Randomized Trial of Fluconazole versus Nystatin for the Prophylaxis of Candida Infection following Liver Transplantation

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A prospective, randomized, multicenter study addressed the safety and efficacy of fluconazole therapy in 143 liver transplant patients. Seventy-six patients received daily oral fluconazole (100 mg), and 67 received nystatin (4 × 10^6 U) during the first 28 days after transplantation. Candida colonization occurred in 25% and 53% of patients in the fluconazole and nystatin groups, respectively (P = .04), and 13% and 34% of patients in the respective groups had Candida infections (P = .022). Of these patients, 10.5% in the fluconazole group and 25.3% in the nystatin group had superficial candidal infections (P = .024). Invasive candidiasis developed in 2 patients in the fluconazole group (2.6%) and 6 in the nystatin group (9.0%) (P = .12). There was no increased hepatotoxicity, cyclosporine interaction, or emergence of clinically relevant resistant Candida strains attributable to fluconazole. Thus, oral fluconazole (100 mg) is safe and reduces Candida colonization and infection after liver transplantation.

Infections remain a major cause of morbidity and mortality in solid-organ transplant recipients [1–3], especially those caused by fungi, even though they are less frequent than bacterial and viral infections [1–4]. Among solid-organ recipients, liver transplantation conveys the highest risk for fungal infections (10%–50%) and 50%–100% associated mortality [2, 5–9]. Candida species account for >75% of fungal infections after liver transplantation and result in 25%–60% mortality [4]. Translocation of Candida in the endogenous gut flora across the intestinal mucosa and disruption of the biliary tract and small bowel during transplantation surgery have been implicated in the pathogenesis of this infection [5, 10, 11]. Bone marrow transplant recipients have a similar incidence of Candida infection and an associated mortality of 80%. Thus, major efforts have been made to reduce the incidence of fungal infection in bone marrow transplant recipients by use of prophylactic antifungal agents [12–14]. Because the pathogenesis and risk factors of bone marrow recipients differ from those of solid-organ transplant recipients, it is difficult to extrapolate prophylaxis regimens between the two types of transplants [4].

Various antifungal prophylactic regimens have been studied in solid-organ recipients, including liver transplant patients. Nystatin reduces oropharyngeal and rectal colonization with Candida organisms, but data are lacking regarding its effectiveness in reducing deep fungal infection in liver recipients [15]. Oral azoles such as ketoconazole also reduce Candida colonization. However, because of potential hepatotoxicity and limitations in gastrointestinal absorption and interaction with cyclosporin A, this agent is a less than ideal prophylactic. Amphotericin B and its liposomal formulation are currently being evaluated, but the inconvenience and cost of parenteral administration plus side effects make its use cumbersome.

Fluconazole has several features that could make it useful as an anti-Candida agent after liver transplantation. It is potent to candida, can be given orally, and is relatively safe at low doses [16]. However, because it potentially could interact with cyclosporin A and tacrolimus and may be hepatotoxic, it needs to be carefully evaluated in liver transplant recipients. In addition, resistant Candida organisms are emerging. In recent studies in neutropenic leukemic patients and in bone marrow transplant recipients, oral fluconazole was safe and reduced colonization and invasive Candida infection [13, 14, 17]. Thus, on the basis of fluconazole’s safety profile and its efficacy as a prophylactic agent in bone marrow transplantation, a randomized controlled study was undertaken in liver transplant recipients. The study determined fluconazole’s potential hepatotoxicity and interaction with cyclosporin A and its usefulness as a prophylactic agent to reduce colonization and infection with Candida organisms.

Patients and Methods

Patient population. Eligible patients were liver transplant recipients ≥8 years old who had their first liver transplantation in the prior 24 h in one of three participant hospitals (Doce de Octubre, Puerta de Hierro, and La Paz). Patients were excluded if they...
had a history of hypersensitivity to azoles, had serum creatinine \geq 3\, mg/dL, had received antifungal agents within 1 week of the study, or had evidence of a preexisting fungal infection. Patients who underwent retransplantation were also excluded from the study before randomization.

