kinetic mechanisms producing these response are far from clear. The basic model for localized drug delivery is shown in Fig. 15.14.

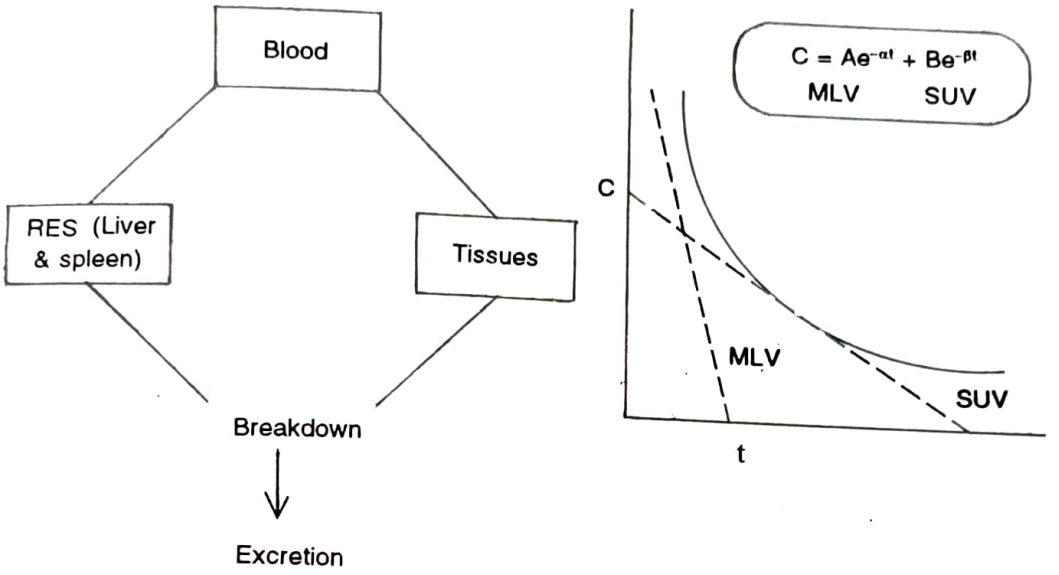

Fig. 15.13. Pharmacokinetic aspects of liposomal drug delivery.

A liposomal drug carrier (1) releases drug into a local compartment (2), from which the drug redistributes into the systemic compartment (3) and then is gradually eliminated. For simplicity, it is assumed that drug efflux from the liposomal carrier (K_1) is irreversible, that the volumes of the compartment are constant, and that the unidirectional rate constants between compartments (2) and (3) are equal, and given by K_t. The equation describing the model are then as follow :-

$$dQ/dt = -K_1.Q_1$$

$$dQ_2/dt = K_1.Q_1 - K_t\,(Q_3 - Q_2) \quad [5]$$

$$dQ_3/dt = -K_{el}.Q_3 + K_t\,(Q_2 - Q_3) \quad [6]$$

$$\text{At } t = 0,\ Q = Q_1,\ Q_2 = Q_3 = 0. \quad [7]$$

Where Q_1, Q_2 and Q_3 are the amounts of the drug in carriers (1) local compartment (2) and systemic compartment (3) respectively; K_{el} is the elimination rate constant.

(iii) Pharmacokinetics of systemic liposomal drugs

A fairly general model for the pharmacokinetic behavior of liposomal drugs injected into the circulatory system is given in Fig. 15.15.

The model deals with the four compartments namely, (1) liposomal drug in blood, (2) free drug in blood, (3) liposomal drug in tissue, and (4) free drug in tissue. It is assumed that the leakage rate of drug from the liposomes is same in blood and tissue, so that $K_{12} = K_{34}$, and unidirectional rate constants of drug exchange between blood and tissue are the same in both directions, so that $K_{24} = K_{42}$. The equations are as follows:

$$dQ_1/dt = -(K_{12} + K_{13})Q_1 \quad [8]$$

$$dQ_2/dt = K_{12}.Q_1 + K_{24}\,.(Q_4 - Q_2) - K_{el}\,Q_2 \quad [9]$$

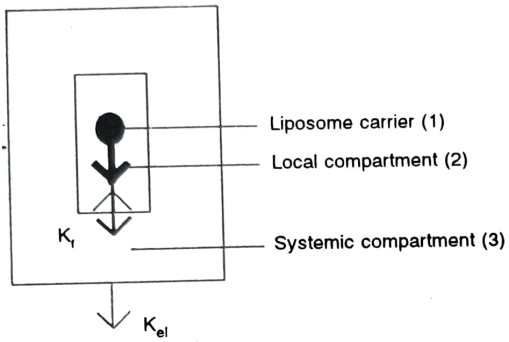

Fig. 15.14. Models for localized drug delivery.

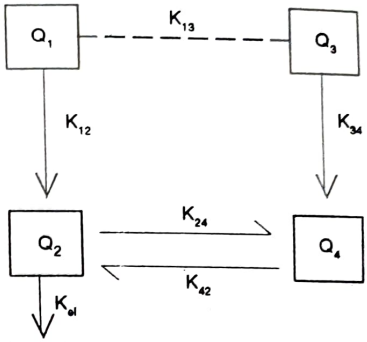

Fig. 15.15. Pharmacokinetic behavior of liposomal drug injected into the circulatory system.

$$dQ_3/dt = K_{13}.Q_1 - K_{12} \cdot Q_3 \quad [10]$$

$$dQ_4/dt = K_{12}.Q_3 + K_{24} .(Q_2 - Q_4) \quad [11]$$

Where Q_1, Q_2, Q_3 and Q_4 are the amounts of drug in different compartments and K_{12}, K_{13}, K_{24}, K_{42} and K_{el} are the rate constants. Once again, these equations are complex and not amenable to single analytical solution. However, even without extensive computation, some points may be deduced about the system.

1. For most drugs, $K_{24} >> K_{12}$ as described in eq. (5-7) if $K_{el} << K_{24}$, free drug will tend to equlibriate between blood and tissue, thus free drug action will be uniform throughout the body. If, on the other hand, $K_{el} >> K_{24}$, then localized drug actions are possible in tissues where large amounts of liposomes accumulate.
2. If $K_{el} >> K_{24}$, both blood and tissue levels of free drug will be low and most of the body burden of drug will be in encapsulated form.

STABILITY OF LIPOSOMES

Liposome stability problems are of course much more severe. Many different changes can occur in liposomes with the passage of time. Liposomal phospholipid can undergo chemical degradation such as oxidation and hydrolysis. Either as a result of these changes or otherwise, liposomes maintained in aqueous suspension may aggregate, fuse or leak their contents.

Methods devised to overcome the problems of liposome instability fall into two categories - those designed to minimize the degradation process which may take place and, secondly, those which contrive to help liposomes survive in the face of conditions which encourage these processes.

(a) Prevention of chemical degradation

The following precautions may be taken to minimize chemical degradation:

1. Start with freshly purified lipids and freshly distilled solvents.
2. Avoid procedure which involves high temperature.
3. Carry out manufacturing in the absence of oxygen.
4. Deoxygenate aqueous solution with nitrogen.
5. Store all liposome suspensions in an inert atmosphere.
6. Include an anti-oxidant as a component of the lipid membrane.

It may also be worthwhile including an iron chelater in the formulation to prevent initiation of the free radical chain reaction. The anti-oxidant in most common use at present time is α-tocopherol

(Vit.-E), a common non-toxic dietary lipid, β, γ, and δ- tocopherols may also be used as they are more effective as long term anti-oxidants (Lambelet & Loliger, 1984). Apart from oxidation problems, the level of oxidizable lipid in the membrane is to be reduced by using saturated lipids instead of unsaturated ones.

Hydrolysis of ester linkage will proceed most slowly at pH values close to neutral. The hydrolysis may be avoided altogether by the use of lipids which contain ether instead of ester linkages such as found in membrane of halophilic bacteria (Kates & Kushwaha, 1976). Hydrolysis *in vivo* as a result of enzymatic attack can be prevented by the use of sphingomyelin, or phospholipid derivatives with the 2-ester linkage replaced by a carbomyloxy function (Bali et al. 1983; Agrawal et al., 1986).

There are number of techniques to analyse liposome stability. Love et al., (1990) used high performance gel permeation liquid chromatography and gel permeation chromatography to analyse stability of liposomes.

(b) Prevention of physical degradation

Leakage and fusion of vesicles can occur as a result of lattice defects in the membrane introduced during their manufacture. Although it is reported particularly in SUVs when liposomes are prepared below the membrane phase transition temperature. There is evidence for packing defects in other types of vesicles (detergent dialysis vesicle, freeze thawed vesicles) even above the phase transition temperature. These irregularities can be dispensed by a process termed 'annealing' which consists simply of incubating the liposomes at a temperature high enough above the phase transition temperature to allow differences in packing density between opposite sides of the bilayers to equalize by trans-membrane flip-flop.

Even in annealing vesicles, aggregation, fusion etc. can take place to significant extents over a long period of time. Aggregation (and sedimentation) of neutral liposomes is brought about by Van der Waals interaction and tends to be more pronounced in large vesicles, where the increased planarity of the membrane allows greater areas of membrane to come into contact with each other. Although factors such as residual solvent and trace elements can enhance the process for uncharged membranes, it is a natural and unavoidable phenomenon and the simplest way to overcome is to impart small quantities of negative charge to the lipid mixture (Larrabee, 1979).

SUVs (<40nm) are prone to fusion as a means of relieving stress arising from the high curvature of the membrane. Since this can occur particularly at the phase transition temperature (Tc), it is advisable to store liposome suspension at a temperature away from the Tc and it could be advantageous to include sufficient cholesterol in the membrane to reduce or completely remove the transition, particularly if it is in a temperature range close to that at which the liposomes will be stored or handled. For liposomes which have negative charge in the membrane, care must be taken to avoid high concentration of metal ions. It may be worthwhile including a metal ion chelater in the suspending buffer.

The large polar or ionic molecules will be retained much more effectively than low molecular weight lipophilic compounds. In general, for both classes of compounds a rigid, more saturated membrane with a high molar ratio of cholesterol is the most stable with regard to leakage of solutes. The permeability can be increased at the Tc, particularly in the presence of high density lipoprotein (HDL), apolipoprotein, tubulin or actin (Weinstein et al., 1981).

Freezing or lyophilization techniques constitute an alternative method of overcoming instability problems by transforming the liposomes into a solid or anhydrous form, where chemical degradation of lipid components or solutes is less likely to occur. However, these techniques are not yet fully perfected. Methods for cryo preservation have achieved some success (Crowe et al., 1986).

The stability of liposomes may also be increased by cross-linking membrane component covalently using methods such as gluteraldehyde fixation, osmification or polymerization of alkyne-containing phospholipids (Leaver et al., 1983). Although, these methods can increase the mechanical strength of the membrane and render them less susceptible to disruption *in vivo* by serum components.

