Liposomal Delivery of Antileishmanial Agents

Mukul Kumar Basu, PhD

Biomembrane Division, Indian Institute of Chemical Biology, Kolkata, India

KEY WORDS: drug delivery, experimental leishmaniasis, glycoside grafted liposomes, antibody coated liposomes, peptide grafted liposomes, pharmacodynamics of liposomal drug

ABSTRACT

Initially, the use of liposomes was mainly confined to model membrane systems, however, recently their use as vehicles for transfer of genetic and other materials into cells in cultures or for drug delivery to desired cellular and subcellular sites in a living system has been the subject of discussion. Liposomes can vary widely in size, chemical composition, and surface characteristics; they can accommodate a remarkable array of pharmacologically active substances, including antitumor and antimicrobial drugs, enzymes, hormones, and vaccines. Currently, specialized liposomes are prepared for site-specific delivery, which has led to considerable interest in the possibility of therapeutic use of liposomes. This paper describes different methods for modifying liposomal surfaces, so that these modified liposomes can be used as efficient macrophage specific drug carriers. Until drug specificity is achieved, liposomes, despite their limitations, will play an important role in optimizing drug action.

INTRODUCTION

As drug delivery-vehicles, liposomes are artificial phospholipid microcapsules where lipids are arranged in a concentric balladeer enclosing an aqueous space. When lipids are suspended in an excess of aqueous solution, they spontaneously give rise to a population of vesicles, which may range in size from tens of nanometers to tens of microns in diameter; the lipid layers always encapsulate an aqueous phase. They can be constructed so that they entrap materials both within their aqueous compartment and within the membrane. Depending on the size and number of lipid bilayers, they are classified as multilamellar large vesicle or small unilamellar vesicle. There are also giant vesicles. Bangham and his co-workers[1,2] have conducted a physical study of liposomes. Phospholipids and stabilizing lipids are the main ingredients needed to form stable liposomes structures, such as cholesterol or ergosterol. Cholesterol decreases permeability of the lipid bilayers to the solutes. A charged amphiphile produces electrostatic repulsion between any two charged bilayers on either side of an aqueous channel. This can greatly increase the volume of the entrapped aqueous solution and hence, the absolute amount of entrapped solute. In addition, the presence of a charged component will confer a positive or negative charge on the surface of liposomes. The liposomes are very similar to the natural membrane, in terms of the membrane fusion, ion-transport, membrane permeability, etc. They are also capable of delivering drugs, proteins, enzymes, and genetic materials to living cells. In addition,

they can serve as an ideal delivery system in medicine, pharmacology, genetic engineering, and cosmetics, as well as the food industry.[3-5]

Drug targeting using liposomes or any other particular drug carriers have two major problems that have not been adequately dealt with. Firstly, other than those having a discontinuous endothelial lining to their capillaries (eg, liver and spleen), these carriers cannot escape from the circulation to tissues because of their size. The second problem concerns the body's immune system recognizing liposomes as foreign particles and their subsequent removal by phagocytic cells of the tissues of the reticuloendothelial system.

Despite these problems, liposomes have been successfully exploited using improved techniques in the treatment of bacterial or parasitic infections of macrophages, and systemic fungal infections. They have also been used in the preparation of vaccines and in the detection of cancer. In the United States, human trials have been conducted on the use of liposomes for the diagnosis of various cancers, and liposomes containing antibiotics for the treatment of infections in cancer patients.

The purpose of this review is to focus on liposomal delivery systems, both classical and modified, that deliver antileishmanial drugs, increase efficacy, and reduce drug toxicity. Drug toxicity is a common adverse effect of antileishmanial agents. Antimonials, although notorious for their toxic effects, remain as the main combating force even now, although current clinical reports indicate that a large proportion of cases of leishmaniasis (15%-25%) are becoming unresponsive to antimonial treatment.[6] The human protozoal pathogen *Leishmania donovani* is the causative agent for visceral leishmaniasis (kala-azar), a fatal disease that is endemic in parts of the tropical world. For leishmaniasis, the target cells are the macrophages of reticuloendothelial origin. Hence, a liposomal delivery system has to be designed in such a way that they are easily recognized by macrophages, because of active targeting. Although a considerable amount of work has been done on passive targeting to the reticuloendothelial system, not much is known about the active targeting to specific subsets of circulating blood cells or to the vascular endothelium. Thus, efficient delivery systems suitable for site-specific delivery are being sought.

Designing of Liposomes as Carriers for Site-Specific Delivery

The idea of specific targeting was developed by Paul Ehrlich, who proposed treatment of infectious diseases with a "magic bullet". According to him, this is a toxin bound to a molecule with a high affinity for a specific site. The combination could result in the destruction of the target cell without affecting the host. For site-specific targeting, liposomes in combination with the therapy are directed to target sites and attach to the surface to alter the normal distribution in vivo. An ideal target would be a cell or tissue that expresses a unique receptor on its surface. It is, therefore, important to identify such receptors on target cells. A receptor is a protein molecule that mediates the internalization of legends via a process called receptor-mediated endocytosis. A large variety of molecules like antibodies, oligosaccharides, lectins, hormones, and nucleic acid have been used as the ligands for targeting liposomes,[7] but the major drawback of liposome targeting is their rapid accumulation by the fixed macrophages of reticuloendothelial system (RES). As such, liposomes have been designed that circulate longer to bypass RES.[8-10] With antileishmanial therapy, the rapid accumulation of liposomes in the RES seems

to be an added advantage, since leishmaniasis is a disease primarily associated with the fixed macrophages of RES. Efforts have been made to promote a higher uptake of liposomes by the RES through modifying liposomal surface and by incorporating or grafting various ligands onto the liposomal surface, so that they are easily recognized by the various receptors located on the macrophages.

Glycolipid/Glycosides Bearing Liposomes

The liposomes are taken up nonspecifically by the RES, however, the uptake can be increased by proper modification of the liposomal surface or liposomal composition. Surolia et al[11] incorporated glycolipids of varying terminal sugars into the liposomes and showed that these glycolipid-bearing liposomes could be used as efficient ligands for specific receptors on the target cells. These specific receptors recognized only the terminal sugar of the glycolipid chain. The two major cell types of the liver, hepatocytes and macrophages, possess distinctly different receptors for galactose and mannose, respectively.[12-13] The glycolipid orientation and the density of glycolipid residue on the liposomal surface, chain length of the oligosaccharide, and the phase transition temperature (Tc) are the parameters that dictate the nature of binding between liposomes and cells.[14]

Moreover, the presence of lectin-like molecules on the surface of hepatocytes and kupffer cells has also been reported. These receptors recognize the terminal galactose, glucose, mannose- or fucose-containing glycosides, and thus can be directed to different liver cell types. A galactose terminating glycoprotein, asialofetuin, has been incorporated into liposomes to direct the liposomes towards the hepatocytes,[15] however, the major disadvantage of using the protein as a ligand/marker stems from the fact that the antibody responses may be elicited against that protein. So the glycolipid/glycosides replaced glycoproteins as the surface marker of liposomes. These glycolipid/glycoside-bearing liposomes can be targeted to different cell-types of liver more efficiently. The glycolipid should be incorporated so that its oligosaccharide portion remains at a high density on the surface of liposomes. After intravenous injection of such liposomes, rapid uptake by liver took place primarily through the endocytotic-process mediated by the galactose receptor. Among the different glycoside residues artificially grafted on the liposomal surface and tested with respect to their uptake by various organs, the grafting of galactoside and mannoside on the liposome surface increased liposome uptake by the liver. The determining factors for the uptake of glycosylated liposomes by the liver include the anomeric form and the density of the glycoside residues on the surface of the liposomes. When plant glycosides, with glucose as end sugars, are incorporated into the liposomes for targeting purposes, the glycoside-bearing liposomes were taken up mainly by the nonparenchymal cells, and uptake was very specific for glucose, although independent of various anomeric forms of glucose.[16-17]

