Efficacy of Ravuconazole in Treatment of Mucosal Candidosis in SCID Mice

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A model of orogastric candidosis in SCID mice, which mimics disease seen in AIDS patients, was used to evaluate ravuconazole in comparison with fluconazole for treatment. Mice were infected orally with Candida albicans and received either no treatment or oral treatment once daily for 12 days with 1, 5, or 25 mg of ravuconazole per kg of body weight per day, 5 or 25 mg of fluconazole per kg per day, or diluent (10% dimethyl sulfoxide in 0.5% carboxymethyl cellulose). The numbers of C. albicans CFU in the esophagus, stomach, small intestine, and cecum on day 25 in mice given no treatment and diluent were equivalent. Both doses of fluconazole significantly reduced numbers of CFU in all four tissues but were equivalent to each other. Ravuconazole showed dose-responsive improvement of clearance of CFU. Ravuconazole at 25 mg/kg was superior in reduction of numbers of CFU in all tissues to controls or 25 mg of fluconazole per kg and to other regimens in at least three tissues. Fluconazole at 25 mg/kg cured no infection in any tissue, whereas 25 mg of ravuconazole/kg cleared infection in all tissues from 50% of mice. Ravuconazole has good efficacy and the potential to cure mucosal candidosis in the absence of a functional immune response.

Mucosal candidosis is often encountered in a variety of patient populations, particularly those that are immunocompromised. Among the most frequently infected persons are those with human immunodeficiency virus or AIDS (1). Treatment of this infection has become more difficult in the past few years with the rising incidence of fluconazole-resistant strains of Candida albicans. Because of this rise in resistance, other treatments, such as oral amphotericin B, have been used (4). However, new therapeutic options are needed.

A murine model of persistent colonization of the gastrointestinal mucosa established in severe combined immunodeficient (SCID) mice has been described (2, 3, 8, 12). This model closely mimics disease found in AIDS patients. The mucosal surfaces are heavily colonized, but systemic dissemination rarely occurs, in contrast to chemotherapy patients receiving agents, such as 5-fluorouracil or methotrexate, that damage the surfaces are heavily colonized, but systemic dissemination consequently to chemotherapy patients receiving agents, such as 5-fluorouracil or methotrexate, that damage the intestinal mucosa, allowing translocation of C. albicans and subsequent dissemination. Because of the similarity to human infection in AIDS patients, the model is useful for the study of new antifungal agents.

Ravuconazole (BMS207147) is a novel triazole currently under development by Bristol-Myers Squibb (Princeton, N.J.) (7). This azole has been shown to have both in vitro and in vivo efficacy against several fungal genera (6, 7, 10, 11, 14, 15). In vitro, ravuconazole has been shown to have good activity against fluconazole- or itraconazole-resistant isolates of C. albicans and Candida dubliniensis (14, 15). Ravuconazole has recently been shown to have good in vitro activity against the endemic pathogens and was lethal at concentrations at or near the MIC (D. A. Stevens, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1514, 1999). In addition, this compound has been shown to reduce numbers of CFU from oral swabs in a rat model of oral candidosis (10). The aim of the present study was to examine the efficacy of orally administered ravuconazole as a treatment for mucosal candidosis in the setting of severe immunodeficiency. Our results, obtained with a model of orogastric candidosis in SCID mice, show that ravuconazole is an effective therapy.

(Materials and Methods)

Inoculum preparation. To prepare the inoculum, C. albicans isolate 5 was revived from storage under water and streaked for isolation on Sabouraud dextrose agar (SDA) plates. The plates were incubated at 35°C for 48 h. The organism was inoculated into sterile bottles containing broth, incubated for 48 h at 35°C in a gyratory shaker (2), and harvested by centrifugation at 1,000 x g. The yeast cells were washed once by centrifugation with saline and then resuspended in saline. The cells were quantified by a hemacytometer, and dilutions were made in sterile water. The final inoculum was 5 x 10^7 CFU per ml of drinking water. Inoculum viability was determined by plating serial 10-fold dilutions onto SDA (with 50 mg of chloramphenicol per liter) plates. The plates were incubated overnight at 35°C, and numbers of CFU were determined.

