The In Vitro Activity of Isavuconazole against *Aspergillus* Species and Zygomycetes According to EUCAST Methodology

SHORT REPORT

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We evaluated the MICs of isavuconazole (ISAV) against 96 isolates of *Aspergillus* species and 36 zygomycetes according to EUCAST methodology. In addition, the in vitro activity was obtained for hyphal inocula. ISAV exhibited good antifungal activity against the tested isolates with the exception of *Aspergillus niger* and *Mucorales*. The in vitro activity of ISAV was comparable to that of voriconazole aside from *Mucorales*. 
Invasive fungal infections (IFIs) are an increasing cause of morbidity and mortality in immunocompromised populations (2, 18). Despite correct antifungal treatment, the mortality rate of patients with IFIs remains high (7, 12). Isavuconazole (ISAV) (formerly known as BAL4815) is a novel and promising broad-spectrum triazole in late-stage clinical development for the treatment of invasive aspergillosis and candidiasis. ISAV has proven in vitro activity against Aspergillus spp. and Candida spp. (13). In an experimental neutropenic murine model of disseminated Aspergillus flavus infection, ISAV treatment resulted in high survival rates compared to control (20).

The aim of this study was the in vitro activity of ISAV against a wide range of non-A. fumigatus species and a subset of various zygomycetes. Susceptibility testing was performed according to the methods of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) (17). In addition, the minimal fungicidal concentrations (MFC) were performed and for a subset of fungi we evaluated the activity of ISAV against hyphae of the various fungi (10, 15).

All fungi were recovered from the Innsbruck Medical University through a period of ten years (1996 to 2006). The isolates were obtained from various specimens such as blood, respiratory tract specimens, biopsies and other deep sites. In totally, we evaluated the minimum inhibitory concentration (MIC) of 132 clinically relevant fungi such as A. fumigatus (n=32), Aspergillus flavus (n=16), Aspergillus terreus (n=35), Aspergillus niger (n=13), Rhizomucor spp. (n=9), Absidia spp. (n=8), Rhizopus spp. (n=7), Cunninghamamella spp. (n=3), and Mucor spp. (n=9). ISAV was kindly provided as reagent-grade powder from Basilea Pharmaceutica (Basel, Switzerland). The azoles voriconazole (VOR) and posaconazole (POS) served as control.

MICs were determined by using the reference procedure of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for spore forming moulds (17). Briefly, testing was performed in flat-bottomed microdilution plates by using RPMI 1640 medium supplemented with 2% glucose and an inoculum size of 2–5 x 10^5 CFU/ml. MIC endpoints were determined visually at 48 h and defined as the lowest drug concentration that resulted in a reduction in growth of 100% compared with that of a drug-free-growth control well. A. fumigatus ATCC 204306 and A. flavus ATCC 204304 were included as
control isolates. The MFCs were evaluated by the method of Espinel-Ingroff (4, 5) and
defined as the lowest drug concentration that resulted in 99% killing.

The MICs for hyphae of Aspergillus spp. and zygomycetes were tested by the method
of Lass-Flörl et al. (10, 15). Briefly, 100 µl of conidial solutions were added onto 96-
well plates (Costar, Vienna, Austria) and incubated at 30°C for 12–20 h to allow the
formation of hyphae. The outgrowth of hyphal length (50 to 70 µm) was determined
by an inverted microscope. Wells were then washed, refilled with 100 µl RPMI 2%G
and the drugs were added and incubated at 37°C for additional 12–18 hours; the
endpoints were read at 100% inhibition. For comparison of hyphal MICs, the
metabolic activity of drug-treated hyphae was determined by their ability to reduce the
tetrazolium compound 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide
(MTT), as described elsewhere (11). All tests run in duplicate and were repeated
twice.

The antifungal activity is expressed as MIC range and MIC at which 90% and 50%
of isolates were inhibited (MIC$_{90}$ and MIC$_{50}$, respectively). Similar is for MFC data.

Our study showed ISAV to be active against a wide range of Aspergillus
conidia and hyphae and demonstrated activity against A. terreus, an amphotericin B-
resistant species (Table 1 and 2). MICs data obtained for POS, VOR and ISAV via
EUCAST methodology are in agreement with MICs evaluated by CLSI methods. The
average geometric means (GMs) of MICs for ISAV against A. fumigatus, A. flavus, A.
terreus and A. niger were 0.63, 0.76, 0.68 and 2.36, respectively. ISAV demonstrated
potent in vitro activity against Aspergillus spp. in a study testing 118 clinical isolates
of A. fumigatus, A. flavus, A. terreus and A. niger according to the CLSI method for
broth dilution antifungal susceptibility testing of filamentous fungi (19). 16
Aspergillus isolates resistant to itraconazole, caspofungin or amphotericin B were
included and ISAV showed no cross-resistance. None of the tested isolates exhibited
ISAV MICs > 2 µg/ml (19, 20). Human pharmacokinetic data show a plasma level of
> 1.7 mg/l (20). In our study, ISAV displays also fungicidal activity and MFCs were
within two dilutions of the MIC for the various aspergilli tested (Table 3) as found by
others (6).

Several A. niger strains tested showed higher MICs when compared to A. fumigatus.
This was not observed in the study by Warn et al. (19) nor by Guinea et al (6). In a
collection of 42 A. niger three isolates were resistant to itraconazole (MIC > 8 µg/ml)
(8).
Zygomycetes are known to be resistant to VOR and echinocandins in vitro and in vivo (1). In our study, ISAV presented limited antifungal effects against zygomycetes (Table 1). Similar data were observed by others using CLSI (3, 14): in comparison with itraconazole, ravuconazole and VOR, ISAV showed partial activity against mucorales. Only 11% of *Rhizomucor* spp. and 28% of *Rhizopus* spp. showed a MIC < 2 µg/ml.

The onset of invasive fungal infection is associated with the appearance of hyphae. Consequently, an agent must be active against the hyphal form in order to be clinically effective (2). ISAV exerted strong activities against the hyphae of *A. fumigatus*, *A. flavus* and *A. terreus* (Table 2). For amphotericin B the MICs of hyphae were two to three dilutions higher compared to conidial MICs (9). Hyphal MICs of zygomycetes against ISAV were similar as for conidia, respectively. For hyphae, comparison of the visually determined endpoints with the results of the MTT method revealed that 87% of the visually determined MICs corresponded to a 95% or greater reduction in metabolic activity.

In conclusion, ISAV demonstrated impressive antifungal activity against hyphae and conidia of *Aspergillus* spp. and EUCAST methodology resulted in similar MIC ranges as with the CLSI reference method.
Reference List


### TABLE 1. In vitro susceptibility of BAL 4815 against moulds according to EUCAST methodology

<table>
<thead>
<tr>
<th>Fungi</th>
<th>No. of isolates</th>
<th>Isavuconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
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**TABLE 2.** MIC data obtained for hyphae of *Aspergillus* spp. and zygomycetes.

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TABLE 3. MFC data obtained for *Aspergillus* spp. and zygomycetes.

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<th>Voriconazole</th>
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