APPLICATIONS OF LIPOSOMES

Liposomes may prove to be efficient carrier for targeting the drug to the site of action, because of being biodegradable, innocuous nature and being identical to biological membrane. Table 15.2 (Gregoriadis & Florence, 1993) shows the liposomes application according to their mode of action.

Table 15.2. Major modes of liposomal action and related application

Mode of Action	*Application*
Intracellular uptake (lysosomes endosomes/cytoplasm)	Microbial diseases.
	Metal storage diseases.
	Gene manipulation.
	Uptake by some tumor cells.
	Macrophages activation to a tumoricidal/microbicidal state.
	Efficient antigen presentation by antigen-presenting cells (vaccines).
Slow release of drug near the target area	Tumor near fixed macrophages.
Circulating reservoirs	Blood surrogates.
Facilitation of drug uptake by certain routes	Drug delivery to skin, lungs, eyes, mucosal tissues.
Avoidance of tissue sensitivity to drugs	Cardiotoxicity of doxorubicin

The following are the few more properties which make liposomes applicable in various fields.

1. *Cell liposome interaction:* The various ways by which SUVs can interact with cells are shown schematically in Fig. 15.16. Stable absorption represents the association of intact vesicles with the cell surface. Such a process could be mediated by non-specific electrostatic, hydrophobic or other forces, or by specific components present at the vesicle and/or cell surfaces. Endocytosis is the uptake of intact vesicles into endocytotic vesicles and results, presumably, in their delivery to the lysosomal apparatus. Fusion is the merging of the vesicle bilayer with the plasma membrane bilayer, with the concomitant release of vesicle contents into the cytoplasmic space. Lipid exchange is the transfer of individual lipid molecules between vesicles and the cell surface, without the cell-association of aqueous vesicle contents (Pagano & Weinstein, 1978)

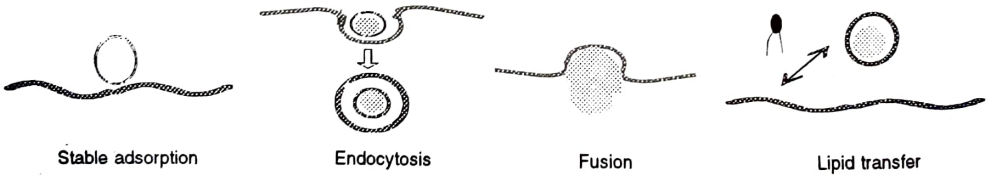

Fig. 15.16. Schematic representation of possible mechanisms of interaction between small unilamellar vesicles and the cell surface (Pagano & Weinstein, 1978)

2. *Localized drug effect:* Liposomes help in depositing the drug within selected sites or selected cell types. This is because of their larger size and low degree of penetration in epithelial and connective tissue barriers which tend to remain at the site. In 1978, Dingle and his colleagues (Dingle et al., 1978) observed an improved effect in the therapy of arthritis with steroid containing liposomes. McCullough & Juliano (1979) were successful in getting organ selective action of antitumor drugs i.e., cytosine arabinoside.

3. *Enhanced drug uptake:* Enhanced drug uptake occurs by vesicle cell fusion or via endocytosis. An interesting example is the antitumor drug cytosine arabinoside triphosphate (Ara CTP) which can be the inhibitor of DNA and is the active principle eventually produced by the administration of the prodrug, cytosine arabinoside.
4. Molecules with wide range of solubility and molecular weight can be accommodated.
5. Flexibility in structural characteristics.

(i) Cancer Chemotherapy and Neoplasia

Anticancer drugs usually are less selective which results into their toxicity to the normal cells. Liposomes have successfully been used to entrap the drug. This provides lot of advantages. First, increases circulation life time ($t_{1/2}$) i.e. drug tends to deposit in the tissue (internalized and remain there for a longer period of time), second, protects the metabolic degradation of drug. Third, altered tissue distribution of drug with enhanced uptake in organs rich in mononuclear phagocytic cells (e.g., liver, spleen and bone marrow) and decrease uptake in kidney, myocardium and brain. Most of the anticancer potency of encapsulated drug have been concentrated on particular specific phase of the cell cycle. They are called as 'cell specific drugs' (Fig. 15.17).

(ii) Liposomes as carriers for vaccines

(a) Liposomes as immunological adjuvants

Studies on cardiolipin (naturally occurring lipids) revealed the importance of bilayer structure in the production of antisera and concluded that liposomes are in fact responsible for the antigenicity of these lipids (Heath et al., 1976). In addition, liposomes also have an adjuvant effect upon protein antigens (diptheria toxoid). Liposomes can serve as an effective vehicle for inducing HI to a wide range of liposomal antigens (Bergers et al., 1995). Advantages in use of liposomes as carriers for vaccines include:

1. A non-immunogenic substance may be converted into immunogenic one.
2. Hydrophobic antigens may be reconstituted.
3. Small amounts of antigen may be suitable as immunogens.
4. Multiple antigen may be incorporated into the single liposomes.
5. Adjuvants may be incorporated with antigens into the liposomes.
6. Longer duration of functional antibody activity may be achieved.
7. Toxicity and allergic reactions of antigens may be reduced or eliminated by inclusion in liposomes.
8. Soluble synthetic antigen may be presented as membrane associated antigens in an insoluble liposomal matrix.

Liposomised diptheria toxoids (both neutral and negatively charged) were equally responsible to produce the same immune response whereas positively charged liposomes produced reduced responses. Therefore, the immune responses with various liposomal antigens are unpredictable with respect to the charge of liposomes.

The size and structure of liposomes may be modulated as required which effect the immunogenicity of liposomised antigens. Studies on HLVs and ULVs of comparable size promoting antibody response revealed that ULVs are more effective than HLVs to entrap BSA (Shek et al., 1983).

Liposomes made from the lipids with Tc above the ambient temperature (37°C) are known to behave differently from liposomes made from lipids whose Tcs are below the ambient temperature when interacting with the cells. Liposomes composed of DPPC and DSPC (Tc: 41.4°C and 54.9°C, respectively) have been reported to be more effective immunogens than those prepared from egg PC because of their greater bilayer stability at physiological temperature and stronger immuno-potentiating property(Hudson, et al., 1979).

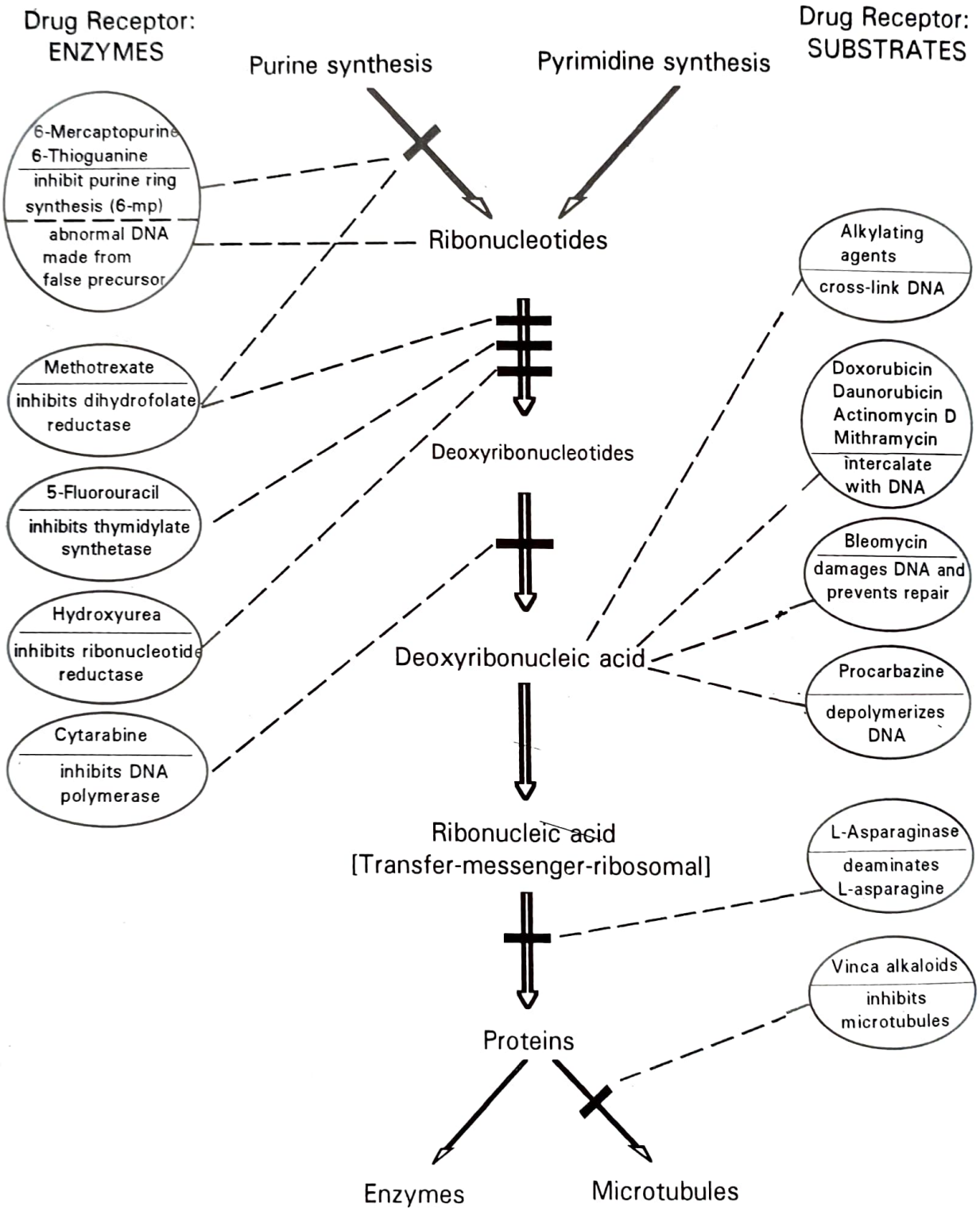

Fig. 15.17. Mechanisms and sites of action of selected cancer chemotherapeutic agents.

The immuno-potentiating effect of liposome associated antigen can be achieved by immunizing animals either by i.v., i.p., i.m., s.c. administration. Strong primary immune response can be induced by i.v. administration, which can be persisted by i.m, s.c. or i.p. administration, as secondary immunization (Heath et al., 1976; Latif & Bachawat, 1984).

(b) Liposomes as carrier of antigens

In addition to their adjuvant effect, liposomes have been recognized as efficient carriers to deliver biologically active material to specific cells. However, on administration of liposomes the major fraction is taken up by the liver and spleen unless steps are taken to retard their uptake. The following criteria help in successful homing of liposomised agent to target cells (Gregoriadis, 1983).

