ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2002, p. 245–247 Vol. 46, No. 1
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.1.245–247.2002

In Vitro Synergy of Caspofungin and Amphotericin B against *Aspergillus* and *Fusarium* spp.

Sevtap Arikan,* Mario Lozano-Chiu, Victor Paetznick, and John H. Rex

Division of Infectious Diseases, Department of Internal Medicine, Center for the Study of Emerging and Reemerging Pathogens, University of Texas Medical School, Houston, Texas 77030

Received 18 June 2001/Returned for modification 18 September 2001/Accepted 16 October 2001

We investigated the in vitro interaction of caspofungin and amphotericin B for clinical isolates of *Aspergillus* and *Fusarium*. Synergy tests were performed using the checkerboard method and following the NCCLS M38-P guidelines in Antibiotic Medium 3 broth supplemented to 2% glucose. Antagonism was not observed for any of the isolates tested. Caspofungin and amphotericin B were synergistic or synergistic to additive for at least half of the isolates.

Echinocandins, amphophilic cyclic hexapeptides with an N-linked acyl side chain, exhibit selective antifungal activity via inhibition of β-glucan synthesis. Although caspofungin has proven to be active in vivo against *Aspergillus* spp. (1, 2; J. Maertens, I. Raad, C. A. Sable, A. Ngai, and R. Berman, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1103, 2000), it has limited in vitro activity when measured using a conventional MIC-0 (complete inhibition of growth) endpoint. Minimum effective concentration (MEC, in micrograms per milliliter) is a microscopic endpoint that may correlate better with the in vivo activity of the echinocandins. The MEC refers to the lowest concentration of the drug that results in the formation of aberrantly growing, unusual hyphal tips (7). We previously demonstrated that the MEC correlates well with the macroscopic MIC-2 (≈50% reduction in turbidity, prominent decrease in growth visually) endpoint (3).

While the distinctive mechanisms of action of caspofungin on *Aspergillus* hyphae make it a good candidate for use in combination with other antifungal agents (C. M. Douglas, J. C. Bowman, G. K. Abruzzo, A. M. Flattery, C. J. Gill, L. Kong, C. Leighton, J. G. Smith, V. B. Pikounis, K. Bartizal, M. B. Kurtz, and H. Rosen, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J-1683, 2000), its behavior in combination has been little studied. In an effort to clarify whether enhanced activity against *Aspergillus* and *Fusarium* is achieved when caspofungin is combined with another antifungal agent acting via a different mechanism, we performed in vitro synergy studies for caspofungin combined with amphotericin B.

Fourteen clinical isolates of *Aspergillus* (*A. flavus* [$n = 4$], *A. fumigatus* [$n = 4$], *A. niger* [$n = 3$], *A. terreus* [$n = 3$]) and six clinical isolates of *Fusarium* (*F. solani* [$n = 4$], *F. oxysporum* [$n = 2$]) were tested. Isolation and identification of the isolates were performed by standard microbiological procedures. Caspofungin and amphotericin B were provided as standard powders from Merck Research Laboratories (Rahway, N.J.) and Bristol-Myers Squibb Co. (Princeton, N.J.), respectively.

The individual caspofungin and amphotericin B MICs (in micrograms per milliliter) were determined initially by using the NCCLS M38-P microdilution methodology (8) in Antibiotic Medium 3 and after 24 and 48 h of incubation. Antibiotic Medium 3 (BBL lot JD4ZSG; Becton Dickinson) was buffered by addition of 1 g of Na_2HPO_4 and 1 g of NaH_2PO_4 to each liter of medium (pH = 7) and then supplemented to 20-g/liter glucose (AM3). It was previously shown that AM3 provided good growth and generated slightly lower amphotericin B MICs than did RPMI medium, particularly for some *Aspergillus* isolates (4). Consistent with data obtained with *Candida* and amphotericin B (9), this lowering effect might help to differentiate caspofungin-susceptible *Aspergillus* isolates from caspofungin-resistant ones, and we thus performed the susceptibility tests with AM3. Also, based on the observations that reading at earlier time points does not reduce, but may increase, the relevance of the observed MIC (5, 10) and that *Aspergillus* spp. other than *Aspergillus nidulans* and *Fusarium* spp. yielded sufficient growth after 24 h (4), we focused on the MICs observed after 24 h of incubation.

