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Evaluation of the efficacy and toxicity of amphotericin B incorporated in lipid nano-sphere (LNS®)

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Abstract

To develop a low-dose therapeutic system for amphotericin B (AmB), the efficacy and toxicity of lipid nano-sphere (LNS®) incorporating AmB (LNS-AmB) were evaluated and compared with those of Fungizone®, the conventional dosage form of AmB with sodium deoxycholate. LNS-AmB and Fungizone showed nearly equal activity against fungal cells both in vitro and in vivo. In contrast to Fungizone, however, LNS-AmB did not cause significant hemolysis. In addition, the vomiting toxicity of Fungizone was largely avoided by the use of LNS-AmB in dogs, in spite of the higher plasma AmB concentrations achieved by LNS-AmB. Therefore, LNS-AmB may be selective for fungal cells over mammalian cells. In a study of its toxicity and toxicokinetics in a regimen of daily 2-h intravenous infusions for 14 consecutive days, LNS-AmB showed less toxicity to the kidney than did Fungizone in spite of the higher plasma AmB concentrations achieved. LNS-AmB, therefore, allows the treatment of systemic fungal infections at low doses without the severe nephrotoxicity of Fungizone. Size-exclusion chromatography provided evidence that, when LNS-AmB was administered to rats, AmB was retained in the LNS particles in the blood circulation, but that when Fungizone was administered, AmB was transferred to high-density lipoproteins (HDL). AmB retained in LNS particles seemed to be less toxic to the kidney than was AmB associated with HDL. Consequently, LNS-AmB has the potential to become a low-dose therapeutic system for AmB, minimizing most of the severe side effects of AmB by decreasing the total dose required.

Keywords: Amphotericin B; Nanoemulsions; Lipid emulsion; LNS

1. Introduction

One of the most ambitious goals in the study of drug delivery systems is to develop a targeted therapy that will allow effective concentrations of a drug to reach the diseased sites. Amphotericin B (AmB) remains the drug of choice for the treatment of systemic mycoses. AmB has been under investigation for a long time because in its conventional dosage form, Fungizone®, an intravenously administered colloidal dispersion with sodium deoxycholate, it has severe side effects such as fever, chills, hemolysis, vomiting, and nephrotoxicity (Gallis et al., 1990).

Recent advances in drug delivery technology have resulted in the development of lipid-based formulations of AmB. After many attempts to decrease the toxicity of AmB by using liposomes, emulsions, and other systems, three lipid formulations of AmB (AmBisome®, Amphocil®, and Abelcet®) were made commercially available (Table 1). The

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Table 1
Characteristics of LNS-AmB and commercial AmB formulations (from Hillery, 1997)

Formulation	Structural characteristics and ingredients	Dose
LNS-AmB	Small lipid emulsion composed of soybean oil and egg lecithin (25–50 nm)	Low
Fungizone®	Colloidal dispersion with sodium deoxycholate (approximately 1 μm)	Medium
AmBisome®	Liposome composed of hydrogenated soy phosphatidylcholine, cholesterol and distearoylphosphatidylglycerol (50–100 nm)	High
Amphocil® (ABCD)	Uniform disk-shaped complex with sodium cholesteryl sulfate (122 nm in diameter and 4 nm thick)	High
Abelcet® (ABLC)	Ribbon-type lipid complex composed of dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol (2–5 μm)	High

toxicity of AmB can be reduced by incorporating it into liposomes or by complexing it to various lipids, and in these less-toxic formulations AmB can be given in higher, more effective doses. However, a high-dose therapeutic system is not necessarily the best formulation since the increased doses of AmB administered will accumulate in the body because of its low rate of elimination (Atkinson and Bennett, 1978). In addition, these lipid-based formulations are substantially more expensive than Fungizone because they require more-expensive ingredients, and the higher costs are a major limitation in clinical practice (Persson et al., 1992). To solve these problems, we set out to develop a low-dose therapeutic system for AmB using conventional ingredients.

