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Evaluation of the in vitro activity of isavuconazole and comparator voriconazole against 2635 contemporary clinical Candida and Aspergillus isolates

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ABSTRACT

Objective: The in vitro activity of isavuconazole was determined for 1677 Candida and 958 Aspergillus isolates from 2012 to 2014 with voriconazole as comparator.

Methods: Aspergillus isolates were screened for resistance using azole-agar. Aspergillus isolates that screened positive and all Candida isolates underwent EUCAST broth microdilution testing. Isolates were categorized as wild-type (wt) or non-wt, adopting EUCAST epidemiological cut-off values (ECOFFs) (where available) or wt upper limits (wtULs; two two-fold dilutions above the MIC50). The CYP51A gene was sequenced for non-wt Aspergillus fumigatus isolates. Itraconazole and posaconazole MICs were found for 13.7/15.2% of non-wt Aspergillus fumigatus MIC50 breakpoints. The reference centre and technical issues. Significant CYP51A alterations were reliably detected applying the isavuconazole breakpoint.

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Introduction

Isavuconazole is a new triazole compound with broad-spectrum in vitro activity against Aspergillus species and a range of other medically important yeasts and moulds [1–5]. In 2015 it was licensed for invasive aspergillosis of adults by the EMA [6] and the FDA [7], following the phase III SECURE trial, which demonstrated non-inferiority compared with voriconazole regarding invasive mould infections [8]. The standard first-line treatment for invasive aspergillosis has until now been voriconazole [9,10]. Voriconazole has variable pharmacokinetics and a narrow therapeutic window, and as such, a proportion of patients are either under-dosed or experience adverse effects due to toxic levels [11]. Drug interactions can pose a problem, as can the cycloheximide content of the intravenous formulation when treating patients who have renal impairment. Isavuconazole provides a welcomed expansion of the available armamentarium against mould infections and offers a

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favourable profile regarding drug interactions, toxicology, pharmacokinetics and with a spectrum at least partially including Mucorales.

Isavuconazole has been shown to be efficacious in oesophageal candidiasis [12] and has been investigated in the phase III clinical ACTIVÉ trial including patients with proven candidaemia or invasive candidiasis. Data evaluation failed to demonstrate non-inferiority compared with caspofungin but did show a similar overall success at the end of treatment, overall mortality and good tolerability (26th European Congress of Clinical Microbiology and Infectious Diseases, abstract 1239). Although these findings support the notion that echinocandins are superior to azoles for the treatment of candidaemia/invasive candidiasis, isavuconazole may remain relevant for selected patients with mixed infections, echinocandin-resistant infections, complicating factors, or for oral step down.

In this study, we investigated the in vitro susceptibility to isavuconazole compared with voriconazole against a large contemporary clinical collection of Aspergillus and Candida isolates received at the Danish mycology reference centre, including azole-resistant isolates. MICs were interpreted using the recently established EUCAST clinical breakpoints and epidemiological cut-off values (ECOFFs).

Materials and methods

Isolates

A total of 958 Aspergillus and 1677 Candida isolates, from 683 and 1487 patients, respectively, were included. The collections contained all isolates from clinical samples or pure cultures received at the mycology reference laboratory at Statens Serum Institut for identification and susceptibility testing during the calendar years 2012–2014. No ethical restraints apply to studies of isolates and MIC distributions were determined:

1. Isolates were categorized as wild-type (wt) or non-wild-type (non-wt) adopting the EUCAST ECOFFs. These have been defined for A. fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus terreus and Aspergillus nidulans (voriconazole and isavuconazole) [16,17] and for Candida albicans, Candida glabrata, Candida kruzie, Candida parapsilosis and Candida tropicalis (voriconazole) [18]. For yeast species without a defined EUCAST ECOFF, a wild-type upper limit (wtUL) was determined as two-fold dilution steps above the MIC50 (MIC50 value is the lowest concentration of the antifungal at which 50% of the isolates were inhibited) with the exception of species for which the entire population of MICs were less than or equal to the lowest concentration tested. In such cases, the lowest concentration tested was chosen as the wtUL [19].

2. The MIC50, MIC distribution range and number of isolates with MICs above the ECOFF/wtUL were determined and compared by species and antifungal compound.

