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Safety and Efficacy of Multilamellar Liposomal Nystatin against Disseminated Candidiasis in Persistently Neutropenic Rabbits

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The activity of liposomal nystatin (L-Nys) against subacute disseminated candidiasis was investigated in persistently neutropenic rabbits. Antifungal therapy was administered for 10 days starting 24 h after intravenous inoculation of 10^3 blastoconidia of *Candida albicans*. Responses to treatment were assessed by the quantitative clearance of the organism from blood and tissues. Treatments consisted of L-Nys at dosages of 2 and 4 mg/kg of body weight/day (L-Nys2 and L-Nys4, respectively) amphotericin B deoxycholate at 1 mg/kg/day (D-AmB), and fluconazole at 10 mg/kg/day (Flu). All treatments were given intravenously once daily. Compared to the results for untreated but infected control animals, treatment with L-Nys2, L-Nys4, D-AmB, and Flu resulted in a significant clearance of the residual burden of *C. albicans* from the kidney, liver, spleen, lung, and brain ($P < 0.0001$ by analysis of variance). When the proportion of animals infected at at least one of the five tissue sites studied was evaluated, a dose-dependent response to treatment with L-Nys was found ($P < 0.05$). Compared to D-AmB-treated rabbits, mean serum creatinine and blood urea nitrogen levels at the end of therapy were significantly lower in animals treated with L-Nys2 ($P < 0.001$) and L-Nys4 ($P < 0.001$ and $P < 0.01$, respectively). L-Nys was less nephrotoxic than conventional amphotericin B and had dose-dependent activity comparable to that of amphotericin B for the early treatment of subacute disseminated candidiasis in persistently neutropenic rabbits.

Candida species are important causes of invasive fungal infections in neutropenic patients (1, 15). Standard treatment with amphotericin B deoxycholate (D-AmB) is associated with a crude mortality rate of 20 to 50% (3, 9, 21, 22, 25, 35), and the crude mortality rate reaches almost 100% in the presence of deep tissue involvement and persistent neutropenia (8, 9, 20). Although the current antifungal azoles have expanded our therapeutic options, they are not considered first-line agents in the neutropenic patient due to deficiencies in either their pharmacokinetics or their antifungal spectra (7, 16). Thus, there is a continuing need for novel antifungal compounds that have potent, broad-spectrum antifungal activity, favorable pharmacokinetic properties, and tolerable toxicity.

Nystatin was discovered in the early 1950s as the first polyene antifungal antibiotic (18). It binds to ergosterol, the main sterol in the cell membrane of fungi and *Leishmania* spp., leading to channel formation, efflux of protons and cations, and concentration-dependent cell death (11, 17, 19). Although nystatin has potent, broad-spectrum fungicidal activity in vitro, problems with solubilization and systemic toxicity precluded its development as a parenteral therapeutic agent (17). In the late 1980s, however, laboratory investigators at the University of Texas incorporated nystatin into a multilamellar liposome preparation consisting of dimyristoyl phosphatidylcholine (DMPC) and dimyristoyl phosphatidylglycerol (DMPG) in a 7:3 molar ratio (23). While it preserved antifungal activity in vitro, the liposomal formulation of nystatin (L-Nys) was considerably less toxic to mammalian cells than the free drug (23) and prolonged survival in a murine model of disseminated candidiasis (24). More recently, a multilamellar liposome formulation of nystatin with the same constituents but with a defined particle size of 0.1 to 3 μm (34) has been launched for clinical development. This liposomal formulation has been shown to improve survival in a murine model of disseminated aspergillosis (28) and to provide effective microbiological clearance in a persistently neutropenic rabbit model of invasive pulmonary aspergillosis (12). It was well tolerated in patients without dose-limiting toxicity at dosages of up to 8 mg/kg of body weight/day (4) and is undergoing phase II and III clinical trials in nonneutropenic patients with candidemia, in neutropenic cancer patients who require empirical antifungal therapy, and as salvage therapy for patients with refractory invasive candidiasis and other invasive mycoses.

Little is known, however, about the in vivo activity of L-Nys against deeply invasive candidiasis in the setting of profound and persistent neutropenia. We therefore investigated the efficacy and safety of this novel polyene formulation in a persistently neutropenic rabbit model of subacute disseminated candidiasis and compared it to therapy with amphotericin B deoxycholate and fluconazole.

MATERIALS AND METHODS

Animals. New Zealand White rabbits (weight, 2.5 to 3.5 kg; Hazleton, Denver, Pa.) were used for all experiments. They were individually housed and were given water and standard rabbit feed ad libitum according to National Institutes of Health guidelines (5). Nontraumatic vascular access was established in each rabbit by the surgical placement of a subcutaneous silastic central venous catheter (31).

