Progressive loss of echinocandin activity following prolonged use for treatment of *Candida albicans* oesophagitis

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Received 5 October 2005; returned 15 December 2005; revised 19 December 2005; accepted 17 January 2006

Objectives: To illustrate the progressive loss of cross-echinocandin activity on *Candida albicans* isolates with strong clonal homology from a patient with advanced HIV infection and chronic oesophagitis progressively resistant to uninterrupted micafungin treatment.

Methods: Antifungal susceptibility profiles for different antifungal agents were determined against serial *C. albicans* isolates retrieved before and during therapy. Multilocus sequencing typing (MLST) was performed on each of the isolates. *FKS1* mutations conferring reduced susceptibility to echinocandin drugs were determined by DNA sequence analysis.

Results: Four *C. albicans* isolates showing identical allelic homology were retrieved from the patient at the initiation and during therapy with micafungin. The progressive lack of clinical response to micafungin therapy was associated with increased MICs of all three echinocandin drugs (caspofungin, micafungin and anidulafungin) in association with the acquisition of mutations in the *FKS1* gene.

Conclusions: This report documents for the first time a progressive loss of activity of all three echinocandin drugs against clonally related *C. albicans* isolates following long-term clinical exposure to this new class of antifungal agents.

Keywords: micafungin, *Candida* infections, antifungal therapy, resistance to echinocandins, HIV infection

Introduction

Echinocandins are a new class of antifungal antibiotics with potent activity against *Candida albicans in vitro*, and with established clinical efficacy in the treatment of oesophageal candidiasis.¹⁻³ As with any new class of antimicrobial agents, the development of clinical resistance is an important concern, particularly given the increasing use of these new agents. Moudgal et al.⁴ recently reported evidence of clinical failure and emergence of multi-echinocandin resistance *in vitro* during therapy in a patient with a *Candida parapsilosis* prosthetic valve endocarditis. However, echinocandins have poor intrinsic *in vitro* activity against *C. parapsilosis*.⁵⁻⁷ Hernandez et al.⁸ have recently reported in a patient with a *C. albicans* oesophagitis emergence of *in vivo* and *in vitro* resistance during prolonged caspofungin treatment. We hereby report failure of micafungin observed during a 10 month course of therapy in a patient with *C. albicans* oropharyngeal and oesophageal candidiasis. Serially obtained isolates during therapy showed increased MIC values of caspofungin, micafungin and anidulafungin in association with the acquisition of mutations in the *FKS1* gene that are characteristic of reduced echinocandin susceptibility.

Patient and methods

Case history

A 38-year-old man with a symptomatic and culture proven *C. albicans* oropharyngeal and oesophageal candidiasis refractory to azole therapy was started on compassionate micafungin. The patient had been HIV seropositive for 17 years, and his course had previously been complicated by *Pneumocystis jiroveci* pneumonia, cytomegalovirus retinitis.

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and repeated symptomatic episodes of *Candida* oesophagitis. These numerous recurrent episodes of oesophagitis were treated in the past with oral fluconazole, oral itraconazole solution and intravenous (iv) amphotericin B in both the desoxycholate and liposomal forms. During the past 12 months, the symptomatic *Candida* oesophagitis persisted and became refractory to these previous medications and also to oral voriconazole, and iv caspofungin combined with oral itraconazole solution. At the time of the initiation of micafungin therapy his medications included tprimanavir, ritonavir, abacavir, didanosine, tenofovir, valganciclovir, daily trimethoprim/sulfamethoxazole, weekly azithromycin and intermittent G-CSF injections whenever his neutrophil count declined to <750 cells/mm³. Three weeks before the initiation of micafungin, his HIV RNA plasma viral load was 44 819 copies/mL and his CD4 lymphocyte count was 5 cells/mm³ of blood. A gastroscopy, 16 days after the initiation of micafungin, detected oesophageal candidiasis and resolution of the oesophageal lesions. Over a period of 10 months, the patient continuously received iv micafungin, 100 mg once daily for the first 2 days, 200 mg daily for 3 weeks and 400 mg daily for the remaining period. The patient noted partial relief of the dysphagia within 2 days of starting therapy and an even greater relief once the dose of micafungin was increased to 400 mg daily. Despite the initial improvement in therapy, by 5 months after the initiation of micafungin, the patient noted increasingly frequent recurrences of oesophageal candidiasis. By 7 months of micafungin therapy, he again complained of progressive dysphagia and weight loss. Oral itraconazole was empirically added with no significant clinical improvement. After nearly 10 months of therapy, surgical implantation of a new venous access was required; the procedure was complicated by a pneumothorax and the patient had an unexpected cardiac arrest and died when a chest tube was inserted. During the course of treatment with micafungin, his HIV infection remained poorly controlled despite several changes in his antiretroviral therapy.

