Recent advances in liposomal drug-delivery systems

Arcadio Chonn and Pieter R Cullis

INEX Pharmaceuticals Corporation, Vancouver and The University of British Columbia, Vancouver, Canada

Liposomal drugdelivery systems have come of age in recent years, with several liposomal drugs currently in advanced clinical trials or already on the market. It is clear from numerous pre-clinical and clinical studies that drugs, such as antitumor drugs, packaged in liposomes exhibit reduced toxicities, while retaining, or gaining enhanced, efficacy. This results, in part, from altered pharmacokinetics, which lead to drug accumulation at disease sites, such as tumors, and reduced distribution to sensitive tissues. Fusogenic liposomal systems that are under development have the potential to deliver drugs intracellularly, and this is expected to markedly enhance therapeutic activity. Advances in liposome design are leading to new applications for the delivery of new biotechnology products, such as recombinant proteins, antisense oligonucleotides and cloned genes.

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Introduction

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of liposomes as drug carriers was recognized more than 25 years ago [1] and, since that time, liposomes have been used in a broad range of pharmaceutical applications (Table 1). This review first highlights some of the key advances of the past decade in the design of liposomes for systemic delivery and then reviews the most recent literature involving specific applications of liposomal drug-delivery systems.

Liposome technology

Preparation of liposomes

Liposomes can be prepared by a variety of methods (extensively reviewed in [2,3]). In general, on the basis of size and lamellarity (number of bilayers present within a liposome), liposomes are classified into three categories: multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs).

Drug loading

Drug loading can be achieved either passively (i.e. the drug is encapsulated during liposome formation) or actively (i.e. after liposome formation). Hydrophobic drugs, such as amphotericin B. taxol or annamycin, can be directly incorporated into liposomes during vesicle formation, and the extent of uptake and retention is governed by drug-lipid interactions. Trapping efficiencies of 100% are often achievable, but this is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs relies on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping efficiencies (generally <30%) are limited by the trapped volume contained in the liposomes and drug solubility. Alternatively, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients [4], which can result in trapping efficiencies approaching 100%

liposomes with prolonged circulation lifetimes

A significant advance in the development of liposomal drugs has come with the use of specialized lipids, such as monosialoganglioside G_{M1} or polyethylene glycol

Abbreviations

CFTR—cystic fibrosis transmembrane receptor; DOPE—dioleoylphosphatidyl ethanolamine; EGF—epidermal growth factor; FDA-Food and Drug Administration; HSV—herpes simpex virus; IL—interleukin; WV-large unilamellar vesicle; MDR—multidrug resistance; MLV—multilamellar vesicle; MTP-PE—muramyl tripeptide phosphatidyl ethanolamine; PEG-P&polyethylene glycol modified phosphatidyl ethanolamine; POPC—1-palmitoyl-2-oleoylphosphatidylcholine; SUV-small unilarnellar vesicle.

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Table 1. Liposomal drugs currently under development or on the market.