Checkerboard tests were employed to determine the fractional inhibitory concentrations (FIC; in micrograms per milliliter) of the combination of caspofungin and amphotericin B for each test isolate. Since the MIC endpoint to be used in caspofungin susceptibility testing is not well established, the MICs of the drugs used individually and their MICs in combination were determined by using both MIC-0 and MEC endpoints, the latter being equivalent in our hands to the macroscopic MIC-2 endpoint for caspofungin (3).

The synergy test results were evaluated by using both MIC endpoints. The FIC of each drug for an individual isolate was calculated as the ratio of the concentration of the drug in combination that achieves the MIC endpoint to the MIC of the drug alone by using that endpoint. For purposes of calculation, off-scale MICs were converted to the next higher dilution. The FIC index (FICI) value for an individual isolate was calculated by adding the FIC of caspofungin to the FIC of amphotericin B and then rounding to the nearest 0.1 unit. FICI values were interpreted as follows: FICI ≤ 0.5, synergistic; $0.5 <$ FICI ≤ 1, synergistic to additive; $1 <$ FICI ≤ 4, indifferent; and FICI > 4, antagonistic.

* Corresponding author. Present address: Mycology Laboratory, Department of Microbiology and Clinical Microbiology, Hacettepe University Medical School, 06100 Ankara, Turkey. Phone: 90 312 3051562. Fax: 90 312 3115250. E-mail: sarikan@metu.edu.tr.

TABLE 1. MIC and FICI results of the test isolates at 24 h

Isolate	MIC (μg/ml)[a]			FICI	Result[b]
	Endpoint	AMB alone	CAS alone[c]		
Aspergillus spp. (n = 14)					
A. fumigatus (n = 4)					
2-160	MIC-0	0.5	>16	0.5	S
	MEC	0.125	0.25	0.6	Ad
7099	MIC-0	0.25	>16	1.0	Ad
	MEC	0.125	0.25	0.7[d]	Ad
7100	MIC-0	0.25	>16	1.0	Ad
	MEC	0.125	0.25	0.7	Ad
2-103	MIC-0	0.25	>16	0.5	S
	MEC	0.06	0.25	1.2	I
A. flavus (n = 4)					
1-74	MIC-0	0.5	>16	2.0	I
	MEC	0.5	>16	1.0	Ad
1-68	MIC-0	1	>16	1.0	Ad
	MEC	0.5	0.5	1.5	I
1-38	MIC-0	1	>16	2.1	I
	MEC	1	>16	0.5	S
1-42	MIC-0	1	>16	1.0	Ad
	MEC	0.5	>16	1.0	Ad
A. niger (n = 3)					
1-44	MIC-0	0.125	1	1.1	I
	MEC	0.125	0.25	0.8	Ad
1-62	MIC-0	0.06	>16	2	I
	MEC	0.03	0.25	2.2[d]	I
1-63	MIC-0	0.06	>16	1.0	Ad
	MEC	0.03	0.25	1.2	I
A. terreus (n = 3)					
1-58	MIC-0	1	>16	1.0	Ad
	MEC	0.5	0.25	0.4	S
1-79	MIC-0	0.125	>16	2.0	I
	MEC	0.06	0.25	2.6	I
1-50	MIC-0	2	>16	1.0	Ad
	MEC	1	0.25	1.3	I
Fusarium spp. (n = 6)					
F. solani (n = 4)					
S-1319	MIC-0	2	>16	2.1	I
	MEC	0.25	16	1.0	Ad
S-1382	MIC-0	0.5	>16	2.0	I
	MEC	0.25	16	0.5[d]	S
S-1237	MIC-0	2	>16	0.5	S
	MEC	1	16	0.5	S
S-1381	MIC-0	8	>16	0.6	Ad
	MEC	1	>16	2.1[d]	I
F. oxysporum (n = 2)					
O-1895	MIC-0	2	>16	0.5	S
	MEC	1	16	NA[e]	NA
O-1739	MIC-0	2	>16	0.5	S
	MEC	1	16	0.5	S

[a] AMB, amphotericin B; CAS, caspofungin; MEC: minimum effective concentration, equivalent to macroscopic MIC-2.
[b] S, synergistic; Ad, additive; I, indifferent.
[c] The high off-scale MIC value, >16 μg/ml, was converted to the next highest concentration, 32 μg/ml, for calculation of FICI.
[d] MEC is not available or is inconsistent; thus, MIC-1 (~80% reduction in turbidity) is used for calculation of the FICI.
[e] NA, not applicable; none of the concentrations of the drug produced turbidity equivalent to that endpoint.