1. Rate of uptake of liposome by RES must be minimized by using small, neutral, unilamellar liposomes having higher Tc and cholesterol.
2. By coating the surface of liposomes which would render liposomes less recognizable by RES.
3. Coupling appropriate molecules (legends) on the liposome surface which can bind to their receptors on the surface of target cells.

Fig. 15.18 illustrates some of the topographical locations where protein may be distributed within liposomes. Proteins may be situated either on the outer surface of the outer lipid bilayer; within the bilayer, that is, as a partly or completely transmembrane protein; or sequestered within the aqueous region inside the liposomes, e.g., in the aqueous interspaces that separate adjacent concentric lipid bilayers.

Table 15.3. Protein antigen entrapped in liposomes to induce immune-response

Antigen	*Liposome composition and nature*	*Route of administration and animal species*	*Major observations*	*References*
Diptheria toxoid	PC:CH:SA/PA/DCP +ve/-ve, MLVs	i.v.,s.c., Mice	-ve liposomes with PA elicited greater absorption. DCP is superior to PA. s.c. and i.m. administration showed much greater absorption.	Allison & Gregoriadis, 1974
Cholera toxins	DMPC:CH:DCP ganglioside GM_1, -ve, MLVs	i.v.,s.c., Rabbits	Toxicity was completely eliminated and antigenicity was enhanced when CT attached to liposomes containing ganglioside GM_1, effective adjuvanticity was observed in liposomes containing lipid A or 2 types of MDP	Alving et al., 1986; Pierce et al.,1984
Herpese simplex virus type I antigen	PC:CH, Neutral, MLVs	i.p., Mice	Enhanced immunogenicity with liposomal antigen. Max. absorption with liposome bound antigen containing lipid A. No CMP with liposome preparations	Naylor et al., 1982

(Contd.)

Antigen	*Liposome composition and nature*	*Route of administration and animal species*	*Major observations*	*References*
Plasmodium falciparum merojoite-enriched antigen	DPPC:CH Neutral MLVs	s.c., Monkey	All immunized monkey survived the challenge only with the adjuvanted liposomes.	Siddique et al., 1978
Mycobacterium leprae antigen	PC:CH:Ganglioside, ULVs	i.d., Human	Liposomised antigen elicited both early (1-2days) and late (3-4 weeks) delayed type hypersensitivity, unlike the soluble antigen alone which elicits only early reaction.	Sengupta et al., 1988
Tuberculin purified protein deritive and peptide	PC, PS, PC:PS	i.m.	Liposomised tuberculoprotein augmented the delayed hypersensitivity	Pimm & Baldwin, 1984
Tetanus toxoid	Various phospholipids, CH	i.m., Mice	Adjuvant effect dependent on liposomal characteristics and source, amount and formulation of IL-2; demonstration of receptor-mediated targeted adjuvanticity.	Gregoriadis et al., 1987 Gregoriadis, 1990 Tan & Gregoriadis, 1989, Davis & Gregoriadis, 1987 Garcon et al., 1988
Salmonella typhimurium	PC/DPPC:CH	i.v., Mice	Protection on challenge with S. typhimurium; DTH	Desiderio & Campbell, 1985
Streptococcus pneumonia type 3	DPPC:CH:SA-hexasaccharide	i.v., Mice	Protection from S. pneumonia type 3	Snippe et al., 1983
Hepatitis B surface antigen	Various phospholipids CH:DCP	s.c., Guinea pig	Adjuvant effect; DTH	Manesis et al., 1979 Kim & Jeong, 1995
Influenza virus (A/PR8/34 strain) glycoprotein	Viral lipid extract	i.n.;i.p.; i.m., Mouse	Superior systemic and local response; protection from virus	El Guink et al., 1989
Poliovirus 3VP2 peptide	Various phospholipids:CH	i.m., Mouse	Adjuvant effect	Xiao et al., 1989
Fibrosarcoma surface antigen	PC:CH;PC:CH:PG	s.c., Mouse	Protection on supralethal tumour challenge	Le Grue, 1984
Tumour(L_2C) antigen	PC:PS	i.d.; Guinea pig	Survival on challenge with tumour cells	Schroit & Key, 1983

PS: Phosphatidyl serine; PC: Phosphatidyl choline; PG:Phosphatidyl glycerol; SA: Stearylamine
CH: Cholesterol; DMPC: Dimyristoyl phosphatidylcholine; DCP: Dicetyl phosphate
DPPC: Dipalmitoyl phosphatidylcholine.

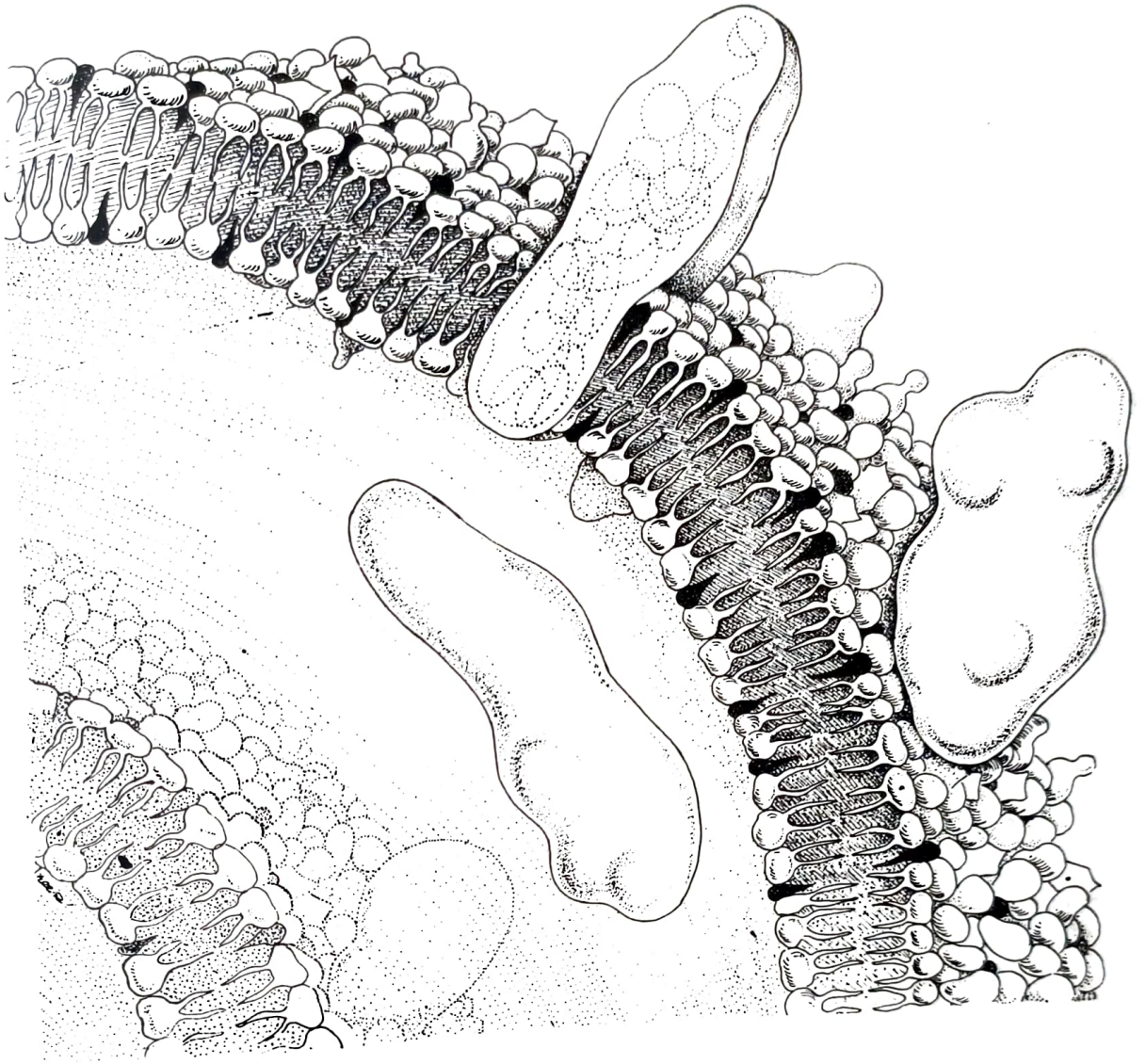

Fig. 15.18. Topographical locations of proteins in liposomes.

(c) Liposomes as carrier of drug in oral treatment

In certain cases, administration of drug by oral route would be preferred not only for convenience but also because it is often important that drugs enter the periphery via portal circulation. Many drugs either remain unabsorbed or are unstable in the gut.

(i) Arthritis

Drugs that are being used in arthritis specially steroids, are destroyed by their peripheral effect. On local administration into the joints, drug diffuses easily from the site of injection and its action on the inflammed area is only transient. Segal et al. (1979) suggested that liposomes could be used in the treatment of local diseases. It is observed that steroids (e.g. cortisol palmitate) can be entrapped into large multilamellar liposomes composed of dipalmitoyl phosphatidyl choline and phosphatidic acid. These preparations, when ingested into rabbits with experimental arthritis, can decrease the temperature as well as the size of the joints to a greater extent than with a similar amount of free steroids.

(ii) Diabetes

The feasibility of using liposomes as a potential delivery system for the oral delivery of insulin has been extensively studied (Dapergolas & Gregoriadis, 1976; Patel & Ryman, 1976; Moufti et al., 1980; Patel et al., 1982; Woddley , 1986). Alteration in blood glucose levels in diabetic animals was obtained by the oral administration of liposome encapsulated insulin (Arrieta-Molero et al., 1982). Dobre et al., (1983) demonstrated a lowering of blood glucose levels in normal rats following the oral administration of insulin entrapped in PC: CH liposomes. Patel & Ryman (1977) observed that, *in vitro*, liposomes exerted protective action in gastric and intestinal areas. Hashimoto & Kawada (1979) confirmed that liposomes had protective effects against proteolytic digestive enzymes (pepsin and pancreatin), but these effects disappeared in the presence of bile salts. However, there is fairly good evidence that liposomes can indeed increase the intestinal uptake of macromolecules (Bridges et al., 1978) and they may be capable of enhancing insulin uptake.

(d) Liposomes for topical applications

Liposomes have shown great potential as a topical drug delivery system. The first report on topical application of liposomally encapsulated drug was presented by Mezei & Guleshekharan (1979). Most of the studies are listed in Table 15.4 showing the use of steroids, which are highly effective in the treatment of skin disorders; and drugs from different categories of antitumor, local anesthetics, antimicrobial, non-steroidal anti-inflammatory drugs and anti-sepsis (Munro, 1976; Patel, 1984; Nishihata, et al., 1987; Moghimi & Patel, 1993; Imbert & Wickett, 1995).

Table 15.4. Different drugs used and results obtained of different studies of liposome for topical application.