Infection of mice. The model of orogastric candidosis established was similar to that described previously (2, 3, 8, 12). In brief, 6-week-old female C.B-17/IcrTac-scid (Taconis Farms, Germantown, N.Y.) mice were used. Because these are SCID animals, with no B- or T-cell-mediated immunity, they were maintained in sterile microisolation cages and given sterilized food and sterilized drinking water ad libitum. All cages were changed twice weekly, and all manipulations of the animals were done in a laminar flow hood under aseptic conditions. Mucosal infection was established by administration of the organism in the drinking water. On the day of infection, the water bottles were removed 8 h prior to replacement with a suspension of 5 x 10^7 CFU per ml of C. albicans 5. The mice were allowed to drink from this suspension for 24 h, at which time the inoculum suspension was removed and replaced with sterile water. (This is considered day 0.) Animal experiments were done under the guidelines and approval of the Institutional Animal Care and Use Committee of the California Institute for Medical Research and Department of Medicine, Division of Infectious Diseases, Santa Clara Valley Medical Center, San Jose, California 95128-2699. Phone: (408) 998-4557. Fax: (408) 998-2723. E-mail: clemons@cimr.org.

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TABLE 1. Recovery of C. albicans from the gastrointestinal tract

<table>
<thead>
<tr>
<th>Treatment (concn [mg/kg])</th>
<th>No. of mice free of infection (n = 10)</th>
<th>Log_{10} geometric mean CFU per tissue [95% CI]</th>
<th>(no. of free of infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Esophagus</td>
<td>Stomach</td>
<td>Small intestine</td>
</tr>
<tr>
<td>1. Untreated</td>
<td>0</td>
<td>2.01 [1.4-2.6] (0)</td>
<td>4.29 [3.7-4.8] (0)</td>
</tr>
<tr>
<td>2. Ravuconazole diluent</td>
<td>1.67 [1.0-2.4] (1)</td>
<td>3.97 [3.5-4.4] (0)</td>
<td>2.40 [2.1-2.7] (0)</td>
</tr>
<tr>
<td>3. Fluconazole (5)</td>
<td>0.450 [0.0-0.9] (6)</td>
<td>2.14 [1.3-2.9] (0)</td>
<td>1.04 [0.4-1.7] (4)</td>
</tr>
<tr>
<td>4. Fluconazole (25)</td>
<td>0.400 [0.1-0.7] (5)</td>
<td>1.79 [1.4-2.2] (0)</td>
<td>1.02 [0.8-1.3] (4)</td>
</tr>
<tr>
<td>5. Fluconazole (1)</td>
<td>1.36 [0.7-2.1] (2)</td>
<td>3.45 [3.0-3.9] (0)</td>
<td>2.14 [1.7-2.5] (0)</td>
</tr>
<tr>
<td>6. Ravuconazole (5)</td>
<td>0.990 [0.03-1.9] (5)</td>
<td>1.83 [1.1-2.5] (0)</td>
<td>1.49 [0.6-2.4] (3)</td>
</tr>
<tr>
<td>7. Ravuconazole (25)</td>
<td>0.00 (10)</td>
<td>0.17 [0.0-0.4] (8)</td>
<td>0.24 [0.0-0.4] (8)</td>
</tr>
</tbody>
</table>

* a P < 0.05 versus group 1, < 0.01 vs. group 2, < 0.001 vs. group 5.  
* b P < 0.05 versus group 1, < 0.01 vs. group 2 or 5.  
* c P < 0.05 versus group 2, < 0.01 vs. group 1 or 5.  
* d P < 0.05 versus group 1 or 2, < 0.01 vs. group 5.  
* e P < 0.05 versus group 1, 2 or 5.  
* f P < 0.05 versus group 2 or 5, < 0.01 vs. group 1.  
* g P < 0.001 vs. group 1.  
* h P < 0.001 vs. group 4 or 6.  
* i P < 0.05 vs. group 3, 4 or 6.  
* j P < 0.001 vs. group 3 or 6, < 0.05 vs. group 4.  
* k P < 0.05 vs. group 3 or 4, < 0.01 vs. group 6.  
* l CI, confidence interval.

Institute for Medical Research, San Jose, using the guidelines of the Office of Laboratory Animal Welfare of the National Institutes of Health.

Treatment groups. Groups of 10 infected mice were treated with 5 or 25 mg of fluconazole per kg of body weight or with 1, 5, or 25 mg of ravuconazole (Bristol-Myers Squibb) per kg of body weight. Ten mice received no treatment (controls), and another 10 received the drug vehicle (0.5% carboxymethyl cellulose with 10% dimethyl sulfoxide [DMSO-CMC]) only. Therapy was initiated on day 12 of infection and was administered orally (0.1 ml) once daily for 12 consecutive days.

