PROCEEDINGS

OF THE

NATIONAL ACADEMY OF SCIENCES, INDIA

1996

VOL. LXVI SECTION–B SPL. ISSUE

Liposomes mediated drug delivery in infectious diseases

GRISH C. VARSHNEY and C.M. GUPTA

Institute of Microbial Technology, Sector 39-A, Chandigarh- 160014, India.

Abstract

Liposome-mediated drug delivery systems alongwith other newer approaches of drug targeting have revolutionized the measures of controlling parasitic infections, including, malaria, leishmania, fungal infections, besides providing a new approach to control several bacterial infections, including Mycobacterial, Salmonella, Pseudomonas, etc., some of which have acquired resistance. These approaches provide definite reduction in drug toxicity, specially in leishmaniasis and control of candidiasis and other pathogenic fungi. Liposomal drugs, like amphotericin-B, would have extensive use in the management of fungal diseases. Liposome mediated drug delivery has an exceptional advantage of biocompatibility, biodegradability, non-immunogenicity and can be used in clinical cases without any side-effects.

(**Key words** : liposomes/drug delivery/parasites/fungal infections/ T.B.)

Introduction

Pathogens have been with us since our existence and the diseases once thought to be conquered or under control have again gathered momentum. This is primarily because of the lacklustre performance of their control measures and the constant evolutionary pressures put up by the microbes on their hosts; recent examples being the emergence of drug resistance[1]. current global estimates of some of the ravaging diseases, e.g, malaria, leishmaniasis, tuberculosis etc., are alarming, emphasizing the importance of their effective treatment and control[2-4].

The last decade has seen a spurt in development of means to deliver antimicrobial agents/drugs to their intended sites of action in the body. Liposomes, first described in early sixties[5], have been extensively investigated for their potential as drug carriers[6]. Because of certain exquisite properties of liposomes, such as biocompatibility, biodegradability,

nonimmunogenicity and versatility in structural constituents, immunerable attempts have been made to use liposomes as drug/enzyme/antigen carriers in biology and medicine. The objectives of this review are two fold: firstly, to summarize various fascinating characteristics of liposomes which make them suitable for use as carriers for drug targeting and secondly, to provide a comprehensive account of the various attempts that have been made to use liposome-based drug delivery systems in treatment of infectious diseases.

Liposomes as drug carriers

Liposomes are spherically closed concentric bilayers which are separated by aqueous compartments, and are formed upon dispersing phospholipids in water. Their major constituents may include natural (phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, cardiolipin, sphingomyelin) or synthetic (dipalmitoylphosphatidylcholine, stearylamine derivatives, dicetylphosphate derivatives) phospholipids, sterols (cholesterol, ergosterol) fatty acids, glycolipids and proteins[7]. Most studies with liposome have been performed, using naturally occurring phospholipids. Depending on the mode of their preparation three types of liposomes can be formed, viz. multilamellar vesicles (MLVs, 200 to 5000 nm in diameter), small unilamellar vesicles (SUVs, 20-100 nm in diameter) and large unilamellar vesicles (LUVs, 100-1000 nm in diameter)[8].A variety of drugs can be encapsulated in liposomes. Highly polar or hydrophilic drugs are entrapped in the internal aqueous compartments (s), non-polar drugs can be intercalated into the liposomal membrane, while the amphipathic drugs partition between the membrane and aqueous phase. Hydrophilic drug entrapment is maximum in LUVs, while MLVs are more effective as lipophilic and non-polar drug carriers where the amount of the drug incorporated is directly proportional to the total mass of the lipid present. Polar drugs are released when the bilayer is broken, but highly non-polar drugs tend to remain associated with the lipid bilayer unless it is disrupted by freezing or by permeation[9]. Also, the release of the drug from liposome is a function of the ambient pH, ionic interactions, liposomes constituents, physicochemical properties of the drug and immunological recognition.

The most common route of liposome administration is intravenous injection following which drugs are rapidly cleared from the blood circulation by the cells of mononuclear phagocyte system (MPS) (also known as reticuloendothelial system, RES). The MPS includes, macrophages and macrophage precursors, specialized endothelial cells surrounding the sinusoids of the liver, spleen, lung and bone marrow and reticular endothelial cells of lymphatic tissue and of bone marrow. Besides, liposomes can also be taken up by circulating blood monocytes within the vascular system[10]. Adsorption and/or endocytosis is the major mechanism of liposome- cell interation. High local concentration of the drug at the intracellular site of infection is usually achieved by endocytosis of liposomes[11,12]. This involves, a) engulfment of adsorbed liposomes into vesicles, called phagosomes, by invagination of the cell membrane, and b) fusion of the phagosome with lysosomes which

contain degradative enzymes. Drugs resistant to lysosomal digestion are released into the cell. This can create a high local concentration of drugs at the intracellular sites of infection. Also, fusion of the outer liposomal membrane with the host cell membrane, and lipid exchange between liposomes and the cell membranes are two other mechanisms which may also contribute, although to a lower extent, towards uptake of the liposomal contents by the cells[8].