To achieve a low-dose therapeutic system, we must find a way to deliver AmB efficiently to the diseased site. We have studied lipid nano-sphere (LNS®), a fine lipid emulsion, as a carrier for lipophilic drugs, and we have shown that LNS can be an excellent carrier for "passive targeting" due to its long circulating lifetime in the blood (Seki et al., 1994). We have also demonstrated an increase in plasma AmB concentrations compared with Fungizone when AmB was administered intravenously in the form of AmB incorporated into LNS (LNS-AmB; Fukui et al., submitted for publication). The aim of the present work was to evaluate the efficacy and toxicity of LNS-AmB and to judge whether the incorporation of AmB into LNS offers a way to reduce the toxicity of AmB.

2. Materials and methods

2.1. Materials

AmB was purchased from Dumex (Copenhagen, Denmark), egg lecithin from QP Corporation (Tokyo, Japan), and 1-amino-4-nitronaphthalene from Aldrich (Milwaukee, WI, USA). All other ingredients were of pharmaceutical grade, and solvents and chemicals were of analytical grade.

Fungizone, a colloidal dispersion of AmB in a micellar solution of deoxycholate, was obtained from Bristol-Myers Squibb (Princeton, NJ, USA). Fungizone (50 mg AmB and 41 mg sodium deoxycholate per vial) was reconstituted with 10 ml of water for injection and diluted 1:9 (v/v) with sterile 5% dextrose in water to an AmB concentration of 0.5 mg/ml. Fungizone was diluted with 5% sterile dextrose if necessary.

2.2. Preparation of LNS-AmB

AmB, egg lecithin and soybean oil were dissolved in chloroform–methanol (2:1, v/v). LNS-AmB contained 50 mg each of egg lecithin and soybean oil in 1 ml of the final dispersion. Organic solvent was removed under a stream of nitrogen gas and then under reduced pressure for 17 h. Complete evaporation resulted in the formation of a lipid paste, to which sterile 5% dextrose was added. The crude dispersion was emulsified with a probe-type sonicator (Sonifier model 250D, Branson Ultrasonic Corporation, Danbury, CT, USA) in an ice–water bath for about 60 min. The final dispersion was obtained by filtration through a 0.2-μm membrane, and the final concentration of AmB in the emulsion was 0.5 mg/ml. LNS-AmB was diluted with 5% sterile dextrose if necessary.

2.3. Animals

Slc:ddY mice (4–5 weeks old), Sprague–Dawley rats (7–10 weeks old) and beagle dogs (9–12 kg) were used. In all experiments, unfasted male animals were used. Mice and rats were purchased from Japan S.L.C. (Hamamatsu, Japan) and dogs from Nihon Nosan

Kogyo (Yokohama, Japan). The animals were allowed to acclimate to a standard environment in the animal-care room for 1 week (mice and rats) or more than 3 weeks (dogs) before the study. All animals were allowed to take water and standard pellet chow ad libitum. Human venous blood was obtained from healthy volunteers from our laboratory who were registered as volunteers for supplying fresh blood for experiments.

2.4. Measurement of AmB concentrations in plasma

The concentration of AmB in the plasma was determined by high-pressure liquid chromatography as described by Otsubo et al. (1999). Venous blood was collected in heparin tubes and plasma prepared by centrifugation at 3000 rpm for 10 min. Plasma (0.1 ml) was deproteinized by vortexing with methanol (1.0 ml) containing 1.0 μg of the internal standard 1-amino-4-nitronaphthalene per ml. After centrifugation at 3000 rpm for 10 min, the supernatant was decanted, dried down under reduced pressure, redissolved in 0.2 ml of methanol, and injected onto a reverse-phase column (L-column®, 4.6 mm × 150 mm; Chemicals Inspection and Testing Institute, Tokyo, Japan). The mobile phase was a mixture of acetonitrile and 10 mM sodium acetate buffer, pH 4.0 (11:17, v/v), and the flow rate was 1.0 ml/min. The eluent was monitored at 408 nm. The detection limit was 5 ng/ml and the interday and intraday coefficients of variation were 5% or less between 5 and 400 ng/ml. If the concentration of AmB was over 400 ng/ml, the sample was diluted with blank plasma.

2.5. In vitro antifungal activity

The in vitro efficacy of LNS-AmB, Fungizone and AmB solubilized by dimethyl sulfoxide (DMSO) was evaluated by measuring the inhibition of growth in *Candida albicans*. Growth inhibition was measured by the decrease in optical density at 540 nm in Sabouraud dextrose broth buffered with 0.165 M 3-morpholinopropanesulfonic acid, pH 7.0, after incubation for 24 h at 35 °C.

