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LIPOSOMES FOR DRUG DELIVERY TO THE RESPIRATORY TRACT

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ABSTRACT

This paper provides a review of the current literature pertaining to the pulmonary delivery of liposomes.

The technological aspects of delivering liposomes to the lung are discussed, including the characterization of liposome-containing aerosols and the potential advantages and disadvantages of the various methods which have been employed for their generation.

Studies have indicated that liposomes can be effectively deposited in the human respiratory tract, wherein they may remain for prolonged periods. A prolonged retention in the airways may markedly alter the pharmacokinetics of liposome associated materials; increasing local concentrations, whilst decreasing levels at sites distant from the lung. The future potential for such

systems is discussed, including the possibilities for selective drug delivery to specific cell populations within the lung.

INTRODUCTION

Liposomes are one of a number of carrier systems currently being investigated for drug delivery to the lung. They would seem particularly appropriate for this application, since they can be prepared from phospholipids found naturally in the lung as components of pulmonary surfactant. Liposome encapsulation may be employed as a means of altering the pharmacokinetics of materials; prolonging their residence time within the airways, decreasing distribution to other sites or as a means of achieving sustained plasma levels.

The tortuosity of the respiratory tract represents a barrier to deep lung particle penetration. Aerosols in the so called "respirable fraction", i.e. capable of efficient penetration to the bronchioles and alveoli require a particle size of 5 to 6μm or less (1). Therapeutic systems for lung delivery must be formulated with this aspect in mind. Currently there are three broad categories of device available for the delivery of materials to the peripheral airways, namely; nebulizers, metered dose inhalers and dry powder inhalers.

DEVICES FOR PULMONARY DELIVERY OF LIPOSOMES

1. Nebulizers

Nebulizers employ compressed gas or ultrasound to generate aerosols from aqueous solutions or suspensions of drugs. All reported studies of pulmonary liposome delivery to humans have used nebulizers for their delivery (2-5). Nebulizers offer a number advantages for such administration:

Formulation of preparations is relatively simple, since liposomes can be

prepared by conventional techniques and following removal of non-vesicle associated material, usually require no further processing. Additionally, whereas metered dose inhalers and dry powder inhalers deliver small unit doses, nebulizers are capable of delivering relatively large dose volumes.

With jet nebulizers, the most important factors determining the final aerosol droplet size produced are; the design of the device (6) and gas flow rate/ pressure (7). Properties of the liquid being nebulized, such as surface tension and viscosity (8) and drug solubility (9) may also affect the final aerosol characteristics.

Taylor and Farr (10) have reported the size characteristics of aerosols generated from liposome preparations by two models of nebulizer, one designed for bronchiolar drug deposition (Hudson), the other for alveolar deposition (Respirgard). At high and low air flow rates, the mass median diameters (MMD) and size distributions were independent of the properties of the liposomes being atomized. The study included vesicles with phospholipid in the gel state, liquid crystalline state and those with bilayers containing cholesterol. By extrusion through polycarbonate membrane filters or probe sonication vesicles were produce with sizes ranging from 100 nm to 6.2 μm. Thus the aerosol characteristics of atomized liposome formulations is determined by the choice of nebulizer and the gas flow rate and is independent of vesicle properties.

The stability of liposome formulations of the hydrophilic drug, sodium cromoglycate to atomization in a Hudson nebulizer was investigated using a multi-stage liquid impinger (11). Egg phosphatidylcholine (PC)/cholesterol (Chol) (1:1) multilamellar liposomes (MLVs) and reverse-phase evaporation

TABLE 1

The Properties of Nebulized PC/Chol Liposomal Formulations of Sodium Cromoglycate (Adapted from REF. 11)