One of the major constraints in using liposomes as the drug delivery systems has been their rapid clearance from the blood circulation by the MPS. This may well be exploited for targeting of drugs to intracellular infections involving the MPS. On the other hand, it poses a serious limitation for delivering drugs to tissues and cells outside the MPS. Circulation time of liposomes depends on their size, charge, composition and dose administered. Large liposomes and negatively charged liposomes are cleared more rapidly than neutral or positively charged vesicles[13,14]. In the last few years, several efforts have been made towards the development of more rigid liposomes having better circulation time[15-18]. In general, *in vivo* stability and rigidity of the liposomes may be enhanced, to some extent, by increasing the percentage of long chain saturated phospholipids, viz. sphingomyelin and sterols in liposomes[8]. It may be further enhanced by including bilayer rigidfying lipids, such as cholesterol, distearoylphosphatidylcholine and sphingomyelin[19]. An elegant approach of mimicking the outer composition of the red blood cell membrane in liposomes, by incorporating monosialo-ganglioside, GMl, and hydrogenated phosphatidylinositol (HPI), resulted in the first generation of long circulating liposomes[20-21]. This paved the way in designing more clinically acceptable second generation liposomes which are formed from phospholipids derivitized with hydrophilic polyethylene glycol (PEG), e.g. PEG-distearoyl phosphatidylethanolamine. These liposomes, more commonly known as sterically stabilized liposomes (S-liposomes), have been well characterized[17,22-25]. S-liposomes have low affinity for the cells of MPS and therefore, show prolonged circulating periods irrespective of the dose administered, surface charge density and the bilayer composition[24,26]. Besides these surface modifications, attempts have also been made to stabilize the liposomes by introducing the fluorinated core within the membrane[27]. Liposomes made of the perfluoroalkylated phosphatidylcholines exhibit higher circulation times in blood, as compared to conventional liposomes of similar size comprising distearoyl phosphatidylcholine (DSPC) or distearoylphoshatidylcholine/cholesterol (DSPC/CH). Blood clearance rate of the fluorinated liposomes was found to be comparable to that of the S-liposomes[27]. More recently, efforts have also been made to combine cyclodextrins and liposomes into a single system[28].

Design of target-specific liposomes

In site-specific targeting of liposomes, usually a ligand (recognition marker), such as lectin[29,30], carbohydrate[31,32], antibody[33-36], paptides[34,37,38] or mannosylated serum albumin

(neoglycoprotein)[39] is attached to the liposomes to make it target specific. The ligand recognises the complementary sites on the target cell and thus increases the efficiency of drug delivery. Monoclonal antibodies (MAbs), armed with high degree of specificity towards certain cell surface antigens, offer the best potential for more precise targeting of the liposomes (referred to as immunoliposomes) to the desired sites in biophase.

Liposome-mediated drug delivery in infectious diseases

One of the major impediments in the effective treatment of the major infectious diseases, e.g. tuberculosis, leishmaniasis, etc., is the intracellular habitat of the causative organisms in macrophages. These organisms get protected from the action of many antimicrobial drugs because of the poor penetration and the poor retention of drug inside the macrophages or because of the decreased intracellular drug activity due to lower intracellular pH[40]. Thus, liposomes mediated targeting of drugs to infected cells holds great promise for the treatment of the various intracellular infections, that are major cause of mortality and morbidity in the third world.

a) *Protozoal infections* : Liposome-mediated drug delivery has been successfully demonstrated in parasitic diseases, particularly in leishmaniasis[37,41-44] and malaria[36,41,45-48].

Treatment of leishmaniasis is usually based on the use of leishmanicidal drugs, principally, pantavalent antimony compounds, such as sodium stibogluconate (Pentostan)[49] and meglumine antimoniate (Glucantime).[50]. Other systemic drugs with proven efficacy in human leishmaniasis are amphotericin B[51] pentamidine[52], itraconazole or miconazole[53] and the orally administered allopurinol ribonucleoside[54]. High toxicity associated with majority of these drugs poses a serious limitations in their systemic use. For instance, WHO[55] recommended dose of 20 mg of pentavalent antimonials/kg/day for nearly 30 days, is often associated with toxic side effect (arthralgias, myalgias and hepatic, cardiac and renal toxicities). These antimonials, when encapsulated in liposomes, proved 200-700–fold more effective than the free drug when compared for single i.v. dose against *L. donovani* rodent models.[56-58] Also, following this mode of delivery, 5-20 fold higher levels of drugs, compared to that when delivered in free form, could be maintained upto 14 days in blood circulation.[56] A variety of other clinically used drugs in liposomes have also been tested against *L.donovani* in animal models. Liposomal amphotericin B has been shown to exhibit much improved efficacy and lower toxicity as compared with free drug in experimental leishmaniasis[59,60].