Product name (if any)	Drug	Company/institution	Phase of development
Conventional drugs			
ABLC (Abelcet)	Amphotericin B	The Liposome Company, Princeton, USA	Marketed in UK and Luxembourg. Awaiting approval for treatment of aspergilfosis
AmBisome	Amphotericin B	NeXstar Pharmaceuticals Inc, Boulder, USA	Marketed in certain countries in Europe
Amphocil	Amphotericin B	Sequus Pharmaceuticals Inc,	Awaiting FDA approval
Doxil (DOX-SL)	Doxorubicin	Menlo Park, USA	FDA-accelerated approval for treatment of Kaposi's sarcoma
TLC D-99	Doxorubicin	The Liposome Company Princeton, USA	Phase III
TLC C-S3	Prostaglandin E_1	The Liposome Company Princeton, USA	Phase II
DaunoXome	Daunorubicin	NeXstar Pharmaceuticals Inc. Boulder, USA	Approval for treatment of Kaposi's sarcoma; in Phase II trials for breast cancer, small cell lung cancer, leukemia and lymphoma
AR-121	Nystatin	Argus Pharmaceuticals Inc,	Phase II
Tretinoin (AR-623)	All-trans retinoic acid	The Woodlands, Texas, USA	Phase II (leukemia) and phase I (Kaposi's sarcoma)
	Annamycin	Argus Pharmaceuticals Inc, The Woodlands, Texas, USA	Phase I
	Vincristine	INEX Pharmaceuticals Corporation, Vancouver, Canada	Phase I
Proteins			
OncoLipin	IL-2	Oncotherapeutics,	Phase II (kidney cancer)
OncoVax	IL-3 and cancer tumor antigen	New Jersey, USA	Phase I
Genes and antisense oligonucleotides			
Allovectin-7	pHLA-B7/b-2	Vical, San Diego and University of Michigan, Michigan, USA	Phase I completed
	pHLA-B7/b-2	Mayo Clinic, Rochester, USA	Phase I
	pHLA-B7/b-2	University of Chicago, Chicago, USA	Phase I
	pHLA-B7/b-2	AZ Cancer Center, USA	Phase I
	CFTR gene	Medical Research Council, UK	Phase I completed
	pKCTR	University of Alabama, Alabama, USA	Phase I, pending FDA approval
	pBMC-neo-hIL-2	University of Miami, Miami, USA	Phase I, pending FDA approval
	pCMV4-AAT	Vanderbilt University, Nashville, USA	Phase I, pending FDA approval
	pMP6-IL-2	Duke University, Durham, North Carolina, USA	Phase I, pending FDA approval

modified phosphatidyl ethanolamine (PEG-PE), that engender long circulation lifetimes when incorporated into liposomes [5-7] Alternatively, the presence of entrapped cytotoxic drug can also lead to extended circulation times [8]. It **has** been demonstrated that increased circulation lifetimes ***enhance the*** opportunity for liposomes, administered systemically, to leave the vascular compartment and enter certain extravascular regions [9-1] Tumors, for example, exhibit leaky blood vessels that have a reduced ability to retain circulating macromolecules [12, 13]. Liposomes can extravasate in these regions, thus leading to preferential accumulation within tumors. Studies have now clearly demonstrated that long-circulating liposomes containing PEG-PE or cytotoxic drugs, such as doxorubicin, accumulate within these sites preferentially compared with conventional liposomes [9,11,14].

Targeted delivery

It is envisioned that the next generation of liposomal pharmaceuticals will consist of drug-loaded liposomes with surface-associated targeting information (Pig. I). Site-directing targeting ligands, such as monoclonal antibodies, can be attached to liposomes by either covalent or non-covalent methods [15-17]. The advent of novel PEG-PE lipids that allow targeting ligands to

be conjugated at the distal ends of the PEG spacer has afforded both effective target binding in vitro and prolonged circulation times [18•,19–21].

To date, only two studies have demonstrated the improved therapeutic activity of liposomal drugs *in* vivo achieved through the use of antibody-mediated targeting [22,23], with both studies employing a monoclonal antibody against lung endothelial thrombomodulin (mAb 34A) and intravenously injected tumor cells. The use of immunoliposomes may be limited because of their potential immunogenicity [24].

In addition to antibodies, glycolipids (e.g. galactose [25] and mannose [26]), proteins (e.g. transferrin [27] and asialofetuin [28•]), and vitamins (e.g. folic acid [18•,29]) have been used to target specific cells via cell surface receptors.

intracellular delivery

Liposomes can facilitate the intracellular delivery of drugs by fusing with the target cell. Alterations in the lipid composition can render liposomes pH sensitive, leading to enhanced fusogenic tendencies in low **pH** compartments such as endosomes [30]. The inclusion of lipids that are able to form non-bilayer phases, such as dioleoylphosphatidyl ethanolamine (DOPE), can promote destabilization of the bilayer, inducing fusion events. DOPE has been particularly useful for cationic liposomes complexed with plasmid DNA for gene delivery [31•,32].

