Comparative Effects of Cilofungin and Amphotericin B on Experimental Murine Candidiasis

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The effectiveness of cilofungin (LY121019, referred to hereafter as LY), a lipopeptide, was studied in a murine candidiasis model. CD-1 mice (5 weeks old) were injected intravenously with \(3 \times 10^6\) Candida albicans yeast cells. Intraperitoneal LY or amphotericin B (Amb) therapy was begun 4 days after infection and was continued daily for 2 weeks. LY and Amb were compared at 62.5, 6.25, and 0.625 mg/kg per day, with the LY dose split into two treatments per day. Mice were observed for 30 days postinfection, and survivors were necropsied. Amb at 62.5 mg/kg per day was lethal in the absence of infection. Cumulative mortality for infected controls was 94% (17 of 18). Survival of mice treated with the control diluent for LY was the same as survival with no treatment. Survival after 0.625 mg of LY per kg per day was the same as that of the controls, and 6.25 or 62.5 mg of LY per kg per day was significantly superior. Amb treatment at 0.625 or 6.25 mg/kg per day was protective and superior to the same LY doses. Atrophied kidneys were common in Amb-treated mice, and mice treated with 6.25 mg of Amb per kg per day appeared ill during therapy. The number of CFU recovered from kidneys and spleens of surviving mice reflected the same relationships between drugs and doses as those described for mortality. C. albicans was not cleared from the kidneys of mice in any group, and only in the 6.25-mg/kg-per-day Amb treatment group was no detectable C. albicans found in the spleens. These data indicate that LY or Amb suppresses candida infection but neither is curative in this model.

Cilofungin (LY121019, referred to hereafter as LY) is a semisynthetic antibiotic with potent antifungal activity in vitro (7, 8, 12). A novel lipopeptide analog of echinocandin B, LY, belongs to a new chemical class of polypeptide antifungal agents which have been reported to inhibit the incorporation of β-1,3-glucan into the fungal cell wall (1, 2, 10). Scanning electron microscopic studies have shown that Candida albicans grown in the presence of LY develops severely damaged cell walls (5). The MICs for 50 and 90% of strains tested (MIC50 and MIC90) of LY were identical to those of amphotericin B (Amb) against 96 isolates of C. albicans (5). LY has potent fungicidal activity against Candida spp. in vitro. Other pathogenic fungi tested are much less susceptible to LY (7, 8). Given the narrow spectrum of LY in vitro and its low toxicity in vivo (LY was shown to have no more than 1/20 the toxicity of Amb in dogs [5]), we compared the effects of LY therapy to those of Amb on intravenously induced murine candidiasis.

MATERIALS AND METHODS

C. albicans. The fungal isolate used in this study (C. albicans 5) was susceptible in vitro to LY (MIC, 0.625 μg/ml) and Amb (MIC, 0.5 μg/ml), as determined by previously described methods (4). Yeast nitrogen base broth (Difco Laboratories, Detroit, Mich.) with 0.5% glucose was inoculated with growth from Sabouraud agar slants. Yeast cells were grown overnight at 35°C without agitation, washed three times in sterile 0.85% saline, and serially diluted in saline.

Inoculation of mice. Five-week-old specific-pathogen-free male CD-1 mice (Charles River Breeding Laboratories, Inc., Portage, Mich.) were used in a model of systemic candidiasis described previously (9, 14).

Groups of 10 to 18 mice (average weight, 30 g) were infected via injection of 0.2 ml of C. albicans suspension into the lateral tail vein (3 \( \times 10^6\) yeast cells per mouse). This inoculum resulted in a 95% lethal dose (LD95) survival curve with no deaths before day 5 or after day 25.

Drugs. Amb for injection (E. R. Squibb & Sons, Princeton, N.J.), containing 50 mg of Amb and 41 mg of sodium deoxycholate buffered with 20.2 mg of sodium phosphates, was reconstituted by the addition of sterile distilled water to the lyophilized powder. Serial dilutions of the concentrate were stored frozen in the dark for daily usage. LY (Eli Lilly & Co., Indianapolis, Ind.) was initially solubilized in 33% polyethylene glycol 300 (PEG 300; Sigma Chemical Co., St. Louis, Mo.), and solutions were stored at 4°C for up to 1 week.

