Evaluation of Renal Toxicity and Antifungal Activity of Free and Liposomal Amphotericin B following a Single Intravenous Dose to Diabetic Rats with Systemic Candidiasis

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Received 30 November 1995/Returned for modification 3 April 1996/Accepted 5 May 1996

Since fungal infections are prevalent in diabetic patients, in whom treatment is often complicated by underlying renal disease and dyslipidemias, the purpose of the present study was to determine if the antifungal activity and nephrotoxic effects of amphotericin B (AmB) and liposomal AmB (L-AmB) are different in nondiabetic (normolipidemic) rats compared with those in diabetic (dyslipidemic) rats with systemic candidiasis. Nondiabetic and diabetic rats infected with *Candida albicans* received a single intravenous dose of either AmB (0.8 mg of AmB per kg of body weight), L-AmB (0.8, 2, or 4 mg of AmB per kg), or an equivalent volume of normal saline (1 ml). Renal function was assessed by inulin clearance, and antifungal activity was determined by measuring the numbers of CFU of *C. albicans* that were present in the right kidney following drug treatment. AmB at 0.8 mg/kg and L-AmB at 0.8, 2, and 4 mg/kg are effective antifungal agents in both diabetic and nondiabetic rats. However, while there was approximately a 4-fold decline in the mean number of CFU per gram of kidney in nondiabetic rats, there was only approximately a 2.5-fold decline for the comparable dose (AmB, 0.8 mg/kg) in diabetic rats. There also appeared to be a similar fold reduction of L-AmB at all of the dosages tested. AmB treatment significantly improved renal function in diabetic and nondiabetic rats with systemic candidiasis. Although L-AmB at all doses tested significantly improved renal function in diabetic rats with systemic candidiasis, only L-AmB at doses of 2 and 4 mg/kg significantly improved renal function in nondiabetic rats with systemic candidiasis. These findings suggest that following administration of a single intravenous dose, AmB and L-AmB appear to be less effective in killing *C. albicans* isolates in diabetic than in nondiabetic rats, while they were found to improve the renal functions of rats in both treatment groups.

For most hydrophilic compounds intravenous administration results in an instantaneous distribution of the compound within the bloodstream (4). This is a consequence of homogeneous mixing of the compound with the aqueous components of the bloodstream. However, when compounds are incorporated into hydrophobic carriers (liposomes), their distribution within the bloodstream is not instantaneous. The interaction of these compounds with nonaqueous components of the bloodstream, including lipoproteins, appears to be an explanation for this observation (23, 24, 26, 27).

Since diabetic patients with fungal infections often display dyslipidemias (i.e., hypertriglyceridemia and hypocholesterolemia) that result in changes in their plasma lipoprotein-lipid composition (3, 5), the interaction of hydrophobic drugs with plasma lipoproteins may be a reasonable explanation for the discrepancies in the pharmacokinetics and pharmacodynamics of these compounds following their intravenous administration into infected patients compared with those after administration into healthy volunteers. Amphotericin B (AmB) is an example of one such hydrophobic drug.

AmB is a polyene macrolide antibiotic used in the treatment of systemic fungal infections caused most notably by *Candida albicans* and *Aspergillus niger* (2). However, its clinical use has been limited by dose-dependent nephrotoxicity, resulting in as much as a 60% reduction in the glomerular filtration rate (1, 22). When AmB is incorporated into liposomes composed of

dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylglycerol (DMPG), AmB is more active against experimental fungal infections (7, 9, 10, 12–15, 18), well tolerated at doses of up to 5 mg/kg of body weight, and effective when conventional AmB therapy fails (10, 11). Although a variety of new lipid-based AmB formulations (i.e., AmBisome [Vestar, San Dimas, Calif.], Abelcet [The Liposome Company Inc., Princeton, N.J.], and AmB colloidal dispersion [Amphocil; SE QUUS Pharmaceuticals, Menlo Park, Calif.]) have recently been used in clinical investigations, in the present study we tested only the DMPC-DMPG-AmB formulation described above.