2.6. In vivo antifungal activity in mice

Mice (10 per group) were systemically infected by intravenous injection of *C. albicans* or *Aspergillus fumigatus* via the tail vein. LNS-AmB or Fungizone was administered intravenously at doses of 0.0625, 0.125, 0.25, or 0.5 mg/kg either 4 h after challenge (single treatment) or 4 and 6 h after challenge (double treatment). Based on the survival rate 14 days after challenge, the ED_{50} and its confidence limits were calculated by Probit analysis. The ED_{50} was based on the single-time dosage even if the test formulation was administered twice.

2.7. Hemolysis

Damage to normal cells was evaluated by assessing the extent of hemolysis essentially as described by Mehta et al. (1984). Erythrocytes were isolated from fresh rat, dog or human blood, washed three times with phosphate-buffered saline (PBS), and suspended in PBS at a hematocrit of 50%. The AmB formulation was added to the suspended erythrocytes and the suspension diluted with PBS to give final AmB concentrations in the range of 1–60 μg/ml and a final hematocrit of 1%. The erythrocytes were then incubated for 20 min in a 37 °C water bath. Cell lysis was stopped by immersion in ice water, the unlysed cells were removed by centrifugation at 3000 rpm for 5 min, and the hemoglobin in the supernatant was determined by its absorbance at 541 nm. Because samples treated with LNS-AmB were too turbid for spectroscopic assay, the release of hemoglobin was evaluated by reference to a set of turbidimetric standards prepared from dilutions of AmB-free LNS. Control samples showing no lysis (with no AmB) and 100% lysis (with distilled water instead of PBS) were used in all experiments.

2.8. Vomiting in dogs

To evaluate the safety of LNS-AmB, vomiting, one of the side effects of Fungizone, was recorded in dogs after intravenous infusion at a rate of 0.1 mg/kg/min. Infusion was carried out for 120 min for LNS-AmB and 60 min for Fungizone, giving total doses of 12 mg/kg for LNS-AmB and 6 mg/kg for Fungizone. The time of vomiting was recorded, and blood samples were taken at scheduled times and at the time of first vomiting. The plasma AmB concentrations were measured by HPLC.

2.9. Toxicity and toxicokinetics of daily intravenous infusion in rats

One week before treatment commenced, a medical-grade catheter was placed in a femoral vein in each rat (five rats per group) and passed subcutaneously to the nape of the neck. The implanted catheter was then attached to a swivel device connected by a length of medical-grade tubing to an infusion pump. LNS-AmB or Fungizone was administered to the rats by intravenous infusion at a daily dose of 0.5 or 2.0 mg/kg for a 2-h period over 14 consecutive days. Dextrose (5%) was administered as a control. The infusion rate was 5.0 ml/kg/h. Blood samples were collected in heparin tubes before administration and at 2 (within 5 min after the end of administration), 3, 8 and 24 h after the start of administration. The body weight was measured and the biochemical characteristics of the serum were evaluated on day 15. The area under the plasma concentration–time curve (AUC) was calculated by a trapezoidal rule, and the significance of intergroup differences between the control and treated groups were assessed with Sheffe's test.

2.10. Serum protein binding

Serum was obtained after intravenous administration of LNS-AmB or Fungizone to rats at a dose of 2.0 mg/kg. To evaluate the binding of AmB to serum proteins, serum samples (80 μl) were applied to a Sepharose CL-6B column (1 cm × 45 cm; Amersham Biosciences, Piscataway, NJ, USA) equilibrated with PBS. The sample was eluted with the same buffer at a flow rate of 0.22 ml/min, and 8-min fractions were collected. The entire chromatographic separation was completed in 4 h. LNS-AmB eluted in the same fraction as very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), while Fungizone eluted in the same fraction as free (unbound) AmB.

3. Results

3.1. In vitro antifungal activity

The antifungal activity of LNS-AmB against *C. albicans* was similar to that of Fungizone and DMSO-solubilized AmB (Fig. 1), showing that AmB retained its antifungal activity in the LNS-AmB formulation.

