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In vitro synergisms obtained by amphotericin B and voriconazole associated with non-antifungal agents against *Fusarium* spp ☆

Tarcieli P. Venturini[a,b], Luana Rossato[b], Tatiana B. Spader[a,b], Giordano R. Tronco-Alves[c], Maria Izabel Azevedo[d], Caroline B. Weiler[a,b], Janio M. Santurio[b,d], Sydney Hartz Alves[a,b,d,*]

[a]*Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal de Santa Maria (UFSM), 97105-900 Santa Maria, RS, Brazil*
[b]*Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Maria (UFSM), 97105-900 Santa Maria, RS, Brazil*
[c]*Hospital Universitário de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[d]*Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), 97105- 900 Santa Maria, RS, Brazil*

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Abstract

Fusarium spp is an opportunistic fungal pathogen responsible for causing invasive hyalohyphomycosis in immunocompromised patients. Due to its susceptibility pattern with a remarkable resistance to antifungal agents the treatment failures and mortality rates are high. To overcome this situation, combination therapy may be considered which must be subjected to in vitro tests.

In vitro activities of amphotericin B, itraconazole, and voriconazole associated with azithromycin, ciprofloxacin, fluvastatin, ibuprofen, metronidazole, and also the combination of amphotericin B plus rifampin against 23 strains of *Fusarium* spp. through the checkerboard technique based on M38-A2 [Clinical and Laboratory Standards Institute (2008). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, 2nd ed. (CLSI document M38-A2) (ISBN 1-56238-668-9). Wayne, PA: CLSI] were evaluated.

The best synergistic interactions with amphotericin B were with ibuprofen (43.5%) (FICI [fractional inhibitory concentration index] range = 0.25–2). Combinations with voriconazole showed synergism, mainly with ciprofloxacin (30.4%) (FICI range = 0.25–3) and metronidazole (30.4%) (FICI range = 0.1–4); however, all the combinations with itraconazole were indifferent. In general, antagonistic interactions were not registered.

Our results showed that in vitro synergisms obtained by some combinations studied deserve attention since they were better than those showed by the antimycotic.

Keywords: *Fusarium* spp.; Susceptibility; Associations; Antifungal agents; Synergism

1. Introduction

Fusarium spp. is a well-known opportunistic fungal agent that can cause important infections in immunocompromised patients. Commonly, it shows primary resistance to most antifungal agents, which explains the high mortality rates which exceed 70% (Boutati and Anaissie, 1997; Nucci and Anaissie, 2002, 2007; Nucci et al., 2004; Raad et al., 2006). Due to the susceptibility pattern of *Fusarium* spp., the antifungal therapy options are limited. In this scenario, the combinations of 2 classes of antifungal agents have been studied in order to search for a better effect based on synergisms. On the other hand, the activity of combinations including antifungal plus a nonantimycotic has been explored for *Candida*, *Cryptococcus*, and *Aspergillus*, but against *Fusarium* spp these interactions are unknown and thus require evaluation. The aim of this study was to evaluate the susceptibility of different species of *Fusarium* to conventional antifungal agents associated with other drug classes. The non-antifungal agents used in this study were

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* Corresponding author. Tel.: +55-55-32208906; fax: +55-55-32208906.

E-mail address: sydneyalves.ufsm@gmail.com (S.H. Alves).

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selected based on other studies which have reported synergism in vitro in combination with conventional antifungal agents against fungi, such as *Candida* spp., *Cryptococcus* spp., and *Aspergillus* spp. (Chin et al., 1997; Clancy and Nguyen, 1998; Clancy et al., 1998; Cury and Hirschfeld, 1997; Edwards et al., 1980; Fujita and Edwards, 1981; Hughes et al., 1984; Kitahara et al., 1976; Kobayashi et al., 1974; Kunin, 1996; Medoff, 1983; Pina-Vaz et al., 2000; Scott et al., 1995; Spader et al., 2009; Stergiopoulou et al., 2008; Stern, 1978).