AmB initially distributes with the high-density lipoprotein (HDL) fraction upon incubation in human serum (24). Over time, AmB redistributes from HDL to low-density lipoprotein (LDL) (24). This redistribution appears to be regulated by lipid transfer protein (LTP) (28), a protein which facilitates the transfer of cholesteryl esters, triglyceride, and phospholipids between different plasma lipoprotein classes (19, 20). However, when AmB is incorporated into liposomes (liposomal AmB [L-AmB]) composed of negatively or positively charged phospholipids, not only is LTP's capability of transferring AmB from HDL to LDL diminished but AmB also remains retained only with the HDL fraction (28).

It has been suggested that AmB's pharmacokinetics may be a result of the slow release of AmB from a tissue or organ site because of AmB's affinity to bind to cholesterol (17) in serum lipoproteins or cell membranes. Furthermore, differences in the pharmacokinetics and tissue distribution of AmB but not those of L-AmB have been demonstrated between healthy rats and rats with diabetes-induced hyperlipidemia, suggesting

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independence of the liposomal delivery mechanism from the diabetic disease state and endogenous triglyceride and cholesterol levels (30).

Since fungal infections are prevalent in diabetic patients, in whom treatment is often complicated by underlying renal disease and dyslipidemias, the purpose of the present study was to determine if the antifungal activities and nephrotoxic effects of free AmB and L-AmB are different in nondiabetic (normolipidemic) rats and diabetic (dyslipidemic) rats with systemic candidiasis.

MATERIALS AND METHODS

Chemicals and lipids. Fungizone (which contains 50 mg of AmB for every 41 mg of sodium deoxycholate), which is reconstituted with sterile water, was provided by Bristol-Myers Squibb (Newark, N.J.). Radiolabeled inulin was purchased from Dupont-NEN Research Products (Boston, Mass.). Chromatographically pure DMPC and DMPG were purchased from Nippon Fine Chemical (Tokyo, Japan). NPH insulin was purchased from the Vancouver Hospital Health Sciences Center pharmacy. Streptozocin was purchased from Sigma Chemical Co. (St. Louis, Mo.).

L-AmB preparation. The method of preparing multilamellar liposomes containing AmB has been described previously (24). Briefly, AmB dissolved in methanol was mixed with chloroform solutions of DMPC and DMPG. The organic solvents were removed by evaporation under vacuum, and the resultant lipid film was dispersed by hand shaking in 0.9% sodium chloride to form liposomes. The final preparation contained a 7:3 (wt/wt) ratio of DMPC:DMPG (4:1 molar ratio of DMPG:AmB). The AmB content in the liposomal preparation was quantitated by removing an aliquot of the preparation, dissolving it in $\text{CHCl}_3\text{-CH}_3\text{OH}$, and quantitating it against a standard curve for AmB by using a UV/VIS spectrophotometer (Beckman) at 405 nm. The incorporation efficiency was greater than 95% for all liposomes. Empty DMPC-DMPG liposomes were prepared as described above, but without the incorporation of AmB. Median liposome size was measured in a Nicomp Submicron Sizer model 370 instrument by using the Gaussian or Nicomp models. These liposomes had a median diameter of 1.08 μm (range, 0.7 to 1.3 μm).

Animal model. All of the animals used in the present study were cared for in accordance with the principles promulgated by the Canadian Council on Animal Care and the University of British Columbia. A total of 60 male albino Sprague-Dawley rats (weight range, 250 to 350 g; Charles River Canada, Montreal, Quebec, Canada) were housed in an animal facility with a 12-h dark-light cycle and controlled temperature and humidity. The availability of water and food (Purina rat chow) was unrestricted throughout the study. The treatment groups were as follows: 25 diabetic rats infected with *C. albicans*, 5 diabetic rats not infected with *C. albicans*, 25 nondiabetic rats infected with *C. albicans*, and 5 nondiabetic rats not infected with *C. albicans*.

(i) Diabetic animals. Thirty rats were randomly chosen and given a single intravenous dose of streptozocin (65 mg/kg) into the femoral vein. The other 30 animals were administered drug-free phosphate buffer vehicle. After 72 h serum and urine samples were obtained and glucose concentrations were measured to determine if the animals were diabetic or not. Diabetes was confirmed if fasting serum glucose concentrations were greater than 300 mg/dl and urine glucose concentrations were greater than 900 mg/dl. Each diabetic rat was maintained on a single subcutaneous dosage of NPH insulin (5 U/kg/day) administered at the start of the active cycle (7:00 p.m.) to maintain partial metabolic control throughout the duration of the study (13 days total). Nondiabetic rats were administered normal saline (NS).

