Differential activity of triazoles in two-drug combinations with the echinocandin caspofungin against Aspergillus fumigatus

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Objectives: We investigated the in vitro activity of various triazoles in two-drug combinations with the echinocandin caspofungin against clinical isolates of Aspergillus fumigatus.

Method: Conidial suspensions were prepared from 20 clinical isolates of A. fumigatus highly susceptible to itraconazole, voriconazole, posaconazole and ravuconazole (MIC-0 range 0.125–1 mg/L), and caspofungin (MIC-0 range 32–64 mg/L). The in vitro susceptibility of A. fumigatus to two-drug combinations of itraconazole, voriconazole, posaconazole and ravuconazole with caspofungin was evaluated by the fractional inhibitory concentration index (FICI) method.

Results: Two-drug combinations of caspofungin with itraconazole (FICI = 0.49 ± 0.04) or posaconazole (FICI = 0.32 ± 0.09) provided synergic interaction. On the other hand, ravuconazole (FICI = 0.61 ± 0.31) and voriconazole (FICI = 1.61 ± 0.42) in combination with caspofungin showed no interaction against A. fumigatus.

Conclusions: Our data show that the in vitro antifungal efficacies of combinations of members from two different classes are not always similar and hence are not predictable.

Keywords: triazoles, antifungal combination, caspofungin, Aspergillus fumigatus

Introduction

Use of combinations of antifungal drugs not only reduces the chance of emergence of drug resistance but may also increase the effectiveness by acting together either synergically or additively.1,2 Although there are several classes of conventional (e.g. polyenes and azoles) and investigational (e.g. echinocandins, nikkomycins, sordarins, morpholines and allylamines) antifungal agents available now, very little is known about the activity of combinations of these antifungal agents against pathogenic filamentous fungi. Voriconazole exhibits excellent in vitro and in vivo activity against a wide spectrum of fungi, including Aspergillus species.3,4 On the other hand, the echinocandin caspofungin shows inhibitory effects on the growth of Aspergillus fumigatus at low concentrations5 with very little killing of the A. fumigatus cells even after prolonged exposure to the drug. Since the echinocandins and the triazoles are different classes of antifungal drugs with distinct modes of action, two-drug combination of anazole with an echinocandin may provide synergic interaction against susceptible microorganisms. We therefore investigated the in vitro susceptibility of 20 clinical isolates of A. fumigatus to four triazoles alone and in two-drug combinations with caspofungin using the fractional inhibitory concentration index method.6

Materials and methods

Antifungal drugs

Voriconazole, itraconazole, posaconazole and ravuconazole were obtained from Pfizer Pharmaceuticals (New York, NY, USA), Janssen Pharmaceutica (Beerse, Belgium), Schering-Plough Research Institute (Kenilworth, NJ, USA) and Bristol-Myers Squibb Institute for Medical Research (Princeton, NJ, USA), whereas caspofungin was obtained from

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Merck and Company (Rahway, NJ, USA), respectively. The triazoles were dissolved in dimethyl sulphoxide to obtain a stock solution of 1 g/L and stored as 0.25 mL lots at −20°C. Caspofungin was dissolved in sterile double-distilled water to obtain a concentration of 10 g/L and stored as 0.25 mL lots at −70°C. The frozen stocks of the antifungal agents were thawed at room temperature and used within 24 h.

**Determination of fractional inhibitory concentration index (FICI)**

Twenty clinical isolates of *A. fumigatus* obtained from the Microbiology Laboratory of the Detroit Medical Center (Detroit, MI, USA) were used in this study. Conidial suspensions from 7-day-old *A. fumigatus* cultures were prepared, standardized by haemocytometry and used as inoculum for susceptibility testing. The *in vitro* susceptibility of *A. fumigatus* to two-drug combinations of caspofungin with various triazoles was evaluated by the FICI method determined by two-dimensional chequerboard using the National Committee for Clinical and Laboratory Standards M38-A broth microdilution technique. This is the reference method for testing filamentous fungi with azoles, amphotericin B and flucytosine, and has not yet been validated for caspofungin. Pair-wise combinations of the required concentrations of caspofungin (antifungal A) and the required triazole (antifungal B) were prepared in two-fold increments in 0.1 mL RPMI 1640 containing 0.165 M MOPS buffer (pH 7.0). Eleven wells in the top row and seven wells in the first column contained various concentrations of caspofungin and the triazole alone, respectively. The well at the top left corner of the microdilution plate contained no drug (drug-free growth control). The other 77 wells in the microdilution plate contained combinations of various concentrations of caspofungin and the triazole alone, respectively. The well at the top left corner of the microdilution plate contained no drug (drug-free growth control). The other 77 wells in the microdilution plate contained combinations of various concentrations of caspofungin and the triazole alone, respectively. The well at the top left corner of the microdilution plate contained no drug (drug-free growth control).

