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MINIREVIEW

Liposomes as Carriers of Antimicrobial Agents

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Liposomes are microscopic vesicles consisting of multiple concentric lipid bilayers formed when an aqueous solution is added to a dried lipid film. Since their initial description by Bangham et al. (5), several types of liposomes have been characterized (41). Multilamellar liposomes or multilamellar vesicles have been extensively used as carriers of antineoplastic and antimicrobial drugs. These multilamellar vesicles have two parts: an extensive lipid compartment that embodies the multiple lipid layers and an aqueous compartment that consists of the spaces between the layers.

As with most drug carriers, liposomes have been extensively used in an attempt to improve the therapeutic index of known active drugs, such as Adriamycin (14). In those studies, the major reduction observed in the target-organ toxicity of the drugs was probably related to a modification in drug distribution. Liposomes are preferentially taken up by organs rich in cells from the reticuloendothelial system. In mice (30) and humans (25), liposomes have been shown to localize in the liver, spleen, lung, and bone marrow.

The biologic and pharmacologic attributes of liposomes have been exploited by several investigators for the delivery of antimicrobial agents (Table 1). Liposome type, size, lipid composition, membrane fluidity, stability and charge, and ease of preparation are factors that must be considered when designing liposomal carriers. Multilamellar vesicles with their large lipid compartment offer an advantage for the incorporation of hydrophobic drugs; in contrast, reverse evaporation vesicles are a better choice for the incorporation of hydrophilic compounds. Usually, naturally occurring, biodegradable, and nontoxic phospholipids, such as lecithin, phosphatidyl serine, and phosphatidyl glycerol, are selected. The fluidity and charge of the liposomal membranes can be altered to facilitate specific objectives, such as in the case of macrophage targeting, in which negatively charged liposomes with more fluid membranes are preferentially phagocytosed. The incorporation of sterols (ergosterol and cholesterol) increases the rigidity and stability of liposomes. However, in the case of amphotericin B (AmpB) the use of sterol-containing liposomes results in decreased antifungal activity. This latter observation is probably related to tight drug-sterol binding which impedes the exchange of the drug with fungal membranes.

AmpB

AmpB, a heptaene antibiotic, is a highly effective but toxic antifungal drug whose activity is related to its capacity to interact with lipids in biologic membranes (3, 17). The high hydrophobicity of AmpB makes it a good candidate for liposomal incorporation. Liposome-incorporated AmpB (L-AmpB) has been studied in a variety of experimental infections. Early work by New et al. (36) demonstrated that L-AmpB was less toxic and more active against experimental leishmaniasis than the free drug (Fungizone). They reported that the lipid composition influenced the toxicity of liposomes, and they observed a decreased toxicity with liposomes composed of sterols, hydrogenated lecithins, or both. Similar results were observed by Graybill et al. (16) against experimental cryptococcosis and by Taylor et al. (42) against experimental histoplasmosis. In both cases, the incorporation of AmpB in liposomes resulted in lower toxicity and at least equal therapeutic efficacy. L-AmpB was also shown to be far less toxic than AmpB but similarly active against experimental candidiasis (Fig. 1; 24, 26, 28). In early studies, the toxicity of AmpB was reduced 10- to 20-fold by encapsulation, thus allowing the use of higher single doses in therapeutic experiments. Furthermore, L-AmpB proved to be superior to AmpB in the treatment and prophylaxis of experimental candidiasis in neutropenic mice (24).

We also observed that the lipid composition played a major role in L-AmpB activity. L-AmpB preparations composed of phospholipids alone were shown to be more effective than those containing ergosterol or cholesterol (27). A possible explanation for these findings was that the sterol-containing L-AmpB preparations combined AmpB more tightly, reducing the potential for drug interaction with fungal membranes.

Clinical studies. Systemic fungal infections are a frequent complication in patients with hematologic malignancies and in immunocompromised patients (10, 15). Although in these patients AmpB is the drug of choice, its usefulness is often limited by its associated toxicity. Maksymiuk et al. (31) observed that of 93 patients with systemic candidiasis, none with persistent neutropenia, including 25 patients who were treated with antifungal therapy, survived the infection. Of the patients with systemic candidiasis who recovered from neutropenia and were treated with antifungal agents, 38% were cured.

