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Liposomal Drug Formulations

Rationale for Development and What We Can Expect for the Future

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Abstract

Liposomes are versatile drug carriers which can be used to solve problems of drug solubility, instability and rapid degradation. Both hydrophilic and hydrophobic drugs can be associated with liposomes and special techniques have been developed for the efficient loading of weak acids and weak bases into liposomes.

Liposomes can function as sustained release systems for drugs and the rate of release can be manipulated. Advantage can be taken of the substantial changes in pharmacokinetics which often accompanies the association of drugs with liposomes. New formulations of liposomes, sterically stabilised with substances like surface-grafted polyethylene glycol have circulating half-lives in humans of up to 2 days. These long circulation times allow concentration of liposomal drug in regions of increased vascular permeability like solid tumours an decreased delivery of drug to normal tissues. Alterations of the biodistribution of drugs, when they are liposomes-associated, in general leads to significant overall decreases in drug toxicity but can also increase toxicity in some tissues. The use of targeting ligands to increase the selectivity of delivery of liposomal drugs to target tissues is currently under development. An understanding of how liposome association can alter drug properties can lead to their rational development in the treatment of many diseases.

Many drugs, either in clinical use or in development, have properties which are far from ideal. They may have poor solubility, rapid metabolism, instability under physiological conditions or unfavourable biodistribution leading to toxicities. One attempt at achieving a solution to these problems has been to associate the drugs with a variety of drug carriers. Liposomes, i.e. phospholipid membrane vesicles, are the most advanced of the drug carriers with several products either approved for clinical use or in advanced clinical trials.[1-4] Table I provides examples of some drug properties which may be improved by liposomal formulations.

Table I. Some biological and physical properties of drugs which may be improved by liposomal drug carriers

Biological	Physical
Low therapeutic index	Low solubility
Rapid metabolism	Lack of stability
Unfavourable pharmacokinetics	Irritant

1. Which Drugs Are Most Suitable for Liposomal Formulation?

Drugs which benefit most from association with liposomal drug carriers are those drugs which have unfavourable pharmacokinetic, biodistribution or toxicity profiles. Anticancer drugs have proven particularly suitable for liposomal delivery.[5,6] Liposomal formulations of several antibacterials, anti-inflammatory drugs and antiviral drugs are also in clinical development.[7-10] Members of other drug

classes whose efficacy is compromised by biological or physical properties, such as those described in table I, may also benefit from liposomal formulations. Liposomal formulations will, in general, be more suitable for drugs with higher, rather than lower, potencies as this will limit the amount of particulate material which must be given in order to achieve a therapeutic concentration of the drug *in vivo*. However, formulation into liposomes will tend to increase the cost of the drug preparation, depending on the cost of the lipid materials and the level of technology required.

Liposomes have the ability to function as carriers for either water-soluble or lipid-soluble drugs. [11] Thus, hydrophilic drugs, such as cytarabine, which have low octanol **:** water partition coefficients (log $P_{oct} < 1.7$), are readily trapped in the liposome interior (fig. 1) and are only slowly released from the liposomes over several hours to several days.[12] Hydrophobic drugs, such as lipophilic derivatives