PCR amplification and sequence analysis of the CYP51A gene

Isolates of A. fumigatus classified as non-susceptible or non-wt to itraconazole, posaconazole, isavuconazole or voriconazole were CYP51A sequenced as part of the routine procedures (as previously described [20]), except for one azole-resistant isolate from 2012, which was not available for CYP51A sequencing. Additionally, a few azole-susceptible isolates were sequenced as controls, or if the patient had previously harboured an azole-resistant A. fumigatus. In total, 57 A. fumigatus isolates were CYP51A sequenced, 45 of which had elevated MICs towards isavuconazole or voriconazole.

Nineteen A. terreus isolates were obtained from one patient with cystic fibrosis known since 2007 to repeatedly harbour isolates with an M217I alteration [21]. Eight of these isolates (one wt and seven resistant) were CYP51A sequenced as previously described [21].

Results

Isolates and MIC distributions

The normally azole-susceptible Candida species had very low and comparable MIC distributions for both compounds (Table 1 and Fig. 1). The following species-specific wtULs for isavuconazole MIC distributions were determined: C. albicans, C. dubliniensis, C. parapsilosis and C. tropicalis: 0.03 mg/L; C. glabrata and Saccharomyces cerevisiae: 0.125 mg/L, and C. kruzie: 0.25 mg/L. For C. glabrata, C. kruzie, S. cerevisiae and other Candida spp., for which the MIC distributions were not truncated, the isavuconazole MIC50 was 0.25 mg/L and for Candida spp. and Aspergillus spp., respective-
and wtULs were one or two dilution steps lower than those for voriconazole. The percentage of non-wt isolates for the two compounds (isavuconazole/voriconazole) was as follow: C. albicans (0.8/1.0), C. dubliniensis (0.1/0.8), C. glabrata (14.9/9.5), C. krusei (2.7/1.4), C. parapsilosis (1.7/1.8), C. tropicalis (14.3/19.1) and S. cerevisiae (10.0/0). For voriconazole, the species-specific wtULs defined in this study were at least two dilution steps lower than the EUCAST ECOFFs for all species, with the exception of C. krusei (Table 1).

For the 306 (32%) Aspergillus isolates that underwent EUCAST susceptibility testing due to a non-fumigatus Aspergillus species identification or growth at the screening agar, the species-specific MIC50 values for isavuconazole and voriconazole were again within one two-dilution step of each other (Table 2). Hence, the isavuconazole MIC distributions were approximately one dilution step higher for A. fumigatus and A. niger (including A. tubingensis) compared with those for voriconazole and one dilution step lower for A. nidulans. The percentage of MICs above the ECOFFs for the two compounds (isavuconazole/voriconazole) were as follows: A. fumigatus (13.7/15.2); A. niger (4.9/0); A. terreus (48.2/22.2; but 27.8/0 if excluding all isolates from a patient with the previously detected M217I alteration); A. flavus (0/0) and A. nidulans (0/0) (Fig. 2). Applying the clinical breakpoints for the 26.3% of A. fumigatus isolates that underwent EUCAST susceptibility testing, the susceptibility profiles were as follows: isavuconazole: 78.2% (susceptible); S/21.8% (resistant); R; voriconazole: 84.8% (S)/6% (intermediate); 6% (I)/8.5% (R); overall, 24.2% were found to be non-susceptible to at least one mould-active azole. The overall isavuconazole and voriconazole non-susceptibility rates among A. fumigatus (when including the 592 isolates found susceptible using theazole agar screenings test) were 5.7% (n = 46) and 4.0%.

Table 1
Total numbers and MIC distributions for Candida species

<table>
<thead>
<tr>
<th>ID</th>
<th>Isavuconazole MIC (mg/L)</th>
<th>Voriconazole (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC50</td>
</tr>
<tr>
<td>C. albicans</td>
<td>≤0.03→&lt;4</td>
<td>≤0.03</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>≤0.03</td>
<td>≤0.03</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>≤0.03→&lt;4</td>
<td>≤0.03</td>
</tr>
<tr>
<td>C. krusei</td>
<td>≤0.03→0.5</td>
<td>0.06</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>≤0.03→0.6</td>
<td>≤0.03</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>≤0.03→&lt;4</td>
<td>≤0.03</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>≤0.03→0.5</td>
<td>≤0.03</td>
</tr>
</tbody>
</table>

