AAC Journals.ASM.org

In Vitro Evaluation of Antifungal Drug Combinations against *Sarocladium* (*Acremonium*) *kiliense*, an Opportunistic Emergent Fungus Resistant to Antifungal Therapies

Fabiola Fernández-Silva,[a] Javier Capilla,[b] Emilio Mayayo,[a] Deanna Sutton,[c] Josep Guarro[b]

Unitat d'Anatomia Patològica[a] and Microbiologia,[b] Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, Reus, Spain; Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, Texas, USA[c]

Sarocladium kiliense, formerly known as *Acremonium kiliense* (1), is a ubiquitous soil saprophyte commonly found in the environment and occasionally infecting humans (2). Its pathogenicity in immunocompetent patients is low and usually is related to inoculation of the fungus via a penetrating injury that often leads to a granuloma formation. However, the presence of underlying immunological disorders can predispose to the development of a usually fatal systemic infection (3). The optimal treatment for these infections is unknown; however, amphotericin B (AMB) seems to be the most efficacious drug, although therapeutic failure has also been reported (3, 4). In addition, this drug shows important side effects that are commonly incompatible with use by patients in poor health. The therapeutic data available are based on a few clinical cases where the etiologic agent was identified only at the genus level or misidentified (5). Antifungal *in vitro* studies have shown that *S. kiliense* is resistant to almost all antifungal drugs (2, 6). In addition, recent murine studies have demonstrated that all of the therapies tested against this fungus, i.e., voriconazole (VRC), posaconazole (PSC), AMB, and anidulafungin (AFG), showed very poor efficacy (7). Regarding that, it is crucial to explore new therapeutic strategies for the treatment of severe invasive infections caused by *S. kiliense*. Therefore, the aim of this study was to evaluate the *in vitro* activity of drug combinations against a set of 12 *S. kiliense* strains from clinical sources previously identified by sequencing of the internal transcribed spacer region of the rRNA gene (2). We determined the individual MICs (MIC-0) of azoles, AMB, and terbinafine (TRB) and the minimal effective concentrations (MECs) of AFG by using the CLSI methodology for filamentous fungi (8). Drug interaction was evaluated in a checkerboard microdilution design based on the CLSI method (9). The combined effects were analyzed by summation of the fractional concentration indexes (FICis). Only for combinations of AFG and azoles, two criteria were used, i.e., the MEC of AFG and the MIC-0 of azoles (criterion A) and the MEC of AFG and the MIC-2 (~50% reduction in turbidity compared to the growth control) of azoles (criterion B) (10–12). Studies were performed in duplicate, and the final results were expressed as the means of these replicates. The FICi was used to classify drug interactions, which were defined as synergistic when the FICi was ≤0.5, as antagonistic when the FICi was >4.0, and absent when the FICi was >0.5 or ≤4 (12).

Table 1 shows the interactions of different combinations. In general, when using criterion A, most of the combinations showed an indifferent effect. Synergism of PSC-TRB was observed in one strain (8.3%) and antagonism of AMB-PSC was observed in two strains (16.6%). When FICis were calculated by using criterion B, AFG-VRC synergism was detected in three strains (25%) and PSC-AFG antagonism was also detected in three strains (16.6%).

Although some of our results showed that the interactions between azoles and echinocandins depend on the endpoint used, the combination of AFG and VRC is promising; however, further experiments evaluating the *in vivo* efficacy of this antifungal combination are warranted in order to provide new therapeutic alternatives for the treatment of infections with this resistant pathogen.

Published ahead of print 18 November 2013

Address correspondence to J. Guarro, josep.guarro@urv.cat.

doi:10.1128/AAC.02131-13

TABLE 1 Interaction showed by antifungal drug combinations against 12 clinical isolates of *S. kiliense*

Antifungal combination	FICi[a,b]	No. (%) of strains by criterion A[c]			FICi[a,b]	No. (%) of strains by criterion B[d]		
		Synergism	Indifference	Antagonism		Synergism	Indifference	Antagonism
AMB-AFG	1.43	0 (0)	12 (100)	0 (0)	ND[e]	ND	ND	ND
AMB-PSC	2.08	0 (0)	10 (83.3)	2 (16.6)	ND	ND	ND	ND
AMB-VRC	2.00	0 (0)	12 (100)	0 (0)	ND	ND	ND	ND
VRC-AFG	1.26	0 (0)	12 (100)	0 (0)	0.90	3 (25)	9 (75)	0 (0)
VRC-TRB	1.43	0 (0)	12 (100)	0 (0)	ND	ND	ND	ND
PSC-AFG	3.27	0 (0)	9 (75)	3 (25)	3.04	0 (0)	9 (75)	3 (25)
PSC-TRB	2.29	1 (8.3)	11 (91.6)	0 (0)	ND	ND	ND	ND

[a] FICi of ≤0.5, synergism; FICi of >0.5 to ≤4, indifference; FICi of >4, antagonism.
[b] Mean FICi determined for 12 *S. kiliense* isolates.
[c] Criterion A: the MEC of AFG and the MIC-0 of azoles AMB and TRB were calculated.
[d] Criterion B: the MEC of AFG and the MIC-2 of azoles were calculated.
[e] ND, not determined.