**Study protocol.** After screening, patients were randomly assigned to receive either 100 mg of fluconazole as a once-daily capsule or a nystatin suspension (10^5\, U every 6 h) from days 3 to 28 after transplantation. Patients who required endotracheal intubation beyond day 3 received the drugs through nasogastric tubes. Randomization was done separately in each center in blocks of 10 patients each. Patients were clinically monitored for safety and for signs and symptoms of fungal infection within 24 h of transplantation, at study enrollment, weekly during the first 4 weeks after transplant, at week 8 and month 3, and when clinically indicated. Any posttransplant event was evaluated to determine drug-related complications or fungal infection.

Samples for mycologic cultures from the oropharynx, feces, and midstream urine were obtained at study enrollment and on days 7, 14, 21, and 28 after transplantation. Samples were cultured, and fungal elements were identified in Sabouraud agar for 48 h using conventional standardized clinical microbiology methods [18]. The same methodology was used in the three centers. In addition, samples from blood, other body fluids, and tissues were obtained as clinically indicated and cultured and analyzed for fungal infection as above. Serum creatinine, electrolytes, aspartate and alanine aminotransferases, alkaline phosphatase, total bilirubin, complete blood cell count and differential, and platelet counts were measured at least weekly during antifungal prophylaxis and at weeks 8 and 16. Cyclosporine levels were measured at least three times a week during antifungal prophylaxis. Decisions to administer empiric amphotericin B were made on a case-by-case basis by the individual investigator. All use of empiric antifungal therapy was recorded. Fluconazole dosage was reduced to 50 mg/day if creatinine clearance was <50 mg/dL. If hemodialysis was required, 100 mg of fluconazole was administered orally after dialysis.

All patients were submitted to the same triple immunosuppression protocol—prednisone, azathioprine, and cyclosporine. Rejection episodes were treated with steroid boluses (1 g for 3 days) followed by OKT3 monoclonal antibody administration (5 mg/day for 10–14 days) if there was no response to steroid treatment. Perioperative antibacterial prophylaxis consisted of parenteral vancomycin and aztreonam during the first 24 h. Thereafter, oral prophylaxis with norfloxacin (400 mg/day) was administered for the first 4 weeks after transplantation.

Fungal colonization was defined as the isolation of fungal organisms from stool, throat, and urine cultures in the absence of clinical findings. Oropharyngeal candidiasis (thrush) was defined by the presence of oral lesions, odynophagia, and a positive culture for candida in the absence of other pathogens. *Candida* cystitis was defined by dysuria, pyuria, and pure growth of candida in the absence of a urinary catheter and, when a urinary catheter was in place, by the presence of dysuria, pyuria, and the isolation of a pure *Candida* growth at least twice (once after urinary catheter replacement). Invasive fungal infection was defined by the presence of organ-specific symptoms and evidence of *Candida* infection in blood, tissues, or bloody fluids. Esophageal candidiasis was identified by a compatible endoscopic appearance and a positive *Candida* culture from endoscopic biopsy specimens.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of liver transplant recipients treated with fluconazole or nystatin.</th>
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<tr>
<td></td>
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<tr>
<td>Mean age (±SD)</td>
</tr>
<tr>
<td>Men/women</td>
</tr>
<tr>
<td>Mean weight (kg ± SD)</td>
</tr>
<tr>
<td>Child C-score</td>
</tr>
<tr>
<td>Emergency transplantation</td>
</tr>
<tr>
<td>Mean rejection episodes/patient (±SD)*</td>
</tr>
<tr>
<td>Relaparotomy*</td>
</tr>
<tr>
<td>Treatment with steroid boluses*</td>
</tr>
<tr>
<td>Treatment with OKT3*</td>
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<tr>
<td>Cytomegalovirus disease*</td>
</tr>
<tr>
<td>Antibiotic treatment for bacterial infection*</td>
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**NOTE.** Data are no. (%), unless otherwise indicated. * After transplantation.

**Statistical analysis.** Statistical analysis was done with an IBM PS2 57 SX personal computer and statistical software (SPSS/PC + V3.0; SPSS, Chicago). \( x^2 \) or Fisher’s exact tests were used when appropriate to compare differences in proportions between the 2 treatment groups. We used the Mann-Whitney \( U \) test to compare mean values and the log-rank test to compare Kaplan-Meier survival curves.

**Results**

A total of 143 eligible consecutive patients were enrolled in the study; 76 were assigned to receive fluconazole and 67 to receive nystatin. Results are reported for all patients enrolled in the study (intention-to-treat analysis). Both patient groups were similar in sex, age, weight, and severity of underlying liver disease before transplant and had similar risk factors identified in previous studies to predispose liver transplant recipients for the development of fungal infections (intensity of immunosuppression, antibiotic use, length of surgery, transfusion requirements, and incidence of cytomegalovirus disease after transplantation; table 1).