Immunosuppressive regimen and supportive care. Cytosine arabinoside (ara-C; Upjohn Pharmaceuticals, Kalamazoo, Mich.) was administered intravenously at 440 mg/m^2 on days 1 through 5 and on days 8 to 9 and 13 to 14 to produce profound and persistent neutropenia, respectively. The mean granulo-

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TABLE 1. Residual *C. albicans* NIH-8621 burden in tissues of untreated control rabbits and rabbits treated with either D-AmB, Flu, L-Nys2, or L-Nys4

Treatment group	Log CFU/g[a]				
	Kidney	Liver	Spleen	Lung	Brain
Controls	6.69 ± 0.28 (8)	6.30 ± 0.17 (8)	6.87 ± 0.38 (8)	4.39 ± 0.34 (8)	2.89 ± 0.81 (6)
D-AmB	0.28 ± 0.28 (1)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)
Flu	0.19 ± 0.19 (1)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)
L-Nys2	1.05 ± 0.71 (2)	1.35 ± 0.89 (2)	0.89 ± 0.67 (2)	0.97 ± 0.64 (2)	0.74 ± 0.55 (2)
L-Nys4	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)	0 ± 0 (0)

[a] All values represent the means ± SEMs for eight rabbits. The number of infected animals is given in parentheses. P was <0.0001 by ANOVA among the five experimental groups.

cyte counts on the day of inoculation ranged from 154 to 237/μl in all five cohorts (differences were not significant by analysis of variance [ANOVA]) and were below 25/μl on days 13 and 17. Concomitant platelet counts ranged from 13,000 to 26,000/μl between cohorts (the difference was not significant). Starting on day 4, ceftazidime (Glaxo Pharmaceuticals, Research Triangle Park, N.C.) was administered at 75 mg/kg intravenously twice daily, gentamicin (Baxter Health Care Corp., Deerfield, Ill.) was administered at 5 mg/kg intravenously every other day, and vancomycin (Eli Lilly & Co., Indianapolis, Ind.) was administered at 15 mg/kg intravenously daily to prevent the occurrence of invasive bacterial infections during neutropenia.

Test strain. *Candida albicans* NIH-8621 was from a granulocytopenic patient with autopsy-confirmed disseminated candidiasis and was used for all experiments. The isolate was subcultured from a frozen stock culture (stored at −70°C in a 10% skim milk, 15% glycerol solution) on Sabouraud dextrose agar plates, incubated for 24 h at 35°C, and maintained during the course of the experiments at 4°C.

The MICs of the antifungal agents for *C. albicans* NIH-8621 was determined by the broth microdilution method of the National Committee for Clinical Laboratory Standards (26). Antibiotic medium 3 (Media Department, Clinical Center, National Institutes of Health) was used instead of RPMI for determination of the MICs of amphotericin B and L-Nys. For determination of the minimum fungicidal concentration (MFC), an aliquot of 100 μl was removed from wells demonstrating no growth after 48 h and was plated and incubated for 24 h on Sabouraud dextrose agar containing chloramphenicol and gentamicin. The MFC was defined as the highest dilution that revealed no growth after 48 h of incubation at 35°C. The MIC at 48 h (MIC_{48}) of L-Nys for *C. albicans* NIH-8621 was 2 μg/ml and those of D-AmB and fluconazole (Flu) were 0.250 and 0.250 μg/ml, respectively. For both L-Nys and D-AmB, the MFC was identical to the MIC.

Preparation of inoculum. The inoculum was grown in Emmon's modified Sabouraud dextrose broth (pH 7.0; Media Department, Clinical Center, National Institutes of Health) as described in detail elsewhere (33), incubated in a gyratory water bath at 37°C for 18 h, and then centrifuged and washed. The concentration of the suspension was adjusted by use of a hemacytometer and was retrospectively confirmed with quantitative cultures of 10-fold serial dilutions. Inoculation was performed on day 6 of the experiment. The inoculum was 10^3 CFU and was administered in a volume of 5 ml of sterile normal saline over 1 min via the indwelling silastic central venous catheter.

