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Short communication

In vitro interactions of antifungal agents against clinical isolates of *Fusarium* spp.

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Abstract

The in vitro activities of amphotericin B (AMB), itraconazole (ITC), voriconazole (VCZ) and terbinafine (TBF) alone and in the combinations AMB + VCZ, TBF + ITC and TBF + VCZ were evaluated against 29 clinical isolates of *Fusarium* spp. (15 *Fusarium solani*, 7 *Fusarium oxysporum*, 2 *Fusarium decemcellulare*, 2 *Fusarium dimerum* and 3 other *Fusarium* spp.). Minimum inhibitory concentrations were determined using the method of the Clinical and Laboratory Standards Institute and the interaction activity was calculated using the fractional inhibitory concentration index. The four antifungal drugs tested alone showed very limited activity against most of the isolates. In contrast, the combination TBF + VCZ showed synergy for 21 isolates. The combination AMB + VCZ showed synergism for only five strains. No interaction or antagonism was observed among the remaining strains. TBF + ITC showed no interaction for 18 strains. The in vitro antifungal activity of the drugs alone and in combination varied for different species. These results corroborate previous in vitro studies in which the combination TBF + VCZ showed synergy against *Fusarium* spp., although further studies are needed to elucidate its potential usefulness for therapy.

Keywords: *Fusarium* spp.; Antifungal susceptibility; Antifungal interactions

1. Introduction

Fusarium spp. can cause severe infection in immunocompromised hosts, including those with haematological malignancies and in transplant recipients, with *Fusarium solani* and *Fusarium oxysporum* being the most frequent species encountered. Mortality from systemic fusariosis in immunocompromised patients ranges from 50% to 80% and treatment modalities are limited [1]. However, the new azoles voriconazole (VCZ), posaconazole and ravuconazole appear to be active in vitro, especially against *F. solani* and *F. oxysporum* [2]. Nevertheless, their in vivo activity in animal models of infection is mediocre, reflecting the high mortality rate associated with this infection in clinical practice [3]. Drug combinations may provide an alternative approach to therapy. Ortoneda et al. [4] determined the in vitro activity of multiple antifungal interactions and reported that the combination of terbinafine (TBF) with VCZ or ravuconazole was mostly synergistic against *Fusarium* spp.

The aim of this work was to evaluate the in vitro activity of four licensed antifungal drugs and their interactions against 29 clinical *Fusarium* spp. strains isolated from hospitalised patients.

2. Material and methods

Twenty-nine clinical *Fusarium* spp. strains (15 *F. solani*, 7 *F. oxysporum*, 2 *Fusarium decemcellulare*, 2 *Fusarium*

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dimerum, 1 *Fusarium incarnatum*, 1 *Fusarium proliferatum* and 1 *Fusarium moniliforme*) were obtained from different sources, including blood culture ($n = 5$), tissue biopsy ($n = 4$), eyes ($n = 16$) and bronchoalveolar lavage ($n = 4$). Identification was based on morphology. *Paecilomyces variotii* ATCC 3257, *Scedosporium prolificans* M96/238, *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as quality control strains for minimum inhibitory concentration (MIC) determination.

Susceptibility testing was based on the Clinical and Laboratory Standards Institute recommendations [5] for microdilution using RPMI-1640 medium with glutamine and without sodium bicarbonate (Gibco BRL–Life Technologies, Grand Island, NY) buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (Sigma Chemical Co., St Louis, MO). Isolates were cultured on potato dextrose agar slants at 35 °C for 3 days and at 28 °C for the following 4 days. The inoculum was prepared by scraping the surface of fungal colonies from the agar growth using a loop and suspending the material in sterile saline solution. The suspension was then filtered to remove the hyphae and adjusted to a final inoculum of $1–5 \times 10^4$ colony-forming units/mL. Antifungal drugs were obtained as standard powders of known potency. Stock solutions of amphotericin B (AMB) (Sigma Chemical Co.), itraconazole (ITC) (Janssen, Buenos Aires, Argentina), TBF (Novartis, Buenos Aires, Argentina) and VCZ (Pfizer, Buenos Aires, Argentina) were prepared by dissolving the drugs in dimethyl sulfoxide (Sigma Chemical Co.) and then diluting further in RPMI-1640 medium. The final concentration range was 0.03–16 μg/mL for AMB, ITC and VCZ and 0.12–64 μg/mL for TBF. All drugs were distributed in 96-well, round-bottomed microtitration plates (Nunclon 167008; Nunc, Naperville, IL).

The microtitration plates were inoculated with 100 μL of inoculated medium and incubated at 35 °C for 72 h. The MIC was defined as the lowest concentration of drug that produced complete inhibition of fungal growth compared with the growth control.