**Candida colonization.** The proportion of patients with fungal colonization before drug prophylaxis was not significantly different in the fluconazole and nystatin groups (20.7% vs. 17.9%, respectively), regardless of site analyzed (table 2). During prophylaxis, 75% of patients in the fluconazole group had absent or decreased fungal colonization compared with 47% of those receiving nystatin (\( P = .04 \)). Overall, 7% of the clinical samples obtained for fungal culture in the fluconazole group yielded a fungus compared with 17% in those treated with nystatin (\( P < .001 \)). When these results were analyzed by site, there was a significant reduction in *Candida* colonization in the urinary tract in the fluconazole group and a similar trend for rectal cultures (table 2).
Table 2. Percentage of patients with a positive fungal culture in throat, urine and feces before and during prophylaxis of Candida infection.

<table>
<thead>
<tr>
<th>Day</th>
<th>Throat</th>
<th>Urine</th>
<th>Feces</th>
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<tbody>
<tr>
<td></td>
<td>Fluconazole</td>
<td>Nystatin</td>
<td>Fluconazole</td>
</tr>
<tr>
<td>0</td>
<td>19</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>7</td>
<td>25^</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>13</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>28</td>
<td>9</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

P = * .03, 1 .008, 1 .01, 1 .02. Statistical analysis compared both prophylactic arms within each day and culture site.

Table 3 shows the colonizing fungi isolated during the study. As expected, Candida albicans was the most frequent species isolated in both groups: It was found in 81% of patients treated with nystatin and in 66% of those given fluconazole. Candida krusei was not isolated in any clinical sample during antifungal prophylaxis. Of the positive cultures in the fluconazole group, 15% were Candida glabrata compared with 3% in the nystatin arm (P = .02). There were no differences in the number of patients colonized during prophylaxis by T. glabrata in either prophylaxis group (5, fluconazole; 4, nystatin); however, 1 fluconazole-treated patient had 5 different samples positive for T. glabrata.

Clinical outcome of prophylaxis. During the antifungal prophylaxis period, Candida infection developed in 9 (12%) of 76 patients receiving fluconazole and in 18 (27%) of 67 receiving nystatin (P = .022). Superficial fungal infection was documented in 8 patients in the fluconazole group (oral thrush, 7; Candida cistitis, 1) and in 17 in the nystatin group (oral thrush, 14; Candida cistitis, 3) (10.5% vs. 25.3%, P = .034).

Documented systemic invasive Candida infection developed in 1 (1.3%) of 76 patients in the fluconazole group and in 4 (6.0%) of 67 in the nystatin group (P = .12) during the 28-day prophylaxis period. Disseminated fungal infection with a fungemia developed in only 1 patient (nystatin treatment group). Candida esophagitis developed in 1 patient in the fluconazole group and in 2 in the nystatin group. An intraabdominal abscess that yielded Enterococcus faecalis and C. albicans developed in an additional patient in the nystatin group. Microbiologic analysis indicated that C. albicans was the etiologic organism in all episodes of invasive candidal infection. Four patients (2 in each group) received amphotericin B because of suspected systemic fungalm infections, thus raising the number of proven and suspected invasive fungal infections to 3 (3.9%) in the fluconazole group and 6 (8.9%) in the nystatin group (P = .13).

From completion of the fungal prophylaxis (day 28 after transplantation) to the end of the follow-up period (day 90), there were 3 episodes of invasive fungal infection (esophagitis): 2 in the nystatin group and 1 in the fluconazole group. Three patients developed aspergillosis after the 28 days of therapy: 2 in the fluconazole group and 1 in the nystatin group.

