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Comparative *in vitro* pharmacodynamic analysis of isavuconazole, voriconazole, and posaconazole against clinical isolates of aspergillosis, mucormycosis, fusariosis, and phaeohyphomycosis

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ABSTRACT

We compared the *in vitro* pharmacodynamics of isavuconazole, voriconazole, and posaconazole against 92 clinical isolates from documented cases of invasive aspergillosis, mucormycosis, fusariosis, and phaeohyphomycosis. Whereas inhibitory and fungicidal concentrations of these triazoles were predictably similar with the exception of *Mucorales*, isavuconazole appeared to have improved pharmacodynamics against *Fusarium solani*.

Isavuconazonium sulfate (ISAV) is a broad-spectrum prodrug triazole antifungal with activity against a wide range of medically important fungi, including *Aspergillus* spp., *Mucorales, Fusarium* spp., *Cryptococcus* species, dimorphic fungi (Thompson et al. 2016), and dematiaceous molds. ISAV is approved for the treatment of invasive aspergillosis and mucormycosis. The drug is associated with fewer toxicities compared to voriconazole (VRC) or amphotericin B-based regimens (Maertens et al. 2016; Marty et al. 2016). The preclinical (*in vitro* and animal infection models) and clinical pharmacodynamics (human studies) of isavuconazole have been characterized for both invasive candidiasis (Lepak et al. 2013a) and aspergillosis (Lepak et al. 2013b; Seyedmousavi et al. 2015; Box et al. 2016; Petraitis et al. 2016; Desai et al. 2017; Kovanda et al. 2017) and are currently used to support clinical breakpoints in susceptibility testing of *Aspergillus* spp. using the EUCAST method (Arendrup et al. 2016). However, studies characterizing ISAV pharmacodynamics for less common molds associated with severe opportunistic mycoses in immunocompromised hosts are scarce.

To address this knowledge gap, we compared the *in vitro* pharmacodynamics of isavuconazole, voriconazole, and posaconazole (PCZ) in a collection of 92 clinical mold isolates from patients treated at the University of Texas M.D. Anderson Cancer Center, Houston, TX, between 1998 and 2017. All isolates were associated with cases of probable or proven invasive aspergillosis, mucormycosis, fusariosis, or phaeohyphomycosis as per EORTC/MSG diagnostic criteria (De Pauw et al. 2008). Isolates were banked at −80 °C using previously described methods (Pasarell and McGinnis 1992). Prior to testing, isolates were thawed and subcultured on Sabouraud dextrose agar to ensure adequate sporulation after 3–5 days of inoculation at 37 °C. In some cases, longer incubation was required.

A standardized inoculum for testing was prepared by covering colonies with 5 mL of sterile water supplemented with 0.1% Tween 20. Conidia were then rubbed with a sterile glass rod and passed through a 40-μm cell strainer to remove residual hyphal fragments. After counting with a hemocytometer, spore suspensions were prepared in sterile culture medium (0.165 M 3-[N-morpholino] propanesulfonic acid plus 2% glucose) at a concentration of 2×10^4 spores/mL. A total of 100 μL of the spore solutions was inoculated into 96-well flat-bottom microtiter trays containing 100 μL of 2-fold serial dilutions of ISAV, VRC, or PCZ (0.031–16 μg/mL in culture medium). Drug stocks were prepared from analytical grade powder provided by the manufacturers and dissolved in dimethyl sulfoxide as previously described (Lewis et al. 2005). After 48 h of incubation at 37 °C, the MIC was read as the drug concentration resulting in complete inhibition of fungal growth according to CLSI M38 guidelines (Clinical Laboratory Standards Institute 2017). All experiments were performed in triplicate.

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To approximate fungicidal activity, experiments were repeated as described above except that hyphal metabolic activity was measured using the XTT reduction assay as reported by Meletiadis et al. (Meletiadis et al. 2001; Antachopoulos et al. 2006) with minor modifications previously described (Lewis et al. 2005). Briefly, after 22 h of incubation, the trays were removed from the incubator, and 50 μL of XTT solution (10 mg/ml) prepared in sterile water containing 125 mM menadione (Sigma) was added to each well. After 2 additional hours of incubation and following brief agitation of the trays, formazan absorbance in each well was read at 460 nm in a microplate spectrophotometer (BIOTEK Power Wave HT, Scimetrics). Absorbance readings were normalized in relation to unconverted XTT in the medium control wells and plate absorbance (692 nm). All experiments were performed in triplicate. Nonconstrained concentration–effect curves were generated by fitting a 3-parameter nonlinear logistic regression model for each drug–species combination to estimate target triazole concentrations associated with a 50% (EC_{50}, half-maximal effective concentration) and 90% (EC_{90}, 90% effective concentration) reduction in 24-h hyphal viability. Model fit was assessed visually and by R^2 and 95% confidence intervals (CIs) of the EC_{50}/EC_{90} estimates.