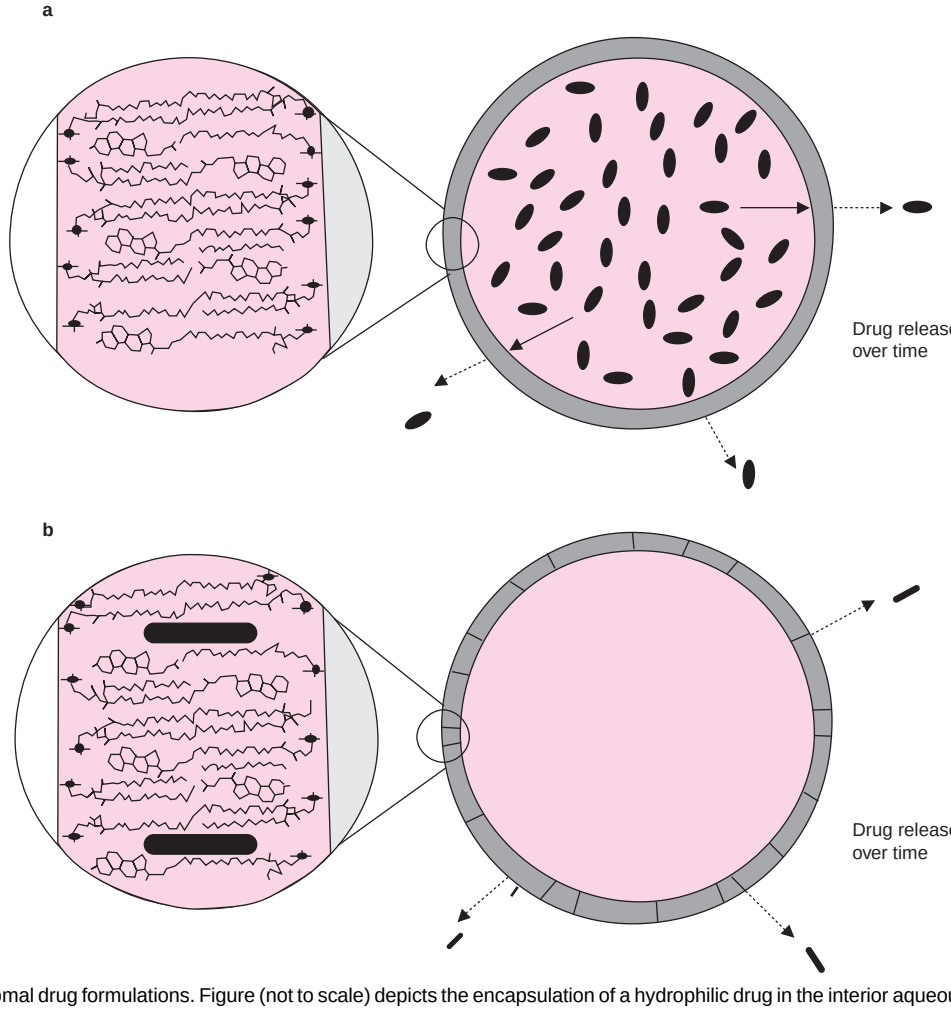

Fig. 1. Liposomal drug formulations. Figure (not to scale) depicts the encapsulation of a hydrophilic drug in the interior aqueous space of a unilamellar liposome (**a**) or association of hydrophobic drugs with the acyl chain region of the liposome bilayer membrane (**b**). In either case, there will be a release of the drug from the liposome at a rate dependent on the liposome characteristics.

of cisplatin or the anthracyclines, which have high octanol : water partition coefficients (log P_{oct} >5), associate rapidly with the hydrophobic interior of the liposome bilayer (fig. 1) and are readily retained in the liposomes with release rates of hours to days.[13-15] Drugs of intermediate solubility, such as doxorubicin, can be more difficult to formulate into liposomes. These drugs rapidly equilibrate between the liposomes and other *in vivo* membranes unless special formulation techniques are used. These techniques involve manipulation of the pH in the liposome interior (fig. 2), or the formation of insoluble molecular complexes with the drug (fig. 2), and are applicable, for example, to drugs which are weak bases or weak acids.[16,17] The resulting formulations have shown excellent retention of contents.[18]

