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Review

Lipophilic drug derivatives in liposomes

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Abstract

The drug molecules can be classified into four categories, i.e. highly hydrophilic, highly lipophilic, amphiphilic and those with biphasic insolubility. These are located differently in the liposomes and exhibit different entrapment and release behaviour. Problems like poor entrapment efficiency in addition to physical as well as chemical instability have been found to be associated with the liposomal entrapment of drug molecules other than those that are highly lipophilic. Therefore, a number of problem drugs have been synthesised into lipophilic derivatives and targeted to the phospholipid bilayer. Some other approaches have also been used for the purpose, which include ion pair formation and pharmacosomes. The present review discusses the advantages of incorporating drugs in the lipid domain of the vesicle. Taking examples of different drug classes, the success and limitations of the approach is discussed.

Keywords: Liposome; Lipophilic; Derivative; Prodrug; Ion-pair; Pharmacosome

1. Introduction

Liposomes are microscopic vesicles composed of one or more lipid layers that enclose aqueous compartments. Due to their high degree of biocompatibility, liposomes have been used as delivery systems for an assortment of molecules. They offer a substantial improvement in the therapeutic indices of the drug molecules entrapped in them. The first few commercial liposomal dosage forms are already in the market, e.g. AmBisome™ (amphotericin B), DaunoXome™ (daunorubicin citrate), and Doxil™ (doxorubicin). Clinical studies are underway for many others (Sharma and Sharma, 1997).

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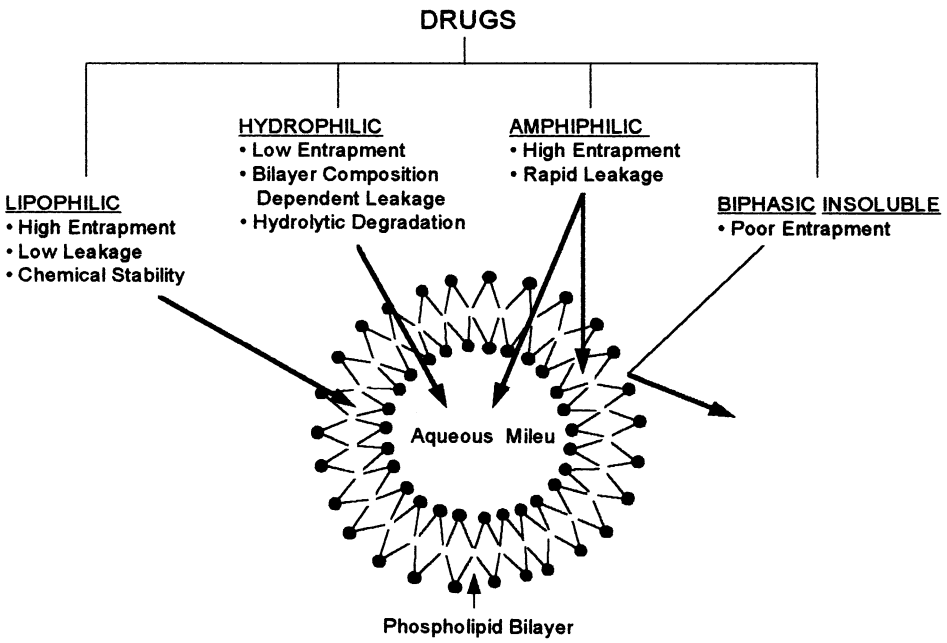


Fig. 1. Types of drugs and site of their incorporation into liposomal vesicle.

Liposomes, because of their biphasic character, can act as carriers for both lipophilic and hydrophilic drugs. Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties (Fig. 1). Based on these parameters, the drugs can be divided into four classes (1) highly hydrophilic, (2) highly lipophilic, (3) amphiphilic drugs that exhibit good biphasic solubility, and (4) drugs that exhibit biphasic insolubility.

Highly hydrophilic drugs with $\log \underline{P} < -0.3$, like cytosine arabinoside and CDP choline, are located exclusively in the aqueous compartments of the liposomes. The movement of such molecules across the liposomal membrane is dependent upon the composition of the bilayer (Mayhew et al., 1979; Fresta et al., 1993a). Highly lipophilic drugs, with $\log P_{oct} > 5$, are entrapped almost completely in the lipid bilayer of the liposomes, e.g. cyclosporin (Vadiei et al., 1989). Since they are very poorly soluble in water, problems like loss of entrapped drug on storage are minimal with this class of drugs. Drugs with intermediate partition coefficients, i.e. $1.7 < \log P_{oct} < 4$, pose a major problem because they partition easily between the lipid and aqueous phases and are very easily lost from the liposomes. Examples are mitomycin C (Sasaki et al., 1984), actinomycin D and vinblastine (Defrise-Quertain et al., 1984). Such molecules form stable liposomal systems only when they form complexes with the membrane lipids. However, the most problematic candidates for liposomal entrapment are the drug molecules which have poor biphasic solubility. Being insoluble in either aqueous or lipid phase, they show only a meager uptake by the liposomes. Typical examples include 6-mercaptopurine (Tsujii et al., 1976), azathioprine (Gulati et al., 1998b) and allopurinol (Philippot and Liautard, 1993).

Of all the above discussed type of liposomal candidates, the lipophilic drugs have been proved to be the best in the terms of cost, stability and utility. A large number of drug molecules belonging to the category of anticancer, antiviral, steroids and diagnostic aids with varying solubility behaviour have been structurally altered to render them more lipophilic in order to achieve their optimal liposomal formulation. The prepara-

tions are at different stages of development with several of them having already reached the stage of clinical trials. Those presently under clinical studies, for example, include liposomal muramyl tripeptide phosphatidyl ethanolamine (MTP-PE) (Gano and Kleinerman, 1995), liposomal neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane-platinum II (L-NDDP) (Perez-Soler et al., 1990; Chase et al., 1991), and liposomal annamycin (Wasan and Morton, 1996).

The topic of lipophilic prodrugs as a means to incorporate drugs into the lipid phase of liposomes was exhaustively discussed by Knight (1981). The author strongly advocated the design of lipophilic prodrugs for the ultimate success of liposomes as drug delivery systems. Since then, a considerable effort has been expended by the research community to get stable liposomal formulations. Apart from using the appropriate hydrophobic anchors, other approaches like complex-formation and pharmacosomes have also been utilised for incorporation of drugs into the lipid phase of liposomes. This review takes a comprehensive look into the developments that have taken place in this area in the last two decades.

2. Advantages of lipophilic modification

The conversion of drugs into lipophilic derivatives and their incorporation into liposomes offers advantages with respect to both improvement in formulation development and the modulation of drug response in-vivo.

2.1. Improvement in formulation development and processing

For the formulation of commercially viable liposomal delivery systems for drugs, the major problems that have been identified include high cost, poor physical and chemical stability, drug leakage, difficulty in sterilization, etc. Most of these problems can be overcome by the use of lipophilic modification of the solute molecules.

2.1.1. Enhancement of drug incorporation

Use of drugs that show encapsulation efficiency of $\approx 100\%$ in liposomes can reduce the amount of costly phospholipids, the basic raw materials for preparation of liposomes. In this regard, the lipophilic drugs carry an advantage that they are taken up in the liposomal bilayer upto their 'solubility' and hence are completely entrapped. Their chances of being lost in the continuous aqueous phase are negligible. Water soluble drugs in comparison show maximum encapsulation efficiency of approximately 70%, which represents their theoretical maximum at lipid concentrations even as high as 600 mmol/ml. This is because at such lipid concentrations, the encapsulation of the aqueous volume is only about 70%, with the remaining 30% representing the unentrapped volume left in the void space due to the curvature of liposomes (Betageri et al., 1993). Stuhne-Sekalec et al. (1986) investigated co-encapsulation of hydrophilic insulin and hydrophobic cyclosporine. It was observed that 2.3 nmol/mmol of insulin and 29.7 nmol/mmol of cyclosporin were entrapped in liposomes. The study hence is a good example showing that lipophilic substances are preferentially uptaken by liposomes as compared to the hydrophilic ones.

The encapsulation efficiency of hydrophilic or other drugs that show poor encapsulation can be improved by making them lipophilic through addition of hydrophobic side chains. A number of successful drug examples are there and a few of them are listed in Table 1. This approach seems to be applicable even in improving the encapsulation of biphasic insoluble drugs, like 6-mercaptopurine (6-MP). The water solubility of this drug is only 0.124 mg/ml. It is also poorly soluble in nonpolar solvents ($\log P = 0.72 \pm 0.01$) (Newton et al., 1982b). Accordingly, the liposomal entrapment of the drug is very small, ranging between 0.10–0.45% (Tsujii et al., 1976). A dramatic improvement in the encapsulation efficiency was observed when 6-(octadecyldithio)purine, its lipophilic prodrug, was subjected to liposomal entrapment. This novel prodrug is reported to be taken up quantitatively by the liposomes (Daniel et al., 1989).

Table 1
Typical examples showing the difference of liposomal encapsulation achieved from the lipophilic derivatives as compared to the drug itself

Drug	Encapsulation of drug (%)	Maximium encapsulation achieved from lipophilic drug derivative (%)	Reference
Asparaginase	12–50	72–100	Fishman and Citri, 1975; Jorge et al., 1994
Arabinosylcytosine	<0.10	99.80	Tokunaga et al., 1988d
5-Fluorouracil	0.03	99.96	Sasaki et al., 1987
Mitomycin	0.10	99.90	Tokunaga et al., 1988a
Triamcinolone acetonide	5.00	85.00	Goundalkar and Mezei, 1984

2.1.2. Ease of processing

During the processing of liposomes, the removal of non-encapsulated drug forms an essential step because the entire purpose of liposomal incorporation of the drug would be defeated if the unentrapped drug is present in the final product. In case the drug in question itself is expensive, the recovery of nonencapsulated drug becomes essential from the economics point of view. However, procedures such as dialysis and passage through exclusion columns which are employed for the removal of non-entrapped material are often time-consuming, tedious and expensive. It is hence best if this step can somehow be eliminated completely. In this respect use of hydrophobic drugs or lipophilic derivatives of drugs is particularly useful as they are quantitatively incorporated into the liposomes.

The use of lipophilic drugs or lipophilic drug derivatives is also important from the aspect of commercial processing of liposomal preparations. The first marketed liposomal preparation meant for parenteral use, i.e. AmBisome (amphotericin B), is supplied in the freeze-dried form (Sharma and Sharma, 1997). Lyophilization assumes importance in development of liposomal formulations, as it is an excellent method to overcome most of the stability problems associated with liposomes, like chemical instability (of phospholipids as well as drugs), leaching, fusion, aggregation, etc. Freeze drying, however, is not absolutely free from problems as it can itself cause damage to the liposomes in different ways: crystallization of the internal water, formation of amorphous material, influence of osmotic forces and dehydration of the lipid bilayers. All these factors can lead to defects in the integrity of the bilayer, thus resulting in the leakage of the material present in the internal aqueous compartment of liposomes. As the association between the liposome and the hydrophobic drug is more stable, the osmotic shock or brief ultrasonication will only release a small fraction of the encapsulated drug. Thus, with lipophilic drugs which are associated with the lipid bilayer, excellent entrapment efficiencies are achievable on rehydration of the lyophilized powder. A typical example is cyclosporine, where 95% encapsulation efficiency was observed 24 h after reconstitution of the freeze-dried powder (Vadiei et al., 1989).

There is another widely employed approach to formulate liposomes in a dry powder form. The phospholipids as solid mixture with an inert water soluble carrier like lactose and sodium chloride form proliposomes on rehydration immediately prior to use. While with water soluble drugs, one can expect usual problems like low entrapment and difficulty in the removal of non-encapsulated drug, the approach ideally suits hydrophobic drugs where the majority of the drug partitions into the lipid membrane and high encapsulation efficiencies can be achieved on hydration. Many lipophilic drugs have been successfully formulated into their proliposomal powders e.g. amphotericin B (Payne et al., 1986) and ibuprofen (Katare et al., 1990). The method has also been used to

Table 2
Typical examples showing comparison of percent drug retained in liposomes containing drugs and their prodrugs

Drug	Prodrug	Time	Percent of drug retained		Reference
			Drug	Prodrug	
Cortisol	Cortisol palmitate	3 days	12.0	71.0	Shaw et al., 1976
5-Fluorouracil	Octadecylcarbamoyl-5-fluorouracil	30 min	3.0	99.9	Sasaki et al., 1987; Fresta et al., 1993b
Mitomycin C	Nonyloxycarbonyl-mitomycin C	1 h	12.8	99.9	Sasaki et al., 1984

prepare liposomal delivery system of MTP-PE, a lipophilic prodrug of MDP. The formulation has been put to clinical trials (van Hoogevest and Frankhauser, 1989).

2.1.3. Sterilization

The liposomal preparations meant for parenteral administration must be sterile. Among the various methods of sterilization, γ-ray treatment has been found to disrupt the liposome membrane (Lanzini et al., 1984). Freeze dried liposomes can be sterilized by exposure to ethylene oxide (Ratz et al., 1989), but the residues of the incompletely removed sterilizing gas and/or contamination caused by it can be toxic (Betageri et al., 1993). The sterilization by filtration can be used for SUVs of size < 0.22 mm in diameter, however, it cannot be used for larger liposomes (Betageri et al., 1993).

Heat sterilization has been used as a convenient alternative for certain types of MLVs and extruded liposomes without much damage to their integrity (Kikuchi et al., 1991). The leakage of various types of drugs on autoclaving was studied by Zuidam et al. (1993). A pronounced leakage was observed with a water soluble compound, calcein. The same behaviour was seen with doxorubicin, an amphipathic compound. The lipophilic bilayer associated prodrug of the latter, *N*-trifluoroacetyl doxorubicin-14-valerate, however, was retained in the liposomes after autoclaving and the liposomes were stable with no aggregation. Similarly, lipophilic perfluoroalkylated bipyridine platinum and palladium complexes in egg phospholipid liposomes showed complete retention after autoclaving (Garelli and Vierling, 1992).

The hydrophobic drugs or the lipophilic drug derivatives thus carry an advantage over water soluble or amphiphilic compounds even with respect to sterilization of liposomal preparations.

2.1.4. Improvement of stability

The liposomal preparations must be stable for a period of 1.5–2 years to be of any clinical utility. The stability problems encountered with liposomes include leakage of entrapped drug, change in liposome structure and the chemical instability of phospholipids and entrapped drug. All these stability problems are more pronounced with liposomes containing low molecular weight, water soluble drugs. On storage as a suspension, there is inevitably a marked loss of hydrophilic drugs from the liposomes, e.g. in case of 5-fluorouracil (5-FU), 15–35% of the encapsulated drug was found to leach out within 6–7 h of storage (Fresta et al., 1993b). On the other hand, leakage of the highly lipophilic drug, tacrolimus was found to be negligible over a period of 40 h (Lee et al., 1995).

