In Vitro Interaction of Caspofungin Acetate with Voriconazole against Clinical Isolates of *Aspergillus* spp.

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The interaction between caspofungin acetate and voriconazole was studied in vitro by using 48 clinical *Aspergillus* spp. isolates obtained from patients with invasive aspergillosis. MICs were determined by the NCCLS broth microdilution method. Synergy, defined as a fractional inhibitory concentration (FIC) index of <1, was detected in 87.5% of the interactions; an additive effect, defined as an FIC index of 1.0, was observed in 4.2% of the interactions; and a subadditive effect, defined as an FIC index of 1.0 to 2.0, was found in 8.3% of the interactions. No antagonism was observed. Animal models are required to validate the in vivo significance of these in vitro data presented for the combination of caspofungin and voriconazole.

*Aspergillus* spp. are common causes of nosocomial pneumonia and disseminated infection in immunocompromised hosts such as bone marrow transplant recipients, patients with hematologic malignancies, solid-organ transplant recipients, AIDS patients, and patients with pulmonary diseases. The most common species include *Aspergillus fumigatus* (approximately 90% of the cases), *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans* (6, 12). The rate of mortality from invasive aspergillosis remains high in severely immunosuppressed patients who receive standard antifungal treatment with amphotericin B. Improved responses have been observed in less immunosuppressed patients sequentially receiving amphotericin B followed by itraconazole and those receiving itraconazole alone (12). Nevertheless, these drugs are not always effective or tolerated in severely ill patients, and therefore, there is still a need for new therapies and new approaches to improve the outcome of this disease. The echinocandin caspofungin acetate (CAS), formerly MK-0991, belongs to a new class of antifungal drugs that inhibit the synthesis of 1,3-β-D-glucan, an essential cell wall polysaccharide that represents a selective target present only in fungal cell walls. It has been shown to have in vitro and in vivo activities against many clinically important fungi such as dimorphic fungi, yeasts, and also opportunistic filamentous fungi, including *Aspergillus* spp. (7, 15).

It has previously been described that combinations of CAS and amphotericin B against *Candida, Cryptococcus, Aspergillus*, and *Fusarium* have additive to synergistic effects against certain isolates, with no evidence of antagonism (3, 4). In the case of VRC, the combination of CAS and terbinafine has been shown to have synergistic activity against *Aspergillus sp.*, *Candida albicans*, and *Candida glabrata* isolates (14, 16; S. Perea, G. Gonzalez, A. W. Fothergill, D. A. Sutton, and M. G. Rinaldi, Abstr. 10th Annu. Focus Fungal Infect., abstr. 28, p. 69, 2000).

The aim of the present study was to investigate the in vitro interaction of CAS and VRC against 48 isolates of *Aspergillus* spp. isolated from patients with invasive aspergillosis.

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Forty-eight isolates of *Aspergillus* spp. (24 *A. fumigatus*, 10 *A. terreus*, 9 *A. flavus*, and 5 *A. niger* isolates) were used throughout the study. All strains evaluated were clinical isolates submitted to the Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, from patients with invasive aspergillosis. The identities of the clinical isolates were confirmed by standard microbiological procedures, and the strains were stored in sterile deionized water at room temperature until they were used in the study. *Paecilomyces variotii* (UTHSC 90-459) was used as the control organism in all experiments.

CAS (Merck, Rahway, N.J.) and VRC (Pfizer Inc., Sandwich, United Kingdom) were obtained in reagent-grade powder form from their respective manufacturers. Stock solutions were prepared in water (CAS) and polyethylene glycol 400 (VRC). Serial twofold dilutions of each antifungal agent were prepared as outlined in document M38-P of the NCCLS (11). Final dilutions were made in antibiotic medium 3 (M3; Difco, Detroit, Mich.) supplemented with 2% glucose (M3-2%).

Drug interactions were assessed by a checkerboard microdi-
lution method that also included the determination of the MIC of each drug alone by using the parameters outlined in the recommendations of the NCCLS (11). The final concentrations of the antifungal agents ranged from 0.25 to 64 μg/ml for CAS and 0.03 to 4 μg/ml for VRC. Inocula were prepared spectrophotometrically and further diluted in order to obtain final concentrations ranging from 0.4 × 10³ to 5 × 10⁴ CFU/ml. Each microdilution well containing 100 μl of the diluted (two times) drug concentrations of both antifungals (CAS and VRC) was inoculated with 100 μl of the diluted inoculum suspension (final volume of each well, 200 μl). The trays were incubated at 35°C, and the results were read at 48 h visually and spectrophotometrically with a spectrophotometric microtiter plate reader (Dynex Technologies Inc, Chantilly, Va.). MIC endpoints were determined as the first concentration of the antifungal agent, either alone or in combination, at which the turbidity in the well was less than 80% of that in the control well. The geometric mean (GM) MICs and the ranges of MICs were analyzed to evaluate the in vitro activities of both drugs, alone and in combination. Both on-scale and off-scale results were included in the analysis. For computation of the GM values, high off-scale MICs were converted to the twofold concentration just above the highest concentration tested. When the MIC was off the bottom of the scale, the MIC was assumed to be the lowest MIC tested.