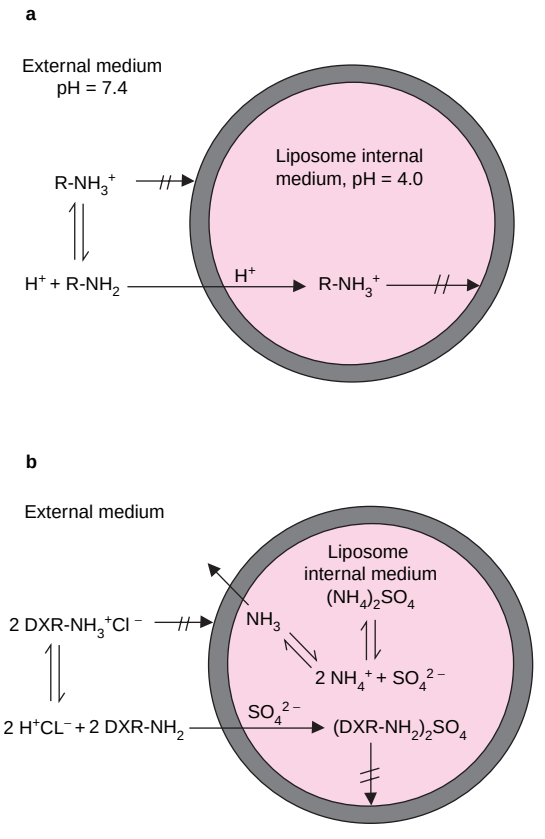

Fig. 2. Loading of drugs into liposomes. Figure (not to scale) shows 2 techniques for loading difficult-to-load drugs into liposomes. In the pH gradient method **(a)**, a weak acid (not depicted) or a weak base in the neutral form ($R\text{-}NH_2$), but not the charged form ($R\text{-}NH_3^+$), will penetrate the liposome bilayer and re-establish an equilibrium in favour of the charged form in the acid environment of the liposome interior. The charged form of the drug is then trapped in the liposome interior, and will be released as the pH gradient dissipates. In the ammonium sulfate gradient method (**b**), often used for doxorubicin (DXR), the drug enters the liposome in the neutral form ($DXR\text{-}NH_2$) and then, in the presence of sulfate ion (SO_4^{2-}), forms a sparingly soluble precipitate of doxorubicin sulfate within the liposome. Doxorubicin sulfate has a very slow leakage rate from liposomes.

2. Some Advantages of Liposomal Drug Formulations

Liposomes are very versatile drug carriers. They can be formed from a great variety of lipid constituents leading to a wide range of physical properties, thus allowing manipulation of properties such as trapping efficiencies of drugs or drug release rates.[19] They can differ greatly in sizes, although the preferred size range for clinical applications is in the order of 50 to 200nm in diameter. Liposomes of this size avoid mononuclear phagocyte system (MPS) uptake better than larger liposomes and are small enough to permit localisation in diseased tissues (see section 2.5), yet large enough to trap useful drug loads.

2.1 Solubility Enhancers

Liposomes are formed from substances which have low intrinsic toxicity. Many of the main components of liposomes occur naturally in mammalian cells e.g. several types of phospholipids, and cholesterol. Thus, liposomes can be excellent solubilising agents for drugs with low solubility and may prevent toxicities associated with some of the more traditional excipients such as propylene glycol, Cremophor EL (a derivative of castor oil and ethylene oxide) surfactants or dimethyl sulfoxide.[14,20,21] Liposomal formulations are also being explored for increasing the delivery of hydrophobic photosensitisers for the photodynamic therapy of tumours.[22]

2.2 Protection from Degradation

Substances associated with liposomes, particularly those in the liposome interior (fig. 1), receive substantial protection from interaction with degradative enzymes, physiological processes or unfavourable pHs which can lead to rapid breakdown of the non-entrapped drug. For example, as a result of the action of the enzyme cytidine deaminase, cytarabine has a half-life of approximately 20 minutes in humans.[23] When the drug is entrapped in liposomes the half-life can be several hours or more, with the exact time depending on the size and composition of the liposome.[12] Another example is topotecan and related structures. The closed α-hydroxy lactone ring moiety in these structures hydrolyses rapidly at physiological pH, thus inactivating the drugs. Encapsulation of these drugs in liposomes having an acidic internal pH (e.g. 5.5), protects the drugs from hydrolysis and significantly extends their half-lives *in vivo*.[24] Many opportunities exist for the use of liposomal drug formulations to increase the *in vivo* lifetime of peptide and cytokine drugs.[17,25-27]

2.3 Sustained Release Systems

Liposomes release their contents over time. The rate of release depends on drug properties, liposome composition, the presence or absence of pH or osmotic gradients and the liposome environment. As has been shown by a number of researchers, disintegration of liposomes can be promoted by plasma proteins, in particular the apoA1 high density lipoproteins.[28-30]

Liposomes can be engineered to release their associated drugs over shorter or longer periods of time, depending on the liposome composition, the presence of pH gradients or molecular complexes in the liposome interior (fig. 2), the *in vivo* environment of the liposomes (in plasma, subcutaneous depot, localised in a solid tumour, etc.) and the *in vivo* stability of the liposomes.