There are several drugs known where conversion of a polar drug to its lipophilic prodrug and subsequent incorporation into liposomes has proved to be effective in preventing the leaching of the drug. One such example is cytosine arabinoside. The liposomes containing this drug lost 40–70% of the incorporated drug within 24 h depending upon the concentration of cholesterol in the bilayer (Mayhew et al., 1979). On the other hand, its lipophilic derivatives showed no leakage from liposomes containing comparable amounts of cholesterol till a period of 30 days (Tokunaga et al., 1988d). More such examples are listed in Table 2.

The effect of lipophilicity of a prodrug on its in-vitro leakage from liposomal preparations has been shown very explicitly by Perez-Soler and Khokhar (1992) for various *cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum compounds. The drug leakage from liposomes containing these lipophilic prodrugs of cisplatin was found to be inversely related to the size of the leaving groups. The compounds with 5, 6, 7, 9 and 10 C-atoms showed drug leakage in a decreasing order of 25.9, 9.7, 11.9, 6.0 and 5.0%, respectively in 6 h.

The use of lipophilic drugs or derivatives has also been shown to be useful in providing protection against hydrolytic decomposition of the drugs. A water soluble drug, when present in the aqueous compartment of liposomes, apart from its tendency to rapidly leach out, is also prone to hydrolytic attack. The hydrophobic drugs/derivatives in comparison are located in the phospholipid bilayers and show resistance to hydrolysis. An interesting case is that of campothecin, an antitumor drug, which is hydrophobic but contains a lactone ring which rapidly hydrolyzes to an inactive carboxylate form. The drug exhibits high binding affinity (97%) in liposomes and its lactone functionality partitions into the bilayer (Burke et al., 1992). By virtue of this, no ring opening was observed for 72 h in liposomes which were composed of phospholipids with low transition temperatures, i.e. DMPC ($T_c \approx 24$°C), which is in its unstable liquid crystal phase at 37°C. There was a significant improvement comparing the 1/2 life of free campothecin which is just 16.6 min (Burke et al., 1993). In a separate study, topotecan, a water soluble analogue of campothecin, when entrapped in liposomes made of DSPC ($T_c \approx 55$°C), which is in the more stable gel phase at 37°C, showed a loss of about 20% of its lactone fraction within 30 h (Burke and Gao, 1994).

The improvement in drug stability on incorporation of lipophilic drug derivatives in liposomes has been observed for several drugs, e.g. cortisone (Arrowsmith et al., 1983c), doxorubicin (Bekers et al., 1989), and cisplatin (Garelli and Vierling, 1992).

2.2. Modulation of drug response in biological milieu

The entrapment of lipophilic drugs or derivatives in lipid bilayers of liposomes alters the biological activity of the drug in a number of ways. Such compounds are released from the liposomes in the biological fluids at slower rates as compared to their water soluble counterparts, resulting in a sustained action of the drug. Moreover, the metabolism of the drugs to their inactive metabolites is slowed down resulting in a longer duration of action.

Inside the body, the biodistribution of the drug is altered when its lipophilic derivatives are used in their liposomal formulation. The therapeutic activity is reported to be enhanced resulting in better therapeutic index. Even the toxic effects normally associated with the therapeutic doses of the drug are removed or reduced in some cases. There are many reports available where the use of liposomal formulations containing lipophilic drug derivatives have succeeded in overcoming the cross-resistance, particularly in the case of antitumor drugs.

2.2.1. Effect on rate of release

It was shown by Juliano et al. (1978) that release of lipophilic material from disrupted liposomes is delayed, but the release of hydrophilic materials is immediate. The lipophilic prodrugs, because of their association with the lipid bilayers, remain entrapped when in contact with the biological fluid and have a potentially longer life in-vivo as compared to their water soluble counterparts which show relatively fast leakage out of the lipid vesicles. Therefore, protection of the drug against metabolic degradation is improved by lipophilic prodrugs and longer lasting therapeutic drug levels can be achieved, e.g. when the stability of *N*-palmitoyl daunorubicin was studied in 10% new-born calf serum for 5 days, no leakage from liposomes was observed (Bard et al., 1982), while more than 10% of the drug was found to leak out in 2 h in buffer medium when the unconverted drug was encapsulated.

In another case, it was found that the stability of cortisone hexadecanoate in the aspirated, cell-

free human rheumatoid synovial fluid was much more as compared to cortisone octanoate, as the association of the latter was not very strong (Arrowsmith et al., 1983c). It is suggested by the authors that even after the liposome integrity is lost in the biological medium, cortisone hexadecanoate remains adhered to the liposomal fragments, thus resulting in prolonged stability.

A lipophilic prodrug of 5-FU, octadecylcarbamoyl-5-FU, when injected intramuscularly as a liposomal formulation was found to be retained at the injection site and in the regional lymph nodes for a considerably longer periods of time as compared to the liposomal formulations of 5-FU (Sasaki et al., 1987). This behaviour is suitable for lymphotropic delivery of the drug to prevent lymph node metastasis in cancer chemotherapy (Tsuruo et al., 1980).

A similar behaviour was observed for mitomycin-C (MMC). After an intramuscular injection of liposomal MMC formulation into the thigh muscle of rats, the drug was rapidly absorbed from the injection site and little drug was found after 30 min (Sasaki et al., 1983a). In comparison, nonyloxycarbonyl derivative of MMC was retained much longer at the site of injection and more than 70% of the dose remained as the prodrug in muscle even 120 min post injection. Moreover, the lymphotropic retention of the liposomal formulation of the lipophilic prodrug was found to be much more as compared to that of the original water soluble drug.

N^4-hexadecyl-1-β-D-arabinofuranosyl cytosine (NHAC), a lipophilic prodrug of 1-β-D-arabinofuranosyl cytosine (Ara-C), in its liposomal formulation was found to be almost completely resistant to deamination in plasma as compared to Ara-C. The formation of Ara-U, the major metabolite of Ara-C, was found to be approximately 42-folds lower when NHAC was formulated into liposomes as compared to the free drug (Horber et al., 1995b).

2.2.2. Altered biodistribution and increase in therapeutic index

The most outstanding and widely studied example of alteration in the biodistribution of liposomal formulation of a drug on its conversion to its lipophilic derivatives is that of doxorubicin and its hydrophobic analog, annamycin. It has been established that when doxorubicin is encapsulated in the aqueous phase of liposomes, its plasma clearance is markedly reduced, distribution into RES is enhanced, and heart drug levels are reduced (Rahman et al., 1990). These changes in biodistribution and tumor targeting are further enhanced when the drug is encapsulated in stealth liposomes composed of phospholipids with high transition temperatures (Gabizon, 1992). However, in case of annamycin, which distributes into the lipid bilayer of liposomes, the stealth liposomes fail to exhibit any tumor targeting advantage as compared to the regular liposomes (Zou et al., 1995). This is explained on the basis of the difference in the driving forces that regulate the bodily disposition of the hydrophilic and lipophilic compounds incorporated into the aqueous phase and lipid bilayers of liposomes, respectively. The bilayer of stealth liposomes is relatively impermeable to the water soluble doxorubicin leading to its longer retention and higher tumor selectivity. Similar behaviour has also been observed for other water soluble antitumor drugs like Ara-C (Allen et al., 1992). However, in case of annamycin, its high affinity for lipid membranes is expected to be an important driving force for its bodily distribution. In a recent study, it was demonstrated that alterations in plasma HDL lipid compositions and HDL structure influences the plasma distribution of annamycin (Wasan et al., 1997). Therefore, the stealth bilayer may not effect its tendency to partition into plasma constituents, blood cells and endothelial cells, etc. and therefore, the pharmacokinetics is not altered much.

Few direct comparisons of in-vivo studies of the liposomal drugs with their liposomal lipophilic derivatives are known. This is expected, because the preparation of the lipophilic derivatives for the purpose of liposomal entrapment has been taken up only in the cases where the liposomal entrapment of the drug molecule, as such, has not proved practical due to various formulation problems. Therefore, in most cases the comparisons have been made with the free drug in its solution form.

The liposomal incorporation of lipophilic prodrug of L-asparaginase brought about a marked increase in the circulation time (MRT, 32 h) as compared with the free prodrug (MRT, 4 h) or the unmodified drug (MRT, 2.9 h) (Jorge et al., 1994).

In case of pindolol, the pharmacosomes prepared from the lipophilic diglyceride derivative of the drug, enhanced the plasma concentrations of the drug 3–5 folds higher as compared to its free form (Vaizoglu and Speiser, 1986). Also, the administration of pharmacosomes was found to result in a lower renal clearance.

This altered biodistribution appears to be the key factor responsible for an increase in the efficacy and a decrease in the toxic effect. NDDP, encapsulated in liposomes has proved to be more active than cisplatin in murine models of experimental liver metastasis and melanoma (Li et al., 1995). Moreover, the in-vitro toxicities of liposomal NDDP were markedly less as compared to those with cisplatin. While the most prominent side effect of cisplatin is severe renal dysfunction, L-NDDP was found to be devoid of any nephrotoxicity, the dose limiting side effect being myelosuppression. This difference in toxicity profile reflects the difference in biodistribution of the two formulations.

The entrapment of various water soluble boron salts into liposomes was found to be successful in tumor targeting (Feakes et al., 1994). However, due to their low entrapment efficiency, lipophilic boron compounds were synthesized and incorporated into liposomes (Feakes et al., 1995). In a unique study, liposomes containing both water soluble salts and the lipophilic compounds in the aqueous phase and lipid bilayer, respectively, were prepared. The biodistribution study showed this combined formulation to be successful in delivering the highest tumor boron concentrations.

Phospholipid analogs of an antiviral drug, AZT, on their incorporation into liposomes exhibited an altered biodistribution showing increased drug levels in liver, spleen and lymph nodes as compared to the free drug (Hostetler et al., 1994a). All these organs being important sites of viral replication, an increase in the antiviral effect was observed with the liposomal formulations of these lipophilic analogs. In a comparative study, a single intraperitoneal injection of liposomal formulation was found to prevent increased spleen weight and reverse transcriptase levels in serum, comparable to that of AZT given continuously in water (Hostetler et al., 1994b).

While studying the mechanism of antiarthiritic activity of phospholipid conjugates of methotrexate (MTX) in their liposomal formulation (MTX-LIPO), it was found that in human blood monocytes, MTX-LIPO strongly inhibited the release of both IL-1-β and TNF, whereas free MTX or empty liposomes failed to show any such effect (Williams et al., 1994). The difference in the course of action was also evident from the fact that free drug showed anti-inflammatory activity if treatment was started on the day of arthiritis induction, but was ineffective in established arthiritis, while MTX-LIPO was ineffective if dosing was started on day 0, but exerted a significant effect on established arthiritis. The hemotoxic effect of MTX was reduced on incorporation of its lipophilic derivatives into liposomes (Williams et al., 1995a).

2.2.3. Overcoming of cross-resistance

The lipophilic prodrugs entrapped in liposomes have also been successful in overcoming the resistance to the original drug. Such an example is explicitly illustrated in the case of Ara-C. A major reason for its treatment failure in leukemia patients is the resistance developed to the drug. The possible mechanisms of Ara-C resistance that have been proposed include low levels of deoxycytidine kinase G (Chu and Fisher, 1965), decrease in number of nucleoside transport sites (Wiley et al., 1982) and a decreased catabolism by cytidine deaminase (Steuart and Burke, 1971). The cytotoxicity of NHAC, the lipophilic prodrug of the drug, was found to be independent of both the nucleoside transporter mechanism and the deoxycytidine kinase activity (Horber et al., 1995b). In Ara-C resistant cells, liposomal NHAC was found to be cytotoxic requiring drug concentration only 1.6-folds higher than the sensitive cells. This behaviour is attributed to the difference in mechanism of cytotoxic action of NHAC and Ara-C. The cytotoxicity of Ara-C is known to be *S*-phase

specific (Gorczyca et al., 1993), NHAC in comparison exhibits no phase specific toxicity at therapeutic concentrations (Horber et al., 1995b).

NDDP, a lipophilic analog of cisplatin, is also shown to overcome cross resistance when delivered in liposomes (Perez-Soler et al., 1988). In in-vitro studies, the lipo-NDDP preparation displayed a significant antitumor effect on human colon carcinoma LoVo cells resistant to cisplatin. Subsequently, a similar effect was seen in-vivo against L-1210/PDD leukemia resistant to cisplatin (Perez-Soler et al., 1989).

Another significant example is that of lipophilic MTX derivative, MTX-γ-DMPE. Liposomes containing MTX in their aqueous compartments were found to partially inhibit the growth of MTX resistant human leukemia cells (CEM/MTX), but only if the cells were exposed to fresh liposomes every day. On the other hand, liposomes containing MTX-γ-DMPE inserted in the lipid bilayer completely by-passed the resistance without recourse to repeated addition of the liposomes (Kinsky et al., 1986). These observations could be explained on the basis of the mechanism of cellular resistance to MTX. The CEM cells become resistant to MTX due to either defective transport or elevated levels of dihydrofolate reductase. The liposomes apparently enter the target cells as a consequence of phagocytosis and not via the MTX transport system and are thus able to overcome both types of resistance.

3. Specific examples of lipophilic transformation of drugs

3.1. Analogues and prodrugs

3.1.1. Anticancer agents

The organ distribution, cellular distribution, cellular uptake and hence the spectrum of activity of the antitumor drugs is largely determined by their lipophilicity. It is, therefore, advantageous to prepare lipophilic analogues of this class of drugs. The lipophilic derivatives are, however, associated with a disadvantage that being water insoluble they cannot be easily presented for intravenous administration. The approach of preparation of lipophilic derivatives and their incorporation into liposomes as a vehicle for intravenous administration has been employed for various antitumor compounds. Examples include cisplatin, anthracyclin antibiotics, cytosine arabinoside, muramyl dipeptide, 5-fluorouracil, etc.

3.1.1.1. Cisplatin. The development of lipophilic analogues of cisplatin was taken up by the group of Perez-Soler in late 1980s. In initial studies, lipophilic cisplatin derivatives, containing platinum complexed to carboxyalkyl, carboxycycloalkyl and to carboxyneoalkyl groups in the range of 5–15 C-atoms were prepared (Khokhar et al., 1989). The liposomal entrapment efficiency of $>80\%$ was observed for this group. The entrapment was correlated directly to the number of C-atoms in the hydrophobic side chain, with compounds containing more than nine C-atoms showing encapsulation efficiencies of $>95\%$ (Perez-Soler et al., 1993). In in-vivo studies, unexpectedly no antitumour activity was seen for the prodrugs, either when in free form or when encapsulated in liposomes composed of dimyristoylphosphatidylcholine (DMPC). Interestingly, however, the activity in-vivo was found to be directly related to the amount of dimyristoylphosphatidylglycerol (DMPG) in the bilayer. The critical role of DMPG was attributed to the formation of drug-DMPG complex. Eventually, based on the study of the antitumor activity of NDDP (Fig. 2) entrapped in liposomes composed of varying ratios of DMPC and DMPG, an active drug-DMPG complex was proposed containing one or two DMPG molecules as side chains (Perez-Soler and Khokhar, 1992).