Antifungal therapy. L-Nys (Nyotran; 50 mg of nystatin USP in a mixture of 350 mg of DMPC and 150 mg of DMPG; Aronex Pharmaceuticals, The Woodlands, Tex.) was provided as a lyophilized powder that was maintained at 4°C. Prior to use the drug was freshly reconstituted with 50 ml of sterile normal saline to a 1-mg/ml solution, as recommended by the manufacturer, and was administered intravenously once daily over 10 min at either 2 mg/kg/day (L-Nys2) or 4 mg/kg/day (L-Nys4). These dosages have been selected for clinical investigation of the efficacy of L-Nys against candidemia and for empirical antifungal therapy in cancer patients with neutropenia and persistent fever. Amphotericin B (50-mg vials; Fungizone, Bristol Myers-Squibb, Princeton, N.J.) was prepared as required and was administered intravenously once daily over 10 min. A dosage of 1 mg/kg/day was used. Flu (2 mg/ml; Diflucan solution for infusion; Pfizer, Groton, Conn.) was administered once daily as a steady intravenous bolus over 1 min at a dosage of 10 mg/kg/day.

Antifungal treatment was begun 24 h after inoculation and was administered daily throughout the experiment. All experiments included treated rabbits and infected but untreated control animals. Each of the five cohorts of the study consisted of eight rabbits.

Assessment of antifungal efficacy. The main endpoint for the assessment of antifungal efficacy of L-Nys was determination of the quantitative clearance of *C. albicans* from tissue. Further endpoints included the time to the clearance of the organism as determined with cultures of blood, the definite clearance of the organism at the end of the experiment as determined with cultures of blood, and survival (in days postinoculation). The pattern of infection of subacute disseminated candidiasis, however, permitted survival of nearly all rabbits until the termination of the experiment.

The presence of candidemia was monitored starting on the day after inoculation for 4 consecutive days and on the last day of the experiment prior to euthanasia by culturing 1 ml of whole blood drawn before dosing on Sabouraud dextrose agar containing chloramphenicol and gentamicin. The duration of survival (in days postinoculation) was recorded for each rabbit. The surviving rabbits were killed 24 h after administration of the 10th dose of antifungal treatment on the 11th day postinoculation by intravenous injection of pentobarbital; and kidney, liver, spleen, lung, and brain tissue as well as cerebrospinal fluid (CSF) were obtained for microbiological cultures. For each rabbit, representative sections of >1 g of tissue were weighed and homogenized in 5 ml of sterile normal saline in Tekmar sterile reinforced polyethylene bags (Tekmar, Cincinnati, Ohio) (29). Each tissue homogenate and each CSF sample was serially diluted 100-fold from 10^{-2} to 10^{-4} in sterile normal saline. A total of 1 ml of whole blood and a 0.1-ml quantity of undiluted tissue homogenate, CSF, and each dilution were separately plated onto Sabouraud dextrose agar containing chloramphenicol and gentamicin. Culture plates were incubated at 37°C for 24 h, after which the number of CFU per gram or per milliliter was calculated for each specimen. This method was sensitive for detection of ≥10 CFU/g or ≥10 CFU/ml.

Monitoring of toxicity. Serum samples were collected from each rabbit before administration of the first dose of ara-C and on days 7, 9, 11, and 17 of the experiment. Samples were stored at −80°C until they were assayed. Clinical chemistry values were analyzed with the last sample drawn from each rabbit and were compared among groups and to the range of values observed in normal, healthy rabbits. A complete blood count was determined before administration of the first dose of ara-C and on day 17 prior to euthanasia.

Statistical considerations. Differences between the means of continuous data among groups were evaluated by nonparametric ANOVA with Bonferroni's correction for multiple comparisons, as appropriate. Differences between proportions were analyzed by the Fisher exact test. All values were tabulated as means ± standard errors of the means (SEMs). A two-tailed P value of ≤0.05 was considered statistically significant.

RESULTS

Antifungal efficacy. L-Nys demonstrated dose-dependent antifungal efficacy in the treatment of subacute disseminated candidiasis. While all eight control animals were heavily infected at the postmortem examination, for four of eight rabbits treated with L-Nys2 ($P < 0.05$ versus controls) and none of 8 rabbits treated with L-Nys4 ($P < 0.001$ versus controls), *C. albicans* was recovered from at least one tissue site ($P < 0.05$ for comparison of both dosage groups). In the cohorts of D-AmB-treated and Flu-treated rabbits, one rabbit each was found to be infected ($P < 0.001$ versus untreated controls; the difference was not significant versus the result for animals treated with L-Nys).

Quantitative assessment of the residual fungal burden in the kidneys, livers, spleens, lungs, and brains of rabbits treated with L-Nys revealed a significant clearance of the infecting organism from these sites compared to clearance from untreated controls ($P < 0.0001$ by ANOVA) (Table 1). There was no statistically measurable difference between rabbits treated with the two dosages of L-Nys or between rabbits treated with L-Nys and animals treated with either D-AmB or Flu. For all 36 rabbits, including 4 control animals, from which CSF could be sampled at the postmortem examination, cultures of CSF remained negative.