Drug interactions were evaluated using a checkerboard titration method for the following combinations: AMB + VCZ; TBF + VCZ; and TBF + ITC. The fractional inhibitory concentration index (FICI) was calculated using the following formula: (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). Drug interactions were considered as synergistic for FICI ≤ 0.5, not interactive when the FICI was between 0.5 and 4 and antagonistic for FICI > 4 [6]. All tests were performed in duplicate.

Table 1
Minimum inhibitory concentrations (MICs) for the antifungal drugs tested

Fusarium spp.	MIC (μg/mL)			
	AMB	TBF	VCZ	ITC
F. solani ($n = 15$)	1–16	>64	4–16	>16
F. oxysporum ($n = 7$)	1 to >16	8 to >64	2–16	>16
F. decemcellulare ($n = 2$)	0.5–1	8	8	>16
F. dimerum ($n = 2$)	1	2–4	8	>16
F. incarnatum ($n = 1$)	16	>64	8	>16
F. proliferatum ($n = 1$)	4	64	8	>16
F. moniliforme ($n = 1$)	8	4	4	>16

AMB, amphotericin B; TBF, terbinafine; VCZ, voriconazole; ITC, itraconazole.

3. Results and discussion

MICs for the antifungal agents alone are summarised in Table 1. FICI values for drug combinations are summarised in Table 2. For the combination TBF + VCZ, synergy was observed for all *F. solani* isolates. For this combination, the MIC for TBF alone was 256 μg/mL and the MIC for VCZ was in the range 2–16 μg/mL, whereas in the combination the MICs were 1 μg/mL and 0.25–1 μg/mL, respectively (data not shown). Antagonism was not observed for *F. solani* for the combinations TBF + ITC and TBF + VCZ, whereas it was seen for seven and five isolates, respectively, from the total 29 tested in strains belonging to the other species.

Fusarium spp. have recently emerged as a cause of disseminated infection. Risk factors include leukaemia, cytotoxic therapy (with or without bone marrow transplantation) and neutropenia. Disseminated fusariosis is associated with high mortality in neutropenic patients and the prognosis is very poor despite antifungal therapy. *Fusarium* spp. are usually resistant to commercially available antifungal agents [7].

The efficacy of conventional and newly available antifungal agents is not always optimal. However, VCZ was approved for the treatment of fusariosis on a compassionate-use basis in patients refractory to other drugs, despite the high in vitro MIC values [8]. One way to enhance treatment is to combine antifungal agents. The efficacy may improve

Table 2
Distribution of the fractional inhibitory concentration index (FICI) values for the *Fusarium* species tested[a]

Fusarium spp.	AMB + VCZ			TBF + ITC			TBF + VCZ		
	S	NI	A	S	NI	A	S	NI	A
F. solani ($n = 15$)	2	12	1	0	15	0	15	0	0
F. oxysporum ($n = 7$)	2	4	1	3	2	2	5	0	2
Other *Fusarium* spp. ($n = 7$)	1	3	3	1	1	5	1	3	3
Total	5	19	5	4	18	7	21	3	5

AMB, amphotericin B; VCZ, voriconazole; TBF, terbinafine; ITC, itraconazole.

[a] S, synergism (FICI ≤ 0.5); NI, no interaction (FICI >0.5–4); A, antagonism (FICI > 4).

due to synergism, dose reduction, decreased side effects and possibly a shorter duration of therapy [9,10]. There are some cases in which drug combination for treatment of fusariosis has been shown to be effective, such as the combination of AMB + caspofungin, followed by suppressive therapy with VCZ [11], or the combination of liposomal AMB + VCZ in acute leukaemia in a patient who was refractory to monotherapy [12].

The aim of this study was to analyse whether antifungal combination might be useful compared with a drug alone. For this purpose, three combinations were evaluated against different *Fusarium* spp. using the microdilution checkerboard technique. The activities of AMB, VCZ, ITC and TBF alone and the combinations AMB + VCZ and TBF + VCZ or ITC were evaluated.

In general, poor antifungal activity was observed for all the drugs tested alone, as previously described for *Fusarium* and other filamentous fungi such as *Scedosporium* or Mucorales [4,8,13].

Synergy was observed for the combination TBF + VCZ against all isolates of *F. solani*, which is in agreement with another study [4]. The recommended dose is 750 mg/day for TBF and 400 mg/day for VCZ, which results in peak levels of 2.7 μg/mL and 3 μg/mL, respectively [14,15]. For these strains, the MIC values for the combination have decreased up to seven dilution steps, being 1 μg/mL for TBF and 0.25–1 μg/mL for VCZ, which are in the range of achievable blood levels, suggesting that the in vitro combination data could be helpful to evaluate drug–drug interactions for some agents.

No interactions were observed for the combination AMB + VCZ for most of the isolates, as described by Ortoneda et al. [4]. The combination of TBF with azole drugs in vitro has been reported to be synergistic against some filamentous fungi such as *Aspergillus* spp. [16] and *S. prolificans* [17] and might be due to the combined effect of these drugs on different targets in the ergosterol biosynthesis pathway. In our case, the combination of TBF with ITC showed no interaction for *F. solani*, while synergy was detected for some isolates of *F. oxysporum*.