L-NDDP, the liposomal prodrug-phospholipid complex, was found to be significantly more cyto-

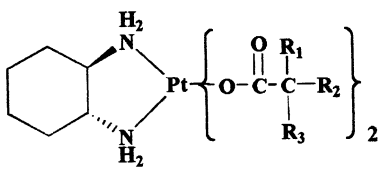


Fig. 2. Structure of NDDP. R_1, R_2, R_3, can be a group of 2–6 carbon atoms to yield a radical with $C_{10}H_{19}O_2$ as an emperical formula.

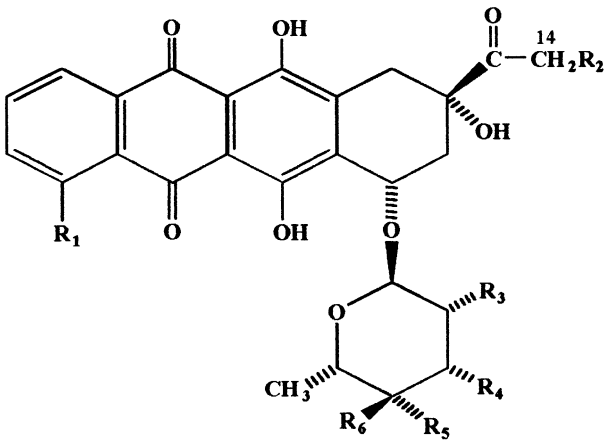
toxic than free NDDP and cisplatin against tumor cell lines sensitive and resistant to cisplatin (Perez-Soler et al., 1988). In in-vivo studies, it was as active as cisplatin against L-1210 leukemia and more active against M-5076 reticulosarcoma (Perez-Soler et al., 1987). In toxicology studies in mice and dogs, L-NDDP proved to be less toxic than cisplatin. The main side effect of L-NDDP was myelosuppression unlike cisplatin which shows severe renal dysfunction.

Studies on the effectiveness of NDDP formulation in conventional liposomes are also reported. The preparation proved to be effective in murine models of experimental liver metastases, attributed to the avid uptake by liver. For treatment of solid tumors outside the liver, NDDP was also formulated in long-circulating liposomes (Li et al., 1995; Mori et al., 1996) composed of phosphatidylcholine (PC), cholesterol, and polyethylene glycol (PEG) conjugated to phosphatidylethanolamine (PE). These were found to localise preferentially in melanomas in mice. The in-vivo toxicities were significantly less than those for cisplatin. On treatment of the tumor with local hyperthermia, after the injection of long-circulating liposomes, the tumor uptake of NDDP was increased by 60% and its tumor inhibitory effect was also found to improve significantly

In a separate study, perfluoroalkylated bipyridine platinum and palladium complexes were encapsulated into liposomes and were found to be remarkably stable in terms of both physical and chemical stability (Garelli and Vierling, 1992). A high fluorophilic character combined with long hydrocarbon spacer gave good incorporation efficiency which was further improved by the introduction of a double bond between the perfluoroalkyl chain and hydrocarbon spacer.

3.1.1.2. Anthracyclin antibiotics. A massive amount of work has been done on the development of liposomal preparations of anthracyclin antibiotics, especially doxorubicin (Gabizon et al., 1989; Rahman et al., 1990). Doxorubicin is very effective for the treatment of acute leukemia, lymphoma, breast carcinoma, osteosarcoma and soft tissue sarcomas (Tan et al., 1973), but its use is severely limited due to its serious side effects, including acute myelosuppression and chronic cumulative cardiotoxicity (Benjamin et al., 1974; Rinehart et al., 1974). On liposomal delivery, reduced cardiotoxicity and increased activity against tumors were observed. The drug, however, being amphiphilic in nature presents formulation problems, as it tends to partition between lipid bilayers and aqueous compartments of liposomes resulting in suboptimal entrapment and significant leakage with time. Rahman and co-workers (Treat et al., 1989; Rahman et al., 1990) observed an entrapment efficiency of 45–55% in liposomes composed of cardiolipin/PC/cholesterol/stearylamine. An efficiency of 60–80% was obtained by Gabizon et al. (1989) in liposomes composed of egg PC/egg derived PG/cholesterol/D-α-tocopherol succinate. On the other hand, the pH driven active drug loading technique was successful in yielding 100% liposomal incorporation of doxorubicin. However, doubts have been expressed on the wide and daily use of the procedure requiring reconstitution of the lyophilized powder with isotonic saline and addition of sodium carbonate solution of pH 10.8–12.0, followed by subsequent heating of the preparation in-situ at 60°C (Creaven et al., 1990).

As an alternate solution to the formulation problem of anthracyclines, Perez-Soler et al. (1993) worked upon the approach of synthesizing different lipophilic analogues of doxorubicin. The compounds were incorporated into MLVs and based on the structure-liposome entrapment relationship studies, 2-iodo-3′-hydroxy-4′-epi-4-demethoxy-doxorubicin (Annamycin) was selected for biological testing. Liposomal annamycin proved to be more active than doxorubicin in L-1210 leukemia and M-5076 reticulosarcoma in mice (Perez-Soler and Priebe, 1990). Also, it was found to be non-cross-resistant in-vitro (Ling et al., 1993a,b) and in-vivo (Zou et al., 1994) in a number of non-resistant and multi-drug-resistant cancer cells. The therapeutic index of annamycin was shown to be dependent on the liposomal size, with SUVs showing better anticancer activity than the large liposomes (Zou et al., 1995). Unlike doxorubicin, annamycin did not show any enhancement in therapeutic effect when encapsulated in long circulating non-leaky liposomes (Zou et al., 1995). Plasma distribution of liposomal annamycin is



Compound	R_1	R_2	R_3	R_4	R_5	R_6
Doxorubicin	OMe	OH	H	NH_2	OH	H
Annamycin	H	OH	I	OH	H	OH
Hydroxyrubicin	OMe	OH	H	OH	OH	H
14-*O*-palmitoyl hydroxyrubicin	OMe	$OCO(CH_2)_{14}CH_3$	H	OH	OH	H
Daunorubicin	OMe	H	H	NH_2	OH	H
N-octanoyl daunorubicin	OMe	H	H	$NHCO(CH_2)_6CH_3$	OH	H
N-palmitoyl daunorubicin	OMe	H	H	$NHCO(CH_2)_{14}CH_3$	OH	H

Fig. 3. Lipophilic prodrugs of doxorubicin, hydroxyrubicin and daunorubicin.

currently being studied (Wasan and Perez-Soler, 1995; Wasan and Morton, 1996).

Bekers et al. (1989) synthesised another highly lipophilic prodrug of doxorubicin, *N*-trifluoroacetyladriamycin-14-valerate, and incorporated it into liposomes. The formulation was found to exhibit both physical as well as chemical stability for a period of 13 weeks at 4°C. The prodrug, when incorporated into long circulating liposomes and immunoliposomes, did not effect either the circulation time or the targetability of the liposomes (Mori et al., 1993).

The effect of lipophilicity on the thermotropic behaviour of DPPC liposomes containing *N*-alkyl derivatives of adriamycin and adriamycin-14-valerate was studied by Constantinidis et al. (1989). The reduction in transition temperature was found to be directly related to the lipophilicity of the alkyl derivatives.

Studies have also been reported on preparation of lipophilic prodrugs of other anthracyclin antibiotics—hydroxyrubicin, daunorubicin, and mitoxantrone. A lipophilic prodrug of hydroxyrubicin, 14-*O*-palmitoyl hydroxyrubicin, showed an entrapment efficiency of $>99\%$ and was stable to leaching for a period of 14 days (Perez-Soler and Priebe, 1992). In comparison to doxorubicin, it showed an enhanced antitumor effect in mice against L-1210 leukemia and M-5076 reticulosarcoma. In case of daunorubicin, its *N*-acyl derivatives with chain length of 2, 4, 8 and 16 C-atoms were prepared and incorporated into liposomes (Bard et al., 1982). While the first two poorly entrapped into the liposomes, *N*-octanoyl and *N*-palmitoyl derivatives showed good entrapment efficiency and the liposomes were found to be highly stable. The liposomal prodrugs were more toxic than the free drug when tested against L-929 cells and also their mode of action was found to be different (Bard et al., 1982). The liposomal incorporation, pharmacokinetic properties, acute toxicity and antitumor activity of a cytostatic complex of mitoxantrone with a lipophilic acid were studied by Schwendener and coworkers (Schwendener, 1990; Schwendener et al., 1991).

The structures of various important liopophilic derivatives of anthracyclin antibiotics are given in Fig. 3.

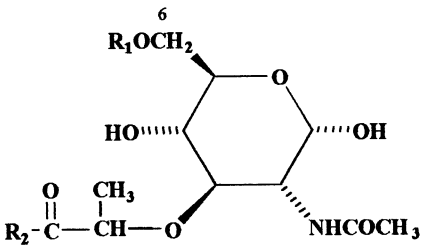
3.1.1.3. Muramyl dipeptide derivatives. The activation of the macrophages to make them tumoricidal through the use of an immunostimulant agent such as muramyl dipeptide (MDP) is one amongst the numerous approaches being employed in cancer research (Fidler, 1986). The in-vivo activation of macrophages by MDP was not very successful in the initial studies as the activator was rapidly cleared from the system, within 60 min after injection (Parant et al., 1979). To decrease the clearance rate, MDP was incorporated into liposomes (Fidler et al., 1981). The liposomal delivery proved useful and the macrophage activation and tumoricidal activity was seen both in-vitro and in-vivo. To further prolong the intracellular residence time, lipophilic derivatives of MDP were prepared (Lopez-Berestein et al., 1983; Phillips et al., 1987). This was also done to decrease leakage of the water soluble peptide from liposomes.

Out of the various derivatives, muramyl tripeptide phosphatidylethanolamine (MTP-PE) worked out to be promising analogue as it could be incorporated into multilamellar liposomes with a high degree of efficiency (Schroit and Fidler, 1982; Fidler, 1986). This synthetic acyl derivative of MDP in liposomes showed a 100-fold enhancement of activity of murine macrophages (Fidler et al., 1982; Fidler, 1986) and human monocytes (Kleinerman et al., 1983). It was also found to give good response in eradication of malignant and highly spontaneous tumors in a variety of animal models (Fidler et al., 1982; Kleinerman et al., 1983, 1995; Talmadge et al., 1986; Mac Ewen et al., 1994; Goldbach et al., 1996). The effect of L-MTP-PE in humans was first studied by Murray et al. (1989), who conducted phase I trial in 28 metastatic cancer patients employing a stable reproducible preparation developed by Ciba-Geigy, Basel (Switzerland) (van Hoogevest and Frankhauser, 1989). Although the preparation expectedly did not give significant antitumor activity, because systemic macrophage activation is effective when the disease is in a minimal state (Fidler, 1988), a clear evidence was provided on in-situ activation of tumoricidal properties (Murray et al., 1989; Sculier et al., 1993; Favaro et al., 1995). Other salient findings were that liposomal preparation of MTP-PE was safe at the dosage schedule used and that the optimal biological dose of MTP-PE was below that recorded for the maximum tolerated dose. A similar finding of the absence of objective antitumoral effect and macrophage activation was made by Sculier et al. (1993) in a pilot study on 11 lung cancer patients.

Apart from MTP-PE, the other lipophilic prodrugs of MDP that were put to investigation based on their satisfactory liposomal encapsulation are MDP-L-alanylcholesterol (Phillips et al., 1985), glycerol dipalmitate derivatives of MDP, *n*-Bu ester of MDP and *N*-acetylmuramyl-D-alanyl-D-isoglutamine (Phillips et al., 1987). These compounds also showed immunomodulating activity. More recently, another lipophilic derivative of MDP, i.e. B30-MDP has been put to investigations (Ando et al., 1995, 1996) but the studies are still at the stage of investigation of chemical stability of the compound in mixed liposomal vesicles.

The structures of MTP-PE and other derivatives of MDP are given in Fig. 4.

3.1.1.4. 1-β-D-Arabinofuranosylcytosine (Ara-C). Ara-C is an effective antimetabolite which is useful in acute myelogenous leukemia (Keating et al., 1982). Its utility is, however, limited by its rapid deamination to an inactive metabolite, 1-β-D-arabinofuranosyluracil (Ho and Frei, 1971). To avoid or delay the enzymatic deamination, different approaches have been employed which include synthesis of its N^4-derivatives (Kanai and Ichino, 1974), concomitant administration of deaminase inhibitors (Kreis et al., 1991), and incorporation of drug into liposomes (Mayhew et al., 1976). The liposomal encapsulation, apart from providing protection against fast enzymatic degradation, was found to enhance the antitumor activity of the drug, which is attributed to the depot function of the liposomes. It was, however, observed that being water soluble, Ara-C was incorporated into the aqueous compartment of the liposomes from where it leaked out very fast resulting in very low entrapment efficiency (Parker et al., 1982). To overcome this problem, a lipophilic prodrug of Ara-C namely, N^4-(*N*-(cholesteryloxycarbon-



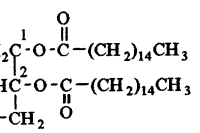
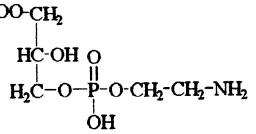
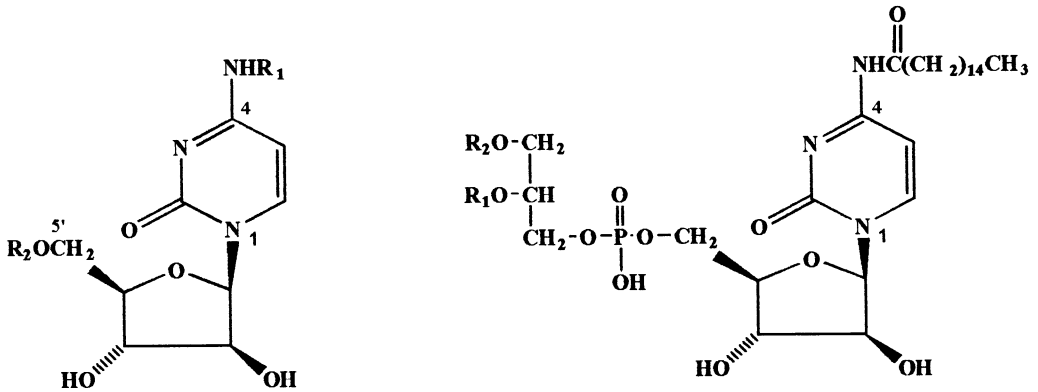
Compound	R_1	R_2
N-acetylmuramyl-L-alanyl-D-isoglutamine (*N*-acetylmuramyl dipeptide, MDP)	H	L-Ala-D-isoGln
6-*O*-(1,2-dipalmitoyl-sn-glyceryl)-MDP		L-Ala-D-isoGln
6-*O*-(1,2-dipalmitoyl-sn-glyceryl)-MDP-n-Bu ester	-do-	L-Ala-D-isoGln-$COOC_4H_9$
6-*O*-(1,2-dipalmitoyl-sn-glyceryl)-*N*-acetylmuramyl-D-alanyl-D-isoglutamine	-do-	D-Ala-D-isoGln
6-O-(2-tetradecylhexadecanoyl)-MDP (B30-MDP)	$COCH(CH_2)_{13}CH_3$	L-Ala-D-isoGln
N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine (Muramyl tripeptide, MTP)	H	L-Ala-D-isoGln-L-Ala
N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanylcholesterol (MDP-L-alanylcholesterol, MTP-cholesterol)	H	L-Ala-D-isoGln-L-Ala-$COOC_{27}H_{45}$
N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanylphosphatidylethanolamine (MTP-phosphatidylethanolamine, MTP-PE)	H	

Fig. 4. MDP, B30-MDP, MTP-PE and other lipophilic derivatives of MDP

yl)glycyl)-Ara-C (COCG-Ara-C), was synthesized by Tokunaga et al. (1988d). The prodrug showed an increased lipophilicity and almost complete entrapment into liposomes. It had a superior antitumor activity against murine L-1210 leukemia as compared to Ara-C. The prodrug bearing liposomes were more efficient than Ara-C in inhibition of growth of human lung adenocarcinoma A 549 xenograft implanted under the renal capsule, showing potential usefulness in cancer chemotherapy.

