

# In Vitro Antifungal Activity of Isavuconazole against *Madurella mycetomatis*

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Currently, therapy of black-grain mycetoma caused by *Madurella mycetomatis* consists of extensive debridement of the infected tissue combined with prolonged antifungal therapy with ketoconazole or itraconazole. In the present study, the *in vitro* activity of the new triazole isavuconazole toward *M. mycetomatis* was evaluated. Isavuconazole appeared to have high activity against *M. mycetomatis*, with MICs ranging from  $\leq 0.016$  to  $0.125 \mu\text{g/ml}$ . Due to its favorable pharmacokinetics, isavuconazole could be a promising antifungal agent in the treatment of mycetoma.

*Madurella mycetomatis* is the most common causative agent of eumycetoma. This chronic, granulomatous infection is often found in the lower extremities, but it has been reported in other parts of the body as well (4). The infection starts as a small subcutaneous nodule, which gradually progresses into chronic inflammatory lesions with multiple sinuses that excrete a purulent and seropurulent discharge containing black grains. To date, treatment of eumycetoma in areas where eumycetoma is endemic consists of a combination of extensive mutilating surgery or amputation of the infected tissue or limb and prolonged antifungal therapy with ketoconazole or itraconazole (1). However, eumycetoma is associated with high recurrence rates, even after amputation of the affected limb. For *M. mycetomatis*, the susceptibility to antifungal agents belonging to the classes of polyenes, azoles, allylamines, and echinocandins has been determined (3, 18, 20). Data from these studies suggest that *M. mycetomatis* is susceptible only to antifungal agents that interfere with ergosterol synthesis. Low MICs were obtained for the azoles.

Currently, isavuconazole is under development as a new azole for the treatment of invasive fungal infections. No *in vitro* susceptibility data of isavuconazole toward *M. mycetomatis* are available. In this study, the *in vitro* activity of isavuconazole to *M. mycetomatis* is evaluated by determining the MICs of 22 *M. mycetomatis* strains (obtained from 21 patients in the Mycetoma Research Centre, University of Khartoum, Sudan, in 1999 and 2000) in duplicate. The strains were identified previously by morphology, PCR-restriction fragment length polymorphism (RFLP), and internal transcribed spacer (ITS) sequencing (2, 19). MICs were determined by using the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide (XTT) assay (3). In brief, *M. mycetomatis* colonies were inoculated in RPMI 1640 medium containing 0.35 g/liter L-glutamine and 1.98 mM 4-morpholinepropanesulfonic acid, sonicated (5 s at 28- $\mu\text{m}$  maximum power, Beun de Ronde, The Netherlands), and incubated for 7 days at 37°C. The mycelia were harvested by another sonication step and 5-min centrifugation at  $2,628 \times g$ . The pellet was washed and resuspended in sterile saline, and the fungal suspension was adjusted to 70% transmission at 660 nm (Novaspec II; Pharmacia Biotech). The drug concentrations tested ranged from  $0.016 \mu\text{g/ml}$  to  $16 \mu\text{g/ml}$  isavuconazole. As comparator agents, ketoconazole and itraconazole were tested at the same concentrations. XTT was administered to facilitate

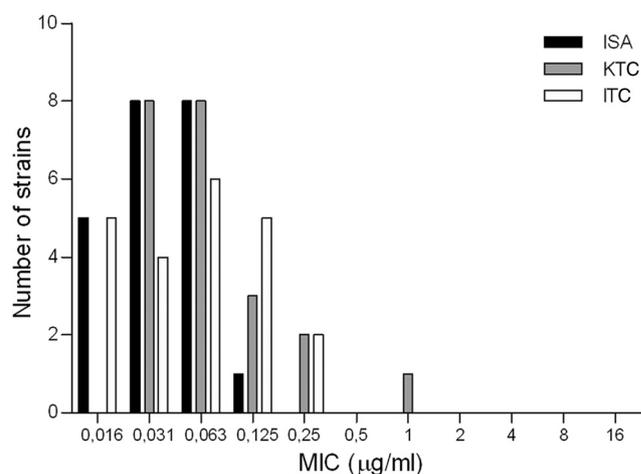


FIG 1 *In vitro* susceptibilities of 22 *Madurella mycetomatis* isolates to isavuconazole (ISA), ketoconazole (KTC), and itraconazole (ITC), according to the XTT assay.

endpoint reading. MICs were determined spectrophotometrically and defined as the lowest concentration of antifungal agent where at least an 80% growth reduction was measured. *Candida parapsilosis* (ATCC 22019) was included in the study as a quality control strain.

