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Activity of Liposomal Nystatin against Disseminated *Aspergillus fumigatus* Infection in Neutropenic Mice

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The purpose of this study was to examine the activity of liposomal nystatin against a disseminated *Aspergillus fumigatus* infection in neutropenic mice. Mice were made neutropenic with 5-fluorouracil and were administered the antifungal drug intravenously for 5 consecutive days beginning 24 h following infection. Liposomal nystatin, at doses as low as 2 mg/kg of body weight/day, protected neutropenic mice against *Aspergillus*-induced death in a statistically significant manner at the 50-day time point compared to either the no-treatment, the saline, or the empty-liposome group. This protection was approximately the same as that for free nystatin, a positive control. Histopathological results showed that liposomal nystatin cleared the lungs, spleen, pancreas, kidney, and liver of *Aspergillus* and that there was no organ damage at the day 5 time point, which was after only three doses of liposomal nystatin. Based on these results in mice, it is probable that liposomal nystatin will be effective against *Aspergillus* infection in humans.

Aspergillus infections represent a major, life-threatening complication in patients undergoing immunosuppressive therapy for organ transplantation and chemotherapeutic treatment of cancer and in AIDS (4). The frequency of these fungal infections has increased greatly due to the expanded use of immunosuppressive drugs and the increasing prevalence of AIDS (13). Amphotericin B, discovered in the 1950s, is considered the first-line therapy for invasive aspergillosis, although its clinical use is limited by its toxic side effects (46). A promising approach to the toxicity problem has been to formulate amphotericin B in lipid suspensions or liposomes. These new lipid formulations have been shown to have clinical activity, with reduced toxicity compared to amphotericin B (33, 45, 49).

Nystatin, a polyene antibiotic derived from *Streptomyces noursei* (20), is similar in structure to amphotericin B. The mechanism of activity of nystatin and amphotericin B has been attributed to the binding to ergosterol in the fungal membrane, resulting in altered membrane permeability which allows the release of K^+, sugars, and metabolites (48). Nystatin is active against a broad spectrum of fungi in vitro (20) and in vivo (9, 11, 16). In addition to its antifungal activity in vitro and in animals, nystatin has been shown to be effective against a variety of fungi in humans, including *Candida* (32), *Aspergillus* (47), *Histoplasma* (36), and *Coccidioides* (32). Nystatin has demonstrated antifungal activity in humans following administration by different routes, including the oral (8, 39), pleural (28, 34), inhalation (14, 40, 43, 50), and topical (25, 35, 47) routes. Nystatin has been used since the 1950s mainly for the treatment of cutaneous, vaginal, and oral candidiases (3).

The intravenous use of nystatin in humans has been reported for a limited number of patients (32, 38). Although nystatin demonstrated antifungal activity in humans (32), it was generally thought that because of toxicity (thrombophlebitis, fever, chills, and nausea) (32, 38), it was not suitable for intravenous therapy. For this reason, an alternative formulation was sought that would allow the intravenous administration of nystatin. Formulation of nystatin into liposomes by Lopez-Berestein and coworkers in the 1980s established that nystatin could be administered intravenously to mice with good evidence of activity and reduced toxicity (30, 31). A liposomal formulation of nystatin is currently in phase III clinical trials for the treatment of human fungal infections (51). Prior to determining the activity of liposomal nystatin against *Aspergillus* infection in neutropenic humans, the efficacy of liposomal nystatin was determined against disseminated *Aspergillus* infection in neutropenic mice.

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MATERIALS AND METHODS

Animals. Male CF-1 mice were obtained from Harlan Sprague-Dawley (Indianapolis, Ind.) and ranged in weight from 20 to 30 g on the day of dosing. Prior to the study, the mice were quarantined for 5 days. The mice were maintained in a room at 22 ± 3°C and a relative humidity of 55% ± 10% with a 12-h light-dark cycle and 12 air exchanges per h. The mice had ad libitum access to standard rabbit chow (Purina rodent chow 5001) and drinking water. Animals were checked once daily for mortality and moribundity. Animals found moribund were sacrificed but were not analyzed for clinical chemistry or histopathological changes. (Animal studies were conducted in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals [31b] and the U.S. Department of Agriculture Laboratory Animal Welfare Act, 24 August 1966 [49a], and subsequent amendment.)