	MLV	REV	REV
Size before nebulization (μm)	5.4	3.4	1.2
Size after nebulization (μm)	2.7	2.5	1.1
Drug lost (%)	50.8	31.9	16.6
(± SE)	(0.5)	(2.6)	(2.4)
MMAD (μm)	2.7	2.8	2.6
σ_g	2.0	1.9	2.0

liposomes (REVs) were unstable to nebulization, which resulted in reduction of mean vesicle sizes (Table 1), causing a loss of entrapped drug. Drug loss was reduced when the size of REVs was reduced to 1.2 μm by extrusion prior to nebulisation. Loss of entrapped material was reduced to less than 4% when MLVs were sonicated (mean size 110 nm) prior to nebulisation (12). The size characteristics of the liposomal sodium cromoglycate aerosols did not differ significantly (Table 1). The mass median aerodynamic diameters (MMAD) and geometric standard deviation (σ_g) for all formulations were in the range that is predicted to be deposited in the peripheral airways.

Other studies have also reported a reduction in vesicle size on nebulisation (2,3), whilst the importance of size for vesicle stability has also been reported

for nebulized carboxyfluorescein containing liposomes (13). Niven et al (14) have investigated how operating conditions such as air pressure, osmotic strength and pH affect vesicle stability to nebulization. The greatest effect was produced by changes in the air pressure. A loss of 1.3% of entrapped carboxyfluorescein increased to 88% when the air pressure used to generate the aerosols was raised from 4 to 50 p.s.i.

These studies suggest that the appropriate liposome formulation for delivery from jet nebulizers will be determined by the partition coefficient and therapeutic dose of the drug. Extruded REVs may be most appropriate for delivering hydrophilic drugs with a large therapeutic dose, being relatively stable to nebulization and capable of entrapping significantly greater amounts of drug than comparable MLV or sonicated liposome preparations (15). Small MLVs are very stable to nebulization and may be the vesicles of choice for delivering hydrophilic drugs with a small therapeutic dose.
Hydrophobic drugs are associated with the phospholipid bilayers of liposomes, consequently, disruption of vesicles may be less significant for these materials.

Ultrasonic nebulizers generate aerosols by vibrations in a piezoelectric crystal. The aerosol characteristics are determined by the ultrasonic frequency and the surface tension and density of the liquid being atomized (16).
PC and small dipalmitoylphosphatidylcholine (DPPC) liposomes were stable to ultrasonic atomization (17). However, DPPC vesicles with diameters of 500 nm increased in size within the nebulizer, suggesting fusion of vesicles. Fusion would be expected to result in losses of significant amounts of entrapped hydrophilic materials.
During ultrasonic nebulization the temperature of the liquid within the nebulizer increases (16). Such an increase in temperature may result in an

increased rate of drug loss from vesicles in the nebulizer, particularly if the transition temperature of the lipid component of vesicles is exceeded.

The droplet size of aerosols produced by ultrasonic nebulizers tends to be larger than from jet nebulizers (18,19). Such devices, although capable of producing aerosols for penetration to the conducting airways, may be inappropriate for applications requiring efficient drug deposition in the alveolar regions.

Prior to delivery of liposomes from a nebulizer, it is usually necessary to remove non vesicle associated material from the preparations. Techniques employed have included dialysis (4), ultrafiltration (5) and gel filtration (2). To be commercially acceptable liposomal formulations for pulmonary delivery should be available in a stable, readily stored form. In most cases stable formulations of liposomes which retain the majority of encapsulated material can not be produced, even at 4°C. In the presence of certain disaccharides liposome preparations can be dehydrated (20). Dry powdered material can be stored for up to 2 years and when rehydrated possesses the same release characteristics as non dehydrated/rehydrated vesicles. Such a strategy may enhance the commercial potential of nebulized liposomal systems.

2. Metered dose inhalers

Pressurized metered dose inhalers (MDIs) are widely used for the administration of anti-asthma drugs. They comprise of drug suspended as a micronized dispersion, or sometimes dissolved in a chlorofluorocarbon (CFC) blend. Upon actuation of the device, a small, precise fraction of the liquid formulation is emitted which is rapidly disrupted into a polydisperse aerosol. As these devices are largely tamper-proof and hold the active drug in a

chemically inert environment during storage they would to offer certain advantages for pulmonary liposomal delivery systems.