Specific receptors were observed on the non-parenchymal or Kupffer's cell that recognized the terminal mannose residue of glycoside bearing liposomes.[18] The enhancing effect of cetylmannoside on the targeting of liposomes to kupffer cells in rats was reported earlier,[19] but according to a recent report, the uptake by cetylmannoside was complement receptor-mediated and not mannose receptor-mediated.[20] The presence of aminomannose receptors on the lungs could enable the liposomes to be directed towards lungs, and the presence of mannose receptor on the glial cell of the

brain could direct the liposomes through the blood-brain barrier.[21] The experimental evidence on the efficacy of glycosylated liposomes in targeting the specific cell types of the liver prompted several researchers to try to find out whether the drugs or therapeutic substances entrapped in these liposomes could be effective in reversing the diseased condition of liver.

Antibody/Peptide-Coated Liposomes

Targeting of various tissues or cells can be achieved by using an antibody or peptide-coated liposomes. As a result of hybridoma technology, it is now possible to obtain a monoclonal antibody for each cell surface protein. The coupling of such antibodies on the liposomal surface would preserve the specificity of the antibody, and the antibody-mediated binding of liposome to the target cells could deliver the liposomal contents into those cells. These antibody-coated liposomes are now used to deliver drugs or other materials to specific cells or tissues (eg, dideoxycytidine triphosphate to human monocyte/macrophage[22] doxorubicin against lung cancer in mice,[23] and chloroquine to *P. berghei* infected mice).[24] The major drawback of such liposomes in vivo is their rapid removal by the RES. As the presence of Fc receptors on the cell surfaces of RES could increase this removal, the use of $F(ab)_2$ portion of the antibody was recommended for coating the liposomal surface for this specific purpose. The binding to lymphocytes was three times greater when the liposomes were prepared with $F(ab')_2$ fragments than with the whole antibody, and as expected, the binding was almost absent using the monovalent F(ab') fragments. Heath, Fraley, and Papahadjopoulos[26] reported that the cell specificity obtained by conjugation of $F(ab')_2$ was vesicle surface specificity. The binding of liposomes to erythrocytes in whole blood or in vivo increased considerably (by at least 20 times) by covalently attaching anti-erythrocyte $F(ab')_2$ to their surface.[27] Huang, Huang and Kennel[29] covalently coupled monoclonal antibodies with fatty acids and in vitro liposome targeting. Cell-specific drug transfer from liposomes bearing monoclonal antibodies was already reported.[30]

A large number of works have been published on the peptide-coated liposomes and their interactions with various cells and possible applications in chemotherapy. Several macrophage activating peptides (eg, tuftstin, N-f-Met-Leu-Phe, N-f Met-Leu-Phe-Phe) were also reported for targeting purposes.[31-34] The tuftsin-bearing liposomes were tested in vitro and their possible chemotherapeutic role was later studied.[35,36]

Routes of Administration of Liposomal Delivery System

The in vivo applicability of liposomal delivery system depends on its routes of administration, oral, intravenous, subcutaneous, dermal, transdermal, intraperitoneal, intramuscular or inhalation through the bronchial track. All these pathways have specific characteristics and limitations. The liposomes, when administered orally, can survive stomach digestion, but are lysed by the lipolytic enzymes in the intestine. It has been shown that they can protect the entrapped materials from such degradation and hence, this route is used for liposomes in oral vaccination. When injected intravenously, liposomes are rapidly cleared from the blood and absorbed mainly by the phagocyte cells of the reticuloendothelial system. Liposomes injected through subcutaneous, transdermal or intramuscular routes may remain in the in circulation longer. Thus, it may act as a depot of drugs and facilitate the slow release of the entrapped materials from the vesicles. One report indicated the supremacy

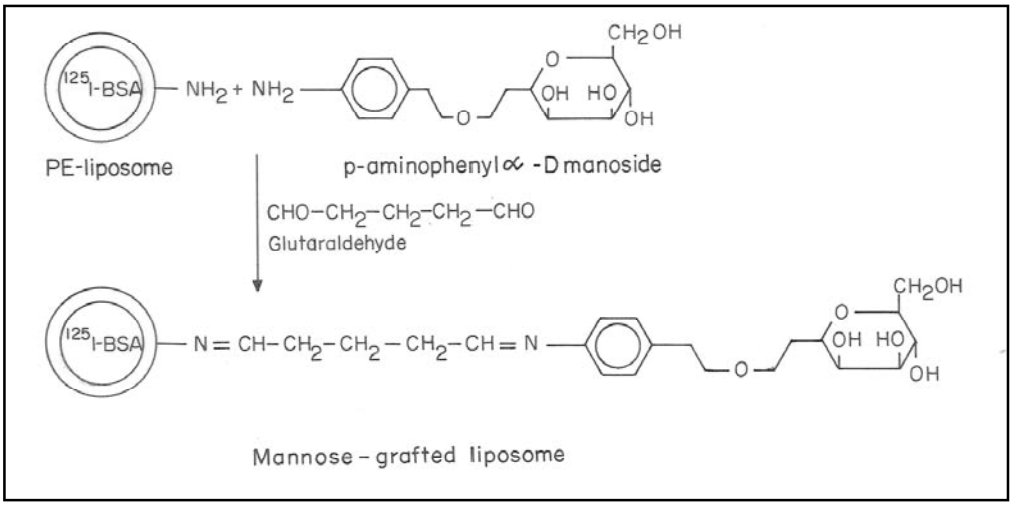

Figure 1. Coupling of p-aminophenyl-□-D-mannoside with PE liposomes.

of the subcutaneous route over other routes.[37]

Pharmacodynamics of Liposome Encapsulated Drugs

The pharmacokinetic behavior of liposome-encapsulated drugs was studied by several researchers.[38,39] They discovered that in vivo behavior (eg, the release characteristics of the encapsulated drug) is dependent on the properties of the liposomes. The specificity of the drug release depends on the quality and composition of the liposomes, which increase the efficiency of drug release to the target cells.[40] Encapsulation of the drug into the liposomes, creates a shield on the drug and thus the rate of metabolism of these drugs is less than the free drugs. Mauk et al[41] showed that SUVs can stay in the system after a subcutaneous injection for up to 600 hours.

In summary, encapsulated drugs tend to demonstrate the following pharmacodynamic properties: retardation of drug clearance from circulation; high drug storage in different RES tissues; longer drug retention in various tissues; and retardation in the rate of drug metabolism and elimination.