Liposomal nystatin. Liposomal nystatin (Nyotran) was manufactured according to Good Manufacturing Practices (31a) as a lyophilized product containing nystatin and dimyristoyl phosphatidyl choline,-dimyristoyl phosphatidyl glycerol (7:3). Following reconstitution with sterile saline, each vial of liposomal nystatin contains 1 mg of nystatin per ml.

Free nystatin. Free nystatin (Lederle) was prepared by dissolving nystatin USP in dimethyl sulfoxide (DMSO) and diluting with 0.9% saline to give a final concentration of 1 mg of nystatin/ml in a 5% DMSO solution.

Amphotericin B desoxycholate. Amphotericin B desoxycholate (Fungizone) was obtained in vials as a sterile lyophilized powder from Apothecon. Each vial contained 50 mg of amphotericin B and 41 mg of desoxycholate. It was made to a final concentration of 1 mg of amphotericin B/ml in sterile water.

Empty liposomes. Empty liposomes were prepared in a manner identical to that for liposomal nystatin, except without nystatin. Rehydration was the same as that for liposomal nystatin, with the final concentration of lipid (dimyristoyl

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TABLE 1. Study timetable

Day	Event
−3	5-FU injection
1	*A. fumigatus* inoculation
2	1 mouse/group sacrificed for cultures; drug dosing begun
5	3 mice/group sacrificed for histopathology; 8 mice/group sacrificed for clinical chemistry and organ culture
19	5 mice/group sacrificed for cultures
50	End of study; available mice sacrificed for clinical chemistry and organ culture evaluation
52	Histopathology on 8-mg/kg-liposomal-nystatin mice

phosphatidyl choline-dimyristoyl phosphatidyl glycerol, 7:3) identical to that in the liposomal nystatin formulation (10 mg of lipid/ml).

Saline. Sterile saline (0.9% NaCl) in water for injection, USP, was obtained from American Regent Laboratories, Inc.

In vitro susceptibility testing of liposomal nystatin and amphotericin B desoxycholate. The in vitro activities of liposomal nystatin and amphotericin B desoxycholate against *Aspergillus* species were determined according to the method of Anaissie et al. (1). The *Aspergillus* species were clinical isolates from M. D. Anderson Cancer Center.

Immunosuppression. Neutropenia was induced with a single intraperitoneal injection of 5-fluorouracil (5-FU) (200 mg/kg; Solopak Laboratories, Inc.). The injection was given 3 days before inoculation with *Aspergillus*.

***A. fumigatus* infection of mice.** An isolate of *Aspergillus fumigatus* was obtained from a human patient at M. D. Anderson Cancer Center and grown on Sabouraud dextrose agar. Mice were injected via the lateral tail vein with the clinical isolate of *A. fumigatus* (3.12×10^3 CFU/0.2 ml/mouse) 3 days following the 5-FU injection.

Drug administration. Conscious mice received daily intravenous injections of 0.9% saline (12 ml/kg of body weight/day), empty liposomes (12 ml of 10-mg lipid per ml of suspension/kg/day), amphotericin B desoxycholate (1 mg of amphotericin B/kg/day), free nystatin in 5% DMSO (4 mg/kg/day), or liposomal nystatin (2, 4, 6, or 8 mg/kg/day) via the tail vein for 5 consecutive days. The doses for all of the groups were split into two injections given 6 h apart. The first dose of each treatment was given 24 h (day 2) after the inoculation with *A. fumigatus*, which was 3 days after the dose of 5-FU (Table 1).

Histopathology. Histology using hematoxylin and eosin was performed on the liver, kidney (plus adrenals), lung, pancreas, and spleen tissues of three mice at day 5 for each group. Histology was performed on mice in the 8-mg/kg-liposomal-nystatin group only at day 52. No control mice were available at day 52 for histological evaluation. A histopathological assessment was made by an independent veterinary pathologist.

Organ culture. Cultures were performed on liver and kidney samples at days 2, 5, 19, and 50. The liver and kidney were aseptically removed, weighed, and homogenized in 5 ml of sterile saline. Serial 10-fold dilutions of the homogenates were plated on dextrose agar, and the plates were incubated at 35°C for 24 h. The number of CFU per gram of tissue was calculated. There were duplicate determinations for each time point per organ.

Clinical chemistry. Blood urea nitrogen (BUN), creatinine, and serum glutamic pyruvic transaminase (SGPT) levels were determined on days 2, 5, and 19. There were an insufficient number of control mice surviving for clinical chemistry determinations at day 50, so no groups were analyzed.