Therapeutic efficacy of the liposomal antileishmanial drugs has been shown to dramatically increase by grafting the cell-specific ligands on the liposomal surface. In this context, tuftsin-bearing liposomes have been found to be highly efficient (at least 2000 times), as compared to free sodium stibogluconate, against the *L. donovani* infections in

mice[37]. Additionally, pretreatment with these liposomes also enhanced the non-specific resistance of the animals against various infections, including leishmaniasis[37]. Exclusive presence of the mannose receptors on the macrophage surface has also been exploited for the site- specific targeting of liposomal drugs[39,43,44,61]. Antileishmanial drug, ureastibamine, encapsulated in mannose-bearing liposomes was found to be more potent than the drug encapsulated in normal liposomes or the free drug, against *L. donovani* in the hamster model[43]. In another approach, a toxic antifungal polyene antibiotic, hamycin, encapsulated in mannosyl serum albumin (neoglycoprotein) containing liposomes was tested in mouse model of visceral leishmaniasis. This preparation at a dose of 1.5 mg/kg/day when given for 4 consecutive days completely cured a 45 days old leishmania infection in BALB/c mice model.[44] In spite of the higher efficacies of the above formulations in animal models, their clinical use was hampered mainly for two reasons; (i) down regulation of the mannose/glucose receptors in *L. donovani* infected macrophages and, ii) the presence of these receptors even in normal uninfected macrophages[62].

Malaria infected erthrocytes have been the other targets for liposomes-mediated drug delivery[36,41,45-48]. Encapsulation of the antimalarial drug chloroquine (CHQ) in liposomes hearing anti-erythrocyte antibody on their surface significantly increased their efficacy against both CHQ-susceptible and CHQ-resistant *Plasmodium berghei* infections in mice[45-47]. The efficacy was further improved by coating CHQ-laden liposomes with infected erythrocyte-specific polyclonal antibodies[48]. In a recent study, it has been shown that coating the CHQ laden liposomes with monoclonal antibody (MAb), specifically recognizing the *P. berghei* infected erythrocytes, significantly controlled not only the CHQ-susceptible but also the CHQ- resistant *P.berghei* infections in mice[36]. This clearly demonstrated that the therapeutic efficacy of CHQ can be significantly increased by delivering it in target-specific liposomes, thus paving the way for the treatment of drug resistant infections using the known drug at lower doses.

b) *Bacterial infections* : Liposome encapsulation of a variety of drugs has been successfully tested in treating several bacterial infections, such as those caused by *Mycobacterium tuberculosis, Mycobacterium avium, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Brucella abortus, Legionella pneumophila* etc[63-67].

Aminoglycosides (e.g. streptomycin, gentamycin, sisomycin, amikacin, tobramycin etc), despite their well documented toxic side effects, remain drugs of choice for the treatment of bacterial infections, because they act on both the gram-negative and gram-positive bacteria, develop only a limited drug resistance during the treatment and do not exhibit the inoculum effect[66,68].

Experimental murine salmonellosis caused by *Salmonella typhimurium,* which resembles the human typhoid causing intracellular bacteria, was effectively treated with

liposomal cephalothin.[69]. Also, liposome-encapsulted gentamycin was effective against similar infections[70]. Further, streptomycin (1.2 mg/kg) upon encapsulation in liposomes significantly enhanced the survival rate of *Salmonella enteritidis*- infected mice, while its 30-times higher dose (40 mg/kg) was only marginally effective in free form[64].

Using liposomal streptomycin, a 10-fold improvement, when compared to free drug, in *in vitro* killing of *E. coli* in J774.2 macrophages cell line was observed[71]. In another *in vitro* study, the liposomes entrapped gentamycin exhibited the enhanced antibacterial activity against clinically isolated resistant strains of *E. coli* and *Pseudomonas aeruginosa*[72]. Thus, the liposome-mediated drug delivery may provide an important approach to overcome bacterial resistance against known drugs.

Effective intracellular killing of *Brucella abortis or Brucella canis* has also been achieved by liposomal streptomycin[63] as well as by liposomal gentamycin[73] . In both the cases, efficacy of liposomal drugs was much superior when compared to free drugs. Similarly, a higher efficacy of the liposomal dihydrostreptomycin towards the intracellular killing of *Staphylococcus aureus* has been reported. The liposomal drug was at least 40-times more effective than the free drug in killing the intraphagosomic *S. aureus*[74]. In fact, the free drug was not much active against this bacteria.

Liposomal delivery of sisomycin has been attempted in experimental legionnaire's disease (caused by *Legionella pneumophila)* in guinea pigs as well as against *in vitro* cultured bacteria in human monocytes[75]. In the *in vitro* experiments, the efficacy was at least ten times higher for the liposomized drug, as compared to free drug, and an intravenous administration of this preparation cured 100% of the infected guinea pigs[75].

Tuberculosis remains a scourge to mankind. Incidence of tuberculosis infection, particularly *Mycobacterium avium- M. intracellulare* complex (MAC), has tremendously increased in the east decade primarily in patients with AIDS[3]. Rifampicin, isoniazid, pyrazinamide, thioautazone, p-aminosalicyclic acid, ethambutol and streptomycin are amongst the major front-line drugs in the treatment of tuberculosis[76,77]. In spite of their high antitubercular activity, use of free drugs is limited because of (i) long duration of chemotherapy (a minimum of 6-12 months)[78] and (ii) several side effects[79,80]. Combination of several drugs given at their maximum tolerated doses have not proven effective in controlling the disseminated disease[81]. Inherent tendency of liposome to localize in the phagocytic cells, that also serve as the host of *M. tuberculosis,* has been well exploited for "passive targeting" of liposomal drugs.

