Voriconazole susceptibility of yeasts isolated from the mouths of patients with advanced cancer

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The in vitro activity of voriconazole was compared with those of fluconazole and itraconazole against 270 clinical isolates of yeasts from the mouths of patients receiving palliative care for advanced cancer. A broth micro-dilution assay as described by the National Committee for Clinical Laboratory Standards was employed for determination of MICs. Of the 270 isolates, 206 (76 %) were fluconazole sensitive and 64 were fluconazole resistant. Voriconazole showed more potent activity than either fluconazole or itraconazole, including against some isolates resistant to both fluconazole and itraconazole. However, for fluconazole-resistant isolates, the MICs of itraconazole and voriconazole were proportionally higher than for the fluconazole-susceptible isolates, suggesting cross-resistance. Voriconazole may be a useful additional agent for the management of oral fungal infections caused by strains resistant to fluconazole and itraconazole, but susceptibility cannot be assumed and in vitro MIC determination is recommended prior to its use.

INTRODUCTION

Many patients receiving palliative care for advanced cancer develop oral fungal infections (Aldred et al., 1991; Clarke et al., 1987; Finlay, 1986) due to the immunocompromised state of such patients. These infections are often seen in conjunction with dry mouth (xerostomia), another frequent complication in hospice patients (Jobbins et al., 1992a; Sweeney et al., 1998). Many patients will receive repeated courses of antifungal medication to deal with recurrent fungal infections. These antifungal drugs are often prescribed without confirmation of the diagnosis of an oral fungal infection by culture. In routine clinical practice, therefore, the identities and antifungal susceptibility profiles of the species involved in the infections are frequently unknown.

It has been suggested that prolonged or repeated exposure to low-dose fluconazole may be associated with emergence of fluconazole resistance among strains of Candida albicans (Johnson et al., 1995; Lopez et al., 2001) and the potential selection of non-albicans species of yeast (Sobel et al., 2001). Since many of the non-C. albicans yeasts, such as Candida glabrata (Arias et al., 1996) and Candida krusei (Berrouane et al., 1996), are inherently less susceptible than C. albicans to fluconazole, such population shifts have treatment implications. Previous studies have reported a diverse oral mycological flora in hospice patients, with a significant proportion of non-C. albicans yeasts (Sweeney et al., 1998; Jobbins et al., 1992b), a result confirmed in a recent study of oral yeast carriage among 120 patients with advanced cancer (Davies et al., 2002). In a recently completed multi-centre study (Bagg et al., 2003), the oral mycological flora of 207 patients receiving palliative care for advanced malignant disease was examined. In total, 194 yeasts were isolated, of which 95 (49 %) were C. albicans. There was a high prevalence of C. glabrata isolates (47) of which 72 % were resistant to both fluconazole and itraconazole by standard National Committee for Clinical Laboratory Standards (NCCLS) susceptibility testing (Bagg et al., 2003).

There is clearly a need to examine new methods for both the prevention and management of fungal infections in patients with advanced cancer. This includes assessment of new antifungal drugs. One such agent is voriconazole, a second-generation azole antifungal, which shows excellent in vitro activity against a wide variety of yeasts and moulds (Johnson & Kauffman, 2003).
It has been reported that voriconazole is active against all Candida species, including C. krusei, strains of C. glabrata that are inherently fluconazole-resistant and strains of C. albicans that have acquired resistance to fluconazole (Johnson & Kauffman, 2003). No breakpoints have yet been agreed for voriconazole, but in general the MICs of voriconazole for C. albicans are 1–2 logs lower than the MICs of fluconazole (Johnson & Kauffman, 2003). However, for some fluconazole-resistant strains of C. albicans, MICs of voriconazole are higher than for fluconazole-susceptible isolates (Johnson & Kauffman, 2003; Ruhnke et al., 1997; Cuenca-Estrella et al., 1999), and for C. glabrata and C. krusei the MICs are higher than for other species, though still in the presumed susceptible range (Johnson & Kauffman, 2003). The potential for drug interactions with voriconazole is high because of its metabolism by CYP450 isoenzymes (Hoffman & Rathbun, 2002), and although it is generally well tolerated, approximately 30% of patients suffer a reversible disturbance of vision, particularly during the first week of therapy (Johnson & Kauffman, 2003).

Based on current knowledge of the oral mycological flora in patients receiving palliative care, the antifungal spectrum of voriconazole suggests that it may be a valuable adjunct to treatment of oral fungal infections in this group. Accordingly, the aim of the present study was to determine the voriconazole susceptibility of a large collection of well-characterized fungal isolates from the oral cavities of patients with advanced cancer.