Drug	*Results*	*References*
Triamcinolone	In epidermis and dermis 4 times higher conc. than control ointment. Decreased urinary excretion of drug.	Mezei & Guleshekharan, 1980
Triamcinolone acetonide in gel	Conc. of drug was 5 times higher in epidermis and 4 times in dermis than control gel.	Mezei & Guleshekharan, 1982
Progesterone	Reduces the rate of hair growth in idiopathic hixsutims.	Rowe et al., 1984; Knepp et al., 1990
Dihydrotestosterone	Negligible systemic absorption, hence no advantage of liposomes to achieve desired biological effects.	
Methotrexate	Reduced percutaneous absorption of drug was obtained. Retention of methotrexate in skin was 2-3 fold higher than free form	Mezei & Guleshekharan, 1980
Potocain cream (Tetracain)	Shows long lasting anesthesia of the skin even at low conc. whereas procain cream was ineffective.	Patel, 1984
Benzocain gel	Shows prolonged anesthesia as compared to plain benzocain cream.	Sharma et al., 1994
Hydrocortisone	Higher conc. of drug in the individual layers of human skin than control ointment.	Wohlrab & Lasch, 1987
Diclofenac gel	Increase conc. of the drug in the subcutaneous tissue as well as increase permeation through the skin	Nishihata et al., 1987 Kriwet & Muller-Goymann, 1995
Corticosteroids	Significant higher blanching response than control preparation	Jacobes et al., 1988
Superoxide dimutase(SOD)	Skin SOD activity after UV exposure was decreased by pre-treatment of skin with liposomal SOD.	Miyachi et al., 1987
Interferon	Reduction of lesion sores whereas application of interferon formulated as a solution or as emulsion was ineffective.	Weiner et al., 1989

Ganesan et al. (1984) found that neither intact liposomes, nor phospholipid of which they are composed, diffuse across the skin. The results showed that the liposomes might be useful for increased local activity while diminishing the percutaneous absorption of the drug. Gehring et al. (1995) demonstrated the effect of anthralin encapsulated liposomes on skin penetration. They observed enhanced effect with anthralin formulated in liposomes.

(e) Liposomes for pulmonary delivery

Liposomes are capable of entrapping an extensive range of solutes from hydrophilic entities to hydrophobic species. Such carriers are available in sizes ranging from 20 nm to greater than 1 μm diameter and of apparent low toxicity, therefore, provide the opportunity of a controlled release drug delivery system for administration to respiratory tract (Kellaway & Farr, 1990). Size is a critical particulate parameter determining the deposition site within the lung (Stahlhofen et al., 1980). Inhalation devices such as nebulizer will produce an aerosol of droplets containing liposomes. Deposition would therefore be seen to be dependent on the size distribution of the aerosol cloud and not the contained liposomes. Variation in lipid composition provides the opportunity of controlling the release rate of entrapped solute (Taylor et al., 1990). Some of the studies on liposomes encapsulated drug for pulmonary administration are reviewed in Table 15.5.

Table 15.5. Studies on some liposome encapsulated drugs administered via pulmonary route.

Drug	*Results*	*References*
Cytosin arabinoside	Free Ara-C was rapidly absorbed into the (Ara-C) systemic circulation whilst liposome encapsulated drug remained within the lung for a considerable time, hence reduces the adverse effects in other tissues.	McCullough & Juliano, 1979; Juliano & McCullough, 1980
6-Carboxy fluorescein (CF)	CF absorption was lipid-dose dependent with high doses producing faster absorption and did not involve transfer of intact liposome into the blood.	Woolfrey et al., 1985, 1988
Antioxidant enzymes	Liposomes containing antioxidant enzymes render it resistant to pulmonary oxygen toxicity	Padmanabhan et al., 1985; Buckley et al., 1987
Pentamidine	No significant difference in organ distribution on comparing free Vs liposome encapsulated drug. Aerosolized product produced substantially higher deposition in the alveolar.	Debs et al., 1987
Enriroxine	Liposome encapsulated drug was observed to be 10 to 50 times less toxic to tissue culture cells than free drug.	Wyde et al., 1988; Gilbert et al., 1988
Atropine glutathione	Maintained much higher level of entrapped drug in the lung than solution form.	Shek et al., 1988
Sodium cromoglycate	Free drug produced peak plasma level more than seven fold higher than the liposomal drug. Showed extended duration of drug plasma level.	Taylor et al., 1990
Benzyl penicillin, Oxytocin	Absorption rate constants of benzyl penicillin and oxytocin were similar for the free and liposome encapsulated drug. AUC was 3 times smaller for liposomal benzyl penicillin and 8 times smaller for liposomal oxytocin compared with free drug.	Mihalko et al., 1988
Metepreterenol	Shorter duration of effect from free sulfate drug compared to the same dose of liposomal drug.	Kamarel et al., 1989; Fielding, 1989; Pettenazzo et al., 1989

(Contd.)

Drug	*Results*	*References*
Tobramycin	Liposome entrapped drug shows pulmonary level 3 times higher than those of the free drug.	Poyner et al., 1995
Salbutamol	Dehydrated liposome entrapped drug ensured an effective sustained release system following inhalation.	Radhakrish et al., 1990
Cytarabine	Liposome encapsulation prolonged the pulmonary retention of intratracheal administered drug. Liposomal drug was cleared with a half-life of 8 hr. whereas free drug cleared with half-life of 40 min. Reduced the distribution to other tissue.	McCullough & Juliano, 1979; Juliano & McCullough, 1980

(f) Leishmaniasis

It is a parasitic disease and is affecting millions of people. It becomes lethal if parasite invades cells of liver and spleen and if it is untreated. Even with the therapy, many people die because the commonly prescribed drugs known as antimonials are related to arsenic. At high concentration they damage the heart, liver and kidney. Encapsulating such drugs in liposomes not only greatly reduces the dose needed to treat the infection but also deposits the conjugates at infected Kupffer cells where atleast some of the liposomes are digested by the parasites (Alving et al., 1978; New et al., 1978; 1981a; 1981b; Taylor et al., 1982). Avila & Rojas (1990) reported elevated cerebroside antibody levels in human visceral and cutaneous leishmaniasis. Desferrioxamine was tested *in vitro* for activity against promastigotes of leishmania donovani and improved effect was noted with liposome encapsulated drug (Segovia et al., 1989).

(g) Lysosomal storage diseases

Lysosomal storage diseases comprise a heterogeneous group of syndromes. However, they all result from genetically determined deficits of particular lysosomal hydrolytic enzymes. There are lysosomal storage diseases which may be suitable candidate for lysosomotropic enzyme replacement. This includes Gaucher's disease (ß-glucosidase deficiency) where the primary site of pathological catabolite accumulation is via RES rather than CNS; and Pomp's disease (α-glucosidase deficiency) where the primary affected tissues are liver and muscle.

Liposomes have the potential for delivery of enzymes to the lysosomal system. A variety of lysosomal enzymes can be readily entrapped in liposomes and infact some enzymes have an affinity for the liposomal membrane. Gregoriadis & Buckland (1973) were able to disperse sucrose filled vacuoles in macrophages and in tissue culture with liposomes containing invertase. Weismann's group has pioneered the use of liposome coated with aggregated immunoglobulins as a mean of introducing enzymes into phagocytic leukocytes (Weismann et al., 1975) and demonstrated the *in vitro* correlation of Tay-Sachs leukocytes using hexosamidase A encapsulated in Ig coated liposome (Cohen et al. 1976). More recently, liposome encapsulated ß-galactoxidase has been used to correct the enzymatic deficiency in GM_1 gangliosidosis fibroblast *in vitro* (Reynolds et al., 1978).

Rymon's group has reported the use of entrapped β-glucosidase in a patient suffering from Pomp's disease. Gregoriadis (1978) has treated at least two Gaucher's disease patients with encapsulated ß-glucosidase.

(h) Cell biological applications

The most obvious use of liposomes in cell biology is to manipulate the status of membrane lipid. It is clear that the exchange of lipids, particularly cholesterol, readily occurs between liposomes and cells; this phenomenon has been studied in detail by Poznansky & Lange (1976,1978). Increments or decrements in the membrane sterol level can lead to the alteration in physical properties (Inbar & Sohinitzky, 1974),

transport properties (Read & McElhaney, 1976) and enzymatic properties (Kimelberg, 1976) of membranes.

Liposomes have been used to carry functional DNA and RNA molecules into cells. Jansons & Mallett (1981) have encapsulated polioviruses in liposomes, thus producing a dramatic extension of host range of the virus (Wilson et al., 1977). Two groups have used liposomes to insert globin mRNA into cells and have demonstrated message translation (Dimitriadis, 1977; Ostro et al., 1978). Another group has used liposome to promote the cellular uptake of metaphase chromosomes and have claimed significant enhancement relative to the uptake of unencapsulated chromosome (Mukerjee et al., 1978). Liposomes will be used to insert functional genes or RNA molecules into the cell for the purpose of studying the control of eukaryotic transcription or translation. Afione et al. (1995) have presented liposomes and receptor-mediated gene transfer technique in gene therapy.

Liposomes are used to insert regulatory molecules such as cyclic AMP, CGMP and enzymatic cofactors into the cell.

(i) Metal storage diseases

The major problem in treating the metal storage diseases with chelating agent is the inability of many chelators (e.g. EDTA, DTPA) to cross cell membranes. As it is found that in these several diseases, metal accumulates in the lysosomes of cells, the lysosomotropic action of liposomes renders this carrier a hopeful approach to therapy (deDuve et al., 1974). Liposomal DTPA was capable of removing significant amounts of plutonium from the liver of mice loaded with the metal (Rahman & Rosenthal, 1973).

A very small amount of chelater (EDTA) is entrapped into the liposomes. A larger proportion of these chelators escape from the carrier during circulation. Therefore, it is desirable to increase entrapment of chelators in liposomes and also prevent their loss by diffusion through lipid lamella. ^{14}C labeled EDTA phosphatidyl ethanolamine complex was synthesized and incorporated quantitatively in liposomes composed of egg PC, cholesterol and phosphatidic acid. The EDTA phospholipid complex has capability of forming liposomes by itself from the blood than that exhibited by labeled EDTA entrapped in similar liposomes.

(j) Ophthalmic delivery of drugs

A major problem in ocular therapeutics is the delivery of an optimal drug concentration at the site of action. The ocular drug bioavailability is often modified by the physical and chemical properties of a drug as well as by physical properties of the vehicle in which the drug is placed. Thus, the selection of vehicles has been limited to the liquid and semisolid varieties, principally because of the anatomical construct of the conjunctival sac and the sensitivity of the cornea to foreign objects. Amongst various vehicles and carriers, liposomes have gained considerable attention for ocular drug delivery (Niesman, 1992; Gregoriadis & Florence, 1993).