(ii) Model for murine *C. albicans* infection. *C. albicans* 336 isolated from a patient with disseminated candidiasis (16) was used to infect the rats. The inoculum was cultivated at 37°C for 18 h on Sabouraud dextrose agar and was then prepared in saline. The animals were injected with 1.35×10^6 CFU ($\pm 10\%$ intraday variability) into the femoral vein on day 10 of the study. The inoculum was prepared fresh daily and was determined with a hemocytometer. We have previously observed that this concentration produces a disseminated seeding of the *C. albicans* isolates (16). On day 12 of the study (48 h after *C. albicans* inoculation) the test drugs were injected into the rats since at this time a well-established systemic infection was present. Although fungal infections in diabetic patients may be due to mucocutaneous infections, there are a number of recent cases in which diabetics have developed candidiasis (21).

Experimental design. Diabetic and nondiabetic rats with systemic fungal infections were randomly assigned to receive a single intravenous dose of either AmB at 0.8 mg/kg (Fungizone; Bristol-Myers Squibb) or L-AmB at 0.8, 2, or 4 mg/kg. Control animals received an equivalent volume of NS (1 ml). A single-dose inulin clearance (CL_{in}) was calculated for each animal 24 h following administration of the AmB or the L-AmB dose. A bolus injection of [^3H]inulin (12.5 μCi) was administered via the penile vein; blood samples (0.05 ml) were collected by tail bleedings at 5, 10, 20, 30, 45, 75, and 90 min following the administration of the dose. Serum was separated from the blood, and 0.01 ml was mixed with 4 ml of scintillation cocktail (Ecolite; ICN Biomedical Inc., Irvine, Calif.) and counted on an LSC 7500 scintillation counter (Beckman Instruments,

TABLE 1. Serum glucose concentrations and serum cholesterol concentrations of diabetic and nondiabetic rats with systemic candidiasis following administration of a single intravenous dose of NS, AmB, or L-AmB^a

Dose	Nondiabetic rats		Diabetic rats	
	Glucose concn (mg/dl)	Cholesterol concn (mg/dl)	Glucose concn (mg/dl)	Cholesterol concn (mg/dl)
NS	151 $\pm$ 18	99 $\pm$ 17	346 $\pm$ 28 ^b	144 $\pm$ 23 ^c
AmB	143 $\pm$ 27	109 $\pm$ 13	370 $\pm$ 37 ^b	134 $\pm$ 9 ^c
L-AmB	150 $\pm$ 22	94 $\pm$ 11	350 $\pm$ 37 ^b	151 $\pm$ 22 ^c

^a NS was used at a volume of 1 ml (equivalent to the volume used in the AmB and LAmB doses). AmB was used at 0.8 mg/kg of body weight. L-AmB contained DMPC-DMPG in a lipid-AmB ratio of 10:1 (wt/wt; 0.8 mg of AmB per kg. Values are means $\pm$ standard deviations ($n = 5$ rats per treatment group).

^b $P < 0.05$ versus the glucose concentration in nondiabetic rats.

^c $P < 0.05$ versus the cholesterol concentration in nondiabetic rats.

Inc., Fullerton, Calif.). After calculation of the CL_{in} , each rat was sacrificed by injecting a single intraperitoneal dose of sodium pentobarbital (300 mg/kg), and 1 ml of blood was collected by cardiac puncture. Serum was separated from the blood to determine glucose and cholesterol concentrations by enzymatic assays. The right kidney was removed, blot dried, and weighed.

Sample analysis. All blood samples were allowed to clot on ice and were centrifuged at $13,000 \times g$ for 2 min. The serum was separated and was placed on ice until it was analyzed. Serum glucose and cholesterol levels were determined by standard enzymatic reactions (Sigma Chemical Co.). The kidney was homogenized with normal saline (2 ml), and a 0.5-ml aliquot of the homogenate (following vortexing for 30 s) was streaked onto a Sabouraud dextrose agar plate. The plates were incubated for 18 h at 37°C, at which time the numbers of CFU were determined and corrected for kidney weight.