Liposomes mediated drug delivery for various antitubercular drugs has been attempted, using animal models as well as the *in vitro* systems. Liposomal-streptomycin exhibited several fold higher concentration in liver and spleens of infected animals, thus reducing the bacterial load significantly, as compared to that achieved with free drug[82,83]. Interestingly,

accumulation of drug in lung and kidney was not dependent on its formulation, suggesting that the free and not liposomal streptomycin accumulates is mouse kidney. Efficacy of liposomal streptomycin has also been tested in mouse model of nontuberculosis mycobacterial (MAC) infections[84,85]. These studies have clearly shown the reduced bacterial growth in liver and spleen compared to untreated controls[86] or the greater inhibition of the bacterial growth with liposomal streptomycin, compared to free drug[85].

Rifampin (RFP) has been extensively used as a potent antitubercular drug. It exhibits little or no cross-resistance with other antimicrobial drugs[87]. Although, the drug is safer, yet the high excretion rate necessitates daily administration in high doses[76]. This results in serious toxic side-effects[88]. Liposomized-RFP given at 10 mg/kg dose has been shown to be more effective than the free drug in controlling the tuberculosis infection[89]. Similar effect was seen when RFP was administered in combination with isoniazid in liposomes[90] Based on the observation that the intermittent but not the continuous treatment with Lip-RFP was around 50 folds more effective than the free drug led to the conclusion that Lip-RFP is preferentially localized in macrophages/monocytes[89]. A change in distribution pattern of Lip-RFP was observed in various tissues of tuberculosis infected animals as compared to normal animals. Comparatively, a higher liposome concentration in lungs, kidneys and blood was observed in tuberculosis-infected animals, whereas in uninfected animals Lip-RFP accumulated mainly in liver.[14] Further, liposomal targeting of RFP to macrophages was significantly enhanced by incorporating tuftsin in the bilayer of Lip-RFP, RFP delivered twice weekly for 2 weeks in tuftsin- bearing liposomes was at least 200 times more effective than the free drug in lowering the load of lung bacilli in infected animals[89].

Liposome encapsulated gentamycin was tested for controlling the disseminated MAC infection and its efficacy was compared to that of free gentamycin[91]. The encapsulated drug was found to be significantly more effective than the free drug alone in reducing the viable cell counts in mouse spleen, liver and lungs. Based on these promising results, phase I/II safety and dose finding study was undertaken using TLC G- 65, a liposome-encapsulated gentamycin[92]. TLCG-65, at different doses, was administered twice weekly for 4 weeks to AIDS patients with MAC infection. This treatment resulted in more than 75% reduction in MAC colony counts in blood[92].

Considerable attention has been focused on the use of liposomal amikacin in the treatment of experimental MAC infection.[86,93] Treatment with liposome encapsulated amikacin was substantially more effective in infected liver, spleen and kidney tissue than with equal amount of free drug given intravenously[86, 93]. A single injection of liposome encapsulated amikacin resulted in the persistence of its antimicrobial activity for at least five weeks, particularly in liver and kidneys[86].

c) *Fungal infections* : Fungal infections are a major cause of mortality of immunocompromised individuals, such as patients of cancer, renal transplant, AIDS etc.[94,95]

Liposome-based drug delivery system has been used in the treatment of several fungal infections, like histoplasmosis, cryptococcosis, aspergillosis and candidiasis[96-99], Polyene antibiotics, like amphotericin- B(Amp-B), nystatin, hamycin, etc., are potent antifungal drugs[96,100,101]. Among these, Amp-B has been the most widely and successfully tested in treatment of the fungal infections. The drug interacts with ergosterol/cholesterol in the fungal cell memberanes, thus generating transmembrane channels that permit the leakage of vital ions and metabolites. The drug, specially at higher doses, is highly nephrotoxic.

Efficacy and associated toxicity of Lip-Amp-B depends on several physical factors, like presence or absence of sterols, phospholipid type, lipid ratio and size of liposomes[102-103]. Due to lower toxicity of Lip-Amp-B, higher doses of the drug are tolerated, resulting in increased therapeutic index. This has been demonstrated in experimental animals[97,98] as well as in human trials[104-106], A follow up of 46 patients who received Lip-Amp-B therapy revealed that 25 patients with a variety of fungal infections were classified as complete responders to the liposomal drug[106]. Similar clinical improvement was noticed using Lip-Amp-B in another study conducted on cancer patients with frugal infections[107].

Majority of the fungal infections in cancer patients are caused by *Candida albicans*[108] Liposomal encapsulation decrease the toxicity associated with the administration of Amp-B while maintaining its therapeutic efficacy in experimental candidiasis[98,109,110]. A ten-fold increase in the dose was achieved with Lip-Amp-B without any apparent toxicity. Unlike free Amp B, Lip-Amp-B fully maintained its effectiveness even at doses of 5-20 mg/kg, providing 100% survival of mice[110]. Recently, it has been shown that the Lip-Amp-B aerosol, given twice-weekly upto 3 weeks, is highly effective in treating the lethal infection of *C. albicans* in mice.[111] Aerosolized Lip-Amp-B was also found effective in treatment of the mice infected intranasally with *Cryptococcus neoformans*[112]. An important observation emanating from the above studies was that Amp-B delivered in this manner to lungs was capable of diffusing to distant organs at therapeutic levels, thus curing the systemic infections.