The ability to control drug release rates, combined with the ability to protect associated drugs from degradation, allows properly formulated liposomes to function as sustained release systems, continually releasing their store of drugs over several hours to several days.[12,31-33] The rate of drug release is believed to be non-linear, i.e., unlike free drug infusion.[12] Manipulation of liposome composition can also result in formulations in which drug release can be triggered at the intended site of action upon exposure of the liposomes to membrane destabilising factors such as fusogenic peptides, transient hyperthermia, local pH changes or ultrasound.[34-38]

2.4 Multidrug Resistance

Evidence is accumulating to indicate that liposome association of drugs may help to overcome multidrug resistance to anticancer drugs mediated by the multidrug resistance-associated protein (MRP) or P-glycoprotein.[39-42] In some instances, liposomal phospholipids like cardiolipin may directly modulate P-glycoprotein transport and in other instances, release of drug in a sustained manner over long time periods (mimicking some aspects of drug infusion) may be involved. In still other instances, fusion of drug-carrying liposomes with the plasma cell membrane, or endocytosis of the liposomal drugs, may deliver the drug to a cell compartment where it cannot readily be pumped out of the cell by the MRP or P-glycoprotein membrane pumps.

2.5 Altered Pharmacokinetics

Perhaps the most compelling property of liposomes is their ability to significantly alter the pharmacokinetics and the biodistribution of many of their associated drugs.[43-45] As long as the drug is associated with its liposomal carrier, it assumes the pharmacokinetics of the carrier, not of the drug. Drugs, when associated with liposomes, are sequestered away from interaction with their normal site of action in the body until they are released from the liposomes. Upon release from the liposome, the released drug becomes free drug and has pharmacokinetic parameters similar to free drug administered at a similar location and at a similar concentration to that of the released drug. As an

example, if we assume that a drug is rapidly released from liposomes in the vasculature, the pharmacokinetic parameters will be very similar to that for the free drug administered intravenously.

For drugs which are very slowly released from liposomes, their pharmacokinetics will be very similar to that of the liposomes themselves and, because of the particulate nature of the carrier, will be characterised by a low volume of distribution (approximately the plasma volume), a slow rate of clearance and a low tendency for distribution into normal tissues with the exception of the MPS (see section 2.6.[44] For drugs with intermediate rates of release, the kinetics will be a combination of the pharmacokinetics of the free drug and that of the carrier. For the purposes of illustration of the substantial changes which can accompany liposome encapsulation of drugs, a comparison of the pharmacokinetics of free doxorubicin and liposomal doxorubicin is given in table II.

2.6 Altered Biodistribution

For much of their early history, the tendency of 'classical' liposomes to alter the biodistribution of their associated drug towards the MPS[43-45] (in particular, fixed macrophages of liver and spleen), was seen as a liability, except in specialised circumstances where one was treating a disease e.g. leishmaniasis, which involved mononuclear phagocytes.[46] Recent years have seen the development of new liposome formulations, having surface coatings such as polyethylene glycol (PEG) (fig. 3), which go a long way towards inhibiting the biodistribution of liposomal drugs to the MPS.[47-49] These formulations, known variously as sterically stabilised liposomes, long-circulating liposomes, cryptosomes, 'Ninjasomes' or Stealth® liposomes result, as their names imply, in an increase in the circulation times of the liposomal drugs and an alteration in the biodistribution of their associated drugs away from the MPS and in favour of some diseased tissues. Although sterically stabilised liposomes are still cleared to a considerable extent by the MPS, this clearance is at a significantly reduced rate and extent than that seen for 'classical' liposomes. Thus, sterically stabilised liposomes show dose-independent, first-order pharmacokinetics, as compared with the non-linear (Michaelis-Menten) pharmacokinetics observed for 'classical' liposomes which saturate their clearance by the MPS at higher doses.[44]