Gross necropsies. Necropsies were performed at days 5 and 19 on three mice in each group. Necropsies were performed on five mice in the 8-mg/kg-liposomal-nystatin group only at day 52. There were no control mice available at day 52.

Statistical analysis. Analysis of survival data was performed by using the Kaplan-Meier test. Analysis of clinical chemistry data was performed by using Student's *t* test.

RESULTS

In vitro susceptibility testing of liposomal nystatin and amphotericin B desoxycholate. The in vitro activities of liposomal nystatin and amphotericin B desoxycholate were determined against three clinical isolates of *A. flavus*, three clinical isolates of *A. fumigatus*, and one clinical isolate of *A. niger* (Fig. 1). Liposomal nystatin was active against all of the clinical isolates, with MICs in the range of 0.25 to 1.0 μg/ml. In all of the isolates, liposomal nystatin was more active than amphotericin B desoxycholate. *A. fumigatus* 2 was used to infect mice in the in vivo study.

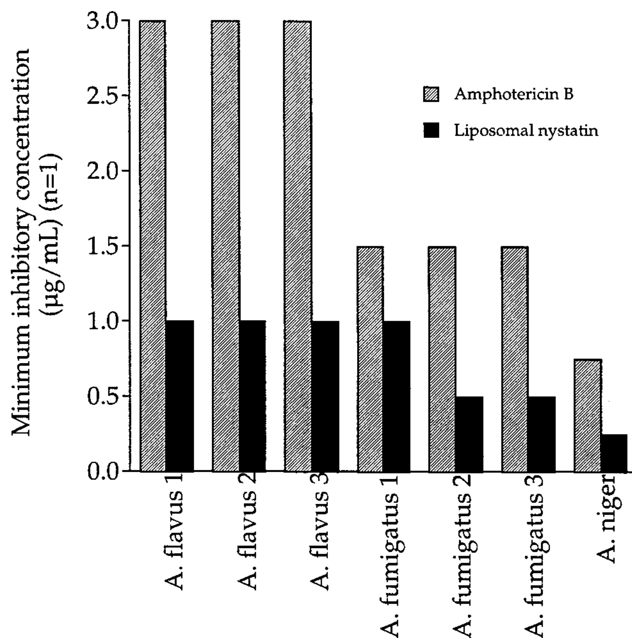

FIG. 1. In vitro susceptibilities of *Aspergillus* species to liposomal nystatin and amphotericin B desoxycholate. The MICs of liposomal nystatin and amphotericin B desoxycholate were determined according to the method of Anaissie et al. (1). The MIC was defined as the lowest concentration of the drug that prevented visible growth. All MIC determinations were performed once. *A. fumigatus* 2 was used for the mouse study.

Immunosuppression. Neutropenia was documented to occur in a separate experiment by 24 to 48 h following 5-FU injection and persisted for at least 9 days (Fig. 2).

Survival. Liposomal nystatin was efficacious in *Aspergillus*-infected, neutropenic mice based on survival (Fig. 3), and all doses were approximately equipotent. Fifty-nine to 70% of mice treated with liposomal nystatin at doses of 2 to 8 mg/kg/

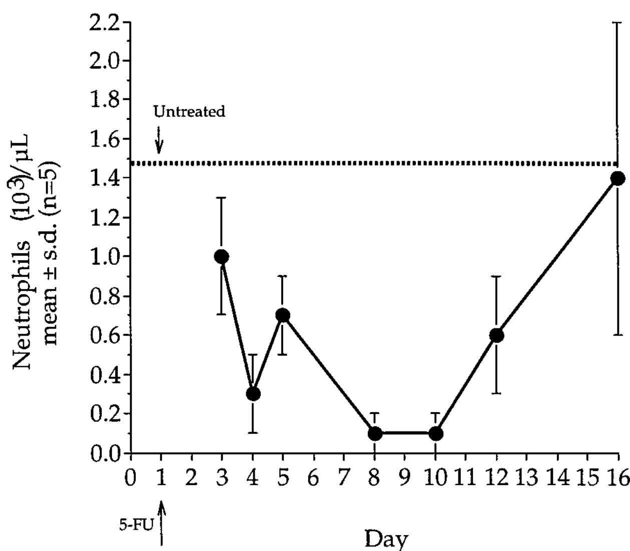

FIG. 2. Neutrophil concentration in mice following 5-FU administration. The degree of immunosuppression was determined in a pilot study prior to administration of drugs. 5-FU was administered on day 1, and absolute neutrophil counts were obtained on days 3, 4, 5, 8, 10, 12, and 16. The concentration of neutrophils in non-5-FU-treated mice was 1.5×10^3/μl.

