[b] Ref, reference value; NS, not significant ($P > 0.05$); blank, statistics not done.

Table 6
Activity of PMNs and micafungin (FK, μg/ml) against *A. fumigatus* hyphae

Conidia time zero	First treatment	Second treatment	ΔA_{450} [a]	% Inhibition	$P<$ [b]				
500 (/well))									
	RPMI	CTCM	630 ± 30	0	Ref				
	RPMI	+PMN (4×10^5)	412 ± 36	31	0.01	Ref			
	+FK 0.05	+FK 0.05	397 ± 85	33	0.01	NS	Ref		
	+FK 0.1	+FK 0.1	329 ±30	43	0.01	0.05	NS	Ref	
	+FK 0.05	+FK 0.05 +PMN	226 ±46	67	0.01	0.01	0.01	0.01	Ref
	+FK 0.1	+FK 0.1 +PMN	214 ± 26	69	0.01	0.01	0.01	0.01	NS
250 (/well)									
	RPMI	CTCM	576 ± 54	0	Ref				
	RPMI	+PMN (4×10^5)	457 ± 115	19	NS	Ref			
	+FK 0.05	+FK 0.05	282 ±74	46	0.01	0.05	Ref		
	+FK 0.1	+FK 0.1	168 ± 46	64	0.01	0.01	0.05	Ref	
	+FK 0.05	+FK 0.05 +PMN	134 ± 45	69	0.01	0.01	0.05	NS	Ref
	+FK 0.1	+FK 0.1 +PMN	117 ± 21	82	0.01	0.01	0.01	0.01	0.05

[a] Conidia were incubated 24 h at 37 °C with or without micafungin. After 24 h, CTCM, same concentration of micafungin, or micafungin +PMNs was added and incubated 24 h more, then the XTT test was performed. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; NS, not significant ($P > 0.05$).

Table 7
Activity of monocytes and micafungin (FK, μg/ml) against *A. fumigatus* hyphae

First treatment	Second treatment	ΔA_{450} [a]	% Inhibition	$P<$ [b]			
RPMI	CTCM	875 ± 54	0	Ref			
+FK 0.01	+FK 0.01	470 ± 36	47	0.01	Ref		
+FK 0.1	+FK 0.1	292 ± 20	66	0.01		Ref	
RPMI	Monocytes	785 ± 87	5	NS			Ref
+FK 0.01	+FK 0.01	332 ± 51	62	0.01	0.01		0.01
+FK 0.1	+FK 0.1	250 ± 52	72	0.01		0.05	0.01

[a] Conidia, 2×10^2 cells were incubated 24 h at 37 °C with or without micafungin. After 24 h, CTCM, same concentration of micafungin, or micafungin + monocytes (2×10^5/well) was added and incubated 24 h more, then the XTT test was performed. Values are means ± standard deviations for quadruplicate cultures.
[b] Ref, reference value; NS, not significant ($P > 0.05$); blank, statistics not done.

4. Discussion

Micafungin has been shown to have antifungal activity against *A. fumigatus*, using the conventional National Committee Clinical Laboratory Standards (NCCLS) method. Here we show that an alternative method, the metabolic XTT assay, can be used to measure the antifungal activity of micafungin against germinating conidia in a concentration-dependent manner. The advantage of this method is that it is an objective, quantitative measurement, amenable to statistical analysis. Moreover, we report here that the activity of micafungin against germlings and hyphae can be measured using this system which is nearly impossible by other methods.

Using the XTT system, we were able to test micafungin in combination with human PMNs, monocytes, and MDMs against tissue forms of *A. fumigatus*. Our results show a collaborative antifungal effect of micafungin and phagocytic

cells against germlings and hyphae of *A. fumigatus*. These findings help explain why micafungin, which is only growth inhibitory in vitro, has good efficacy in murine models of pulmonary or systemic aspergillosis, i.e., due to the collaboration between micafungin and phagocytic cells [10,11].

Previously, caspofungin (MK-0991), another echinocandin, was reported to inhibit growth of germlings, e.g. 35% with 0.05 μg/ml and 57% with 0.10 μg/ml [3]. Micafungin had similar antifungal activity against germlings at these concentrations, i.e. 58% and 61%, respectively. Another echinocandin, anidulafungin (LY 303366), inhibited growth of germlings. Anidulafunglin at 0.1 μg/ml inhibited growth of germlings by 43% [2]. These findings indicate that all three echinocandins, at these concentrations, have comparable inhibitory growth activity against germlings.

We show for the first time, that micafungin and PMNs together have a powerful additive antifungal activity against germlings. Caspofungin on the other hand, in similar types of experiments, did not have such an additive antifungal effect against germlings [3]. The possible collaboration of PMNs and anidulafungin against germlings, to our knowledge, has not been reported.

MDMs inhibit growth of germlings deposited on MDM monolayers and collaborate with micafungin for potent additive antifungal activity (95%). These findings are comparable to those reported for collaboration of MDMs and caspofungin (90% inhibition) in similar types of experiments [3]. It is not known at this time if MDMs can collaborate with anidulafungin against germlings for additive antifungal activity.

The strongest additive growth inhibitory activity by PMNs plus micafungin against hyphae (99–100%) was demonstrated when hyphae were generated in the presence of micafungin. Comparable additive antifungal activity of PMNs plus anidulafungin against hyphae generated in the presence of anidulafungin has been reported [2]. These results indicate that exposure of developing hyphae to these echinocandins is important for subsequent collaborative antifungal activity of PMNs and drug.

Monocytes significantly increased the antifungal activity of micafungin against hyphae developed in the presence of micafungin compared with micafungin alone. In another study with monocytes and anidulafungin [2], the results were similar to those reported here. Possible collaborative activity of monocytes with caspofungin against caspofungin-derived hyphae remains to be determined [3].

Mouse models of aspergillosis frequently use cyclophosphamide for immunosuppression in order to establish infection. Cyclophosphamide is a frequently used antineoplastic drug. This drug interferes with growth of susceptible neoplasms but also causes leukopenia, e.g. neutropenia, which puts patients at risk for opportunistic infections. Although there is information about how it affects B-cell and T-cell functions, there is less information about the effects on macrophages [13–15]. Matsumoto et al. [11] reported an interesting in vivo experiment about micafungin. They immunosuppressed mice with cyclophosphamide (200 mg/kg, i.p. at day –4 and day +1 post-pulmonary infection) and evaluated the effect of micafungin. Neutropenia persisted for 6 days after cyclophosphamide treatment. All mice treated with micafungin (1 mg/kg, i.v.) daily for 4 days post-infection were alive at day 22, whereas all mice not receiving micafungin were dead by day 9. Since micafungin is only growth inhibitory against *A. fumigatus* in vitro, it is difficult to explain its efficacy against pulmonary aspergillosis in neutropenic mice. Our data offer a possible explanation for the in vivo efficacy of micafungin in mouse models against tissue forms of *A. fumigatus*. We speculate that innate host defenses that are not severely affected by cyclophosphamide, i.e. tissue macrophages, collaborate with micafungin during the neutropenic phase of immunosuppression. Upon recovery from neutropenia, neutrophils, which are very active against hyphae of *A. fumigatus*, could provide resistance and recovery from infection.

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