Data analysis. CL_{in} was calculated by standard noncompartmental methods. The elimination rate constant (k_e) was iterated by nonlinear regression (PCNONLIN; Human Systems Dynamics) of the terminal time points. The area under the serum concentration-time curve (AUC) was calculated by the trapezoidal rule and was extrapolated to infinity by the following equation: $\text{AUC} = \text{AUC}_{0-t} + C_t/k_e$, where AUC_{0-t} is the AUC from time zero to time t and C_t is the counts per minute of ^3H in the blood sample obtained at the last time point. CL_{in} was estimated by the following equation: $\text{CL}_{\text{in}} = D/\text{AUC}$, where D is the counts per minute of injected solution multiplied by the volume injected (0.25 ml). The degree of antifungal activity was determined by measuring the numbers of CFU of *C. albicans* in the kidney.

Statistical analysis. CL_{in} , the numbers of CFU in the kidney, and glucose and cholesterol concentrations were compared between groups by analysis of variance (PCANOVA; Human Systems Dynamics). Critical differences were assessed by the Newman-Keuls post hoc tests. A difference was considered significant if the probability of chance explaining the results was reduced to less than 5% ($P < 0.05$). All data were expressed as means $\pm$ standard deviations.

RESULTS

Biochemical parameters. No significant differences in body weight or kidney weight were observed between diabetic and nondiabetic rats with systemic candidiasis (data not shown). Consistent with the lipid-diabetes model (30), serum glucose and total serum cholesterol concentrations were significantly higher in diabetic rats than in nondiabetic rats with systemic candidiasis for all treatment groups (Table 1).

Antifungal activity. To assess the antifungal activities of AmB and L-AmB in nondiabetic and diabetic rats with systemic candidiasis, rats were randomly assigned to receive a single intravenous bolus of either NS (1 ml), AmB (0.8 mg/kg), or L-AmB (0.8, 2.0, or 4.0 mg/kg). At 25.5 h after the injection, the animals were sacrificed and the numbers of CFU per gram of kidney were determined as described above. AmB and L-AmB at 0.8 mg of AmB per kg of rat body weight significantly decreased the numbers of CFU of *C. albicans* in the kidneys of diabetic ($P = 0.05$) and nondiabetic ($P = 0.10$) rats compared with the numbers in the kidneys of saline-treated controls (Fig. 1). Furthermore, L-AmB at 2 and 4 mg/kg significantly decreased the numbers of CFU of *C. albicans* in the kidneys of

FIG. 1. The numbers of CFU of *C. albicans* per gram of kidney (kidney weight [KW]) in infected diabetic and nondiabetic rats following the administration of single intravenous doses of NS (1 ml; S), AmB at 0.8 mg/kg (A 0.8), or L-AmB at 0.8 mg/kg (LA 0.8), 2.0 mg/kg (LA 2), or 4.0 mg/kg (LA 4). C, uninfected diabetic or nondiabetic rat controls. Values are means $\pm$ standard deviations ($n = 5$ rats in each treatment group). *, $P < 0.05$ compared with diabetic rats treated with NS; @, $P < 0.05$ compared with nondiabetic rats treated with NS; @@, $P < 0.10$ compared with nondiabetic rats treated with NS.

both diabetic and nondiabetic rats ($P = 0.05$) compared with the numbers in the kidneys of saline-treated controls (Fig. 1). Since our previous studies have shown that AmB doses of greater than 1.0 mg/kg resulted in animal death (16, 24), no comparisons of AmB with higher doses of L-AmB could be made. Empty DMPC-DMPG liposomes did not alter the numbers of CFU of *C. albicans* in the kidneys of diabetic and nondiabetic rats with systemic candidiasis (data not shown).

Renal toxicity. To assess the renal toxicities of AmB and L-AmB in diabetic and nondiabetic rats with systemic candidiasis, rats were randomly assigned to receive a single intravenous bolus of either NS (1 ml), AmB (0.8 mg/kg), or L-AmB (0.8, 2.0, or 4.0 mg/kg). Twenty-four hours after the adminis-

tration of the dose a CL_{In} measurement was made to determine renal function. AmB treatment significantly decreased CL_{In} in diabetic rats with systemic candidiasis compared with that in diabetic rats without candidiasis ($P = 0.05$). L-AmB at all doses tested significantly increased CL_{In} in diabetic rats compared with that in saline-treated controls ($P = 0.05$) (Fig. 2). AmB treatment significantly increased CL_{In} in nondiabetic rats compared with that in saline-treated controls ($P = 0.10$) (Fig. 2). L-AmB at doses of 2 and 4 mg/kg significantly increased CL_{In} in nondiabetic rats compared with that in saline-treated controls ($P = 0.10$) (Fig. 2). Empty DMPC-DMPG liposomes did not alter the CL_{In} in diabetic and nondiabetic rats with systemic candidiasis (data not shown).