The potential of liposomes in topical ocular drug delivery was first foccused by Smolin et al. (1981); Schaeffer & Krohn (1982) and Schaeffer et al. (1983). Liposomes offer advantages over most ophthalmic preparations in being completely biodegradable and relatively non-toxic. Smolin et al., (1981) reported the treatment of acute and chronic herpetic keratitis in albino rats, idoxuridine entrapped in liposomes was more effective than a comparable therapeutic regimen of unentraped drug. Schaeffer et al., (1983) reported that transcorneal flux of Penicillin G, Indoxol and Carbachol were approximately double when these drugs were presented to the corneal surface in liposomal form. In direct contrast to these findings, Startford et al., (1983a, 1983b) observed a reduction in the fraction of epinephrine and inulin absorbed into aqueous humor in liposomes.

A potential advantage of liposomes is their ability to intimately contact with the corneal and conjunctival surfaces, thereby, increasing the probability of ocular drug absorption. The ability is especially desirable for drugs that are poorly absorbed, for example, drugs with low partition coefficient or those with medium to high molecular weights. In principle, by varying the phospholipid composition

of the liposomes (Schaeffer et al., 1983) or by incorporating into liposomes legends for which receptors are present at the absorption surface (Megaw et al., 1981; Schaeffer et al., 1983), one can control the degree of liposome accumulation at the absorption surface and at the same time the rate of drug release from these liposomes.

Liposomes could offer another therapeutic advantage i.e., to protect a drug from the metabolic enzymes present at the tear/corneal epithelium interface. However, it is doubtful that liposomes can protect a drug from the metabolic enzymes in the corneal epithelial cells themselves, since liposomes probably do not diffuse across the cornea intact (Silverstein et al., 1977).

The effectiveness of liposomes in ocular drug delivery depends on a number of factors including (Lee et al., 1985) :

1. drug encapsulation efficiency,
2. size and charge of liposomes,
3. distribution of a drug within liposomes,
4. stability of liposomes in the conjunctival sac and ocular tissues,
5. retention of liposomes in the conjunctival sac; and
6. affinity of liposomes exhibited towards the corneal surface.

Schaeffer & Krohn (1982) demonstrated that the binding of liposome to the cornea *in vitro* decreased in the order of +ve, -ve, and neutral liposomes. These investigators showed that there was no significant difference in the binding of multi- and unilamellar liposomes to the corneal surface *in vitro*. The multilamellar, neutral liposome are loosely bound at the corneal surface (Lee et al., 1984). Schaeffer & Krohn (1982) and Guo et al., 1989/1990., have investigated the interaction of liposomes with corneal surface and observed following order of interaction: +ve MLV > +ve SUV > -ve SUV > MLV > SUV. However order of benzyl penicillin transport across the cornea was +ve SUV > -ve MLV > +ve MLV > -ve SUV > MLV. Ahmed & Patton (1985) and Guo et al., (1989/1990) confirmed the importance of +ve charge on corneal retention of liposomes, presumably as a result of association with the polyionic corneal and conjunctival mucoglycoprotein. The corneal surface is saturable with liposomes, the half-life of clearance being 2 hr. This was confirmed by McCalden & Levy (1990) with liposomes containing benzyl-dimethyl stearyl ammonium chloride, but was extended to 4 hr. with liposomes containing dimethyl-dioctyl decyl ammonium chloride.

The targeting of liposomes to the corneal surface was first pursued by Megaw et al. (1981) using lecithin, an approach used later by Schaeffer & Krohn (1982). Immunoliposomes bearing antibody against cell surface viral glycoprotein have been suggested as targeting carriers in the treatment of ocular herpetic keratitis (Norley et al., 1986).

Liposomes have been used as vehicles for subconjunctival and intravitreal injection of both cytotoxic drugs and antibiotics. Because of a substantial reduction in the retinal toxicity of cytarabine in liposomal form, it has been suggested that this combination offers promise in the treatment of ocular proliferative disorders (Liu et al., 1989) as an alternative to fluorouracil, which is intrinsically less toxic. Liposomal fluorouracil produced significantly higher concentration of drug in the vitreous humour after bilateral intravitreal injection in rabbit eyes and to a lesser extent after subconjunctival injection when compared to drug injected in phosphate buffered saline (Fishman et al., 1989). Several liposome entrapped drugs have been studied to deliver the drug via this route (Rao et al., 1989; Peyman et al., 1989; Alvarado, 1990; Khoobehi et al., 1988, 1989,1990). Khoobehi et al., (1989) quantitated the delivery of liposome encapsulated cytosine arabinoside and 5-fluorouridine into eye following hyperthermia. The studies showed significant higher drug level in the treated versus the untreated eye without significant damage to ocular structure. Some of the liposomes encapsulated drugs studied are shown in Table 15.6.

Table 15.6. Liposomes encapsulated drugs studied for ophthalmic administration

Drug	*Results*	*References*
Idoxuridine	Improved efficacy of liposomes encapsulated drug in the treatment of herpes simplex keratitis.	Smolin et al., 1981
Triamcinolone acetonide	Observed significant higher conc. (>2 fold) of drug in ocular tissues compared with a suspension, for upto 5 hr.	Singh & Mezei, 1983
Dihydrostreptomycin	Liposomal encapsulation reduces drug penetration.	Singh & Mezei, 1984
Benzyl penicillin Indoxol	Ocular bioavailability enhanced by delivery in liposomes.	Schaeffer & Krohn, 1982
Adrenalin (epinephrine)	Observed 50% reduction in adrenalin absorption.	Stratford et al., 1983a
Inulin	Absorption greatly enhanced.	Lee et al., 1984; 1985
Benzyl-dimethyl stearyl ammonium chloride	Half-life of clearance was observed to be 2 hr.	McCalden & Levy, 1990
Dimethyl-dioctyldecyl ammonium chloride	Half-life of clearance extended to 4 hrs.	McCalden & Levy, 1990
Penicillin G	Flux was enhanced by +ve charged unilamellar liposomes.	Schaeffer & Krohn, 1982

The more detailed literature on liposomal technology are available in the spate of books (Gunstone, 1967; Ansell et al., 1973; Papahadjopoulos, 1978; Dingle & Jaques, 1979; Tom & Six, 1980; Gregoriadis & Allison, 1980; Gregoriadis, 1979; 1984; 1993; Gregoriadis et al., 1981; 1993; Knight, 1981; Leserman & Barbet, 1982; Nicolau & Parag, 1982; Ostro, 1983,1987; Bangham, 1983, Chapman, 1983; Schmidt, 1986; Yagi, 1986.; Shek & Barber, 1988; Shek, 1995).

CONCLUSIONS

Twenty five years of research into the use of liposomes in drug delivery have led to vastly improved technology in terms of drug capture, vesicle stability on storage, scale-up production and the design of formulations for special tasks. In parallel, remarkable advances have been made in understanding and controlling liposomes behavior *in vivo*. This has facilitated the application of a wide range of liposomal drugs in the treatment and prevention of diseases in experimental animals and clinically. In case of sterile production of liposomes, reproducibility of preparation, pyrogen content, method of sterilization and its impact on the stability of the product, integrity of lipid, potentiality cost, quality control methods and regulatory issues, as well as acute, subacute and chronic toxicity are seldom addressed. Further, the utility of liposome formulation technology at the floor of industry is to be explored by simplyfying, standardizing and optimizing methods of preparation and enhancing the storage stability of the product. The safety of liposome formulations must be specifically and categorically demonstrated. A comprehensive programme of relevent toxicity testing will be required before any liposome formulation is accepted for widespread clinical investigation.

The recent research is concentrated on the use of liposome to deliver hemoglobin and act as a red blood cell substitute. These liposome based red blood cells appear most promising as second generation delivery system. Another field where liposomes may find application in the industry is recombinant DNA technology for the synthesis of exotic chemicals, including drugs. Because, liposomes may transfer normally non-penetrating molecules into cells and organelles, they could be particularly useful for transferring fragments of genetic material from cell to cell. The scientists are also engaged in designing of liposomal prodrug, using principle of specific enzymatic cleavage and facilitated spontaneous hydrolysis.

Another field of liposomal research, which is not discussed in this chapter, is producing sterically stabilized liposomes (SSL) for prolonged circulation in blood stream. These SSL have ability to reduce *in vivo* recognition and phagocytic uptake, resulting in prolonged circulation and localization in tumors as well as other sites of pathology . This has no doubt restored their potential as a widely useful delivery system but has also created new challenges; the need of the agent associated with the liposome during incubation and distribution followed by control of its release for activity. The efforts at targeting and cell delivery of SSL are in infancy, but a rapid expansion of activities in this area can be expected. The pathological sites which can be approached for selective delivery, controlled by ligand targeting, must be addressed.

Yet another area that continuously demands significant efforts is designing the liposome which can be used for non-invasive route of administration. This will no doubt continue to contribute significantly to more efficient use of 'old' drugs with better and established therapeutic index vis a vis minimum side effects.

REFERENCES

Afione, S.A.; Conrad, C.K.; Flotte, T.R. (1995) Clin. Pharmacoki., 28:281.

Agrawal, K.; Bali, A.; Gupta, C.M. (1986) Biochim. Biophys. Acta, 856:36.

Ahmed, I.; Patton, T.F. (1985) Invest. Ophthalmol. Vis. Sci., 26:584.

Allison, A.C.; Gregoriadis, G. (1974) Nature, 252:252.

Alonso, A.; Saez, R.; Villena, A.; Goni, F.M. (1982) J. Membrane Biol., 67:55.

Alvarado, J.A. (1990) Trans. Am. Ophthalmol., 187:489.

Alving, C.R.; Richards, R.L.; Moss, J., Alving, L.I.; Clements, R.D.; Shiba, T.; Kotani, S.; Wirtz, R.A.; Hockmeyer, W.T. (1986) Vaccine, 4:166.

Alving, C.R.; Steck, E.A.; Hanson, W.L.; Loizeaux, P.S.; Chapman, W.L. Jr.; Waits, U.B. (1978) Life Sci., 22:1021.

Ambramson, M.B.; Katzman, R.; Gregor, H.P. (1964) J. Biol. Chem., 239:70.

Amselem, S.; Cohen, R.; Barenholz, Y. (1993) Chem. Phys. Lipids, 64:219.

Ansell, G.E.; Hawthorve, J.W.; Dawson, M. Eds., (1973) *Form and Function of Phospholipids*, Elsevier Scientific Publishing Co. Amsterdam.

Aoki, H.; Sun, C.; Fuji, K.; Miyajima, K. (1995) Int. J. Pharm., 115:183.

Arnardottir, H.B.; Sveinsson, S.L.; Kristmundsdottir, T. (1995) Int. J. pharm., 117:237.

Arrieta-Molero, J.A.; Aleck, K.; Sinha, M.K.; Brownscheidle, C.M.; Shapiro, L.J.; Sperling, M.A. (1982) Hormone Res., 16: 249.

Avila, J.L.; Rojas, M. (1990) Am. J. Trop. Med. Hyg., 43:52.

