In Vitro and In Vivo Efficacy of Amphotericin B Combined with Posaconazole against Experimental Disseminated Sporotrichosis

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We evaluated the combination of posaconazole with amphotericin B in vitro and in a murine model of systemic infections caused by Sporothrix brasiliensis and Sporothrix schenckii sensu stricto. In vitro data demonstrated a synergistic effect, and although posaconazole alone was effective against sporotrichosis, efficacy in terms of survival and burden reduction was increased with the combination. This combination might be an option against disseminated sporotrichosis, especially when itraconazole or amphotericin B at optimal doses are contraindicated.

Disseminated infection is the most severe manifestation of sporotrichosis, occurring mainly in immunocompromised patients, although it has also been reported in immunocompetent people (1, 2). The Sporothrix schenckii complex encompasses four species able to cause sporotrichosis in humans (3, 4). Treatment of disseminated infections is carried out with amphotericin B and maintenance with itraconazole, showing various outcomes (5–7). Posaconazole has shown low MICs against Sporothrix spp. (8), and although only one study has explored its clinical role, its safety and efficacy in animal models indicate that this compound could be a good alternative for the treatment of disseminated sporotrichosis (5, 9). Seeking to enhance the treatment against sporotrichosis, posaconazole was evaluated in combination with amphotericin B against murine systemic infections by Sporothrix brasiliensis and S. schenckii sensu stricto.

Two strains of S. brasiliensis (FMR 8319 and FMR 8326) and two strains of S. schenckii sensu stricto (FMR 8606 and FMR 8609) in the mold phase were included in the study. The MICs of amphotericin B (Sigma-Aldrich Co., St. Louis, MO, USA) and posaconazole (Schering-Plough, Kenilworth, NJ, USA) were determined from 7-day-old cultures, in accordance with the CLSI guidelines (10), and the activity of the drug combination was tested using the checkerboard method. The fractional inhibitory concentration index (FICI) was calculated, and the combination was defined as synergistic at an FICI of ≤0.5, indifferent at an FICI of >0.5 and ≤4.0, and antagonistic at an FICI of >4.0 (11). The tests were carried out in duplicate.

For the in vivo study, the inocula were prepared from the filamentous growth by flooding the surface of the cultures with saline solution and scraping the sporulating mycelia. The resulting conidial suspension was transferred to potato dextrose broth and incubated in an orbital shaker at 150 rpm and 30°C for 5 days. The cultures were then filtered through sterile gauze and centrifuged at 325 × g. The conidial suspension was adjusted to the desired concentrations by hemocytometer counting (9). Four-week-old OF-1 male mice (Charles River, Critffa S.A., Barcelona, Spain) with a mean weight of 30 g were infected intravenously (i.v.) via the lateral tail vein with 2 × 10⁶ CFU in 0.2 ml of sterile saline. Six groups of 15 animals/group, 10 for survival and 5 for tissue burden studies, were established for each strain. Treatment groups received amphotericin B (Sagalés-Xalabarder Pharmacy, Barcelona, Spain) at 0.3 mg/kg of body weight i.v. or posaconazole (Noxafil; Schering-Plough Ltd., Hertfordshire, United Kingdom) at 2.5 or 5 mg/kg twice a day (BID) by gavage. The combined treatments consisted of posaconazole at 2.5 or 5 mg/kg BID together with amphotericin B at 0.3 mg/kg. Additionally, mice infected with S. brasiliensis (FMR 8319) received posaconazole at 10 mg/kg alone or combined with amphotericin B at 0.3 mg/kg. All treatments began 1 day after infection and lasted for 18 days, with the control groups receiving no treatment. When control mice started to die at 12 days postinfection, five mice from each group were euthanized, and the liver and the spleen, which are the most affected organs in experimental systemic sporotrichosis (12), were mechanically homogenized and placed on potato dextrose agar (PDA) to calculate the CFU per gram of tissue. All animal care procedures were carried out in duplicate and supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Ethics Committee.