FIG. 2. CL_{In} per gram of kidney (kidney weight [KW]) in infected diabetic and nondiabetic rats receiving single intravenous doses of NS (1 ml; S), AmB at 0.8 mg/kg (A 0.8), or L-AmB at 0.8 mg/kg (LA 0.8), 2.0 mg/kg (LA 2), or 4.0 mg/kg (LA 4). C, uninfected, diabetic or nondiabetic rat controls. Values are means $\pm$ standard deviations ($n = 5$ rats in each treatment group). *, $P < 0.05$ compared with control diabetic rats; #, $P < 0.10$ compared with diabetic rats treated with NS; **, $P < 0.10$ compared with nondiabetic rats treated with NS.

DISCUSSION

The present study demonstrates that AmB at a dose of 0.8 mg/kg and L-AmB at doses of 0.8, 2, and 4 mg/kg are effective antifungal agents in both diabetic and nondiabetic rats with systemic candidiasis. Furthermore, AmB at 0.8 mg/kg and L-AmB at 2 and 4 mg/kg improves renal function in both diabetic and nondiabetic rats with systemic candidiasis.

A critical factor which should not be overlooked when interpreting these findings is that the diabetic rats are probably immunocompromised and are less likely than nondiabetic rats to clear the infecting *Candida* isolates. Thus, the nondiabetic immunocompetent rats would demonstrate substantially lower numbers of CFU per gram of kidney than the diabetic rats simply because of the intact immune systems of the nondiabetic rats (Fig. 1). While there was approximately a 4-fold decline in the mean numbers of CFU per gram of kidney in nondiabetic rats, there was only approximately a 2.5-fold decline for the comparable AmB dose (0.8 mg/kg) in diabetic rats. There also appeared to be a similar fold reduction for L-AmB at all doses tested. These findings suggest that AmB and L-AmB appear to be less effective in killing *C. albicans*.

A possible hypothesis to explain the decreased antifungal activities of AmB and L-AmB following the onset of diabetes (which increases total [Table 1] serum HDL and LDL cholesterol levels [8]) may be a result of the drug's predominant distribution within the plasma HDL cholesterol fraction upon entrance into the bloodstream (24). This increased level of association of AmB with plasma HDL may also explain the changes in the pharmacokinetics and tissue distributions of AmB in diabetic rats compared with those in nondiabetic rats observed in a previous study (30).

The decreased AUC for AmB, the increased clearance of AmB from serum, and the increased volume of distribution of AmB at steady state (30) in diabetic rats compared with those in nondiabetic rats suggest that following its administration AmB is cleared more rapidly from the systemic circulation into peripheral tissues of diabetic rats than those of nondiabetic rats. This could be explained by AmB's interaction with HDL. Since HDL has a terminal half-life of 1 to 2 h and is found mostly in the peripheral tissues of rats (6), increasing levels of circulating HDL cholesterol could result in an increase in the amount of AmB removed from the systemic circulation by way of its association with HDL. Furthermore, the interaction of AmB with HDL may decrease AmB's interaction with systemic and tissue fungal cells, resulting in an overall decrease in the antifungal activities of AmB and L-AmB. However, one caveat to this hypothesis is that without doing a dose-response analysis of AmB in diabetic rats, we cannot be sure if the decrease in the numbers of CFU is a result of the activity of AmB or is simply the result of the fact that immune-modulating properties help to reduce the numbers of CFU without having any effect on the pharmacokinetics of AmB.