Nineteen patients with systemic fungal infections complicating their hematologic malignancies have been treated (22a, 23). Of these, 18 had been previously treated with antifungal agents, including AmpB, and 1 patient had a bona fide history of anaphylactic reaction to AmpB. All had histologic and cultural evidence of progressive fungal infection at the start of therapy with L-AmpB. Nine patients were cured, six had partial responses, and four did not respond to treatment (Table 2). Nine patients were neutropenic throughout treatment; of these, one was cured, four had partial responses, and four did not respond to therapy. Responses were observed in patients with pulmonary, sinus, hepatosplenic, and renal infections. Fever and chills were seen infrequently and responded well to meperidine or diphenhydramine. No evidence of chronic renal or central nervous system toxicity has been observed in patients with

TABLE 1. Liposomes as drug carriers for antimicrobial agents

Drug(s)	Disease	Reference(s)
AmpB	Leishmaniasis	37, 38
	Histoplasmosis	42
	Cryptococcosis	16
	Candidiasis	24, 26, 27
Antimonials	Leishmaniasis	1, 2, 7, 8, 36, 37, 43
Cephalothin	Salmonellosis	11, 12
Primaquine phosphate	Malaria	39, 40
Immunomodulators		
Muramyl dipeptide	Herpes	20
	Candidiasis	13
Interferon	Hepatitis	22
Other antibiotics		4, 6, 21, 35

follow-up periods greater than 1 year. In none of the nine patients cured who received additional chemotherapy for their leukemia (of these patients, two had bone marrow transplantation) was there evidence of recurrence of the fungal infection.

Pharmacologic considerations. The mechanisms involved in the enhanced therapeutic index observed with L-AmpB are not well understood, but tissue localization may play a role. We observed that when AmpB was given in liposomes, higher concentrations were found in the liver, spleen, lung, and kidney tissue of mice infected with *Candida albicans* (30). However, tissue localization alone may not be the explanation, since AmpB concentrations exceeding 160 μg/g of tissue (liver and spleen) were noted in patients with hepatic candidiasis failing to respond to AmpB (9).

This observation raises the possibility that L-AmpB may be delivered to the infected sites via either capillary leakage or secondary transport by phagocytes. In vitro, it has been shown that L-AmpB fungicidal activity was maintained, although its toxicity to erythrocytes was abrogated (33). However, the presence of serum reduces the toxicity of AmpB; therefore, the conditions present in the biologic

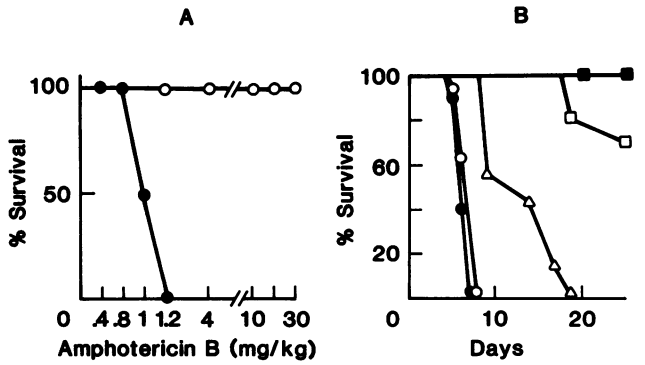

FIG. 1. Toxicity and antifungal activity of free AmpB versus L-AmpB in mice. All drugs were administered intravenously in 0.2 ml of 0.9% NaCl solution. (A) Toxicity. Symbols: ●, free AmpB; ○, L-AmpB. (B) Antifungal activity. The drugs were injected 2 days after inoculation of yeasts. Symbols: ●, untreated infected control; ○, empty liposomes; △, free AmpB at 0.8 mg/kg; □, free AmpB at 0.8 mg/kg daily for 5 days; ■, L-AmpB at 4 mg/kg. Modified from reference 28.

TABLE 2. Summary of clinical trials with L-AmpB in treatment of systemic fungal infections

Disease	No. of patients		
	Cured	Partial response	No response
Candidiasis	7	2	1
Aspergillosis	2	3	2
Other	0	1	1

microenvironment were not accurately reflected in these experiments. We also observed that the suppressive effect of AmpB on macrophages (32) was also nullified by AmpB incorporation in liposomes (34). Thus, a preponderant exchange of AmpB from sterol-free liposomes to ergosterol-containing fungal membranes may also be a factor.