Farr et al (21) proposed the in situ formation of liposomes from phospholipid/CFC solutions following aerosol deposition onto a moist surface. This was demonstrated in vitro using a multi-stage liquid impinger. However, the respirable fractions of emitted aerosols were small and directly related to the phospholipid concentration within the formulation. Drug entrapment within liposomes formed following the hydration of aerosol droplets emitted from MDIs containing a solution of the drug and phospholipid, is dependent on the physico-chemical character of the drug molecule (22). Thus negligible incorporation of an ionised, hydrophilic drug salbutamol was demonstrated. In contrast, the entrapment of a model hydrophobic compound, hydrocortisone octanoate was highly efficient. These data purport to the utility of this approach for the controlled release of hydrophobic but not hydrophilic agents. With the latter category of drugs, a second formulation strategy based on the conventional suspension system, may be more appropriate. Radhakrish et al (23) describe the preparation of dehydrated liposomes containing a drug such as salbutamol. These are suspended in a CFC blend exhibiting poor solubilising capacity for phospholipids. The advantage of this approach is that liposomal drug entrapment may be maximised before inclusion into the pressurised system, ensuring an effective sustained release system following inhalation.

3. Dry powder inhalers

Dry powder inhalers, developed in the 1970s (24,25) have received renewed interest in recent years as propellant free alternatives to MDIs. Drug is micronized to less than 5 μm and packaged into the device directly or via a

gelatin capsule or foil disk. The powder is aspirated into the airways as a patient inhales.

Such a device can be employed for pulmonary liposome delivery (23). Drug containing vesicles may be spray dried to produce particles less than 2 μm and filled into a capsule. Powder can then be discharged into an airstream drawn through the device. This approach to pulmonary vesicle delivery relies on rehydration of vesicles in situ.

The employment of a dry powder system avoids the stability problems associated with delivering aqueous suspensions from nebulisers. It is likely that a similar strategy could be employed to deliver a freeze dried liposome formulation.

To achieve efficient and uniform filling of a dry powder into an inhalation device or capsule requires it to be free flowing. The flow properties of a powder depend on particle size, surface characteristics and its cohesive and adhesive properties (24). The ability of a powder to flow freely will also govern the efficiency of its transfer from the device into the airstream. Consequently, commercial formulations for dry powder inhalation frequently include lactose having a larger particle size than the drug. This acts as a "flow aid" or "carrier", improving the flow properties of the powder. The use of a flow aid may be required to optimise liposomal delivery by this approach.

THE FATE OF LIPOSOMES IN THE LUNG

The fate of liposomes following pulmonary administration is an important issue impinging on the overall efficacy of the drug delivery system. For

example, the stability and/or residence of the vesicles in the lung will greatly influence the effective duration of drug release in vivo. There is a wealth of information regarding the clearance/turnover of natural lung surfactant, from which it is possible to surmise the potential fate of liposomal drug delivery systems (26). Early studies by Oyarzun et al (27) showed that radio-labelled DPPC small unilamellar vesicles (SUVs) administered to rabbits by intratracheal administration became rapidly associated with lung parenchyma. Similar kinetics of uptake were confirmed in rats for DPPC/Chol SUVs (28). Liposome composition has been shown to modify the rate and extent of lung uptake, where considerably higher rates are achieved with phospholipid vesicles containing phosphatidylglycerol (27). Generally, however, phospholipid administered in liposomal form is cleared from the lung more slowly than comparable doses of lung surfactant (29). Type II epithelial cells are able to remove exogenous phospholipid from the alveolar airspace, and it has been demonstrated that this process is augmented by surfactant apoproteins (30-33). Interestingly alveolar surfactant protein SP-A has been shown to dramatically enhance lipid uptake following incubation of liposomes with isolated type II cells and with alveolar macrophages (32). Other studies have also alluded to the importance of these latter cells in liposome accumulation (34).

