Ibuprofen Potentiates the In Vivo Antifungal Activity of Fluconazole against Candida albicans Murine Infection

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Candida albicans is the most prevalent cause of fungemia worldwide. Its ability to develop resistance in patients receiving azole antifungal therapy is well documented. In a murine model of systemic infection, we show that ibuprofen potentiates fluconazole antifungal activity against a fluconazole-resistant strain, drastically reducing the fungal burden and morbidity. The therapeutic combination of fluconazole with ibuprofen may constitute a new approach for the management of antifungal therapeutics to reverse the resistance conferred by efflux pump overexpression.

The pursuit of knowledge regarding the efflux pump mechanism in Candida arises from the homology between yeasts and human cells. In eukaryotic neoplastic cells, ATP-dependent drug efflux pumps, such as P-glycoprotein (P-gp), which is encoded by the MDRI gene, are important mediators of resistance, contributing to the failure of cancer therapy. In the human kidney, ibuprofen can inhibit metothrexate efflux transporters (17). A similar effect has been described for FK506 (tacrolimus), a potent immunosuppressive agent that shows a synergistic effect when combined with antineoplastic agents on tumor cells, decreasing or even suppressing multidrug resistance by competing with cytotoxic drugs for the P-glycoprotein (18–20). Thus, a similar approach could be applied to C. albicans cells.

To investigate the potential clinical application of fluconazole and ibuprofen, in vivo experiments were conducted in a murine candidosis model approved by the Directorate General of Food and Veterinary Medicine of the European Union (authorization no. 6411).

Female specific-pathogen-free BALB/c mice (age, 6 to 8 weeks; weight, 17 to 20 g; Charles River Laboratories) were injected with 5 x 10^5 cells of the CaS or the CaR strain in 0.1 ml of sterile saline via the lateral tail vein (21). The fluconazole effective dose that reduced by 50% the pathological effects of intravenous (i.v.) C. albicans challenge relative to untreated mice (control) was defined as the 50% effective dose (ED_50) (8). Therapy was initiated 3 h after the yeast challenge and was administered daily for a total of 3 days (8, 21). The mice were treated intraperitoneally with fluconazole (8 to 60 mg/kg of body weight/day) (8, 21), ibuprofen (10 or 20 mg/kg/day) (11, 22), or fluconazole (8 to 60 mg/kg of body...
TABLE 1 *In vitro* development of azole resistance and its reversion by ibuprofen

<table>
<thead>
<tr>
<th>Strain</th>
<th>FLC (μg/ml) phenotype</th>
<th>FLC + Ibu</th>
<th>VRC</th>
<th>VRC + Ibu</th>
<th>PSC</th>
<th>PSC + Ibu</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaS</td>
<td>1/S</td>
<td>1/S</td>
<td>0.06/S</td>
<td>0.06/S</td>
<td>0.03/S</td>
<td>0.06/S</td>
</tr>
<tr>
<td>CaR</td>
<td>&gt;64/R</td>
<td>2/S</td>
<td>&gt;8/R</td>
<td>&gt;8/R</td>
<td>&gt;8/R</td>
<td>0.125/R</td>
</tr>
</tbody>
</table>

*MICs to azoles, namely, fluconazole (FLC), voriconazole (VRC), and posaconazole (PSC), alone and in combination with subinhibitory concentration of ibuprofen (Ibu) (100 μg/ml). S, susceptible; R, resistant.*

weight/day) plus ibuprofen (10 or 20 mg/kg/day). The weight of each mouse was registered daily, and at day 4 postinfection, the mice were euthanized and the kidneys aseptically removed. The fungal burden was calculated as the number of CFU per gram of tissue, and the isolates were preserved for later MIC determination. For histological studies, the kidneys were processed for periodic acid-Schiff (PAS) staining.

In mice infected with the CaS strain and treated with 30 mg/kg of body weight/day (ED₅₀) of fluconazole, a significant reduction (*P* < 0.001) in yeast colonization in the kidney was found compared with that in the untreated mice (Fig. 1). In mice infected with the CaR strain, no significant reduction in fungal burden was achieved, even when treated with 60 mg/kg of fluconazole (data not shown). Mice infected with the CaS and CaR strains and treated with 10 or 20 mg/kg/day of ibuprofen did not show a reduction in fungal burden. However, when fluconazole was administered with ibuprofen, even at the lower concentration, a significant reduction in CaR fungal burden (*P* < 0.001) was observed (Fig. 1).

Mice infected with the CaR strain and treated with fluconazole plus ibuprofen showed the lowest weight loss (Fig. 2).

Interestingly, yeasts recovered from mouse kidneys retained their susceptibility profile, i.e., susceptible or resistant for the CaS or CaR strain, respectively.

Histopathological sections of mouse kidneys confirmed the fungal burden quantitative analysis. Infection caused by CaR was evident in untreated and fluconazole-treated mice (Fig. 3A to E). At day four postinfection, untreated mice infected with the CaR strain revealed a dramatic increase in fungal colonization by different types of cells, yeasts and hypha, extensive tissue damage, and necrosis (Fig. 3A). An identical scenario was found in kidneys collected from mice treated with 30 mg/kg of fluconazole (Fig. 3B to E). In Fig. 3E, fungal cells were predominantly in the hyphal form and were apparently intact, forming a clear barrier to the progression of inflammatory leukocytes. Notably, in mice treated with fluconazole plus ibuprofen, the kidney tissue architecture was preserved, and the fungal cells were rare, all displaying a yeast form (Fig. 3F and G).

The phenotypic switching between yeast and hypha in *C. albicans* is often described as the major virulence factor, as hyphal formation is associated with elevated secretion of hydrolytic enzymes, direct tissue invasion, and adherence to host surfaces (23). Hyphal morphotypes are more invasive, and their extension is essential for dissemination and the subsequent events responsible for the gross damage of tissues, which are commonly observed in the kidneys of infected mice (24, 25).

Mice treated with fluconazole and ibuprofen experienced clearance of infection, recovery of body weight, and the conservation of tissue architecture, having scarce fungal cells in the kidneys, being predominantly yeast forms. The hypothesis that the presence of ibuprofen may target the regulation of the morphological switch from yeast to hypha thus deserves more attention and should be a subject of future in-depth research.

*C. albicans* cells recovered from mice treated with fluconazole plus ibuprofen still displayed a fluconazole-resistant phenotype. Consequently, we can conclude that the presence of ibuprofen is crucial and mandatory for the reversion of azole resistance. The *in vivo* assays clearly demonstrate that ibuprofen potentiates the antifungal activity of fluconazole and reduces the virulence of *C. albicans*. Since it is not immunosuppressive, its anti-inflammatory activity has advantages over FK506 (26). Further studies are being addressed to uncover the mechanism of ibuprofen on yeast cell physiology and to assess its influence on the dynamics of antifun-
gal resistance induction and reversion. Ibuprofen in combination with fluconazole might play a relevant role in a therapeutic strategy for severe fungal infections.

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