2. Materials and methods

Twenty-three isolates of *Fusarium* spp. were studied: *F. chlamydosporum* (3), *F. oxysporum* (6), *F. solani* (10), *F. solani* ATCC 36031 (1), and *F. proliferatum* (3). These strains were obtained from different sources, including blood culture (n = 13), tissue biopsy (n = 5), cornea (n = 3), and sediment of the dialysate from continuous ambulatory peritoneal dialysis (n = 1). Isolation and identification of the isolates were performed by standard microbiological and molecular techniques. Molecular analyses were performed to confirm the identity of the *Fusarium* spp. A DNA fragment comprising an internal transcribed spacer (ITS) was amplified using primers ITS1 (5′-TCCGTAGGTGA-ACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGAT-ATGC-3′) (O'Donnell et al., 2007). The amplified fragments were sequenced, and the sequences were compared with DNA sequences of *Fusarium* obtained from GenBank whose accession numbers were HQ696899, HQ696900, and HQ696908 for *F. chlamydosporium*; HQ696888, HQ696889, HQ696890, HQ696893, HQ696894, and HQ696895 for *F. oxysporum*; HQ696874, HQ696875, HQ696876, HQ696877, HQ696878, HQ696879, HQ696880, HQ696881, HQ696882, and HQ696883 for *F. solani*; and HQ696886, HQ696887, and GQ149771 for *F. proliferatum*.

The antifungal agents amphotericin B (AMB), itraconazole (ITZ), and voriconazole (VRZ), as well as non-antifungal agents, azithromycin (AZM), ciprofloxacin (CIP), fluvastatin (FVS), ibuprofen (IBP), metronidazole (MTZ), and rifampicin (RIF), were obtained from their manufacturers as pure powder. The stock solutions were prepared in dimethyl sulfoxide, except for FVS, which was prepared in sterile distilled water. The concentrations of antifungal agents tested were 0.0625 to 8 μg mL^{-1} for AMB and 0.125 to 16 μg mL^{-1} for ITZ and VRZ; the non-antifungal agents were tested at concentrations ranging from 0.25 to 32 μg mL^{-1}. The antifungal agents were tested alone and in association with non-antifungal agents. The interactions evaluated were as follows: AMB + AZM, AMB + CIP, AMB + FVS, AMB + IBP, AMB + MTZ, AMB + RIF, VRZ + AZM, VRZ + CIP, VRZ + FVS, VRZ + IBP, VRZ + MTZ, ITZ + AZM, ITZ + CIP, ITZ + FVS, ITZ + IBP, and ITZ + MTZ.

The susceptibility tests were determined by a microdilution technique (CLSI M38-A2) (Clinical and Laboratory Standards Institute, 2008), and *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, and *Aspergillus flavus* ATCC 204304 were included as quality control strains. The interactions of the drugs, based on the same document, were evaluated by the chequerboard method (Cuenca-Estrella, 2004), where 50 μL of each drug dilution was combined with another 50 μL of each dilution of the second drug, and to this volume was added 100 μL of inoculum in RPMI 1640 medium. The concentration ranges of the antifungal agents and non-antifungal agents were the same as those used for the susceptibility testing of each agent alone. The inoculum preparation was based on CLSI M38-A2 (Clinical and Laboratory Standards Institute, 2008).

To evaluate the interaction between drugs, the fractional inhibitory concentration (FIC) was calculated for each agent by dividing the MIC of each drug in the combination by the MIC of the drug alone. FIC values were then summed to determine the fractional inhibitory concentration index (FICI) resulting from the combination. Synergism was defined as FICI ≤0.5. Indifference was defined as 0.5 < FICI ≤ 4, whereas antagonism was defined when FICI was >4 (Cuenca-Estrella, 2004; Mukherjee et al., 2005).

3. Results

ITZ, AZM, CIP, FVS, IBP, MTZ, and RIF when tested alone showed no activity against *Fusarium* spp. The antifungal activity of AMB showed MIC values ranging from 0.125 to 4.0 μg mL^{-1}. Twenty (87%) of 23 isolates showed MIC ≤1.0 μg mL^{-1} for AMB and only 3 isolates (13%) showed MIC >1.0 μg mL^{-1}. Only 1 isolate of *F. solani* and 1 isolate of *F. oxysporum* required MIC = 4.0 μg mL^{-1} to AMB.