ANTIMONIAL DRUGS

An excellent example of the use of liposomes to carry antimicrobial agents was provided by the studies of Alving et al. (1, 2) in the treatment of experimental leishmaniasis. Leishmanias are intracellular parasites of phagocytic cells of the reticuloendothelial system, offering a natural target for liposomal delivery. These investigators showed that liposome-incorporated antimicrobial agents and 8-aminoquinolines were far superior to the free drugs against this parasite.

CEPHALOTHIN

Liposome-incorporated cephalothin has been shown to be effective in the treatment of other facultative intracellular bacteria. Desiderio and Campbell (11, 12) showed that liposome-encapsulated cephalothin was more effective than the free drug in the treatment of experimental murine salmonellosis. Mice treated with the liposomal form of the drug had a reduced number of salmonellae in the liver and spleen.

PRIMAQUINE

Liposome encapsulation of primaquine phosphate has been demonstrated to reduce the acute toxicity related to the drug while allowing it to maintain its efficacy against murine malaria (39, 40). Although drug targetting was not the major objective in these experiments, its therapeutic index was enhanced. Primaquine phosphate is toxic mainly to the lungs, heart, kidneys, and brain; by incorporation in liposomes, its distribution is radically modified.

IMMUNOMODULATORS

Macrophages are a major component of the defenses of a host against microbial diseases. Several synthetic and biologic products can render macrophages activated to destroy microorganisms and neoplastic cells. Liposome-incorporated macrophage activators have been shown to be more effective than free agents in the activation of macrophages for antitumor activity (29). A similar rationale was used to treat herpes (18–20), hepatitis (22), and fungal (13) infections.

PROSPECTS

Liposomes are but one of the many new and promising drug-carrier systems being developed. Their successful use

in the treatment of several experimental diseases demonstrates that, with the selection of the right drug, the right carrier, and the right disease target, a solid rationale for the clinical development of liposomes as drug carriers can be established. Understanding of liposome behavior in the body and of the physicochemical mechanisms involved in the interactions of liposome, drug, and cellular targets is essential for their future application. Technology has recently been developed for large-scale production of liposome-incorporated drugs, so their promise will soon be verified in extensive clinical trials.