Application in Chemotherapy: As Carrier of Antileishmanial Agent

The protozoal pathogen *Leishmania donovani* is the causative agent of visceral leishmaniasis (kala-azar). It has a digenic life cycle. The flagellated promastigotes or the vector forms are converted to the aflagellated amastigotes, which reside and multiply within the host cell (ie, macrophages of the liver and spleen). When injected into the blood stream, the superior efficacy of a liposomal drug compared to a free drug is mainly due to the accumulation of liposomes in the liver and spleen. For leishmaniasis therapy, the antimonials are the drugs of choice, although notorious for their toxic effects. The superior efficacy of various antimonial drugs like meglumine antimoniate or sodium stibogluconate in liposomal form has been compared to their respective free drugs.[42,43] In addition to visceral leishmaniasis, New, Chance, and Heath[44] extended their observations to show that liposomes also enhance the activity of sodium stibogluconate against experimental cutaneous leishmaniasis, when the parasites are located in the macrophages in peripheral tissues, rather

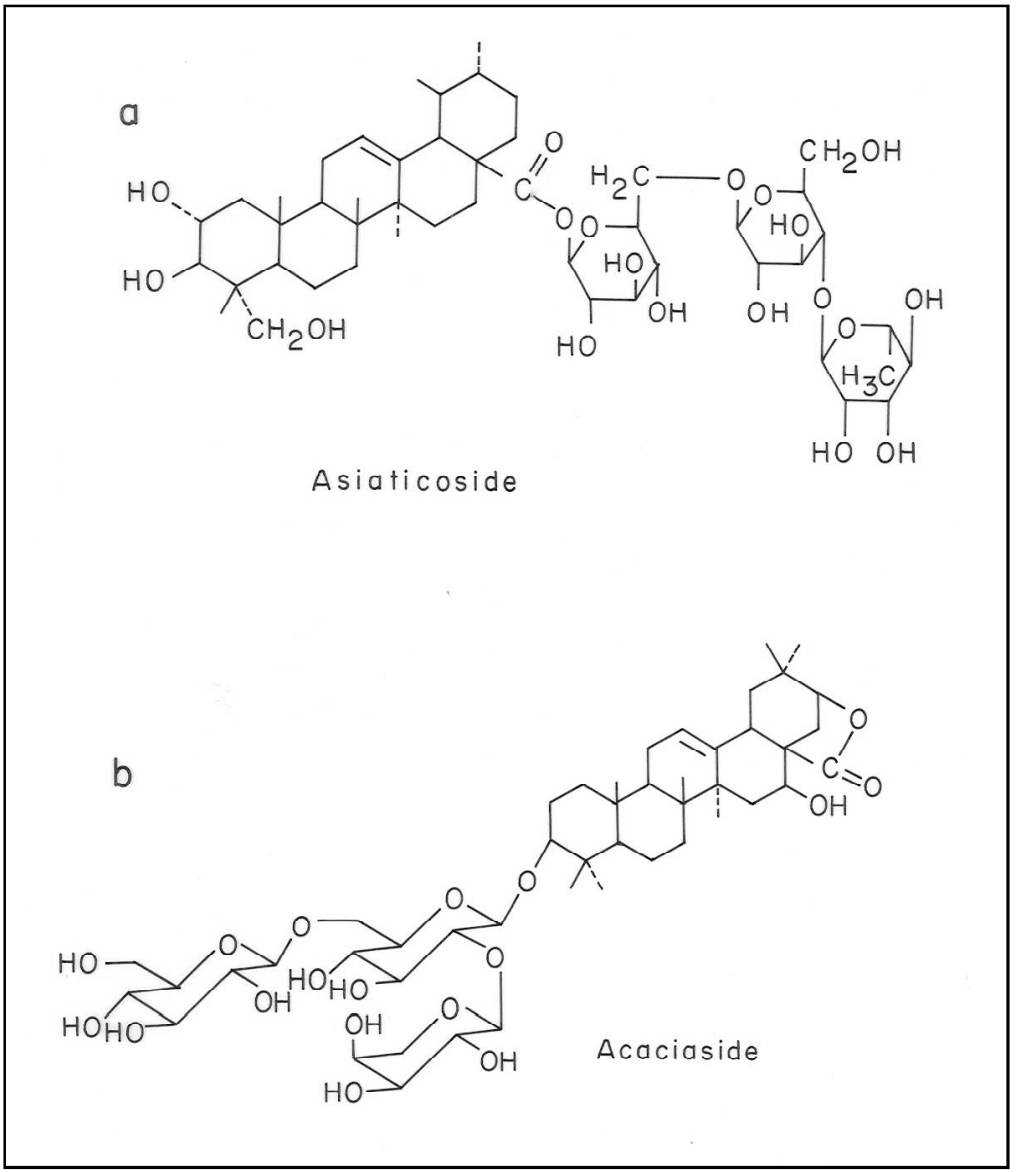

Figure 2. A). Asiaticoside. B). Acaciaside.

than in the liver. Recently a classical drug urea-stibamine was used in the liposomal and in the mannose-grafted liposomal forms to combat experimental leishmaniasis in a hamster model. The mannose-grafted liposomal form was judged more efficient in transporting the drug to the specific site;[45] the efficacy and toxicity of this drug have also been critically analyzed and compared using different sugar bearing liposomes. Mannose-bearing liposomes have proved more efficient in the transportation of drugs compared to those bearing glucose or non-bearing liposomes (ordinary liposomes) (Figure 1). Toxicity studies also show no apparent drug toxicity in either of the two sugar bearing liposomal forms. Very recently, Tampon et al,[46] in an in vitro approach, used the

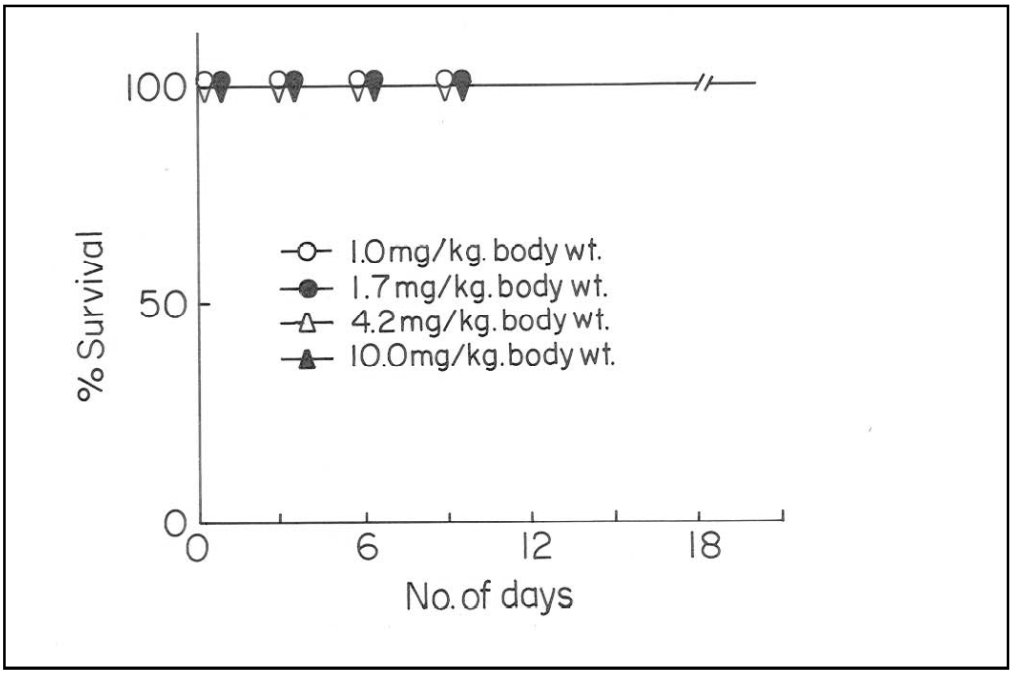

Figure 3. Survival of hamsters after treatment with free asiaticoside.

negatively charged lipid phosphatidylserine-liposome-entrapped antimony to improve targeting to *L. chagasi* infected macrophages through the interaction with the scavenger receptor of macrophages. Besides the antimonials, imidines, aminoquinolines or various antibiotics, they are also being used for the treatment of both visceral and cutaneous leishmaniasis. This group of drugs has gained their importance because most of the *Leishmania* parasites are antimony resistant and the number of new resistant cases is alarming to scientists worldwide.