Bali, A.; Dhawan, S.; Gupta, C.M. (1983) FEBS Lett., 154:373.

Bangham, A.D., Ed., (1983) *Liposome Letters*, Academic Press, London.

Bangham, A.D.; Hill, M.V.; Miller, N.G. (1974) Methods Membr. Biol., 1:1.

Bangham, A.D.; Standish M.M.; Watkins, J.C. (1965) J. Mol. Biol., 13:238.

Barenholtz, Y.; Gibbes, D.; Litman, B.J.; Goll, J.; Thompson, T.E.; Carlson, F.D. (1977) Biochemistry, 16:2806.

Barenholtz, Y.; Amselem, S.; Lichtenberg, D. (1979) FEBS Lett., 99:210.

Bartlett, G.R.J. (1959) J. Biol. Chem., 234:466.

Batelle Memorial Inst. (1979) British patent Appl. No. 2001929A.

Batzri, S.; Korn, E.D. (1973) Biochim. Biophys. Acta, 298:1015.

Bergelson, L.D. (1979) Methods Membr. Biol., 9:275.

Bergers, J. J.; Ten Hagen, T.L.; Van etten, E.W.; Bakker-Woudenberg, I.A. (1995) Pharmacy World & Science, 17:1.

14

Niosomes as Drug Carriers

Prof. N. Udupa
College of Pharmaceutical Sciences, K.M.C., Manipal-676119, India

1. INTRODUCTION

Niosomes, the nonionic surfactant vesicles reported recently, can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials. It is reported to attain better stability than liposomes. It can prolong the circulation of the entrapped drugs. Because of the presence of nonionic surfactant with the lipid, there is better targeting of drugs to tumour, liver and brain. It may prove very useful for targeting the drug for treating cancer, parasitic, viral and other microbial diseases more effectively (Udupa & Pillai, 1991).

Non-phosholipid vesicular systems have been studied for many years and have largely involved investigation of dialkyl dimethyl ammonium salts and other ionic amphiphiles. Most of the amphiphiles are toxic and are unsuitable for use as drug carriers. (Kunitake & Okahata, 1976; Murakumi et al., 1979; Okahata et al., 1981).

Azmin et al. (1985) and Rogerson et al. (1988) used nonionic hydrophilic surfactants like Tween-80 for making niosomes entrapped with methotrexate and studied the pharmacokinetics of methotrexate after i.v. injection of niosomes to the mice. Chandraprakash et al., (1990, 1993a, 1993b) made nonionic surfactant vesicles using lipophilic surfactants like Span-40, Span-60 and Span 80 entrapped with methotrexate. The tissue distribution of methotrexate was improved after entrapping with niosomes. These vesicles were also found to be osmotically stable. Rogerson et al, (1988) and Baillie et al. (1984) have used niosomes as drug carriers for doxorubicin and sodium stilbogluconate for better targeting property.

Cook & Florence (1988) have shown that Tween 80 enhanced the cytotoxic effect of podophyllotoxin derivative, etoposide. Nonionic surfactants increased both fluidity and permeability of biological membranes. Chitnis et al., (1984) studied the effect of adriamycin dissolved in Tween 80 and compared its antitumour activity with adriamycin dissolved in water on mice bearing lymphocytic leukaemia and found that antitumour activity of adriamycin dissolved in aqueous solution of Tween 80 was higher than that of adriamycin dissolved in water (Baillie et al., 1984; Azmin et al., 1988).

Chandraprakash et al. (1990, 1993) could make niosomes of mean diameter of 4.5 μm using Spans and Tweens. The entrapment of methotrexate was better with Span 60 compared to Tween 80 which may be due to increased liphophilicity. The plasma levels of methotrexate were observed to be significantly higher with niosomes entrapped methotrexate injection, than when methotrexate was given at the same dose in the form of solution in normal saline in tumour bearing mice. The elimination of methotrexate from the plasma of mice bearing S-180 tumours was slower when given in the form of niosome entrapped methotrexate made with Span-60. A notable increase in the area under the plasma methotrexate concentration time curve could be noticed after the injection of niosome entrapped methotrexate as

compared to free methotrexate injection. There was a significant increase in the mean residence time (MRT) of methotrexate after the injection of niosome encapsulated drug. The apparent volume of methotrexate distribution was also decreased with niosome with methotrexate as compared to free methotrexate injection (Table 14.1). It was also observed that surfactant vesicles made with Span-60 entrapped with methotrexate were more effective for tumour regression as compared to plain methotrexate injection (Table 14.2) (Chandraprakash et al., 1993).

Table 14.1. Pharmacokinetic parameters of MTX in mice transplanted with S-180 tumours

Parameter	*Free Drug*	*Niosomal MTX*	*MDP + Niosomal MTX*
$[AUC]_0$ ($\mu gh^{-} mL^{-1}$)	2.17	64.57	202.23
$[AUMC]_0$ ($\mu gh^{-} mL^{-1}$)	54.74	11,464.76	22,989.64
MRT (h)	25.275	177.53	113.68
K_{ss} (h^{-1})	0.0396	0.0056	0.0088
Vd_{ss} (mL)	1.11	0.287	0.056
Cl (ml h^{-1})	0.0441	0.0016	0.0005

Table 14.2. Effect of niosomal encapsulation of MTX and macrophage activation on tumour volume

Days after tumour inoculation macrophage	MTV ± SD control	MTV ± SD commercial	1st group TVR (Nio MTX) after macrophage activation	2nd group MTV ± SD	2nd group TVR (Nio MTX)	3rd group MTV ± SD	3rd group TVR
	mm^3	mm^3	T/C	mm^3	T/C	mm^3	T/C
7	398±5.67	317±5.12	0.792	342±5.76	0.859	406±6.95	1.021
8	145±6.52					399±5.30	0.961
9	426±4.27	324±3.92	0.759	315±2.68	0.739	356±3.42	0.836
10	449±2.12					330±2.39	0.735
11	452±8.21	327±6.27	0.723	283±4.63	0.626	302±4.92	0.668
12	458±5.92					276±6.95	0.602
13	460±6.73	331±3.93	0.718	255±3.02	0.554	249±4.08	0.540
14	459±3.51					220±3.16	0.478
15	463±7.50	334±2.73	0.722	233±5.87	0.503	193±4.88	0.417
16	463±4.12					167±5.20	0.478
17	466±3.99	336±4.23	0.722	210±4.33	0.450	138±2.36	0.296
18	467±5.43					109±4.86	0.233
19	468±7.58	341±5.29	0.728	178±3.88	0.380	88±3.12	0.188
20	470±2.21					64±4.25	0.136
21	472±4.27	348±6.72	0.737	153±4.63	0.324	27±5.72	0.056
22							
23	478±4.21	350±4.76	0.733	125±6.16	0.261	-	-

(Contd.)

Days after tumour inoculation macrophage	MTV ± SD control	MTV ± SD commercial	1st group TVR (Nio MTX) after macrophage activation	2nd group MTV ± SD	2nd group TVR (Nio MTX)	3rd group MTV ± SD	3rd group TVR
	mm^3	mm^3	T/C	mm^3	T/C	mm^3	T/C
24							
25	482±7.95	347±4.59	0.721	58±4.71	0.119	-	-
26							
27	489±5.13	343±3.31	0.702	21±3.20	0.042	-	-
28							
29	494±6.37	340±2.21	0.668	-	-	-	-
30							
31	502±4.32	332±3.12	0.661	-	-	-	-
32							
33	559±4.21	326±4.72	0.583	-	-	-	-
34							
35	562±3.97	320±4.85	0.569	-	-	-	-

Niosomes can be exploited to attain different drug distribution and release characteristics with the help of surfactants. Azmin et al.,(1985) made niosomes of smaller size around 120nm but Rogerson et al.,(1988) made larger vesicles of 800-900 nm diameter. Earlier work in mice has suggested that niosomes may suffer uptake by the RES and methotrexate and sodium stilbogluconate were found to accumulate in liver following administration as niosome. The absence of accumulation of drugs in the liver supports the evidence of sustained plasma levels of drugs resulting from slow release of circulating rather than trapped vesicles. The larger niosomes may not accumulate in the liver or spleen and may be filtered out in the passage through the lung capillary network. In the treatment of parasitic infection of liver, spleen and bone narrow, niosomes made with surfactants may be very useful. (Table 14.3) (Chandraprakash, et al., 1990).

The cardiotoxicity of adriamycin may be reduced by administering it as niosomes made with surfactants without the loss of therapeutic efficacy. Surfactants like Tween-80 also increased brain level of methotrexate. Analgesic effect and brain level of D-Kyotorphen was enhanced by Tween-80. Nonionic surfactants like Triton-X, Triton-N, Brij and Tweens inhibited the motility of few microorganisms.

The handling and storage of the surfactants require no special conditions. Niosomes behave *in-vivo* like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. Inclusion of cholesterol in the preparation of niosomes has been demonstrated to alter the properties of niosomes and markedly decreasing the efflux of entrapped solid.

Non-ionic surfactant vesicles as vehicles for drug formulation may reduce the systemic toxicity of many anticancer and antiinfective drugs. Secondly, as carriers for enhanced delivery to specific cells niosomes may improve the therapeutic index by restricting drug effects to target cells. They are nontoxic and biodegradable. Many nonionic surfactants like Tweens and Spans, cationic surfactants like cetrimide, sodium dodecyl sulphate are used with cholesterol to entrap drugs in vesicles.

Liver can act as a depot for many drugs where niosomes containing drugs may be taken up and broken down by lysosomal lipase slowly to release the free drug and re-enter the circulation. Niosomes are slowly degraded providing a more sustained effect.

Niosomes are found to have selective drug delivery potential for cutaneous application of 5-alpha dihydrotestosterone, triamicinolone acetonide and intravenous administration of methotrexate for cancer treatment and sodium stilbogluconate in the treatment of leishmaniasis etc.

