Interactions between Triazoles and Amphotericin B in Treatment of Disseminated Murine Infection by *Fusarium oxysporum*

Mery Ruiz-Cendoya, Marçal Mariné, M. M. Rodríguez, and Josep Guarro*

Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, IISPV, Universitat Rovira i Virgili, Reus, Spain

Received 4 December 2008/Returned for modification 7 January 2009/Accepted 25 January 2009

Disseminated disease is the most frequent and challenging clinical form of fusariosis (13), showing a poor response to antifungals (3, 8, 13, 14). For a long time, conventional therapy for invasive fusariosis has consisted of an amphotericin B (AMB)-based regimen (2, 4, 12). However, clinical practice has demonstrated that this drug has poor activity and shows renal toxicity. Other treatment options include voriconazole (VRC) and posaconazole (PSC), which are indicated for patients with refractory disease or those who are intolerant to certain antifungal agents (15, 16). However, the use of a drug alone to treat systemic fusariosis has not been completely satisfactory (13). Combined therapy could be another option, although its usefulness for the treatment of fusariosis has not yet been investigated. The combination of AMB with VRC has demonstrated some beneficial effects in a murine infection by *Fusarium oxysporum* (19). Clinical data confirmed the usefulness of that combination since it led to clinical improvement before neutropenia was reduced in two clinical cases (9, 18). We have evaluated the activity of triazoles (PSC and VRC) in combination with AMB in a murine model of systemic infection by *Fusarium oxysporum*. This species is the second most frequently reported cause of human fusariosis (1).

Two isolates of *F. oxysporum*, FMR 5205 and FMR 10281, were used in this study. They were subcultured on potato dextrose broth at 30°C for 5 days. The in vitro susceptibilities of both strains were tested by a microdilution reference method (6). Antifungal interactions were assessed by a checkerboard microdilution method (7). The fractional inhibitory concentration index (FICI) was used to classify drug interactions (10). For all of the drugs tested and their combinations, we used a MIC-0 (100% inhibition of growth) endpoint criterion.

Male OF1 mice were immunosuppressed with a single intraperitoneal injection of 200 mg of cyclophosphamide/kg of body weight plus a single intravenous injection of 150 mg of 5-fluorouracil/kg 1 day before challenge (20). The procedure standards were approved by the Animal Welfare Committee of the Rovira i Virgili University.

Mice were challenged with $1 \times 10^7$ CFU in 0.2 ml of sterile saline injected into the lateral tail vein. Preliminary experiments demonstrated that this inoculum was appropriate for producing an acute infection, with 90% of the animals dying within 11 days (data not shown). Groups of 20 mice were established for each strain and each treatment. Ten mice were randomly chosen for survival, and the other 10 were used for tissue burden studies.

The different groups were treated once daily as follows. AMB at 3 mg/kg of body weight/dose was given intraperitoneally, and VRC at 60 mg/kg and PSC at 100 mg/kg were both given orally. In addition, AMB was combined with VRC or PSC at the same doses described above. All treatments began 1 day after challenge, and the therapy lasted 10 days. From 3 days prior to infection, the mice that received VRC were given 50% grapefruit juice in place of water (5). Mouse survival was evaluated daily for 20 days after challenge. For tissue burden studies, mice were euthanized on day 6 of treatment and the spleens and kidneys were aseptically removed; the entire organs were homogenized in 1 ml of sterile saline. Serial 10-fold dilutions of the homogenates were plated on potato dextrose agar and incubated for 48 h at 30°C.

Mean survival times were estimated by the Kaplan-Meier method and compared among groups by using the log rank test. CFU counts were analyzed by the Mann-Whitney U test. SPSS version 15.0.1 and GraphPad Prism version 4.0 for Windows were used. A $P$ value of $\leq 0.05$ was considered statistically significant.

The in vitro activities of the drugs tested were very poor, and the combinations showed indifferent interactions (Table 1).

The monotherapies exerted very poor in vivo efficacy. Only PSC was able to prolong the survival of mice infected with strain FMR 10281. The combination of AMB with VRC only improved the survival of mice infected with strain FMR 10281. However, the combination of AMB with PSC prolonged the survival of mice infected with either strain (Fig. 1). AMB reduced significantly the fungal burdens of both strains only in spleen tissue. VRC reduced the fungal burden of strain FMR 5205 only in kidney tissue. PSC significantly reduced the fungal loads of both strains in spleen tissue and the load of strain...
FMR 5205 only in kidney tissue. The combination of AMB plus VRC was able to reduce the spleen and kidney tissue burdens only of strain FMR 5205. Finally, the combination of AMB plus PSC reduced significantly the fungal loads of both strains in spleen tissue and the load of strain FMR 5205 in kidney tissue with respect to the control group and the mono-therapies (Fig. 2).

The lack of efficacy of AMB and VRC observed in our study agrees with clinical data, where approximately 55 to 70% of the patients of fusariosis receiving such drugs died (13). However, in our study, PSC was able to reduce the fungal load in the spleen, the target organ in this experimental model. In a study of salvage treatment for invasive fusariosis, PSC showed an overall efficacy of 48% in patients with neutropenia, although the species of Fusarium involved in these cases were not determined (17). Although, in general, PSC shows high MICs against all of the fusaria tested, this drug is apparently more active against F. oxysporum than against F. solani (1, 21).

Combined therapy with AMB and triazoles appears to be a promising alternative against fusariosis. However, up to now there were no data regarding the use of AMB plus PSC in experimental fusariosis. In our study, despite the high doses of both

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (µg/ml)</th>
<th>AMB</th>
<th>VRC</th>
<th>PSC</th>
<th>AMB + VRC</th>
<th>MICs (µg/ml)</th>
<th>FICI</th>
<th>Effect</th>
<th>AMB + PSC</th>
<th>MICs (µg/ml)</th>
<th>FICI</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMR 5205</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2/4</td>
<td>2</td>
<td>2/2</td>
<td>1</td>
<td>I</td>
<td>2</td>
<td>2/2</td>
<td>1</td>
<td>I</td>
</tr>
<tr>
<td>FMR 10281</td>
<td>4</td>
<td>16</td>
<td>&gt;16</td>
<td>4/16</td>
<td>2</td>
<td>4/32</td>
<td>2</td>
<td>1</td>
<td>4/32</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* I, indifference.

![Graph](image-url)
drugs used, the results were only modest, although the combination was more effective than AMB plus VRC. By contrast, Lewis et al. (11) reported a lack of efficacy of AMB plus PSC in a case of human fusariosis. However, according to those authors, the failure resulted from the subtherapeutic PSC levels achieved in serum due to the patient’s extremely poor diet.

In conclusion, the combination of AMB with PSC could be an alternative therapy for *F. oxysporum* infections. Further studies are needed to ascertain the real clinical relevance of this combination.

This work was supported by a grant from the Fondo de Investigaciones sanitarias of the Ministerio de Sanidad y Consumo of Spain (PI 050031).

REFERENCES


![FIG. 2. Effects of antifungal treatments on colony counts of *F. oxysporum* strains FMR 5205 (A) and FMR 10281 (B) in the kidneys and spleens of infected mice. AMB was used at a dose of 3 mg/kg. VRC was used at 60 mg/kg. PSC was used at 100 mg/kg. *a*, *P* < 0.05 versus the control. *b*, *P* < 0.05 versus all treatments. The horizontal lines in the scatterplots indicate mean values.](http://aac.asm.org/)

VOL. 53, 2009 AZOLES AGAINST *FUSARIUM OXYSPORUM* 1707

Downloaded from http://aac.asm.org/ on July 9, 2017 by guest