The results of the MIC and pharmacodynamic testing are summarized in Table 1. With few exceptions (*Alternaria* spp. and *Curvularia* spp.), ISAV and PCZ MICs were 2–4-fold lower than VRC for most molds tested. Notably, all 5 *Alternaria* isolates tested were nonsusceptible to PCZ, whereas MICs of VRC (0.4 μg/mL) and ISAV (0.5 μg/mL) were relatively low, corroborating previously reported wide variations in the *in vitro* susceptibility of *Alternaria* spp. to triazole antifungals (Pastor and Guarro 2008). In line with higher MICs, XTT assays revealed substantially higher estimated EC_{50} (4.74 μg/mL) and EC_{90} (6.70 μg/mL) for PCZ than VRC and ISAV.

While lower EC_{50} and EC_{90} values for PCZ against *A. fumigatus* and ISAV against *A. flavus* and *A. terreus* were found, the 95% CIs for the studied triazoles overlapped and all EC_{50}/EC_{90} estimates were within clinically achievable serum exposures. As expected, VRC was less active against *Mucorales* compared to ISAV and PCZ with markedly higher estimated EC_{50} and EC_{90} values for *Rhizopus arrhizus, Rhizomucor* spp., *Mucor* spp., and *Cunninghamella bertholletiae*. PCZ had lower EC_{50}/EC_{90} estimates than ISAV for *Rhizomucor* spp. and *C. bertholletiae*, but ISAV EC_{50} and EC_{90} values remained within achievable average serum concentrations, which are 4–6 times higher than for PCZ (Cornely et al. 2016; Cornely et al. 2017; Desai et al. 2017).

Among 7 *Fusarium solani* complex isolates tested, ISAV displayed the lowest estimated EC_{50} (1.16 μg/mL) and EC_{90} (2.49 μg/mL) values that were within achievable serum exposures. The broad range of observed VRC and PCZ EC_{50} and EC_{90} values for *F. solani* was consistent with more variable activity against this frequently multidrug-resistant species. All 3 triazoles exhibited similarly low estimated EC_{50}/EC_{90} values and steep concentration–response curves against the 5 isolates of *Scedosporium apiospermum*. None of the 3 triazoles appeared to substantially reduce the viability of *Lomentospora prolificans* over a range of clinically achievable drug concentrations, and the estimates for EC_{50}/EC_{90} were relatively imprecise due to poorer fit of the logistic regression models ($R^2 = 0.45$-0.60). However, the limited activity is consistent with the well-established clinical observation of high treatment failure rates of all antifungals with this species (Pellon et al. 2018).

Taken together, the *in vitro* pharmacodynamics of VRC, PCZ, and ISAV were largely similar for *Aspergillus* species. Predictably, the potency of VRC against mucormycosis was substantially lower than PCZ and ISAV. We unexpectedly found that PCZ was less active compared to other triazoles against *Alternaria* isolates. Unlike other triazoles, ISAV EC_{90} values for *F. solani* fell within clinically achievable serum concentrations, suggesting that ISAV may provide pharmacodynamic advantages in the treatment of this multidrug-resistant mold. This observation would merit future PK/PD bridging studies and comparative therapeutic evaluation of ISAV *versus* other triazoles in animal models of fusariosis.

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Table 1
MIC, EC_{50}, and EC_{90} of isavuconazole, voriconazole, and posaconazole against 92 clinical mold isolates.