TABLE 2. Effects of L-Nys on parameters of renal function as determined by analysis of serum in comparison to effects of no treatment or treatment[a] with either AmB-D or Flu

Treatment group	Creatinine concn (mg/dl)	BUN concn (mg/dl)	Potassium concn (mmol/liter)	Magnesium concn (mmol/liter)	Bicarbonate concn (mmol/liter)
Controls	1.76 ± 0.17[b] (1.10–2.40)	23.5 ± 2.2[b] (12.0–34.0)	3.15 ± 0.15[c] (2.20–3.60)	0.76 ± 0.03[c] (0.59–0.90)	20.7 ± 0.9 (18.1–25.9)
D-AmB	4.91 ± 0.69 (1.80–7.00)	133.6 ± 17.7 (62.0–215)	4.01 ± 0.24 (3.20–5.30)	0.95 ± 0.02 (0.85–1.05)	17.1 ± 1.4 (11.7–23.1)
Flu	1.16 ± 0.05[b] (1.00–1.40)	21.1 ± 4.8[b] (7.0–53.0)	4.34 ± 0.5[d] (3.70–5.10)	0.79 ± 0.02 (0.72–0.91)	23.2 ± 0.7[c] (20.3–26.3)
L-Nys2	1.45 ± 0.16[b] (1.00–2.40)	22.2 ± 2.6[b] (12.0–38.0)	3.93 ± 0.27 (2.50–4.90)	0.84 ± 0.05 (0.65–1.06)	21.2 ± 1.1 (17.9–28.0)
L-Nys4	2.76 ± 0.48[e] (1.20–5.00)	48.0 ± 10.2[b] (19.0–101)	3.94 ± 0.11 (3.50–4.50)	0.96 ± 0.05[f] (0.75–1.15)	19.2 ± 1.6 (11.7–25.3)
Normal values	0.72 ± 0.02 (0.50–1.00)	18.0 ± 0.71 (13.0–26.0)	4.50 ± 0.08 (3.80–5.40)	91 ± 0.01 (0.82–1.05)	26 ± 0.29 (22.0–29.0)

[a] All values for experimental rabbits represent the means ± SEMs for eight rabbits; normal values (means ± SEMs) are derived from 24 healthy, catheterized rabbits. Values in parentheses are ranges. All *P* values were determined by ANOVA and Bonferroni's correction for multiple comparisons.
[b] $P < 0.001$ versus D-AmB-treated animals.
[c] $P < 0.05$ versus D-AmB-treated animals.
[d] $P < 0.01$ versus untreated control animals.
[e] $P < 0.01$ versus D-AmB-treated animals.
[f] $P < 0.05$ versus controls and Flu-treated animals.

The bloodstreams of all treated rabbits were cleared of *C. albicans* within 24 h; however, despite the presence of the indwelling silastic catheter and extensive tissue infection, only two of eight untreated control animals exhibited persistent candidemia for the investigated time of 96 h postinoculation. Also, while three additional animals in the control group had positive blood cultures at the time of the termination of the experiment, all blood cultures for animals that received antifungal therapy remained negative.

Toxicity. Treatment with L-Nys was associated with significantly less nephrotoxicity than treatment with D-AmB, as assessed by the mean serum creatinine and blood urea nitrogen levels at the end of treatment ($P < 0.01$ to $P < 0.001$ for all comparisons) (Table 2). No significant differences between treatment groups were noted with regard to serum potassium levels. There were, however, increases in serum magnesium levels in D-AmB- and L-Nys4-treated rabbits, which reached statistical significance in the latter group, and a trend toward low serum bicarbonate levels in D-AmB- and L-Nys4-treated rabbits. These observations may reflect impaired renal clearance of magnesium and early renal tubular acidosis, respectively.

With the exception of one rabbit each in both the D-AmB- and the Flu-treated groups which sustained moderate elevations in serum transaminase levels, rabbits that received antifungal treatment had normal bilirubin, alanine aminotransferase, and alkaline phosphatase levels at the end of treatment (Table 3). By comparison, untreated control animals had significantly elevated mean bilirubin and hepatic transaminase levels as the means of expression of the uninhibited fungal infection of the liver ($P < 0.001$ for all comparisons).

There was a significant decrease in the mean serum albumin level in untreated control rabbits at the end of treatment. No differences in the mean serum cholesterol level were noted between groups. In comparison to untreated controls and animals treated with D-AmB, however, rabbits that had received Flu or L-Nys had significantly lower mean serum trigliceride levels (Table 3).

No differences in the change in hemoglobin levels or platelet counts from the baseline to the end of therapy or in the absolute mean end-of-therapy values were observed among the five cohorts. Also, there were no clinically apparent infusion-related toxicities and no significant differences regarding weight changes during the course of the experiment.

