Combined Therapies in a Murine Model of Blastoschizomycosis

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Received 6 February 2007/Returned for modification 23 March 2007/Accepted 6 April 2007

In a murine model of blastoschizomycosis, amphotericin B combined with micafungin, flucytosine or voriconazole did not improve the efficacy of fluconazole. However, such combinations can constitute therapeutic options for those cases where fluconazole fails.

The yeast Blastoschizomyces capitatus (formerly known as Geotrichum capitatum and Trichosporon capitatum) causes severe systemic infections in immunocompromised patients, mainly in those with hematological malignancies (6, 12, 13) but also in immunocompetent patients (14, 21). Up to now, the use of a single drug to treat systemic blastoschizomycosis has not been completely satisfactory (1, 2, 3, 12, 16). Despite the administration of amphotericin B (AMB) or fluconazole (FLC), this infection is almost always fatal (2, 3). However, combined therapy has not yet been investigated. Previously we observed a low efficacy of all antifungals tested, with the exception of FLC (18). Here, we have evaluated the activity of AMB combined with micafungin (MFG), flucytosine (5FC), or voriconazole (VRC).

Two clinical strains of B. capitatus, IHEM 5666 from a blood culture of a patient with leukemia and IHEM 16105 from sputum of a patient with cystic fibrosis of the pancreas, were used. The isolates were stored at −80°C, and prior to testing they were subcultured on Sabouraud dextrose agar (SDA) at 35°C. The resulting suspensions were adjusted to the desired inoculum based on hemocytometer counts and by serial plating on SDA to confirm viability.

The in vitro antifungal susceptibilities of the strains were tested by a reference microdilution method (15). The interactions of the drugs were assessed by a checkerboard method (5, 11). For all the drugs and their combinations we used a MIC-0 end point criterion, defined as the lowest concentration resulting in 100% inhibition of growth.

Male OF1 mice weighing 30 g (Charles River, Criffa S.A., Barcelona, Spain) were used in this study. Animals were housed under standard conditions. All animal care procedures were supervised and approved by the Universitat Rovira i Virgili Animal Welfare Committee. Animals were immunodepressed by intraperitoneal administration of a single dose of 200 mg of cyclophosphamide per kg plus intravenous administration of 150 mg of 5-fluorouracil per kg on the day of infection (9). Mice were challenged with $2 \times 10^6$ CFU in 0.2 ml into the lateral tail vein. Groups of 15 mice were established for each strain and each treatment. Ten mice were used for survival and five for tissue burden studies, with the latter being identified before the study started. The different groups were treated with the following: FLC (Pfizer Inc., Madrid, Spain) at doses of 40 mg/kg given orally twice a day (80 mg/kg/day); AMB (Fungizona) at 1 mg/kg/day given intraperitoneally; VRC (Vfend) at 40 mg/kg/day given orally; 5-FC (Sigma-Aldrich, St Louis, MO) at 60 mg/kg/day dissolved in the sole source of drinking water (8, 10); MFG (Astellas Pharma Inc., Tokyo, Japan) at 5 mg/kg given subcutaneously twice a day (10 mg/kg/day); and the combinations AMB plus MFG, AMB plus VRC, and AMB plus 5-FC at the same doses described above. All treatments began 24 h after challenge, and the therapy lasted for 6 days. From 3 days prior to infection, the mice receiving VRC were given grapefruit juice in place of water (8, 19, 20). Mouse survival was evaluated daily for 30 days after challenge. One day after the treatment was finished, the liver, spleen, and kidneys were aseptically removed and were homogenized in 1 ml of sterile saline. Serial 10-fold dilutions of the homogenates were plated on SDA and incubated 48 h at 35°C. The numbers of CFU per gram of tissue were calculated.

<table>
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<tr>
<th>Strain*</th>
<th>MIC, μg/ml (FICI$^b$)</th>
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<tbody>
<tr>
<td></td>
<td>FLC</td>
</tr>
<tr>
<td>IHEM 16105</td>
<td>8</td>
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<tr>
<td>IHEM 5666</td>
<td>16</td>
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$^a$ IHEM, Scientific Institute of Public Health, Louis Pasteur Institute, Brussels, Belgium.

$^b$ FICI, fractional inhibitory concentration index ($\leq$0.5, synergistic; >0.5 and $\leq$4, indifferent; >4, antagonistic) (11).

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Published ahead of print on 23 April 2007.
Mean survival time (MST) was 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. The in vitro results are shown in Table 1. Tables 2 and 3 show the effects of the different treatments on mouse survival and tissue burden. In general, monotherapies showed results similar to those obtained in a previous study (18), with no significant differences, which proved the reproducibility of the model. Once again, FLC at 80 mg/kg/day showed the highest efficacy. The combined treatments were unable to improve the efficacy of FLC in survival prolongation and tissue burden reduction. For strain IHEM 16105, all the treatments prolonged survival with respect to that of the control group, with the exception of MFG alone and AMB combined with VRC (P = 0.4957 and P = 0.3897, respectively). Only the combination of AMB with MFG prolonged the MST versus the respective monotherapies.

For strain IHEM 5666, all the treatments prolonged the MST with respect to that of the control group, with the exceptions of MFG and 5-FC given alone (P = 0.5215 and P = 0.1259, respectively). The combinations of AMB plus VRC and AMB plus 5-FC prolonged the MST with respect to the monotherapies.

For strain IHEM 16105, all the treatments reduced significantly the fungal load versus that in controls, with the exception of MFG (Table 3). The three combinations tested were also able to reduce significantly the fungal burden with respect to the monotherapies in the three organs studied, with the exception of the combination of AMB with VRC in spleen. For strain IHEM 5666, MFG and 5-FC showed no efficacy in tissue burden, reduction and AMB and VRC reduced the fungal load in only two of the three organs tested. FLC was able to reduce significantly the fungal load only with respect to the other single drugs. The three combinations studied were able to reduce significantly the tissue burden versus their respective monotherapies in the three organs tested, but not with respect to FLC.

This is the first time that combined therapy was tested in experimental B. capitatus infections. In general, the combinations tested showed good results, and although they could not improve the efficacy of FLC, they were always more effective with respect to the control group and in almost all cases were better than their respective monotherapies. It is well known that azole-resistant isolates are increasing among clinically relevant yeasts (4, 7, 17), and in the case of B. capitatus infections, several cases of FLC failure have been documented (2, 3, 16). The combinations tested here can offer alternative therapies in infections caused by azole-resistant isolates, as has been suggested by Christakis et al. (3). Further studies are needed to ascertain the real clinical relevance of these combinations.
This work was supported by a grant from Fondo de Investigaciones Sanitarias from the Ministerio de Sanidad y Consumo of Spain (PI 050031).

REFERENCES