Rubas et al. (1986), synthesized different lipophilic acyl derivatives of Ara-C, namely N^4- and 5′-oleyl-1-β-D-arabinofuranosyl cytosine (N^4- and 5′-oleyl-Ara-C) and N^4-palmitoyl-1-β-D-arabinofuranosylcytosine (N^4-palm-Ara-C). Encapsulation efficiency in the range of 85–97% could be obtained with these prodrugs. The in-vivo antitumor activity of these liposomal prodrugs against L-1210 leukemia and B16 melanoma was found to be superior to that of the pure drug. Liposomes containing N^4-oleyl-Ara-C were targeted by means of biotinylated antibodies and were found to show selectivity towards the cells that were recognized by these antibodies (Schott et al., 1988). In a phase I/II study, the resistance of N^4-oleyl-Ara-C to enzymatic degradation to Ara-U was considered to be insufficient (Schwendener



Compound	R_1	R_2
1-β-*D*-arabinofuranosyl-cytosine (Ara-C)	H	H
N^4-palmitoyl-Ara-C	$CO(CH_2)_{14}CH_3$	H
N^4-oleoyl-Ara-C	$CO(CH_2)_7CH=CH(CH_2)_7CH_3$	H
5'-oleoyl-Ara-C	H	$CO(CH_2)_7CH=CH(CH_2)_7CH_3$
N^4-hexadecyl-Ara-C (NHAC)	$(CH_2)_{15}CH_3$	H
N^4-[*N*-(cholesteryloxy-carbonyl)glycyl]-Ara-C (COCG-Ara-C)	$COCH_2NHCO-OC_{27}H_{45}$	H

Compound	R_1	R_2
Phospholipid-N^4-palmitoyl-1-β-*D*-Ara-C conjugates	$CO(CH_2)_{14}CH_3$	$CO(CH_2)_{14}CH_3$
	$CO(CH_2)_{17}CH_3$	$CO(CH_2)_{17}CH_3$
	$CO(CH_2)_{14}CH_3$	$CO(CH_2)_{17}CH_3$

Fig. 5. Lipophilic derivatives and phospholipid conjugates of Ara-C

et al., 1989). Therefore, an alkyl lipophilic derivative of Ara-C, i.e. N^4-hexadecyl-1-β-D-arabinofuranosylcytosine (NHAC) was synthesized and entrapped into liposomes (Schwendener and Schott, 1992). The liposomal preparation of NHAC was shown to exert stronger antitumor activity than Ara-C in the murine L-1210 leukemia even at single dose schedules (Schwendener and Schott, 1992; Schwendener et al., 1995). NHAC, in its liposomal form, was shown to exhibit altered cellular pharmacology in various cell-lines like HL-60, K-562 and U-937 (Horber et al., 1995a,b). This was attributed to its highly lipophilic nature. The liposomal preparation was also shown to overcome Ara-C resistance, resulting in a 23-fold lower 50% inhibitory concentration as compared to Ara-C in Ara-C resistant H-60 cells (Horber et al., 1995c).

In a recent study, N^4-palm-Ara-C was conjugated to phospholipid and the conjugates in liposomes were found to exhibit significantly higher antitumor activity than N^4-palm-Ara-C or Ara-C (Schott and Schwendener, 1996b). Lately, the synthesis of NHAC-phospholipid conjugates and structure-activity studies in-vivo on liposomal N^4-palm-Ara-C and NHAC conjugates have been reported (Schott and Schwendener, 1996a).

Fig. 5 shows the structures of various prodrugs and phospholipid conjugates of Ara-C.

3.1.1.5. 5-Fluorouracil (5-FU). 5-FU, a widely used antitumor agent shows serious side effects like gastrointestinal problems and bone marrow toxicity (Chabner, 1982). To raise its therapeutic index, numerous attempts have been made to develop its liposomal delivery systems (Ozer and Talsma, 1989; Fresta et al., 1993b). Because of its low lipophilicity, encapsulation efficiency beyond

10% could not be achieved even after optimising the lipid composition and preparation procedures. A considerable leakage of the drug, about 15–35%, from the liposomal preparation was observed within 6–7 h of storage (Fresta et al., 1993b).

The lipophilic prodrugs of 5-FU have been prepared and incorporated into liposomes. Sasaki et al. (1987) prepared four alkylcarbamoyl derivatives of 5-FU, viz. butyl, hexyl, octyl and octadecylcarbamoyl-5-FU and incorporated them into liposomes. Degree of incorporation in liposomes was found to be proportional to the lipophilicities of the prodrugs. The most lipophilic derivative, octadecylcarbamoyl 5-FU (C18FU) was found to be the optimal liposomal drug as it showed the slowest release and highest antitumor activity in its liposomal form against L-1210 leukemia. In another study, a lipophilic prodrug with a cholesterol promoeity, cholesteryl-5-(5-fluorouracilcarbamoyl)capronate (ChFU) was synthesized and compared with C18FU (Hashida et al., 1988). Though ChFU and C18FU showed almost complete incorporation into liposomes, the release of 5-FU from liposomal formulation of C18FU was slower as compared to that of ChFU. The liposomal formulation of C18FU also exhibited superior in-vivo antitumor activity than that of ChFU.

Similarly, pentyl-5-fluorouracil-1-acetate (PFA) and hexyl-5-fluorouracil-1-acetate (HFA) were synthesised and incorporated into proliposomes (Jee et al., 1995). The cytotoxicity, stability, antitumor activity, in-vivo pharmacokinetics and tissue distribution have been investigated (Jee et al., 1995; Lee and Jee, 1996a,b).

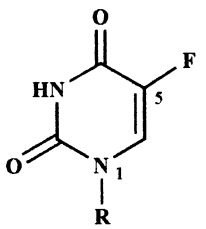
Reports also exist on the preparation and activity of lipophilic prodrugs of 5-fluoro-2′-deoxyuridine (5-FUdR), a highly cytotoxic metabolite of 5-FU (Schwendener et al., 1984, 1985; Supersaxo et al., 1988; van Borssum et al., 1992, 1993; Mori et al., 1995). The prodrugs, 3′-*O*-palmitoyl-5-FUdR, 5′-*O*-palmitoyl-5-FUdR and 3′,5′-*O*-dipalmitoyl-5-FUdR, were incorporated into liposomes of various compositions (Schwendener et al., 1984). Both were quantitatively entrapped. The activity of these preparations against L-1210 leukemia was not very significant, but they proved to be about 10 times more effective than the parent drug in solid tumors like colon 38 and mamma carcinoma 13/C. The in-vivo distribution of dipalmitoyl derivative was strongly dependent upon liposomal composition and drug/lipid ratio (van Borssum et al., 1992). However, lipid composition had little effect on the therapeutic activity when tested against a number of murine models like P388 leukemia, Lewis lung carcinoma, B16 melanoma and C26 adenocarcinoma. The liposomal preparation of the dipalmitoyl prodrug showed antitumor activity at 100–600 times lower doses than the free drug.

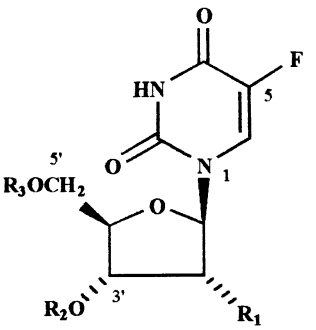
Similar studies have also been reported on 5-fluorouridine (5-FUR). A phospholipid analogue of 5-FUR, dipalmitoylphosphatidylfluorouridine (DPPF), was incorporated into RES avoiding liposomes (Doi et al., 1994). The long-circulating liposomal preparation of DPPF was found to be effective in reducing tumors and prolonging survival time as compared to free DPPF or DPPF in conventional liposomes. Recently, a report has described the antitumor activity of liposomes and immunoliposomes containing 5′-palmitoyl- and 5′-succinyl-5-FUR (Crosasso et al., 1997). The immunoliposomal preparation containing 5′-palmitoyl-5-FUR was found to posses the best antitumoral activity when injected i.p. in athymic mice grafted with human HT-29 cell line.

The structures of prodrugs of 5-FU, 5-FUdR and 5-FUR are listed in Fig. 6.

3.1.1.6. Mitomycin C (MMC). MMC, like some other antitumor agents, is amphiphilic in nature. It shows very little entrapment into liposomes (Sasaki et al., 1984). Moreover, the entrapped drug leaks out to an extent of 87.2% out of the liposomes within 1 h.

In the earliest studies, five lipophilic 1a-*N*-substituted derivatives of MMC, possessing an aromatic promoeity with different linkage structures, i.e. benzyl, benzoyl, benzylcarbonyl, benzyloxycarbonyl and benzyloxymethyl groups were synthesized (Sasaki et al., 1983b). These prodrugs exhibited characteristic antitumor activities after being regenerated to the parent drug. Among the five linkage structures, alkoxycarbonyl type linkage was found to be the best because of its





Compound	R
5-Fluorouracil (5-FU)	H
Cholesteryl 5-(5-FU-carbamoyl)capronate (ChFU)	$CONH(CH_2)_5COO-C_{27}H_{45}$
Octadecylcarbamoyl-5-FU (C18FU)	$CONH(CH2)_{17}CH_3$
Pentyl 5-FU-1-acetate	$CH_2COO(CH_2)_4CH_3$
Hexyl 5-FU-1-acetate	$CH_2COO(CH_2)_5CH_3$

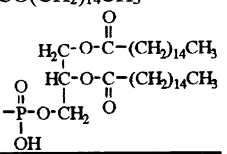
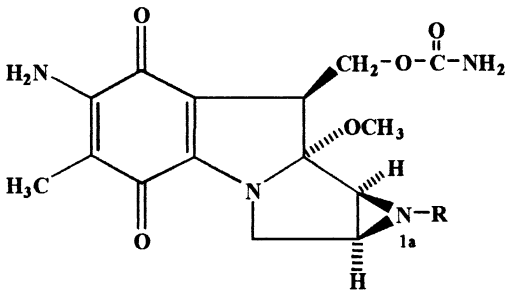
Compound	R_1	R_2	R_3
5-Fluoro-2'-deoxyuridine (5-FUdR)	H	H	H
3'-*O*-palmitoyl-5-FUdR	H	$CO(CH_2)_{14}CH_3$	H
5'-*O*-palmitoyl-5-FUdR	H	H	$CO(CH_2)_{14}CH_3$
3',5'-*O*-dipalmitoyl-5-FUdR	H	$CO(CH_2)_{14}CH_3$	$CO(CH_2)_{14}CH_3$
5-Fluorouridine (5-FUR)	OH	H	H
5'-*O*-succinyl-5-FUR	OH	H	$COCH_2CH_2COOH$
5'-*O*-palmitoyl-5-FUR	OH	H	$CO(CH_2)_{14}CH_3$
Dipalmitoylphosphatidyl fluorouridine (DPPF)	OH	H	

Fig. 6. Structures of derivatives of 5-FU, 5-FUdR and 5-FUR

chemical stability and biological lability (Sasaki et al., 1983c). Therefore, more alkoxycarbonyl type prodrugs with different promoieties were synthesized (Sasaki et al., 1983a). These derivatives showed an increase in lipophilicity with an increase in the length of the side chain. They were also found to possess significant antitumor activity and were biolabile. These lipophilic prodrugs were then formulated into liposomes (Sasaki et al., 1984). Based on their lipophilic character, all the compounds were efficiently entrapped into liposomes. Almost complete incorporation was achieved in the case of nonyloxycarbonyl and cholesteroyloxycarbonyl-MMC, the compounds with the highest oct/water partition coefficient. The in-vitro release of the drug was also found to be negligible for these two compounds. However, antitumor activity against L-1210 leukemia was seen in all alkoxycarbonyl type of derivatives except cholesteroyloxycarbonyl-MMC. This exception could be attributed to its failure to undergo regeneration to MMC in buffer as well as biological media. The nonyloxycarbonyl prodrug resulted in sustained release of drug from the injection site in the rat thigh muscle and showed a longer stay in the lymph nodes (Sasaki et al., 1985b). On the whole, however, these alkoxycarbonyl derivatives were shown subsequently to be rapidly released in in-vitro studies on addition of rat plasma to buffer (Sasaki et al., 1985a). Eventually, the behaviour was also confirmed in-vivo with prodrugs getting rapidly removed from the circulation (Sasaki et al., 1985b).