Statistical analysis was done using GraphPad Prism 5 for Windows (GraphPad Software, Inc., La Jolla, CA). The mean survival time was estimated by the Kaplan-Meier method and compared among the groups by using the log rank test. The colony counts from the tissue burden studies were analyzed using the Mann-Whitney U test (P values of ≤0.05 statistically significant).

The in vitro combination of posaconazole with amphotericin B was synergistic for all the isolates (FICI, ≤0.5 for S. brasiliensis and ≤0.28 for S. schenckii) (Table 1). All isolates caused systemic infection with 100% death in control animals, with no
significant differences between species or between strains of the same species \((P \geq 0.09,\) in multiple comparisons). Treatments consisting of 0.3 mg/kg amphotericin B prolonged the survival of animals in comparison to their respective controls \((P \leq 0.043).\) However, all animals receiving posaconazole alone at any concentration or in combination with 0.3 mg/kg amphotericin B survived through the experimental period (Fig. 1).

The results of the tissue burden studies correlated with those of the survival studies, i.e., amphotericin B reduced the burden significantly, but posaconazole alone or combined at any dose did so more efficiently (Fig. 2). Posaconazole administered at 5 mg/kg was more effective in reducing burdens than when administered at 2.5 mg/kg in all cases \((P \leq 0.0001).\) The efficacy of posaconazole was better when combined with amphotericin B, with posaconazole at 5 mg/kg plus amphotericin B being the treatment that showed the highest burden reduction \((P \leq 0.03\) in comparison to the other treated and untreated groups). Interestingly, the data obtained demonstrated an equivalent efficacy in reducing the fungal burden with 5 mg/kg of posaconazole alone and 2.5 mg/kg of posaconazole plus amphotericin B against \(S.\) schenckii and a trend to equivalence against \(S.\) brasiliensis. The combination of 10 mg/kg of posaconazole amphotericin B against \(S.\) brasiliensis strain FMR 8319 did not further improve the efficacy over that with monotherapy with posaconazole at 10 mg/kg or 5 mg/kg even when combined with amphotericin B \((P \geq 0.061).\)

The FICI values were lower against \(S.\) schenckii than against \(S.\) brasiliensis, and the combination was more effective against the strains of \(S.\) schenckii than against \(S.\) brasiliensis, correlating the FICI with the animal outcome. There are several reported cases of systemic sporotrichosis having a fatal outcome despite having used the recommended treatments (13–15), which makes it desirable to explore new therapeutic options. Among them, voriconazole and terbinafine might be an option. Voriconazole has shown efficacy against \(S.\) schenckii but not against \(S.\) brasiliensis (16), and terbinafine has not been evaluated against systemic infections by \(Sporothrix\), although it has demonstrated activity \(in vitro\) (17, 18). Posaconazole has also demonstrated efficacy against systemic sporotrichosis, but in the present study, we show that such efficacy can be enhanced in combination with amphotericin B at low doses. Amphotericin B plus itraconazole at high doses has proven efficacious in an experimental disseminated infection by \(S.\) brasiliensis (19), although this combination failed in a clinical case due to toxic effects. Therapy was then changed to amphotericin B in combination with posaconazole, resulting in a dramatic clinical improvement (5). The combination of posaconazole and suboptimal doses of amphotericin B deserves attention as an alternative, especially in those patients suffering disseminated sporotrichosis who do not respond to treatment or when

<table>
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<th>Species</th>
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<th>MIC (mg/liter) of AMB/PSC</th>
<th>FICI Effect</th>
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<tr>
<td></td>
<td></td>
<td>Alone</td>
<td>In combination</td>
<td></td>
</tr>
<tr>
<td>(S.) brasiliensis</td>
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<tr>
<td></td>
<td>8609</td>
<td>4/2</td>
<td>1/0.03</td>
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</tbody>
</table>

*TABLE 1 In vitro interaction of amphotericin B and posaconazole against \(S.\) brasiliensis and \(S.\) schenckii*
itraconazole or high doses of amphotericin B are contraindi-
cated.

ACKNOWLEDGMENTS

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