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LITERATURE CITED

1. **Alving, C. R., E. A. Steck, W. L. Chapman, Jr., V. B. Waits, L. D. Hendricks, G. M. Swartz, Jr., and W. L. Hanson.** 1980. Liposomes in leishmaniasis: therapeutic effects of antimonial drugs, 8-aminoquinolines, and tetracycline. Life Sci. **26**:2231–2238.
2. **Alving, C. R., E. A. Steck, W. L. Hanson, P. S. Loizeaux, W. L. Chapman, Jr., and V. B. Waits.** 1978. Improved therapy of experimental leishmaniasis by use of a liposome-encapsulated antimonial drug. Life Sci. **22**:1021–1026.
3. **Andreoli, T. E.** 1974. The structure and function of amphotericin B-cholesterol pores in lipid bilayer membranes. Ann. N.Y. Acad. Sci. **235**:448–468.
4. **Bakker-Woudenberg, I. A. J. M., A. F. Lokerse, J. C. Vink-van den Berg, F. H. Roerdink, and M. F. Michel.** 1986. Effect of liposome-entrapped ampicillin on survival of *Listeria monocytogenes* in murine peritoneal macrophages. Antimicrob. Agents Chemother. **30**:295–300.
5. **Bangham, A. D., M. W. Hill, and N. G. A. Miller.** 1974. Preparation and use of liposomes as models of biological membranes. Methods Membr. Biol. **1**:1–68.
6. **Bonventre, P. F., and G. Gregoriadis.** 1978. Killing of intraphagocytic *Staphylococcus aureus* by dihydrostreptomycin entrapped within liposomes. Antimicrob. Agents Chemother. **13**:1049–1051.
7. **Chapman, W. L., Jr., W. L. Hanson, C. R. Alving, and L. D. Hendricks.** 1984. Antileishmanial activity of liposome-encapsulated meglumine antimonate in the dog. Am. J. Vet. Res. **45**:1028–1030.
8. **Chapman, W. L., Jr., V. B. Waits, and W. L. Hanson.** 1984. Liposomes in leishmaniasis: effects of parasite virulence on treatment of experimental leishmaniasis in hamsters. Ann. Trop. Med. Parasitol. **78**:279–286.
9. **Christiansen, K. J., E. M. Bernard, J. W. M. Gold, and D. Armstrong.** 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. **152**:1037–1043.
10. **Degregorio, M. W., W. M. F. Lee, C. A. Linker, R. A. Jacobs, and C. A. Ries.** 1982. Fungal infections in patients with acute leukemia. Am. J. Med. **73**:543–548.
11. **Desiderio, J. V., and S. G. Campbell.** 1983. Intraphagocytic killing of *Salmonella typhimurium* by liposome-encapsulated cephalothin. J. Infect. Dis. **148**:563–570.
12. **Desiderio, J. V., and S. G. Campbell.** 1983. Liposome-encapsulated cephalothin in the treatment of experimental murine salmonellosis. RES J. Reticuloendothel. Soc. **34**:279–287.
13. **Fraser-Smith, E. B., D. A. Eppstein, M. A. Larsen, and T. R. Matthews.** 1983. Protective effect of a muramyl dipeptide analog encapsulated in or mixed with liposomes against *Candida albicans* infection. Infect. Immun. **39**:172–178.
14. **Gabizon, A., A. Dagan, D. Goren, Y. Barenholz, and Z. Fuks.** 1982. Liposomes as *in vivo* carriers of Adriamycin: reduced cardiac uptake and preserved antitumor activity in mice. Cancer Res. **42**:4734–4739.
15. **Gold, J. W. M.** 1984. Opportunistic fungal infections in patients with neoplastic disease. Am. J. Med. **76**:458–463.
16. **Graybill, J. R., P. C. Craven, R. L. Taylor, D. M. Williams, and W. E. Magee.** 1982. Treatment of murine cryptococcosis with liposome-associated amphotericin B. J. Infect. Dis. **145**:748–752.
17. **Hamilton-Miller, J. M. T.** 1973. Chemistry and biology of the polyene macrolide antibiotics. Bacteriol. Rev. **37**:166–196.
18. **Koff, W. C., and I. J. Fidler.** 1985. The potential use of liposome-mediated antiviral therapy. Antiviral Res. **5**:179–190.
19. **Koff, W. C., I. J. Fidler, S. D. Showalter, M. K. Chakrabarty, B. Hampar, L. M. Ceccorulli, and E. Kleinerman.** 1984. Human monocytes activated by immunomodulators in liposomes lyse herpesvirus-infected but not normal cells. Science **224**:1007–1009.
20. **Koff, W. C., S. D. Showalter, D. A. Seniff, and B. Hampar.** 1983. Lysis of herpesvirus-infected cells by macrophages activated with free or liposome-encapsulated lymphokine produced by a murine T cell hybridoma. Infect. Immun. **42**:1067–1072.
21. **Kozulitsina, T. I., O. A. Uvarova, V. I. Golyshevskaya, and M. P. Elshanskaya.** 1980. Experimental study of chemotherapeutic activity of various antibacterial drug combinations. Probl. Tuberk. **7**:53–57.
22. **LaBonnardiere, C.** 1978. Donnees preliminaires sur l'effet protecteur de l'interferon couple a des liposomes dans le modele souris-virus de l'hepatite murine. Ann. Microbiol. (Paris) **129A**:397–402.

22a. **Lopez-Berestein, G., G. P. Bodey, L. S. Frankel, and K. Mehta.** 1987. Treatment of hepatosplenic fungal infections with liposomal-amphotericin B. J. Clin. Oncol. **5**:310–317.