Conventional drugs

A vast literature describes the feasibility of formulating a wide range of conventional drugs in liposomes, often resulting in enhanced therapeutic activity and/or reduced toxicity compared with the free drug. In general, altered pharmacokinetics for liposomal drugs can lead to enhanced drug bioavailability to specific target cells that reside in the circulation, or more importantly, to extravascular disease sites such as tumors. Recent

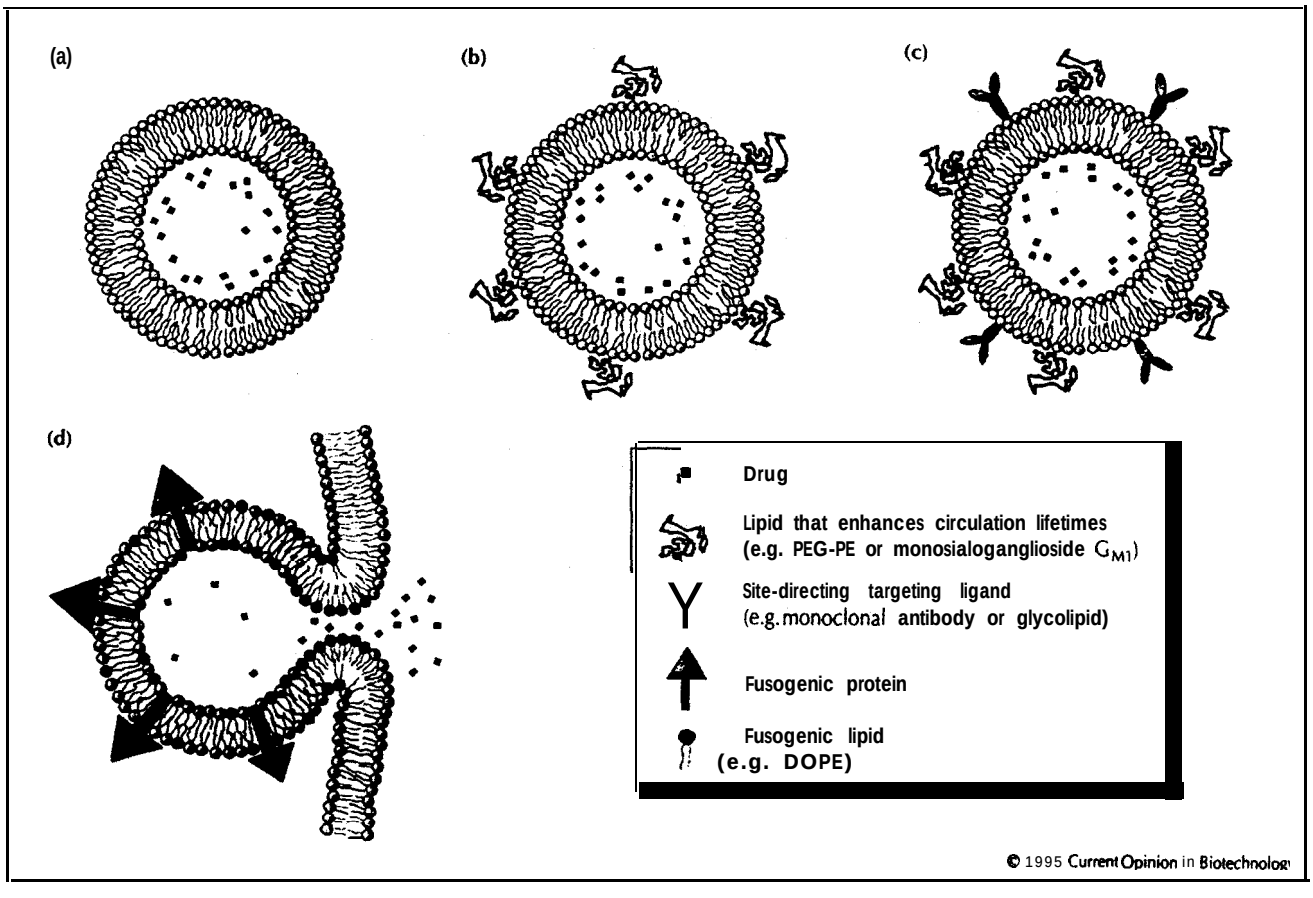

Fig. 1. Types of liposomal delivery. (a) Liposomes prepared from natural or synthetic phospholipids containing an encapsulated drug. This type of drug delivery reduces toxicity, maintains or enhances activity and facilitates accumulation in the disease site. (b) Conventional liposomes that incorporate lipids enhancing circulation lifetimes. Delivery in these molecules improves access to the disease site and reduces interaction with phagocytic cells of the reticulo-endothelial system. (c) Conventional liposomes with lipids that enhance circulation lifetimes and have surface-associated targeting information. Drug delivery using this type of liposome results in an improved therapeutic index and target cell specific delivery. (d) Fusogenic liposomes with DOPE or fusogenic proteins. This method allows intracellular drug delivery.

advances include liposomal formulations of all-trans retinoic acid [33, 34] and daunorubicin [35-38], which has recently received Food and Drug Administration (FDA) approval as a first-line treatment of AIDS-related advanced Kaposi's sarcoma. Notable examples are given below.

Amphotericin B

Liposomal amphotericin B drugs are presently approved for sale in certain European countries and are nearing regulatory approval in North America. Acute toxicities associated with amphotericin B are markedly reduced with liposomal formulations, without losing broad-spectrum antifungal activity. Early studies on a variety of formulations of liposomal amphotericin B demonstrated the successful treatment of fungal infections in mice [39,40]. Recent studies have focused on understanding the possible mechanisms for reduced toxicities, which include altered pharmacokinetics [41, 42] and increased association with high-density lipoproteins [43,44].