Table 14.3. Disposition of MTX in organs following i.v. administration (μg/mL) of organ homogenate

	Hours								
	1			2			4		
	Free MTX	*Nio MTX MDP*	*Nio MTX+*	*Free MTX*	*Nio MTX MDP*	*Nio MTX+*	*Free MTX*	*Nio MTX MDP*	*Nio MTX+*
Liver	4.449	5.590	4.480	0.280	5.802	5.137	0.117	5.756	3.957
Kidney	12.137	3.173	2.679	4.316	2.418	2.016	4.316	2.019	1.502
Spleen	4.438	12.354	10.129	2.547	11.473	10.674	1.442	9.648	7.823
Lung	3.423	0.769	1.467	1.304	0.713	1.208	0.683	0.633	1.065
Brain	0.046	0.142	0.232	0.075	0.185	0.239	0.052	0.161	0.127
Tumor	8.579	10.218	13.582	8.916	11.532	17.583	9.127	14.583	23.640
	Hours	8			12			24	
Liver	0.032	3.917	5.026	0.017	1.321	1.118	0.005	0.058	0.026
Kidney	3.429	1.591	1.079	2.015	1.263	0.897	0.812	0.867	0.385
Spleen	3.128	7.815	10.014	2.953	9.769	9.692	2.761	10.643	6.785
Lung	0.938	0.498	1.049	0.977	0.435	0.620	0.402	0.358	0.184
Brain	0.029	0.087	0.119	0.022	0.074	0.077	0.019	0.068	0.053
Tumor	10.581	18.269	30.586	10.928	25.216	40.283	11.282	40.125	50.149

2. METHODS OF PREPARATION, EVALUATION AND CHARACTERIZATION

Niosomes can be formulated by lipid layer hydration method, reverse phase evaporation techniques or by trans-membrane pH gradient uptake process (remote loading). Niosomes can be characterized by their size distribution studies (small niosomes: 100 nm -200nm, large niosomes: 800 nm-900nm, big niosome: 2 μm - 4 μm). Drug entrapment efficiency, drug stability, drug leakage in saline and plasma on storage, pharmacokinetic aspects, toxicity studies and drug targeting efficiency etc. are some of the other evaluation parameters.

The use of niosomes as drug delivery vehicles naturally assumes an ability to efficiently load the niosomal systems with the drug of choice. Passive trapping technique, and active trapping procedures (remote loading) are the two members employed for loading drug into niosomes. Selection of an encapulation protocol is largely dictated by concerns such as encapsulation efficiency, drug/lipid ratio drug retention, base I preparation, sterility, cost efficiency as well as drug stability.

2.1. Passive trapping techniques

Passive trapping procedures include almost all techniques that have been employed to entrap drugs in niosomal systems. Passive trapping means that the drug and the lipid are codispersed with a fraction of the drug being entrapped according to hydrophobic and electrostatic considerations. Entrapped hydrophilic drug will be associated with internal aqueous spaces, hydrophobic drug will partition primarily to the hydrocarbon region of the membrane and enhanced entrapment of charged drug partitioning into the lipid-water interface regions if lipids of opposite charge are present in the niosome bilayer.

Of the different passive trapping techniques, only the freeze thaw procedure can provide trapping efficiencies in the range of 90%, and that only when high lipid concentrations are used provided the agent to be entrapped should be highly water soluble. If not, it is difficult to achieve high drug/lipid ratios by this procedure. The SUV produced by French Press procedures, can exhibit trapping efficiencies upto 25%, but this value decreases for higher molecular weight compounds. Advantages include the lack of protein denaturation and longer retention times for entrapped solute.

Freezing and thawing SUV results in dramatic increase in vesicle size and trapped volume. This procedure can also be used to produce MLV exhibiting trapping efficiencies of 50% or more without affecting the activity of agents such as DNA and protein. Similarly, encapsulation efficiencies of 50% for MLV can also be obtained by dehydration-rehydration procedures. The advantages of this freeze-thaw and dehydration-rehydration are :

(i) large trapping efficiencies,
(ii) can be applied to large number of biologically active agents,
(iii) produce MLV that exhibit stable solute retention, characteristics.
(iv) are relatively simple,
(v) do not require organic solvents or detergents and
(vi) display extended storage stabilities in the frozen to dried state.

A negative point of these procedures is that niosomes formed are of heterogeneous size distributions. More homogeneous sized vesicles can be achieved by subsequently extruding the MLV dispersions through filters of various pore size under moderate pressures. The trapping efficiencies of these vesicles by extrusion techniques can be substantial, the vesicles display excellent retention of entrapped solute, degradation of protein and DNA is negligible, extrusion renders the preparation sterile (extruded through 0.2 μm) and scale up would appear straight forward.

Solvent evaporation technique employing ether (reverse phase evaporation) are useful for entrapping drugs in LUV. Lipids dissolved in ether are mixed with aqueous phase (containing the agent to be entrapped) and removal of the organic solvent by heat, by reduced pressure or by both, induces the spontaneous formation of LUV. This procedure yields trapping efficiencies upto 45% for a wide range of solvents. A disadvantage of solvent based liposomes is that some macromolecules are denatured by this procedure.

2.2. Active trapping techniques

An exciting recent development concerning loading of lipophilic cationic drugs into niosomes is the active trapping that can be achieved in response to ion gradients placed across niosomal membranes. This ability, which is also referred to as remote loading, allows drug entrapment after the niosomal carrier has been generated. The mechanism of drug uptake induced by pH gradients is probably similar to pH gradient dependent transmembrane redistribution of other weak bases. This procedure offers a superior methods for loading lipophilic cationic drugs into niosomes nearing 100% entrapment efficiency. In addition, it results in drug-lipid ratios which are significantly greater than possible by any other method. Besides being extremely cost efficient, this method of entrapment also decreases the rate of drug efflux from the vesicles by as much as 30 fold. The fact that this procedure entraps drug subsequent to formation of the liposome offers additional advantage for labile drugs when it may be important to encapsulate the agents immediately before use.

The properties of niosomes, their charge, chemical reactivity, stability and biological characteristics are determined by preparation processes and choices of chemical constituents. There are three major types of niosomes - multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV).

Multilamellar vesicles that result in increased trapped volumes and equilibrium solute distributors include hand shaken MLV with variations in lipid compositions, hydration from organic solvent, dehydration-rehydration procedure and freeze-thawing.

Small unilamellar vesicles are commonly produced by sonication and French Press procedures. Ultrasonic electrocapillary emulsification or solvent dilution techniques can be used to prepare SUV.

The injection of lipids solubilized in an organic solvent into an aqueous buffer can result in the spontaneous formation of LUV. But the better method that is widely accepted for generating LUV is by reverse-phase evaporation. LUV can also be formed by solubilizing lipid in an aqueous buffer that contains detergents.

Methotrexate was encapsulated in nonionic surfactant vesicles made with cholesterol, dicetyl phosphate and Span-60 by reverse phase evaporation techniques. Similarly, diclofenac sodium was encapsulated in niosomes containing Span-60 and Tween-60 and Poloxamer. Vincristine sulfate was encapsulated in niosomes made with Span-40 and cholesterol (1:1) and 90% entrapment could be achieved using transmembrane pH gradient (inside acidic) drug uptake (Parthasarthi et al., 1994).

3. NIOSOMES AS CARRIERS FOR TARGETING OF ANTI CANCER AND OTHER DRUGS

Niosome encapsulation of vincristine enhanced the antitumour activity against Ehrlich's ascites and Sarcoma-180 models. The niosomal/free drug mean survival time values as high as 1.89 were achieved in Ehrlichs ascites infected animals. Multiple doses of niosomal vincristine and methotrexate increased the survival rate in mice. Tumour volume doubling time of S-180 tumours increased significantly in niosomal drug treated animals. An increase in doubling time reflects the decreased rate of proliferation of sarcoma. Subsequent to macrophage activation using encapsulated muramyl dipeptide a more quantitative delivery of anti-cancer drugs to the tumour site could be achieved. Tumour drug level was increased after macrophage activation (Table 14.4, 14.5).

Table 14.4. Concentration of VCR in tumour at different time intervals after different treatments

Treatment	*Tumour drug accumulation ng/mL*						
	1h	*2h*	*4h*	*8h*	*12h*	*24h*	*48h*
FVCR	6.10	6.41	6.93	6.50	5.81	3.62	0.55
NVCR	10.34	11.20	12.48	13.01	13.32	12.26	5.71
MDP+NVCR	10.62	12.71	16.94	16.31	15.50	11.21	3.62

FVCR free vincristine
NVCR niosomal vincristine
MDP muramyl dipeptide

Table 14.5. Anti tumour activity of niosomal VCR subsequent to macrophage activation in BALB/c mice bearing Ehrlich's Ascites cells

S.No.	*Sample*	*Survival*	*Time (days)*	*ILS*
Gp I	Control	0/10	19.1	-
Gp II	NVCR	0/10	26.1	36.64
Gp III	NVCR + MDP	6/10	59.4	210.99[a,b]
Gp IV	NVCR + Tuftsin	0/10	44.9	135.07[a]

NVCR Niosomal Vincristine
MDP Muramyl dipeptide
Compared to control all the treatment are significant ($P<0.05$)
a: significant at $P<0.001$, when compared to only group II.
b: significant different from group IV at $p<0.01$.

Toxicological effects of anticancer drugs after entrapping in niosomes were compared with those of free drug. The 50% lethal dose of 2.8 mg/kg in BALB/c mice observed for free vincristine increased to 4.8 mg/kg with niosomal vincristine. Thirty day dose response survival studies in mice indicated that niosomal anticancer drugs were less toxic than free drugs (Table 14.6).

Table 14.6. Antitumor activity of multiple dose free and niosomal VCR in BALB/c mice bearing Ehrlichs Ascites cells

Sample	*Dose mg/kg*	*Survival 40 day*	*Time (days) mean*	*ILS*	*N/F*[a]
Free	-	0/06	19.0	-	-
VCR[b]	0.5	0/10	21.20	11.0	-
	0.75	0/10	21.6	11.0	-
	1.0	0/10	21.50	11.0	-
Niosomal VCR[b]	0.5	0/10	25.43	29.4[d]	1.16[c]
	1.0	0/10	37.60	93.20[d]	1.74[c]
	1.5	0/10	37.30	71.59[d]	1.54[c]
Control	-	0/06	19.0	-	-
Free VCR[e]	0.5	0/10	23.25	18.2	-
	0.75	0/10	22.9	18.2	-
	1.0	0/10	23.1	18.2	-
Niosomal VCR[e]	0.5	0/10	32.1	64.3[d]	1.38[c]
	1.0	1/10	39.1	101.5[d]	1.70[c]
	1.5	3/10	37.5	85.9[d]	1.57[c]

a: N/F, Niosomal/Free mean survival time
b: BALB/c mice were given injections (i.v.) of the indicated doses of drug 1, 3 and 5 i.p. injection of 1 million Ehrlichs cells per mouse.
c: Significant at $p<0.05$ level
d: Significantly different from control at $p<0.05$
e: BALB/c mice were given injections i.v. of the indicated doses of drug on days 1, 5 and 9 post i.p. injection of 1 million Ehrlichs cells per mouse.

Histopathological studies of skeletal muscle, spinal cord and aciatic nerve of niosomal vincristine treated rats confirmed the less toxic potential of the encapsulated vincristine. Unimpaired locomotor activity evident from roto-rod performance and unaltered gait patterns of niosomal vincristine treated rats further confirmed the safety profile of niosomal vincristine (Table 14.7). The suppression of peripheral and bone marrow WBC counts upon niosomal anticancer drug treatment of mice though slightly increased was not significant.

