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In Vitro Combination of Isavuconazole with Micafungin or Amphotericin B Deoxycholate against Medically Important Molds

Aspasia Katragkou,[a] Matthew McCarthy,[a] Joseph Meletiadis,[b] Vidmantas Petraitis,[a] Patriss W. Moradi,[a] Gittel E. Strauss,[a] Monique M. Fouant,[c] Laura L. Kovanda,[c] Ruta Petraitiene,[a] Emmanuel Roilides,[d] Thomas J. Walsh[a,e,f]

Transplantation-Oncology Infectious Diseases Program, Division of Infectious Diseases, Department of Medicine, Weil Cornell Medical Center of Cornell University, New York, New York, USA[a]; Clinical Microbiology Laboratory, Attikon University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece[b]; Astellas Pharma Global Development, Inc., Northbrook, Illinois, USA[c]; Infectious Disease Unit, 3rd Department of Pediatrics, Faculty of Medicine, Aristotle University School of Health Sciences, Hippokration Hospital, Thessaloniki, Greece[d]; Department of Pediatrics, Weill Cornell Medical College of Cornell University, New York, New York, USA[e]; Department of Microbiology and Immunology, Weill Cornell Medical College of Cornell University, New York, New York, USA[f]

Whether isavuconazole, an extended-spectrum triazole, possesses synergistic activity in combination therapy with echinocandins or amphotericin B for the treatment of invasive molds infections has not been studied. Our *in vitro* combination studies showed that isavuconazole and micafungin are synergistically active against *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, and *Cunninghamella bertholletiae*. These results suggest that isavuconazole, in combination with micafungin, may have a role in the treatment of invasive aspergillosis and warrants further investigation.

Invasive mold infections are important causes of morbidity and mortality in immunocompromised hosts. Response to treatment with a single antifungal agent is often inadequate, as acquired resistance and breakthrough infections have been reported among patients with long-term exposure to even the newest of antifungal agents (1–3). Combination antifungal therapy provides a potential strategy to improve antimicrobial activity and clinical outcomes (4, 5). Isavuconazole is an extended-spectrum antifungal triazole with favorable pharmacokinetic and safety profiles and *in vitro* activity against a wide variety of fungal pathogens, including non-*Aspergillus* spp., *Fusarium* spp., *Scedosporium* spp., and *Mucorales* (6, 7). Whether isavuconazole possesses synergistic activity when combined with echinocandins or polyenes is unknown. Herein, we examine the combination of isavuconazole with micafungin or amphotericin B deoxycholate against medically important mold isolates.

A collection of 11 mold isolates was used in this study. *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), and *Aspergillus flavus* (ATCC 204304) were used for quality control. Amphotericin B deoxycholate (AMB) (Sigma-Aldrich, St. Louis, MO), micafungin (MFG) (Astellas Pharma US, Inc., Northbrook, IL), and isavuconazole (ISA) (Basilea Pharmaceutica, Basel, Switzerland) were used in this study. The antifungal agents were obtained in lyophilized powder form and were prepared and preserved according to the manufacturer's instructions. Inoculum preparation and MICs were determined according to the Clinical and Laboratory Standards Institute reference method for broth dilution antifungal susceptibility testing (CLSI M38-A2) (8).

The *in vitro* combination testing between ISA and MFG or AMB was performed using a two-dimensional (8-by-12) checkerboard microdilution method in sterile 96-well flat-bottom microtitration plates. The choice of the appropriate range of drug concentrations was based on the MIC findings on each individual drug and isolate. ISA and MFG or AMB were added in plate wells as has been described elsewhere (9, 10).

The combined effects of the antifungal agents were quantified after incubation for 48 h by using the metabolic reduction assay XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]2*H*-tetrazolium-5-carboxanilide) and 25 μM menadione as the final electron acceptor agent, as described previously (11). Interactions between the antifungal agents were analyzed with the Loewe additivity model (12) using the fractional inhibitory concentration (FIC) index and the Bliss independence model (9, 13–17).

The results of the FIC index model are summarized in Table 1. For the ISA-MFG combinations, the median FIC indices of all the strains ranged from 0.28 to 1.06. These indices for *A. fumigatus*, *A. flavus*, *Aspergillus terreus*, *Cunninghamella bertholletiae*, *Lichtheimia corymbifera*, and *Scedosporium apiospermum* indicate synergy. In addition, a mean FIC value of >1.25 for all replicates was not noted in any of the mold isolates tested, indicating that no antagonism was found. For the ISA-AMB combinations, the median FIC indices of all strains, with the exception of those for *A. fumigatus*, *A. flavus*, and *Fusarium oxysporum*, ranged from 1 to 1.18, indicating an additive effect. However, a mean FIC value of <1, indicating synergism, was not noted in any of the isolates tested.