Although most applications involve am intravenous route of administration to treat ***systemic*** fungal infections, liposomal amphotericin B can also **lbe** administered in an aerosolized form, resulting in treatment of systemic ***Candida albicans*** or *Cryptococcus neoformans* infections in mice [45,46].

Doxorubicin

Phase III clinical trials on liposomal doxorubicin are ongoing. As demonstrated in several pre-clinical and clinical reports, the administration of liposomal doxorubicin significantly reduces drug-associated cardiotoxicity beause cardiac uptake of liposome-encapsulated doxorubicin is substantially reduced compared with the free drug. A variety of lipo:some-doxorubicin formulations have been described. These include PEG-stabilized liposomes [14], as well as conventional egg phosphatidylcholine/cholesterol LUVs [8]. Recently described formulations that extend the circulation haIf-life of doxorubicin include dipalmitoylphosphatidylcholine/cholesterol (1 :l) liposomes containing 10 mol% paImityl-D-glucuronide, a uronic acid derivative [47], and fluorinated liposomes [48]. Targeted liposomal doxorubicin systems have recently been described **[29,49•];** however, their efficacy, compared with non-targeted systems in vivo, has yet to be determined.

Of the above types of formulation, reports of PEG-coated liposomal doxorubicin dominate the recent literature **[50•,51–61].** Much attention has focused on the use of liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma [55-58,62]. In the prolonged use of liposomal doxorubicin for AIDS-related Kaposi's sarcoma, hand-foot syndrome may be a limiting toxicity [55]. With regard to liposomal doxorubicin-induced toxicities, a recent report indicates that the depletion and impairment of phagocytic activity of rat liver macrophages by Iiposomal doxorubicin can be substantial [63]. Whether this finding applies to humans remains to be seen. To date, severe hepatic toxicities have not been reported in any clinical trial.