In a murine model of cryptococcosis, which included intraperitoneal, intratracheal, or intracerebral challenges with *C. neoformans,* the efficacy of Lip-Amp B was compared with that of micellar Amp-B deoxycholate suspension (ABDS, Fungizone). Doses of Lip-Amp-B formulation that were five fold higher than the maximum tolerated dose of ABDS, were found to be more efficacious regardless of the site of inoculation of the organism[97]. Amp-B colloidal dispersion (ABCD) is another lipid based complex, consisting of Amp-B and cholesteryl sulfate in a 1:1 molar ratio, and has been proposed as an equally but less toxic alternative to ABDS, for the treatment of disseminated cryptococcosis[113].

Aspergillosis is one of the most common airborne systemic fungal infections which can be fatal for immunodebilitated patients[114]. Lip-Amp-B has been used to control aspergillosis in experimental animals[115] and humans[116]. Lip-Amp-B at a dose of 0.5 mg/kg

body weight is more effective & less toxic than the free drug in the treatment of experimental aspergillosis in BLAB/c mice[115]. Further, it was observed that inclusion of cholesterol into phosphatidylcholine liposomes increased the LD50 of AMP-B from 5.3. to 8.5 mg/kg body weight[117]. Moreover, the efficacy of liposomal AMP-B or hamycin could be further improved by grafting on the liposomes surface mannose[99] or tuftsin[38].

Coda

Targeting drugs specifically to diseased cells in the body has been one of the coveted goals in clinical therapy. Liposomes so far constitute the best known system and have been widely used in the delivery of several kinds of drugs. The last about ten years have seen a spurt in the efforts towards the improvement of this mode of drug delivery. Liposomes can now be designed with more circulation time and commercial acceptability. It is now clear that passive targeting of liposomes encapsulated drugs effectively cures MPS associated intracellular infections. This has led to the successful use of liposomal drugs in human patients with drug resistant visceral leishmaniasis, fungal infections and bacterial diseases. However, clinical data for liposome mediated drug delivery in infections outside MPS is sparse. Making liposomes more specific to diseased cells, without affecting normal cells, is another area demanding serious attention. Using parasite infected cell surface specific monoclonal antibodies for coating the drug laden liposomes may further provide specificity to this mode of drug delivery.

Acknowledgement

We are grateful to Ms. Apollina Goel and Ms. Amita Sharma for their help in preparing the manuscript. This is a communication no. 030/95 from Institute of Microbial Technology, Chandigarh, India.

References

1. Gibbon, A. (1992) *Science* **257 :** 1036.
2. Ashford, R.W., Desjeux, P. & DeRaaft, P. (1992) *Parasitol. Today* **8 :** 104.
3. Bloom, B.R. & Murray, C.J.L. (1992) *Science* **257 :** 1055.
4. Lederberg, J. (1993) *Trends Microbiol.* **1 :** 43.
5. Bangham, A.D., Standish, M.M. & Watkins, J.C. (1965) *J. Mol. Biol.* **13 :** 238.
6. Gregoriadis, G. & Florence, A.T. (1993) *Drugs* **45 :** 15.
7. DeGier, J., Mandersloot, J.S. & Vandeenen, L.L.M. (1986) *Biochim. Biophys. Acta* **860 :** 666.
8. Ostro, M.J. (1987) *Sci. Am.* **256 :** 102.
9. Stamp, D. & Juliano, R.L. (1979) *Can. J. Physiol. Pharmcol.* **57 :** 535.
10. Poste, G. (1983) *Biol. Cell.* **47 :** 19.
11. Ostro, M.J., Giacomoni, D., Lavelle, D., Pasetron, W. & Dray, S. (1978) *Nature* **274 :** 921.

