Efficacy of Cilofungin Therapy Administered by Continuous Intravenous Infusion for Experimental Disseminated Candidiasis in Rabbits

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Cilofungin has potent in vitro activity against Candida albicans, but previous in vivo models using twice daily intermittent dosing regimens have not consistently demonstrated in vivo efficacy. Because of the pharmacokinetics of cilofungin in rabbits, it has been suggested that administration by continuous intravenous infusion might be more effective. We compared the in vivo efficacy of continuous intravenous infusion of cilofungin with that of amphotericin B in a rabbit model of disseminated candidiasis. Cilofungin prepared as previously described in phosphate-buffered 33% polyethylene glycol was lethal to infected rabbits in this model, as was phosphate-buffered 33% polyethylene glycol alone. In contrast, cilofungin in 26% polyethylene glycol and sterile water administered by continuous intravenous infusion was tolerated by rabbits, was significantly more effective than amphotericin therapy in reducing candida colony counts in kidney tissue, and was as effective as amphotericin therapy in lung and spleen tissue and in cardiac valvular vegetations. The dosage regimen and diluent used in some previous studies may have adversely affected outcome of treatment with cilofungin.

There is little debate over the need to develop effective antifungal chemotherapeutic agents that have improved ease of administration and safety relative to those of amphotericin B. Cilofungin, one such potential agent, is a (1–3)-β-glucan synthase noncompetitive inhibitor (13) with potent in vitro fungicidal activity limited primarily to Candida species, including Candida albicans (4–6, 14).

Despite its promising in vitro activity, previous reports have not consistently demonstrated clear efficacy in vivo of cilofungin for the treatment of serious candida infections. Morrison and Stevens (9), in a murine model of candida pyelonephritis and splenitis, found mice treated twice daily with cilofungin to have significantly worse survival and more residual microorganisms in kidney and spleen 2 weeks after completing therapy than did mice treated with amphotericin B in equivalent doses. Smith and coworkers (12), also in a murine model, reported no significant decrease in mortality among mice treated with cilofungin in doses up to 100 mg/kg of body weight twice daily compared with that in untreated mice, and among survivors cilofungin was less effective than amphotericin B in reducing the number of candida cells in kidney tissue.

In a rabbit model of experimental endocarditis, Padula and Chambers (10) reported no survivors after 4 days of treatment with cilofungin at 50 mg/kg given twice daily compared with 100% survival in animals treated with amphotericin B. Perfect et al. (11), in a rabbit model of disseminated candidiasis, reported that cilofungin at 50 mg/kg twice daily was effective in reducing colony counts of candida in renal cortex assessed 24 h after completing therapy and in sterilizing vitreous humor compared with those in untreated animals but found no statistically significant effect on quantitative cultures of cardiac valve vegetations compared with that in no treatment.

Cilofungin was administered twice daily in all of the studies described above. Where stated in these reports, the diluent was 33% polyethylene glycol (PEG) 300 but was not described further. In mice, the elimination half-life of cilofungin was reportedly 30 min (9). Lee and colleagues (7) in a detailed study of cilofungin pharmacokinetics in rabbits reported an elimination half-life of 12.9 min and striking nonlinear kinetics. In Lee’s study, tissue penetration was as much as 89 times higher when cilofungin was administered by continuous intravenous infusion rather than by intermittent dosing, with highest tissue-to-plasma ratios in liver and bile.

Preliminary studies in our laboratory with continuous-infusion cilofungin reconstituted in 33% PEG and phosphate buffer, as recommended by the manufacturer, suggested the possibility that the phosphate buffer itself was toxic to rabbits and responsible for increased mortality among cilofungin-treated animals. Therefore, our study was designed to (i) study the effect of cilofungin diluent on survival of infected rabbits and (ii) compare the efficacy of cilofungin administered by continuous infusion with that of amphotericin B in clearing candida from lung, spleen, and kidney tissue and cardiac valve vegetations by using a rabbit model of disseminated candidiasis.

MATERIALS AND METHODS

The organism used in this study was a clinical isolate of C. albicans kindly provided by John Washington, Cleveland Clinic. In vitro susceptibility tests were performed with an inoculum of 10^5 CFU in Sabouraud dextrose broth at pH 5.6 and 35°C by using a macrodilution method as previously described (8). Candida for inoculation into rabbits were prepared in Sabouraud dextrose broth and incubated overnight at 35°C in room air. The broth culture was diluted to a concentration of 5 × 10^5 candida blastospores per ml. The inoculum was prepared fresh prior to each use, with confirmation of the inoculum size by quantitative culture.

Cilofungin was prepared initially according to the manu-
Efficacy of Continuous-Infusion Cilofungin Therapy

TABLE 1. Results of cilofungin and amphotericin B treatment of experimental disseminated candidiasis in rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (no./total)</th>
<th>Log_{10} CFU/g of tissue*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valve</td>
<td>Kidney</td>
</tr>
<tr>
<td>Phosphate-buffered PEG</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cilofungin in phosphate-buffered PEG</td>
<td>8/9</td>
<td>ND</td>
</tr>
<tr>
<td>No treatment</td>
<td>5/7</td>
<td>5.90 ± 1.62</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>6/8</td>
<td>4.24 ± 1.82*</td>
</tr>
<tr>
<td>Cilofungin in sterile water-P EG</td>
<td>10/12b</td>
<td>2.73 ± 1.48*</td>
</tr>
</tbody>
</table>

* ND, not done. Values represent the means ± standard deviation in surviving rabbits.