Antagonism was not observed for the combination TBF + ITC or VCZ for *F. solani*, whereas it was observed for the other strains. The mechanisms underlying the antagonism observed in *Fusarium* is not known but might reflect decreased ergosterol in the membrane induced by VCZ. Alternatively, TBF might act as a competitive inhibitor because it acts one step ahead of VCZ.

Antagonism was observed for the combination AMB + VCZ in only 5 of the 29 strains tested. The combination of AMB either with azoles or TBF was observed to be antagonistic against *Aspergillus* spp. and this was considered to be due to the use of different methods and the influence on the FICI of different end points for the drugs tested [16].

The sterol biosynthetic pathway varies between different strains: nuclear demethylations occur before side-chain alkylation in *Candida glabrata* and *Saccharomyces cerevisiae*, but this reaction sequence is reversed in some *Candida albicans* strains [18]. Such differences might explain why antagonism was seen in some strains but not in others.

In conclusion, the results observed from this work show that drug combinations, especially TBF + VCZ, could be effective against infections cause by *Fusarium* spp. Systemic fusariosis is associated with a high mortality rate, therefore it is important to optimise early and rapid diagnosis, including species-level identification since different interactions have been observed for different species tested. Further determination of susceptibility tests of drugs alone and/or in combination could be helpful. However, further in vitro and in vivo studies are needed to elucidate the clinical significance of these in vitro findings in the clinical context in order to determine adequate therapy.

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References

[1] Dignani MC, Anaissie E. Human fusariosis. Clin Microbiol Infect 2004;10(Suppl.1):67–75.

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

[3] Torres HA, Kontoyiannis DP. Hyalohyphomycoses. In: Dismukes WE, Pappas PG, Sobel JD, editors. Oxford textbook of clinical mycology. New York: Oxford University Press; 2003. p. 252–70.

[4] Ortoneda M, Capilla J, Javier Pastor F, Pujol I, Guarro J. *In vitro* interactions of licensed and novel antifungal drugs against *Fusarium* spp. Diagn Microbiol Infect Dis 2004;48:69–71.

[5] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard. Document M38-A. Wayne, PA: NCCLS; 2002.

[6] Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother 2003;52:1.

[7] Fleming RV, Walsh TJ, Anaissie EJ. Emerging and less common fungal pathogens. Infect Dis Clin North Am 2002;16:915–33, vi–vii.

[8] Arikan S, Lozano-Chiu M, Paetznick V, Nangia S, Rex JH. Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of *Aspergillus* and *Fusarium* species. J Clin Microbiol 1999;37:3946–51.

[9] Ghannoum MA, Rice LB. Antifungal agents: mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clin Microbiol Rev 1999;12:501–17.

[10] Polak A. Combination therapy with antifungal drugs. Mykosen Suppl 1988;2:45–53.

[11] Vagace JM, Sanz-Rodriguez C, Casado MS, Alonso N, Garcia-Dominguez M, de la Llana FG, et al. Resolution of disseminated fusariosis in a child with acute leukemia treated with combined antifungal therapy: a case report. BMC Infect Dis 2007;7:40.

[12] Durand-Joly I, Alfandari S, Benchikh Z, Rodrigue M, Espinel-Ingroff A, Catteau B, et al. Successful outcome of disseminated *Fusarium* infection with skin localization treated with voriconazole and amphotericin B-lipid complex in a patient with acute leukemia. J Clin Microbiol 2003;41:4898–900.

[13] Pujol I, Guarro J, Sala J, Riba MD. Effects of incubation temperature, inoculum size, and time of reading on broth microdilution susceptibility

test results for amphotericin B against *Fusarium*. Antimicrob Agents Chemother 1997;41:808–11.

[14] Groll AH, Piscitelli SC, Walsh TJ. Antifungal pharmacodynamics: concentration–effect relationships *in vitro* and *in vivo*. Pharmacotherapy 2001;21:133S–48S.

[15] Perfect JR, Marr KA, Walsh TJ, Greenberg RN, DuPont B, de la Torre-Cisneros J, et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. Clin Infect Dis 2003;36:1122–31.

[16] Te Dorsthorst DT, Verweij PE, Meis JF, Punt NC, Mouton JW. Comparison of fractional inhibitory concentration index with response surface modeling for characterization of in vitro interaction of antifungals against itraconazole-susceptible and -resistant *Aspergillus fumigatus* isolates. Antimicrob Agents Chemother 2002;46: 702–7.

[17] Meletiadis J, Mouton JW, Meis JF, Verweij PE. In vitro drug interaction modeling of combinations of azoles with terbinafine against clinical *Scedosporium prolificans* isolates. Antimicrob Agents Chemother 2003;47:106–17.

[18] Margalith PZ. Steroid microbiology. Springfield, IL: Charles C. Thomas; 1986.