Table 2
Total numbers and MIC distributions for Aspergillus species

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Isavuconazole MIC (mg/L)</th>
<th>Voriconazole (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range</td>
<td>MIC50</td>
</tr>
<tr>
<td>A. fumigatus sensu stricto</td>
<td>211 (69.0)</td>
<td>0.125→16</td>
</tr>
<tr>
<td>A. niger species complex</td>
<td>41 (13.4)</td>
<td>1→8</td>
</tr>
<tr>
<td>A. terreus species complex</td>
<td>57 (16.6)</td>
<td>0.05→2</td>
</tr>
<tr>
<td>A. nidulans species complex</td>
<td>5 (1.6)</td>
<td>0.125→0.25</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>1 (0.3)</td>
<td>4</td>
</tr>
<tr>
<td>A. quadrilineatus</td>
<td>1 (0.3)</td>
<td>&lt;0.125</td>
</tr>
<tr>
<td>A. tamarii</td>
<td>1 (0.3)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not available; MIC50, the MIC value inhibiting the growth of ≥50% of isolates.

Species distribution (isolates submitted to either azole screening or EUCAST susceptibility testing): A. fumigatus (803), A. niger (60), A. terreus (41), A. flavus (35), A. nidulans (6), A. nidulans var. echinulatus (4), A. tubingensis (2), A. sydowii (2), A. calidoustus (1), A. ochraceus (1), A. tamarii (1), A. versicolor (1), and A. quadrilineatus (1).

Fig. 1. MIC distributions for selected Candida species.
Six of the 17 isolates (35.3%) harboured a zole ECOFF, whereas six were non-wt applying the voriconazole breakpoint. One patient with cystic fibrosis displayed non-wt MICs for isavuconazole (isavuconazole MIC of 2 mg/L). In comparison, one TR34/L98H/S297T/F495I was classified as voriconazole susceptible, illustrating that MICs. However, for C. krusei and C. glabrata, our wt distributions were two to three dilution steps lower than those reported by CLSI, which may reflect the inter-laboratory variability previously associated with MIC testing of these species (C. glabrata especially [2]).

### Discussion

Overall, the in vitro activities of isavuconazole and voriconazole against Candida and Aspergillus isolates were comparable and consistent with previously published EUCAST [2] and CLSI [15,23,24] MICs. However, for C. krusei and C. glabrata, our wt distributions were two to three dilution steps lower than those reported by CLSI, which may reflect the inter-laboratory variability previously associated with MIC testing of these species (C. glabrata especially [2]).

Elevated MICs were not uncommon. Indeed, up to 15% of C. glabrata and C. tropicalis displayed non-wt MICs for isavuconazole, whereas this was the case for 5.7% of all included A. fumigatus isolates. Reports of azole resistance rates in A. fumigatus vary. In an international surveillance study from 2009 to 2011 the prevalence in Denmark was 3.3% and equal to the overall average [25]. A subsequent retrospective laboratory-based documented an increase in azole resistance from 1.4% to 6% in clinical samples in the time period 2010–2014, suggesting that the resistance rate in Denmark may be increasing [26].

The proportion of isolates with an isavuconazole MIC of 2 mg/L (categorized as resistant but still within the wt MIC range) was comparable to the proportion reported from a recent multicentre study (~8%; 34/401 isolates), but higher than in the data set used for setting the EUCAST ECOFFS (3%) [2,17]. This may reflect the selected proportion of isolates undergoing EUCAST susceptibility testing after azole agar screening (for which a higher proportion of resistance is expected) and the fact that isolates received at a reference laboratory constitute a selected subset of isolates that may not be fully representative for the national epidemiology.

All A. fumigatus isolates with CYP51A mutations consistently known to confer voriconazole or pan-azole resistance were classified as resistant applying the isavuconazole breakpoint. One M220K alteration resulted in discreet MIC elevations (isavuconazole MIC of 2 mg/L), but whether this is clinically relevant is unclear. However, one TR34/L98H was found within the wt MIC range (isavuconazole MIC of 2 mg/L). In comparison, one TR34/L98H/S297T/F495I was classified as voriconazole susceptible, illustrating
that perfect discrimination of susceptible and mutant isolates is challenging to achieve due to the overlapping MIC distributions for wt and mutant isolates for both compounds. For A. terreus, we found a high proportion of non-wt isolates for both drugs and notably higher (27.8%) for isavuconazole compared with voriconazole (0%), even after excluding a patient known to harbour isolates with an M217I alteration. These differences are most likely not reflecting efficacy differences between the two compounds, but rather technical issues related to MIC testing and categorization, as the MIC distributions were symmetric with an MIC₉₀ of 1 mg/L and produced an almost identical categorization if an isavuconazole ECOFF of 2 mg/L was applied.