Two studies have employed the technique of gamma scintigraphy to determine the fate and clearance of ^{99m}Tc labelled liposomes in the lung.

DPPC MLVs and SUVs were produced by Farr et al (2) with their outer surface labelled with ^{99m}Tc. These were atomized with a Hudson nebulizer and inhaled by healthy volunteers. The pulmonary distribution of activity with both formulations was dependant on the aerosol characteristics and was independent of vesicle size and type. Clearance of both vesicle types was comparable

TABLE 2

The Properties of Nebulized ^{99m}Tc Labelled DPPC Liposomes and their Pulmonary Retention in volunteers (Adapted from REF. 26)

	MLV	SUV
Size before nebulization (μm)	5.7	0.07
Size after nebulization (μm)	2.9	0.07
MMAD (μm)	3.7	3.2
σ_g	1.5	1.5
6 h. retention (%)	87.5	76.8
(±SE)	2.1	5.1

(Table 2). Short term clearance (within 6 h) was typical for particles cleared by the mucociliary clearance process. Activity remaining in the lung at 20 h was assumed to represent alveolar deposited vesicles. The measured values for alveolar retention were consistently less than values predicted by extrapolating the short term clearance profiles. This may indicate removal of vesicles from the alveolar regions, or a time dependant dissociation of the surface associated radio-label.

Barker et al (5) used a Respirgard nebulizer to generate aerosols (MMD = 1.8 μm) from DPPC/Chol (1:1) MLVs having mean vesicle size of 0.9 μm, containing the aqueous marker ^{99m}Tc-DTPA. These were inhaled by healthy volunteers. SPECT tomographic imaging 1 h post inhalation indicated

widespread distribution of activity throughout the lungs. Forty five per cent of the originally pulmonary deposited activity remained after 24 hours. This represented the fraction of radiolabel remaining in intact alveolar deposited vesicles, since free radio-label was removed from the airways with a half-life of 95 minutes.

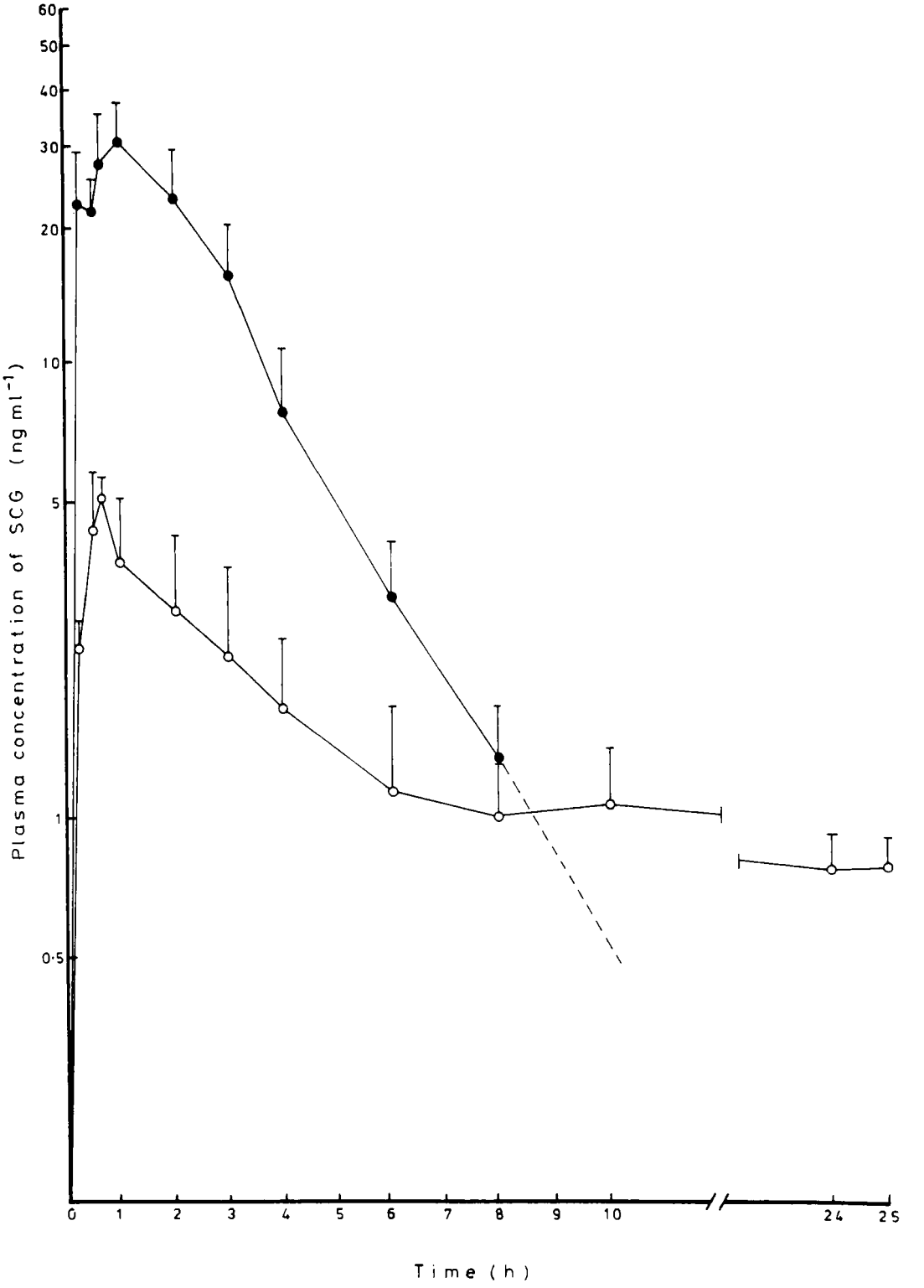
Together these studies demonstrate that liposomes can be deposited in the peripheral airways of man, where they are retained for prolonged periods.