Subsequent to i.v. administration, the niosome encapsulated anticancer drugs were cleared from the plasma much more slowly than the free drug. A markedly enhanced plasma drug concentration was achieved in mice when it was administered in niosome (Fig. 14.1) Encapsulation of anticancer drugs also caused marked alteration in the tissue disposition of injected drugs. The tumour drug level enhanced significantly. This correlates well with the increased antitumour activity of niosomal anticancer drugs. The decreased drug levels in small intestine and skeletal muscle reflect the reduced gastrointestinal and myological toxicity of niosomal anticancer drugs. The decreased partitioning of niosomal vincristine to nonactive sites resulted in a significant amelioration of the toxic side effects, myological and intestinal impairment of drugs. A more quantitative delivery of anticancer drugs to tumour site is possible after macrophage activation. Use of niosomes as anticancer drug carrier will be certainly promising approach in cancer therapy in future.

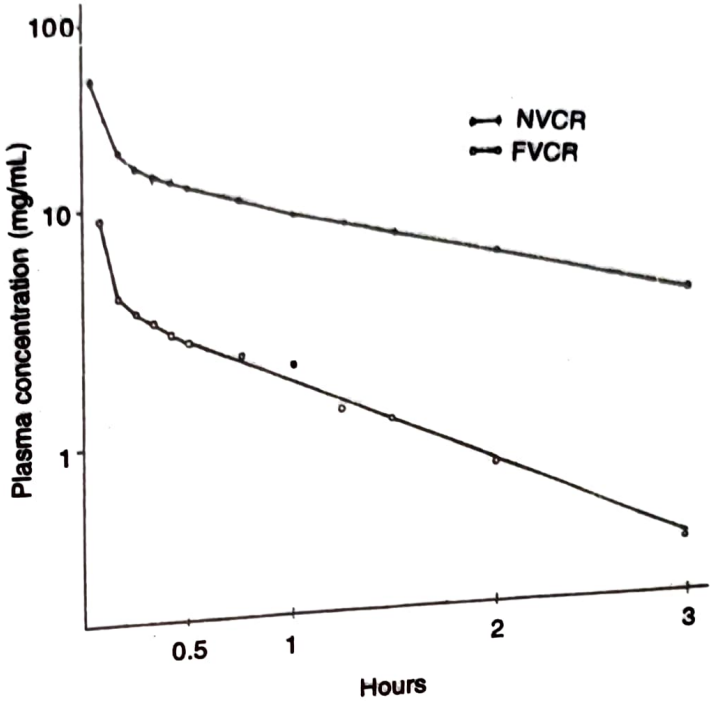

Fig. 14.1

Table 14.7. Neurotoxicity evaluation.

Passive avoidance data			
Time tested after	*Minutes spent in safe side ± S.E. (four trials per rat)* *Control*	*FVCR*	*NVCR*
2 minutes	291.93 ± 4.14	187.83 ± 29.17	268.33 ± 16.33
24 hours	259.95 ± 9.72	174.12 ± 34.82	233.77 ± 23.33
30 days	82.23 ± 6.56	56.65 ± 7.61	53.05 ± 5.54
Average nerve conduction and rotorod performance			
S.No	*Treatment*	*Average nerve conduction velocity (m/sec) + S.D. n=10*	*Average time spent on rotorod (sec) n=10*
Group I	Control	67.80 ± 9.08	> 300
Group II	FVCR	50.52 ± 2.90	30.6 ± 1.86a
Group III	NVCR	63.30 ± 8.95	242.00 ± 8.52b
Giat patterns of control and treated rats			
Group	*Width (mm)*	*Length (mm)*	*Sine of angle*
Control	29.82 ± 1.60[a]	142.7 + 4.10[a]	0.8076 + 0.026[a]
NVCR treated	29.10 ± 1.82[a,b]	137.63 ± 3.51[a,b]	0.8201 ± 0.073[a,b]
FVCR Treated	34.80 ± 2.28	125.81 ± 5.38	0.8708 ± 0.064

FVCR : Free Vincristine; NVCR : Niosomal Vincristine; a: significantly different from control ($p<0.05$); b: non-significant from control at $p< 0.05$

Intraperitoneal, transdermal and oral administration of niosomal diclofenac sodium resulted in better antiinflammatory activity compared to free drug administration. Thus, niosomal delivery of antinflammatory drug may be promising in the therapy of arthritis and other inflammatory conditions (Rajanaresh, et al. 1993, 1994).

4. IN VITRO AND IN VIVO EVALUATION AND CORRELATION

After i.v. administration, niosomes are first filtered and after passage through the lung distributed to the body, where they are cleared mainly by the liver and spleen, although most organs take up niosomes and their content to a limited extent. In general, large niosomes are cleared more readily than small ones. At the cellular level niosomes can interact with cells in at least four ways.

1. fusion of the outer bilayer of the niosome with the plasma membrane,
2. stable adsorption to the cell surface, either non-specific or via ligand,
3. transfer lipid molecules between the outer monolayer of niosome and cell without direct association of the two entities, and
4. endocytosis.

Many factors, including the size, chemical composition and surface charge of niosomes like liposomes, can alter their properties. Positive, negative or neutral charge that the niosomes carry on surface can alter their pattern of distribution in the body. The glycolipid composition of liposome can also alter their affinity for various tissues. Coating the outermost surface of niosomes with polysaccharide derivatives is another way for niosomes to be utilized as targetable drug carriers. Furthermore, depending on their structure, niosomes can be made to release entrapped drugs at increased temperature, thus increasing the potential for regional therapy because parts of the body can be made hyperthermic with various techniques. Because of the pH of the tumour base being low, niosomes were prepared that would release their content more efficiently at pH 5-6 rather than at physiological pH of 7.4 with the intent of releasing drug only at the specific site of tumors (Connor et al., 1986). The local release of drug can also be triggered by the enzymatic degradation of niosomal lipids. The efficacy of niosomal arabinoside-C was prolonged in the treatment of leukaemia (Connor et al., 1986). A number of cancer chemotherapeutic agents have been entrapped in liposomes and niosomes and tested for therapeutic activity including in some cases for toxicity in animal models. They include actinomycin D (Rahmann et al., 1974), adriamycin (Gregoriadis & Neerunjun, 1975a; Gregoriadis & Neerunjun, 1975b; Rahmann et al., 1978; Rahmann et al., 1980; Foresson et al., 1979; Olson et al., 1982; Storm et al., 1987), asparginase (Gregoriadis et al., 1977), BCNU, cisplatin (Kaledin et al et al., 1981), cytosine arabinoside (Mayhew et al., 1978; Rustum et al., 1979), 5-fluorouracil, mechlorethamine (Freise et al., 1979), melphalan, methotrexate (Kosloski et al., 1978; Patel, et al., 1982) etc.

The use of liposomes or niosomes in cancer chemotherapy can be attributed to the following properties.

(a) They prolong drug effect due to longer circulation time than with non-encapsulated drugs.
(b) They are sequestered as particles to the target tumour location.
(c) Toxicity is reduced.
(d) Drugs are protected from metabolism and immune attack until they reach their targets.
(e) They are confined to a chosen anatomical compartment.
(f) They are directed to target cells by attaching an antibody or other ligand.
(g) They are directed to their natural targets such as the phagocytic cells or liver, spleen and other organs.
(h) Therapeutic effects are amplified by incorporation of numerous drug molecules in each target directed particles.

(i) Selective local release from carrier is a function of physical factor such as the local temperature or pH.

(j) Permeability barriers are circumvented by endocytosis or fusion of carriers with cells.

(k) Delivery of drugs is designed to be active after endocytic uptake.

5. COMPARISON WITH OTHER DRUG DELIVERY SYSTEMS

Liposomes and niosomes have certain disadvantages, one of which relates to their chemical instability. The phospholipids used are more prone to oxidative degradation and hence they must be handled and stored in nitrogen atmosphere. The phospholipids used will interact with the serum components, the high density lipoproteins in particular. High density lipoproteins remove phospholipid molecules from lipid bilayer structure, an event which allegedly leads to their destruction resulting in the leakage of the entrapped drugs. It is highly unlikely that such leaky niosomes would turn out to be useful for any therapeutic or diagnostic applications if they reach their target site after loosing entrapped drug. They tend to fuse with one another on standing. This behavior can pose a serious constraint on the commercial manufacture. They are suitable for parenteral administration but oral administration is not possible because of inability of niosomes to survive to the action of bile salts and phospholipase. The cost and variable purity of natural phospholipids is another limiting factor. As compared to liposomes, about 50% of phospholipids can be replaced with nonionic surfactant in case of niosome preparation, the vesicle stability may be slightly improved. Due to presence of nonionic surfactants in niosomes, there may be improvement in the permeation and release of the drugs entrapped through various barriers of body and organs which may improve the targeting efficiency of the drugs. The drug targeting efficiency of niosomes may be improved using suitable surface modification with the help of many other adjuvants. Thus, niosomes may be better alternative carrier for various drugs compared to microspheres, liposomes and nanoparticles, with improved efficacy.

6. CONCLUSIONS - FUTURE PROSPECTS

There is lot of scope to encapsulate toxic anticancer drugs, antiinfective drugs, anti AIDS drugs, antiinflammatory drugs, anti-viral drugs, etc. in niosomes and to use them as promising drug carriers to achieve better bioavailability and targeting properties and for reducing the toxicity and side effects of the drugs. The ionic drugs carriers are relatively toxic and unsuitable whereas niosomal carriers are safer. In addition, handling and storage of niosomes require no special conditions. Vesicular drug carriers like niosomes can be transported by macrophages which are known to infiltrate tumour cells. It may be possible to take advantage of these activated macrophage system in delivering the antitumour agents within vesicles more quantitatively to tumour sites. So far only animal experimentation of this targeted drug delivery system is reported but further clinical investigations in human volunteers, pharmacological and toxicological investigations in animals and human volunteers may help to exploit niosomes as prosperous drug carriers for targeting drugs more efficiently, for treating cancer, infection and AIDS etc.

REFERENCES

Azmin, M.N.; Florence, A.T.; Handjani Vila, R.M.; Stuart, J.F.B.; Vanlerberghe.G.; Whittaker, J.S. (1985) *J. Pharm. Pharmacol.*, (Suppl) *J. Pharm. Pharmacol.* 37:237.

Baillie.A.J.; Florence, A.J.; Hume, L.R.; Murihead, G.T; Rogerson, A. (1984) *J. Pharm. Pharmacol*, 38:863. 36, 48.

Florence, A.T.; Hume, L.R.; Muirhead, G.T.; Rogerson, A. (1985) *J. Pharm. Pharmacol.*, 38:502.

A.J.; Coombs, G.H.; Dolan, T.F.; Laurie, J. (1986) *F.E.B.S. letters*, 38:863.

olz, Y.; Amselem, S.; Lichtenberg, D. (1979) *J. Urol.*, 135:612.

, J.B.; Anderson, R.U.; Tacker, J.R.(1986)