Two A. niger complex isolates (4.9%) were non-wt for isavuconazole, one of which belonged to the intrinsically less susceptible cryptic species A. tubingenesis. This is in line with a previous report and within the percentage of wild-type isolates expected to be above the ECOFF [2,27].

Technical challenges regarding MIC endpoint reading were encountered. For C. tropicalis, we found a tail of high MIC values for both compounds, primarily consisting of isolates with trailing phenotype, i.e. with residual growth over a broad range of MIC values. Whether such isolates are truly resistant is still unresolved and the phenomenon defies any attempt at a precise and random susceptibility classification. Moreover, the growth curves for C. krusei were less steep for isavuconazole around the 50% growth inhibition target, resulting in less reproducible MIC determination (and less well-defined normal distributions for isavuconazole than for voriconazole). This may, at least in part, explain the higher degree of inter-laboratory variation previously observed for this species [2]. Finally, the MIC distributions for the two compounds against A. terreus mirrored one another with a modal MIC of 1 mg/L. In contrast, the modal MIC from the data set used to set EUCAST ECOFFs for isavuconazole was 0.5 mg/L and CLSI modal MICs are reported to be 0.25–0.5 mg/L [1,23]. These discrepancies (+ one dilution) are all within the expected variation for susceptibility testing, yet lead to interpretative disagreement when a restrictive endpoint is adopted. Of note, inter-laboratory discrepancies in MIC testing of A. terreus in particular have previously been reported, but the underlying mechanism remain unclear [2].

Our study has limitations. For Candida species, the truncation of the MIC distribution interfered with definitive MIC and wtUL determination, particularly for the normally azole-susceptible species. The discrepancy between wtULs for isavuconazole and voriconazole ECOFFs did result in some non-wt classification differences. For C. glabrata, the difference is most likely artificial and due to our strict definition of an wtUL, in combination with a truncated MIC interval. If we applied our strict definition of an wtUL on voriconazole, 15% of isolates would be non-wt for both compounds, suggesting comparable activity of the two.

One concern could be that we may have overlooked resistant A. fumigatus isolates, as confirmatory EUCAST AFST was only performed for isolates that grew on the screening agar (in which isavuconazole is not incorporated). We have EUCAST susceptibility tested one-quarter of all A. fumigatus isolates as they were originally screening positive, but only confirmed resistance in under one-quarter. Moreover, a recent multicentre study confirmed a specificity of 95%–100% (27th European Congress of Clinical Microbiology and Infectious Diseases, abstract P1750). On this background, we find it unlikely that the isavuconazole resistance rate was underestimated despite not performing EUCAST microdilution testing on all isolates. However, as isavuconazole was not licensed in our country in the years of isolate collection, it remains to be seen if isavuconazole mono-resistance mutations may occur once the compound is in clinical use.

In conclusion, the in vitro activity of voriconazole and isavuconazole is similar, with observed differences in Candida species being primarily due to methodological issues. This suggests that isavuconazole will be a promising new alternative in most cases where voriconazole is indicated, particularly in patients with invasive aspergillosis, and that susceptibility testing is important for clinical management.

Transparency declaration

This study was supported by an unrestricted grant from Basilea. Some of these data were presented at the 26th ECCMID 2016 in Amsterdam, the Netherlands, 9–12 April 2016 (#O227). Outside this study, the authors declare the following potential conflicts of interest: MCA has received research grants or speaker honoraria from Amplyx, Astellas, Basilea, Cidara, F2G, Gilead, MSD, Novartis, Pfizer and T2Biosystems. She is the current chairman for the EUCAST-AFST and before this served on advisory boards for MSD (until 2014), and Pfizer (until 2012). KMTA has received travel grants from Pfizer and Gilead. RKH has received research grant from Gilead and travel grants from Astellas, MSD and Pfizer.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.03.023.

References