Improvement in renal function following the administration of AmB to diabetic and nondiabetic rats may be due to a significant decrease in the numbers of CFU of *C. albicans* in the kidneys. The lack of improvement in renal function observed following the administration of L-AmB at a dose of 0.8 mg/kg to nondiabetic rats may be a result of a decrease in the numbers of CFU of *C. albicans* in the kidneys that is not ample enough to alter kidney function. However, the increased renal function found following the administration of L-AmB at a dose of 0.8 mg/kg to diabetic rats may be a result of a significant decrease in the numbers of CFU of *C. albicans* in the kidneys (Fig. 1) and an increasing association of L-AmB with HDL (24, 27). Previous studies have demonstrated that HDL-

associated AmB is less toxic to renal cells than LDL-associated AmB (29). Therefore, increasing AmB's association with HDL by incorporating it into a liposome appears to decrease its renal toxicity. In addition, although plasma HDL and LDL concentrations were not measured and correlated with toxicity and fungicidal activity in the present study, we have preliminary findings which suggest that patients with higher serum LDL cholesterol concentrations are more susceptible to AmB-induced renal toxicity than patients with lower serum LDL cholesterol concentrations (25).

These studies suggest that the presence of diabetes in rats with systemic candidiasis appears to modify the therapeutic indices of AmB and L-AmB by decreasing their effectiveness at killing the fungi while improving renal function in both treatment groups. Further work is warranted to determine the mechanism(s) that may explain the discrepancies in the antifungal activities of AmB and L-AmB administered to diabetic rats compared with those of the drugs administered to nondiabetic rats with systemic candidiasis.

ACKNOWLEDGMENTS

Funds to complete this work were provided by Tilak Technologies and the Medical Research Council of Canada. K.M.W. is a fellow of the American Society of Pharmacology and Experimental Therapeutics.

REFERENCES

1. Cabor, G. G., R. Pazdur, F. A. Valeriote, and L. H. H. Baker. 1989. Pharmacokinetics and toxicity of continuous infusion of amphotericin B in cancer patients. *J. Pharm. Sci.* **78**:307-310.
2. Cipolle, R. J., and J. S. Solomkin. 1986. Clinical application of therapeutic drug monitoring, p. 321-328. In W. J. Taylor and M. H. Diers Caviness (ed.), *Amphotericin B*. Diagnostics Division, Abbott Laboratories, Irving, Tex.
3. Feingold, K. R., R. M. Krauss, M. Pang, W. Doerrler, P. Jensen, and C. Grunfeld. 1993. The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low density lipoprotein subclass pattern B. *J. Clin. Endocrinol. Metab.* **76**:1423-1427.
4. Gibaldi, M., and D. Perrier. 1982. Pharmacokinetics, p. 1-41. Marcel Dekker, Inc., New York.
5. Grunfeld, C., M. Pang, W. Doerrler, J. K. Shigenaga, P. Jensen, and K. R. Feingold. 1992. Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J. Clin. Endocrinol. Metab.* **74**:1045-1052.
6. Harmony, J. A. K., A. L. Aleson, and B. M. McCarthy. 1986. Study of lipids and lipoproteins, p. 403-430. In A. M. Scanu and A. A. Spector (ed.), *Biochemistry and biology of plasma lipoproteins*. Marcel Dekker, Inc., New York.
7. Janoff, A. S., L. T. Boni, M. C. Popescu, et al. 1988. Unusual lipid structures selectively reduce the toxicity of amphotericin B. *Proc. Natl. Acad. Sci. USA* **85**:6122-6128.
8. Laakso, M., and K. Pyorala. 1988. Lipid and lipoprotein abnormalities in diabetic patients with peripheral vascular disease. *Atherosclerosis* **74**:55-63.
9. Lopez-Berestein, G. 1988. Liposomes as carriers of antifungal drugs. *Ann. N. Y. Acad. Sci.* **544**:590-597.
10. Lopez-Berestein, G., G. P. Bodey, V. Fainstein, M. Keating, L. S. Frankel, B. Zeluff, L. Gentry, and K. Mehta. 1989. Treatment of systemic fungal infections with liposomal amphotericin B. *Arch. Intern. Med.* **149**:2533-2536.
11. Lopez-Berestein, G., V. Fainstein, R. L. Hopfer, et al. 1985. Liposomal amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary study. *J. Infect. Dis.* **154**:704-710.
12. Lopez-Berestein, G., R. L. Hopfer, R. Mehta, K. Mehta, E. M. Hersh, and R. L. Juliano. 1984. Liposome-encapsulated amphotericin B for the treatment of disseminated candidiasis in neutropenic mice. *J. Infect. Dis.* **150**:278-283.
13. Lopez-Berestein, G., R. L. Hopfer, R. Mehta, K. Mehta, E. M. Hersh, and R. L. Juliano. 1984. Prophylaxis of *Candida albicans* infection in neutropenic mice with liposome-encapsulated amphotericin B. *Antimicrob. Agents Chemother.* **25**:366-367.
14. Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. Juliano. 1983. Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposomal-encapsulated amphotericin B. *J. Infect. Dis.* **147**:939-945.
15. Lopez-Berestein, G., M. G. Rosenblum, and R. Mehta. 1984. Altered tissue distribution of amphotericin B by liposomal encapsulation; comparison of normal mice to mice infected with *Candida albicans*. *Cancer Drug Delivery* **1**:199-205.