Second line drugs, (eg, amphotericin-B, pentamidine isethinonate) are too toxic for use as first-line therapy on a large scale. Increasing the dosage of antimony may at least temporarily overcome parasite resistance, but at the same time would increase the risk of serious side effects. Therefore, new drugs or delivery systems have been sought. Thus, amphotericin-B (in the liposomal form), despite its limitations, was tried to combat leishmaniasis both in model disease[47] and also in clinics by several groups of researchers.[48] Croft et al[49] used liposomal amp-B (AmBisome) in the treatment of patients suffering from multidrug resistance visceral leishmaniasis. In a non-empirical approach to antileishmanial drug design and delivery, lipoprotein mediated antileishmanial chemotherapy was studied.[50]The idea of utilization of a specific receptor by low density lipoprotein (LDL) and acetylated LDL on *Leishmania* infected macrophages was exploited in vitro to selectively deliver the anti-leishmanial drug adriamycin.

One of the 8-aminoquinoline groups of compounds, primaquine, known for its antimalarial effect, had a limited leishmanicidal effect.[51] Whether the efficacy of primaquine could be increased a reasonable extent using an appropriate delivery system, remains an open question. The effectiveness of these drugs could be increased significantly by prop-

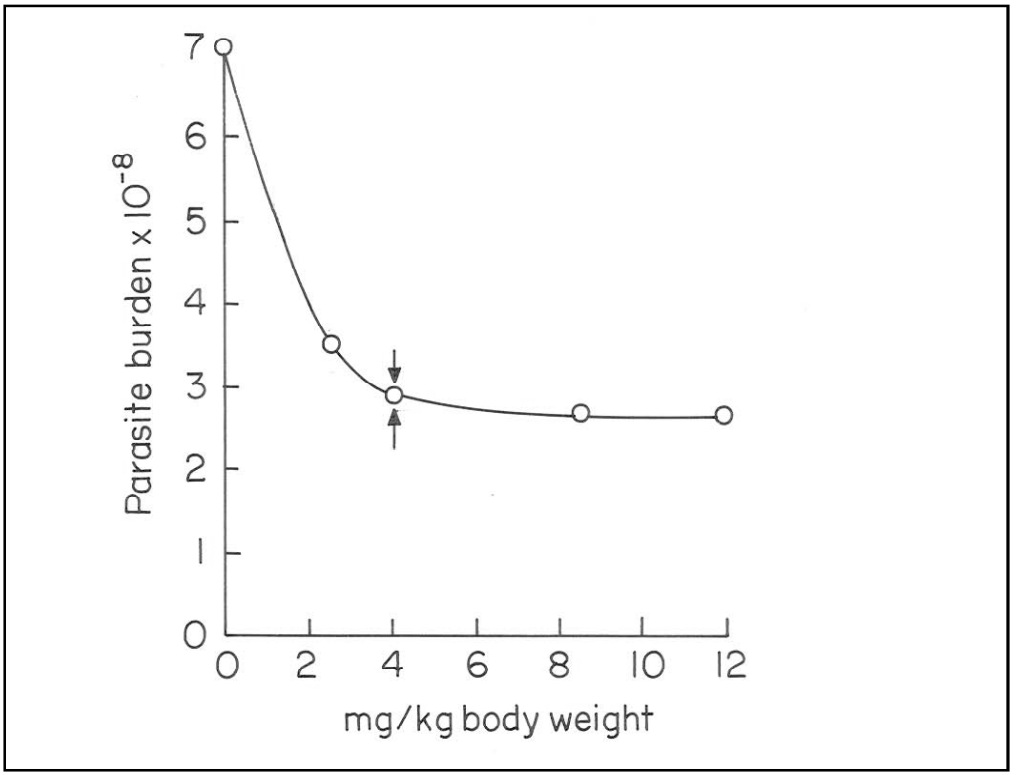

Figure 4. Dose-dependent reduction of parasite burden in spleen of hamsters treated with asiaticoside.

er manipulation of the liposomal composition or modification of the liposomal surface by incorporating or grafting different ligands, appropriate for the receptors on the host-cell surface.[17,35,45] Lately, it was demonstrated that primaquine encapsulated in peptide-grafted liposomes or f Met-Leu-Phe-grafted liposomes was more effective in lowering the spleen parasite load in experimental leishmaniasis compared with its efficacy in either the free form or encapsulated in ungrafted liposomes.[52] Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin was reported, but normochromic anemia and neutropenia developed as side effects.[53]

Very recently, different sugar-coated liposomal delivery systems were designed, either by incorporating plant glycosides or by grafting synthetic glycosides, and their specificity toward macrophages was tested in vitro using appropriate inhibitors.[17] The neoglycosylated liposomes were also used as efficient ligands for the estimation of specific sugar receptor status of macrophages in heath and in experimental leishmaniasis.[54] Some plant glycosides (eg, amarogentin isolated from Indian Medicinal Plant, *Swertia chirata,*[55] and Bacopasaponin C isolated from Indian Medicinal Plant, *Bacopa monniera*[56]) were reported to have antileishmanial properties. Thus, after incorporation in the liposomes, they serve two purposes, 1) the end sugar of the hydrophilic sugar chain, which sticks out of the liposomal surface, acts as ligands for appropriate receptors on the macrophage surface; and (2) because of their leishmanicidal properties, they also act as an indigenous antileishmanial drug. Hence, these atypi-

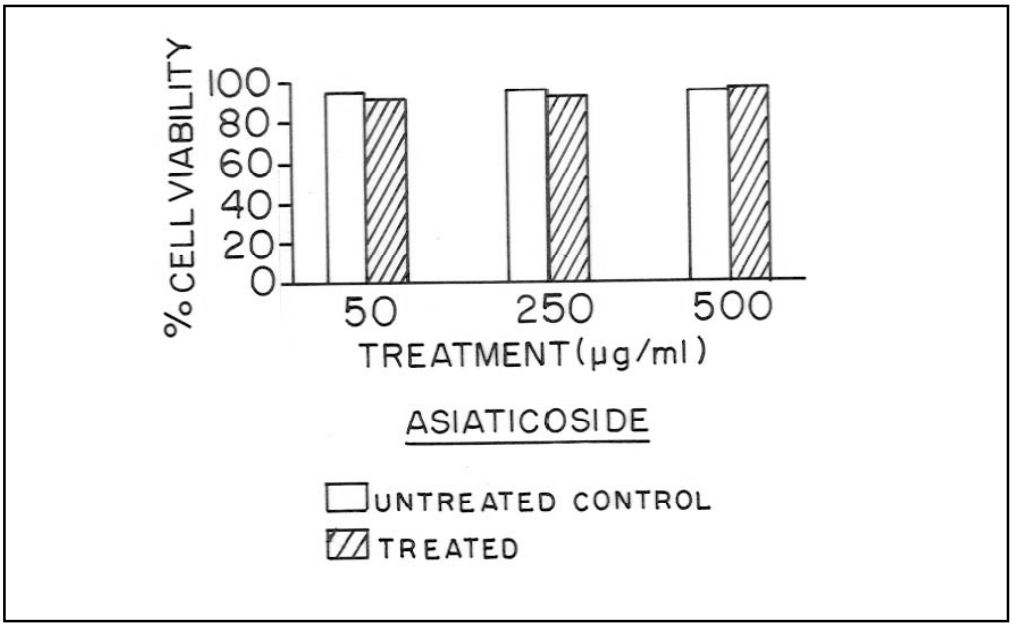

Figure 5. Trypan blue exclusion test to assess host cell viability against asiaticoside.

cal plant glycosides are appropriate for site-specific delivery to combat leishmaniasis, and because of their indigenous and nontoxic nature, they are expected to be useful in clinical applications.