In concordance with previously published data, *M. mycetomatis* was strongly inhibited by low concentrations of ketoconazole and itraconazole (18), the antifungal agents currently used to treat mycetoma in the areas where mycetoma is endemic. MICs for ketoconazole and itraconazole ranged from  $0.031 \mu\text{g/ml}$  to  $1 \mu\text{g/ml}$  and  $\leq 0.016 \mu\text{g/ml}$  to  $0.25 \mu\text{g/ml}$ , respectively (Fig. 1).

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TABLE 1 Susceptibility of *M. mycetomatis* to ketoconazole, itraconazole, and isavuconazole

Antifungal agent	GM <sup>a</sup> MIC (μg/ml)	MIC (μg/ml)		
		Range	50%	90%
Ketoconazole	0.070	0.031–1	0.063	0.25
Itraconazole	0.054	≤0.016–0.25	0.063	0.125
Isavuconazole	0.037	≤0.016–0.125	0.031	0.063

<sup>a</sup> GM, geometric mean.

Concentrations of 0.25 μg/ml and 0.125 μg/ml were needed to inhibit 90% of the isolates (MIC<sub>90</sub>) for ketoconazole and itraconazole, respectively (Table 1). Compared to ketoconazole, significantly lower MICs were obtained for isavuconazole, ranging from ≤0.016 μg/ml to 0.125 μg/ml, with a MIC<sub>90</sub> of 0.063 μg/ml (Mann-Whitney, *P* = 0.018). Compared to previously determined MICs of posaconazole (18) and voriconazole (20), MICs of isavuconazole were significantly lower than those of voriconazole (*P* = 0.0002) but not those of posaconazole.

The *in vitro* susceptibility of *M. mycetomatis* to isavuconazole is comparable to that of *Exophiala* spp., also a well-known cause of black-grain mycetoma (6). In contrast, white-grain mycetoma agents, like *Pseudallescheria boydii* and *Fusarium* spp., have higher MICs of isavuconazole than *M. mycetomatis* does (6, 10). Various studies on the *in vitro* activity of isavuconazole on *Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans*, *Cryptococcus gattii*, and other fungi conclude that isavuconazole has at least similar or better activity than comparable antifungal compounds, like posaconazole and voriconazole (5–9, 12, 13, 16, 17, 21). Isavuconazole has even shown high activity against *Aspergillus* spp. resistant to itraconazole, caspofungin, and amphotericin B (21) and to *Candida* spp. resistant to fluconazole (24). In addition, in *in vivo* animal models of fungal infections, such as invasive aspergillosis and disseminated candidiasis, isavuconazole showed good therapeutic activity, comparable to that of voriconazole and itraconazole (11, 22, 23).

Although the activity of isavuconazole is often comparable to the activity of voriconazole, it is already known from phase I and II studies that isavuconazole has several pharmacokinetic properties that are advantageous over currently available azoles (14, 15). The prodrug BAL8557 (or isavuconazonium) is water soluble in contrast to currently used triazoles, including itraconazole and voriconazole, which require cyclodextrin to achieve solubility. Furthermore, the prodrug is rapidly converted into the active compound BAL4815, which has a longer half-life than other currently available triazoles. Also, its toxicity is lower than ketoconazole or itraconazole, and it has extensive tissue distribution and high plasma binding capacity (15). Isavuconazole is under investigation in different phase III studies on the safety and efficacy in treatment of fungal infections caused by *Candida* spp., *Aspergillus* spp., other filamentous fungi, rare molds, yeasts, and dimorphic fungi (ClinicalTrials.gov identifiers NCT00413218, NCT00634049, and NCT00412893). Furthermore, in the study published by Schmitt-Hoffmann et al., in which both oral and intravenous treatment regimens in a multiple-dose study in healthy volunteers were tested, excellent bioavailability with maximum drug concentration levels in serum of >1.85 μg/ml was found (15). This serum level is much higher than the MICs obtained for *M. mycetomatis* in the present study (MIC<sub>90</sub> of 0.063

μg/ml). These studies will give us a good understanding whether isavuconazole has an equal or even better therapeutic efficacy than the currently used azoles in the treatment of invasive fungal infections.

In conclusion, isavuconazole appears highly active against *M. mycetomatis* with significantly lower MICs than the MICs of ketoconazole. While no clinical data are available yet, the known pharmacokinetic properties of isavuconazole and the data presented in the current report suggest that isavuconazole is a promising antifungal agent in the treatment of mycetoma caused by *M. mycetomatis*.

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