**RESULTS AND DISCUSSION**

**Yeast isolates**

The identities of the 270 isolates studied are shown in Table 1. There were a significant number of isolates of non-C. albicans yeasts, reflecting the heterogeneous oral mycological flora in patients with advanced cancer.

**Table 1. Identity of 270 yeast species isolated from the mouths of patients with advanced cancer**

<table>
<thead>
<tr>
<th>Species</th>
<th>No. (%) isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>160 (59)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>51 (19)</td>
</tr>
<tr>
<td>Candida dubliniensis</td>
<td>20 (7)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>16 (6)</td>
</tr>
<tr>
<td>Candida tropicalis</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Candida guilliermondii</td>
<td>2 (&lt; 1)</td>
</tr>
<tr>
<td>Candida lusitaniae</td>
<td>2 (&lt; 1)</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>2 (&lt; 1)</td>
</tr>
<tr>
<td>Candida famata</td>
<td>1 (&lt; 1)</td>
</tr>
</tbody>
</table>
Fluconazole, itraconazole and voriconazole susceptibilities

The comparative in vitro susceptibilities of the yeast isolates to the three antifungal agents are shown in Table 2. Overall, 206 (76 %) of the isolates were susceptible to fluconazole (MIC $\leq 8 \, \mu g \, ml^{-1}$). Twenty-five isolates were susceptible dose-dependent and 39 isolates (14 %) were fully resistant to fluconazole (MIC $> 64 \, \mu g \, ml^{-1}$). The comparative in vitro susceptibilities of the 64 isolates with a fluconazole MIC of $> 8 \, \mu g \, ml^{-1}$ are shown in Table 3; seven were susceptible to itraconazole, 16 were susceptible dose-dependent and 41 were itraconazole resistant.

Table 2. Comparative in vitro susceptibilities of 270 yeasts isolated from the mouths of 199 patients with advanced cancer

<table>
<thead>
<tr>
<th>Species*</th>
<th>Antifungal agent</th>
<th>MIC (µg ml$^{-1}$)$^+$</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC$^{50}$</td>
<td>MIC$^{90}$</td>
<td>Range</td>
</tr>
<tr>
<td>All organisms (270)</td>
<td>Fluconazole</td>
<td>1</td>
<td>64</td>
<td>0.125–64</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.125</td>
<td>2</td>
<td>0.015–16</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016</td>
<td>4</td>
<td>0.016–8</td>
</tr>
<tr>
<td>C. albicans (160)</td>
<td>Fluconazole</td>
<td>0.5</td>
<td>16</td>
<td>0.125–64</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.06</td>
<td>0.5</td>
<td>0.015–16</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016</td>
<td>0.25</td>
<td>0.016–8</td>
</tr>
<tr>
<td>C. glabrata (51)</td>
<td>Fluconazole</td>
<td>32</td>
<td>64</td>
<td>0.5–64</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>1</td>
<td>16</td>
<td>0.06–16</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>4</td>
<td>8</td>
<td>0.016–8</td>
</tr>
<tr>
<td>C. dubliniensis (20)</td>
<td>Fluconazole</td>
<td>0.25</td>
<td>2</td>
<td>0.125–8</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.06</td>
<td>0.25</td>
<td>0.015–0.5</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016</td>
<td>0.5</td>
<td>0.016–4</td>
</tr>
<tr>
<td>S. cerevisiae (16)</td>
<td>Fluconazole</td>
<td>4</td>
<td>64</td>
<td>1–64</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.5</td>
<td>2</td>
<td>0.03–16</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.125</td>
<td>1</td>
<td>0.016–2</td>
</tr>
<tr>
<td>C. tropicalis (13)</td>
<td>Fluconazole</td>
<td>1</td>
<td>64</td>
<td>0.125–64</td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.25</td>
<td>0.5</td>
<td>0.03–1</td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.125</td>
<td>4</td>
<td>0.016–8</td>
</tr>
<tr>
<td>C. krusei (3)</td>
<td>Fluconazole</td>
<td>16–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.25–0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016–1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. guilliermondii (2)</td>
<td>Fluconazole</td>
<td>4–8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.5–16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.125–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. lusitaniae (2)</td>
<td>Fluconazole</td>
<td>1–8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.125–0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis (2)</td>
<td>Fluconazole</td>
<td>2–64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.125–16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016–0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. famata (1)</td>
<td>Fluconazole</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Itraconazole</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Voriconazole</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of isolates.