3.2. In vivo antifungal activity in mice

In systemic candidiasis in mice, the protective effect of LNS-AmB was stronger than that of Fungizone, irrespective of the number of treatments (Table 2).

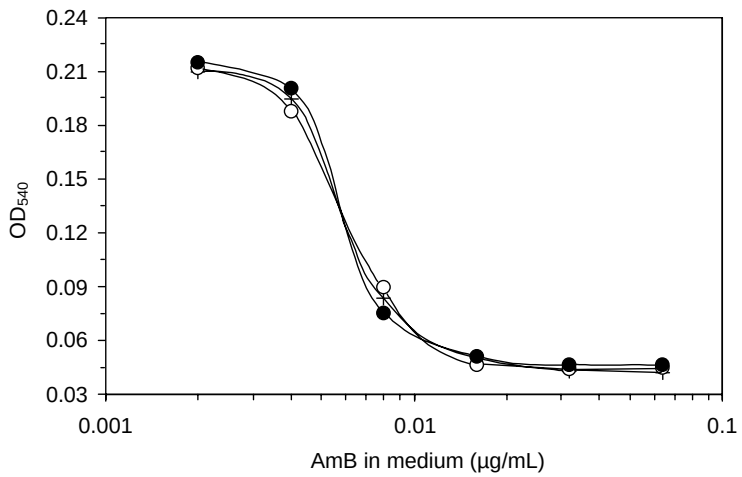

Fig. 1. Antifungal activity of LNS-AmB, Fungizone and DMSO-solubilized AmB in vitro. The growth inhibition of *Candida albicans* was measured by the decrease in optical density at 540 nm in SD-MOPS broth after a 24-h incubation at 35 °C. The results are the mean of two experiments. Closed circles, LNS-AmB; open circles, Fungizone; +, DMSO-solubilized AmB.

Table 2
Protective effect of LNS-AmB and Fungizone against systemic infection in mice

Organism	Inoculum size (cells/mouse, i.v.)	Formulation	Number of treatments	ED_{50} (mg/kg)	95% confidence limit (mg/kg)
Candida albicans	2.0×10^6	LNS-AmB	1[a]	0.14	0.06–0.23
		Fungizone	1	0.31	0.19–0.49
	4.0×10^6	LNS-AmB	2[b]	0.23	0.01–0.50
		Fungizone	2	0.31	0.10–0.70
Aspergillus fumigatus	4.0×10^6	LNS-AmB	1	0.42	0.30–0.59
		Fungizone	1	0.42	0.30–0.61

[a] In the single treatment, drugs were administered intravenously 4 h after challenge of the organism.
[b] In the double treatment, drugs were administered intravenously 4 and 6 h after challenge of the organism.

In contrast, in systemic aspergillosis, their protective effect was the same.

3.3. Hemolysis

Severe erythrocyte lysis was observed in vitro with Fungizone, but not with LNS-AmB, in all species tested (Fig. 2).

3.4. Vomiting in dogs

The mean time of first vomiting after the beginning of intravenous infusion was substantially later for LNS-AmB (159 min) than for Fungizone (50 min) (Fig. 3), and the respective plasma AmB levels at those times were 162 and 7 μg/ml.

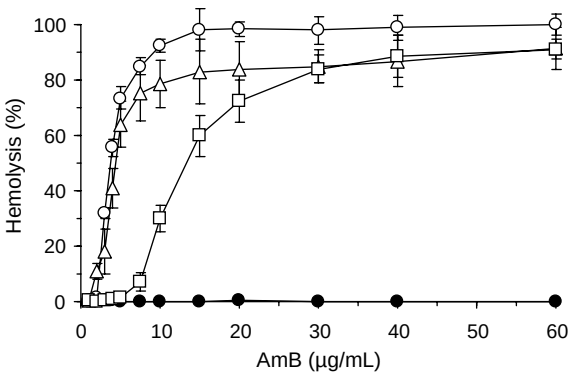

Fig. 2. Erythrocyte lysis by LNS-AmB and Fungizone. Each point represents the mean ± S.D. of three experiments. Closed circles, LNS-AmB (rat, dog, human); open circles, Fungizone (rat); open triangles, Fungizone (dog); open squares, Fungizone (human).