No animals died during the course of the study. On day 25, all mice were euthanized using CO₂ asphyxia. Standardized samples of the esophagus, stomach, small intestine, and cecum were aseptically removed (5). Each tissue sample was mechanically homogenized (Tissumizer; Tek Mar Co., Cincinnati, Ohio) in 5 ml of saline with 100 U of penicillin and 100 μg of streptomycin per ml. Serial dilutions of the homogenate were plated onto SDA with 50 mg of chloramphenicol per liter for quantitative determination of the number of yeasts in the tissue samples. All yeast burdens were expressed as the log_{10} number of viable yeasts. Statistical analyses of the comparative burdens of C. albicans recovered from the tissues in the various treatment groups were analyzed using a Mann-Whitney U test (GBSTAT version 6.0; Dynamic Microsystems, Silver Spring, Md.).

In vitro susceptibility. The in vitro activities of ravuconazole and fluconazole against the strain of C. albicans used in the in vivo model and five additional clinical isolates of C. albicans were tested by broth macrodilution using NCCLS methods (13, 16). Minimal fungicidal activities (≥96% killing) were ascertained by dilution on agar as described previously (9, 16).

RESULTS

In vitro activities. The MICs of both drugs for this strain proved to be difficult to ascertain by the NCCLS method because of clumping of the organism, which makes the tubes difficult to read. The MICs of fluconazole were assayed 9 times, and those of ravuconazole were assayed 10 times. The modal MICs were 2 and 0.25 μg/ml, respectively, and were within 1 tube dilution of the modal MIC in six assays for each drug, but the clumping could have led to difficulties in interpretation, even possibly yielding interpretations of resistance in two and four assays, respectively, because of the clumping. Interpretation difficulties did not occur with NCCLS broth microdilution methods (13), where the susceptibility to both drugs was confirmed (D. Bonner and J. Fung-Tomc, personal communication). The minimal fungicidal activities of both drugs were >32 μg/ml. To further examine the activity of ravuconazole, five additional random clinical isolates of C. albicans were tested. Each of these isolates was sensitive to fluconazole, with a MIC of ≤0.5 μg/ml, and to ravuconazole, with a MIC of ≤0.125 μg/ml.

In vivo activities. The results of our study indicate that animals either left untreated or given the DMSO-CMC diluent were, in all tissues examined, equally infected, with no significant differences in numbers of CFU (Table 1). Both dosages of fluconazole were significantly effective in reducing numbers of CFU recovered from all four tissues compared with controls (Table 1). However, fluconazole at 25 mg/kg was not significantly better in clearing infectious burdens than fluconazole at 5 mg/kg (Table 1). Ravuconazole proved to have dose-responsive efficacy (Table 1). At 1 mg/kg, it caused nonsignificant reduction of the numbers of CFU from the esophagus and cecum compared with both untreated and diluent-treated controls; this was also true for the small intestine compared with the DMSO-CMC-treated, but not the untreated, controls (Table 1). At 1 mg/kg, ravuconazole caused a significant reduction in numbers of CFU recovered from the stomach compared with no treatment. At a dosage of 5 mg/kg, it significantly reduced numbers of CFU from the esophagus, stomach, and cecum compared with no treatment and from the stomach and cecum compared with DMSO-CMC treatment. At 5 mg/kg it was equivalent in efficacy to controls in the small intestine. Ravuconazole at 25 mg/kg caused a significant reduction in numbers of CFU from all tissues compared with controls (Table 1). Ravuconazole proved to be more efficacious than fluconazole, with the 25-mg/kg dosage being superior to all other regimens (Table 1). Ravuconazole at 1 mg/kg was the least effective, with both 5 and 25 mg of fluconazole per kg and 25 mg of ravuconazole per kg being superior in all tissues; 5 mg of ravuconazole per kg was superior to 1 mg/kg in the stomach and cecum. Five mice were cleared of infection in all four tissues by the 25-mg/kg regimen of ravuconazole; 80% of these mice were free of infection in three tissues (Table 1). In comparison, fluconazole at 25 mg/kg cleared no mice of detectable...
infection and only five of esophageal infection (all other tissues were infected).

**DISCUSSION**

Mucosal candidosis is a common affliction of patients with severe immunosuppression. With the increasing prevalence of azole-resistant isolates of *C. albicans*, better therapeutic options are needed. The aim of the present study was to examine the efficacy of the new triazole ravuconazole in the treatment of mucosal candidosis in the setting of severe immunosuppression.