The susceptibility to VRZ showed MIC ≥4.0 μg mL^{-1} for 87% of isolates and MIC >16.0 μg mL^{-1} for ITZ for 100% of the isolates.

All combinations of non-antifungal agents with AMB showed synergistic interactions, and antagonisms were not observed in these combinations (Table 1). The best combinations with AMB were AMB + IBP (43.5%), followed by AMB + CIP (39.1%). The best combinations with VRZ were VRZ + CIP (30.4%) and VRZ + MTZ (30.4%). The concentrations of non-antifungal agents in all the synergistic interactions were ≤0.25 μg mL^{-1}. Antagonistic interactions were not observed. All combinations with ITZ showed indifferent interactions for 100% of the *Fusarium* spp. isolates.

4. Discussion

The typical profile of the antifungal susceptibility of *Fusarium* spp. is the resistance to most antifungal agents.

Table 1
Number, percentage of synergistic interactions, and FICI range obtained by combinations of amphotericin B and voriconazole with non-antifungal agents against *Fusarium* spp.

Fusarium spp. (*n*)	Antifungal agents	Non-antifungal agents					
		Number (percentage of synergistic interactions) FICI range					
		AZM	CIP	RIF	MTZ	IBP	FVS
F. chlamydosporum (*n* = 3)	AMB	2 (66.7) 0.25–1	0 (0) 1–2	1 (33.3) 0.1–1	1 (33.3) 0.5–2	0 (0) 1–2	1 (33.3) 0.5–2
F. oxysporum (*n* = 6)	AMB	3 (50) 0.5–1	3 (50) 0.5–2	1 (16.7) 0.25–1	1 (16.7) 0.5–2	0 (0) 1–2	1 (16.7) 0.5–4
F. solani (*n* = 11)	AMB	1 (9.1) 0.5–2	5 (45.5) 0.5–1	4 (36.4) 0.25–2	5 (45.5) 0.5–2	8 (72.7) 0.25–1	4 (36.4) 0.5–2
F. proliferatum (*n* = 3)	AMB	0 (0) 1–2	1 (33.3) 0.5–1	2 (66.7) 0.5–1	1 (33.3) 0.5–1	2 (66.7) 0.5–1	2 (66.7) 0.5–1
Total	AMB	6 (26.1) 0.25–2	9 (39.1) 0.5–2	8 (34.8) 0.1–2	8 (34.8) 0.5–2	10 (43.5) 0.25–2	8 (34.8) 0.5–4
F. chlamydosporum (*n* = 3)	VRZ	2 (66.7) 0.5–1	2 (66.7) 0.5–1	(–)	1 (33.3) 0.5–1	1 (33.3) 0.5–1	0 (0) 1
F. oxysporum (*n* = 6)	VRZ	2 (33.3) 0.5–1	4 (66.7) 0.25–1	(–)	4 (66.7) 0.5–1	2 (33.3) 0.5–1	1 (16.7) 0.5–1
F. solani (*n* = 11)	VRZ	1 (9.1) 0.5–3	1 (9.1) 0.5–3	(–)	2 (18.2) 0.1–4	2 (18.2) 0.1–4	2 (18.2) 0.1–1
F. proliferatum (*n* = 3)	VRZ	0 (0) 1–2	0 (0) 1	(–)	0 (0) 1	0 (0) 1	0 (0) 1
Total	VRZ	5 (21.7) 0.5–3	7 (30.4) 0.25–3	(–)	7 (30.4) 0.1–4	5 (21.7) 0.1–4	3 (13) 0.1–1

AMB = Amphotericin B; VRZ = voriconazole; AZM = azithromycin; CIP = ciprofloxacin; RIF = rifampicin; MTZ = metronidazole; IBP = ibuprofen; FVS = fluvastatin; (–) = not performed.

However, different species may have different patterns of sensitivity. The susceptibility of *F. solani* and *F. oxysporum* has been studied more than that of other *Fusarium* species because they are more prevalent in immunocompromised patients (Córdoba et al., 2008; Nucci and Anaissie, 2007). Species such as *F. chlamydosporum*, *F. nygamai*, *F. proliferatum*, and *F. sporotrichoides* are rarely reported in human infections; thus the susceptibility of these species is almost unknown.