3.5. Toxicity and toxicokinetics of daily intravenous infusion in rats

On day 15, there was a significant decrease in body weight in the group receiving 2.0 mg/kg of Fungizone compared to controls (Table 3). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum of rats receiving either LNS-AmB or Fungizone were not significantly greater than control levels. However, significant increases in blood urea nitrogen (BUN) were noted at high doses of both formulations.

Without exception, higher plasma AmB levels were observed for LNS-AmB than for Fungizone for the same dose at all time-points. At the same dose, $AUC_{2-24\,h}$ was greater in rats receiving LNS-AmB than in those receiving Fungizone (Fig. 4). At 2.0 mg/kg of Fungizone, $AUC_{2-24\,h}$ was higher for days 7 and 14 than for day 1 (1.23 and 1.45 times, respectively). $AUC_{2-24\,h}$ for 0.5 mg/kg of LNS-AmB was about the same as for 2.0 mg/kg of Fungizone.

3.6. Serum protein binding

When serum samples were analyzed by size-exclusion chromatography, the elution position of the AmB peak observed after administration of LNS-AmB was consistent with that of LNS-AmB itself (Fig. 5). In contrast, the AmB peak observed after administration of Fungizone was in the plasma lipoprotein (high-density lipoproteins, HDL) fraction. Free (unbound) AmB was not detected after the administration of either LNS-AmB or Fungizone. The elution position of the AmB peak did not change with time after the administration of either formulation.

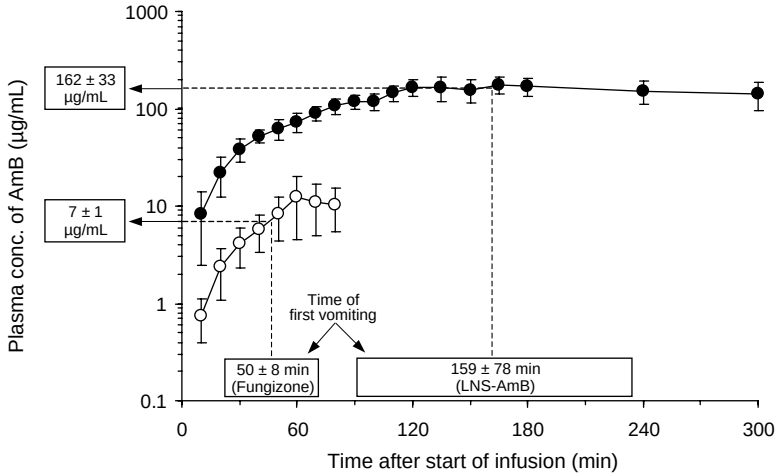

Fig. 3. Plasma concentrations of AmB and times of first vomiting during constant intravenous infusion of LNS-AmB or Fungizone to dogs. The total doses were 12 mg/kg for LNS-AmB and 6 mg/kg for Fungizone. The mean times of first vomiting ± S.D. of three animals are shown on the horizontal axis and the plasma AmB levels ± S.D. at those times on the vertical axis. Closed circles, LNS-AmB; open circles, Fungizone.

Table 3
Body weight and biochemical characteristics of serum obtained on day 15 after a daily 2-h intravenous infusion for 14 consecutive days in rats

	Control (5% dextrose)	LNS-AmB		Fungizone	
		0.5 mg/kg	2.0 mg/kg	0.5 mg/kg	2.0 mg/kg
Body weight (g)	316 ± 14	311 ± 17	291 ± 16	296 ± 21	281 ± 14[a]
AST[b] (IU/l)	106 ± 21	128 ± 31	109 ± 24	111 ± 21	105 ± 16
ALT[b] (IU/l)	29 ± 3	27 ± 3	29 ± 6	29 ± 7	28 ± 7
BUN[b] (mg/dl)	25 ± 3	29 ± 5	49 ± 10[a]	32 ± 7	69 ± 14[a]

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen. Each value is the mean ± S.D. for five rats.
[a] Statistically significant when compared with control at $P < 0.05$.
[b] Normal ranges in rats are 97 ± 28 IU/l for AST, 38 ± 14 IU/l for ALT, and 20 ± 3 mg/dl for BUN (from Wolford et al., 1986).

