Comparison of disc diffusion assay with the CLSI reference method (M27-A2) for testing in vitro posaconazole activity against common and uncommon yeasts

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Objectives: To evaluate the suitability of disc diffusion (DD) assay for testing posaconazole activity and to corroborate its activity against recently isolated yeasts by the CLSI reference microdilution M27-A2 method.

Methods: A total of 224 yeast isolates (7 species with 52 to 11 isolates each, and 15 species with 1 to 6 isolates) were evaluated, 125 were recent bloodstream isolates, 30 isolates from other sources and six ATCC isolates that included amphotericin B-resistant Candida albicans ATCC 200955, Candida lusitaniae (ATCC 200950, 200951, 200952 and 200953) and amphotericin B- and itraconazole-resistant Candida tropicalis ATCC 200956. MICs were determined at 24 and 48 h by following the CLSI guidelines, document M27-A2. DD testing was performed by following CLSI M44-A document with 5 μg posaconazole discs. Inhibition zone diameters were measured at the transition point at which growth decreased at both 24 and 48 h.

Results: DD showed very good reproducibility, with coefficient of variability median value 4.56. Posaconazole demonstrated good in vitro activity against all clinical isolates, including the emerging species and amphotericin B-resistant ATCC isolates except for C. tropicalis ATCC 200956 (posaconazole MIC ≥ 16 mg/L). Only 1.5% and 4.1% of isolates were inhibited by >2 mg/L posaconazole at 24 and 48 h. Good correlation was obtained between methods (R = 0.763 at 24 h and 0.602 at 48 h). DD detected posaconazole-resistant isolates (MIC > 2 mg/L).

Conclusions: DD could be an alternative to the microdilution reference method, as no major discrepancies were detected.

Keywords: susceptibility, M44-A, azoles

Introduction

Posaconazole (Noxafil®) is a new triazole agent approved in the European Union for invasive aspergillosis, fusariosis, chromoblastomycosis and coccidioidomycosis treatment in patients with refractory diseases or intolerance to amphotericin B or itraconazole. It has been licensed by the FDA (September 2006) for prophylaxis of invasive Aspergillus and Candida infections in adult patients. To date, it is the first and only antifungal agent approved for the prevention of invasive fungal infections caused by Aspergillus species. Posaconazole has a broad spectrum of activity against both yeasts and filamentous moulds, including zygomycetes, although isolates with decreased susceptibility have also been described.1–5 Its efficacy in vivo has been proven in clinical trials.5 The CLSI (formerly NCCLS) reference method (document M27-A2) is time consuming for use in the
clinical laboratory. Although, the disc diffusion (DD) method is easier to perform, it has only been standardized for fluconazole and voriconazole (document M44-A). Unlike the problems with availability in the US, posaconazole discs are readily available in Europe, making this test an attractive alternative to microbroth dilution testing.

The aim of this study was dual: (i) to evaluate the suitability of the DD method for testing posaconazole by correlating disc results with MICs obtained by the microdilution reference method and (ii) to corroborate posaconazole activity against common and uncommon recent clinical yeast isolates.

Materials and methods

Isolates

A total of 224 isolates were evaluated and included 125 recent bloodstream isolates, 30 isolates from other sources and six ATCC isolates amphotericin B-resistant Candida albicans (ATCC 200955), Candida lusitaniae (ATCC 200950, 200951, 200952 and 200953) and amphotericin B- and itraconazole-resistant Candida tropicalis (ATCC 200956).8,9

Susceptibility testing

Broth microdilution susceptibility tests were performed according to CLSI guidelines.8 Posaconazole (Schering-Plough, Kenilworth, NJ, USA) was dissolved in DMSO and final drug dilutions were prepared in standard RPMI-1640 medium (Sigma-Aldrich, Madrid, Spain). Posaconazole concentrations ranged from 0.03 to 16 mg/L. The MIC2 and MIC0 (≥50% and 100% growth reduction, respectively) were determined visually and by spectrophotometer at both 24 and 48 h.

Disc diffusion tests were carried out following the M44-A Document guidelines on Mueller–Hinton agar supplemented with 2% dextrose and 0.5 mg/L methylene blue (10 cm plates, with 25 mL medium). Plates were inoculated by dipping sterile cotton swabs into the standard inoculum suspension (10⁶ cfu/mL) and evenly streaking the entire surface with a rotor device (Retro C80, AB Biodisk, Solna, Sweden). After drying for 15–20 min, posaconazole discs (5 μg, Becton–Dickinson and Co., Sparks, MD, USA) were applied to the inoculated agar. Inhibition zone diameters were measured in millimetres at the transition point at which growth decreased at 24 and 48 h. Quality control (QC) isolates Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were included in each set of tests performed. Both disc and microbroth dilution tests were run in parallel.