†MIC$^{50}$ and MIC$^{90}$ results are the concentrations of each antifungal agent necessary to inhibit 50 % and 90 %, respectively, of the isolates.

Overall, 160 (59 %) of the isolates were susceptible to itraconazole (MIC $< 0.125 \, \mu g \, ml^{-1}$). Sixty-one isolates were susceptible dose-dependent and 49 isolates (18 %) were fully resistant to itraconazole (MIC $\geq 1 \, \mu g \, ml^{-1}$). Of the 49 isolates that were resistant to itraconazole, 41 were also resistant to fluconazole and eight were susceptible.

The distribution of the MICs to the three antifungal drugs for the 206 fluconazole-sensitive strains is illustrated in Fig. 1(a). The median values (and interquartile ranges) for fluconazole, itraconazole and voriconazole, respectively, were 0.5 $\mu g \, ml^{-1}$ (0.25–2 $\mu g \, ml^{-1}$), 0.125 $\mu g \, ml^{-1}$ (0.06–0.25 $\mu g \, ml^{-1}$) and 0.016 $\mu g \, ml^{-1}$ (0.016–0.125 $\mu g \, ml^{-1}$).
Fig. 1(b) shows the distribution of the MICs to the three antifungal drugs for the 64 fluconazole-resistant strains. The median values and interquartile ranges for fluconazole, itraconazole and voriconazole respectively were 64 μg ml$^{-1}$ (32–64 μg ml$^{-1}$), 1 μg ml$^{-1}$ (0.5–8 μg ml$^{-1}$) and 1 μg ml$^{-1}$ (0.125–8 μg ml$^{-1}$).

In total, 41 (15 %) of the isolates were fully resistant to both fluconazole and itraconazole. Of these, 23 were C. glabrata, 12 were C. albicans, four were Saccharomyces cerevisiae, one was Candida parapsilosis and one was Candida tropicalis. The MICs of voriconazole for these isolates are shown in Fig. 2.

### Table 3. Comparative in vitro susceptibilities of 64 yeasts that were susceptible dose-dependent or resistant to fluconazole

<table>
<thead>
<tr>
<th>Species*</th>
<th>Antifungal agent</th>
<th>MIC (μg ml$^{-1}$)†</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All organisms (64)</td>
<td>64 64</td>
<td>16–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1 16</td>
<td>0.03–16</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>1 8</td>
<td>0.016–8</td>
<td></td>
</tr>
<tr>
<td>C. glabrata (34)</td>
<td>64 64</td>
<td>16–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>1 16</td>
<td>0.125–16</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>4 8</td>
<td>0.125–8</td>
<td></td>
</tr>
<tr>
<td>C. albicans (17)</td>
<td>64 64</td>
<td>16–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>4 16</td>
<td>0.03–16</td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.031 2</td>
<td>0.016–8</td>
<td></td>
</tr>
<tr>
<td>S. cerevisiae (6)</td>
<td>Fluconazole</td>
<td>16–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.5–16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.016–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. tropicalis (3)</td>
<td>Fluconazole</td>
<td>32–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.25–1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.062–2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. krusei (3)</td>
<td>Fluconazole</td>
<td>16–64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.25–0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.016–1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. parapsilosis (1)</td>
<td>Fluconazole</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Itraconazole</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of isolates.
†MIC50 and MIC90 results are the concentrations of each antifungal agent necessary to inhibit 50 % and 90 %, respectively, of the isolates.

Fig. 1(b) shows the distribution of the MICs to the three antifungal drugs for the 64 fluconazole-resistant strains. The median values and interquartile ranges for fluconazole, itraconazole and voriconazole respectively were 64 μg ml$^{-1}$ (32–64 μg ml$^{-1}$), 1 μg ml$^{-1}$ (0.5–8 μg ml$^{-1}$) and 1 μg ml$^{-1}$ (0.125–8 μg ml$^{-1}$).

In total, 41 (15 %) of the isolates were fully resistant to both fluconazole and itraconazole. Of these, 23 were C. glabrata, 12 were C. albicans, four were Saccharomyces cerevisiae, one was Candida parapsilosis and one was Candida tropicalis. The MICs of voriconazole for these isolates are shown in Fig. 2.