4. Discussion

In a previous report (Fukui et al., submitted for publication), we suggested that LNS-AmB, which is prepared with the inexpensive ingredients egg lecithin and soybean oil, could become a low-dose therapeutic system for AmB due to the high plasma concentrations achieved. In this study, we examined the efficacy and toxicity of LNS-AmB.

In in vitro antifungal-activity experiments, LNS-AmB maintained the potent activity of AmB against fungal cells even though the AmB was incorporated into LNS particles. In vivo, LNS-AmB showed a stronger protective effect against candidiasis than did Fungizone, but the protective effect of both formulations against aspergillosis was the same. This difference reflects the difference in the minimum inhibitory concentration (MIC) of the formulations: in a separate experiment, we found that MIC against *C. albicans* was 0.125 μg/ml for LNS-AmB and 0.25 μg/ml for Fungizone, whereas MIC against *A. fumigatus* was 0.5 μg/ml for both formulations. In an emulsion formulation composed of lecithin and soybean oil, AmB is located in the lecithin emulsifier monolayer and not in the bulk of the oil droplet (Washington et al., 1988). We, therefore, believe that AmB can be easily transferred from the LNS particles to ergosterol in the membranes of the fungal cells, while size-exclusion

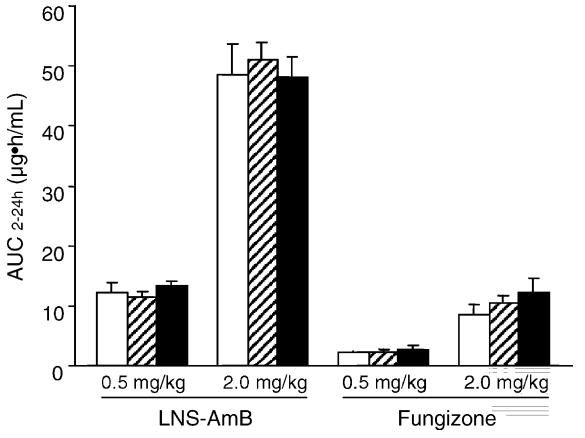

Fig. 4. $AUC_{2–24\,h}$ profiles after daily 2-h intravenous infusion for 14 consecutive days in rats. Each set of three bars represents $AUC_{2–24\,h}$ on days 1, 7 and 14 (left to right). Each value is the mean ± S.D. of $AUC_{2–24\,h}$ for five animals.

chromatography demonstrates that in the blood of normal rats LNS-AmB retains the AmB.

An emulsion formulation of AmB is as active as Fungizone against *Candida* cells (Egito et al., 1996; Shadkhan et al., 1997), but there are different opinions regarding liposome formulations. Anaissie et al. (1991) examined over 100 pathogenic clinical yeast isolates of *Candida* and *Cryptococcus* species and showed that AmBisome retains sufficient antifungal activity, but Pahls and Schaffner (1994) found that AmBisome had only one-eighth to one-quarter of the antifungal activity of Fungizone against four isolates of *C. albicans*. Multilamellar liposomal AmB prepared from dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol is less active in vitro against yeast strains than is Fungizone (Ralph et al., 1991). High-dose therapy necessitated by a decrease in activity can lead to toxicity, so it is very important for new formulations of AmB to be evaluated for their balance of toxicity and efficacy.

Though LNS-AmB has a long circulating lifetime while maintaining the potent activity of AmB, in order to assess its therapeutic potential it is necessary to study its toxicity towards mammalian cells. LNS-AmB showed no erythrocyte lysis in three species tested, whereas Fungizone caused severe lysis in all three species. Liposomal and emulsion formulations show less toxicity to erythrocytes than do solubilized formulations with methyl formamide and Pluronic (Mehta et al., 1984; Forster et al., 1988). In particular, Forster et al. (1988) reported that a lecithin-stabilized emulsion showed very low toxicity compared with a Pluronic-stabilized emulsion. They also considered that the low toxicity of the liposomes and the lecithin-stabilized emulsion was due to the strong AmB–phospholipid interaction demonstrated by binding studies (Bolard et al., 1980; Witzke and Bittman, 1984; Jullien et al., 1990). Therefore, using lecithin as the stabilizer in LNS-AmB may be the key to its low toxicity towards mammalian cells.