Most liposomal drug formulations are presently being developed for intravenous administration, although the subcutaneous, pulmonary and intraperitoneal routes may also be suitable for liposomal formulations. The topical and transdermal routes have recently been generating considerable interest.[50-52] The experience with liposomal formulations of drugs for oral administration has, to date, been disappointing, although uptake of liposomes by Peyer's patches is attracting attention.[53] Liposomes do not appear to cross the intact, healthy blood-brain barrier to a significant extent, although there is evidence that liposomes may cross this barrier in disease.[54-56]

Because liposomes and their associated drug are confined largely to the central compartment, uptake of the drug into normal tissues is decreased, leading to decreased toxicities in some sensitive normal tissues.[57] For example, when entrapped in liposomes, the anthracycline class of anticancer drugs, which includes doxorubicin and daunorubicin, has much lower cardiac toxicity than the respective free drugs, thus allowing for higher cumulative doses of the drug to be administered.[58-61] It was thought that altered distribution of liposomal

Table II. A comparison of the pharmacokinetics of free doxorubicin and liposomal doxorubicin in humans at a dose of 25 mg/m^2

Formulation	$t_{½\alpha}$ (h)	$t_{½\beta}$ (h)	AUC (mg • h/l)	C_{max} (mg/L)	CL (ml/min)	V_d (L)
Free drug[18]	0.07	8.7	1.0	3.3	755	254
Liposomal drug[18]	3.2	45.2	609	12.6	1.33	4.1

AUC = area under the plasma concentration-time curve from 0 to ∞; **CL** = total body clearance; $\mathbf{C_{max}}$ = peak plasma concentration; $\mathbf{t_{½\alpha}}$, $\mathbf{t_{½\beta}}$ = initial and terminal half-lives; $\mathbf{V_d}$ = volume of distribution.

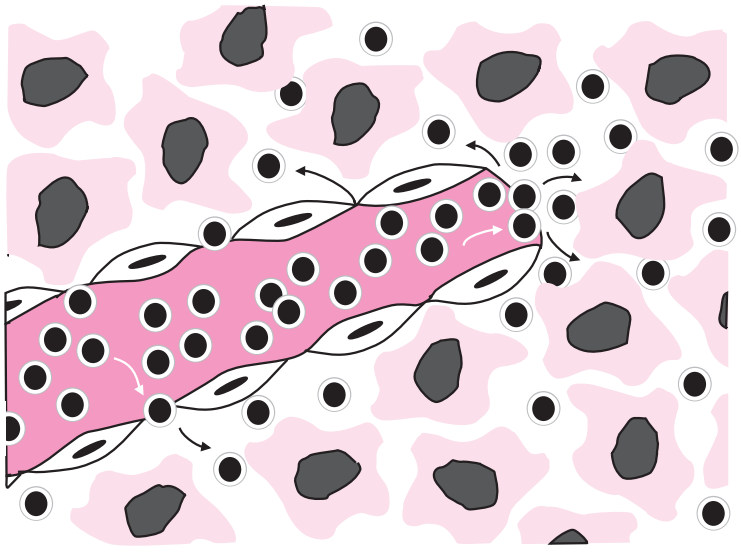

Fig. 3. Passive targeting of liposomes. Figure (not to scale) depicts the passive migration of small (0.1μm diameter) drug-containing liposomes from a leaky tumour capillary into the tumour interstitium. Leakage of drug from the liposomes, with uptake of the released drug by the tumour cells, is thought to be the mechanism of cytotoxicity.

drugs towards the MPS, relative to free drugs, might lead to increased MPS impairment.[62-63] To date, experience has not borne this out. Bone marrow toxicity appears to be similar or less for the liposomal anthracyclines as for the free drugs and liver and spleen toxicities have not been evident for the liposomal formulations.