Survival. All rabbits treated with L-Nys2 and L-Nys4 survived until the termination of the experiment. There was one premature death, on day 10 postinoculation, in each of the D-AmB- and Flu-treated cohorts. Both animals had completely cleared the organism from their bloodstreams and tissues, and there was no evidence of bacterial superinfection. The serum creatinine level was elevated in the D-AmB-treated rabbit but not in the Flu-treated animal. As to be expected by the study design, the majority of untreated control rabbits (n = 5 of 8 [62.5%]) survived until the end of the experiment.

DISCUSSION

The results of this study demonstrate that L-Nys2 or L-Nys4 is as active as standard dosages of AmB-D or Flu for the early treatment of subacute disseminated candidiasis in persistently neutropenic rabbits. Responses to treatment with L-Nys were dosage dependent, with complete microbiological clearance of all investigated tissue sites by treatment with L-Nys4. Overall, L-Nys was less nephrotoxic than conventional amphotericin B, and rabbits treated with L-Nys displayed no apparent hepatic, hematological, or metabolic toxicities.

These findings are important, as experimental data on the in vivo antifungal activity of the currently developed liposomal formulation of nystatin against *Candida* species are scant, are limited to survival studies, and have not been published. Our study demonstrates for the first time the principal ability of L-Nys to clear *Candida* spp. from blood and tissues in a manner equivalent to that of standard therapies. This lends important scientific support to the evaluation of the compound in clinical trials with patients with proven or documented invasive *Candida* infections. Indeed, in an ongoing phase II and III multicenter study with nonneutropenic patients with candidemia, L-Nys2 showed promising antifungal activity, and the rate of successful treatment with L-Nys4 was comparable to the rate of successful treatment with conventional amphotericin B in a historical control group of patients (10, 27).

The pharmacokinetics of L-Nys in plasma after intravenous administration to rabbits (13) are overall comparable to those in the blood of human subjects reported previously (6). As shown previously (13), on the basis of the area under the plasma concentration-versus-time curve from time zero to 24 h and the observation that concentrations in plasma are approximately twice as high as the corresponding levels in blood (14), a 4-mg/kg dose in a healthy rabbit approximately corresponds to a 2-mg/kg dose in humans and a 6-mg/kg dose in the rabbit approximately corresponds to a 3-mg/kg dose in humans. The up to twofold lower projected mean peak levels in the plasma

TABLE 3. Effects of L-Nys on parameters of hepatic and metabolic function as determined by analysis of serum in comparison to the effects of no treatment or treatment with either D-AmB or Flu

Treatment group	Bilirubin concn (mg/dl)	Alk.Phos. concn (U/liter)	ALT concn (U/liter)	AST concn (U/liter)	Albumin concn (g/dl)	Triglyceride concn (mg/dl)	Cholesterol concn (mg/dl)
Controls	0.98 ± 0.19 (0.14–1.65)	38.7 ± 7.9 (8–69)	166.8 ± 36.2 (68–396)	218.8 ± 47.8 (70–454)	2.21 ± 0.14 (1.60–2.70)	257.8 ± 35.7 (82–398)	110.7 ± 9.3 (75–146)
D-AmB	0.15 ± 0.00[b] (0.11–0.19)	41.1 ± 5.0 (23–62)	33.2 ± 18.6[b] (8–163)	83.1 ± 73.5 (6–598)	3.30 ± 0.19[b] (2.60–4.10)	193.1 ± 26.1 (56–312)	137.2 ± 9.2 (108–191)
Flu	0.16 ± 0.00[b] (0.12–0.20)	41.5 ± 6.5 (18–63)	37.6 ± 7.5[b] (20–84)	33.7 ± 19.5 (10–170)	3.63 ± 0.13[b] (3.10–4.10)	53.8 ± 7.1[b,c] (32–89)	110.3 ± 10.5 (73–156)
L-Nys2	0.15 ± 0.00[b] (0.13–0.18)	38.2 ± 3.75 (14–47)	27.5 ± 3.7[b] (15–41)	10.8 ± 1.7[d] (6–22)	3.34 ± 0.16[b] (2.50–3.90)	81.5 ± 21.5[b] (26–221)	117.2 ± 14.0 (67–169)
L-Nys4	0.18 ± 0.01[b] (0.12–0.23)	42.6 ± 6.7 (20–77)	22.0 ± 2.6[b] (11–35)	8.2 ± 0.5[e] (6–10)	3.51 ± 0.18[b] (2.40–4.00)	115.1 ± 21.8[e,f] (45–234)	119.6 ± 14.0 (59–156)
Normal values	0.11 ± 0.00 (0.10–0.19)	108 ± 6.6 (64–235)	43 ± 5.8 (20–163)	16 ± 2.0 (8–45)	3.93 ± 0.11 (3.40–6.40)	46 ± 2.66 (25–79)	44 ± 2.05 (29–67)