The prognosis of fusariosis in immunocompromised patients is directly related to the immune status of patients (Nucci and Anaissie, 2007), with high mortality rates (over 70%) in this population (Raad et al., 2006). Proper treatment of disseminated infections is still not fully understood. AMB and VRZ are the most effective drugs; however, numerous therapeutic failures have been registered (Pujol et al., 1997).

Here, we evaluated the in vitro interactions between AMB, ITZ, and VRZ with non-antifungal agents (AZM, CIP, FVS, IBP, and MTZ) as well as the combination between AMB + RIF against 23 *Fusarium* spp. isolates.

The combinations with AZM showed synergism with AMB (26.1%) and VRZ (21.7%) but were indifferent with ITZ. Clancy and Nguyen (1998) have reported the synergism AMB + AZM for *Fusarium* spp.; however, the combinations with VRZ (VRZ + AZM) and ITZ (ITZ + AZM) have not been reported until now. AZM has no antifungal activity due to its inability to penetrate the membrane of fungal cell. However, the damage caused by AMB may facilitate the entry of AZM which may inhibit the synthesis of protein (Clancy and Nguyen, 1998). No studies emphasizing the combination VRZ + AZM against *Fusarium* spp. have been found.

When we tested combinations with CIP, the synergisms showed AMB (39.1%), VRZ (30.4%), and indifference with ITZ. Stergiopoulou et al. (2008) reported a synergism between AMB + CIP against *Candida albicans* and *A. fumigatus* as well as between VRZ + CIP against *A. fumigatus*. No other studies focusing on these combinations against *Fusarium* spp. have been performed. CIP has no intrinsic antifungal activity but it may interact with antifungal agents and inhibit DNA gyrase (topoisomerase II), which is abundant in fungi species (Stergiopoulou et al., 2008).

The synergisms observed by the combinations with FVS were AMB + FVS (34.8%) and VRZ + FVS (13%); the combinations with ITZ + FVS showed indifference. The synergisms between FVS with antifungal agents have been reported by other studies: Chin et al. (1997) reported synergism between FVS plus fluconazole or ITZ against *Candida* spp. and *Cryptococcus neoformans*. Natesan et al. (2008) observed that FVS increased the effect of caspofungin but did not show synergistic interactions with VRZ or AMB against *A. fumigatus*. The combinations AMB + FVS, ITZ + FVS, and VRZ + FVS have not been tested against *Fusarium* species yet. The mechanism of antifungal action of FVS in combination with antifungal agents is still unclear. It has been hypothesized that the inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase could lead to a decrease of ergosterol levels or another cytosolic precursor of ergosterol required in the fungal cell membrane synthesis (Nash et al., 2002).

The combination AMB + IBP showed synergism for 43.5% of the *Fusarium* spp. isolates, which was the highest rate of synergism observed in this study. The synergism of the combination VRZ + IBP was 21.7%; however, the indifference (100%) was the interaction showed by ITZ + IBP. Scott et al. (1995) and Pina-Vaz et al. (2000) have reported synergism for the fluconazole + IBP combination against *Candida* spp. These authors concluded that high doses of IBP are fungicidal and directly damage the cytoplasmatic membrane, whereas at low concentrations the combination (fluconazole + IBP) is fungistatic and does not affect the cytoplasmic membrane of the fungi. Another

point emphasized by those authors was the rapid potassium efflux induced by IBP. These mechanisms may explain our results; however, the combinations AMB + IBP, VRZ + IBP, and ITZ + IBP have not been reported until now.

The associations with MTZ showed synergism for AMB + MTZ (34.8%) and VRZ + MTZ (30.4%), but the interaction ITZ + MTZ showed indifference (100%). Cury and Hirschfeld (1997) reported synergism for the combination AMB + MTZ against *Candida albicans*. The mechanism of interaction between MTZ and antifungal agents is not well understood. To date, no studies encompassing combinations between antifungal agents plus MTZ against *Fusarium* spp. have been reported.