Two indigenous glycosides, one having glucose as an end sugar in the hydrophilic sugar chain (eg, acaciaside) (Figure 2A) and the other having rhamnose as an end sugar with no tissue specificity but having leishmanicidal property (eg, asiaticoside) (Figure 2B), were incorporated together into the liposomes in definite molar proportion to combat experimental leishmaniasis in animal models. The multiple dose response curve of free asiaticoside on the survival of hamsters is shown in Figure 3. The dose-dependent reduction of parasite burden in the spleens of hamsters when treated with asiaticoside alone is shown in Figure 4. Thus, the optimum dose for chemotherapy was 4 mg/kg of body weight. The host cell viability at varying concentrations of asiaticoside is shown in Figure 5. In the treated group, at the 500 µg/mL concentration, the drug demonstrated 97% viability, compared to untreated controls, where the drug demonstrated 96% viability. After chemotherapy, both acaciaside- and asiaticoside-incorporated liposomes were more efficient in lowering the spleen parasite load compared to asiaticoside incorporated liposomes or to free asiaticoside used as an antileishmanial agent (Table 1). Toxicity studies involving the levels of normal liver function enzymes also indicated the apparent nontoxic nature of the drug (ie, asiaticoside) when co-incorporated with acaciaside in the liposomes (Table 2).

The non-antimonial classical drugs, pentamidine isethionate, and their analogues were also examined in vitro for antileishmanial activities.[57,58] When encapsulated in mannose bearing liposomes, therapeutic efficacy and resultant toxicity were critically analyzed.[59] Moreover, hamycin, a polyene antibiotic, was examined in our laboratory for antileishmanial activity. When tested in vivo against experimental leishmaniasis, in a hamster model, the liposomal

Table 1. Effect of Asiaticoside on a 30-day Infected Hamster Model of *L. donovani* (AG83 strain)*

Treatment	Liver wt (gm) (mean ± SD)	Parasite load in spleen ($X10^{-9}$)	% Suppression of parasite load in spleen
No drug (control)	4.05 ± 0.25	2.9 ± 0.9	—
Free drug	3.50 ± 0.20	1.5 ± 0.5	47
Liposomal drug	2.35 ± 0.30	0.6 ± 0.3	62
Acaciaside-coated liposomal drug	2.05 ± 0.35	0.2 ± 0.8	92

*The reduction of parasite load, by administration of empty liposomes, both regular and acaciaside-coated, was approximately 12% to 14%. Results are shown as Mean ± SD (n = 3).

hamycin and mannose-bearing liposomal hamycin were more potent than regular hamycin; the mannose bearing liposomal hamycin was the most effective in reducing the spleen parasite load. Toxicity of the drug was reduced in the liposomal forms, as determined by hemoglobin levels and the level of specific enzymes related to normal liver function. Toxicity was reduced reduced when sterol-rich liposomes were used as a delivery system.[60]

Cationic liposomes have been used to deliver DNA to target cells. Using an in vitro approach, it was demonstrated that cationic liposome-encapsulated antisense oligonucleotides, complimentary to the *Leishmania* universal miniexon sequence, mediate efficient killing of intracellular *Leishmania*.[61] Prior to that study, a significant leishmanicidal effect was reported using phosphorothioate oligonucleotides encapsulated in maleyated albumin coated liposomes.[62] Enhanced activity of antisense phosphorothioate oligos against *Leishmania* amastigotes was reported later.[63] An augmented uptake of oligo, ribunuclease activation, and efficient target intervention were also observed under altered growth conditions. Recently, an antisense oligonucleotide, targeted to the parasite □-tubulin gene and encapsulated in cationic liposomes, was used to test its antileishmanial activity in vitro. Cationic liposomes containing dioleyl trimethyl ammonium propane (DOTAP) had higher antileishmanial activity (88% at 4 μM oligonucleotide) compared to other liposomes with stearyl amine (SA) and cetyl trimethyl ammonium bromide (CTAB) as cations. This work also showed that antisense oligonucleotides targeted to the □-tubulin gene of *Leishmania donovani* inhibit □-tubulin synthesis, which stop intracellular parasites from multiplying.[64]

Immunoliposomes have also been developed for combating experimental leishmaniasis. Characterization of *Leishmania donovani* antigens encapsulated in liposomes induced protective immunity in BALB/c mice.[65,66] With maximum induction by positively charged liposomes, followed by neutral liposomes and lastly by the negatively charged liposomes, the extent of protection induced by the same antigens var-

Table 2. Specific Enzyme Level in Sera of Hamsters Undergoing Experimental Leishmaniasis*

Groups	Enzyme activity	
	Alkaline phosphates†	SGPT‡
Normal	13.4 ± 2.1	76.0 ± 12.0
Infected control	16.9 ± 2.7	104.1 ± 19.1
Free drug	14.2 ± 1.0	99.1 ± 24.7
Liposomal asiaticoside	13.9 ± 1.0	78.8 ± 28.8
Acaciaside-coated liposomal drug	13.9 ± 2.1	76.6 ± 17.1

*Results are shown as Mean ± SD (n = 4).
†µmol of p-nitrophenol released/min/dL of serum.
‡µmol of sodium pyruvate/min/L of serum.

ied depending on the charge of the vesicles. Effective immunization against cutaneous leishmaniasis was noted when defined membrane antigens were reconstituted into liposomes.[67] Liposomes coated with neoglycolipids constructed with mannopentose and dipalmitoyl phosphatidyl ethanolamine (Man5-DPPE) induced cellular immunity against antigens encapsulated in the liposomes (using *L. major* infection in susceptible balb/c mice).[68] These results indicate that Man5-DPPE-coated liposome-encapsulated antigen could serve as a vaccine to trigger protection against infection. Several immunomodulators have been studied for active or passive targeting. Active targeting of muramyl dipeptide (MDP) to macrophages was studied by conjugation with the neoglycoprotein, mannosyl serum albumin (mannose-HSA), using visceral leishmaniasis as the model disease.[69] Conjugation did not decrease the affinity of the neoglycoprotein for macrophage mannose receptor. Mannose-HSA-MDP was 50 times more efficient than free MDP in inhibiting the growth of *Leishmania donovani* inside the peritoneal macrophages. Kole et al[70] in attempting active targeting noticed a synergistic effect of interferon-gamma and doxorubicin, when incorporated in mannosylated liposomes for therapy of experimental visceral leishmaniasis. But, a similar synergistic effect in natural targeting of glucantine and liposome-encapsulated muramyl dipeptide analog was reported earlier.[71]Targeting of immunostimulatory DNA, encapsulated in mannose-grafted liposomes reported to cure experimental visceral leishmaniasis through nitric oxide up regulation and T cell activation.[72] Liposomes bearing surface-attached antibodies, raised against parasite-specific antigen, were a better approach in combating the disease in a more precise manner.[73]