Reproducibility study

The reproducibility of the DD method was evaluated by testing 20 of the study isolates at least three times on different days. Percentages of reproducibility were obtained by determining the coefficient of variability and standard deviations.

Data analysis

All data (total 267) were included in the analysis, MIC values ≤0.03 mg/L were left as 0.03 and those ≥16 mg/L were raised to 32 mg/L. MIC ranges, MIC50, MIC90, as well as ranges and means of inhibition zone diameters were calculated for each species and test method. The correlation between both methods was determined by plotting the inhibition zones against their respective MICs and a linear regression analysis (least-square method) was performed. The goodness of adjustment was determined by the Pearson correlation coefficient (R). Break points for posaconazole are not available. To assess the suitability of the disc diffusion in identifying resistant isolates, we used voriconazole break points (susceptible, ≤1 and ≥17 mm; susceptible dose dependent, 2 mg/L and 14–16 mm; and resistant, ≥4 mg/L and ≤13 mm) to perform the categorical agreement analysis between the methods. Very major errors were defined as when the isolate was resistant by broth microdilution and susceptible by disc diffusion, major errors when the isolate was susceptible by broth microdilution and resistant by disc diffusion and minor errors were identified when there were shifts between susceptible and susceptible dose-dependent or between susceptible dose-dependent and resistant.

Results and discussion

MICs for the QC strains were within the expected range.10–12 Posaconazole disc diffusion method showed very good reproducibility: the median value of the coefficient of variability was 4.56 (24 h) and 5 (48 h) ranging from 0 to 12, and the inhibition diameters were within two standard deviations of the mean diameter in all strains tested.

Table 1 summarizes the results by the two methods obtained for each Candida species. Posaconazole susceptibility data for emerging species are scarce; consequently, the results for these species are shown separately in the table. By the microdilution method, posaconazole presented good in vitro activity against all clinical isolates, including the emerging species and most ATCC isolates resistant to amphotericin B; posaconazole MIC ≥16 mg/L was obtained for the amphotericin B- and itraconazole-resistant C. tropicalis ATCC 200956, suggesting cross-resistance between itraconazole and posaconazole. The overall MIC90 was 0.25 and 1 mg/L at 24 and 48 h, respectively, in agreement with data published by other authors.12,4,13,14 Our results were in agreement with those of Sabatelli et al.,14 in which of the isolates inhibited by posaconazole only 1.5% and 4.1% were inhibited by >2 mg/L at 24 and 48 h, respectively. The incubation time did not significantly influence MIC values (two C. albicans, one C. krusei and one Candida guilliermondii changed categorical classification at 48 h), suggesting that posaconazole MICs could be determined at 24 h. The MIC90 was equal to or within two dilutions higher than the MIC2 for 69.3% and 60.9% of the strains at 24 and 48 h, respectively.

The comparison of MICs by the microdilution method with the results of the inhibition zone exhibited good correlation (R = 0.763 at 24 h and 0.602 at 48 h) (Figure 1), the statistical regression analysis being significant (P < 0.01). Our correlation values are slightly lower than those obtained by Sims et al.13 for Candida spp. It could be due to the fact that our study included more species of Candida and other yeast species. Nevertheless, our correlation results were similar to those reported for testing moulds with posaconazole or other antifungal agents.14–16 The lowest correlation obtained at 48 h could be due to the heavy trailing growth of some C. albicans and C. tropicalis isolates at 48 h. The categorical agreement between the methods was good (96.6%). One major error (0.37%) was found for a Candida glabrata isolate (MIC 1 mg/L and inhibition zone of 12 mm), the percentage of minor errors was 3% whereas no very major errors were detected (Figure 1).

This study confirms the results of previous studies and extends the evaluation of the posaconazole disc diffusion method to
uncommon species. Consequently, the disc method could be an alternative to the microdilution reference method in the clinical laboratory to determine susceptibility of yeasts to posaconazole. Nevertheless, there are not enough data to affirm the ability of the disc diffusion method to detect posaconazole-resistant isolates because only ≤3% of the isolates evaluated in this and other studies were inhibited by >2 mg/L. On the contrary, there are a lot of data for susceptibility screening by the disc diffusion method.13–16

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Transparency declarations

None to declare.

References