RIF was combined only with AMB which showed synergism for 34.8% of *Fusarium* spp. isolates. Some studies showed that RIF interacts synergistically in vitro with AMB against *Candida* spp., *Cryptococcus neoformans*, *Aspergillus* spp., and *Histoplasma capsulatum* (Clancy et al., 1998; Edwards et al., 1980; Fujita and Edwards, 1981; Hughes et al., 1984; Kitahara et al., 1976; Kobayashi et al., 1974; Kunin, 1996; Medoff, 1983). According to Stern (1978), this combination was synergistic for most of the *Fusarium* spp. isolates, which was shown by Spader et al. (2009) who reported high rates of synergism (68.7%) against *Fusarium* spp. Guarro et al. (1999) have also reported a synergism between AMB + RIF against *Fusarium solani*. Clancy et al. (1998) suggest that AMB damages the fungal cell membrane allowing the entry of RIF, which exerts its antifungal effect by inhibiting the synthesis of RNA.

F. solani and *F. oxysporum* are the most prevalent *Fusarium* species and therefore deserve to be emphasized. Among all the combinations tested, the highest rate of synergism against *F. solani* was shown by AMB + IBP (72.7%). For *F. oxysporum*, for best results, 2 combinations were obtained: VRZ + CIP and VRZ + MTZ, both showing 66.7% synergisms. The plasmatic concentrations of ibuprofen (61.1 ± 5 μg mL^{-1}), ciprofloxacin (2.5 ± 1 μg mL^{-1}), and metronidazole (11-14 μg mL^{-1}) (Brunton et al., 2007) are at least 10 times more elevated than the MIC values observed in combination with amphotericin B or voriconazole. Considering that ibuprofen, ciprofloxacin, and metronidazole are well-known and secure drugs, we believe these synergisms deserve in vivo studies in order to confirm our findings. If these anti-*Fusarium* activities are confirmed, they may be useful in some refractory cases of fusariosis as salvage therapy.

Based on previous reports, our results confirm that combinations between antifungal agents plus non-antifungal agents may be promising and instigating further studies focusing on in vivo applications in an experimental *Fusarium* infection deserves to be pursued.

References

Boutati EI, Anaissie EJ (1997) *Fusarium*, a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. *Blood* 90:999–1008.

Brunton LL, Lazo JS, Parker KL (2007) , 11th ed.Rio de Janeiro: MacGraw-Hill Interamericana, pp. 1848.

Chin NX, Weitzman I, Della-Latta P (1997) In vitro activity of fluvastatin, a cholesterol-lowering agent, and synergy with fluconazole and itraconazole against *Candida* species and *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 41:850–852.

Clancy CJ, Nguyen MH (1998) The combination of amphotericin B and azithromycin as a potential new therapeutic approach to fusariosis: in vitro demonstration of synergy. *J Antimicrob Chemother* 41: 127–130.

Clancy CJ, Yu YC, Lewin A, Nguyen MH (1998) Inhibition of RNA synthesis as a therapeutic strategy against *Aspergillus* and *Fusarium*: demonstration of in vitro synergy between rifabutin and amphotericin B. *Antimicrob Agents Chemother* 42:509–513.

Clinical and Laboratory Standards Institute (CLSI) (2008) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard-2nd ed., M38-A2. Wayne, PA: CLSI.

Córdoba S, Rodero L, Vivot W, Abrantes R, Davel G, Vitale RG (2008) In vitro interactions of antifungal agents against clinical isolates of *Fusarium* spp. *Int J Antimicrob Agents* 31:171–174.

Cuenca-Estrella M (2004) Combinations of antifungal agents in therapy — what value are they? *J Antimicrob Chemother* 54:854–869.

Cury AE, Hirschfeld MP (1997) Interactions between amphotericin B and nitroimidazoles against *Candida albicans*. *Mycoses* 40:187–192.

Edwards JE, Morrison J, Henderson DK, Montgomerie JZ (1980) Combined effect of amphotericin B and rifampin on *Candida* species. *Antimicrob Agents Chemother* 17:484–487.

Fujita NK, Edwards JE (1981) Combined in vitro effect of amphotericin B and rifampin on *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 19:196–198.

Guarro J, Pujol I, Mayayo E (1999) In vitro and in vivo experimental activities of antifungal agents against *Fusarium solani*. *Antimicrob Agents Chemother* 43:1256–1257.