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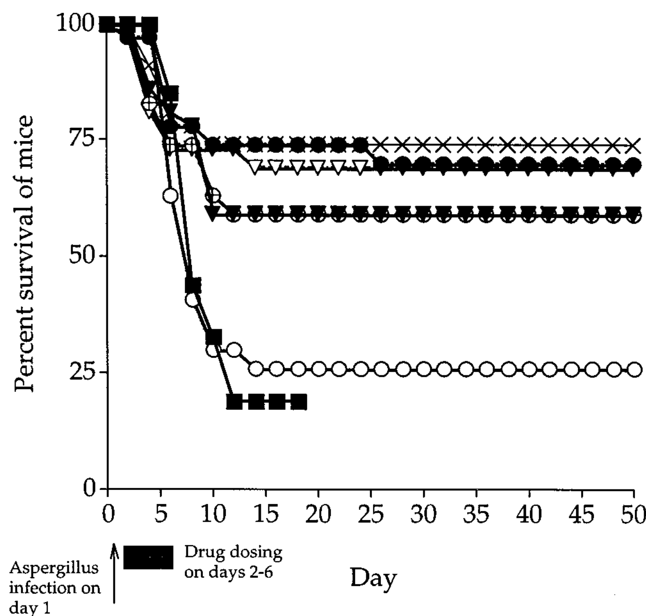

FIG. 3. Survival of *Aspergillus*-infected, neutropenic mice following treatment with liposomal nystatin. Mice were made neutropenic by using 5-FU and inoculated with *A. fumigatus* as described in Materials and Methods. Survival was monitored over 50 days. ■, no treatment; ○, empty liposomes; ×, nystatin (4 mg/kg/day); ●, ▼, ⊕, and ▽, liposomal nystatin at 2, 4, 6, and 8 mg/kg/day, respectively. Liposomal nystatin protected mice against *Aspergillus*-induced death in a highly significant manner at all doses compared to the controls (Kaplan-Meier test).

day survived 50 days after inoculation, compared to only 19 to 33% of mice in the control groups ($P < 0.0001$). Nystatin at a dose of 4 mg/kg/day provided approximately the same protection (74% survival). Ninety-three percent of mice treated with amphotericin B desoxycholate at a dose of 1 mg/kg/day survived to day 50, and this was statistically different from survival after liposomal-nystatin treatment ($P < 0.006$).

Clinical chemistry. The BUN, creatinine, and SGPT levels were determined on days 2, 5, and 19. The data for day 2 represent results for mice that were immunosuppressed with 5-FU and inoculated with *Aspergillus* but prior to treatment with liposomal nystatin. The data are shown in Table 2. There were no significant differences in the levels of BUN or creatinine at day 5 or 19 compared to the predose level. However, there was a statistically significant difference ($P < 0.05$; Student's *t* test) between the SGPT levels at day 5 and the predose levels in the untreated and empty-liposome groups. These values were much higher than those of drug-treated mice. Clinical chemistry was not analyzed at day 50 due to the unavailability of control mice.

Organ clearance (cultures). Preliminary experiments showed little or no *Aspergillus* infection in the lungs and spleen compared to the liver and kidney (data not shown). Therefore, organ clearance was analyzed only for the liver and kidney in the present experiment.

(i) Liver. *Aspergillus* could be detected in the liver in all groups at day 2 (pretreatment) and in most groups at day 5 (following three doses of liposomal nystatin) but was absent in all surviving mice except the empty-liposome and amphotericin B desoxycholate groups on day 19 (Table 3). *Aspergillus* was also absent in all groups except for one mouse in the liposomal-nystatin 4-mg/kg group at day 50. These data indicate that in either control or drug-treated mice which survived, the amount of *Aspergillus* in the liver was greatly reduced or absent on days 19 and 50 compared to days 2 and 5. These data also demonstrate that even in the groups in which survival was enhanced by using either liposomal nystatin, nystatin, or amphotericin B, the mice harbored little or no *Aspergillus* in the liver. (Note that control mice that were available at day 50 were used for the determination of organ clearance. Because the control mice were used for organ clearance, there were no control mice available for clinical chemistry or histopathology.)