### Antifungals in oral fungal infections

Antifungal drugs are essential tools in the management of oral candidosis among debilitated patients. Fluconazole has been widely used to good effect. It is found in saliva at concentrations comparable to those in blood after single or multiple doses (Debruyne, 1997). Itraconazole, both as capsules and as the cyclodextrin solution, has also been shown to be effective in the treatment of oral candidosis (Cross et al., 2000) and the oral solution generates effective levels in both plasma and saliva (Reynes et al., 1997). However, the increasing incidence of colonization and infection with non-C. albicans yeasts displaying reduced susceptibility to the first generation azoles (fluconazole and itraconazole) has raised concerns for the future (Johnson et al., 1995). In the present study of a group of patients who we believe to be representative of those receiving palliative care for advanced cancer, 24 % of the isolates were not susceptible to fluconazole at standard doses. Consideration of the possible role of newer antifungal drugs in this group of patients is therefore important.

The data presented in this paper indicate that voriconazole is more potent than either fluconazole or itraconazole against yeasts isolated from the mouths of patients with advanced cancer. This finding is in keeping with published work on yeasts from a range of other patient groups and body sites (Cuenca-Estrella et al., 1999; Pelletier et al., 2002; Laverdiere et al., 2002). Of particular relevance is the voriconazole susceptibility of the isolates that were resistant to fluconazole and itraconazole. Many of these strains displayed low MICs to voriconazole, confirming reports by others that this agent is of value in treating some infections involving fluconazole-resistant Candida species (Pelletier et al., 2002; Laverdiere et al., 2002). However, the MICs of voriconazole for flucona-
zole-resistant isolates were higher than those for fluconazole-susceptible isolates, as reported by others (Cuenca-Estrella et al., 1999).

Whilst no interpretive breakpoints have yet been agreed for voriconazole, the pharmacokinetics of the drug suggest that adequate serum levels are available to treat infecting organisms with MICs of $\leq 2 \mu g ml^{-1}$ (Pelletier et al., 2002). Forty-four of the fluconazole-resistant strains had voriconazole MICs of $\leq 2 \mu g ml^{-1}$, but 18 of the remaining 20 strains had MICs of $8 \mu g ml^{-1}$. The pharmacokinetics of voriconazole appear suitable for the management of oral candidosis. A recent study showed that the pharmacokinetic profiles for saliva followed a pattern similar to those observed for plasma, with a highly significant correlation between plasma and saliva voriconazole concentrations (Purkins et al., 2002).

C. glabrata in oral fungal infections

The frequent isolation of C. glabrata is worthy of note. Of the 51 isolates of C. glabrata, 34 were fluconazole-resistant, with correspondingly high MICs to voriconazole. C. glabrata therefore constituted 53% of the 64 fluconazole-resistant yeasts isolated. Although the numbers were small, one other group has reported a relatively lower success rate for voriconazole treatment of C. glabrata infections (Perfect et al., 2003). These data indicate the value of accurate identification of isolates to species level, ideally accompanied by antifungal susceptibility testing, when managing florid oral fungal infections in those with advanced disease.

Biofilms in oral fungal infections

One further issue relevant to the management of oral fungal infections is that the yeasts are present in the form of a biofilm. It is well recognized that antimicrobial agents are often far less effective against sessile, as opposed to planktonic, organisms (Ramage & López-Ribot, 2004). These concepts are now being considered in relation to antifungal drug susceptibility testing (Ramage et al., 2001) and are to be examined in more detail in future work by our group.

Conclusions

In summary, 75% of the oral isolates were susceptible to fluconazole and this drug is likely to continue as the mainstay...
of treatment for oral fungal infections in the terminally ill for the foreseeable future. A significant proportion of yeasts showed reduced susceptibility to fluconazole and itraconazole, many of which were inhibited by low concentrations of voriconazole. By contrast, some of the fluconazole-resistant isolates, in particular *C. glabrata*, demonstrated reduced susceptibility to voriconazole. These data suggest a potential role for voriconazole in management of some refractory oral fungal infections in those with advanced cancer. However, more comprehensive studies of voriconazole are required to determine the correlation between *in vitro* activity and clinical outcome *in vivo*.

**ACKNOWLEDGEMENTS**

This study was funded by Pfizer (UK) who also provided the fluconazole and voriconazole free of charge. The Janssen Research Foundation (Belgium) provided the itraconazole free of charge. Professor David Coleman, University of Dublin, is thanked for his help in confirming the identifications of isolates of *C. dubliniensis*. We would like to acknowledge the support of Professor David Beighton, GKT Dental Institute.

**REFERENCES**