[a] All values represent the means ± SEMs for eight rabbits each; Normal values (means ± SEMs) are derived from 24 healthy, catheterized rabbits. Values in parentheses are ranges. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Alk.Phos. alkaline phosphatase. ALL *P* values were determined by ANOVA and Bonferroni's correction for multiple comparisons.
[b] $P < 0.001$ versus untreated controls.
[c] $P < 0.01$ versus D-AmB-treated animals.
[d] $P < 0.05$ versus untreated controls.
[e] $P < 0.0$ versus untreated controls.
[f] $P < 0.05$ versus D-AmB-treated animals.

of humans after the administration of equivalent doses may be accounted for mainly by the relatively rapid infusion rate of 10 min in the rabbit but also by the high interindividual variability in human patients (13). These pharmacokinetic considerations may serve to validate the efficacy data from our experiments with regard to their implications for clinical studies. In a broader sense, they also underscore the essential need for integration of pharmacokinetic data into the preclinical evaluation of investigational drugs.

With the introduction and maintenance of profound neutropenia, broad-spectrum antibiotic therapy, a relatively low inoculum, and the start of treatment within 24 h after inoculation, our infection model most closely resembles the clinical situation in a neutropenic patient who presents with persistent fever and early, occult *Candida* infection or early phases of overt candidemia, which is probably the most frequently encountered clinical situation. The results of this study cannot be directly applied to preventive modalities and more advanced stages of acute disseminated candidiasis and chronic disseminated candidiasis, for which separate experimental investigations would be required (30). It bears notice, however, that cultures of blood from only three of the five surviving untreated animals were positive for *C. albicans* at the termination of the experiment, despite uncontrolled and extensive tissue infection. This observation may serve to emphasize the fact that overt candidemia represents only part of the clinical spectrum of invasive candidiasis (2, 9) and that new, non-culture-based diagnostic approaches continue to be urgently needed.

In healthy rabbits, a dosage of 6 mg of L-Nys per kg per day administered intravenously for 14 days produced only mild increases in serum creatinine levels, which did not exceed 1.5 times the baseline levels, and no increases in the blood urea nitrogen values (13). The 4-mg/kg/day dosage was tolerated without signs of nephrotoxicity and was therefore used as the maximum dosage in this study. Although nephrotoxicity occurred in some animals that received 4 mg/kg/day in the setting of disseminated candidiasis, dehydration, catabolism, immunosuppression, and vancomycin and gentamicin therapy, L-Nys was overall significantly less nephrotoxic than D-AmB. Our toxicity data are overall consistent with results from a clinical phase I study with 32 neutropenic patients with hematological malignancies and fever refractory to broad-spectrum antibiotics. The patients received multiple doses of L-Nys at escalating dosages of up to 8 mg/kg/day without dose-limiting nephrotoxicity (4). Similarly, in the ongoing phase II and III study with nonneutropenic patients with candidemia who are receiving L-Nys2, no patient has experienced therapy-limiting nephrotoxicity (27). A doubling of the serum creatinine levels from those at the baseline was observed for 14% of patients; this rate is less than that reported for patients treated with D-AmB (10) and is approximately equivalent to that for neutropenic patients treated with liposomal amphotericin B at a dosage of 3 mg/kg/day and investigated in the setting of empirical antifungal treatment (32).

In conclusion, L-Nys demonstrated potent, dose-dependent antifungal activity in the early treatment of subacute disseminated candidiasis in persistently neutropenic rabbits. It was as effective as Flu and amphotericin B but was less nephrotoxic than the latter. Its demonstrable efficacy and safety as well as its broad spectrum of fungicidal activity support the further clinical investigation of L-Nys for the treatment of candidemia and for empirical antifungal therapy in persistently febrile neutropenic patients.

