Treatment of Murine Invasive Candidiasis with Amphotericin B and Cilofungin: Evidence for Enhanced Activity with Combination Therapy

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The in vivo interactions of cilofungin, an echinocandin antifungal agent, and amphotericin B, a polyene derivative, in a murine model of disseminated candidiasis have been investigated. While single therapy with either drug alone prolonged survival of infected mice, kidney colony counts were not appreciably reduced. In contrast, combination therapy, especially at higher doses of both drugs, resulted in significant prolongation of survival and suppression of growth of yeast cells in the kidneys. Combination therapy of experimental candidiasis with cilofungin and amphotericin B did not result in antagonism; rather, additive or synergistic effects were seen. Future preclinical work with other echinocandin and polyene derivatives should include studies evaluating the in vivo interactions of both classes of compounds.

Cilofungin is a derivative of echinocandin and displays potent antifungal activity in animal models of candidiasis, with restricted in vitro activity against Candida albicans and C. tropicalis (3, 6, 7). While in vitro data have documented the occurrence of antagonism of antifungal effect when cilofungin is combined with amphotericin B (1), in vitro susceptibility tests are unreliable in predicting in vivo activities of antifungal drugs (2). For this reason, animal models of mycoses have been used to evaluate the potential utility of new compounds for use as antifungal drugs (8). Cilofungin has demonstrated activity in animal models of candidiasis (5, 9). While the present paper was in review, a recent study evaluating the interactions of amphotericin B and cilofungin in a murine model of candidiasis was published (4). We also have been interested in the in vivo interactions of various antifungal agents. In this study, we evaluated the effects of cilofungin alone and in combination with amphotericin B in the treatment of disseminated candidiasis in mice. Our results, confirming those of Hanson et al. (4), demonstrate that antagonism between amphotericin B and cilofungin did not occur and that, at certain concentrations, synergy could occur.

ICR male mice, 3 to 4 weeks old, were purchased from Harlan Sprague Dawley (Indianapolis, Ind.). At the start of each experiment, the mice weighed 22 to 25 g. Mice were housed, eight per cage, in filter-topped cages and fed standard mouse chow and water ad libitum. Each group in the survival experiments consisted of eight mice. Cohorts of eight additional mice per group were added for the study on kidney colony counts.

C. albicans 64 was maintained on Sabouraud dextrose agar slants at 4°C. A large loopful of organisms was suspended in fresh Sabouraud dextrose broth and incubated for 24 h at 37°C. Blastocystidia were harvested and washed twice in sterile buffered saline (pH 7.4) by centrifugation. Cells were counted in a hemacytometer and adjusted to a concentration of 5 × 10⁶/ml. The number of yeast cells administered to the mice was determined by plating the same inoculum on blood agar plates. Colonies were counted 24 to 48 h later.

Mice were injected through a lateral tail vein with 0.1 ml of sterile saline containing 5 × 10⁶ blastoconidia. Preliminary studies indicated that this inoculum usually resulted in an 80 to 100% lethal dose (LD₉₀ to LD₁₀₀) in 10 days.

Cilofungin was provided as a sterile powder by Lilly (Indianapolis, Ind.). The drug was suspended at the appropriate concentration in 33% polyethylene glycol 300 daily. Mice were treated with cilofungin by intraperitoneal injection twice daily. Mice received 12.5 or 50 mg/kg of body weight per day. Amphotericin B (Sigma, St. Louis, Mo.) was prepared fresh daily in sterile water and administered by injection of 0.1 ml containing 0.1 or 1 mg/kg once daily into a lateral tail vein. Therapy was begun 24 h following inoculation of mice with C. albicans and was continued for a total of 10 or 14 consecutive days. Mice were weighed weekly and drug doses were adjusted accordingly. Control groups received sterile 5% glucose in water intraperitoneally, once daily. Cages were observed twice daily for deaths. Polymethylene glycol 300 was not used as a control, as previous investigations have shown no effect of this compound.

Randomly selected mice were sacrificed on days 1, 14, and 30 of the experiment, and the right kidney of each was aseptically removed and homogenized in 3 ml of sterile saline by using an electric tissue homogenizer (Tekmar Company, Cincinnati, Ohio). Serial 10-fold dilutions were plated onto blood agar. After incubation at 37°C for 24 h, the number of colonies on each plate was determined.

Gehan’s Wilcoxon test and t test were performed where appropriate, using a commercially available statistics program suitable for use on IBM PC-compatible equipment (Stat Soft, Tulsa, Okla.). Significance was defined as P < 0.05.