16. **Luke, D. R., K. M. Wasan, R. R. Verani, L. J. Brunner, K. L. Berens, K. Vadie, and G. Lopez-Berestein.** 1991. Attenuation of amphotericin B nephrotoxicity in the candidiasis rat model. *Nephron* **59**:139–144.
17. **Medoff, G., J. Brajtburg, and G. S. Kobayashi.** 1983. Antifungal agents useful in therapy of systemic fungal infections. *Annu. Rev. Pharmacol. Toxicol.* **23**:303–313.
18. **Mehta, R. T., T. J. McQueen, A. Keyhani, and G. Lopez-Berestein.** 1994. Phagocyte transport as a mechanism for enhanced therapeutic activity of liposomal amphotericin B. *Exp. Chemother.* **40**:256–264.
19. **Morton, R. E.** 1990. Interaction of lipid transfer protein with plasma lipoproteins and cell membranes. *Experientia* **46**:552–560.
20. **Morton, R. E., and D. B. Zilversmit.** 1983. Inter-relationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258**:11751–11757.
21. **Nicholl, T., P. Phillips, and P. J. Jewesson.** 1994. Amphotericin B use in a major acute care hospital. *Clin. Ther.* **16**:28–40.
22. **Tolins, J. P., and L. Rajj.** 1989. Adverse effect of amphotericin B administration on renal hemodynamics in the rat neurohumoral mechanisms and influence of calcium channel blocker. *J. Pharmacol. Exp. Ther.* **245**:594–599.
23. **Venkataram, S., W. M. Awni, K. Jordan, and Y. E. Rahman.** 1990. Pharmacokinetics of two alternative dosage forms for cyclosporine: liposomes and intralipid. *J. Pharm. Sci.* **79**:216–219.
24. **Wasan, K. M., G. A. Brazeau, A. Keyhani, A. C. Hayman, and G. Lopez-Berestein.** 1993. Role of liposome composition and temperature on the distribution of amphotericin B in serum lipoproteins. *Antimicrob. Agents Chemother.* **37**:246–250.
25. **Wasan, K. M., and J. S. Conklin.** Enhanced amphotericin B nephrotoxicity in intensive care patients with elevated low-density cholesterol. *Clin. Infect. Dis.*, in press.
26. **Wasan, K. M., V. B. Grossie, Jr., and G. Lopez-Berestein.** 1994. Concentrations in serum and distribution in tissue of free and liposomal amphotericin B in rats during continuous intralipid infusion. *Antimicrob. Agents Chemother.* **38**:2224–2226.
27. **Wasan, K. M., and G. Lopez-Berestein.** 1993. The influence of serum lipoproteins on the pharmacokinetics and pharmacodynamics of lipophilic drugs and drug carriers. *Arch. Med. Res.* **24**:395–401.
28. **Wasan, K. M., R. E. Morton, M. G. Rosenblum, and G. Lopez-Berestein.** 1994. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoproteins: role of lipid transfer protein. *J. Pharm. Sci.* **83**:1006–1010.
29. **Wasan, K. M., M. G. Rosenblum, L. Cheung, and G. Lopez-Berestein.** 1994. Influence of lipoproteins on renal cytotoxicity and antifungal activity of amphotericin B. *Antimicrob. Agents Chemother.* **38**:223–227.
30. **Wasan, K. M., K. Vadie, G. Lopez-Berestein, and D. R. Luke.** 1990. Pharmacokinetics, tissue distribution and toxicity of free and liposomal amphotericin B in a diabetic rat model. *J. Infect. Dis.* **161**:562–566.