The results of Bliss independence drug interaction analysis for the *in vitro* interactions of ISA-MFG and ISA-AMB are summarized in Table 2. The ISA-MFG combination resulted in a synergistic interaction in *A. fumigatus*, *A. flavus*, *A. terreus*, and *C. bertholletiae*. The degree of synergy was the highest among the *Aspergillus* species, ranging from 29.98% to 65.59%. Only for *F. oxysporum* was the ISA-MFG interaction antagonistic, while for *Mucor circinelloides*, *Rhizopus microsporus*, and *Rhizopus oryzae*, the combined ISA-MFG effect resulted in an indifferent interaction. For *Fusarium solani*, *L. corymbifera*, and *S. apiospermum*, the type of interaction was drug concentration dependent.

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Address correspondence to Thomas J. Walsh, thw2003@med.cornell.edu.

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TABLE 1 *In vitro* interactions by FIC indices of ISA in combination with MFG or AMB against different mold isolates[a]

Isolate (strain)	FIC index (median [range])	
	ISA-MFG	ISA-AMB
Aspergillus fumigatus (4215)	0.75 (0.51–1.06)	1.5 (0.53–2.5)
Aspergillus flavus (10B)	0.59 (0.51–1.007)	1.5 (0.14–2.25)
Aspergillus terreus (95644)	0.625 (0.31–1.015)	1.18 (0.14–2.12)
Fusarium oxysporum species complex (34)	1.015 (0.507–1.5)	1.37 (1.004–2.5)
Fusarium solani species complex (26)	1.06 (1.007–1.5)	1.06 (1–2.5)
Cunninghamella bertholletiae (182)	0.59 (0.52–2)	1.007 (0.5–2.12)
Lichtheimia corymbifera (187)	0.75 (0.5–2.5)	1.12 (0.56–2.5)
Mucor circinelloides (234)	1.06 (1–1.5)	1 (0.5–1.24)
Rhizopus microsporus (230)	1.06 (1–1.5)	1.03 (0.5–2.1)
Rhizopus oryzae (98)	1.06 (1–1.5)	1.09 (0.56–2.25)
Scedosporium apiospermum (142)	0.28 (0.12–1)	1.06 (0.5–1.25)

[a] FIC, fractional inhibitory concentration; ISA, isavuconazole; MFG, micafungin; AMB, amphotericin B deoxycholate.

The ISA-AMB combination resulted in antagonistic interactions in *A. fumigatus*, *A. flavus*, *F. solani*, and *R. microsporus*, while for *S. apiospermum*, the interaction was synergistic. For *L. corymbifera*, *M. circinelloides*, and *R. oryzae*, the ISA-AMB combination resulted in indifferent interactions. The type of ISA-AMB interaction against *A. terreus*, *F. oxysporum*, and *C. bertholletiae* appeared to be concentration dependent.

Overall, our results show that the ISA-MFG combination has consistent synergistic effects against *A. terreus*, *A. fumigatus*, *A. flavus*, and *C. bertholletiae*. In addition, the ISA-AMB combination has low-grade antagonistic effects against *A. fumigatus*, *A. flavus*, *F. solani*, and *R. microsporus*. Moreover, the ISA-MFG combination was found to be concentration and organism dependent (i.e., for *C. bertholletiae* and *L. corymbifera* at ISA concentrations near the MIC, the interactions were synergistic, and at low ISA concentrations, the interactions were antagonistic). By comparison, for *S. apiospermum*, synergistic ISA-MFG interactions were noted at low ISA concentrations and antagonistic interactions at high ISA concentrations. From the mechanistic and clinical perspectives, the former findings about *S. apiospermum* ISA-MFG interactions seem intriguing. However, the low magnitude of Bliss antagonism between ISA and MFG, which was beyond the sensitivity of the XTT assay to detect fungal growth and may also be due to the variation of background absorbance (18), makes this interaction not clinically considerable. From a clinical perspective, the range of *in vitro* ISA concentrations at which the synergistic interactions were observed fell within achievable plasma concen-

TABLE 2 *In vitro* interactions by Bliss independence analysis of ISA in combination with MFG or AMB against different mold isolates[a]

Isolate (strain)	ISA-MFG Bliss interaction		ISA-AMB Bliss interaction	
	Type of interaction[b]	Mean %ΔE value (range)	Type of interaction	Mean %ΔE value (range)
Aspergillus fumigatus (4215)	SYN	29.98 (21.23 to 51.57)	ANT	−4.64 (−0.84 to −19.72)
Aspergillus flavus (10B)	SYN	65.59 (19.58 to 128.35)	ANT	−1.41 (−0.10 to −24.28)
Aspergillus terreus (95644)	SYN	33.73 (0.74 to 88.11)	ANT[c]	−0.04 (−0.01 to −0.25)
			SYN[c]	1.35 (0.14 to 4.26)
Fusarium oxysporum species complex (34)	ANT	−38.57 (−25.95 to −56.34)	ANT[c]	−6.45 (−0.89 to −21.2)
			SYN[c]	2.69 (2.47 to 3)
Fusarium solani species complex (26)	ANT[d]	−17.15 (−5.31 to −29.65)	ANT	−0.49 (−0.03 to −4.93)
	SYN[d]	18.65 (9.72 to 28.11)		
Cunninghamella bertholletiae (182)	SYN	19.31 (4.54 to 35.68)	ANT[c]	−0.31(−0.13 to −0.54)
			SYN[c]	9.15 (6.39–16.23)
Lichtheimia corymbifera (187)	ANT[d]	−15.56 (−6.39 to −25.70)	INT	
	SYN[d]	26.32 (7.43 to 39.74)		
Mucor circinelloides (234)	INT		INT	
Rhizopus microsporus (230)	INT		ANT	−3.04 (−0.11 to −50.59)
Rhizopus oryzae (98)	INT		INT	
Scedosporium apiospermum (142)	ANT[d]	−0.61 (−0.21 to −1.7)	SYN	11.16 (0.08 to 23.1)
	SYN[d]	8.95 (5.22 to 16.46)		

