In vitro interaction of posaconazole with calcineurin inhibitors and sirolimus against zygomycetes

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Objectives: Zygomycosis is an uncommon but devastating disease with few therapeutic options. Calcineurin inhibitors and sirolimus (mTOR inhibitor), commonly used in transplant patients as immunosuppressives, have antifungal activity. They are known to demonstrate synergy with triazoles against certain fungi, though limited data exist about their activity against zygomycetes. Our aim was to study the in vitro interaction of posaconazole with calcineurin inhibitors and sirolimus against zygomycetes.

Methods: Drug interactions were assessed with chequerboard dilution for posaconazole with calcineurin inhibitors and sirolimus according to the CLSI M38-A2 method for filamentous fungi. Twenty-eight clinical isolates were studied, including Rhizopus arrhizus, Rhizopus microsporus, Rhizomucor pusillus, Mucor sp., Cunninghamella bertholletiae, Myocladus corymbifera and Apophysomyces elegans. Combinations of posaconazole with tacrolimus, cyclosporin A or sirolimus were used. Experiments were performed in duplicate. Mean fractional inhibitory concentration indices were calculated.

Results: Posaconazole with calcineurin inhibitors demonstrated consistent synergy against C. bertholletiae, M. corymbifera and A. elegans, whereas synergy or no interaction was primarily observed against R. arrhizus, R. microsporus, R. pusillus and Mucor. Antagonism was seen with the combination of posaconazole and sirolimus. Strain variability was noted among the same species.

Conclusions: The clinical significance of these findings is unclear, but further studies are warranted given the potential for concomitant use of these agents in transplant patients treated for zygomycosis.

Keywords: azole antifungals, fungal susceptibility, mucormycosis

Introduction

Zygomycosis is an uncommon but frequently fatal mycosis caused by members of the class zygomycetes, seen primarily as an opportunistic disease in immunocompromised hosts. The majority of zygomycosis in humans is caused by members of the family Mucoraceae. Among the common genera seen clinically are Rhizopus, Rhizomucor, Mucor, Myocladus (formerly Absidia) and Cunninghamella.1 Calcineurin inhibitors and sirolimus are commonly used immunosuppressive agents, particularly in solid organ transplant recipients. Calcineurin is a Ca²⁺/calmodulin-activated protein phosphatase that is highly conserved in eukaryotes. It plays an essential role in morphogenesis and virulence in Candida albicans and Cryptococcus neoformans as well as controlling physiological processes including cell cycle progression, cation homeostasis and morphogenesis.2 Cyclosporin A and tacrolimus are commonly used calcineurin inhibitors, though their immunosuppressive effects outweigh any specific antifungal activity in vivo. Emerging data show that the antifungal attributes of these agents may have a role in the development of novel antifungal strategies, in particular as combination therapies.3 Sirolimus acts through a distinct mechanism of action, inhibition of the mTOR pathway.3 It was originally developed as an antifungal drug, but was found to have potent immunosuppressive properties.

Combination therapies with calcineurin inhibitors have been shown to be active in vitro against Candida albicans, Cryptococcus neoformans and Aspergillus spp.3–5 In the present study we investigated the in vitro antifungal activity of calcineurin inhibitors and sirolimus with posaconazole in combination against 28 strains of zygomycetes.

Materials and methods

Zygomycete strains used: Rhizopus arrhizus (5); Rhizopus microsporus (5); Rhizomucor pusillus (3); Mucor sp. (5); Myocladus (formerly Absidia)
Discussion

Zygomycosis is an uncommon, but often refractory, mycosis, usually seen in immunocompromised patients. The standard therapy for decades has been amphotericin B, though lipid formulations are increasingly recommended as front-line therapy, given their reduced nephrotoxicity with the high doses required to treat these infections. Posaconazole has emerged as a potential alternative, with good clinical activity and excellent tolerability compared with amphotericin B. Despite these advances, morbidity and mortality remain high for these infections and novel therapeutic strategies are needed.

Calcineurin inhibitors have been shown to have antifungal properties and have been shown to be synergistic in combination with other antifungal agents against fungi such as Candida, Cryptococcus and Aspergillus. In a recent study by Dannaoui et al., the combination of triazoles with calcineurin inhibitors and sirolimus was tested against 10 isolates of zygomycetes. They found variable synergy with each combination, and occasional antagonism only with sirolimus and itraconazole.

In this study, we tested the effects of posaconazole with immunosuppressive drugs against 28 isolates of seven clinically important species of zygomycetes. We found synergistic activity against each of the species using calcineurin inhibitors, though some were more consistent than others. Importantly, significant antagonism was not observed with calcineurin inhibitors, suggesting that, particularly in solid organ transplant patients receiving tacrolimus or cyclosporin A, posaconazole may be given without affecting its antifungal activity. No significant antagonism was observed with itraconazole either. In contrast, sirolimus in combination with posaconazole resulted in consistent and significant antagonism with most species isolated.

While we observed similar synergistic activity with calcineurin inhibitors and posaconazole to that in the Dannaoui et al. study, our finding of strong antagonism with sirolimus was unique. This may be in part due to the different endpoint readings used. According to CLSI M38-A2, the endpoint reading for posaconazole against filamentous fungi is 100% inhibition, which may have led to lower MICs and also affected the resulting FICIs.

The clinical significance of these interactions is unknown at present. While the immunosuppressive effects of cyclosporin A and tacrolimus alone clearly outweigh the antifungal effects, it is not known how potent synergy may affect therapy in systemic fungal infections. A recent study by Singh et al. of solid organ transplant patients demonstrated that the use of tacrolimus was associated with a protective effect from developing synergies.

Table 1. Results of synergy testing of posaconazole, calcineurin inhibitors and sirolimus

<table>
<thead>
<tr>
<th>Zygomycete (n)</th>
<th>FICI, range (median)</th>
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<tbody>
<tr>
<td></td>
<td>posaconazole+cyclosporin A</td>
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<tr>
<td>R. arrhizus (5)</td>
<td>0.19–3 (0.62)</td>
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<tr>
<td>R. microsporus var. rhizopodaformis (5)</td>
<td>0.5–2 (0.59)</td>
</tr>
<tr>
<td>R. pusillus (3)</td>
<td>0.31–2 (0.47)</td>
</tr>
<tr>
<td>Mucor sp. (5)</td>
<td>0.13–5 (0.56)</td>
</tr>
<tr>
<td>M. corymbifera (4)</td>
<td>0.09–0.37 (0.3)</td>
</tr>
<tr>
<td>C. bertholletiae (5)</td>
<td>0.06–1.5 (0.09)</td>
</tr>
<tr>
<td>A. elegans (1)</td>
<td>0.05–0.06 (0.06)</td>
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zygomycosis. In our study, some intrinsic antifungal activity was seen with tacrolimus, suggesting that this agent may have some clinical activity as well. The antagonism seen with sirolimus is concerning, though any clinical correlation is lacking at present. Additional in vivo studies are warranted to confirm this effect. Non-immunosuppressive cyclosporin A and tacrolimus analogues are available that have potent antifungal properties. These should also be studied for synergy, as cyclosporin A and tacrolimus are unlikely to be used clinically in non-transplant patients. In addition, the molecular basis of this mechanism in these fungi needs to be further elucidated.

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**References**