However, toxicities which have been described for sustained, high-dose therapy with the free drugs may also be observed for liposomal drugs. For example, long-circulating liposomal doxorubicin (Caelyx®) at higher dose regimens causes desquamation of the skin in areas of stress (e.g. hands and feet), a toxicity which has also been described for high dose free doxorubicin.[58,64]

More anticancer, antibiotic and anti-inflammatory drugs are being developed as liposomal formulations than other classes of drugs because of a particularly advantageous property of liposomes. Small liposomes (approximately 100nm or less in diameter) will, if they circulate long enough, extravasate into regions of enhanced capillary permeability, e.g. solid tumours undergoing angiogenesis, or regions of infection or inflammation (fig. 4).[65-67] This enhanced ability of liposomes to localise in tissues with leaky capillaries results in an increased delivery of drug to these tissues and enhanced efficacy.[68,70] Thus liposomes can increase the therapeutic index for drugs by decreasing toxicity and/or increasing efficacy. Access of liposomes to sites of action which involves passage of the liposomes across normal, non-leaky vasculature remains problematic.

3. Future Considerations

Now that several liposomal formulations have received clinical approval, the path is open for the appearance of many additional liposomal drugs in the market. Additional anticancer drugs which are in clinical development include liposomal vincristine, cisplatin, paclitaxel annamycin and hydrophobic derivatives of cisplatin. As new antineoplastic drugs appear on the market, their suitability for liposome encapsulation should be considered.

Liposomal formulations may be particularly useful for rapidly degraded drugs such as cytokines, proteins and peptides, and there is much interest in this area. Similarly, liposomal formulations of antibacterials may allow antibacterial drugs to be directed more selectively towards regions of gross infection, or may allow the antibacterial drug to more effectively reach intracellular organisms. We may even be able to revive some previously discarded drugs whose pharmacokinetic, solubility or toxicity profiles made them unsuitable for use as free drugs.

Until now, the principal mechanisms of action of liposomal drug formulations have been through sustained release of drug and/or passive targeting to areas of increased capillary permeability. For some applications, we may wish to manipulate the liposomal drug formulation further in order to increase the selectivity of interaction between the drug carrier and the target cell. A number of investigators are exploring ligand-targeted liposomes where antibodies or other ligands are attached at the surface of the liposome to increase the binding of liposomes to specific epitopes or receptors at the target cell surface (fig. 5).[70-71] Results to date suggest that this approach may be particularly useful for targets within the vasculature, e.g. haematological malignancies, where the targeted liposomes have unimpeded access to the target cells.[72] Use of antibodies, antibody fragments or ligands against internalising epitopes are thought to be of particular advantage, as binding of the liposome to its target will trigger the entry of the entire drug package into the target cell interior (fig. 5).[70]

A question which is assuming increasing importance is how to manipulate the rate and extent of drug release from the liposomes once they reach their site of action. Research into various ways to trigger release of liposomal drugs at their target tissues is currently underway, and may result in

