In Vitro Activities of Isavuconazole and Other Antifungal Agents against Candida Bloodstream Isolates

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Isavuconazole is the active component of the new azole antifungal agent BAL8557, which is entering phase III clinical development. This study was conducted to compare the in vitro activities of isavuconazole and five other antifungal agents against 296 Candida isolates that were recovered consecutively from blood cultures between 1995 and 2004 at a tertiary care university hospital. Microdilution testing was done in accordance with CLSI (formerly NCCLS) guideline M27-A2 in RPMI-1640 MOPS (morpholino propane sulfonic acid) broth. The antifungal agents tested were amphotericin B, fluconazole, fluconazole, itraconazole, voriconazole, and isavuconazole. C. albicans was the most common species, representing 57.1% of all isolates. There was no trend found in favor of non-Candida albicans species over time. In terms of MIC50s, isavuconazole was more active (0.004 mg/liter) than amphotericin B (0.5 mg/liter), itraconazole (0.008 mg/liter), voriconazole (0.03 mg/liter), fluconazole (0.125 mg/liter), and fluconazole (8 mg/liter). For isavuconazole, MIC50s/MIC90s ranged from 0.0002/0.004 mg/liter for C. albicans to 0.25/0.5 mg/liter for C. glabrata. Two percent of isolates (C. glabrata and C. krusei) were resistant to fluconazole; C. albicans strains resistant to fluconazole were not detected. There were only two isolates with MICs for isavuconazole that were >0.5 mg/liter: both were C. glabrata isolates, and the MICs were 2 and 4 mg/liter, respectively. In conclusion, isavuconazole is highly active against Candida bloodstream isolates, including fluconazole-resistant strains. It was more active than itraconazole and voriconazole against C. albicans and C. glabrata and appears to be a promising agent against systemic Candida infections.

Over the past two decades, the incidence of Candida bloodstream infections has increased dramatically (3, 15), primarily due to the increase in the number of at-risk patients. Mortality rates associated with systemic Candida infections remain high (1, 4). Several Candida spp., such as C. glabrata and C. krusei, exert reduced susceptibility to fluconazole, the first available triazole antifungal agent (8). Recently, a new generation of triazoles, including posaconazole, voriconazole, ravuconazole, and isavuconazole, has been developed. As a prodrug, BAL8557 is the water-soluble triazole precursor suitable for oral and intravenous administration (11). In vitro, the active moiety isavuconazole shows broad-spectrum activity against all major opportunistic fungi (e.g., Candida, Cryptococcus, Aspergillus, Absidia, Rhizopus, and Rhizomucor species) and the dimorphic fungi (13, 16). In rat models, the active drug is highly effective against systemic candidiasis and disseminated Aspergillus flavus infection (14).

In this study, we compared the in vitro activity of isavuconazole with those of fluconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 296 clinical isolates of Candida spp. from bloodstream infections.

MATERIALS AND METHODS

Organisms. A total of 296 bloodstream isolates of Candida spp. obtained consecutively at a single university center over a 10-year period between 1995 and 2004 were selected for testing. The collection included C. albicans (166 isolates), C. glabrata (46 isolates), C. krusei (11 isolates), C. parapsilosis (23 isolates), C. tropicalis (25 isolates), and other Candida spp. (25 isolates, including 6 isolates of C. lusitaniae; 4 of C. guilliermondii; 4 of C. kefyr; 3 of C. dubliniensis; 2 each of C. famata and C. pulcherrima; and 1 each of C. catenulata, C. inconspicua, C. lipoelitica, and C. rugosa) Table 1). All isolates were identified to the species level by CHROMagar Candida (Mast Diagnostica GmbH, Reinfelden, Germany) and with the VITEK 2 automated identification system (bioMérieux, Marcy l’Étoile, France) using VITEK 2 YST cards in accordance with the guidelines of the manufacturers. Identification of rare species was confirmed by API Candida (bioMérieux). Prior to testing, each isolate was subcultured at least twice on Sabouraud dextrose agar plates to ensure purity and optimal growth.

Antifungal susceptibility testing. Broth microdilution was performed by the reference method described by the CLSI (formerly National Committee for Clinical Laboratory Standards) in accordance with guideline M27-A2 (5), with a final inoculum concentration of 0.5 x 10^5 to 2.5 x 10^5 cells per ml and RPMI 1640 medium (Sigma, Steinheim, Germany) buffered to pH 7.0 with 0.165 M MOPS (morpholino propane sulfonic acid) buffer (Merck, Darmstadt, Germany). Microtiter plates containing dehydrated antifungal agents were provided by Merlin Diagnostica (Bornheim-Hersel, Germany). The antifungal agents and concentration ranges tested in twofold steps were as follows: amphotericin B, 0.03 to 32 mg/liter; fluconazole, 0.03 to 64 mg/liter; fluconazole, 0.06 to 128 mg/liter; itraconazole, 0.004 to 8 mg/liter; voriconazole, 0.004 to 8 mg/liter; and isavuconazole, 0.00025 to 8 mg/liter. Plates were incubated in air at 35°C for 24 to 48 h. Plates were observed for the presence or absence of growth at 24 h and reexamined at 48 h if sufficient growth was not obtained at 24 h. The MIC was determined visually as the lowest concentration of drug showing no growth for the azoles compared to the drug-free growth control. C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were used as quality control strains.