In vivo drug treatment. Four days postinfection, intraperitoneal LY or Amb treatment was initiated and continued for 2 weeks. Effects of LY or Amb treatment (0.2 ml per dose per mouse) were compared at 62.5, 6.25, or 0.625 mg/kg per day and 6.25 or 0.625 mg/kg per day, respectively. One group of 12 mice served as a diluent control for LY-treated mice and received only PEG 300 diluent containing no drug; another group of 18 mice received no treatment. The Amb and LY doses and regimens were known to be safely administered from prior studies (data not shown). Amb at 62.5 mg/kg per day was, in contrast, lethal in the absence of infection. LY (31.25 mg/kg per day [half of the daily chronic dose]) produced peak concentrations in the serum of these mice as determined by previously described bioassay methods (9) of 46 μg/ml 0.5 h after administration, well above the MIC of LY for the challenge isolate (L. H. Hanson, K. V. Clemons, and D. A. Stevens, unpublished data). The daily LY dose was given divided twice a day because of its short
half-life (30 min) in mice, as noted in our studies (L. H. Hanson et al., unpublished data).

Mice were observed twice daily for 30 days postinfection, and survivors were necropsied. The gross pathology of kidneys and spleens was recorded. Kidneys and spleens were homogenized in sterile 0.85% saline (tissue homogenizer; Tekmar Co., Cincinnati, Ohio). Homogenates were serially diluted in sterile 0.85% saline and pipetted on blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) for enumeration of CFU after incubation at 35°C for 48 h.

Statistical analyses. The Wilcoxon test was used to determine statistical significance for survival between experimental groups. The Student t test was used for group comparisons of quantitative data (CFU per organ). For these comparisons, a conservative assumption was made; it was assumed that each mouse dying from infection had an organ burden in CFU equal to that of the survivor of the group with the highest burden. In all likelihood, this assumption minimized the differences between groups. Values of P < 0.05 were considered significant.

RESULTS

Effect of LY or AmB treatment on mouse survival during systemic candidiasis. The results are shown in Fig. 1. By day 30, only 6% (1 of 18) of untreated control mice survived. Survival after treatment with PEG 300 diluent alone or 0.625 mg of LY per kg per day was not significantly different from that of the untreated control group. Survival after 6.25 mg of LY per kg per day was 30% (3 of 10), and the survival curve was superior to that of controls (P < 0.002). Treatment with 62.5 mg of LY per kg per day resulted in 90% survival, and this curve was superior to that of controls (P < 0.0002) and to that of lower doses of LY (0.625 mg/kg per day, P < 0.0004; 6.25 mg/kg per day, P < 0.006).

AmB therapy at 0.625 or 6.25 mg/kg per day resulted in the survival of 100% of infected mice (P < 0.0002). The 0.625- or 6.25-mg/kg-per-day dose of AmB was clearly superior to an equivalent dose of LY in regard to animal survival. Whereas 62.5 mg of AmB per kg per day could not be assayed because of lethality, an equivalent dose of LY was highly protective (90% survival).

Not evident from these data is the observation that surviving mice receiving 62.5 mg of LY per kg per day appeared to be well throughout the study whereas mice receiving a log lower dose of AmB (6.25 mg/kg per day) appeared ill (i.e., ruffled fur, huddled into groups without activity, shaking). The AmB (6.25 mg/kg per day)-treated mice eventually recovered from these signs after AmB administration was stopped. These observations indicate that LY may be less toxic than AmB to mice, particularly since the 62.5-mg/kg-per-day LY group showed greater infection (but appeared healthy) than the 6.25-mg/kg-per-day AmB group, as described below.

Effect of LY or AmB treatment on gross pathology of mouse organs. Mice surviving at day 30 postinfection were necropsied and examined for gross organ pathology. All control mice and all mice treated with LY at doses of 0.625 or 62.5 mg/kg per day had lesions in one or both kidneys (found previously [9, 13] to represent residual sites of infection). Kidney lesions were apparent in two of three (67%) surviving mice treated with 6.25 mg of LY per kg per day. In contrast, 3 of 10 (30%) or 2 of 10 (20%) mice treated with 0.625 or 6.25 mg of AmB per kg per day, respectively, had apparent kidney lesions. In all five survivors treated with AmB with lesions, only one kidney each had visible lesions. In contrast, in only 33% of the mice treated with 62.5 mg of LY per kg per day was only one kidney involved. Moreover, scoring of lesions on a subjective, semiquantita-
tive scale from 0 to 4+ suggested that the one kidney involved after AmB treatment was relatively less diseased (1 of 40 was 2+ or more) than those from LY-treated mice (15 of 26 were 2+ or more).