In the treatment of systemic mycoses, Fungizone is infused intravenously over a 3-h period to avoid acute side effects. Nevertheless, the clinical use of Fungizone is often limited by acute side effects such as fever, chill, and vomiting. The vomiting toxicity of LNS-AmB in dogs was lower than that of Fungizone after intravenous infusion in spite of the higher plasma AmB concentrations achieved. Since LNS-AmB also showed no hemolytic activity in vitro, this formulation may be selective for fungal cells over mammalian cells. Though the reason for this selectivity is not clear, we are confident that these favorable characteristics of LNS-AmB will allow the development of a low-dose AmB therapy for internal fungal infections. We have reported that, after intravenous bolus-injection at a dose of 1.0 mg/kg, LNS-AmB yields plasma AmB concentrations up to 10-fold higher than does Fungizone (Fukui et al., submitted for publication). In the vomiting experiment, even though we administered a higher dose than normal to ensure vomiting with intravenous infusion, the difference in plasma AmB concentrations between the formulations was approximately consistent with the difference observed after intravenous bolus-injection at a dose of 1.0 mg/kg.

Renal damage is the most significant toxic effect of AmB. In rats, we observed BUN increases at high doses (2.0 mg/kg) of both formulations after daily 2-h intravenous infusion for 14 consecutive days. Fungizone especially showed severe nephrotoxicity. Although LNS-AmB increased BUN at 2.0 mg/kg, $AUC_{2–24\,h}$ for LNS-AmB was about five times that of Fungizone at the same dose. This shows that the nephrotoxicity of LNS-AmB was not related to the concentration of AmB in the plasma. Since there were no significant changes in BUN at 0.5 mg/kg of LNS-AmB, which corresponds to 2.0 mg/kg of Fungizone in terms of $AUC_{2–24\,h}$, we believe that low-dose