Interpretive criteria for susceptibility to amphotericin B (MIC, ≤1 mg/liter), fluconazole (MIC, ≤4 mg/liter), fluconazole (MIC, ≤8 mg/liter), and itraconazole (MIC, ≤0.125 mg/liter) were those published by Rex et al. (9) and CLSI (5).

RESULTS AND DISCUSSION

The number of Candida bloodstream infections at our hospital remained stable from 1995 to 2002, ranging from 20 to 29 episodes per year; it increased, however, to 49 and 47 episodes...
in 2003 and 2004, respectively. The species distribution is illustrated in Fig. 1. *C. albicans* was the most common species, with 56.1% of all isolates; followed by *C. glabrata*, accounting for 15.5% of isolates; *C. tropicalis* (8.4%); and *C. parapsilosis* (7.8%). These data are in agreement with previously reported findings (6, 7).

Table 1 summarizes the MIC distributions and in vitro susceptibilities of 296 bloodstream isolates of *Candida* spp. to isavuconazole in comparison to other azole antifungal agents, amphotericin B, and fluconazole. Isavuconazole showed good activity against all *Candida* spp., including those species that are inherently less susceptible to fluconazole (e.g., *C. glabrata* and *C. krusei*). Overall, on the basis of MIC$_{90}$, isavuconazole was as active as amphotericin B, itraconazole, and voriconazole (each at 0.5 mg/liter) and more active than fluconazole (2 mg/liter). In terms of MIC$_{50}$, isavuconazole was more active (0.004 mg/liter) than amphotericin B (0.5 mg/liter), itraconazole (0.008 mg/liter), voriconazole (0.03 mg/liter), fluconazole (0.125 mg/liter), and fluconazole (8 mg/liter). For isavuconazole, MIC$_{50}$/MIC$_{90}$ ranged from 0.002/0.004 mg/liter for *C. albicans* to 0.25/0.5 mg/liter for *C. glabrata*. Using tentative breakpoints, all isolates were susceptible to amphotericin B, whereas 92.6% of isolates were susceptible to fluconazole. Nonsusceptibility to fluconazole was noted for 0.6%
of C. albicans, 2.2% of C. glabrata, 100% of C. krusei, and 36% of C. tropicalis isolates.

Candida isolates with MICs of >32 mg/liter, >0.5 mg/liter, and >2 mg/liter for fluconazole, itraconazole, and voriconazole, respectively, are considered resistant (5, 9). Of the 296 isolates studied, 6 (3 of C. krusei, 2 of C. glabrata, and 1 of C. lusitaniae) were resistant to fluconazole (2%), 5 (all C. glabrata) were resistant to itraconazole, and 2 (both C. glabrata) were resistant to voriconazole. Consistent with previous reports (10), isolates with elevated MICs for one azole were generally less susceptible to all azoles (Table 2). There were only two isolates with MICs for isavuconazole that were >0.5 mg/liter: both were C. glabrata isolates, and the MICs were 2 and 4 mg/liter, respectively. MICs of other azoles for these two isolates were as follows: fluconazole, 32 and 128 mg/liter; itraconazole, 1 and 2 mg/liter; and voriconazole, 0.25 and 4 mg/liter, respectively.

Twenty-one isolates (11 of C. glabrata, 8 of C. krusei, and 1 each of C. famata and C. inconspicua) were inhibited by 16 to 32 mg/liter fluconazole (dose dependently susceptible). Of note, none of the C. albicans isolates was resistant to fluconazole, and the highest MIC recorded was 4 mg/liter. The number of isolates resistant or dose dependently susceptible to fluconazole was far less than in other recent studies (2, 6, 7).

Isavuconazole efficacy in mice, similarly to those of other azoles, is driven by the area under the concentration-time curve (AUC) (12), often expressed as ratio of daily AUC over the MIC. Assuming that pharmacokinetics/pharmacodynamics in humans follow similar rules, at steady state the ratio of the daily AUC over the MIC90 will be 80 or 160 for maintenance doses of 100 or 200 mg, respectively (11). Correction of the daily AUC by the free fraction in plasma of 0.02 will result in ratios greater than 1 for the vast majority of bloodstream isolates. Limitations of our study include the fact that the number of isolates was small and represents only a single medical center. Larger studies are needed to confirm the potent activity of the drug against fluconazole-resistant strains.

In conclusion, isavuconazole exhibited good activity against 296 Candida bloodstream isolates obtained over a period of 10 years. Isavuconazole was more potent than fluconazole against all organisms tested and often more potent than itraconazole, voriconazole, amphotericin B, and fluconazole, confirming its potential as a useful agent for patients with serious systemic Candida infections.

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REFERENCES