As a solution to the problems with the alkoxycarbonyl derivatives, 1a-*N*-stearoyl MMC and six 1a-*N*-substituted derivatives of MMC possessing the cholesterol moiety with different spacers, viz. cholesteryloxycarbonyl, *N*-(cholesteryloxycarbonyl)glycyl, cholesteryloxyacetyl, *N*-(cholesteryloxycarbonyl)-4-aminomethylbenzoyl, *N*-(cholestery-



Compound	R
Mitomycin C	H
1a-*N*-stearoyl-MMC	$CO(CH_2)_{16}CH_3$
Nonyloxycarbonyl-MMC	$COO(CH_2)_8CH_3$
Cholesteryloxyacetyl-MMC	$COCH_2OC_{27}H_{45}$
Cholesteryloxycarbonyl-MMC	$COOC_{27}H_{45}$
N-(Cholesteryloxycarbonyl) glycyl-MMC (COCG-MMC)	$COCH_2NHCOOC_{27}H_{45}$
N-(Cholesteryloxycarbonyl)-4-aminomethylbenzoyl-MMC	$COPhCH_2NHCOOC_{27}H_{45}$
N-(Cholesteryloxycarbonyl)-4-aminophenylacetyl-MMC	$COCH_2PhNHCOOC_{27}H_{45}$
3-(Cholesteryloxycarbonyl) propanoyl-MMC	$COCH_2CH_2COOC_{27}H_{45}$

Fig. 7. Mitomycin C and its lipophilic deivatives

loxycarbonyl)-4-aminophenylacetyl and 3-(cholesteryloxycarbonyl)propanoyl-MMC, were synthesized (Tokunaga et al., 1988a). All these lipophilic prodrugs showed an almost quantitative entrapment into liposomes. The susceptibility of the compounds to hydrolysis was found to be dependent on the spacer structure. Some prodrugs like cholesteryloxyacetyl-MMC were converted to MMC by chemical hydrolysis while others, like cholesteryloxycarbonyl-MMC and *N*-(cholesteryloxycarbonyl)-4-aminophenyl acetyl-MMC, did not regenerate MMC even in rat serum. Entrapment of the prodrugs into liposomes provided stability against both chemical and enzymatic hydrolysis. The prodrugs with cholesteryl moiety were found to be associated more firmly with liposomes in circulation than stearoyl MMC. Based on this report, liposomal formulations of *N*-(cholesteryloxycarbonyl)glycyl-MMC (COCG-MMC) and cholesteryloxyacetyl-MMC were studied in-vivo in mice (Tokunaga et al., 1988b). Sustained blood levels of MMC could be achieved by the use of both the preparations. Liposomes containing COCG-MMC showed less activity and less toxicity as compared to the parent drug when tested against P-388 leukemia, colon 26 adenocarcinoma and human mammary carcinoma MX-1 (Tokunaga et al., 1988c).

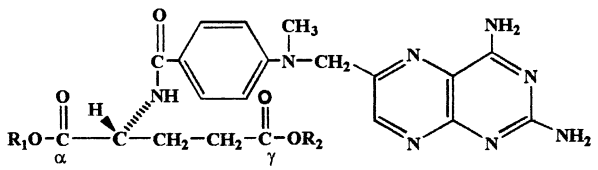
Fig. 7 gives the structures of various MMC lipophilic derivatives.

3.1.1.7. Methotrexate (MTX). Methotrexate, a potent cytotoxic agent, inhibits cell-growth by inhibiting dihydrofolate reductase. The drug penetrates the cells via the folate transport system and results in generalized non-specific cytotoxicity. There are a number of reports available in literature where MTX has been encapsulated in the aqueous compartments of antibody bearing liposomes (Singh et al., 1989, 1991, 1996) to target the drug to the cancer tissue. The liposomal drug is proposed to enter the cells by endocytosis of the liposomes (Leserman et al., 1981).

However, the incorporation of this water soluble drug is accompanied by problems like low entrapment, low stability and in-vivo leakage

leading to non-specific cellular toxicity. Encapsulation efficiency of MTX in liposomes of various compositions using charged lipids, cholesterol and α-tocopherol was studied. The maximum encapsulation efficiency achieved was only 42% (Kim and Han, 1995). Its lipophilic derivatives were hence synthesized. MTX was coupled through its free carboxylic functions to phosphatidylethanolamine (PE-NH_2) to yield three MTX derivatives of dimyristoylphosphatidylethanolamine, i.e. MTX-γ-DMPE, MTX-α-DMPE and MTX-α,γ-diDMPE and their corresponding glycerophosphorylethanolamine (glycero-PE) analogs (Hashimoto et al., 1985a).

The incorporation of these phospholipid associated drugs (Fig. 8) was found to be practically quantitative in contrast to the low encapsulation of the water soluble parent drug (Noe et al., 1988). The liposomes containing phospholipid



Compound	R_1	R_2
Methotrexate (MTX)	OH	OH
MTX-α-dimyristoylphosphatidyl-ethanolamine (MTX-α-DMPE)	DMPE*	OH
MTX-γ-DMPE	OH	DMPE
MTX-α,γ-diDMPE	DMPE	DMPE
MTX-α-glycerophosphoryl-ethanolamine (MTX-α-glyceroPE)	GPE*	OH
MTX-γ–glyceroPE	OH	GPE
MTX-α,γ-diglyceroPE	GPE	GPE

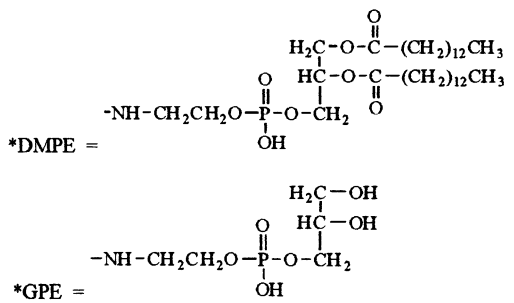


Fig. 8. Structure of MTX lipophilic derivatives

analogues were more resistant than conventional liposomes to repeated cycles of freezing and thawing. The growth inhibitory effect of MTX-liposomes as well as of free MTX was reversible by either thiamin pyrophosphate (Tpp) or N^5-formyltetrahydrofolate (F-THF), while the effect due to MTX-DMPE liposomes was reversed only by F-THF. This ability of Tpp which competes with MTX for entry into cells, to protect against MTX-liposomes, but not MTX-DMPE liposomes, confirms that the former type of liposomes can leak their contents, leading to inhibitory effects on non-targeted cells (Noe et al., 1988). Of the three MTX-DMPE derivatives and their glycero-PE analogues, MTX-γ-DMPE and its glycero-PE analogue proved to be the most effective in inhibiting cell proliferation (Hashimoto et al., 1985b). They were, however, less effective than free MTX. This has been attributed to the fact that free MTX enters the cells by folate transport system, while liposomal MTX-DMPE is taken up by phagocytosis of the liposomes. Liposomes containing MTX-γ-DMPE were shown to be equally effective against human leukemia cells that were sensitive or resistant to MTX. The resistant cells were not significantly effected by the water soluble glycero-PE analogues (Kinsky et al., 1986). Subsequently, a few more phospholipid analogues, namely MTX-γ-dihexadecylphosphatidylethanolamine and MTX-γ-hexadecylphosphatidylethanolamine were synthesized (Kinsky et al., 1987). Both these derivatives, when incorporated into liposomes proved to be much less effective than liposomal MTX-γ-DMPE. It was suggested that these derivatives are not hydrolyzed by phospholipases and are, therefore, unable to revert back to MTX.

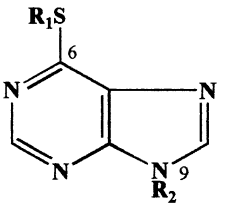
In a separate study, protein adsorption characteristics of liposomes containing MTX covalently linked to liposome phospholipids were studied in relation to their cytotoxic activity (Hernandez et al., 1990).

The liposomal preparations of the above discussed DMPE derivatives of MTX, viz. MTX-γ-DMPE, MTX-α-DMPE and MTX-α,γ-diDMPE, were evaluated by Williams et al. (1992) for an entirely new use—as antiarthritic agents. In an ex-vivo study, the macrophages from MTX-LIPO

(liposomal methotrexate phospholipid conjugate) treated arthritic rats, on lipopolysaccharide stimulation, were found to produce significantly less tumor necrosis factor (TNF) and PGE_2, than did the macrophages from saline treated controls (Williams et al., 1994). Similarly, in human blood monocytes, MTX-LIPO proved to be a potent inhibitor of both IL-1-β and TNF release, whereas empty liposomes and free MTX showed no such effect (Williams et al., 1995b). In an in-vivo study in rats, free MTX showed anti-inflammatory activity if treatment was started on the day of arthritis induction, but was ineffective to an established arthritis (Williams et al., 1995a). Conversely, MTX-LIPO did not effect the progression of arthritis when dosing was started on day 0, but exerted a significant effect on established arthritis. MTX-LIPO was also found to be less hemotoxic than free MTX. Encouraged by the results the authors subsequently studied intra-articular delivery of MTX-γ-DMPE (Williams et al., 1996). The compound was incorporated into MLVs and SUVs and administered as single intra-articular injection in the inflamed knee of rats 7 days after arthritis induction. Control animals were treated with saline, free MTX, and empty liposomes. While the liposomal preparations were effective in suppressing inflammation, no significant effect was seen in rats in the control groups. Among the two liposomal formulations, MTX-MLVs were much more effective than MTX-SUVs. The effect is attributed by the authors to the better retention of MLVs within the joint space than SUVs (Williams et al., 1996).

3.1.1.8. Purine antimetabolites. 6-Mercaptopurine (6-MP) is a widely used anticancer and immunosuppressive agent. However, like other antimetabolites, the drug is associated with severe cytotoxic effects (Present et al., 1989). To reduce the toxicity, attempts were made to incorporate it into liposomes (Tsujii et al., 1976). The drug was found to be poorly entrapped, which was ascribed to its inherent low biphasic solubility (Newton et al., 1982a).

Tsujii et al. (1976) probed the possibility of enhancing liposomal encapsulation of this insoluble drug through formation of polar charge trans-



Compound	R_1	R_2
6-Mercaptopurine (6-MP)	H	H
6-(Octadecyldithio)-purine (6-ODP)	$S(CH_2)_{17}CH_3$	H
S^6,9-bisoctanoyloxy-methyl-6-MP	$CH_2OCO(CH_2)_6CH_3$	$CH_2OCO(CH_2)_6CH_3$
S^6,9-bislaureoyloxy-methyl-6-MP	$CH_2OCO(CH_2)_{10}CH_3$	$CH_2OCO(CH_2)_{10}CH_3$

Fig. 9. Lipophilic prodrugs of 6-MP

fer complexes. In initial studies chloranil was employed as an electron acceptor. The complex resulted in enhanced entrapment, however, it was very unstable. Also, since chloranil itself possessed toxic pharmacological effects, complexation of 6-MP was thereafter tried with cyanocobalamine, a non-toxic electron acceptor (Kano and Fendler, 1977). Even this complex decomposed readily to its constituents and the desirable entrapment efficiency could not be achieved.

The synthesis of lipophilic prodrugs is the other approach that has been tried as a solution to the problem of low entrapment of 6-MP. Müller (1988) synthesised 6-(octadecyldithio)purine (Fig. 9) by linking the drug to a C_{18} thiol through a disulfide bond. In in-vitro studies, the liposomal formulation of the prodrug exhibited immunosuppressive potencies comparable with those of 6-MP (Daniel et al., 1989). Waranis and Sloan (1987) have reported synthesis of lipophilic bisacyloxymethyl derivatives of 6-MP. The liposomal incorporation of these derivatives is being investigated in our laboratory. Preliminary investigations have shown enhanced entrapment of the drug in liposomes (Gulati et al., unpublished results).

3.1.1.9. L-Asparaginase (L-ASNase). A high molecular weight enzyme, L-asparaginase (L-asparagine amido hydrolase) obtained commercially from *E. coli* is indicated for treatment of a variety

of acute and chronic human leukemias (Asselin et al., 1989). Being a foreign protein, its activity is accompanied by several immunogenic side effects ranging from skin rashes to fatal anaphylaxis (Charles and Bono, 1981). The allergic reaction is shown sometimes even after a negative reaction to the intradermal test. Rarely, the skin test itself results in an anaphylactic shock.

Lipsomes have been tried as carriers to circumvent the serious side effects of the proteinaceous enzyme. Since the enzyme is water soluble, the encapsulation efficiency achieved with the native protein has been quite low, ranging between 12–50% (Fishman and Citri, 1975; Neerunjun and Gregoriadis, 1976; Oshawa et al., 1985). The use of large liposomes was also tried to improve the extent of the encapsulation, but it resulted in short circulation times. In addition, size reduction by sonication was tried but it did not help either, as it lead to a decrease in both enzyme activity and entrapment efficiency.

In an attempt to obtain better liposomal formulation, a lipophilic derivative, palmitoyl-L-asparaginase, was prepared by the acylation of the enzyme (Martins et al., 1990). This acylated protein was found to incorporate well into the liposomes and showed an increased catalytic activity as compared to the native enzyme. Subsequently, trials were made on liposomes of different phospholipid combinations (Cruz et al., 1994; Jorge et al., 1994). The best lipidic compositions that emerged from in-vitro studies were PC/Chol/PI and PC/Chol/SA, in molar ratios of 10:5:1 and 7:2:0.5, respectively. Liposomes of these lipidic compositions were prepared before (MLVs) and after the extrusion procedure (VET_{200}) and subjected to in-vivo study of pharmacokinetics, toxicity and antitumor activity. A significant increase in MRT and AUC values and a decrease in plasma clearance was observed with both the preparations and there were no changes in steady state distribution values. The results of toxicity studies were further revealing as negatively charged liposomal formulation composed of PC/Chol/PI prepared by extrusion method (VET_{200}) showed elimination of acute toxicity, whereas the PC/Chol/SA formulation prepared in a similar manner showed enhanced toxicity both when used as a sensitizing and challenging agent. The PC/Chol/PI formulation showed good antitumor activity above 2000 U/kg and, therefore, is suggested as a substitute for the native enzyme in the treatment of lymphoblastic leukemia.

3.1.1.10. Boron neutron capture therapy (BNCT). The BNC binary cancer therapy is based on ionisation tracking, cellular damage and cytotoxicity caused by ^{7}Li and ^{4}He, the two energetic fission ions, formed on irradiation of boron-10 with thermal neutrons. The effective distance of travel of the two ions in tissue is limited to one cell diameter which advantageously results in localized destruction of the malignant cells without any detrimental effect on the neighboring cells. The successful therapy, however, requires site-specific delivery of significant quantities of boron-10 isotope in cancer cells (15–20 mg of B/g of tumor mass) (Fairchild and Bond, 1985). This is a problem area in BNC therapy. The strategies employed for selective transport of the boron compounds to tumours include use of boron derivatives having natural affinity to tumours (Hatanaka, 1975), attachment of boron containing species to porphyrins (Hill et al., 1992), conjugation with tumor specific monoclonal antibodies (Pak et al., 1995; Primus et al., 1996), and incorporation of borane ions in liposomes (Shelly et al., 1992).