REFERENCES

1. **Anaissie, E. J., J. H. Rex, O. Uzun, and S. Vartivarian.** 1998. Predictors of adverse outcome in cancer patients with candidemia. Am. J. Med. **104:**238–245.
2. **Berenguer, J., M. Buck, F. Witebsky, F. Stock, P. A. Pizzo, and T. J. Walsh.** 1993. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis. Diagn. Microbiol. Infect. Dis. **17:**103–109.
3. **Besnard, M., O. Hartmann, D. Valteau-Couanet, M. Robert, L. Brugieres, and J. Lemerle.** 1993. Systemic Candida infection in pediatric BM-autotransplantation: clinical signs, outcome and prognosis. Bone Marrow Transplant. **11:**465–470.
4. **Boutati, E., H. C. Maltezou, G. Lopez-Berestein, S. E. Vartivarian, and E. Anaissie.** 1995. Phase I study of maximum tolerated dose of intravenous liposomal nystatin for the treatment of refractory febrile neutropenia in patients with haematological malignancies, abstr. LM22, p. 330. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
5. **Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.** 1996. Guide for the care and use of laboratory animals. National Academy Press, Washington, D.C.
6. **Cossum, P. A., J. Wyse, Y. Simmons, T. L. Wallace, and A. Rios.** 1996. Pharmacokinetics of Nyotran (liposomal nystatin) in human patients, abstr. A88, p. 17. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
7. **Edwards, J. E., G. P. Bodey, R. A. Bowden, T. Buchner, B. E. de Pauw, S. G. Filler, M. A. Ghannoum, M. Glauser, R. Herbrecht, C. A. Kauffman, S. Kohno, P. Martino, F. Meunier, T. Mori, M. A. Pfaller, J. H. Rex, T. R. Rogers, R. H. Rubin, J. Solomkin, C. Viscoli, T. J. Walsh, and M. White.** 1997. International Conference for the Development of a Consensus on the Management and Prevention of Severe Candidal Infections. Clin. Infect. Dis. **25:**43–59.
8. **Goodman, J. L., D. J. Winston, R. A. Greenfield, P. H. Chandrasekar, B. Fox, H. Kaizer, R. K. Shadduck, T. C. Shea, P. Stiff, and D. J. Friedman.** 1992. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N. Engl. J. Med. **326:**845–851.
9. **Goodrich, J. M., E. C. Reed, M. Mori, L. D. Fisher, S. Skerrett, P. S. Dandliker, B. Klis, G. W. Counts, and J. D. Meyers.** 1991. Clinical features and analysis of risk factors for invasive candidal infection after marrow transplantation. J. Infect. Dis. **164:**731–740.
10. **Gordon, D., I. Baird, R. Darouiche, V. Fainstein, L. Juaregui, C. Levy, and P. Lewis.** 1997. Liposomal nystatin vs. amphotericin B for candidemia in non-neutropenic patients: a historical comparison, abstr. 144, p. 98. *In* Program and abstracts of the 35th Meeting of the Infectious Diseases Society of America. Infectious Diseases Society of America, Arlington, Va.
11. **Gosh, B. K., and A. Gosh.** 1967. The effects of antibiotics on Leishmania donovanii. Dermatol. Int. **6:**154–160.
12. **Groll, A. H., C. E. Gonzalez, N. Giri, K. Kligys, W. Love, J. Peter, E. Feuerstein, J. Bacher, S. C. Piscitelli, and T. J. Walsh.** 1999. Liposomal nystatin against experimental pulmonary aspergillosis in persistently neutropenic rabbits: efficacy, safety, and non-compartmental pharmacokinetics. J. Antimicrob. Chemother. **43:**95–103.
13. **Groll, A. H., D. Mickiene, R. Petraitiene, V. Petraitis, M. Candelario, A. Field-Ridley, J. Bacher, S. Piscitelli, and T. J. Walsh.** 1998. Compartmental pharmacokinetics and tissue distribution of liposomal nystatin in rabbits, abstr. J-60, p. 468. *In* Program and abstracts of the 38h Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
14. **Groll, A. H., D. Mickiene, K. Werner, S. C. Piscitelli, and T. J. Walsh.** High-performance liquid chromatographic determination of liposomal nystatin in plasma and tissues for pharmacokinetic and tissue distribution studies. J. Chromatogr., in press.
15. **Groll, A. H., P. M. Shah, C. Mentzel, M. Schneider, G. Just-Nuebling, and K. Huebner.** 1996. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. J. Infect. **33:**23–32.
16. **Groll, A. H., S. C. Piscitelli, and T. J. Walsh.** 1998. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. Adv. Pharmacol. **44:**343–500.
17. **Hamilton-Miller, J. M. T.** 1973. Chemistry and biology of the polyene macrolide antibiotics. Bacteriol. Rev. **37:**166–196.