Overall, the results of our model showed that both triazoles were efficacious. Dose escalation of fluconazole from 5 to 25 mg/kg did not result in a significantly improved reduction of infectious burdens in any of the tissues sampled. This is similar to and extends our previous data showing no difference in efficacy between doses of 5 and 10 mg of fluconazole per kg (3). The reasons for the flatness of the response curve for fluconazole in the present study and the previous study (3) are unknown. However, it might be related to a reduced gastrointestinal absorption of the drug in the infected animals, which would lower concentrations of the compound in serum and tissue. Another possibility is that treatment in this model is partially topical (i.e., infection of the orogastric mucosa with the drugs administered orally) and that fluconazole in the form administered is not as effective topically.

In contrast to the results with fluconazole, ravuconazole showed a clear improvement in efficacy with dose escalation. Ravuconazole at 25 mg/kg was superior to fluconazole. This is also demonstrated by the number of tissues cleared of detectable infection: 50% of mice given ravuconazole at 25 mg/kg were free of detectable *C. albicans*, whereas no mice given 25 mg of fluconazole per kg cleared *C. albicans* in all four tissues studied. Given the apparent flatness of the response curve of fluconazole in this model, it seems that an improvement in efficacy is not possible, even with substantial increases in the amount of drug administered. It seems unlikely that the half-life of fluconazole versus that of ravuconazole in mice contributes to the differences in efficacy, since in normal mice they are both about 4 h (11). However, pharmacokinetic studies with infected animals using our model system would be needed to identify any differences that could account for the differential efficacies.

No toxicities were observed either during dosing or at necropsy of the ravuconazole-treated mice. Thus, 25 mg of ravuconazole per kg per day was well tolerated. However, efficacy might be improved using a diluent that improves the solubility of the ravuconazole, since it was noted that in the DMSO-CMC diluent all regimens of ravuconazole were colloidal suspensions rather than clear solutions. However, it is possible that the colloidal nature of the drug was beneficial in this setting of mucosal infection.

Overall, the 5-mg/kg doses of ravuconazole and fluconazole were equally efficacious, but ravuconazole might be considered fivefold more effective, since 5 mg of ravuconazole per kg was equivalent to 25 mg of fluconazole per kg in the treatment of orogastric candidosis. A 25-mg/kg dose of ravuconazole was superior to the most effective dose of fluconazole, 5 or 25 mg/kg. Similarly, the comparative efficacies of the two drugs have been examined in pulmonary and systemic models of infection with *C. albicans* (10, 11). In those studies, ravuconazole was found to be as effective as fluconazole and more effective than itraconazole in a systemic model of candidosis (11). In studies of pulmonary candidosis, Hata et al. (10) reported that ravuconazole significantly reduced the numbers of fluconazole-sensitive *C. albicans* organisms recovered from the lungs and was equal to or better than fluconazole and itraconazole. Against fluconazole-resistant *C. albicans*, the same authors found ravuconazole to be superior to both of the other azoles (10). However, no indications were given that any animals were free of detectable infection (10).

Our results substantiate and extend a previous report of the efficacy of ravuconazole in a rat model of oral candidosis (10). In that study, normal immunocompetent rats were used and the course of treatment began after only 2 days of infection (10). Ravuconazole at 1 or 4 mg/kg was found to be efficacious and better than itraconazole but not better than fluconazole, and no indication was given that any animals had been cleared of *C. albicans* in the oral cavity (10). Similarly, in our model, ravuconazole was efficacious in reducing mucosal infection and appeared to be superior to fluconazole. Furthermore, the activity of ravuconazole was demonstrated in the setting of no acquired immune response from the host. In contrast to the rat study (10), we found that prolonged treatment at the highest dose, 25 mg/kg, resulted in clearance of *C. albicans*, whereas fluconazole did not.

Presumably, the concentrations of drug attained locally are sufficient to reduce or eradicate the infection. The majority of isolates of *C. albicans* tested are exquisitely susceptible to ravuconazole (5, 6, 15). We also found this result with the five additional clinical isolates we tested. However, fluconazole-resistant (MIC of >32 μg/ml) as well as fluconazole-susceptible dose-dependent isolates (MIC of 16 to 32 μg/ml) have been reported to be less susceptible to ravuconazole, with MIC ranges of 0.007 to 16 μg/ml (5).

In conclusion, we have clearly demonstrated the efficacy of orally administered ravuconazole for the treatment of mucosal candidosis. Additional studies could be designed to look for increased efficacy using a different diluent, dose escalation, or longer durations of treatment to determine whether a complete cure in all treated mice can be attained and to examine the efficacy attained by administration via the parenteral route.

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