Alternatives to amphotericin B for Candida rugosa infection

Steve Hernandez1*, Gloria M. Gonzalez 1,2, Dora I. McCarthy 3, Arnaldo Lopes Colombo 4, Laura K. Najvar 1, Rosie Bocanegera 1 and John R. Graybill 1

1Department of Medicine, Division of Infectious Diseases, and 2Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; 3Departamento de Microbiologia, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Mexico; 4Division of Infectious Diseases, Universidade Federal de São Paulo, São Paulo, Brazil

Received 28 August 2003; returned 5 November 2003; revised 18 May 2004; accepted 25 May 2004

Objective: Amphotericin B failure is frequently seen in patients with candidaemia caused by Candida rugosa. We evaluated amphotericin B, fluconazole, posaconazole and voriconazole as alternative treatments against infection in mice with two isolates of C. rugosa.

Methods: Neutropenic mice were inoculated intravenously with C. rugosa. Amphotericin B, fluconazole, posaconazole and voriconazole were administered for 7 days after infection. Efficacy of the antifungal treatment was assessed by survival and tissue burden of C. rugosa.

Results: All of the four drugs significantly prolonged survival over controls. With both isolates, kidney counts were reduced significantly below controls for amphotericin B, fluconazole and posaconazole. However, voriconazole was less effective than the other antifungals.

Conclusion: Despite poor clinical response to amphotericin B, in vivo data indicate that amphotericin B increases organ clearance and survival over untreated controls. However, although voriconazole improved survival over controls, increased tissue clearance was not seen. This discrepancy may be caused by rapid clearance of voriconazole in mice. These studies suggest fluconazole, posaconazole or voriconazole may be useful alternatives to amphotericin B in therapy of C. rugosa infection.

Keywords: murine model, antifungals, C. rugosa

Introduction

Haematogenous candidiasis is the fourth most common cause of nosocomial bloodstream infections in the USA.1 These infections are generally difficult to diagnose and have an attributable mortality rate of 38%.

Candida albicans is still considered the most common agent of haematogenous candidiasis, but the emergence of non-albicans species is clearly a concern. The Candida species causing invasive fungal infections appear to vary in different regions of the world. In the USA, Canada and some European countries, Candida glabrata is a major agent of candidaemia. In contrast, Candida parapsilosis and Candida tropicalis account for most non-albicans Candida systemic infections documented in Latin America.2

The emergence of non-albicans species as aetiological agents of invasive infections has major clinical implications, given the decreased susceptibility to some of the currently available antifungal agents.3 Recently, a cluster of Candida rugosa candidaemia involving six patients hospitalized in an intensive care unit was reported in Brazil. During this outbreak, four out of six patients died despite receiving antifungal treatment with amphotericin B.4 Dubé et al.5 reported 15 episodes of candidaemia due to C. rugosa in a burn unit where topical nystatin use was associated with the increased fungaemia caused by nystatin-resistant C. rugosa isolates. Subsequent to these reports, suggesting that C. rugosa infection may be refractory to polyene antifungal drugs, we evaluated other classes of antifungal drugs for efficacy against C. rugosa in a mouse model. In the present studies, we compared amphotericin B with fluconazole,
Materials and methods

Pathogens

Animals were challenged with C. rugosa strains 1 and 2 isolated from failed patients treated with amphotericin B during an outbreak of candidaemia reported in São Paulo, Brazil.4 The yeast identification of the isolates was based on microscopic morphology of the isolates on corn-meal agar, and the biochemical profile obtained by using the commercial system ID-32C (BioMérieux, France). The susceptibility profile of the two isolates against amphotericin B, fluconazole, voriconazole and posaconazole, was obtained by using the NCCLS microdilution method.6

Animals

All animal research procedures were approved by the Institutional Animal Care and Use Committee at The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA. Outbred male ICR mice were obtained from Harlan Sprague-Dawley. Mice weighed ~30–35 g and had access to food and water ad libitum. Mice were housed four to a cage. Each treatment group consisted of 8–12 mice. C. rugosa is not lethal in immunocompetent mice. Since C. rugosa fungemia may occur in neutropenic patients, mice were immunocompromised with cyclophosphamide at 200 mg/kg intraperitoneally (ip) administered the day before and the day after infection with C. rugosa. Cyclophosphamide reduces the absolute neutrophil count to < 100/mm³ >10 days.

Infection

C. rugosa clinical isolates 1 and 2 were each cultured on Sabouraud dextrose agar plates, 2 days before infection. On the day of infection, the colonies were scraped off with a sterile loop. C. rugosa was then centrifuged in sterile isotonic saline at 2000 rpm for 15 min. Organisms were washed three times. The stock suspension was counted in a haemocytometer. This was diluted in sterile saline to a final concentration of viable inoculum at 2.6 × 10⁴–5.6 × 10⁶ cfu/mouse. Neutropenic mice were inoculated intravenously (iv), using a 0.2 mL volume. The inoculum of viable organisms was determined by serial dilution quantitative cultures.