(ii) Kidney. The amount of *Aspergillus* in the kidneys was determined at day 2 (pretreatment), day 5 (following three doses of liposomal nystatin), day 19, and day 50 (conclusion of the experiment). Although liposomal-nystatin-treated mice at day 5 had variable levels of *Aspergillus* infection in their kidneys, by day 19 liposomal-nystatin treatment had resulted in

TABLE 2. Clinical chemistry

Group (mg/kg)	BUN (mg/dl)	Creatinine (mg/dl)	SGPT (IU/liter)
Day 2, Untreated	22.2 ± 1.2	0.5 ± 0.1	52 ± 13
Day 5			
Untreated	20.3 ± 2.7	0.4 ± 0.1	185 ± 183
Saline	21.5 ± 3.3	0.4 ± 0.1	174 ± 307
Empty liposomes	21.6 ± 5.3	0.4 ± 0.1	600 ± 585
Amphotericin B desoxycholate (1)	25.7 ± 23.4	0.4 ± 0.1	29 ± 2
Nystatin (4)	23.4 ± 2.8	0.5 ± 0.3	28 ± 7
Liposomal nystatin			
2	19.3 ± 2.8	0.4 ± 0.2	54 ± 41
4	22.0 ± 4.8	0.6 ± 0.1	32 ± 13
6	22.4 ± 3.2	0.7 ± 0.3	30 ± 13
8	22.9 ± 7.2	0.4 ± 0.1	26 ± 8
Day 19			
Untreated	65.6 ± 44.3	0.7 ± 0.2	43 ± 17
Saline	59.7 ± 32.9	0.6 ± 0.2	57 ± 38
Empty liposomes	31.7 ± 3.2	0.5 ± 0.1	53 ± 23
Amphotericin B desoxycholate (1)	30.3 ± 3.6	0.5 ± 0.1	65 ± 33
Nystatin (4)	31.5 ± 4.3	0.5 ± 0.1	109 ± 124
Liposomal nystatin			
2	34.0 ± 3.8	0.5 ± 0.1	81 ± 60
4	33.2 ± 1.7	0.5 ± 0.1	41 ± 10
6	31.0 ± 4.0	0.5 ± 0.1	70 ± 31
8	30.9 ± 2.9	0.4 ± 0.1	58 ± 29

TABLE 3. Clearance in liver

Group (mg/kg)	CFU/g (mean ± SD)[a]			
	Day 2	Day 5	Day 19	Day 50
Untreated	782	66 ± 78	0 ± 0	NA
Saline	135	22 ± 30	0 ± 0	0 ± 0
Empty liposomes	406	54 ± 67	18 ± 28	0 ± 0
Amphotericin B desoxycholate (1)	296	0 ± 0	4 ± 8	0 ± 0
Nystatin (4)	228	55 ± 58	0 ± 0	0 ± 0
Liposomal nystatin				
2	442	0 ± 0	0 ± 0	0 ± 0
4	122	43 ± 68	0 ± 0	87 ± 195
6	328	0 ± 0	0 ± 0	0 ± 0
8	502	44 ± 50	0 ± 0	0 ± 0

[a] Determined as described in Materials and Methods. There was one mouse per group at day 2. There were two to five mice per group at days 5, 19, and 50. NA, no mice available.

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TABLE 4. Clearance in kidneys

Group (mg/kg)	CFU/g (mean ± SD)[a]			
	Day 2	Day 5	Day 19	Day 50
Untreated	0	37 ± 83	439 ± 228	NA
Saline	0	38 ± 85	295 ± 327	0 ± 0
Empty liposomes	0	117 ± 171	0 ± 0	0 ± 0
Amphotericin B desoxycholate (1)	0	0 ± 0	17 ± 38	0 ± 0
Nystatin (4)	297	76 ± 171	0 ± 0	0 ± 0
Liposomal nystatin				
2	0	0 ± 0	0 ± 0	481 ± 1,076
4	0	81 ± 112	14 ± 32	0 ± 0
6	0	0 ± 0	0 ± 0	0 ± 0
8	237	87 ± 121	0 ± 0	0 ± 0

[a] Determined as described in Materials and Methods. There was one mouse per group at day 2. There were two to five mice per group at days 5, 19, and 50. NA, no mice available.

little or no kidney infection compared to untreated and saline-treated controls (Table 4). *Aspergillus* was absent in all groups at day 50, except in the 2-mg/kg-liposomal-nystatin group, in which one mouse was infected.