The increase in therapeutic index of liposomal doxorubicin most likely results from the 'passive* targeting to tumor sites of liposomes, which because of the leaky vasculature, exhibit increased extravasation. This is particularly relevant for liposomes with long circulation lifetimes. At the tumor site, liposomes appear to act as a depot for slow release of drug. This model is supported by the findings of Suzuki et al. [64] indicating that liposomal doxorubicin remaining on the cell surface is more cytotoxic than endocytosed liposomal doxorubicin. Furthermore, several reports indicate that hyperthermia induces the release of doxorubicin from long-circulating liposomes and enhances their antitumor efficacy [59-61].

Natural or acquired resistance to doxorubicin may limit the clinical use of liposomal doxorubicin. Different ways of overcoming multidrug resistance, including the use of modulators that can inhibit drug efflux mediated by P-glycoprotein [65], have been explored and have proved effective in in ***vitro systems. Several*** successful attempts have also been described, at least ***in*** vitro, to overcome multidrug resistance by employing structurally different analogs of anthracyclines entrapped in liposomes **[66,67•].** For instance, the non-cross-resistant anthracycline antibiotic, annamycin, formulated in dimyristoylphosphatidylcholine/dimyristoylphosphatidylglycerol SUVs or MLVs, is more effective than doxorubicin against several tumor models, and multidrug resistance shows only partial cmssresistance to annamycin, both ***in vitro*** and in vivo **[67•].**

Vincristine

The benefits of prolonged drug bioavailability as a result of administering the drug in a liposomal form is perhaps best exemplified by liposomal vincristine, an important anticancer drug effective against a wide variety of neoplasms. Vincristine is a cell cycle specific drug that arrests cell growth exclusively during metaphase by attaching to the growing ends of microtubules and inhibiting their assembly. As such, prolonged exposure of neoplastic cells to vincristine should greatly enhance its therapeutic index. Indeed, increased drug retention and increased circulation longevity, as achieved by encapsulating vincristine in distearoylphosphatidylcholine/cholesterol LUVs with an internal pH of 2.0, act synergistically to significantly **enhance** the circulation lifetime of encapsulated vincristine, the extent and duration of tumor exposure to vincristine, and ultimately, the therapeutic activity of vincristine **[68•,69].**

The development of a liposomal formulation of vincristine, employing sphingomyelin/cholesterol LUVs with an internal pH of 4.0 or 2.0 has recently been de-

scribed [70••]. This formulation displays significantly enhanced stability and antitumor properties compared with distearoylphosphatidylcholine/cholesterol LW systems [70••]. Substantially increased vincristine accumulation, compared with the free drug, is observed in both peritoneal ascitic murine P388 tumors and subcutaneous solid A431 human xenograft tumors. In addition, a recent report of a liposomal vincristine formulation employing PEG-PE shows an enhanced therapeutic index for vincristine entrapped in liposomes against subcutaneously or intraperitoneally implanted P388 tumor cells [71].

As is the case for several toxic conventional drugs, liposomal vincristine exhibits reduced toxicity compared with the free drug [72]. Particularly notable is the greatly enhanced efficacy that can be achieved for liposomal vincristine compared with equivalent doses of the bee drug. Liposomal vincristine is currently in clinical trials.

Proteins and peptides

The majority of current hposomal protein formulations are still in various preclinical research stages (recently reviewed in [73]), with one Iiposomal interleukin (IL)-2 drug entering a phase II clinical trial for kidney cancer. For the production of artificial blood substitutes, the use of liposomes to encapsulate hemoglobin is actively being investigated for the ***in vivo*** delivery of hemoglobin without many of the inherent toxicities associated with the delivery of the free molecule (recently reviewed in [74-76]). Another area of intense research is the application of liposomes exhibiting improved adjuvancy for vaccine development.