Mortality. No significant difference in overall mortality was observed between the 2 groups up to 90 days after treatment. Among the fluconazole recipients, 10 (13%) of 76 died as did 9 nystatin recipients (13%). Of the deaths in the fluconazole group, 3 were associated with early development of multiorgan

Table 3. Number of patients and specimens of different Candida species isolated during prophylaxis with fluconazole and nystatin.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Fluconazole</th>
<th></th>
<th>Nystatin</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Specimens</td>
<td>Patients</td>
<td>Specimens</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10 (13)</td>
<td>41 (66)</td>
<td>25 (37)</td>
<td>102 (81)</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>5 (7)</td>
<td>9 (15)</td>
<td>4 (6)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Candida parapsilosis</td>
<td>3 (4)</td>
<td>6 (10)</td>
<td>4 (6)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Candida pseudotropicalis</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td>3 (4)</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>1 (1)</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Candida species (not determined)</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td>3 (4)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>19*</td>
<td>62*</td>
<td>35*</td>
<td>126*</td>
</tr>
</tbody>
</table>

NOTE. No Candida krusei were found. Data are no. (%) of patients colonized patients) and no. (%) total Candida isolates from each prophylaxis group (specimens).

* Some patients were colonized by >1 Candida species.
failure, 2 with related cytomegalovirus disease, 2 with bacterial sepsis with disseminated intravascular coagulation, and 1 each with graft failure, disseminated aspergillosis, and cardiac arrest of unknown cause. Of the 9 patients who died in the nystatin group, 3 died of severe bacterial infection, 2 had graft and 2 had multiorgan failure, and 1 had myocardial infarction. Among nystatin recipients, death was attributed to Candida infection in 1 patient receiving nystatin. No death among fluconazole recipients was directly attributed to Candida infection during or after active prophylaxis with the drug.

**Adverse events and abnormal laboratory values.** There were no significant differences between fluconazole and nystatin patients in whom adverse events were ascribed to antifungal prophylaxis (33% vs. 25%, respectively, \(P > .1\)). Mild gastrointestinal symptoms (nausea, vomiting), the most common clinical side effect, were more frequent in the nystatin (19%) than in the fluconazole group (9%) \(P = .09\). Drug was discontinued in only 1 patient (fluconazole group, day 21 after transplantation) due to a potential drug-related adverse event (confusion). This patient did not receive additional antifungal prophylaxis and was free of fungal infection at day 90 after transplantation. Leukopenia was recorded in 15% of patients in the fluconazole group and in 19% in the nystatin group. Acute renal failure developed in 13% of patients in the fluconazole arm and in 12% in the nystatin arm but did not require discontinuation of prophylactic drug. Close monitoring of liver function in this cohort of liver transplant recipients enabled determination of the potential hepatotoxicity of fluconazole. There were no differences in mean aminotransferase, alkaline phosphatase, and bilirubin levels at each biochemical evaluation between treatment groups. Moreover, no episode of liver dysfunction was attributed to drug toxicity.

Potential cyclosporine-fluconazole interactions were measured during study drug administration by comparing the mean cumulative dosage of cyclosporine required to maintain serum levels in the therapeutic range. No differences were found in the mean total dose of cyclosporine administered between the fluconazole and nystatin groups \(8.2 \pm 3.9\) vs. \(7.8 \pm 2.9\) g, respectively. As an indirect measure of cyclosporine-fluconazole interaction, no differences were found in the mean creatinine levels of both groups at each biochemical evaluation.

**Discussion**

Deep Candida infection remains a major problem in liver transplant recipients. Our study indicates that antifungal prophylaxis with fluconazole is safe and effective in preventing candidal infection in liver transplant recipients. Many liver transplant centers treat patients with nonabsorbable polyene compounds in an attempt to reduce Candida colonization within the endogenous flora of the gastrointestinal tract [4]. One advantage of fluconazole is that due to its favorable pharmacologic properties, it can reduce Candida colonization in locations other than the gut (e.g., urine, skin, and oral mucosa), sites in which yeast colonization correlates with the development of deep fungal infection in other immunosuppressed patients [19, 20]. Alternatives to oral absorbable agents include parenteral administered systemic agents (e.g., amphotericin). Some studies [21] but not others [22] have shown the efficacy of low-dose (10 mg/day) intravenous amphotericin B in preventing fungal infection. Nephrotoxicity, cyclosporine-amphotericin interactions, and myelotoxicity are major drawbacks to the generalized use of amphotericin B in solid-organ transplant recipients but may, in part, be overcome by use of liposomal amphotericin B [23].

Our study indicates that oral fluconazole administered at low doses (100 mg/day) is a safe alternative for the prophylaxis of Candida infection in liver recipients. The greater efficacy of fluconazole than of nystatin in reducing the incidence of oral thrush may be due to its excellent oral absorption and penetration into saliva [24]. This may also pertain for the reduction of urinary colonization, ultimately influencing Candida cystitis. These findings may be relevant for other types of solid-organ transplant patients (e.g., renal system/pancreas) in whom urinary tract infections with Candida organisms are more frequent than in liver recipients [25] and in gut transplantation where systemic antifungal levels may be pertinent.