The study on liposomal incorporation by Shelly et al. (1992) involved encapsulation of hydrolytically stable borane anions like $(B_{10}H_{10})^{2-}$, $(B_{12}H_{11}SH)^{2-}$, $(B_{20}H_{17}OH)^{4-}$, $(B_{20}H_{19})^{3-}$ and the normal form and the photoisomer of $(B_{20}H_{18})^{2-}$ (as their soluble sodium salts) in SUVs. The vesicles were observed to selectively deliver therapeutic quantities of boron to tumors. Among the different borane anions, the two isomers of $(B_{20}H_{18})^{2-}$ gave most favourable results. The polyhedral borane ion, $(n-B_{20}H_{18})^{2-}$, was chosen for further investigations. It was reacted with liquid ammonia in presence of a suitable base to produce an apical-equatorial (*ae*) isomer of the $(B_{20}H_{17}NH_3)^{3-}$ ion, $(1\text{-}(2'\text{-}B_{10}H_9)\text{-}2\text{-}NH_3B_{10}H_8)^{3-}$ (Feakes et al., 1994). The sodium salt of both these ae and a^2 isomers were encapsulated in SUVs and investigated as boron delivery agents.

Excellent tumor uptake and selectivity was observed at very low injected doses of the two agents. However, the encapsulation efficiency of these hydrophilic boron compounds was $\approx 3\%$. Moreover, the encapsulated aqueous phase containing the hydrophilic boron salts was hypertonic in nature which limited the use of these liposomes due to their instability in plasma.

To overcome these formulation problems a lipophilic nido-carborane species, K(nido-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$) was synthesized which could be embedded in the liposome bilayer with an efficiency of 53% (Feakes et al., 1995). The efficiency improved to about 80% when liposomes were prepared containing a hypertonic HBS-buffered solution encapsulated in the aqueous milieu of the vesicles. Based on the previous experience of hydrophilic boron compounds yielding hypertonic aqueous phase, the hypertonic buffer solution was eventually replaced by Na_3C_{ae}-$B_{20}H_{17}NH_3$) solution. This lead to incorporation of both the hydrophilic and the lipophilic boron-containing compounds within the same liposomal formulation and also resulted in dose enhancement for a given volume of liposome bolus. The biodistribution studies eventually proved that the combined formulation gave the higher tumor boron concentrations than obtained by any other means.

3.1.2. Antiviral drugs

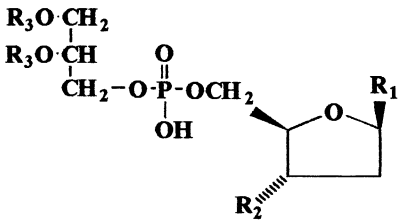
Liposomes appear to be an attractive delivery system for antiviral drugs by virtue of their property of passive targeting to the reticuloendothelial system (RES). This property has already been exploited in the cure of diseases involving RES. A successful example is liposomal Amphotericin B (AmBisome), recommended for the treatment of leishmaniasis and invasive fungal infections (Gulati et al., 1998a). Ambisome is already in the market for commercial use.

In AIDS, though the symptoms are absent in the early stages, a substantial level of HIV replication takes place in the lymphoid tissue (Pantaleo et al., 1993). Since bulk of the parenterally administered liposomes are taken up by the lymphoid system, the liposomal delivery offers to improve in-vivo efficacy, lower toxicity and carries prospects of improved pharmacokinetics, prolonged effect, etc. Similarly, targeting of the antiviral drugs to the liver in hepatitis B is anticipated to enhance the effectiveness and reduce the nonhepatic toxicity.

The physicochemical properties of antiviral drugs are similar to anticancer drugs and hence pose same types of incorporation problems in liposomes. A number of antiviral drugs have been modified chemically to render them more lipophilic and their liposomal preparations have been subjected to in-vitro and in-vivo studies.

3.1.2.1. Nucleoside analogs. The major work on lipophilic derivatization of antiviral nucleoside drugs, azidothymidine (AZT) and dideoxynucleotides, has been carried out by the group of Hostetler et al. (1990, 1994a,b). In a preliminary investigation, they synthesized phosphatidyl-AZT, AZT diphosphate dipalmitin, phosphatidyl-ddC and phosphatidyl-ddT (Hostetler et al., 1990). These novel phospholipid prodrugs readily incorporated into liposomal phospholipid bilayers and showed antiretroviral activity in HIV infected U937 and CEM cells. In a subsequent study, a fluorescent analogue, 3′-deoxythymidine diphosphate-1-myristoyl-2-(10-pyren-1-yldecanoyl)glycerol was prepared (van Wijk et al., 1992). The purpose was to gain an insight into the membrane association of the prodrugs and the spontaneous and protein mediated intermembrane transfer. The compound was found to incorporate readily into the ethanol-injection vesicles. The biodistribution of liposomal dioleoylphosphatidyl-ddC (DOP-ddC) and dipalmitoylphosphatidyl-AZT (DPP-AZT) was studied in rauscher leukemia virus-infected rats (Hostetler et al., 1994b). The liposomal DOP-ddC was shown to provide higher levels of drug in plasma, spleen and lymphoid tissue as compared to ddC and AZT. Similarly, an intraperitoneal single dose of DPP-AZT liposomes prevented increased spleen weight and reverse transcriptase levels in serum comparable to that of AZT given continuously in drinking water.

When tested in hepatitis B virus (HBV)-infected human hepatoma cells, liposomal ddC was less



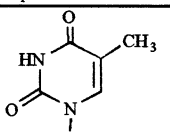
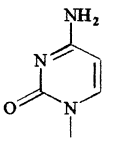
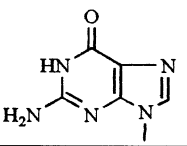
Compound	R_1	R_2	R_3
Dimyristoylphosphatidyl-AZT (Phosphatidyl-AZT, DMP-AZT)		N_3	$CO(CH_2)_{12}CH_3$
Dipalmitoylphosphatidyl-AZT (DPP-AZT)	-do-	N_3	$CO(CH_2)_{14}CH_3$
Dimyristoylphosphatidyl-ddT (Phosphatidyl-ddT, DMP-ddT)	-do-	H	$CO(CH_2)_{12}CH_3$
Dimyristoylphosphatidyl-ddC (Phosphatidyl - ddC, DMP-ddC)		H	$CO(CH_2)_{12}CH_3$
Dioleylphosphatidyl-ddC (DOP-ddC)	-do-	H	$CO(CH_2)_7CH{=}CH(CH_2)_7\,CH_3$
Dipalmitoylphosphatidyl-ddG (DPP-ddG)		H	$CO(CH_2)_{14}CH_3$

Fig. 10. Phosphatidyl derivatives of AZT, ddT, ddC and ddG

toxic than ddC (Hostetler et al., 1994a). On intraperitoneal administration in mice, the liposomal prodrug showed increased drug levels in liver, spleen and lymph nodes, the important sites of HBV replication. In comparison to free ddC, the plasma levels of DOP-ddC remained six times longer above the ED_{90}. Based on the encouraging results from this study, further work was carried out to find out the most potent and selective liver targeted anti-HBV nucleoside conjugate for treatment of woodchuck hepatitis virus. Two types of conjugate series, one involving lipophilic 1,2-dipalmitoyl-sn-glycerol-3-phosphate conjugates of enantiomers of 2′,3′-dideoxy-3′-thiacytidine, and the other involving phosphatidyl derivatives of 2′,3′-dideoxyguanosine (ddG) especially 1,2-dipalmitoyl phosphatidyl-dideoxyguanosine (DPP-ddG), were prepared and incorporated into liposomes (Xie et al., 1995; Korba et al., 1996). The given data suggests that the use of lipid prodrugs encapsulated in liposomes for liver targetting is a useful approach in enhancing antiviral therapy of hepatitis.

The structures of AZT, ddT, ddC and ddG derivatives are given in Fig. 10.

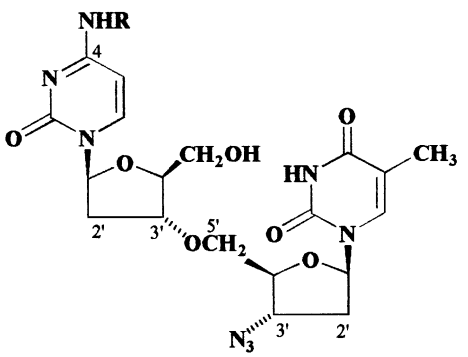
Schott et al. (1994) in a separate study has reported the synthesis of amphipathic dinucleoside AZT derivatives, N^4-palmitoyl-2′-deoxycytidinyl(3′,5′) - 3′ - azido - 2′,3′ - dideoxythymidine and N^4-hexadecyl-2′-deoxycytidinyl(3′,5′)-3′-azido-2′3′-dideoxythymidine (Fig. 11). The products were prepared by masking the polar

AZT-5′-monophosphate with lipophilic deoxycytidine residues of variable stability. The drug derivatives were reported to be well-incorporated into liposomes and exhibited anti-HIV activity in-vitro.

Reports of similar type exist on acyclovir, another promising antiviral agent. An amphipathic nucleolipid containing acyclovir as the hydrophilic group and a 17-C side chain as the lipophilic part has been shown to form stable liposomes with cholesterol or dicetyl phosphate (Rosenmeyer et al., 1985). The preparation of lipophilic laurate and palmitate esters of acyclovir (Fig. 11) is also described (Tong et al., 1992).

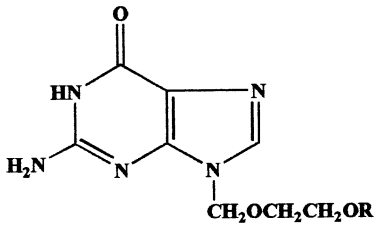
Acyclovir palmitate liposomes proved to be more effective against *Herpes simplex* virus in African green monkey cultures as compared to the parent drug. Recently, another lipophilic prodrug, acyclovir diphosphate dimyristoylglycerol (ACVDP-DG) (Fig. 12) was tested for antiviral activity. In human cytomegalovirus (HCMV) infected MRC-5 cells, ACVDP-DG was 9-fold more effective than the drug. The ocular toxicity and pharmacokinetics after intravitreal injections of liposomal ACVDP-DG in rabbit eyes are also reported (Shakiba et al., 1995).

3.1.2.2. Phosphonoacids. Phosphonoacids are relatively new antiviral agents. These are reported to



Compound	R
N^4-palmitoyl-2'-deoxycytidinyl-(3',5')-3'-azido-2',3'-dideoxythymidine	$CO(CH_2)_{14}CH_3$
N^4-hexadecyl-2'-deoxycytidinyl-(3',5')-3'-azido-2',3'-dideoxythymidine	$CO(CH_2)_{15}CH_3$

Fig. 11. Structures of dinucleoside AZT derivatives



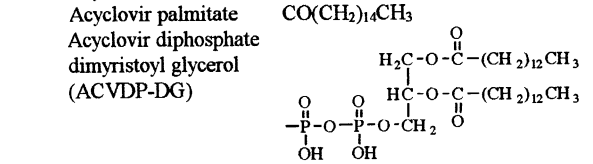
Compound	R
Acyclovir	H
Acyclovir laurate	$CO(CH_2)_{10}CH_3$
Acyclovir palmitate	$CO(CH_2)_{14}CH_3$
Acyclovir diphosphate dimyristoyl glycerol (ACVDP-DG)	

Fig. 12. Lipophilic prodrugs of acyclovir

be effective against a number of viruses, including HIV, CMV and herpes simplex virus (Aweeka et al., 1989; Jacobson et al., 1989). The major drugs belonging to the category are sodium salts of phosphonoformic acid (phosphono-formate trisodium, foscarnet sodium) and phosphonoacetic acid (phosphonoacetate disodium monohydrate, fosfonet sodium).

The work on preparation of lipophilic derivatives of these phosphonoacids was done by Hostetler and Kumar (1993, 1995). The prodrugs were prepared by linking the acids (through the phosphate or carboxyl group) to a lipid, selected from among the sphingolipids, phospholipids, glycerolipids and fatty acids. The lipid prodrugs, in general, were effective in improving the efficacy by prolonging the antiviral activity (Hostetler and Kumar, 1995). In particular, a derivative, 1-*O*-octadecyl-sn-glycero-3-phosphonoformate (Fig. 13), is shown to be very effective against HIV (Hostetler et al., 1996). There is, however, no report yet on liposomal incorporation of this compound.

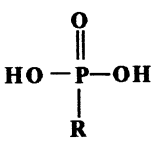
3.1.3. Steroids

The idea of using liposomes to improve the local therapy of rheumatoid arthritis with corticosteroids was first introduced by Shaw et al. (1976). This approach was employed to deliver

liposomes containing corticosteroids directly into the synovial cavity in animals and in arthritic patients (Dingle et al., 1978). It was found that the unsonicated liposomes remained, by virtue of their size, in the enclosed joint cavity, thus facilitating the uptake of the steroid by the target synovial cells. This also reduced the escape of the drug into the systemic circulation thus decreasing the exposure of the non-target sites and eliminating the undesirable side effects.

Steroids, as such, however, are not retained by liposomes under normal conditions. The drugs are required to be modified chemically to render them more lipophilic. Many lipid soluble derivatives have been successfully incorporated into liposomes.

3.1.3.1. Cortisol. Cortisol is incorporated into liposomes only in a low concentration and is rapidly leached out. The lipophilic esters, cortisol octanoate and cortisol palmitate, were shown to give much better incorporation and retention characteristics (Shaw et al., 1976). Among the two, cortisol palmitate was found to be retained longer. This was confirmed using differential scanning calorimetry (Fildes and Oliver, 1978). On intra-articular delivery of the liposomal preparation of the ester, much greater anti-inflammatory activity than an equivalent dose of microcrystalline cortisol acetate was seen on testing in rabbits. The decrease in inflammation was shown to persist upto 7 days and a dose as low as 2 mg of steroid per joint was found to be effective. Cortisol palmitate, when incorporated into negatively charged DPPC liposomes was found to be very effective in low doses in the clinical trials (de-Silva et al., 1979).



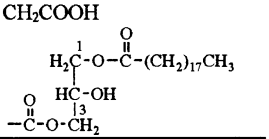
Compound	R
Phosphonoformic acid	COOH
Phosphonoacetic acid	CH_2COOH
1-*O*-octadecyl-sn-glycero-3-phosphonoformate	

Fig. 13. Phosphonoacids and a lipophilic derivative

3.1.3.2. Cortisone. The lipophilic esters of cortisone ranging in the side chain from acetate to hexadecanoate were incorporated into liposomes (Arrowsmith et al., 1983a). An increase in the length of the side group resulted in an increase in the entrapment. Maximum incorporation occured in the case of cortisone hexadecanoate. The in-vitro release from liposomes became slower as the acyl chain length increased (Arrowsmith et al., 1983b). The relationship between efflux rate constant and number of C-atoms was linear within a group of compounds having side chain length 6–14 carbons. For compounds with 16–22 C-atoms in the side chain, the release rates were faster than expected.