REFERENCES

1. **Summerbell RC, Gueidan C, Schroers HJ, de Hoog GS, Starink M, Rosete YA, Guarro J, Scott JA.** 2011. *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. Stud. Mycol. **68:**139–162. http://dx.doi.org/10.3114/sim.2011.68.06.
2. **Perdomo H, Sutton DA, García D, Fothergill AW, Cano J, Gené Summerbell JRC, Rinaldi MG, Guarro J.** 2011. Spectrum of clinically relevant *Acremonium* species in the United States. J. Clin. Microbiol. **49:** 243–256. http://dx.doi.org/10.1128/JCM.00793-10.
3. **Khan Z, Al-Obaid K, Ahmad S, Ghani AA, Joseph L, Chandy R.** 2011. *Acremonium kiliense*: reappraisal of its clinical significance. J. Clin. Microbiol. **49:**2342–2347. http://dx.doi.org/10.1128/JCM.02278-10.
4. **Mattei D, Mordini N, Lo Nigro C, Gallamini A, Osenda M, Pugno F, Viscoli C.** 2003. Successful treatment of *Acremonium* fungemia with voriconazole. Mycoses **46:**511–514. http://dx.doi.org/10.1046/j.0933-7407.2003.00924.x.
5. **Novicki TJ, LaFe K, Bui L, Bui U, Geise R, Marr K, Cookson BT.** 2003. Genetic diversity among clinical isolates of *Acremonium strictum* determined during an investigation of a fatal mycosis. J. Clin. Microbiol. **41:** 2623–2628. http://dx.doi.org/10.1128/JCM.41.6.2623-2628.2003.
6. **Guarro J, Gams W, Pujol I, Gené J.** 1997. *Acremonium* species: new emerging fungal opportunists *in vitro* antifungal susceptibilities and review. Clin. Infect. Dis. **25:**1222–1229. http://dx.doi.org/10.1086/516098.
7. **Fernández-Silva F, Capilla J, Mayayo E, Sutton DA, Hernández P, Guarro J.** 2013. Antifungal therapies in a murine model of disseminated infection by the emerging opportunistic fungus *Sarocladium* (*Acremonium*) *kiliense*. Antimicrob. Agents Chemother. http://dx.doi.org/10.1128/AAC.01484-13.
8. **Clinical and Laboratory Standards Institute.** 2008. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard—second edition. CLSI document M38-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
9. **Dannaoui E, Lortholary O, Dromer F.** 2004. *In vitro* evaluation of double and triple combinations of antifungal drugs against *Aspergillus fumigatus* and *Aspergillus terreus*. Antimicrob. Agents Chemother. **48:**970–978. http://dx.doi.org/10.1128/AAC.48.3.970-978.2004.
10. **Calvo E, Pastor F, Salas V, Mayayo E, Guarro J.** 2012. Combined therapy of voriconazole and anidulafungin in murine infections by *Aspergillus flavus*. Mycopathologia **173:**251–257. http://dx.doi.org/10.1007/s11046-011-9507-6.
11. **Shalit I, Shadkchan Y, Samra Z, Osherov N.** 2003. In vitro synergy of caspofungin and itraconazole against Aspergillus spp.: MIC versus minimal effective concentration end points. Antimicrob. Agents Chemother. **47:**1416–1418. http://dx.doi.org/10.1128/AAC.47.4.1416-1418.2003.
12. **Cuenca-Estrella M, Gomez-Lopez A, Garcia-Effron G, Alcazar-Fuoli L, Mellado E, Buitrago MJ, Rodriguez-Tudela JL.** 2005. Combined activity *in vitro* of caspofungin, amphotericin B, and azole agents against itraconazole-resistant clinical isolates of *Aspergillus fumigatus*. Antimicrob. Agents Chemother. **49:1232**–1235. http://dx.doi.org/10.1128/AAC.49.3.1232-1235.2005.