The empty liposomes showed apparent antifungal activity in the kidney (Table 4) but did not have activity against organ clearance in liver (Table 3) and did not have survival-promoting activity (Fig. 3). The effect of empty liposomes in the kidney may be an anomaly or possibly an additional beneficial effect of the lipids which are contained in the liposomal nystatin.

Necropsy and histopathology. There were no gross lesions found in any animal at necropsy. Mycotic splenitis, nephritis, and hepatitis were observed in the no-treatment control, saline, and empty-liposome groups at day 5. Liposomal-nystatin-treated mice had no evidence of *Aspergillus* infection at either day 5 in all of the dosing groups or at day 52 in the 8-mg/kg-liposomal-nystatin group (the only group examined at day 52). There were no control mice available for the day 52 histopathology.

DISCUSSION

Amphotericin B desoxycholate became the first-line therapy for the treatment of patients with progressive and potentially threatening fungal infections, although it exhibited clinical activity and toxicity similar to those of nystatin in early human studies (26). Unlike amphotericin B, nystatin was never developed as an intravenous drug. However, there are a number of favorable data to support the use of nystatin systemically, including a broad spectrum of antifungal activity, differences in biological actions compared to amphotericin B, and proven antifungal activity in animals and humans. A brief discussion of these follows.

(i) Nystatin is active against a broad spectrum of fungi in vitro (17, 20, 24). In addition to activity against yeast (20), nystatin has activity in vitro against a variety of *Aspergillus* species, including *A. fumigatus* (20, 30, 42), *A. flavus* (30, 42), *A. niger* (42, 44), and *A. terreus* (42). Preliminary data indicate that the in vitro spectrum of activity of liposomal nystatin is the same as that of nystatin (unpublished data). Several studies have suggested that nystatin has a broader spectrum of activity than amphotericin B. It has been reported that nystatin is effective against *Geotrichum*, *Torulopsis*, *Candida krusei*, and *Beauvaria*, whereas amphotericin B has no activity (44). *Candida* fungal isolates from patients with AIDS showed significantly higher in vitro 50% inhibitory concentrations of amphotericin B, itraconazole, and flucytosine but no loss of susceptibility to nystatin (24). A similar resistance to amphotericin B has been reported for severely immunocompromised patients undergoing allogeneic or autologous bone marrow transplantation and/or myelosuppressive chemotherapy (37). Thus, there is some evidence that nystatin may have antifungal activity not possessed by amphotericin B in certain clinical situations.

(ii) Data indicate that nystatin and amphotericin B have different biological properties. (i) The rate at which nystatin causes K^+ release (i.e., membrane disruption) from *Candida albicans* in vitro is much higher than that of amphotericin, but the rate at which fungal death occurs is higher for amphotericin B than for nystatin (10). However, in 24-h susceptibility assays, both drugs have nearly identical MICs. (ii) Nystatin has been reported to be markedly less toxic to mammalian cells than amphotericin at equimolar concentrations (48). Kinsky (23) has reported that amphotericin B (5 μg/ml) produced 78% lysis in human erythrocytes in 40 min whereas nystatin (5 μg/ml) produced no lysis in 40 min. Hemolytic activity is known to correlate approximately with mammalian toxicity (18). (iii) K^+ and NH_4^+ reverse amphotericin B-induced inhibition of fungal glycolysis but do not reverse nystatin-induced inhibition of fungal glycolysis (18). Inhibition of glycolysis is a sequela to polyene-induced K^+ leakage. (iv) Differences in the abilities of nystatin and amphotericin B to inhibit ATPase have been seen (48). (v) Amphotericin B-resistant fungi are sometimes not cross resistant to nystatin. Hebeka and Solotorovsky (21) developed two *Candida albicans* strains that had 50-fold increases in resistance to amphotericin B but normal sensitivity to nystatin. Broughton et al. (6) reported the isolation of a *Candida albicans* strain that was resistant to amphotericin B but not to nystatin. An ergosterol-deficient, nystatin-resistant mutant of *Candida albicans* has been shown to be cross resistant to amphotericin B, but in contrast, an ergosterol-producing, amphotericin B-resistant mutant of *Candida albicans* was not cross resistant to nystatin (6). However, there are also examples in which resistance in *Candida* develops simultaneously against both nystatin and amphotericin B (53). Thus, it can be demonstrated that certain fungal isolates can be resistant to either nystatin or amphotericin B or to both agents.