Antifungal agents

Amphotericin B was obtained commercially and dissolved with 5% dextrose. Fluconazole for iv administration was purchased commercially and diluted in sterile water. Posaconazole was obtained from Schering Plough Research Institute in the oral liquid formulation commercially and diluted in sterile water. Voriconazole was purchased commercially and diluted in sterile water. Fluconazole for iv administration was purchased commercially and diluted in sterile water. Voriconazole was purchased commercially and diluted in sterile water.

Treatment

Therapy began 1 day after infection and continued for 10 days in the survival studies for isolates 1 and 2. The mice were monitored for a total of 15 days. For tissue burden studies, treatment began 1 day after infection and continued for 7 days post-infection. Posaconazole was given at 1 or 20 mg/kg/day orally by gavage in a volume of 0.2 mL. Fluconazole was given orally by gavage at 0.5 or 10 mg/kg twice daily in 0.2 mL/dose. Amphotericin B was given at 0.3 or 1 mg/kg/day ip in a 0.2 mL volume. Voriconazole was also given orally by gavage in a 0.2 mL volume at 0.5 or 10 mg/kg twice daily. In addition, the voriconazole group received grapefruit juice orally by gavage in a volume of 0.2 mL. In this study, there were two control groups. In one control group, sterile water (0.2 mL) was orally administered once daily by gavage. The second control group (for the voriconazole recipients) received grapefruit juice (0.2 mL) orally by gavage once daily, beginning 3 days before voriconazole therapy was started. Kidneys and spleens were cultured from mice succumbing before day 8 and from survivors on day 8. Organs were removed, weighed and homogenized in 2 mL of saline containing piperacillin and amikacin at 60 mg/L, to suppress bacterial growth. Tissue burden was measured by serial 10-fold dilution colony counting. In the situation of low initial counts, the entire homogenate was plated to determine complete clearance of C. rugosa. All mice were included in determination of median tissue burden, which was expressed as log₁₀ cfu/g weight of organ.

Statistical analysis

For survival studies, the Logrank Test was used. For tissue burden studies, the Mann–Whitney test was used. P ≤ 0.05 determined significance of comparisons.

Results

MICs

The MIC values in Table 1 indicate that all three triazoles were active against both of the C. rugosa isolates.4 Amphotericin B was also active against both isolates.

Outcome in mice

As shown in Table 2, for isolate 1, groups of mice inoculated with an average of 1.1 × 10⁶ cfu/mouse and treated with posaconazole (1, 20 mg/kg), fluconazole (0.5, 10 mg/kg twice daily), amphotericin B (0.3, 1 mg/kg) or voriconazole (0.5, 10 mg/kg twice daily), all survived significantly longer than controls, with P values of ≤ 0.0167 compared with controls. This clearly showed that all four antifungal drugs were effective in prolonging survival. Table 3 shows two tissue burden studies conducted with isolate 1. In the first study, mice, inoculated with 3 × 10⁶ cfu/mouse and treated with posaconazole (20 mg/kg), fluconazole (10 mg/kg twice daily) and amphotericin B (1 mg/kg), had significantly lower kidney burdens than controls (P ≤ 0.0003). In contrast, voriconazole (10 mg/kg twice daily) did not reduce kidney burden counts when compared with a grapefruit juice control (P = 0.1605). Furthermore, the grapefruit juice control was very similar to the untreated control group.

Table 1. Minimum inhibitory concentrations for Candida rugosa isolates 1 and 2 at 24/48 h incubation

<table>
<thead>
<tr>
<th></th>
<th>Isolate 1 24/48 h (mg/L)</th>
<th>Isolate 2 24/48 h (mg/L)</th>
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<tbody>
<tr>
<td>AmB</td>
<td>0.5/0.5</td>
<td>0.25/0.25</td>
</tr>
<tr>
<td>POS</td>
<td>≤ 0.015/≤ 0.015</td>
<td>≤ 0.015/≤ 0.015</td>
</tr>
<tr>
<td>FLU</td>
<td>0.25/2</td>
<td>0.5/1</td>
</tr>
<tr>
<td>VORI</td>
<td>≤ 0.015/≤ 0.015</td>
<td>≤ 0.015/≤ 0.015</td>
</tr>
</tbody>
</table>