Chemotherapy: Experimental to Clinical

Although miltefosine is being used for treating visceral leishmaniasis in India,[74] no report of ligand directed active drug targeting in the field of leishmaniasis in humans has been reported. But, cases of natural targeting to macrophages involving some familiar antileishmanial drugs, (eg, AmBisome) are very common.[75,76]

Successful treatment with liposomal amphotericin B for antimony resistant cutaneous leishmaniasis,[77] visceral leishmaniasis infected with HIV,[78] and AIDS[79] were also reported. Apparently, successful treatment of post kala-azar dermal leishmaniasis (PKDL) with liposomal amphotericin B has been reported.[80] Sundar and Murray[81] were successful in curing antimony unresponsive Indian Visceral leishmaniasis with amphotericin B lipid complex. Thicker et al[82] conducted a randomised dose finding study to compare three treatment regimens with AmBisome for visceral leishmaniasis in India. Bodhe et al[83] introduced a 10-day courses of L-amp B-LRC (Bombay), a new liposomal amphotericin B, to treat visceral leishmaniasis in India. Unfortunately, no other formulations proposed so far have the potential to be used as widely as the amphotericin B in the liposomal form. Thus, new drugs and delivery systems need to be developed to combat epidemics on leishmaniasis.

EXPERT OPINION AND CONCLUSION

Due to their biodegradability, biocompatibility, non-toxic, and non-immunogenic nature, ease of administration, and capability of long-term sustained release, liposomes have shown great promise in the delivery of therapeutic substances. But despite extensive research, liposome-mediated therapy in humans is not a reality. However, at present with a much clearer understanding of the limitations of liposome technologies, as well as a better technical understanding of designing and manufacturing these phospholipid based vesicles, companies are conducting an impressive number of drug trials in humans. But to date, liposomes have been most effective against cancer and to some extent against fungal and parasitic infections.

Moreover, the time has come when the efficacy of liposomes needs to be tested against other classic drug carriers (eg, neoglycoproteins, niosomes, etc). The biggest challenge will come from polymeric delivery vehicles (eg, microspheres and nanoparticles), which are made of cost effective natural or synthetic biodegradable polymers. Because of their small size and highly stable nature, the use of nanoparticles as effective drug carriers has been explored in experimental leishmaniasis using a series of antileishmanial compounds.[84,85] The efficacy in reducing the spleen parasite load, as well as reducing the hepatotoxicity and renal toxicity compared to free drugs or drugs incorporated in other delivery vesicles varied according to the delivery vesicles used. Best efficacy was shown by nanoparticles, followed by niosomes, liposomes, microspheres and free drug.

Thus, it is obvious that, the feasibility of the use of nanoparticles in clinics is much better than that of liposomes and/or any other existing drug delivery vesicles. However, the future role of liposomes in newly designed delivery systems has yet to be determined.

ACKNOWLEDGEMENT

Financial support was provided in the form of an Emeritus Scientist's Scheme to the author by the Council of Scientific and Industrial Research, India. The author is grateful for technical assistance provided by Dr. Sanchaita Lala in article preparation.

REFERENCES

1. Bangham AD. Physical structure and behaviour of lipids and lipid enzymes. *Adv Lipid Res.* 1963;1:65-69.
2. Bangham AD, Standish MM, Watkins JC. Diffusion of equivalent ions across the lamellae of swollen phospholipids. *J Mol Biol.* 1965;19:238-252.
3. Gregoriadis G. The carrier potential of liposomes in biology and medicine. *N Eng J Med*. 1976;295:704-710.

4. Gregoriadis G. Liposomes for drugs and vaccine. *Trends Biotechnol.* 1985;3:235-240.

5. Gregoriadis G. Liposomes in therapeutic and preventive medicine: the development of the drug carrier concept. *Ann NY Acad Sci.* 1978;308:343-370.

6. Modabber F. Antimonials: large-scale failure in leishmaniasis alarming. *TDR New.* 1990;34:1-7.

7. Pojansky MJ, Juliano RL. Biological approaches to the controlled delivery of drugs: A critical review. *Pharmacol Rev.* 1984;36:277-336.

8. Allen TM, Chonn A. Large unilamellar liposomes with low uptake into the reticuloendothelial system. *Febs Lett.* 1987;223:42-46.

9. Gabizon A, Apahadjopoulos D. The role of surface charge and hydrophilic groups on liposome clearance in vivo. *Biochim Biophys Acta.* 1992;1103:94-100.

10. Allen TM, Hansen O, Rutledge J. Liposomes with prolonged circulation times, factors affecting uptake by reticuloendothelial and other tissues. *Biochim Biophys Acta.* 1989;981:27-35.

11. Surolia A, Ahmad A, Bachhawat BK. Affinity chromatography of galactose containing biopolymers using covalently coupled Ricinus communis lectin to sepharose 4B. *Biochim Biophys Acta.* 1975;404:83-92.

12. Krantz MZ, Hotzhman NA, Stowells CP, Lee YC. Attachment of thioglycosides to proteins: enhancement of liver membrane binding. *Biochemistry.* 1976;15:3962-3968.

13. Stahl PD, Rodman JS, Miller MJ, Schlesinger PH. Evidence for receptor mediated binding for glycopropane, glycoconjugates and lysosomal glycosidases by alveolar macrophages. *Proc Acad Natl Sci U S A*. 1978;75:1399-1403.

14. Surolia A, Bachhawat BK. The effect of lipid composition on liposome lectin interaction. *Biochim Biophys Res Commun.* 1978;83:779-784.

15. Hara TL, Ishihara H, Aramakiy, Tsuchiya S. Characteristics of the binding of asialofetuin-labeled liposomes to isolated rat hepatocytes. *Int J Pharmaceut.* 1991;67:123-129.

16. Das N, Bachhawat BK, Mahato SB, Basu MK. Plant glycosides in liposomal drug delivery systems. *Biochem J.* 1987;247:359-361.

17. Medda S, Mukherjee S, Das, Naskar K, Mahato SB, Basu MK. Sugarcoated liposomes for increased drug efficacy and reduced drug toxicity. *Biotechnol Appl Biochem.* 1993;17:37-47.

18. Ghosh P, Bachhawat BK. Grafting of different glycosides on the surface of liposomes and its effect on the tissue distribution of ^{125}I-labelled gamma globulin encapsulated in liposomes. *Biochem Biophys Acta.* 1980;632:562-572.

19. Yamashita C, Matsuo H, Akiyama K, Kiwada H. Enhancing effect of Cetylmannoside on targeting of liposomes to Kupffer cells in rats. *Int J Pharmaceut.* 1991;70:225-233.

20. Matsuo H, Funato K, Harashim A, Kiwadah H. The complement but not mannose receptor mediated phagocytosis is involved in the hepatic uptake of cetylmannoside-modified liposomes *in situ*. *J Drug Target.* 1994;2:141-146.