Antifungal therapy was continued for 4 days. Twelve hours after the last dose of drug, surviving animals were sacrificed with a lethal dose of sodium pentobarbital. The aortic valve and attached vegetations, a cross-section of the middle lobe of the right lung, a wedge section of the right kidney, and the distal 1 cm of the spleen were removed aseptically, weighted, and homogenized. Quantitative cultures were performed by serially diluting tissue homogenates and culturing 750-μl aliquots of each dilution in 10 ml of Sabouraud dextrose agar by using a pour plate technique. The fungal culture results were expressed as the log_{10} CFU of candida per gram of tissue.

Blood for bioassay of cilofungin or amphotericin B concentration in serum was obtained from the rabbits on day 3 of treatment. Blood for amphotericin B assay in serum was obtained 1 h after administration. Aspergillus montevideensis A35137 was used as the test organism for cilofungin assay, and Paecilomyces variotii ATCC 36257 was used as the test organism for amphotericin B assay (1, 4).

Survival results were compared by using Fischer’s exact test. Quantitative culture results were compared statistically by using the Student-Newman-Keuls test.

RESULTS

The MICs of amphotericin B and cilofungin for the strain of C. albicans used in vivo were 0.32 and 0.16 μg/ml, respectively. In treated animals, the concentration in serum of amphotericin B and cilofungin in PEG 300 and sterile water diluent were 1.3 ± 0.2 and 105 ± 21 μg/ml (mean ± standard deviation) respectively.

Table 1 shows the results of treatment. Among animals treated with the phosphate-buffered cilofungin formulation or phosphate-buffered PEG alone, no animals survived longer than 2 days in contrast to survival after 4 days of 71% among untreated animals and 83% among animals treated with cilofungin reconstituted in sterile water and 26% PEG 300 (P < 0.05). Cilofungin in sterile water and 26% PEG 300 by continuous infusion was more effective (P < 0.05) than no treatment in reducing candida colony counts in all tissues studied. Cilofungin was significantly more active (P < 0.05) than amphotericin B in reducing candida colony counts in kidney tissue. Differences among colony counts in cardiac valve vegetations, spleen, or lung tissue (Table 1) among cilofungin- or amphotericin B-treated animals were not statistically significant. The lack of statistical significance, particularly for cardiac valve vegetations, may be related in part to the small size used in this study.
DISCUSSION

In our study, we found that rabbits with disseminated candidiasis treated with continuous-infusion cilofungin prepared in phosphate-buffered 33% PEG or phosphate-buffered 33% PEG alone did not survive longer than 48 h. These results are similar to the observation by Padula and Chambers (10) of high mortality in rabbits with candida endocarditis treated with intermittently dosed cilofungin prepared in 33% PEG and phosphate-buffered saline (2). Comparison of survival among rabbits treated with this formulation of cilofungin (0 of 7) and among untreated rabbits (5 of 7) suggests that deaths were related to toxicity associated with administration of the cilofungin preparation. Poor survival among rabbits treated with cilofungin reported in earlier studies may have been due in part to toxicity of the phosphate-buffered formulation of cilofungin. In contrast, rabbits in our study treated with cilofungin prepared in 26% PEG with sterile water diluent had a survival rate after 4 days of treatment similar to that among amphotericin B-treated animals. Among surviving animals, cilofungin at 100 mg/kg daily administered by continuous-intravenous infusion was more effective than was amphotericin B at 1 mg/kg in reducing candida cell counts in kidney tissue and as effective as amphotericin B in cardiac valvular vegetations and in lung and spleen tissue.

Walsh et al. (14) recently reported the results of treatment with multiple intermittent and continuous-intravenous infusion dosing regimens of cilofungin in a granulocytopenic rabbit model of disseminated candidiasis. They reported toxicity of cilofungin in 33% PEG and phosphate-buffered saline which was attributed to the PEG component infused in volumes greater than 10 ml/kg. Data supporting this hypothesis were not included, and the toxicity was reportedly alleviated by increasing the concentration of cilofungin to 50 mg/ml. Unfortunately, no standard treatment arm was included for comparison with the outcome of treatment with cilofungin. This study also differed in the concomitant administration of cefazidine and vancomycin (potential drug-drug interactions affecting efficacy, pharmacokinetics, or toxicity were not investigated), the presence of granulocytopenia, and interruption of continuous infusion of cilofungin for 6 h daily during the 6-day treatment period. A dose-response relationship was observed with significantly improved efficacy of cilofungin dosing regimens achieving nonlinear saturation kinetics.

In our study the efficacy of cilofungin in reducing candida cell counts in cardiac valvular vegetations (a measure of fungicidal activity) contrasts with previous reports of lack of efficacy with intermittent twice-daily cilofungin administration (10, 11). Differences between our results and those of earlier studies could be due to differences in strains of candida, treatment duration, or other parameters of the model. We believe, however, that the in vivo effects of cilofungin therapy in our study are most likely due to sustained cilofungin concentrations in serum and improved cilofungin tissue penetration when the drug is administered by continuous intravenous infusion, as suggested by Lee and coworkers (7) and Walsh et al. (14).

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2. Chambers, H. F. Personal communication.