AmB, amphotericin B; POS, posaconazole; FLU, fluconazole; VORI, voriconazole.
In the spleen, only amphotericin B (1 mg/kg) significantly reduced tissue burden counts. In the second study with isolate 1, inoculated at 2.4 x 10^6 cfu/mouse, all treated groups significantly reduced kidney counts when compared with appropriate controls (Table 3). Also, amphotericin B (1 mg/kg) again was the only treatment with both isolates. For isolate 2, the azoles, especially voriconazole, were highly effective in the spleens. In contrast, posaconazole (20 mg/kg) or fluconazole (10 mg/kg twice daily) did not significantly reduce counts in kidneys or spleens. In the second study with isolate 2, inoculated at 5.6 x 10^6 cfu/mouse and treated with posaconazole (20 mg/kg), fluconazole (10 mg/kg twice daily) or amphotericin B (1 mg/kg), the kidney colony counts were significantly below the control group. Also, voriconazole (10 mg/kg twice daily) showed no significant differences when compared with the grapefruit juice control group. Posaconazole (20 mg/kg) and amphotericin B (1 mg/kg) were superior when compared with the spleen control group. Voriconazole (10 mg/kg twice daily) and fluconazole (10 mg/kg twice daily) showed no significance in decreasing spleen counts.

**Discussion**

Despite amphotericin B failure of the two patients in the *C. rugosa* outbreak, the *C. rugosa* isolates were inhibited by amphotericin B in vitro. Amphotericin B MIC values >0.8—1.0 mg/L tend to be associated with clinical failure. However, in this study, mice responded well to therapy with amphotericin B in that survival was prolonged, and both the kidneys and spleen showed reduced fungal burden. Therefore, there is discrepancy between clinical experience and animal response.

We were encouraged by the results with both fluconazole and posaconazole. Both drugs in modest to low doses significantly prolonged survival and decreased renal tissue counts for infection with both isolates. For isolate 2, the azoles, especially posaconazole, were highly effective in the spleens. In contrast, voriconazole was equally effective in prolonging survival, but significantly reduced organ fungal burden in only one of four situations (i.e. the kidney for isolate 1).

Voriconazole is rapidly metabolized in mice. This rapid clearance may be due to intestinal mucosal cytochrome activity. This results in metabolism of voriconazole to less active metabolites...
before it can be absorbed and distributed haematogenously. In an effort to circumvent this problem, we used grapefruit juice as an inhibitor of these mucosal cytochrome systems. This delays clearance of the drug, allowing concentration of voriconazole to persist in the bloodstream for a longer period. 9,10 However, an even longer tissue dwelling time may be required to lower tissue burden of fungi in mice. The present studies demonstrated that fluconazole, posaconazole and voriconazole all offer effective alternatives to amphotericin B for treatment of C. rugosa infection. Posaconazole appears to be the most potent of the three triazoles tested.

References


Table 4. Isolate 2 fungal burdens in the kidneys/spleen of mice infected with Candida rugosa, median (range) log10 cfu/g weight of organ

<table>
<thead>
<tr>
<th>Treatment group (mg/kg)</th>
<th>Kidneys; inoculum 2.6×10^4 cfu/mouse; study 3</th>
<th>Spleen; inoculum 2.6×10^4 cfu/mouse; study 3</th>
<th>Kidneys; inoculum 5.6×10^6 cfu/mouse; study 4</th>
<th>Spleen; inoculum 5.6×10^6 cfu/mouse; study 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls n = 8</td>
<td>5.8 (4.1–7.5)</td>
<td>3.3 (2.2–3.7)</td>
<td>5.5 (4.1–7.0)</td>
<td>2.8 (2.0–3.9)</td>
</tr>
<tr>
<td>POS 20 n = 8</td>
<td>4.4 (1.9–6.0)*</td>
<td>1.1 (0.6–1.9)*</td>
<td>0.9 (0.5–1.7)*</td>
<td>2.3 (0.7–2.8)*</td>
</tr>
<tr>
<td>FLU 10 twice daily n = 8</td>
<td>2.7 (2.5–4.2)*</td>
<td>1.2 (0.6–1.5)*</td>
<td>3.0 (2.0–3.5)*</td>
<td>2.3 (1.5–3.2)*</td>
</tr>
<tr>
<td>AmB 1 n = 8</td>
<td>4.4 (3.0–5.0)*</td>
<td>1.5 (0.6–2.0)*</td>
<td>3.9 (2.4–4.7)*</td>
<td>0.5 (0.4–1.1)*</td>
</tr>
<tr>
<td>Controls with Gj n = 8</td>
<td>6.0 (2.6–7.3)</td>
<td>2.2 (1.9–3.8)</td>
<td>4.8 (4.3–5.1)</td>
<td>2.1 (1.4–2.6)</td>
</tr>
<tr>
<td>VORI 10 twice daily n = 8</td>
<td>5.3 (2.8–6.2)</td>
<td>2.1 (0.8–3.3)</td>
<td>5.3 (2.1–5.7)</td>
<td>2.4 (1.8–3.5)</td>
</tr>
</tbody>
</table>

POS, posaconazole; FLU, fluconazole; AmB, amphotericin B; VORI, voriconazole; Gj, grapefruit juice; n, number of mice/group.

*P < 0.05, significant reduction compared with controls.