Fungal isolates

Isolates of *C. albicans* were recovered from the patient’s oropharyngeal cultures on three different occasions as follows: at the time of initiation of micafungin therapy, at week 6 and at week 36 of treatment. At week 36, two phenotypically different isolates were recovered from culture.

The isolates were all identified as *C. albicans* by means of germ tube and chlamydospore formation and by the Vitek YBC identification system (Biomerieux).

Antifungal susceptibility testing of each isolate was performed by a broth microdilution method. For amphotericin B, itraconazole, fluconazole, voriconazole, ravuconazole and posaconazole, susceptibilities were determined according to the CLSI in document M27-A.9 Susceptibilities to caspofungin, micafungin and anidulafungin were performed according to the guidelines recommended by Odds et al.10 MIC endpoints were determined using the visual ‘prominent growth reduction’ (MIC-2) criteria measured at 24 h in RPMI 1640 medium. *C. parapsilosis ATCC 22019 and Candida krusei ATCC 6258* were used for quality control.

*FKS1* mutations conferring reduced susceptibility to echinocandin drugs were determined by DNA sequence analysis of a region of the *CaFKS1* that was recently shown to confer reduced susceptibility in both laboratory strains and clinical isolates of *C. albicans.*11 The sense and antisense primers used for PCR and sequencing, based on *CaFKS1* sequence (GenBank accession no. D88815), were 5'-CATTGCTGGGCCACCTTATG-3' and 5'-GGTCAAAATCATG-GAAAACCG-3', respectively. The PCR products were purified, quantified by fluorescence labelling (Pico Green, Molecular Probes) and sequenced in both the 5' and 3' directions using the DTCS Quick Start Kit (Beckman Coulter).

Multilocus sequencing typing (MLST) was performed on each of the isolates as previously described.12,13 For each gene, distinct alleles were identified and numbered using the non-redundant databases program (http://c albicans.mlst.net/). The alleles at each of the six loci constituted a strain’s allelic profile, i.e. diploid sequence type (DST). Each distinct allelic profile was considered a unique DST or genotype. An unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the pair-wise differences in the allelic profiles of the six genes was constructed using the START program (http://www.medawar.ox.ac.uk/maiden/software.shtml).

The discriminatory power was measured with Simpson’s index of diversity. Genotypes for MLST were based on individual DSTs.