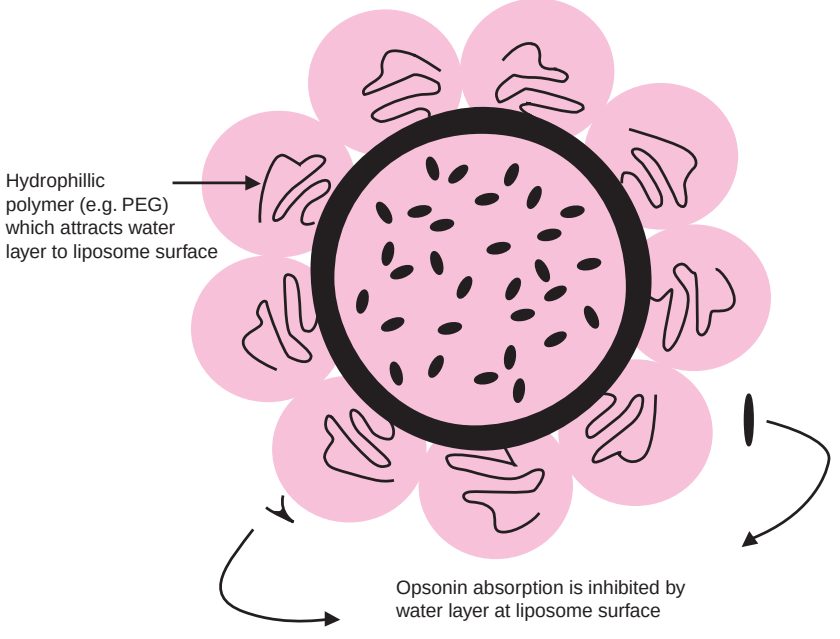

Fig. 4. Long-circulating liposomes. Figure (not to scale) depicting a drug-containing liposome with a surface coating of a hydrophilic polymer. This coating attracts water to the liposomes surface, presenting a barrier to the adherence of protein opsonins. A decrease in opsonisation of the liposomes in turn leads to a decreased rate and extent of liposome uptake into the mononuclear phagocyte system, resulting in increased circulation half-lives. The hydrophilic barrier also retards disintegration of the liposomes through exchange and/or transfer of liposomal phospholipids to high density lipoproteins. **PEG** = polyethylene glycol.

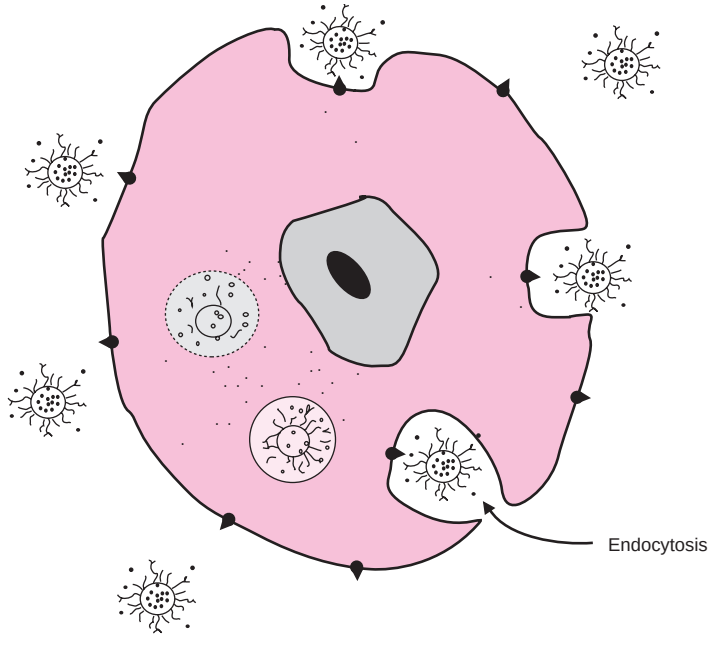

Fig. 5. Antibody-targeted liposomal drugs. Figure (not to scale) depicts the binding of antibody-targeted liposomes (immunoliposomes) to an epitope expressed at the surface of a target cell. In the situation where the epitope is an internalising one, the binding of the antibody-targeted liposome to the epitope triggers internalisation of the liposome-drug package into cellular lysosomes. Over time, if the drug survives the environment of the lysosome, it will be released into the cell to find its cellular site of action.

further improvements in the therapeutic efficacy of liposomal drugs.

Liposomal carriers are also generating considerable interest for their use in vaccines, or as non-viral vectors for gene therapy, but these applications are beyond the scope of this review.[73-76]

In conclusion, an appreciation of how the pharmacokinetics of the drug is changed by the carrier is essential to the development of successful liposomal drug formulations. Also, in order to develop the most appropriate applications for liposomes, as should be apparent from the above discussion, it is necessary to have a good understanding of the physical properties and characteristics of both the liposomal carrier and the drug to be delivered, as well as a clear understanding of the biology and physiology of the disease to be treated. Liposomal drug formulations are not a panacea but, used with imagination and creativity in the appropriate circumstances, they can increase the therapeutic indices for a variety of drugs by increasing the efficacy and/or decreasing the toxicities of their associated drugs.

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