	Isavuconazole mg/L			Voriconazole mg/L			Posaconazole mg/L		
Pathogen (number of isolates)	Geometric mean MIC (range)	EC_{50} (95% CI)	EC_{90} (95% CI)	Geometric mean MIC (range)	EC_{50} (95% CI)	EC_{90} (95% CI)	Geometric mean MIC (range)	EC_{50} (95% CI)	EC_{90} (95% CI)
Aspergillus fumigatus ($n = 10$)	0.5 (0.25–1)	0.44 (0.34–0.54)	1.26 (0.93–1.58)	2.0 (2–2)	0.72 (0.56–0.88)	1.64 (0.99–2.30)	0.4 (0.25–1)	0.17 (0.12–0.21)	0.53 (0.27–0.79)
Aspergillus flavus ($n = 10$)	0.8 (0.5–1)	0.38 (0.31–0.45)	0.67 (0.45–0.86)	2.1 (2–4)	0.82 (0.54–1.09)	2.67 (1.00–4.32)	1.2 (0.5–2)	0.62 (0.41–0.82)	1.98 (0.76–3.20)
Aspergillus terreus ($n = 10$)	0.4 (0.125–1)	0.31 (0.21–0.41)	0.81 (0.53–1.10)	1.5 (0.25–4)	0.53 (0.33–0.73)	1.63 (0.55–2.70)	1.9 (0.5–4)	0.80 (0.31–1.30)	3.10 (1.65–4.52)
Rhizopus arrhizus ($n = 10$)	1.1 (0.5–2)	1.08 (0.84–1.32)	2.08 (1.24–2.92)	5.7 (4–8)	3.47 (2.73–4.21)	6.07 (3.78–8.37)	0.7 (0.5–1)	0.87 (0.72–1.01)	1.23 (0.95–1.51)
Rhizomucor spp. ($n = 10$)	0.5 (0.25–1)	1.15 (0.71–1.59)	3.39 (1.81–4.97)	6.5 (4–8)	4.28 (3.51–5.05)	6.34 (4.47–8.21)	0.4 (0.25–0.5)	0.87 (0.69–1.04)	1.23 (0.88–1.58)
Mucor spp. ($n = 10$)	2.5 (2–4)	1.87 (1.10–2.63)	2.36 (0.34–4.44)	6.5 (2–16)	6.34 (4.99–7.79)	12.43 (7.28–17.60)	3.0 (1–4)	0.22 (0.13–0.31)	0.59 (0.24–0.94)
Cunninghamella bertholletiae ($n = 5$)	1.7 (1–2)	1.28 (1.28–1.28)	1.78 (1.18–1.99)	10.6 (8–16)	9.75 (5.76–13.75)	15.42 (4.45–26.40)	0.6 (0.5–1)	0.61 (0.35–0.87)	1.11 (0.28–1.93)
Alternaria spp. ($n = 5$)	0.5 (0.5–0.5)	0.44 (0.01–0.82)	0.51 (0.22–0.79)	0.4 (0.25–1)	0.13 (0.07–0.20)	0.42 (0.17–0.67)	12.1 (8–16)	4.74 (3.46–6.01)	6.70 (2.81–10.58)
Curvularia spp. ($n = 5$)	1.7 (1–2)	0.79 (0.12–0.55)	1.67 (0.82–2.50)	0.8 (0.5–1)	0.32 (0.21–0.42)	0.62 (0.36–0.88)	0.5 (0.125–0.5)	0.32 (0.21–0.43)	0.58 (0.37–0.80)
Fusarium solani ($n = 7$)	2.7 (2–4)	1.16 (0.81–1.51)	2.49 (1.35–3.62)	8.0 (8–8)	4.11 (3.11–5.12)	8.22 (4.67–11.79)	4.9 (4–8)	1.55 (0.89–2.21)	6.76 (2.94–10.58)
Lomentospora prolificans ($n = 5$)	10.6 (8–16)	7.97 (4.39–11.57)	18.40 (4.28–28.59)	7.0 (4–8)	2.53 (1.10–3.96)	8.60 (1.58–15.6)	12.1 (8–16)	41.21 (22–79.9)	64.0 (55.2–128.2)
Scedosporium apiospermum ($n = 5$)	0.5 (0.5–0.5)	0.33 (0.21–0.46)	0.85 (0.35–1.35)	0.2 (0.125–0.25)	0.15 (0.09–0.20)	0.32 (0.15–0.50)	0.5 (0.25–1)	0.28 (0.12–0.44)	0.79 (0.31–1.26)

Declarations of interest

REL has received research support from Merck & Co and has served on advisory boards for Astellas Pharma and Cidara Therapeutics. NDB has received research support from and is on the advisory board of Astellas Pharma. DPK reports research support from Astellas Pharma and honoraria from Merck & Co, Amplyx Pharmaceuticals, Astellas Pharma, Gilead Sciences, Cidara Therapeutics, and Mayne Pharmaceuticals. He has served as a consultant for Astellas Pharma, Merck & Co, and Pfizer. All other authors report no potential conflicts of interest.

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