21. Umezwa F, Eto Y. Liposomes targeting to mouse brain: mannose as a recognition marker. *Biochem Biophys Res Commun.* 1988;153:1038-1044.

22. Betageri GV, Black CD, Szebeni J, Wahl LM, Weinstein JN. Fc-receptor mediated targeting of antibody bearing liposomes containing dideoxy cytidine triphosphate to human monocyte/macrophages. *J Pharm Pharmacol*. 1993;45:48-53.

23. Ahmed I, Longereeker M, Samuel J, Allen TN. Antibody targeted delivery of doxorubicin entrapped in sterically stabilized liposomes can eradicate long cancer in mice. *Cancer Res*. 1993;53:1484-1490.

24. Chandra S, Agarwal AK, Gupta CM. Chloroquine delivery to erythrocytes in *Plasmodium barghei* infected mice assay antibody bearing liposomes as drug vehicles *J Biosci.* 1991;16:137-144.

25. Weinstein JN, Blumenthal R, Sharrow SO, Henkart PA. Antibody-mediated targeting of liposomes: binding to lymphocytes does not ensure incorporation of vesicle contents into the cells. *Biochim Biophys Acta.* 1978;509:272-288.

26. Heath TD, Fraley RT, Papahadjopoulos D. Antibody targeting of liposomes: cell specificity obtained by conjugate of F (ab')$_2$ to vesicle surface. *Science.* 1980;210:539-543.

27. Singhal A, Gupta CM. Antibody mediated targeting of red cells *in vivo. FEBS Lett*. 1988;201:321-326.

28. Singhal A, Bali A, Gupta CM. Antibody mediated targeting of liposomes to erythrocytes in whole blood. *Biochim Biophys Acta.* 1986;880:72-77.

29. Huang A, Huang L, Kennel SJ. Monoclonal antibody covalently coupled with fatty acids a reagent for *in vitro* liposome targeting. *J Biol Chem*. 1980;255:8015-8022.

30. Lesserman MP, Barbet J. Cell specific drug transfer from liposomes bearing monoclonal antibodies. *Nature.* 1981;293:226-228.

31. Schiffmann E, Corcoran BA, Wahl SM. N-formyl methionyl peptides as chemoattractants for leukocytes. *Proc Natl Acad Sci U S A*. 1975;72:1059-1062.

32. Niedel J, Wilkinson S, Cuatrecasus P. Receptor mediated uptake and degradation of ^{125}I-chemotactic peptide by human neutrophils. *J Biol Chem.* 1979;254:10700-10706.

33. Snyderman R, Fudman EJ. Demonstration of a chemotactic factor receptor on macrophage. *J Immunol.* 1980;124:791-796.

34. Snyderman R, Goetzel EJ. Molecular and Cellular mechanism of leukocyte chemotaxis. *Science.* 1981;213:830-837.

35. Guru PY, Agarwala AK, Singha UK, Singhal A, Gupta CM. Drug targeting in *Leishmania donovani* infections using tuftsin bearing liposomes as drug vehicles. *FEBS Lett.* 1989;245:204-205.

36. Chillari E, Arcoleo F, Dieli M, et al. The macrophage-activating tetrapeptide tuftsin induces nitric oxide synthesis and stimulates murine macrophages to kill leishmania parasites in vitro. *Infect Immun.* 1994;62:2649-2652.

37. Allen TM, Hansen CB, Guo LS. Subcutanous administration of liposomes: a comparison with the intravenous and intraperitoneal routes of injection. *Biochim Biophys Acta.* 1993;1150:9-16.

38. Kimelberg H. Protein-liposome interactions and their relevance to the structure and function of cell membranes. *Mol Cell Biochem*. 1976;1:171-190.

39. Freund O, Amedee J, Roux D, Laversanne R. *In vitro* and *in vivo* stability of new multilamellar vesicles. *Life Sci*. 2000;67:411-419.

40. Unezaki S, Maruyama K. Takahashi N, et al. Enhanced delivery and antitumor activity of doxorubicin using long circulating thermosensitive liposomes containing amphipathic polyethylene thermosensitive with local hyperthemia. *Pharm Res.* 1994;11:1180-1185.

41. Mauk MR, Gamble RC,Baldeschwieler M. Vesicle targeting: Timed release and specificity for leukocytes in mice by subcutaneous injection. *Science.* 1980;207:309-311.

42. Alving CR, Steck EA, Hansen WL, Loizeaux PS, Chapman WL Jr, Waits VB. Improved therapy of experimental leishmaniasis by use of a liposome-encapsulated antimonial drug. *Life Sci.* 1978;22:1021-1026.

43. Alving CR, Steck EA, Champman WL, et al. Therapy of leishmaniasis: superior efficacies of liposome-encapsulated drugs. *Proc Natl Acad Sci U S A.* 1978;75:2559-2563.

44. New RRC, Chance ML, Heath S. The treatment of experimental cutaneous leishmaniasis with liposome-entrapped pentostam. *Parasitology.* 1981;83:519-527.

45. Das N, Mahato SB, Naskar K, Ghosh DK, Basu MK. Targeting of urea stibamine encapsulated in liposomes to reticuloendothelial system for the treatment of experimental leishmaniasis. *Biochem Med Metab Biol.* 1990;43:133-139.

46. Tempone AG, Perez D, Rath S, Vilarinho AL, Mortara RA, De Andrade HF Jr. Targeting of *Leishmania (L.) chagasi* amastigotes through macrophage scavenger receptors: the use of drugs entrapped in liposomes containing phosphatidyl serine. *J Antimicrob Chemother.* 2004;54:60-68.

47. Ahmad I, Agarwal A, Pal A, Guru PY, Bachhawat BK, Gupta CM. Tissue distribution and antileishmanial activity of liposomal amphotericin B in Balb/c mice. *J Biosci.* 1991;16:217-221.

48. Giacchino R, Giambartolomei G, Tasso L, et al. Treatment with liposomal amphotericin B of a child affected with drug-resistant visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 1993;87(3):310.

49. Croft SL, Davidson RN, Thornton EA. Liposomal amphotericin B in the treatment of visceral leishmaniasis. *Antimicrob Chemother.* 1991;28:111-118.

50. Hart DT. Leishmania host-parasite interactions: the development of chemotherapeutic targets and specific drug delivery systems. I Lipoprotein mediated antileishmanial activity. In: Chang KP, Suary D, eds. *Host Parasite Cellular and Molecular Interactions in Protozoa Infections.* Berlin, Germany: Springer-Verlag; 1987:203-211.

51. New RRC, Chance ML, Heath S. Liposome therapy for experimental cutaneous and visceral leishmaniasis. 1983;47:59-64.

52. Banerjee G, Medda S, Basu MK. A novel peptide-grafted liposomal delivery system targeted to macrophages. *Antimicrob Agents and Chemother.* 1978;42:348-351.

53. Proulx ME, Desormeaux A, Marquis J, Olivier M, Bergeron MG. Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin. *Antimicrob Agents Chemother.* 2001;45:2623-2627.

54. Dutta M, Bandyopadhyay R, Basu, MK. Neoglycosylated liposomes as efficient ligands for the evaluation of specific sugar receptors on macrophages in health and in experimental leishmaniasis. *Parasitol.* 1994;109:139-147.