Immunomodulaton: interleukins

The feasibility of formulating cytokines in MLVs [77-791 and in sterically stabilized SUVs [80,81•] has recently been demonstrated. These Iiposomal cytokines show great promise as immunoadjuvants for vaccine development. IL-2 enapsulated in sterically stabilized SUVs (65 nm in diameter) is significantly more effective than free IL-2 both in increasing leukocyte number in the blood and spleen and in triggering spleen lymphokine-activated killer-cell activity [81•]. Co-injection of phosphatidylcholine/cholesterol (1:l) MLVs containing IL-6 (50 000 U IL-6 mouse-l) or 65 kDa heat-shock protein antigen (0.03 μg mouse-r or 0.3 μg mouse-r) significantly enhanced secondary antibody responses at antigen dosages where other adjuvants (e.g. Ribi or dimethyldioctadecylammonium-bromide) exhibit no adjuvant activity [79]. Liposomal formulations of IL-7 have been shown to enhance the immune responses of mice vaccinated with either alum-associated or liposome-formulated recombinant HIV envelope protein env-2-3SF2 [82]. Antibody titers resulting from vaccination with liposome-formulated antigen were higher than those with alum-associated antigen, and these antibody responses were enhanced by concurrent administration of IL-7 liposomes. In addition, immunogenicity of alum-associated herpes simplex virus (HSV) gD antigen can be enhanced by a recombinant IL-7 liposomal formulation, resulting in a significantly reduced severity and course of primary HSV-2 infection. The sustained release of IL-7, over a period of >6 days, contributes to the observed effects [83•].

Recent reports also indicate that unencapsulated cytokines, at relatively low doses, augment the therapeutic effects of liposomal reagents [84•,85]. For instance, unencapsulated recombinant IL-2 administered intraperitoneally (10 000 U day^{-1}), in combination with intravenously administered phosphatidylcholine/phosphatidylserine (1: 1) MLVs containing a synthetic peptide derived from C-reactive protein (RS-83277), significantly inhibited tumor metastases and prolonged survival of C57B1/6 mice bearing established pulmonary metastases of fibrosarcoma T241. The combination therapy was accompanied by an increase in the number of $Thy1.2^{+}$ cells in the lungs of RS-83277 MLV/IL-2 treated animals compared with those receiving RS-83277 MLVs alone.

Liposomal muramyl tripeptide

Muramyl tripeptide phosphatidyl ethanolamine (MTP-PE) is a synthetic lipophilic analog of muramyl dipeptide, the smallest component of a mycobacterium capable of stimulating the immune system. MTP-PE is a potent monocyte/macrophage activator and is currently under clinical investigation against metastatic melanoma and osteosarcoma (reviewed in [86]). Of major interest to the development of liposomal MTP-PE immunomodulators is the recent finding that repeated per os (oral) administration of l-palmitoyl-2-oleoylphosphatidylcholine (POPC) MLVs containing a synthetic muramyl tripeptide, CGP 19835A (Ciba-Geigy, Basel, Switzerland), increased the tumoricidal activity of alveolar and peritoneal macrophages against renal cell carcinoma [87••]. Liposomes were rapidly absorbed in the intestine and reached the systemic circulation within 4 h, as determined by the biodistribution of radioactively labeled, or N-4-nitrobenzo-2-oxa-1,3-diazolephosphatidyl ethanolamine fluorescently labeled POPC-CGP 19835A liposomes. The mechanism of liposomal muramyl tripeptide antitumor activity is linked to its activation of monocyte/macrophage tumoricidal function, as shown by several recent reports [88-90].