Fifty percent of kidneys from AmB-treated mice were atrophied in appearance and, as 75% of these kidneys had no grossly evident fungal disease, this may be due to AmB nephrotoxicity (11, 15). Atrophied kidneys were rarely noted in control mice or in mice receiving LY and then only in the presence of 4+ lesions.

No grossly evident disease was noted in the livers, stomachs, or abdomens of infected mice. Two mice treated with 6.25 mg of AmB per kg per day presented with enlarged spleens, and these were the only mice receiving this drug dose which also demonstrated obvious kidney lesions.

**Effect of LY or AmB treatment on C. albicans CFU recovered from kidneys or spleens of infected mice.** Each spleen and pair of kidneys were removed from mice surviving to day 30 and were homogenized, serially diluted, and cultured for the determination of CFU (Fig. 2).

Each group of surviving untreated control, PEG 300 diluent-treated, or LY-treated (0.625 mg/kg per day) mice had a mean of 10⁶ to 10⁷ CFU per two kidneys (the range of individual values was 1.06 × 10⁶ to 3.51 × 10⁶ CFU). Mice treated with 6.25 or 62.5 mg of LY per kg per day had a mean of 10³ to 10⁵ CFU per two kidneys (range, 3.95 × 10³ to 2.23 × 10⁵), whereas mice treated with 0.625 or 6.25 mg of AmB per kg per day had a mean of 9 × 10³ CFU (range, 2.1 × 10³ to 2.8 × 10⁴) or 5 × 10² CFU (range, 0 to 2.1 × 10³), respectively. Only one mouse treated with 6.25 mg of AmB per kg per day had sterile kidneys; this was the only experimental animal which was apparently microbiologically cleared of all *Candida* infection by any treatment (this mouse also had a sterile spleen, as described below). The clearance in all treatment groups except the 0.625-mg/kg-per-day LY group was significantly superior to that in controls (untreated plus PEG 300 diluent treated) (P < 0.001). The higher AmB dose regimen was significantly superior to each LY regimen in terms of clearance, and the lower AmB regimen was significantly superior to the lower two LY regimens (0.625 and 6.25 mg/kg per day) (P < 0.001 for all comparisons). The higher AmB dose was superior to the lower AmB dose (P < 0.01), and the lower LY regimen was significantly inferior to the two higher LY regimens (P < 0.001).

All untreated control, PEG 300-treated, and LY-treated (0.625 and 6.25 mg/kg per day) mice had residual splenic fungal disease after specimens were taken from them for culture (Fig. 2). Eighty percent of spleens from surviving mice treated with LY (62.5 mg/kg per day) or AmB (0.625 mg/kg per day) were culture positive, whereas no spleens from mice treated with AmB (6.25 mg/kg per day) were infected.

Each group of mice treated with 0.625 or 6.25 mg of LY per kg per day had a mean of 10⁵ to 10⁶ CFU per spleen (range, 80 to 2,405). Mice treated with 62.5 mg of LY per kg per day or 0.625 mg of AmB per kg per day and controls had a mean of 10⁴ to 10⁵ CFU per spleen (range, 0 to 120). Only mice treated with 6.25 mg of AmB per kg per day had no detectable *C. albicans* in the spleen. The clearance in each treatment group was significantly superior to that in the controls (P < 0.001). The higher AmB dose regimen was superior to the lower two LY regimens (P < 0.001) or the

![FIG. 2. CFU of *C. albicans* recovered from organs of mice surviving experimental systemic candidiasis to day 30. Each point represents either CFU from both kidneys (●) or the spleen (▲) of one mouse. Bars represent the mean CFU recovered from the kidneys or spleen of surviving mice in each experimental group.](http://aac.asm.org/fig2.jpg)
higher LY regimen ($P < 0.005$), and the lower AmB regimen was significantly superior to the lower two LY regimens ($P < 0.001$) but, as with the comparison with residual kidney infection, not significantly different from the 62.5-mg/kg-per-day LY regimen. The two AmB regimens were not significantly different from each other with respect to spleen disease; the highest LY regimen was superior to the lower LY regimens ($P < 0.001$), and the 6.25-mg/kg-per-day LY regimen was superior to the 0.625-mg/kg-per-day LY regimen ($P < 0.05$).