18. **Hazen, E. L., and R. Brown.** 1950. Two antifungal agents produced by a soil actinomycete. Science **112:**423.
19. **Kleinberg, M. E., and A. Finkelstein.** 1984. Single-length and double-length channels formed by nystatin in lipid bilayer membranes. J. Membr. Biol. **80:**257–269.
20. **Klingspor, L., G. Stintzing, A. Fasth, and J. Tollemar.** 1996. Deep Candida infection in children receiving allogeneic bone marrow transplants: incidence, risk factors and diagnosis. Bone Marrow Transplant. **17:**1043–1049.
21. **Lecciones, J. A., J. W. Lee, E. E. Navarro, F. G. Witebsky, D. Marshall, S. M. Steinberg, P. A. Pizzo, and T. J. Walsh.** 1992. Vascular catheter-associated fungemia in patients with cancer: analysis of 155 episodes. Clin. Infect. Dis. **14:**875–883.
22. **Marina, M. N., P. N. Flynn, G. K. Rivera, and W. T. Hughes.** 1991. Candida tropicalis and Candida albicans fungemia in children with leukemia. Cancer **68:**594–599.
23. **Mehta, R. T., R. L. Hopfer, L. A. Gunner, R. L. Juliano, and G. Lopez-Berestein.** 1987. Formulation, toxicity, and antifungal activity in vitro of liposome-encapsulated nystatin as therapeutic agent for systemic candidiasis. Antimicrob. Agents Chemother. **31:**1897–1900.
24. **Mehta, R. T., R. L. Hopfer, T. McQueen, R. L. Juliano, and G. Lopez-Berestein.** 1987. Toxicity and therapeutic effects in mice of liposome-encapsulated nystatin for systemic fungal infections. Antimicrob. Agents Chemother. **31:**1901–1903.
25. **Meunier, F., M. Aooun, and N. Bitar.** 1992. Candidemia in immunocompromised patients. Clin. Infect. Dis. **14**(Suppl. 1)**:**S120–S125.
26. **National Committee for Clinical Laboratory Standards.** 1997. Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard. NCCLS document M27A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
27. **Rolston, K., I. Baird, D. R. Graham, and L. Jauregui.** 1998. Treatment of refractory candidemia in non-neutropenic patients with liposomal nystatin (Nyotran™), abstr. LB-1, p. 24. *In* Program and abstracts of the 38h Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
28. **Wallace, T. L., V. Paetznick, P. A. Cossum, G. Lopez-Berestein, J. H. Rex, and E. Anaissie.** 1997. Activity of liposomal nystatin against disseminated *Aspergillus fumigatus* infection in neutropenic mice. Antimicrob. Agents Chemother. **41:**2238–2243.
29. **Walsh, T. J., C. McEntee, and D. M. Dixon.** 1987. Tissue homogenization with sterile reinforced polyethylene bags for quantitative culture of *Candida albicans*. J. Clin. Microbiol. **25:**931–932.
30. **Walsh, T. J., J. W. Lee, E. Roilides, P. Francis, J. Bacher, C. A. Lyman, and P. A. Pizzo.** 1992. Experimental antifungal chemotherapy in granulocytopenic animal models of disseminated candidiasis: approaches to understanding investigational antifungal compounds for patients with neoplastic diseases. Clin. Infect. Dis. **14**(Suppl. 1)**:**S139–S147.
31. **Walsh, T. J., P. Bacher, and P. A. Pizzo.** 1988. Chronic silastic central venous catheterization for induction, maintenance, and support of persistent granulocytopenia in rabbits. Lab. Anim. Med. **38:**467–470.
32. **Walsh, T. J., R. W. Finberg, C. Arndt, J. Hiemenz, C. Schwartz, D. Bodensteiner, P. Pappas, N. Seibel, R. N. Greenberg, S. Dummer, M. Schuster, J. S. Holcenberg, and W. E. Dismukes.** 1999. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia. N. Engl. J. Med. **340:**764–761.
33. **Walsh, T. J., S. Aoki, F. Mechinaud, J. Bacher, J. Lee, M. Rubin, and P. A. Pizzo.** 1990. Effects of preventive, early, and late antifungal chemotherapy with fluconazole in different granulocytopenic models of experimental disseminated candidiasis. J. Infect. Dis. **161:**755–760.
34. **Wasan, K. M., M. Ramaswami, S. M. Cassidy, M. Kazemi, F. W. Strobel, and R. L. Thies.** 1997. Physical characteristics and lipoprotein distribution of liposomal nystatin in human plasma. Antimicrob. Agents Chemother. **41:**1871–1875.
35. **Winston, D. J., P. H. Chandrasekar, H. M. Lazarus, J. L. Goodman, J. L. Silber, H. Horowitz, R. K. Shadduck, C. S. Rosenfeld, W. G. Ho, and M. Z. Islam.** 1993. Fluconazole prophylaxis of fungal infections in patients with acute leukemia. Results of a randomized, placebo-controlled, double-blind multicenter trial. Ann. Intern. Med. **118:**495–503.