55. Medda S, Mukhopadhyay S, Basu MK. Evaluation of the in vivo activity and toxicity of amarogentin, an antileishmanial agent in both liposomal and niosomal forms. *J Antimicrob Chemother.* 1990;44:791-794.

56. Sinha J, Raay B, Das N, et al. Bacopasaponin C: critical evaluation of antileishmanial properties in various delivery modes. *Drug Del.* 2002;9:55-62.

57. Nandi G, Mukherjee S, Basu MK, Mahato SB. Synthesis, spectroscopic properties and antileishmanial activity of pentamidine analogues. *J Ind Chem Soc.* 1993;70:527-531.

58. Mahato SB, Nandi G, Mukherjee S, Basu MK. Chemotherapy of leishmaniasis: urea stibamine and some pentamidine analogues. In: Bhaduri AN, Basu MK, Sen AK, Kumar S, eds. *Current Trends in Leishmaniasis Research*. New Delhi, India: Publication & Information Directorate, Council of Scientific and Industrial Research; 1993:246-253.

59. Banerjee G, Nandi G, Mahato SB, Pakrashi A, Basu MK. Drug delivery system: targeting of pentamidines to specific sites using sugar-grafted liposomes. *J Antimicrob Chemother.* 1996;38:145-150.

60. Banerji G, Bhaduri AN, Basu MK. Mannose-coated liposomal hamycin in the treatment of experimental leishmaniasis in hamster. *Biochem Med Metab Biol.* 1994;53:1-7.

61. Chakraborty R, Dasgupta D, Adhya S, Basu MK. Cationic liposome-encapsulated antisense oligonucleotide mediates efficient killing of intracellular leishmania. *Biochem J.* 1999;340:393-396.

62. Chowdhury G. Scavenger receptor-mediated delivery of antisense miniexon phosphorothioate oligonucleotide to *Leishmania* infected macrophages. Selective and efficient elimination of the parasite. *Biochem Pharmacol.* 1997;53:569-580.

63. Mishra M, Porter-Kelley JM, Singh PK, Bennet JR, Chaudhury G. Enhanced activity of antisense phosphorothioate oligos against leishmania amastigotes: augmented uptake of oligo, ribonuclease H activation, and efficient target intervention under altered growth conditions. *Biochem Pharmacol.* 2001;62:569-580.

64. Dasgupta D, Adhya S, Basu MK. The effect of beta tubulin specific antisense oligonucleotide, encapsulated in different cationic liposomes on the suppression of intracellular L. donovani parasites in vitro. *J Biochem*. 2002;132:23-27.

65. Afrin F, Rajesh R, Anam K, Gopinath M, Pal S, Ali N. Characterization of *Leishmania donovani* antigens encapsulated in liposomes that induce protective immunity in Balb/c mice. *Infect Immun.* 2002;70:6697-6706.

66. Afrin F, Anam K, Ali N. Induction of partial protection against *Leishmania donovani* by promastigote antigens in negatively charged liposomes. *J Parasitol.* 2000;86:730-735.

67. Russel DG, Alexander J. Effective immunization against cutaneous *leishmania* with defined membrane antigens reconstituted into liposomes. *J Immunol.* 1988;140:1274-1279.

68. Shimizu Y, Yamakami K, Gomi T, et al. Protection against *Leishmania* major infection by oligomannose coated liposomes. *Bioorg Med Chem.* 2003;11:1191-1195.

69. Sarkar K, Das PK. Protective effect of neoglycoprotein-conjugated muramyl dipeptide against *Leishmania donovani* infection. The role of cytokines. *J Immunol.* 1997;158:5357-5365.

70. Kole L, Das L, Das PK. Synergistic effect of interferon gamma and mannosylated liposome incorporated doxorubicin in the therapy of experimental visceral leishmaniasis. *J Infect Dis.* 1999;180:811-820.

71. Adinolfi LE, Bouventre PF, Yander PAS M, Eppstein DA. Synergistic effect of glucantine and a liposome encapsulated muramyl dipeptide analog in therapy of experimental visceral leishmaniasis. *Infect Immun.* 1985;48:409-416.

72. Datta N, Mukherjee S, Das L, Das PK. Targeting of immunostimulatory DNA cures experimental visceral leishmaniasis through nitric oxide up regulation and T-cell activation. *Eur J Immunol.* 2003;33:1508-1518.

73. Mukherjee S, Das L, Kole S, Karmakar S, Datta N, Das PK. Targeting of parasite specific immunoliposome-encapsulated doxorubicin in the treatment of experimental visceral leishmaniasis. *J Infect Dis*. 2004;189:1024-1034.

74. Davies CR, Kaye P, Croft SL, Slundar S. Leishmaniasis: new approaches to disease control. *Brit Med J*. 2003;326:377-382.

75. Davidson RN, Croft SL, Scott A, Maini M, Moody AH, Bryceson AD. Liposomal amphotericin B in drug resistant visceral leishmaniasis. *Lancet* 1991;337:1061-1062.

76. DiMartino L, Raimondi F, Scotti S. Davidson RN, Gradoni L, Giacchino R. Efficacy and tolerability of liposomal amphotericin B in Italian infants with visceral leishmaniasis. *Trans R Soc Trop Hyg.* 1993;87:477.

77. Torre-Cisneros J, Prada JL, Villanueva JL, Valvarde F, Sanchez-Guijo P. Successful treatment of antimony-resistant cutaneous leishmaniasis with liposomal amphotericin B. *Clin Infect Dis.* 1994;18:1024-1025.

78. Russo R, Nigro LC, Minniti S, et al. Visceral leishmaniasis in HIV infected patients: treatment with high dose amphotericin B (AmBisome). *J Infect Dis.* 1996;32:133-137.

79. Lazanas MC, Tsekes, GA, Pappandreou S, et al. Liposomal amphotericin B for leishmaniasis treatment of AIDS patients unresponsive to antimonium compounds. *AIDS.* 1993;7:1018-1019.

80. Hashim FA, Khalil EA, Ismail A, eL Hassan AM. Apparently successful treatment of two cases of post kala-azar dermal leishmaniasis with liposomal amphotericin B. *Trans R Soc Trop Med Hyg.* 1995;89:440.

81. Sundar S, Murray HW. Cure of antimony unresponsive Indian visceral leishmaniasis with amphotericin B lipid complex. *J Infect Dis.* 1996;173:762-765.

82. Thakur CP, Pandey AK, Sinha GP, Roy S, Behbehani K, Olliaro P. Comparison of three treatment regimens with liposomal amphotericin B (AmBisome) for visceral leishmaniasis in India: a randomized dose finding study. *Trans R Soc Trop Med Hyg.* 1996;90:319-322.

83. Bodhe PV, Pathare AV, Kshirsagar NA, Pandya SK. Treatment of visceral leishmaniasis with a 10 days course of L-amp B–LRC (Bombay), a liposomal amphotericin B. *J Assoc Physicians India.* 1996;44:222.

84. Sarkar S, Mandal S, Sinha J, Mukhpadhyay S, Das N, Basu MK. Quercetin: Critical evaluation as an antileishmanial agent in vivo in hamsters using different vesicular delivery modes. *J Drug Target.* 2002;10:573-578.

85. Lala S, Pramanik S, Mukhopadhyay S, Bandyopadhyay S, Basu MK. Harmine: evaluation of its antileishmanial properties in various vesicular delivery systems. *J Drug Target.* 2004;12:165-175.