Hughes CE, Harris C, Moody JA, Peterson LR, Gerding DN (1984) In vitro activities of amphotericin B in combination with four antifungal agents and rifampin against *Aspergillus* spp. *Antimicrob Agents Chemother* 25:560–562.

Kitahara M, Seth VK, Medoff G, Kobayashi GS (1976) Antimicrobial susceptibility testing of six clinical isolates of *Aspergillus*. *Antimicrob Agents Chemother* 9:908–914.

Kobayashi GS, Cheung SC, Schlessinger D, Medoff G (1974) Effects of rifamycin derivatives, alone and in combination with amphotericin B, against *Histoplasma capsulatum*. *Antimicrob Agents Chemother* 5:16–18.

Kunin CM (1996) Antimicrobial activity of rifabutin. *Clin Infect Dis* 22: S3–S14.

Medoff G (1983) Antifungal activity of rifampin. *Rev Infect Dis* 5:614–620.

Mukherjee PK, Sheehan DJ, Hitchcock CA, Ghannoum MA (2005) Combination treatment of invasive fungal infections. *Clin Microbiol Rev* 18:163–194.

Nash JD, Burgess DS, Talbert RL (2002) Effect of fluvastatin and pravastatin, HMG-CoA reductase inhibitors, on fluconazole activity against *Candida albicans*. *J Med Microbiol* 51:105–109.

Natesan SK, Chandrasekar PH, Alangaden GJ, Manavathu EK (2008) Fluvastatin potentiates the activity of caspofungin against *Aspergillus fumigatus* in vitro. *Diagn Microbiol Infect Dis* 60:369–373.

Nucci M, Anaissie E (2002) Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clin Infect Dis* 35:909–920.

Nucci M, Anaissie E (2007) *Fusarium* infections in immunocompromised patients. *Clin Microbiol Rev* 20:695–704.

Nucci M, Marr KA, Queiroz-Telles F, Martins CA, Trabasso P, Costa S, Voltarelli JC, Colombo AL, Imhof A, Pasquini R, Maiolino A, Souza CA, Anaissie E (2004) *Fusarium* infection in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 38:1237–1242.

O'donnell K, Sarver BA, Brandt M, Chang DC, Noble-Wang J, Park BJ, Sutton DA, Benjamin L, Lindsley M, Padhye A, Geiser DM, Ward TJ

(2007) Phylogenetic diversity and microsphere array-based genotyping of human pathogenic fusaria, including isolates from the multistate contact lens-associated U.S. keratitis outbreaks of 2005 and 2006. *J Clin Microbiol* 45:2235–2248.

Pina-Vaz C, Sansonetty F, Rodrigues AG, Martinez-de-Oliveira J, Fonseca AF, Mardh PA (2000) Antifungal activity of ibuprofen alone and in combination with fluconazole against *Candida* species. *J Med Microbiol* 49:831–840.

Pujol I, Guarro J, Gené J, Sala J (1997) In vitro antifungal susceptibility of clinical and environmental *Fusarium spp*. strains. *J Antimicrob Chemother* 39:163–167.

Raad II, Hachem RY, Herbrecht R, Graybill JR, Hare R, Corcoran G, Kontoyiannis DP (2006) Posaconazole as salvage treatment for invasive fusariosis in patients with underlying hematologic malignancy and other conditions. *Clin Infect Dis* 42:1398–1403.

Scott EM, Tariq VN, McCrory RM (1995) Demonstration of synergy with fluconazole and either ibuprofen, sodium salicylate, or propylparaben against *Candida albicans* in vitro. *Antimicrob Agents Chemother* 39:2610–2614.

Spader TB, Venturini TP, Cavalheiro AS, Mahl CD, Mario DN, Lara VM, Santurio J, Alves SH (2009) In vitro interactions between amphotericin B and other antifungal agents and rifampin against *Fusarium* spp. *Mycoses* 54:131–136.

Stergiopoulou T, Meletiadis J, Sein T, Papaioannidou P, Tsiouris I, Roilides E, Walsh TJ (2008) Isobolographic analysis of pharmacodynamic interactions between antifungal agents and ciprofloxacin against *Candida albicans* and *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 52:2196–2204.

Stern GA (1978) In vitro antibiotic synergism against ocular fungal isolates. *Am J Ophthalmol* 86:359–367.