Drug interactions were classified as synergistic, additive, or antagonistic on the basis of the fractional inhibitory concentration (FIC) index. The FIC index is the sum of the FICs of each of the drugs and is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone. The interaction was defined as synergistic if the FIC index was <1, additive if the FIC index was 1.0, subadditive if the FIC index was between 1.0 and 2.0, indifferent if the FIC index was 2, and antagonistic if the FIC index was between 1.0 and 2.0, indifferent if the FIC index was between 1.0 and 2.0, indifferent if the FIC index was <1, and antagonistic if the FIC index was 1.0, subadditive if the FIC index was 1.0, indifferent if the FIC index was 2.
endpoints for measuring the in vitro activities of these new class of antifungals (MIC_{50}, MIC_{50}, and minimum effective concentration) have been published previously (2, 4, 5, 8, 13). Because of the technical variability, data reported thus far on the activity of CAS against Aspergillus show a wide range of MICs. Pfaller et al. (13) used the NCCLS microdilution method and reported MIC_{50}s after 72 h of incubation of 0.12 μg/ml, using an MIC endpoint of a 75% reduction in growth (MIC_{75}). Del Poeta et al. (5) used the microdilution methodology proposed by the NCCLS for yeasts (M27-A) (11a), an inoculum size of 0.5 × 10^{3} to 2.5 × 10^{3} CFU/ml, and an MIC endpoint. The GM MICs for A. fumigatus and A. flavus were ≤0.09 and 0.2 μg/ml, respectively (5). Espinel-Ingroff (9) used the MIC_{50} and found GM MICs for A. fumigatus, A. flavus, and A. terreus of 2.15, 0.5, and 0.5 μg/ml, respectively. Arikan et al. (2) reported GM MICs, obtained after 48 h of incubation with M3 medium and by the NCCLS M38-P methodology (11), of 3.7, 2.83, 0.26, and 0.25 μg/ml for A. flavus, A. fumigatus, A. niger, and A. terreus, respectively.

Because of the lack of standardization in the methodology used to perform in vitro antifungal susceptibility testing for CAS, we arbitrarily chose to use M3-2% medium and to calculate the endpoint as the lowest drug concentration that showed a significant reduction of growth (approximately 80% reduction). For the other variables (inoculum preparation, preparation of drugs, broth inoculation, and incubation) the NCCLS M38-P methodology was followed (11).

The MICs obtained in the previous studies are lower than the ones obtained in the present study. The CAS MICs obtained in the present study support and extend the findings presented in a previous report of a study which evaluated the activity of CAS against Aspergillus spp. by the same methodology, that is, with M3-2% medium, with reading of the MIC endpoint as the lowest drug concentration that showed a significant reduction of growth (approximately 80% reduction), and by the NCCLS methodology (M38-P) (Flately et al., 40th ICAAC). In that study, the MIC_{50} ranges for A. fumigatus, A. flavus, A. niger, and A. terreus were 32, >64, ≤0.03, and 32, respectively. With respect to the VRC MICs, our results are similar to those published recently by Espinel-Ingroff (8) when M3-2% medium, reading of the MIC endpoint as MIC_{50}, and the NCCLS M38-P methodology (11) were used.

The results obtained in the present study with a combination of agents, which showed either synergy or additive but no evidence of antagonistic effects when both agents were used in combination, are very encouraging. Although the exact mechanism of the interaction between the two agents is unknown, one theoretical explanation for such an effect would be the simultaneous disruption of the fungal cell membrane by VRC and disruption of the cell wall by CAS, ultimately decreasing the cell stability and leading to the death of the fungal cell.

In conclusion, our results indicate that a combination of CAS and VRC might be effective against infections caused by Aspergillus spp. However, improvements to the methodology used to determine the in vitro susceptibility of the fungi to CAS, as well as in vitro-in vivo correlation studies, are required before clinical studies can be conducted.

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REFERENCES