**Results and discussion**

We used 20 clinical isolates of *A. fumigatus* randomly selected from our collection (*n* = 619) obtained from the Detroit Medical Center (Detroit, MI, USA). All 20 isolates were highly susceptible to itraconazole (MIC-0 range 0.25–0.5 mg/L), voriconazole (MIC-0 range 0.25–0.5 mg/L), posaconazole (MIC-0 range 0.125–0.25 mg/L), ravuconazole (MIC-0 range 0.25–1 mg/L) and much less susceptible to caspofungin (MIC-0 range 32–64 mg/L). Although the MIC-0 of caspofungin was relatively high, this antifungal drug produced prominent growth inhibition [MIC-2 (50% inhibition of growth compared with the drug-free control)] range 0.031–0.062 mg/L] at low concentration. Since FICI is a ratio of the MICs of the component drugs alone and in two-drug combination, we used the MIC-0 of all drugs for the calculation of the FICI to avoid subjectivity in end-point determination. The MIC-0 of caspofungin was decreased 32-fold (64 to 2 mg/L) in the presence of posaconazole. Similarly, in the presence of caspofungin, the posaconazole MIC-0 was decreased four-fold (0.25 to 0.062 mg/L) indicating synergic interaction. On the other hand the MIC-0 of caspofungin was not affected at all in the presence of voriconazole and vice versa suggesting that caspofungin plus voriconazole provides indifferent interaction. A summary of the results of drug interactions obtained for all 20 isolates of *A. fumigatus* is shown in Table 1. Both itraconazole and its analogue posaconazole showed synergic *in vitro* interaction whereas voriconazole and its analogue ravuconazole showed no interaction. We found no evidence of antagonism for the azole plus echinocandin combination.

The primary fungal target of action for all azoles, including triazoles, is believed to be the cytochrome P450-dependent lanosterol 14α-demethylase, an enzyme essential for the synthesis of ergosterol. The differential interaction of triazoles with caspofungin (inhibitor of cell wall synthesis by interfering with the function of glucan synthase) could not have been predicted based on the modes of action of these two classes of antifungal drugs. The fact that these two classes

**Table 1. In vitro interaction of caspofungin in two-drug combinations with various triazoles against clinical isolates (*n* = 20) of *A. fumigatus***

<table>
<thead>
<tr>
<th>Drug combination used</th>
<th>FICI ± s.d.</th>
<th>Drug interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspofungin + itraconazole</td>
<td>0.49 ± 0.04</td>
<td>synergy</td>
</tr>
<tr>
<td>Caspofungin + posaconazole</td>
<td>0.32 ± 0.09</td>
<td>synergy</td>
</tr>
<tr>
<td>Caspofungin + voriconazole</td>
<td>1.61 ± 0.42</td>
<td>no interaction</td>
</tr>
<tr>
<td>Caspofungin + ravuconazole</td>
<td>0.61 ± 0.31</td>
<td>no interaction</td>
</tr>
</tbody>
</table>

Note: The FICI was determined by a two-dimensional chequerboard as described in Materials and methods. The data were obtained from two independent experiments.
of drugs act on different fungal targets would have suggested a possible synergic interaction against *A. fumigatus*. However, our results show that only two of the four triazoles we used produced synergic results against *A. fumigatus*.

The reason(s) for the differential *in vitro* activity of triazoles in two-drug combinations with caspofungin is at present only a matter of speculation. However, it is possible that the effect occurs only *in vitro* and has no *in vivo* correlation. The azoles may have additional less prominent mode(s) of action and such a minor mode of action is enhanced prominently in combination with caspofungin in the case of certain triazoles resulting in increased efficacy. Moreover, it is possible that the uptake of azoles is selectively interfered with or enhanced in the presence of caspofungin. Alternatively, it is possible that differential lipophilicity of the four triazoles may play a role in their differential interaction in combination with caspofungin. Itraconazole and posaconazole with their long hydrophobic aliphatic tail region will be more lipid soluble than voriconazole and ravuconazole. The lipophilic itraconazole and posaconazole interact synergically with caspofungin, a cyclic lipohexapeptide compound, whereas voriconazole and ravuconazole are unable to do so. These results indicate that the *in vitro* interactions of members from two drug classes are not always identical and hence are not predictable.

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References