12. Dimitriadis, G.J.(1978) *Nature* **274 :** 923.
13. Mehta, K., Lopez-Berestein, G., Hersh, E.M., Juliano, R.L. (1982) *Reticuloendothelial Soc.* **32 :** 155.
14. Orozco, L.C., Forero, M., Wasserman, M. & Ahumada, J.J. (1990) *Tubercle,* **71 :** 209.
15. Allen, T.M., Hansen, C. & Rutledge, J. (1989) *Biochem.Biophys. Acta* **981 :** 27.
16. Gabizon, A. & Papahadjopoulos, D. (1992) *Biochem. Biophys. Acta* **1103 :** 94.
17. Allen, T.M. (1994) *Trends Pharmacol. Sci.* **15 :** 215.
18. Bakker-Woudenberg, I.A.J.M., Storm, G. & Woodle, M.C. (1994) *J. Drug Targeting* **2 :** 363.
19. Robbins-Roth, (1991) *Bioventure View* **6 :** 1.
20. Allen, T.M. & Chonn, A. (1987) *FEBS Lett.* **223 :** 42.
21. Gabizon, A. & Papahadjopoulos, D. (1988) *Proc. Natl. Acad. Sci. USA* **85 :** 6949.
22. Klibanov, A.L., Maruyaa, K., Torchilin, V.P. & Huang, L. (1990) *FEBS Lett.* **268 :** 235.
23. Allen, T.M., Hansen, C., Martin, F., Redemann, C. & Yau-Young, A. (1991) *Biochem. Biophys. Acta* **1066 :** 2.
24. Papahadjopoulos, D., Allen, T.M., Gabizon, A., Mayhew, E., Mathay, K., Huang, S.K., Lee, K.D., Woodle, M.C., Lasic, D.D., Redemann, C. & Martin, E.J. (1991) *Proc. Natl. Acad. Sci. USA* **88 :** 11460.
25. Blume, G. & Cevc, G. (1993) *Biochem. Biophys. Acta* **1146 :** 157.
26. Allen, T.M. & Hansen, C. (1991) *Biochem. Biophys. Acta* **1068 :** 133.
27. Santaella, C., Frizard, F., Vierling, P. & Riess, J.G. (1993) *FEBS Lett.* **3 :** 481.
28. McCormack, B. & Gregoriadis, G. (1994) *Int. J. Pharmaceutics* **112 :** 249.
29. Hutchinson, F.J. & Jones, M.N. (1988) *FEBS Lett.* **234 :** 493.
30. Bagdanov, A.A., Gordeeva, L.V., Torchilin, V.P. & Margolis, L.B. (1989) *Exp. Cell. Res.* **181 :** 326.
31. Ghosh, P. & Bachhawat, B.K. (1980) *Biochem. Biophys. Acta* **632 :** 562.
32. Das, N., Bachhawat, B.K., Mahato, S.B. & Basu, M.K. (1987) *Biochem. J.* **247 :** 359.
33. Singhal, A. & Gupta, C.M. (1986) *FEBS Letts.* **201 :** 321.
34. Singhal, A., Bali, A. & Gupta, C.M. (1986) *Biochem. Biophys. Acta.* **880 :** 72.
35. Senior, J. & Gregoriadis, G. (1989) *Biochem. Biophys. Acta* **1003 :** 58.
36. Owais, M., Varshney, G.C., Choudhury, A., Chandra, S. & Gupta, C.M. (1995) *Antimicrob. Agents Chemother.* **39 :** 180.
37. Guru, P.Y., Agrawal, A.K., Singha, U.K., Singhal, A. & Gupta, C.M. (1989) *FEBS Lett.* **245 :** 204.
38. Owais, M., Ahmad, I., Krishnakumar, B., Jain, R.K., Bacchawat, B.K., & Gupta, C.M. (1993) *FEBS, Lett.* **326 :** 56.
39. Chakraborty, P., Bhaduri, A.N. & Das, P.K. (1990) *Biochem. Biophys. Res. Commun.* **166 :** 404.
40. Scwab, J.C. & Mandell, G.L. (1989) *Infect. Dis. Clin. North Am.* **3 :** 461.
41. Alving, C.R. (1986) *Parasitol. Today* **2 :** 101.
42. Dolan, T.F., Hunter, C.A., Laakso, T., Coombs, G.H., Baillie, A.J., Sjarnkvist, P. & Sjoholm, I. (1989) in *Leishmaniasis- the current status & new strategires for control,* ed. Hart, D.T., Serier, A., Vol. 163, p. 793.
43. Medda, S., Mukherjee, S., Das, N., Naskar, K., Mahato, S.B. & Basu, M.K. (1993) *Biotechnol. Appl. Biochem.* **17 :** 37.

44. Kole, L., Sarkar,K., Mahato, S.B. & Das, P.K. (1994) *Biochem. Biophys. Res. Commun.* **200 :** 351.

45. Agrawal, A.K., Singhal, A. & Gupta, C.M. (1987) *Biochem. Biophys. Res. Commun.* **148 :** 357.

46. Peeters, P.A.M., Huiskamp, C.W.E.M., Eling, W.M.C. & Crommelin, D.J.A. (1989) *Parasitology* **98 :** 381.

47. Chandra, S., Agarwal, A.K. & Gupta, C.M. (1991) *J. Biosci* **16 :** 137.

48. Singh, A.M., Owais, M. & Varshney, G.C. (1993) *Ind. J. Biochem. Biophys.* **30 :** 411.

49. Herwaldt, B.L. & Berman, J.D. (1992) *Am. J. Trop. Med. Hyg.* **46 :** 296.

50. World Health Organization (1991) *Tropial disease – Progress in research,* TDR Tenth Programme Report, WHO, Geneva, 1989-90, p. 79.

51. Marsden, P.D. & Jones, T.C. (1985) in *Leishmaniasis,* ed. Chang, K.P., Bray, R.S., Elsevier, London, p. 183.

52. Thakur, C.P., Kumar, M. & Pandey, A.K. (1991) *Am. J. Trop. Med. Hyg.* **45 :** 435.

53. Saenz, R.E., Paz, H. & Berman, J.D. (1990) *Am. J. Med.* **89 :** 147.

54. Chunge, C.N., Gachihi, G., Muigai, R., Wasunna, K., Rashid, J.R., Chulay, J.D., Anabwani,G., Oster, C.N., & Bryceson, A.D.M. (1985) *Trans. R. Soc. Trop. Med. Hyg.* **79 :** 715.

55. World Health Organization (1984) *The Leishmaniasis,* Report of a WHO Expert Committee, Technical Report Series No. 701, WHO, Geneva.

56. Black, C.D.V., Watson, G.J. & Ward, R.J. (1977) *Trans. R. Soc. Trop. Med. Hyg.* **71 :** 551.

57. Alving, C.R., Steck, E.A., Chapman, W.L. Jr., Waits, V.B., Hendricks, L.D., Swartz, G.M. Jr. & Hanson, W.L. (1978) *Proc. Natl. Acad. Sci. USA* **75 :** 959.