In further studies, the release rates of cortisone hexadecanoate from liposomes were determined in the presence of rat skeletal muscle homogenate (Arrowsmith et al., 1983c). The presence of the biological material was found to have little effect on the stability of the liposomal prodrug. The stability of DPPC liposomes in the rabbit blood was rather enhanced by the presence of cortisone hexadecanoate. No such effect was shown by cortisone octanoate. Saket et al. (1984) studied the thermodynamics of partitioning of these esters in liposomes formed from DMPC. The esterification of the steroids at 21-hydroxyl position was found to increase the liposomal partitioning. In an in-vivo study in rabbits (Arrowsmith et al., 1984), the biodistribution of liposomes containing cortisone octanoate and cortisone hexadecanoate was studied after i.m. injection. The liposomes were found to possess a clearance $T_{1/2}$ of 8.5 days.

3.1.3.3. Triamcinolone acetonide. The entrapment efficiency of triamcinolone acetonide in liposomes is only around 5% (Goundalkar and Mezei, 1984). To improve its encapsulation, 21-palmitate ester of the steroid was synthesized which exhibited an entrapment efficiency of 85%. This lipophilic prodrug was then studied for its biodistribution on incorporation in liposomes with neutral, negative and positive charge (Abraham et al., 1984). The

liposomes with different charges behaved differently in the body and those with positive charge exhibited the maximum circulation time.

3.1.3.4. Dexamethasone. The palmitate ester of dexamethasone was incorporated into liposomes (Benameur et al., 1993). An optimum efficiency of 75% was observed in liposomes made from PC alone. The conformational analysis indicated that dexamethasone inserted into the bilayer with its carbonyl group oriented towards the aqueous surface.

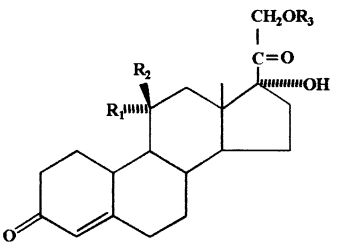
The structures of lipophilic derivatives of various steroidal drugs are given in Fig. 14.

3.1.4. Miscellaneous

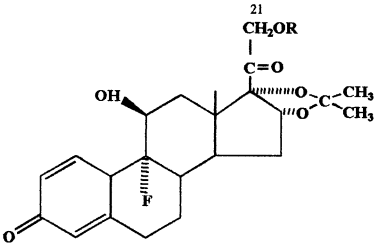
Metronidazole, a water soluble antiprotozoal drug, shows very poor entrapment efficiency and low stability in liposomes. Therefore, its lipophilic myristic and lauric esters (Fig. 15) were synthesized (Hou et al., 1990). The encapsulation efficiency and stability of the prodrugs were found to be 10-folds or more in liposomes as compared to the original drug. The biological activity of liposome entrapped lauric ester was tested against *Trichomonas vaginalis*. The prodrug was found to be much more effective than the original drug entrapped in liposomes (Hou et al., 1994).

The prodrugs of para aminobenzoic acid (PABA) ranging from methyl to butyl PABA (Fig. 16) were prepared to study the effect of alkyl chain length on the bilayer/water partition coefficient (Ma et al., 1991). The increase in the lipophilicity of the compound by the addition of methylene groups resulted in an increase in the bilayer partitioning.

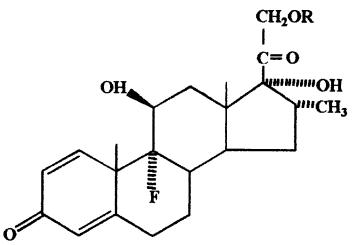
Two enkephalin-lipid conjugates were prepared and immobilised on polymerized vesicles (Tetsui et al., 1992). The liposomes containing these immobilised conjugates were tested as ligands of specific opioid receptors in bovine brain homogenates. In one conjugate, the enkephalin molecule was directly linked to the lipid, while in the other the linkage was via an intervening spacer. The receptor affinity of the liposomes containing enkephalin-lipid conjugate with an intervening spacer was higher than the corresponding liposomes containing a conjugate without a spacer.



Compound	R_1	R_2	R_3
Cortisol	H	OH	H
Cortisol octanoate	H	OH	$CO(CH_2)_7CH_3$
Cortisol palmitate	H	OH	$CO(CH_2)_{14}CH_3$
Cortisone		=O	H
Cortisone octanoate		=O	$CO(CH_2)_6CH_3$
Cortisone hexadecanoate		=O	$CO(CH_2)_{15}CH_3$



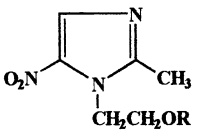
Compound	R
Triamcinolone acetonide	H
Triamcinolone acetonide-21-palmitate	$CO(CH_2)_{14}CH_3$



Compound	R
Dexamethasone	H
Dexamethasone palmitate	$CO(CH_2)_{14}CH_3$

Fig. 14. Steroid drugs and their lipophilic derivatives

Castelli et al. (1990) linked 4-biphenylacetic acid (Felbinac, Fig. 17) to a α-β-poly(*N*-hydroxyethyl)-DL-aspartamide. The macromolecular prodrug was incorporated into liposomes and thermotropic properties were studied. The interaction of the prodrug with the apolar moiety of the lipid layer was attributed to the lipophilic nature of the polymer bound felbinac.



Compound	R
Metronidazole	H
Metronidazole laurate	$CO(CH_2)_{10}CH_3$
Metronidazole myristate	$CO(CH_2)_{12}CH_3$

Fig. 15. Metronidazole and derivatives

The synthesis of octadecydiatrizoate (Fig. 18) and use of its liposomal preparation as a contrast medium is discussed (Charles and Robinson, 1986).

Insulin, a peptide drug, shows a very low entrapment efficiency, i.e. 2.3 nmol/mmol of lecithin (Stuhne-Sekalec et al., 1986). To improve its entrapment characteristics, it was converted to phosphatidyl ethyl insulin and incorporated into liposomes (Wu, 1980).

3.2. Charge transfer complexes

Charge transfer complexes have been successfully utilised for increasing the lipophilicity of the drugs for various applications (Gasco et al., 1985). This approach has also proved to be effective for increasing the liposomal encapsulation of magnetic resonance imaging agents and other drugs.

3.2.1. Magnetic resonance imaging agents (MRI agents)

The paramagnetic ions are used as agents for magnetic resonance imaging. Liposomes can act as suitable delivery system for these contrast agents. However, for the entrapment of these ions into liposomes, a carrier moiety is required.

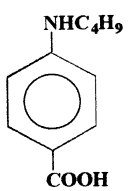


Fig. 16. *n*-Butyl PABA

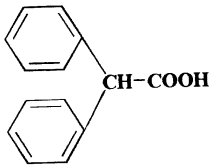


Fig. 17. Felbinac

Grant et al. (1989) attached phosphatidyl ethanolamine through its amino head group with the chelating agent, diethylenetriamine pentaacetic acid (DTPA) (Fig. 19). The complex readily assembled into the lipid bilayers of liposomes. The complex was primarily taken up by the liver and spleen and was excreted through biliary route 24 h post injection. Another complex of DTPA, i.e. DTPA-stearate (DTPASA), as a lipophilic chelate for liposomal entrapment of paramagnetic metal ions, Gd, Mn and Fe is also reported (Schwendener et al., 1990). The Fe- and Mn-DTPASA complexes were not very stable, whereas Gd-DTPASA liposomes were stable and showed strong signal enhancement of the organs of mononuclear phagocyte system. The complex had an elimination 1/2 life of 61 h, thus proving itself to be an efficient and specific MRI contrast agent for upper abdomen.

Using acetylacetone, a lipophilic chelate, high levels of Indium(III) into liposomes were achieved (Hwang, 1978; Beaumier and Hwang, 1982).

Elgavish and Kim (1990) evaluated highly lipophilic polyaminopolycarboxylic acid complexing agents with long side chains as carrier systems for Gd ions. The 1:1 complex was shown to be stable by NMR relaxivity measurements and incorporated well into the liposomes. For liposomal delivery of Mn^{2+}, complexes with lipophilic chelating agents having long acyl chains were synthesized by reacting 3-decylamino-1,2-propane

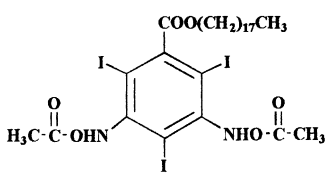


Fig. 18. Octadecyldiatrizoate

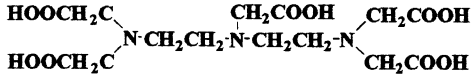


Fig. 19. Diethylenetriaminepentaacetic acid (DTPA)

diol (DDP) with EDTA anhydride and reacting the resulting compound with $MnCO_3$ (Unger and Shen, 1991). Mn-EDTA-DDP was incorporated into liposomes and was successful as a contrast agent for imaging of hepatic tumors in rats.

Tilcock et al. (1992) discussed general design considerations for the vesicle associated MR contrast agents based on paramagnetic chelates, either entrapped within the vesicle interior or attached to the membrane surface.

3.2.2. Others

Bard et al. (1983) investigated liposomes containing a lipophilic chelator which could complex a variety of β-emitting radionuclides as vehicles for radioisotopes in radiosynovectomy. The chelate, 3-cholesteryl-6-(*N'*-iminobis(ethylenenitrilo)tetraacetic acid)hexyl ether, was complexed with the γ-emitting tracer, ^{51}Cr and incorporated into liposomes. The liposomal preparation showed much better distribution and retention of ^{51}Cr as compared with colloidal and water soluble preparations.

The formation of ion pair between methantheline bromide (Fig. 20), a quaternary ammonium compound, and trichloroacetate is reported to result in a 3-fold increase in encapsulation of the drug in multilamellar liposomes (Jay and Digenis, 1982). This is attributed to increased lipophilicity of the ion pair, which results in better solubility in the bilayer. Similarly, an ion pair formed between warfarin and sodium ions has been shown to increase the partioning of the drug into the lipid

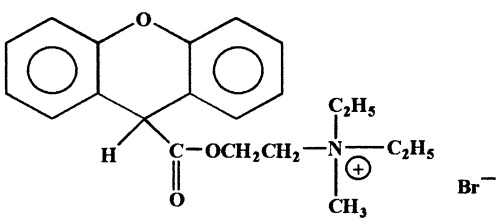
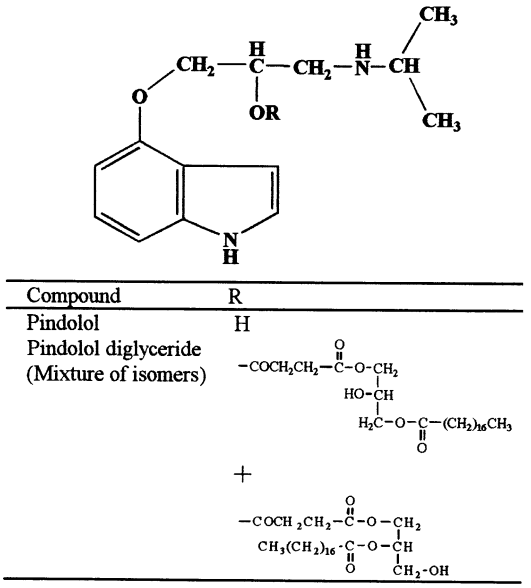


Fig. 20. Methantheline bromide



Compound	R
Pindolol	H
Pindolol diglyceride (Mixture of isomers)	$-COCH_2CH_2-C(=O)-O-CH_2$, $HO-CH$, $H_2C-O-C(=O)-(CH_2)_{16}CH_3$ + $-COCH_2CH_2-C(=O)-O-CH_2$, $CH_3(CH_2)_{16}-C(=O)-O-CH$, CH_2-OH

Fig. 21. Pindolol diglyceride

layers of reverse phase evaporation vesicles (Cools and Janssen, 1984). The increase in Na^+ concentration, however, did not effect the vesicle permeability. Lee et al. (1988) observed an improvement of encapsulation of isopropamide iodide by ion-pairing with taurodeoxycholate. The resulting complex being more lipophilic showed a 3-fold increase in the encapsulation efficiency.

3.3. Pharmacosomes

Pharmacosomes can be defined as colloidal dispersion of drugs covalently linked to lipids. The idea for the development of pharmacosomes was based on the surface and bulk interactions of lipids with water (Carey and Small, 1970). Pharmacosomes are designed to avoid the usual problems associated with the liposomal entrapment of polar drug molecules like low drug incorporation, leakage and poor stability. Any drug can be attached directly or through a spacer to the hydroxyl group of a lipid molecule.

This approach of forming vesicles by the use of drug molecules linked to lipids was utilised to produce pharmacosomes of pindolol, a β-receptor blocking agent (Vaizoglu and Speiser, 1986). The

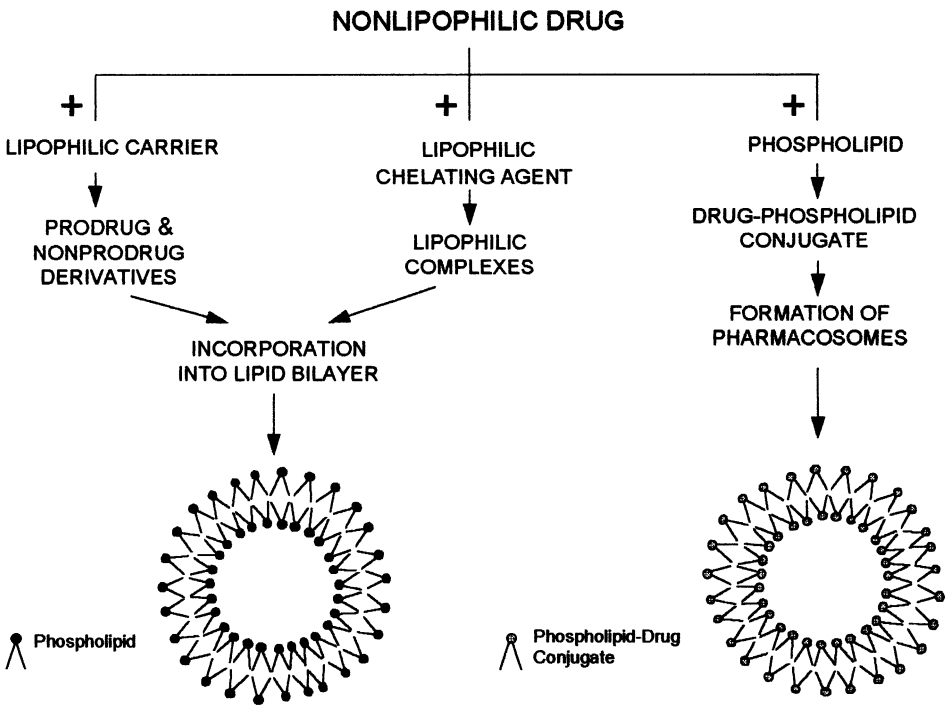


Fig. 22. The approaches for targetting of drugs to the phospholipid bilayer of liposomes

drug was converted to pindolol diglyceride (Fig. 21). It was combined with tween-80 and MLVs could be prepared by the film method. Tween-80 was not found necessary for dispersion in preparing liposomes by the injection method.