MODIFICATION OF THE PHARMACOKINETICS OF DRUGS ENTRAPPED IN PULMONARY DEPOSITED LIPOSOMES

A number of liposome-associated materials have been administered to the respiratory tracts of humans and animals. These include cytotoxic agents (35,36), anti-asthma drugs (4,37,38), antimicrobials (3,39-41), drugs delivered for a systemic action (40,42,43) in addition to markers of the lipid (2) and aqueous (4,44) phases of liposomes.

Studies in animals have shown that liposomal encapsulation of the hydrophilic drug, cytarabine prolonged the pulmonary retention of intratracheally administered drug (35,36). Free drug was cleared rapidly with a half-life of 40 min and entered the systemic circulation. Liposomal drug was cleared with a half-life of 8 h, with reduced distribution to other tissues.

Similar results were reported when liposome associated hydrophobic atropine was administered to the lungs of rabbits. Four per cent of drug administered as a solution remained associated with lung tissue after 48 h (43). Twenty one per cent of liposome associated drug remained in the lung after the same time period.



In a healthy volunteer study, inhaled sodium cromoglycate encapsulated in DPPC/Chol (1:1) REVs gave detectable plasma levels of drug 24 and 25 h after inhalation (4). An equivalent dose of drug inhaled as a solution, produced peak plasma concentrations seven times greater than liposomal sodium cromoglycate, but could not be detected in plasma after 24 and 25 h (Fig. 1). The absorption half-life of inhaled liposomal sodium cromoglycate was 57 h, approximately three times faster than predicted from in vitro release experiments. This suggests that simple diffusion of entrapped drug across vesicle bilayers is not the primary factor determining drug release and hence plasma levels. Enhancement of drug release and subsequent absorption may result from vesicle degradation within alveolar macrophages, fusion with epithelial cells, or fusion and/or phospholipid exchange with constituents of pulmonary surfactant.