In one experiment (Fig. 1), mice were intravenously injected with 10⁶ Candida blastoconidia. Twenty-four hours after inoculation, treatment was begun and continued daily for 14 days, with the total daily doses noted in the figure. Of the mice receiving no treatment, 88% were dead by day 9 (Fig. 1A; figures are divided into two parts for clarity). In contrast, all of the mice receiving the low dose of cilofungin...
alone were dead by the fifth day. These mice all died early in the treatment course, suggesting either drug toxicity or trauma related to intraperitoneal injections as the cause. Similar increases in mortality with low doses of cilofungin were not seen in two other experiments, arguing against drug toxicity as a cause of the increased mortality. Treatment with amphotericin B alone at low (0.1-mg/kg/day) and high (1-mg/kg/day) doses resulted in improved survival compared with that of controls (38 and 25 versus 12%, respectively), but these results did not achieve statistical significance. Similarly, 50% of the mice receiving high doses of cilofungin survived (Fig. 1B).

When cilofungin was combined with amphotericin B, low doses of both drugs were not superior to low doses of amphotericin B alone (Fig. 1A). However, all of the mice treated with high-dose combination therapy survived (Fig. 1B; \(P < 0.003\)), thus demonstrating an additive or synergistic effect of the two drugs.

In a second experiment, mice received \(3.8 \times 10^6\) yeast cells. Treatment was begun 24 h after inoculation and continued for 10 days. By day 11, 75% of the control mice were dead (Fig. 2A). Low-dose cilofungin slightly increased survival. However, the best treatment results were seen with high-dose combination therapy (Fig. 2B; \(P = 0.01\) compared with control, \(P = 0.058\) compared with high-dose cilofungin alone, and \(P = 0.14\) compared with high-dose amphotericin B alone), followed by low-dose combination therapy (\(P = 0.003\) compared with control, \(P = 0.015\) compared with low-dose cilofungin alone, and \(P = 0.073\) compared with low-dose amphotericin B alone).

In the same experiment, the times required to reach the LD\(_{50}\) were 4 days in the control mice; 9 and 16.5 days in mice receiving low- and high-dose cilofungin, respectively; and 15 days in mice receiving low-dose amphotericin B. An LD\(_{50}\) was not achieved in either of the combination therapy groups or in the high-dose amphotericin B group. Thus, by this criterion, combination therapy resulted in improved survival compared with cilofungin alone and low-dose amphotericin B alone.

Results of kidney cultures obtained during the experiment whose results are presented in Fig. 2 confirm the increased activity of combination therapy compared with that of treatment with each drug individually (Table 1). On day 14, 3 days after therapy was discontinued, a decrease in yeasts recovered from kidneys was found only in the combination therapy groups (\(P < 0.041\)). An approximate one-log increase in CFU was seen in the other groups (\(P > 0.001\)). At the end of therapy, a large decrease in CFU recovered from the kidneys was found only in both groups that received combination therapy (\(P < 0.001\)). A decrease in numbers of colonies was also seen in both amphotericin B groups (\(P < 0.001\)). Cilofungin at either dose appeared to have a fungistatic effect, since the numbers of organisms recovered on the first day of therapy were similar to those recovered at the completion of therapy.

This study clearly demonstrates that in a murine model of disseminated candidiasis, therapy with the echinocandin analog cilofungin in combination with amphotericin B results in a better outcome than that obtained with either drug used alone. It is particularly noteworthy that there was no antagonism observed, in contrast to results obtained from in vitro studies (1). The additive or synergistic activity was observed both in improvements in survival and in colony counts from kidneys, a major target organ in experimental candidiasis. These results support the conclusions of Hanson et al., who, with a similar model of disseminated candidiasis in mice, concluded that combination therapy was superior to monotherapy, as measured by survival or colony counts in the kidneys and spleen (4). However, in contrast to Hanson et
al., who found that 2 of 12 mice had sterile kidneys when treated with cilofungin (6.25 or 62.5 mg/kg/day) plus amphotericin B (0.625 mg/kg/day), we did not find sterile kidneys in our study.

While clinical investigation of cilofungin has been discontinued, echinocandin derivatives are attractive agents since they have a mode of action different from that of the currently available major antifungal classes, polyenes and azoles. The finding that combination therapy with cilofungin and amphotericin B can be more effective than monotherapy suggests that future studies evaluate the interactions of the different classes of drugs in experimental models of fungal diseases in an attempt to discern potentially clinically useful drug combinations.

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REFERENCES