23. **Lopez-Berestein, G., V. Fainstein, R. L. Hopfer, K. Mehta, M. P. Sullivan, M. Keating, M. G. Rosenblum, R. Mehta, M. Luna, E. M. Hersh, J. Reuben, R. L. Juliano, and G. P. Bodey.** 1985. Liposomal-amphotericin B for the treatment of systemic fungal infections in patients with cancer: a preliminary report. J. Infect. Dis. **4**:704–710.
24. **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.
25. **Lopez-Berestein, G., L. Kasi, M. G. Rosenblum, T. Haynie, M. Jahns, H. Glenn, R. Mehta, G. M. Mavligit, and E. M. Hersh.** 1984. Clinical pharmacology of ^{99m}Tc-labeled liposomes in patients with cancer. Cancer Res. **44**:375–378.
26. **Lopez-Berestein, G., T. McQueen, and K. Mehta.** 1985. Protective effect of liposomal-amphotericin B against candida infection in mice. Cancer Drug Delivery **2**:183–189.
27. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mehta, E. M. Hersh, and R. Juliano.** 1983. Effects of sterols on the therapeutic efficacy of liposomal amphotericin B in murine candidiasis. Cancer Drug Delivery **1**:37–42.
28. **Lopez-Berestein, G., R. Mehta, R. L. Hopfer, K. Mills, L. Kasi, K. Mehta, V. Fainstein, M. Luna, E. M. Hersh, and R. L. Juliano.** 1983. Treatment and prophylaxis of disseminated infection due to *Candida albicans* in mice with liposome-encapsulated amphotericin B. J. Infect. Dis. **147**:939–944.
29. **Lopez-Berestein, G., L. Milas, N. Hunter, K. Mehta, E. M. Hersh, C. G. Kurahara, M. Vanderpas, and D. A. Eppstein.** 1984. Prophylaxis and treatment of experimental lung metastases in mice after treatment with liposome-encapsulated 6-*O*-stearoyl-*N*-acetylmuramyl-L-α-aminobutyryl-D-isoglutamine. Clin. Exp. Metastasis **2**:127–137.
30. **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 **3**:199–205.
31. **Maksymiuk, A. W., S. Thongprasert, R. Hopfer, M. Luna, V. Fainstein, and G. P. Bodey.** 1984. Systemic candidiasis in cancer patients. Am. J. Med. **77**(Suppl. 4D):20–27.
32. **Mehta, K., P. Claringbold, and G. Lopez-Berestein.** 1986. Amphotericin B inhibits the serum-induced expression of tissue transglutaminase in murine peritoneal macrophages. J. Immunol. **136**:4206–4212.
33. **Mehta, R., G. Lopez-Berestein, R. Hopfer, K. Mills, and R. L.**

Juliano. 1984. Liposomal-amphotericin B is toxic to fungal cells but not to mammalian cells. Biochim. Biophys. Acta **770**:230–234.

34. **Mehta, R. T., K. Mehta, G. Lopez-Berestein, and R. L. Juliano.** 1985. Effect of liposomal amphotericin B on murine macrophages and lymphocytes. Infect. Immun. **47**:429–433.
35. **Morgan, J. R., and K. E. Williams.** 1980. Preparation and properties of liposome-associated gentamicin. Antimicrob. Agents Chemother. **17**:544–548.
36. **New, R. R. C., M. L. Chance, and S. Heath.** 1981. Antileishmanial activity of amphotericin and other antifungal agents entrapped in liposomes. J. Antimicrob. Chemother. **8**:371–381.
37. **New, R. R. C., M. L. Chance, S. C. Thomas, and W. Peters.** 1978. Antileishmanial activity of antimonials entrapped in liposomes. Nature (London) **272**:55–56.
38. **Panosian, C. B., M. Barza, F. Szoka, and D. J. Wyler.** 1984. Treatment of experimental cutaneous leishmaniasis with liposome-intercalated amphotericin B. Antimicrob. Agents Chemother. **25**:655–656.
39. **Pirson, P., R. Steiger, and A. Trouet.** 1982. The disposition of free and liposomally encapsulated antimalarial primaquine in mice. Biochem. Pharmacol. **31**:3501–3507.
40. **Pirson, P., R. F. Steiger, A. Trouet, J. Gillet, and F. Herman.** 1980. Primaquine liposomes in the chemotherapy of experimental murine malaria. Ann. Trop. Med. Parasitol. **74**:383–391.
41. **Szoka, F., and D. Papahadjopoulos.** 1980. Comparative properties and methods of preparation of lipid vesicles (liposomes). Annu. Rev. Biophys. Bioeng. **9**:465–506.
42. **Taylor, R. L., D. M. Williams, P. C. Craven, J. R. Graybill, D. J. Drutz, and W. E. Magee.** 1982. Amphotericin B in liposomes: a novel therapy of histoplasmosis. Am. Rev. Respir. Dis. **125**:610–611.
43. **Weldon, J. S., J. F. Munnell, W. L. Hanson, and C. R. Alving.** 1983. Liposomal-chemotherapy in visceral leishmaniasis: an ultrastructural study of an intracellular pathway. Z. Parasitenkd. **69**:415–424.