58. New, R.R.C., Chance, M.L., Thomas, S.C. & Peters, W. (1978) *Nature* **272 :** 55.

59. New, R.R.C., Chance, M.L. & Health, S. (1981) *J. Antimicrob. Chemoth.* **8 :** 371.

60. Berman, J.D., Hanson, W.L., Chapman, W.L., Alving, C.R. & Lopez- Berestein, G. (1986) *Antimicrob. Agents. Chemother.* **30** : 847.

61. Sett, R., Sarkar, K. & Das, P.K. (1993) *J. Infec. Dis.* **168 :** 994.

62. Basu, N., Sett, R. & Das, P.K. (1991) *Biochem. J.* **277 :** 451.

63. Fountein, M.W., Weiss, S.J., Fountein, A.G., Shen, A. & Lenk, A.P. (1985) *J. Infect. Dis.* **152 :** 529.

64. Tadakuma, T., Ikewaki, N., Yasuda, T., Tsufsumi, M., Saito, S. & Saito, K. (1985) *Antimicrob. Agent Chemother.* **28 :** 28.

65. Bermudez, L.E.M., Yau-Young, A.O., Liu, J.P., Cogger, J. & Young, L.S. (1990) *J. Infect. Dis.* **161 :** 1262.

66. Karlowsky, J.A. & Zhanel, G.G. (1992) *Clin. Infect. Dis.* **15 :** 654.

67. Bakker-Woudenberg, I.A.J.M., Lokerse, A.F., Tenkate, M.T., Melissen, P.M.B., Vianen, W.V. & VanEtten, E.W.M. (1993) *Eur. J. Clin. Microbial. Infect. Dis. Suppl.* **11 :** 61.

68. Zhanel, G.G. & Ariano, R.E. (1992) *Renal failure* **14 :** 1.

69. Desiderio, J.V. & Capbell, S.G. (1983) *J. Infect. Dis.* **148 :** 563.

70. Swenson, C.E., Stewart, K.A., Hammett, J.L., Fitzsimmons, W.E. & Ginsberg, R.S. (1990) *Antimicrob. Agents. Chemother.* **34 :** 235.

71. Stevenson, M., Baillie, A.J. & Richards, R.M.E. (1983) *Antimicrob. Agents Chemother.* **24 :** 742.

72. Nacucchio, M.C., Gatto-Bellora, M.J., Sordelli, D.O., D' Aquino, M. (1988) *J. Microencapsul.* **5 :** 303.

73. Dees, C., Fountain, M.W., Taylor, J.R. & Schultz, R.D. (1985) *Vet. Immunol. Immunopathol.* **8 :** 171.

74. Bonventre, P.F. & Gregoriadis, G. (1978) *Antimicrob. Agents Chemother.* **13 :** 1049.

75. Sunamoto, J., Goto, M., Iida, T., Hara, K., Saito, A. & Tomonaga, A. (1984) in *Receptor mediated targeting of drugs,* eds. Gregoriadis, G., Poste, G., Senior, J. & Trouet, A., Plenum Press, New York, p. 359.

76. Raleigh, J.W. (1984) in *The Mycobacteria : a source book,* eds. Kubica, G.P. & Wayne, L.G., Microbiology Series, Library of congress cataloging in publication data, Vol. 15, p. 1007.

77. Mitchison, D.A., Ellard, G.A. & Grosset, J. (1988) *Br. Med. Bull.* **44 :** 757.

78. Chaulet, P. (1989) *World Health Forum* **10 :** 16.

79. Girling, D.J. (1978) *Tubercle* **59 :** 13.

80. Zierski, M. & Bek, E. (1980) *Tubercle* **61 :** 41.

81. Agins, B.D., Berman, D.S., Spicehandler, D.E., Sadr, W., Simberkoff, M.S. & Rahal, J.J. (1989) *J. Infect. Dis.* **159 :** 784.

82. Vladimirsky, M.A. & Ladigina, G.A. (1982) *Biomedicine* **36 :** 375.

83. Ladigina, G.A. & Vladimirsky, M.A. (1986) *Biomed. Pharmacother.* **40 :** 416.

84. Duzgunes, N., Ashtekar, D.R., Flasher, D.C., Ghori, N., Debs, R.J., Friend, D.S. & Gangadharam, P.R.J. (1991) *J. Infect. Dis.* **164 :** 143.

85. Ashtekar, D., Duzgunes, N. & Gangadharam, P.R.J. (1991) *J. Antimicrob. Chemother* **28 :** 615.

86. Duzgunes, N., Perumal, V.K., Kesavulu, L., Goldstein, J.A., Debs, R.J. & Gangadharan, P.R.J. (1988) *Antimicrob. Agents Chemother.* **32 :** 1404.

87. Konno, K., Oizumi, K., Ariji, F., Yamaguchi, J. & Oka, S. (1973) *Am. Rev. Resp. Dis.* **107 :** 1002.

88. Raleigh, J.W. (1972) *Am. Rev. Resp. Dis.* **105 :** 397.

89. Agarwal, A., Kandpal, H., Gupta, H.P., Singh, N.B. & Gupta, C.M. (1994) *Antimicrob. Agents. Chemother.* **38 :** 588.