The FICI values obtained for each test isolate at 24 h are shown in Table 1. Overall synergy results of *Aspergillus* and *Fusarium* isolates at 24 h are summarized in Table 2. Caspofungin and amphotericin B in combination appeared synergistic or synergistic to additive for more than half of the isolates of both *Aspergillus* and *Fusarium* spp. In general, caspofungin MICs in combination decreased dramatically (three to nine twofold dilutions, often from an off-scale endpoint) while amphotericin B MICs decreased very slightly (one twofold dilution) or remained the same. The results were qualitatively similar whether based on MIC-0 or MEC endpoints.

TABLE 2. Overall results obtained for *Aspergillus* and *Fusarium* at 24 h using the two endpoints

Genus (n)	No. of isolates showing indicated synergy result[a] at endpoint:							
	MIC-0				MEC[b]			
	S	Ad	I	An	S	Ad	I	An
Aspergillus (14)	2	7	5	0	2	6	6	0
Fusarium[c](6)	3	1	2	0	3	1	1	0

[a] S, synergistic; Ad, additive; I, indifferent; An, antagonistic.
[b] MEC, minimum effective concentration; equivalent to macroscopic MIC-2.
[c] Turbidity equivalent to the MEC endpoint could not be achieved for one *Fusarium* isolate, and the result for this isolate was thus omitted.

Importantly, antagonism was not observed for any of the isolates tested. There was no obvious species-related or endpoint-related variation of the results. Despite caspofungin's limited activity when used alone, its synergistic-to-additive interaction with amphotericin B against *Fusarium* was of great interest.

At 48 h, the overall results were similar qualitatively. For *Aspergillus*, the number of strains showing synergy rose from two to five by the MEC endpoint and fell from two to one with the MIC-0 endpoint. Similar small shifts were observed for *Fusarium* (data not shown). Overall, at least half of the isolates showed a synergistic or synergistic-to-additive interaction under each of the tested conditions (24 versus 48 h, MEC versus MIC-0).

Data on both the in vitro interaction of caspofungin with other antifungal drugs and the in vivo use of caspofungin in combination therapy against fungal pathogens are as yet limited. Our report is the first to demonstrate the favorable in vitro interaction of caspofungin and amphotericin B against *Aspergillus* and *Fusarium* spp. With results similar to our findings with caspofungin, Stevens (D. A. Stevens, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 151, 1999) reported a favorable in vitro interaction of another echinocandin, FK463, with liposomal amphotericin B against *Aspergillus*. Franzot et al. (6) have demonstrated that caspofungin enhanced the in vitro activity of amphotericin B against *Cryptococcus neoformans* isolates. This finding is of special interest since caspofungin alone has no meaningful activity against *C. neoformans*. This is similar to our finding of additive or synergistic effect of the caspofungin-amphotericin B combination against *Fusarium* isolates, despite the lack of activity of caspofungin alone against this particular fungus. The mechanism of synergy and additive effect is unknown. The decrease in the MICs of amphotericin B used in combination compared to the MIC of the drug used alone may be attributed to the increased activity of amphotericin B due to its enhanced penetration to the cell membrane following the effect of caspofungin on the cell wall. However, it remains difficult to explain how caspo-

fungin MICs are lowered when the drug is used in combination with amphotericin B. Further investigation is required to clarify the actual mechanism underlying the interaction between the two drugs.

In vivo data reported so far on treatment of aspergillosis with the amphotericin B-echinocandin combination are in accordance with the available in vitro data. Combination therapy with amphotericin B and FK463 in a murine pulmonary aspergillosis model resulted in a higher survival rate and more favorable pathological findings than those obtained with FK463 or amphotericin B therapy alone (M. Nakajima, S. Tamada, K. Yoshida, Y. Wakai, T. Nakai, F. Ikeda, T. Goto, Y. Niki, and T. Matsushima, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1685, 2000). Similarly, amphotericin B-FK463 combination therapy resulted in a significantly higher survival rate than that obtained by monotherapy with FK463 or amphotericin B in another murine invasive pulmonary aspergillosis model (S. Kohno, S. Maesaki, J. Iwakawa, Y. Miyazaki, K. Makamura, H. Kakeya, K. Yanagihara, H. Ohno, Y. Higashimyama, and T. Tashiro, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1686, 2000).