Invasive fungal (Candida) infection was lower in our study than in previous liver transplantation studies that reported 15%–20% infection rates [5, 7–10, 23, 26]. In our study, the incidence of deep fungal infection caused by Candida organisms was 8.9% in the nystatin group and 2.6% in the fluconazole group. This may in part be due to the short follow-up period (\(\leq\)3 months), when we would expect to observe an effect of an antifungal drug given during the first month after transplantation. Due to the low incidence of deep fungal infections in this early posttransplant period, it was not possible to determine statistical significance between groups. Thus, a larger sample will be required to confirm the trend towards a significant reduction of invasive Candida infection by fluconazole prophylaxis.

Only 2 cases of proven deep candidal infection occurred in the fluconazole group versus 6 (including the only case of disseminated candidiasis associated with death) in the nystatin group. Of interest, 3 episodes of deep Candida infection (1 in the fluconazole group, 2 in the nystatin group) developed after discontinuation of antifungal prophylaxis. The 3 patients shared several well-recognized risk factors for the development of fungal infections (surgical reinterventions, high doses of steroids for allograft rejection, and prolonged use of antibacterial antibiotics). This observation suggests that specific fungal prophylaxis should be given not only to patients with risk factors before and intratransplant (fulminant hepatitis, prolonged surgical intervention, or need for large volumes of blood product transfusions) but also to those who develop posttransplant risk factors. In the latter group, a more prolonged course with or without an increase in dosage should be considered. As expected, in our study there was less aspergillosis than candidal
infection. Aspergillosis occurred equally between groups and after the first month of therapy.

Primary goals of our study were to determine the safety and drug interactions of fluconazole in solid-organ transplant recipients. Fluconazole was well tolerated, resulting in good patient compliance. No major differences in the number of adverse events possibly attributed to fluconazole were observed. The drug was discontinued in only 1 patient (who exhibited confusion); the patient had normal cyclosporine levels. Moreover, no hepatotoxicity was observed [27]. As expected during the first 2 weeks after transplantation, abnormal liver function test results were common; however, there was no clinical, biochemical, or histologic evidence of fluconazole liver toxicity as indicated by the similar numbers of liver biopsies done per patient in both treatment arms.

Pharmacologic interactions between imidazole compounds (e.g., ketoconazole, itraconazole, and cyclosporine) have been extensively reported [28–30]. These antifungal agents increase serum levels of CsA through the inhibition of the cytochrome P-450 enzyme system. Such interaction is more controversial in the case of fluconazole [31–33], although it has been suggested that fluconazole doses of <200 mg/day should not warrant close monitoring of CsA concentrations [34]. In our study, 100 mg/day of fluconazole did not result in any significant interaction with cyclosporine in liver transplant patients.

The potential for the selection or development of flucona-zole-resistant Candida organisms, namely C. krusei and T. glabrata, needs to be considered. Wingard et al. [35] reported an increase of C. krusei colonization and infection when bone marrow transplant recipients were given 400 mg/day of fluconazole to prevent fungal infection. However, other studies in neutropenic leukemia and bone marrow transplant recipients did not find increased fluconazole-resistant yeasts during fluconazole prophylaxis, and even in those in whom there was an increased incidence of C. krusei colonization, there was no associated significant morbidity or mortality [13, 14, 36]. In our study, in which we gave lower doses of fluconazole for a shorter period, no significant increase in the colonization and infection by fluconazole intrinsically resistant yeasts was observed except in 1 patient in the fluconazole group from whom T. glabrata was frequently isolated, although not leading to disease.

In conclusion, fluconazole prophylaxis was well tolerated and effective in preventing Candida infection in liver transplant recipients. A dose of 100 mg/day did not select for intrinsically resistant organisms, cause disease, or interact with cyclosporine. From the data derived from this study, it can be inferred that fluconazole should be a valid alternative agent in the prophylaxis of Candida infection after liver transplantation. It could be given immediately after transplant to persons with known pre- and intratransplant risk factors for development of Candida infections. In addition, occurrence of posttransplantation risk factors for C. albicans should trigger its use or prolong its administration.

Acknowledgments

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References