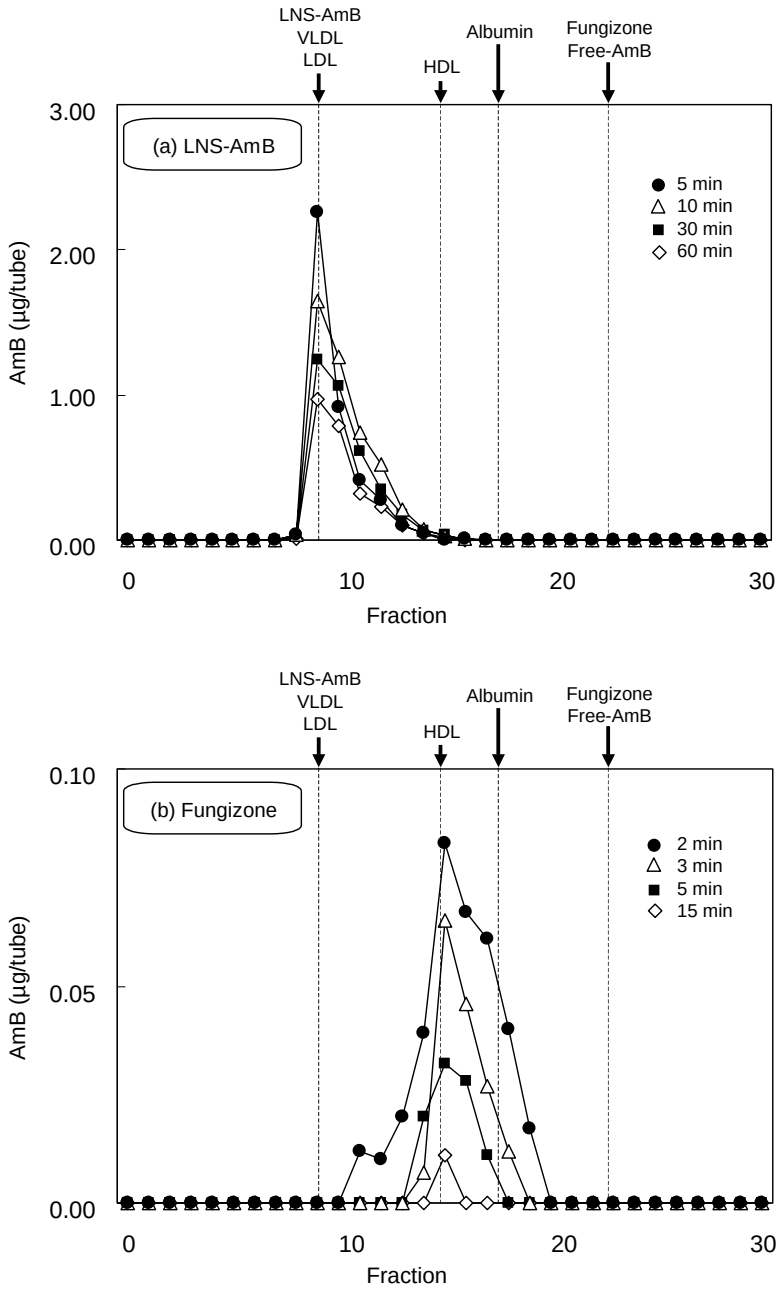

Fig. 5. Size-exclusion chromatograms of serum after intravenous administration of LNS-AmB (a) or Fungizone (b) at a dose of 2.0 mg/kg to rats. The arrows represent the elution position of LNS-AmB, Fungizone, free AmB or serum components on a Sepharose CL-6B column. VLDL, very-low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

therapy with LNS-AmB would enable AmB to retain its efficacy while decreasing its side effects.

To evaluate the binding of AmB to serum proteins, we initially carried out equilibrium dialysis against serum obtained from rats receiving LNS-AmB or Fungizone and found no free (unbound) AmB. Bekersky et al. (2002) showed by both ultrafiltration and equilibrium dialysis that AmB was bound to the extent of >95% when human plasma was spiked with AmB solubilized in DMSO. After confirming the extent of binding of AmB, we used size-exclusion chromatography to identify the components to which AmB bound in the serum after administration of LNS-AmB or Fungizone to rats. We concluded that AmB is associated with the LNS particles after administration of LNS-AmB and with HDL after administration of Fungizone. AmB is equally distributed between HDL and LDL after incubation with human plasma for 1 h at 25 °C (Brajtburg et al., 1984). However, Wasan et al. (1993) showed that when Fungizone is incubated in pooled human serum for 1 h at 37 °C, more than 75% of the AmB is recovered in the HDL fraction, and they proposed that the change in AmB distribution at 37 °C was related to the transition temperature of lipoprotein, which is between 27 and 34 °C (Cushley et al., 1987). The transfer of AmB from Fungizone to HDL that we observed is consistent with these results.

Wasan et al. (1994) showed that HDL-associated AmB is less toxic than LDL-associated AmB in a pig kidney epithelial cell line and concluded that the reduced nephrotoxicity associated with HDL is related to a decreased uptake of AmB by renal cells because of their low level of expression of HDL receptors. We believe that AmB retained in LNS particles is even less toxic than that associated with HDL, because the renal damage caused by LNS-AmB is less than that caused by Fungizone in spite of the higher plasma AmB concentrations delivered by LNS-AmB.

In our vomiting- and renal-toxicity experiments, we did not separately measure free and bound AmB in the plasma. However, as stated above, we did not detect free AmB in the serum of rats after intravenous administration of LNS-AmB or Fungizone. Furthermore, Bekersky et al. (2002) showed in a phase IV study of AmBisome that the concentrations of free AmB in the plasma were very low, due to its limited solubility. Therefore, we do not believe that the difference in toxicity between LNS-AmB and Fungizone reflects any difference in the concentrations of free AmB in the plasma, but rather reflects the difference in toxicity between AmB incorporated into LNS and AmB bound to lipoproteins.

In this study, we have evaluated the efficacy and toxicity of LNS-AmB by comparing it with Fungizone. We have demonstrated that LNS-AmB retained the potent activity of AmB as well as its selectivity for fungal over mammalian cells. In addition, the nephrotoxicity of LNS-AmB did not increase in proportion to the plasma AmB concentrations yielded. LNS-AmB has the potential to become a low-dose therapeutic system for AmB, and most of the severe side effects of AmB may be minimized by decreasing the total dose of AmB required. A detailed comparison of LNS-AmB with commercial lipid-based formulations is the subject of a separate report from our laboratory.

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