The phospholipid analogs containing azidothymidine and other antiviral agents as the polar head groups, which were discussed above, are also reported to form lipid bilayers (Hostetler et al., 1990).

Müller-Goymann and Hamann (1991) produced pharmacosomes by dilution of lyotropic liquid crystals formed by amphiphilic drugs. Fenoprofen salt and acid were formed into vesicles by this modified technique.

4. Conclusions

Lipophilic derivatization of the drug molecules to improve their entrapment characteristics, which was propounded by Knight (1981), has proved to be very useful for liposomal encapsulation of many problem candidates. The summary list drawn in Table 3 gives a good idea on the success level of the approach highlighting the range of drugs and diagnostic agents which have been converted and subjected to studies. The major achievement is that a good number of liposomal preparations containing lipophilic derivatives have reached the clinical trial stage.

In total, three approaches have emerged during the years for increasing the lipophilicity of the drugs with the purpose of enhancing their liposomal encapsulation. These are preparation of prodrug and non-prodrug lipophilic derivatives, formation of lipophilic complexes, and formation of pharmacosomes (Fig. 22).

It has been observed that lipophilicity cannot be taken as the only criterion for the solubility of a drug molecule into the phospholipid bilayers. Geometrical constraints of the lipophilic molecules also govern their distribution into the liposomes (Barenholz and Crommelin, 1988). Some very hydrophobic compounds tend to phase-separate and form microscopic and macro-

Table 3
List of the drugs of which lipophilic derivatives have been prepared alongwith the objectives achieved and the stage of development of the liposomal preparation

Drug	Promising lipophilic derivative	Objectives achieved	Stage of development	Reference
Acyclovir	Nucleolipid 17-C side chain	↑Encapsulation efficiency	Physiochemical studies	Rosenmeyer et al., 1985
	Laurate and palmitate esters	↑Encapsulation efficiency	Cell-culture studies	Tong et al., 1992
	Diphosphate dimyristoyl glycerol derivatives	↑Encapsulation efficiency ↑Antiviral effect	Cell-culture studies	Shakiba et al., 1995
Arabinosylcytosine	N^4-(*N*-(cholesteryloxy carbonyl)glycyl) derivative	↑Encapsulation efficiency ↑Antitumor effect	Animal studies	Tokunaga et al., 1988d
	N^4- and 5′-oleyl- and N^4-palmitoyl derivatives	↑Encapsulation efficiency ↑Antitumor effect	Clinical trials	Rubas et al., 1986; Schwendener et al., 1989
	N^4-hexadecyl derivative (NHAC)	↑Encapsulation efficiency ↑Antitumor effect effect Non-cross resistant	Preclinical studies	Schwendener and Schott, 1992; Horber et al., 1995a,b,c; Schwendener et al., 1995
	Phospholipid-N^4-palmitoyl conjugates	↑Encapsulation efficiency	Animal studies	Schott and Schwendener, 1996b
	Phospholipid-N^4-hexadecyl conjugates	↑Encapsulation efficiency	Animal studies	Schott and Schwendener, 1996a
L-Asparaginase	Palmitoyl derivative	↑Encapsulation efficiency ↑Antitumor activity ↓Toxicity	Animal studies	Martins et al., 1990; Cruz et al., 1994; Jorge et al., 1994
Azidothymidine	Phospholipid derivatives	↑Encapsulation efficiency ↓Toxicity	Animal studies	Hostetler et al., 1990, 1994a,b
	N^4-palmitoyl and N^4-hexadecyl-2′-deoxy cytidinyl-(3,5′)-3′-azido-2′,3′-dideoxythymidine	↑Encapsulation efficiency	Cell-culture studies	Schott et al., 1994
Boron	K(nido-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$)	↑Encapsulation efficiency	Animal studies Antitumor effect	Feakes et al., 1995
Cisplatin	Neodecanoato-*trans*-*R*,*R*-1,2-diamino cyclohexane platinum II)	↑Encapsulation efficiency ↑Antitumor effect ↓Toxicity	Clinical trials	Perez-Soler et al., 1987, 1988, 1989, 1990; Chase et al., 1991; Khokhar et al., 1989; Perez-Soler and Khokhar, 1992
Cortisol	Alkyl esters	↑Encapsulation efficiency ↑Physical stability Anti-inflammatory activity	Clinical trials	Shaw et al., 1976; Fildes and Oliver, 1978; de-Silva et al., 1979
Cortisone	Alkyl esters	↑Encapsulation efficiency ↑Physical stability Alteration in biodistribution	Animal studies	Arrowsmith et al., 1983a,b,c, 1984
^{51}Cr	Complex with 3-cholesteryl-6-(*N*′-iminobis(ethylenenitrilo)tetraacetic acid)hexyl ether	↑Encapsulation efficiency Better biodistribution	In vitro studies	Tilcock et al., 1992
Daunorubicin	*N*-acyl derivatives	↑Encapsulation efficiency ↑Physical stability ↑Antitumor effect	Cell-culture studies	Bard et al., 1982
Dexamethasone	Palmitate ester	↑Encapsulation efficiency	Physicochemical studies	Benameur et al., 1993
Diatrizoic acid	Long chain esters	↑Encapsulation efficiency Better contrast medium	Physicochemical studies	Charles and Robinson, 1986

Table 3 (Continued)

Drug	Promising lipophilic derivative	Objectives achieved	Stage of development	Reference
Doxorubicin	2-Iodo-3′-hydroxy-4′-epi-4-demethoxy-doxorubicin (annamycin)	↑Encapsulation efficiency ↑Antitumor effect Non-cross-resistant	Clinical trials	Perez-Soler and Priebe, 1990; Ling et al., 1993a,b; Zou et al., 1994, 1995; Wasan and Perez-Soler, 1995; Wasan and Morton, 1996
Enkephalin	DPPE-enkephalin conjugate	↑Encapsulation efficiency	Physicochemical studies	Tetsui et al., 1992
Felbinac	α-β-Poly(*N*-hydroxyethyl)-DL-aspartamide conjugate	↑Encapsulation efficiency	Physicochemical studies	Castelli et al., 1990
Fenoprofen	Pharmacosomes	↑Encapsulation efficiency	Physicochemical studies	Müller-Goymann and Hamann, 1991
5-Fluorouracil	Alkylcarbamoyl derivatives	↑Encapsulation efficiency ↑Antitumor effect	Animal studies	Sasaki et al., 1987
	Pentyl- and hexyl- 5-fluorouracil-1-acetate	↑Encapsulation efficiency	Physicochemical studies	Jee et al., 1995
	Palmitoyl and dipalmitoyl derivatives of 5-fluoro-2′-deoxyuridine	↑Encapsulation efficiency ↑Antitumor effect	Animal studies	Schwendener et al., 1984; van Borssum et al., 1992; Mori et al., 1993
	Dipalmitoylphosphatidyl fluorouridine	↑Encapsulation efficiency ↑Antitumor effect	Animal studies	Doi et al., 1994
	5′-Palmitoyl- and 5′-succinyl derivatives of 5-fluorouridine	↑Encapsulation efficiency ↑Antitumor effect	Animal studies	Crosasso et al., 1997
Foscarnet sodium	Sphingolipid-, glycerolipid-, phospholipid- and fatty acid analogs	↑Encapsulation efficiency Prolonged antiviral activity	Cell-culture studies	Hostetler and Kumar, 1993, 1995
	1-*O*-octadecyl-sn-glycero-3-phosphonoformate	↑Encapsulation efficiency ↑Antiviral activity	Cell-culture studies	Hostetler et al., 1996
Fosfonet sodium	Sphingolipid-, glycerolipid-, phospholipid- and fatty acid analogs	↑Encapsulation efficiency Prolonged antiviral activity	Cell-culture studies	Hostetler and Kumar, 1993, 1995
Gadolinium	DTPA-stearate complex	↑Encapsulation efficiency ↑Physical stability Signal enhancement of MRI	Animal studies	Schwendener et al., 1990
Hydroxyrubicin	14-*O*-palmitoyl derivative	↑Encapsulation efficiency ↑Physical stability ↑Antitumor effect	Animal studies	Perez-Soler and Priebe, 1992
Indium	DTPA-stearate complex	↑Encapsulation efficiency	Animal studies	Grant et al., 1989
Insulin	Phosphatidyl ethanolamine derivative	↑Encapsulation efficiency	In vitro studies	Wu, 1980
Isopropamide iodide	Ion pairs with taurodeoxy cholate	↑Encapsulation efficiency	In-vitro studies	Lee et al., 1988
Manganese	Mn-EDTA-DDP	↑Encapsulation efficiency	Animal studies	Unger and Shen, 1991
6-Mercaptopurine	(6-Octadecyldithio)purine	↑Encapsulation efficiency	In-vitro studies	Müller, 1988
Methantheline bromide	Ion pairs with trichloroacetic acid	↑Encapsulation efficiency		Jay and Digenis, 1982
Methotrexate	α- and γ- DMPE and α, γ-di DMPE derivatives	↑Encapsulation efficiency Non-resistant anticancer effect	Cell-culture studies	Hashimoto et al., 1985a,b; Kinsky et al., 1986, 1987; Noe et al., 1988
		↑Antiarthiritic activity	Animal studies	Williams et al., 1992, 1994, 1995a,b, 1996

Table 3 (Continued)

Drug	Promising lipophilic derivative	Objectives achieved	Stage of development	Reference
Metronidazole	Myristic and lauric esters	↑Encapsulation efficiency ↑Antiprotozoal activity	Cell-culture studies	Hou et al., 1990, 1994
Mitomycin C	Benzyl, benzoyl, benzylcarbonyl, benzyloxy-carbonyl and benzyloxymethyl derivatives	↑Encapsulation efficiency	Animal studies	Sasaki et al., 1983a,b,c
	Alkoxycarbonyl derivatives	↑Encapsulation efficiency Altered biodistribution	Animal studies	Sasaki et al., 1984, 1985a,b
	Stearoyl MMC and cholesterol derivatives with different spacers	↑Encapsulation efficiency ↑Stability Sustained release	Animal studies	Tokunaga et al., 1988a,b,c
Mitoxantrone	Lipophilic complex	↑Encapsulation efficiency	Animal studies	Schwendener, 1990; Schwendener et al., 1991
Muramyl dipeptide (MDP)	Muramyl tripeptide ethanolamine (MTP-PE)	↑Encapsulation efficiency ↑Physical stability ↑Immunomodulating effect	Clinical trials	Kleinerman et al., 1983, 1995; Murray et al., 1989; Sculier et al., 1993
	MDP-L-alanyl-cholesterol (MDP-CHOL)	↑Encapsulation efficiency ↑Immunomodulating effect	Cell-culture studies	Phillips et al., 1985
	Glycerol dipalmitate derivatives of MDP, MDP-*n*-butyl ester and *N*-acetyl-muramyl-D-alanyl-D-isoglutamine	↑Encapsulation efficiency ↑Immunomodulating effect	Cell-culture studies	Phillips et al., 1987
PABA	Ester derivatives	↑Encapsulation efficiency	Physicochemical studies	Ma et al., 1991
Pindolol	Pharmacosomes	↑Encapsulation efficiency Altered biodistribution	Animal studies	Vaizoglu and Speiser, 1986
Triamcinolone acetonide	Myristic and lauric esters	↑Encapsulation efficiency	Cell-culture studies	Goundalkar and Mezei, 1984
Warfarin	Ion pairs with sodium ions	↑Encapsulation efficiency	Physicochemical studies	Cools and Janssen, 1984

scopic subphases. Examples include triacylglycerols and cholesterol esters which are poorly incorporated into the phospholipid bilayers (Barenholz and Crommelin, 1988). There exist other reported cases where also the strong role of structure of the lipophilic product on its liposomal entrapment is highlighted. Hashida et al. (1988) prepared two lipophilic prodrugs of 5-fluorouracil and both showed complete incorporation into liposomes. However, on studying the release characteristics, the release 1/2 life of FU from one was found to be only about 1 h, while it was more than 24 h for the other. This happened despite that the partition coefficient of the first was much higher than second. Sampedro et al. (1994) similarly studied the liposomal entrapment behaviour of eight lipophilic anticancer drugs. While good liposomal preparations were obtained with hexamethylmelamine, penclomedine, mitindomide and fazarabine, presence of either free drug crystals or microaggregates of lipid/drug complex were seen in liposomes containing taxol, batracylin, trimelamol and diaziquone.

It is also shown that the increase in the lipophilicity of the drug and its successful incorporation into liposomes does not guarantee that the final liposomal preparation will give the desired effect in-vivo. Several factors tend to play a significant role, these include formulation factors like the nature and composition of the phospholipid, the type of liposomes, etc., and the pharmacodynamic/pharmacological factors like mechanisms of action, toxicity, uptake, etc. The influence of these factors was shown in several of the drug cases discussed above. For example, the lipophilic derivatives of cisplatin showed encapsulation efficiencies of 80–95%, but no antitumour activity was seen from the liposomes formed from DMPC (Perez-Soler et al., 1989). Good in-vivo activity was observed when DMPC was replaced with DMPG as the phospholipid material. Similarly, in L-asparginase, a strong effect of the charge on liposomes was observed. While the negatively charged liposomes showed elimination of toxicity, the positive charged were rather more toxic (Jorge et al., 1994). In the same manner, the effect of the type of liposomes was observed with methotrexate. The lipophilic complex of the drug with γ-DMPE was found to be more effective in suppressing the knee joint inflammation when delivered in MLVs than in SUVs (Williams et al., 1996). When the MTX-γ-DMPE complex and its glycerol-PE analogs were tested for cytotoxic activity, they were found to be less active in comparison to the free drug. In this case the difference in mechanisms of uptake of the drug and liposomes is reported to be the governing factor (Kinsky et al., 1987).

The observations as above suggest that more such studies that identify the influence of side chain and linkage/spacer structures and the effect of formulation and other in-vivo factors need to be carried out to optimise the liposomal delivery sytems containing lipophilic drug derivatives.

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