(iii) Nystatin has demonstrated antifungal activity against a variety of fungi in animals. Nystatin has demonstrated activity against *Candida albicans* (7, 19, 31), *Cryptococcus neoformans* (41), *Histoplasma capsulatum* (9, 11), and *Coccidioides immitis* (11, 16) in animals.

(iv) Nystatin has demonstrated antifungal activity in humans. Nystatin has demonstrated antifungal activity against *Candida*, *Aspergillus*, *Histoplasma*, and *Coccidioides* infections in humans (4), as discussed above. In addition, as previously mentioned, nystatin has demonstrated antifungal activity in humans when administered by intravenous, oral, pleural, inhalation, or topical routes. In conclusion, these data suggest that the mechanisms of action of nystatin and amphotericin B are different in certain fungal strains and that these drugs have a number of biological activities that are distinguishable.

Liposomes have been used as carriers for a variety of antitumor and antimicrobial drugs (12, 52) and are particularly useful for drugs that are water insoluble and have clinical toxicity that is dose limiting. Two such drugs are amphotericin B and nystatin. Liposomal encapsulation of amphotericin B has resulted in the successful treatment of human fungal infections at higher doses than could be achieved with nonliposomal amphotericin B, with reduced toxicity (27). Nystatin, a drug that is virtually insoluble in water and in numerous pharmaceutical excipients, was formulated into liposomes by

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Lopez-Berestein and coworkers in order to determine if the liposomes would allow the use of nystatin intravenously. It was found that liposomal nystatin was active against a variety of fungi in vitro (30) and was active against *Candida albicans* in vivo (31). The in vivo toxicity of liposomal nystatin was greatly reduced compared to that of free nystatin (31).

The results of the present study show that nystatin and liposomal nystatin protect mice against *Aspergillus*-induced death in a highly statistically significant manner and that both drugs cleared *Aspergillus* from organs after approximately three doses. This is the first demonstration of the activities of nystatin and liposomal nystatin against *Aspergillus* in an animal model of fungal infection. The use of animal models is of significant importance for the screening of antifungal drugs, since the correlation between in vitro testing (e.g., MIC) and clinical response is poor in most cases (15). Indeed, in the present study liposomal nystatin was more potent than amphotericin B desoxycholate in vitro but not in vivo. A large number of published studies have demonstrated that the activities of antifungal drugs in in vivo experimental animal models infections are qualitative predictors of activities in human fungal infections (2, 22). Whether liposomal nystatin will have a clinical benefit over amphotericin B cannot be predicted from this study alone, since there are other factors that contribute to clinical outcome, such as pharmacokinetics, spectrum of antifungal activity, and toxicity. However, liposomal nystatin has already been reported to be active in some human patients who have failed therapy with amphotericin B (5).

The data in this report show that liposomal incorporation of nystatin provides an effective and well-tolerated therapy in a neutropenic-mouse model of disseminated *Aspergillus* infection. One obvious question arising from these data is whether the antifungal activity of liposomal nystatin that was seen in the present *Aspergillus*-infected-mouse study will be predictive of antifungal activity in human patients infected with *Aspergillus*. Based on the predictive value of antifungal testing in animals that has been cited in numerous published reports (2, 22), it is probable that liposomal nystatin will have antifungal activity in humans against *Aspergillus*. This is further supported by both the demonstration of antifungal activities of nystatin and liposomal nystatin against *Aspergillus* in vitro and the demonstration of the antifungal activity of aerosolized nystatin against pulmonary *Aspergillus* in humans (4).

Another obvious question is whether the dosages required for antifungal activity or the degree of efficacy that was seen in this study in *Aspergillus*-infected mice will also be seen in humans. No published study of antifungal drugs has reported that the order of potency of antifungal drugs in animals, effective animal dosages, or degree of efficacy in animals can be directly extrapolated to humans. There are a large number of factors, including pharmacokinetics and the site of infection, that may affect antifungal activity. Thus, although animal studies are valuable for assessing potential antifungal activity, there is a limit to how the results can be utilized to predict drug behavior in humans.

In summary, liposomal nystatin, given 24 h following infection at doses as low as 2 mg/kg/day for 5 days, protected neutropenic mice against *Aspergillus*-induced death. The survival and histopathological results provide evidence of the efficacy of liposomal nystatin.

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