Antisense oligonucleotides, ribozymes and genes

Antisense molecules and ribozymes present interesting challenges for delivery systems. The efficacy of these

drugs is dependent on their ability to gain entry into cells in an intact form; however, they are particularly susceptible to degradation by nucleases in the biological milieu and usually cannot cross the target cell membrane. For example, in general, phosphodiester antisense oligonucleotides have been reported to have little or no inhibitory effect in culture because they are rapidly degraded in the culture medium. In addition, these molecules are highly charged and can activate the complement system, resulting in the generation of anaphylatoxins and other immunomodulators. The potential of liposomes to encapsulate antisense oligonucleotides or DNA, protecting them from nucleases and complement, represents a great advantage over other drug carriers, such as polymers or immunoconjugates. The further potential for fusogenic liposomes to promote intracellular delivery of these compounds is also of major importance. The application of liposomes to deliver antisense oligonucleotides, ribozymes and genes is an area of intense research.

Antisense oligonucleotides and ribozymes

Several reports demonstrate the feasibility of employing liposomal systems to deliver antisense oligonucleotides, with the accompanying significant enhancement of efficacy in vitro and in vitro [91,92,93•,94•]. Cellular uptake of fluorescently labeled oligonucleotides is significantly enhanced by cationic liposomes, as assessed by confocal laser scanning microscopy, flow cytofluorometry and laser-scanning microscopy. Intact oligonucleotides are found in the cytoplasm and nucleus only when they are delivered by cationic liposomes.

The overwhelming conclusion from studies to date is that liposomes are able to resolve the problems of extracellular degradation by nucleases and poor membrane permeability that are inherent for oligonucleotide drugs. This has been achieved using a variety of liposomal compositions, with the majority employing cationic lipids and DOPE [93•,95–97]. A recent report describes an extensive physicochemical study of the aggregation and **fusion reactions that occur** during the formation of oligonucleotide and cationic liposomal complexes in solution [98]. Furthermore, several approaches to encapsulate antisense oligonucleotides have been described. A probe sonication method employing phosphatidylcholine/cholesterol/dipalmitoylphosphatidylethanolamine covalently coupled to L-polylysine (5.5:3.0:1.5) has recently been shown to result in liposomes with a diameter of 1 10-140nm and encapsulation efficiencies ranging from 55% to 100% depending on the oligonucleotide [99]. The use of immunoliposomes has also been described [100]. Aigner and Caroni [101•] report the use of liposomes composed of phosphatidylcholine/phosphatidylserine (10:1) and myelin proteins derived from adult rat spinal cord or sciatic nerve to deliver antisense oligonucleotides to dorsal root ganglion neurons. In addition, liposomes containing viral fusion proteins, derived from Sendai virus, have been used to promote fusion with target cells [94•]. Wang et al. [102••] describe the use of phosphatidylcholine/cholesterol(3:2) containing 0.5mol% folate conjugated to PEG-distearoylphosphatidyl ethanolamine to deliver antisense oligonucleotides against human epidermal growth factor (EGF) (up to 2.0x 107 molecules $cell^{-1}$) in a folate-specific manner, as free folic acid competes with EGF uptake.

An interesting approach to increase the association of antisense oligonucleotides with liposomes involves coupling antisense oligonucleotides to cholesterol via a reversible disulfide bond [103•,104–107]. Using this method, the association of oligonucleotides with immunoliposomes is improved by a factor of -10. The capacity of modified oligonucleotides directed against the *tat* gene of HIV-1 to inhibit HIV-l proliferation in acutely infected cells has been found to be the same as the unmodified oligonucleotide on an equimolar basis (IC_{50} =0.1 μM) [103•].

To date, only a few papers have reported the use of cationic liposomes to deliver ribozymes, a class of RNA molecule that possesses enzymatic cleavage activity [108•,109,110•,111•]. Ribozymes, being RNA molecules, are highly susceptible to nuclease digestion. Their stability is markedly increased ***in vitro*** in the presence of cationic liposomes, with >30% remaining intact after a 60min incubation in medium containing 10% fetal bovine serum. The feasibility of using a variety of cationic liposomes. to deliver ribozyrnes into cultured cells in vitro has recently been described for ribozymes directed against leukocyte-type 12-lipoxygenase mRNA [108•], bcr-abl mRNA [110•], or multiple drug resistance (MDR)-1 mRNA [111•]. Liposome-mediated transfer of ribozymes against MDR-1 mRNA was shown to reverse the MDR phenotype of adriamycin-resistant and vindesine-resistant human pleural mesothelioma cell lines and restored sensitivity to chemotherapeutic drugs [Ill']. As with antisense oligonucleotides, it is likely that liposomal systems will provide significant advantages to the delivery of ribozyme molecules in vivo. The development of such liposomal formulations is advancing rapidly