### Results and discussion

In a patient with *C. albicans* oesophagitis refractory to triazole and polyene therapy, the use of an echinocandin appears warranted. Micafungin (which had not yet been approved at the time it was started) was selected because of its higher tolerable dosage.14,15 The MIC values for sequential *C. albicans* isolates recovered at baseline and during therapy are shown in Table 1. At baseline, the MICs of micafungin, caspofungin and anidulafungin were all ≤0.06 mg/L. Within 6 weeks of treatment, MIC values of all three echinocandins increased >33-fold and remained constant throughout the remaining 7.5 months of micafungin treatment. DNA sequencing of the known mutational ‘hot spot’ regions of the *FKS1* gene correlated with the increases in the MIC values (Figure 1). Two prominent Fks1p mutations, S645F and R1361H, known to confer reduced echinocandin susceptibility in *C. albicans*11 were identified in the week 6 and week 36 isolates. All four fluconazole-resistant isolates had a homozygous F145L mutation in the triazole target gene *ERG11* and 11- to 74-fold increases in transcript levels of the multidrug efflux pump genes *CDR1* and *CDR2* relative to the azole-susceptible reference strain ATCC 90028 (Table 2). Strong clonal homology of all four isolates was supported through MLST analysis showing identical allelic profiles genetically distinct from five other geographically diverse strains (Figure 2).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Baseline</th>
<th>Week 6</th>
<th>Week 36 no. 1</th>
<th>Week 36 no. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
<td>≥16</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>32</td>
<td>64</td>
<td>256</td>
<td>≥512</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>≥16</td>
</tr>
<tr>
<td>Ravuconazole</td>
<td>4</td>
<td>4</td>
<td>≥16</td>
<td>≥16</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Micafungin</td>
<td>0.03</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>0.06</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>0.03</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Exposure to antimicrobial agents is an important triggering factor in the emergence of drug resistance. Little is known about the development of *C. albicans* echinocandin resistance...
Echinocandin loss of activity

Table 2. Summary of isolates azole resistance mutations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Azole susceptibility</th>
<th>Echinocandin susceptibility</th>
<th>ERG11 mutation</th>
<th>CDR1</th>
<th>CDR2</th>
<th>ERG11</th>
<th>FLU1</th>
<th>MDR1</th>
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</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>resistant</td>
<td>susceptible</td>
<td>F145L</td>
<td>11.4</td>
<td>34.8</td>
<td>0.9</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Week 6</td>
<td>resistant</td>
<td>reduced</td>
<td>F145L</td>
<td>14.7</td>
<td>69.5</td>
<td>1.0</td>
<td>1.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Week 36 no. 1</td>
<td>resistant</td>
<td>reduced</td>
<td>F145L</td>
<td>21.8</td>
<td>48.6</td>
<td>1.1</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Week 36 no. 2</td>
<td>resistant</td>
<td>reduced</td>
<td>F145L</td>
<td>18.2</td>
<td>74.8</td>
<td>1.0</td>
<td>1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>ATCC 90028</td>
<td>susceptible</td>
<td>susceptible</td>
<td>None</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Figure 1. Summary of isolates’ echinocandin reduced susceptibility mutations.

Figure 2. MLST dendrogram. An unweighted pair group method with arithmetic mean (UPGMA) dendrogram based on the pair-wise differences in the allelic profiles of the six genes was used to assess the genetic relatedness among the four C. albicans isolates and five geographically distinct strains. of activity was manifested 14 weeks before the documentation of micafungin clinical failure despite no obvious change in the patient’s antiretroviral therapy or general immunosuppressive status.

In conclusion, echinocandins are potent, well-tolerated antifungal drugs with good clinical efficacy and low toxicity profiles. Given the paucity of antifungal drugs, their use in clinical situations represents an attractive alternative. Our report and that of others raise concern for potential loss of activity following extensive clinical exposures.

Acknowledgements

The work was partially supported by grants to R. G. L. from the Fonds de Recherche en Santé du Québec, Réseau sida and the Canadian HIV Trials Network. Micafungin was provided free of charge by Astellas Canada Inc. We thank Christiane Restieri for her support.

Transparency declarations

M. L. has acted as a member of the Canadian medical advisory boards of Astellas Canada, Merck-Frosst Canada, Schering Canada Inc. and Pfizer Canada; has received unrestricted educational grants from Merck-Frosst Canada Ltd, Fujisawa Canada, Pfizer Canada and Wyeth Canada; has received research grants from Bio-Rad Laboratories, Fujisawa Canada, Merck & Co. and Schering Plough; and has received clinical trial research contracts from Astellas Healthcare Inc., Merck-Frosst Canada, Pfizer Canada, Schering Canada Inc. and Vicuron Inc.
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