[a] ISA, isavuconazole; MFG, micafungin; AMB, amphotericin B deoxycholate.

[b] SYN, synergistic interaction; ANT, antagonistic interaction; INT, indifferent interaction.

[c] The type of ISA-AMB synergy was drug concentration dependent for *A. terreus*, *F. oxysporum*, and *C. bertholletiae*; that is, treatment of *A. terreus* showed antagonistic interactions at high AMB (1 to 8 mg/liter) and ISA (1 to 16 mg/liter) concentrations, while at low AMB (0.125 to 0.5 mg/liter) and ISA (0.250 to 2 mg/liter) concentrations, the interactions were synergistic. For *F. oxysporum*, synergistic interactions were noted with a low concentration of ISA (0.0625 mg/liter) and with a range of AMB concentrations (from 0.5 to 4 mg/liter). On the contrary, the combination of high concentrations of ISA (8 to 32 mg/liter) and AMB at a range (from 0.125 to 8 mg/liter) resulted in antagonistic interactions. For *C. bertholletiae*, low-level synergistic interactions were noted at low concentrations of AMB (0.125 to 0.5 mg/liter) and at high concentrations of ISA (4 to 32 mg/liter), while at higher concentrations of AMB (1 to 8 mg/liter) and ISA (from 2 to 32 mg/liter), the interactions were antagonistic.

[d] The type of ISA-MFG synergy was drug concentration dependent for *F. solani*, *L. corymbifera*, and *S. apiospermum*; that is, treatment of *F. solani* resulted in antagonistic interactions at low concentrations of ISA (0.03 to 0.125 mg/liter) and in synergistic interactions at high concentrations of ISA (1 to 8 mg/liter), treatment of *L. corymbifera* resulted in antagonistic interactions at low concentrations of ISA (0.015 to 0.125 mg/liter) and in synergistic interactions at higher ISA concentrations (0.25 to 2 mg/liter), and treatment of *S. apiospermum* with low concentrations of ISA (0.062 to 0.25 mg/liter) and MFG resulted in synergistic interactions, while treatment with high concentrations of ISA (2 to 32 mg/liter) and MFG resulted in weak antagonistic interactions.

trations of healthy volunteers (19). However, the antagonistic interactions occurred at concentrations that exceeded safely achievable levels. Furthermore, the degree of the ISA-MFG antagonistic interactions was low enough (mean %Δ*E* value, −0.61%) (Table 2) as to be clinically negligible.

Drug-drug interactions between antifungal agents may not necessarily be the same at all concentrations. The power of Bliss surface analysis reveals that, at certain concentrations, synergy may be present, while at other concentrations, antagonism may occur. For a given species, clinicians may assess these concentration-dependent interactions by assessing the predominant direction of percent synergy or percent antagonism. For example, for *S. apiospermum*, the predominant interaction is synergy (ΔE = 8.95% [range, 5.22% to 16.46%]), while antagonism is only minimally detectable (ΔE = −0.61% [range, −0.21% to −1.7%]). Yet, depending on the relative concentrations of the antifungal agents, synergy or antagonism may be observed at different time points over the dosing interval. Within a given tissue site, these dynamic changes of synergy and antagonism may occur. The final *in vivo* outcome will be determined by the predominant drug interaction of synergy (+Δ*E*), indifference ($\Delta E = 0$), or antagonism (−ΔE).

The correlation of *in vitro-in vivo* results has long been an issue of controversy. Our combination studies of invasive aspergillosis in a persistently neutropenic rabbit model have been correlated with *in vitro* combination studies (20–23). The key to correlation between *in vitro* and *in vivo* outcomes of invasive aspergillosis is the presence of circulating neutrophils. Specifically, while neutrophils contribute to the antifungal effect *in vivo*, they are absent in *in vitro* combination studies. As murine models of aspergillosis may be transiently neutropenic, their correlation with *in vitro* combination studies may be poor.

In conclusion, the *in vitro* findings of the present study collectively indicate that the isavuconazole-micafungin combination is synergistic for *A. fumigatus*, *A. flavus*, *A. terreus*, and one of the most virulent *Mucorales* species, *C. bertholletiae*. This *in vitro* synergism may prove effective for the treatment of difficult-to-treat *Aspergillus* infections. Animal model and clinical studies are warranted to further elucidate the potential of the isavuconazole-echinocandin combination for difficult-to-treat filamentous fungal infections.

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