Genes

Several reviews on the use of liposomes to deliver genes have appeared recently (see [112-l 14]; this issue, Cunliffe, Thatcher and Craig, pp 709-713). Although the utility of cationic liposomes in the delivery of reporter genes was noted in the early 1980s, we are only now beginning to characterize these systems and to understand the cellular processes -that are required. For instance, the role of DOPE in mediating cytosolic delivery of plasmid DNA has now been elucidated [32,115]. It has been shown that the principal route of cationic liposome-mediated gene transfer occurs after endocytosis [116•]. Recent electron microscopy studies have attempted to reveal the structural features of plasmid

DNA-cationic liposomal complexes [117,118], which remain relatively poorly characterized. As with all liposomal drugs, well characterized liposomal systems will be the DNA carrier of choice. Procedures to produce well defined liposomal systems with encapsulated DNA, to protect the DNAfrom nuclease degradation, are at early stages of development.

The major barriers in the cellular processing of liposome-DNA complexes have recently been described [119••]. On average, COS-1 cells take up 3x 105 plasmids after 6 h of incubation in the presence of N-[1-(2,3-dimyristyloxy)propyl]- N, N-dimethyl- N-(2-hydroxyethyl) ammonium bromide/DOPE-DNA complexes; however, after 24 h, the majority of the DNA-lipid complexes aggregate into large perinuclear complexes, with only a small amount of DNA in the cytoplasm of most cells. Another important factor is that the lipid and DNA must dissociate before transcription can occur. The maturation of liposomes as a viable systemic gene delivery vehicle in vivo will thus require the following steps: first, liposomes should be targeted to endocytic receptors in order to enhance the rate of endocytosis; second, fusion processes (mediated by lipids or proteins) should be optimized in order to enable efficient escape from the endosome and entry into the cytoplasm; and third, cytoplasmic stability and nuclear targeting of the plasmids should be enhanced.

Results from a phase I clinical study on cationic liposome-mediated cystic fibrosis transmembrane regulator (CFTR) gene transfer to the nasal epithelium of patients with cystic fibrosis has recently been reported [120•]. No adverse clinical effects were observed from cationic liposome-mediated gene transfer to nasal epithelia.

Conclusions

After three decades of development, liposomes are Willing their promise as a drug delivery vehicle with general applications. Liposomal drugs exhibit reduced toxicities and retain, or gain enhanced, efficacy compared with their free counterparts. Liposomes that allow enhanced drug delivery to disease sites, by virtue of long circulation residence times, are now achieving clinical acceptance. Also at hand are liposomes that promote targeting to particular diseased cells within the disease site. Finally, liposomes are showing particular promise as intracellular delivery systems for proteins/peptides, antisense molecules, ribozymes and DNA. The development of liposomes that can be administered systemically and exhibit targeted and fusogenic properties appears to be increasingly within our grasp.

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A Chonn, INEX Pharmaceuticals Corporation. 1779 West 75th Avenue, Vancouver, British Columbia V6P 6P2, Canada.
E-mail: achonn@inexpharm.com

PR Cullis, Department of Biochemistry and Molecular Biology, The University of British Columbia, 2146 Health Sciences Mali, Vancouver, British Columbia V6T1Z3, Canada and INEX Pharmaceuticals Corporation, 1779 West 75th Avenue, Vancouver, British Columbia V6P 6P2, Canada.
E-mail: pcullis@inexpharm.com