**DISCUSSION**

LY and AmB have been demonstrated to have equally potent antifungal activities in vitro (5, 13). Preliminary studies have indicated that a correlation exists between the in vitro and in vivo antifungal activities of LY (5). We chose to examine and compare the effects of LY and AmB on experimentally induced systemic candidiasis by using an isolate of *C. albicans* susceptible to both LY and AmB.

We found that treatment of mice with LY at 6.25 or 62.5 mg/kg per day significantly prolonged the survival of infected mice. LY was less effective than AmB on a milligram-per-kilogram-per-day basis, since treatment with either 0.625 or 6.25 mg of AmB per kg per day resulted in 100% survival (compare with the same LY doses). Estimates of relative effectiveness suggest that AmB may be at least 100 times more effective than LY in terms of milligrams per kilogram per day (i.e., AmB at 0.625 mg/kg per day was somewhat more effective for survival, diminution of kidney lesions, and recoverable CFU from organs than was LY at 62.5 mg/kg per day).

LY appears to be less toxic than AmB since treatment with 62.5 mg of AmB per kg per day in the absence of infection was lethal while the same dose of LY significantly prolonged the survival of infected mice. The observed atrophy of kidneys in 40% of AmB-treated mice (even though 75% of those kidneys showed no evidence of fungal disease) indicates that AmB may be nephrotoxic in mice. Additional evidence of toxicity was observed, as mice receiving AmB appeared ill during therapy. Mice that were treated with LY (62.5 mg/kg per day) and that survived appeared to be well throughout the study. LY has been reported to have 1/20 the renal toxicity of AmB in dogs (5), and our observations indicate that LY has comparatively less toxicity than AmB in mice as well. The difference in toxicity we observed could be estimated to be 100-fold, since mice given 0.625 mg of AmB per kg per day or 62.5 mg of LY per kg per day lacked the toxic signs of 6.25-mg/kg-per-day AmB-treated mice, and some members of the 0.625-mg/kg-per-day AmB group also had atrophied kidneys.

LY treatment also lessens residual disease in the kidneys (6.25 or 62.5 mg/kg per day) and spleens (62.5 mg/kg per day) of survivors, as demonstrated by a log fewer recoverable *Candida* organisms in drug-treated versus control mice. AmB was more effective than LY at reducing recoverable CFU from the kidneys (3 logs lower for AmB at the same doses) or spleens (1 log lower for AmB at 0.625 mg/kg per day and 2 logs lower for AmB at 62.5 mg/kg per day, with the latter dosage resulting in complete sterilization).

Previous studies have suggested that LY was effective against *C. albicans* in a model of intraperitoneally induced systemic candidiasis. LY administered orally at a dose of 6.25 mg/kg per day reduced the recovery of *Candida* organisms from kidneys by 2 logs (5). The drug was given either 4 h before infection or 24 h after infection and for 5 days thereafter. Our treatment regimen allowed for the establishment of infection for 4 days prior to the initiation of treatment. This regimen may therefore more closely approximate the clinical course of disease and therapy. The therapeutic effectiveness of LY for the reduction of CFU recovered from mouse kidneys was somewhat better in the previous study (5), in which CFU from kidneys were reduced by 2 logs, relative to our own study, in which LY treatment resulted in a reduction of CFU from kidneys by 1 log. These differences may be due to the delay before initiation of treatment in our system or to the different route of infection employed (intraperitoneal versus intravenous).

The antifungal effects of LY have been ascribed to its inhibition of fungal cell wall β-1,3-glucan biosynthesis (5). Since this mode of action is different from that of the conventional antifungal drugs such as AmB, which binds to membrane sterols causing leakage (6, 11), or the azoles, which inhibit sterol synthesis and have other actions (3, 12, 16), it would be of interest to determine whether synergistic therapies could be developed between LY and other antifungal agents. Alternatively, the administration of LY concentrations slightly higher than those tested in our study (greater than 62.5 mg/kg per day) would be of interest to examine, since significant toxicity (hepatotoxicity) does not appear to occur until doses of 100 mg/kg are administered (5). Synergistic drug regimens may prove to be the most effective, however, since LY requires active metabolism by candidal cells for antifungal effectiveness (5) while AmB or the azoles are also effective against nonmetabolizing fungi (6, 11, 16).

We conclude that LY may prove to be of therapeutic value alone or in combination with other antifungal agents in the treatment of systemic candidal infection; however, while LY and AmB were effective in suppressing candidal infection, neither drug alone was curative in our model.

**LITERATURE CITED**