The checkerboard method that we have employed is useful for an initial analysis of drug-drug interactions. Time-kill studies would provide additional data on the nature of these interactions and would be useful for further analysis of the interaction between caspofungin and amphotericin B.

In conclusion, our results indicate that a combination of amphotericin B and caspofungin might be effective in infections due to *Aspergillus* and *Fusarium* spp. Animal models similar to those accomplished for amphotericin B and FK463 are required to validate the in vivo significance of these in vitro data presented for the amphotericin B-caspofungin combination.

(This work was presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, 17 to 20 September 2000, Toronto, Ontario, Canada, abstract no. J-932.)

This study was supported by a grant to Sevtap Arikan from the Turkish Scientific and Technical Research Council (TUBİTAK) and by a grant from Merck Research Laboratories.

REFERENCES

1. **Abruzzo, G. K., C. J. Gill, A. M. Flattery, L. Kong, J. G. Smith, V. B. Pikounis, K. Bartizal, and H. Rosen.**2000. Efficacy of the echinocandin caspofungin against disseminated aspergillosis and candidiasis in cyclophosphamide-induced immunosuppressed mice. Antimicrob. Agents Chemother. **44:**2310–2318.
2. **Abruzzo, G. K., A. M. Flattery, C. J. Gill, L. Kong, J. G. Smith, V. B. Bikounis, J. M. Balkovec, A. F. Bouffard, J. F. Dropinski, H. Rosen, H. Kropp, and K. Bartizal.**1997. Evaluation of the echinocandin antifungal MK-0991 (L-743,872): efficacies in mouse models of disseminated aspergillosis, candidiasis, and cryptococcosis. Antimicrob. Agents Chemother. **41:** 2333–2338.
3. **Arikan, S., M. Lozano-Chiu, V. Paetznick, and J. H. Rex.**2001. In vitro susceptibility testing methods for caspofungin against *Aspergillus* and *Fusarium* isolates. Antimicrob. Agents Chemother. **45:**327–330.
4. **Arikan, S., M. Lozano-Chiu, V. Paetznick, S. Nangia, and J. H. Rex.**1999. Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of *Aspergillus* and *Fusarium* species. J. Clin. Microbiol. **37:**3946–3951.
5. **Arthington-Skaggs, B. A., D. W. Warnock, and C. J. Morrison.**2000. Quantitation of *Candida albicans* ergosterol content improves the correlation between in vitro antifungal susceptibility test results and in vivo outcome after fluconazole treatment in a murine model of invasive candidiasis. Antimicrob. Agents Chemother. **44:**2081–2085.
6. **Franzot, S. P., and A. Casadevall.**1997. Pneumocandin L-743,872 enhances the activities of amphotericin B and fluconazole against *Cryptococcus neoformans* in vitro. Antimicrob. Agents Chemother. **41:**331–336.
7. **Kurtz, M. B., I. B. Heath, J. Marrinan, S. Dreikorn, J. Onishi, and C. Douglas.** 1994. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-β-D-glucan synthase. Antimicrob. Agents Chemother. **38:**1480–1489.
8. **National Committee for Clinical Laboratory Standards.** 1998. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi; proposed standard. NCCLS document M38-P. National Committee for Clinical Laboratory Standards, Wayne, Pa.
9. **Rex, J. H., C. R. Cooper, Jr., W. G. Merz, J. N. Galgiani, and E. J. Anaissie.** 1995. Detection of amphotericin B-resistant *Candida* isolates in a broth-based system. Antimicrob. Agents Chemother. **39:**906–909.
10. **Rex, J. H., P. W. Nelson, V. L. Paetznick, M. Lozano-Chiu, A. Espinel-Ingroff, and E. J. Anaissie.**1998. Optimizing the correlation between results of testing *in vitro* and therapeutic outcome *in vivo* for fluconazole by testing critical isolates in a murine model of invasive candidiasis. Antimicrob. Agents Chemother. **42:**129–134.