90. Orozco, L.C., Quintana, F.O., Beltran, R.M., Moreno, I.D., Wasserman, M. & Rodriguez, G. (1986) *Tubercle* **67 :** 91.

91. Klemens, S.P., Cynamon, M.H., Swenson, C.E. & Ginsberg, R.S. (1990) *Antimicrob. Agents Chemother.* **34 :** 967.

92. Nightingale, S.D., Saletan, S.L., Swenson, C.E., Lawrence, A.J., Watson, D.A., Pilkiewicz, F.G., Silverman, E.G. & Cal, S.X. (1993) *Antimicrob. Agents Chemother.* **37 :** 1869.

93. Bermudez, L.E.M., Wu, M. & Young, L.S. (1987) *J. Infect. Dis.* **156 :** 510.

94. Korting, H.C., Ollert, M., Georgii, A. & Froschl, M. (1988) *J. Clin. Microbiol.* **26 :** 2626.

95. Meunier, F. (1988) in *Clinical approach to infection in the compromised host,* eds. Rubin, R.H. & Young, L.S., 2nd Ed., New York, Plenum Press, p. 193.

96. Taylor, R.L., Williams, D.M., Craven, PC., Graybill, J.R., Drutz, D.J. & Magee, W.E. (1982) *Am. Rev. Resp. Dis.* **125 :** 610.

97. Graybill, J.R., Craven, P.C., Taylor, R.L., Williams, D.M. & Magee, W.E. (1982) *J. Infect. Dis.* **145 :** 748.

98. Tremblay, C., Barza, M., Fiore, C. & Szoka, F. (1984) *Antimicrob. Agents Chemother.* **26 :** 170.

99. Ahmad, I., Sarkar, A.K. & Bacchawat, B.K. (1991) *J. Clin. Biochem. Nutr.* **10 :** 171.

100. Juliano, R.L., Lopez-Berestein, G., Hopfer, R., Mehta, R., Mehta, K. & Mills, K. (1985) *Ann. N.Y. Acad. Sci.* **446 :** 390.

101. Mehta, R.T., Mcqueen, T.J., Keyhani, A. & Lopez-Berestein, G. (1991) *J. Infect. Dis.* **164 :** 1003.

102. Lopez-Berestein, G. (1988) *Ann. N.Y. Acad. Sci.* **544 :** 590.

103. Szoka, F.C., Milholland, D. & Barza, M. (1987) *Antimicrob. Agents Chemother.* **31 :** 421.

104. Lopez-Berestein, G., Fainstein, V., Hopefer, R., Mehta, K., Sullivan, M.P., Keating, M., Rosenblum, M.G., Mehta, R., Luna, M., Hersh, E.M., Reuben, J., Juliano, R.L. & Body, G.P. (1985) *J. Infect. Dis.* **151 :** 704.

105. Lopez-Berestein, G., Body, G.P., Frankel, L.S. & Mehta, K. (1987) *J. Clin. Oncol.* **5 :** 310.

106. Lopez-Berestein, G., Body, G.P., Fainstein, V., Keating, M., Frankel, L.S., Zeluff, B., Gently, L. & Mehta, K. (1989) *Arch. Intern. Med.* **149 :** 2533.

107. Sculier, J.P., Coune, A., Meunier, F., Blassine, C., Laduron, C., Hollaert, C., Collette, N., Heyman, C. & Klustersky, J. (1988) *Eur. J. Cancer Clin. Oncol.* **24 :** 527.

108. Bodey, G.P. (1977) *Curr. Prob. Cancer.* **1 :** 3.

109. Lopez-Berestein, G.,Mehta, R., Hopfer, R.L., Mills, K., Kasi, L., Mehta, K., Fainstein, V., Luna, M., Hersh, E.M. & Juliano, R. (1983) *J. Infect. Dis.* **147 :** 939.

110. Gondal, J.A., Swartz, R.P. & Rahman, A. (1989) *Antimicrob. Agents Chemother.* **33 :** 1544.

111. Gilbert, B.E., Wyde, P.R., Lopez-Berestein, G. & Wilson, S.Z. (1994) *Antimicrob. Agents Chemother.* **38 :** 356.

112. Gilbert, B.E., Wyde, P.R. & Wilson, S.Z. (1992) *Antimicrob. Agents Chemother.* **36 :** 1466.

113. Hostetler, J.S., Clemons, K.V., Hanson, L.H. & Stevens, D.A. (1992) *Antimicrob. Agents Chemother.* **36 :** 2636.

114. Medoff, G., Brajtburg, J., Kobayashi, G.S. & Bolard, J. (1983) *Annu. Rev. Pharmacol. Toxicol.* **23 :** 303.

115. Ahmad, I., Sarkar, A.K. & Bacchawat, B.K. (1989) *Indian J. Biochem. Biophys.* **26 :** 351.

116. Weber, S.R. & Lopez-Berestein, G. (1987) *Larynegoscope* **97 :** 937.

117. Ahmad, I., Sarkar, A.K. & Bacchawat, B.K. (1990) *Biotech. Appl. Biochem.* **12 :** 550.