Woolfrey et al (44) suggested that in rats, pulmonary absorption of liposome entrapped carboxyfluorescein was lipid dose dependant. The rate of absorption was greatest with the highest doses of phospholipids, which the authors suggested might be due to the higher doses initiating a local biological response which enhanced the removal of entrapped dye. Encapsulated dye was absorbed more than twice as quickly from negatively charged vesicles than from comparable neutral vesicles. A greater fraction of the administered dose of dye was available from the charged liposome formulation.

FIGURE 1

Plasma levels following nebulization of 20 mg sodium cromoglycate to volunteers. (●) Free drug (n = 5), (○) Liposomal drug (n=4). Each point is a mean ± SE. (Reproduced with permission from REF. 4).

These studies indicate that liposomal encapsulation can prolong the pulmonary retention of materials, altering their pharmacokinetics. This can result in a localised drug action within the respiratory tract, whilst decreasing activity at sites distant from the lung. For instance, pulmonary administration of cytarabine solutions inhibited DNA synthesis in the gastro-intestinal tract and bone marrow in addition to the lung. Liposome entrapped cytarabine inhibited DNA synthesis in the lung, with insignificant activity in other tissues (35). Liposome entrapped orciprenaline produced prolonged bronchodilation against histamine induced bronchoconstriction, without the cardiovascular side-effects observed with free drug at the same dose (37).

CONCLUSION

This paper has illustrated the potential of liposomes as a carrier system for drug delivery to the respiratory tract. A number of types of delivery device exists capable of delivering intact liposomes to the human lung, where they can be retained for prolonged periods.

The ability to prepare vesicles from compounds endogenous to pulmonary surfactant is frequently quoted as a major advantage of liposomes over other colloidal carrier systems. To date, however, there has been only one controlled, objective study of the potential acute effects of nebulized liposome administration to healthy volunteers (45). No changes were observed up to 6 hours post administration, suggesting that liposomes are innocuous to the lung. Some consideration should be given to the potential effects following chronic administration, particularly in relation to pulmonary surfactant turnover and specific cell accumulation. However, chronic studies in mice where animals were subjected to 1 hour exposure 5 days per week for 1 month demonstrated

no differences in histology or macrophage phagocytic index compared to saline treated controls (34).

Vesicles deposited at the alveolar surface provide a sustained release of encapsulated material. The extent to which the rate of release can be controlled has yet to be established. Inclusion of cholesterol into vesicle bilayers generally increases their in vivo stability, decreasing the rate of drug loss (46), whilst studies with exogenous surfactant suggest that inclusion of phosphatidylglycerol increases spreading at the alveolar surface (27), potentially decreasing vesicle stability and promoting drug release.
If fusion of vesicles with the alveolar surface and/or lipid exchange between vesicle bilayers and surfactant are major mechanisms of drug release, then liposomes with more than one bilayer are likely to provide a more sustained release of entrapped materials than unilamellar vesicles.

This paper has reported on studies aimed at depositing liposomes in the lung. Some attempt has been made to target specific cell populations within the lung. Since liposomes are rapidly taken up by phagocytic cells, this property might be exploited to target alveolar macrophages, for instance to deliver immunomodulators or antimicrobial compounds. In vitro studies have demonstrated rapid alveolar macrophage uptake of liposomes (34). This has allowed a liposomal formulation of amikacin to be used in the treatment of *Mycobacterium avium-intracellulare* infected alveolar macrophages (47). The liposome formulation increased drug activity approximately 100 fold as compared to an equivalent dose of amikacin in solution.
Targeting to (non-phagocytic) lung epithelial cells provides a more difficult problem, although modulation of liposome composition by inclusion of alveolar surfactant protein SP-A has been shown to dramatically improve vesicle uptake into alveolar type II cells (32).

Although offering a great deal of promise for pulmonary drug delivery, to date published in vivo studies have employed rodents or healthy volunteers. The usefulness of such systems has yet to be established in disease states which may result in obstructed airways, mucociliary disfunction, elevated enzyme levels and altered airways permeability.

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