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International Journal of Pharmaceutics 173 (1998) 117–125

international journal of pharmaceutics

Nebulisers for the generation of liposomal aerosols

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Received 6 January 1998; accepted 29 June 1998

Abstract

Multilamellar liposomes and latex spheres have been atomised using a range of jet and ultrasonic nebulisers. Studies with spheres indicated that relatively large particles can be delivered from nebulisers, although the smaller the suspended particle size, the more efficiently they are delivered from all nebulisers. The size of aerosols generated from liposome suspensions, as measured by laser diffraction, was determined by the lipid concentration and the nebuliser used, but was independent of liposome size and bilayer composition. Generation of aerosols by both jet and ultrasonic nebulisers resulted in damage to liposome structures, with a consequent reduction in the measured vesicle sizes. The Respirgard II jet nebuliser produced aerosols with the smallest median droplet size and caused the greatest damage to liposomes. However, the size of droplets produced by the nebuliser is not the only determinant of liposome stability during atomisation. Relatively fluid EggPC liposomes experienced the greatest disruption during nebulisation. Inclusion of cholesterol or DPPC in the liposome bilayers rendered them more resistant to the disruptive forces to which they were exposed during either jet or ultrasonic nebulisation.

Keywords: Aerosol; Liposome; Nebuliser; Particle size; Stability; Suspension

1. Introduction

A wide range of liposome-associated materials have been administered to the airways of both animals and humans, including anti-asthma (Taylor et al., 1989) and antimicrobial compounds (Gilbert, 1996), antioxidants (Suntres and Shek, 1995) cytotoxic drugs (Juliano and McCullough, 1980), insulin (Liu et al., 1993) and recombinant genes for gene therapy in cystic fibrosis (Caplen et al., 1995). Together, such studies have demonstrated that drug encapsulation in liposomes prior to administration can produce modulated absorption, resulting in localised drug action in the respiratory tract, a prolonged drug presence in the circulation and decreased systemic side effects.

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0378-5173/98/$ - see front matter
PII S0378-5173(98)00212-9

A major function of the airways, resulting from its tortuous structure, is the prevention of inhaled particle deposition in the deep lung. The ability of a particle to access the peripheral airways depends principally on its size. To reach the respiratory bronchioles or alveoli, generally requires a size less than about 5–6 μm, with particles less than 2 μm preferable for alveolar deposition (Stahlhofen et al., 1980). Thus, control of the size of aerosols is critical for effective inhalation therapy. It is also necessary to consider the distribution of sizes about the mean, changes in the size of formulations as they pass through the highly humid airways and pathophysiological changes in the airways resulting from disease.

Currently, drug delivery to the human respiratory tract is achieved by three principle types of devices: the first type of devices are the pressurised metered-dose inhalers (MDIs), which comprise solutions or suspensions of drug in liquefied propellants, currently almost exclusively chlorofluorocarbons (CFCs). Phospholipids dissolved in CFCs will spontaneously form vesicles in an aqueous environment, such as occurs in the airways (Farr et al., 1987). However, the new, non-ozone depleting hydrofluoroalkane replacements for CFCs, such as Propellant 134a, are very poor solvents for phospholipids (Byron et al., 1994). Thus the future approach to formulation of liposomes into MDIs is likely to involve the suspension of freeze-dried or spray-dried vesicles in propellants.

The second type of aerosol delivery devices are the dry powder inhalers, from which drug is dispersed into the patient's airstream as a fine powder during inhalation. Freeze-dried (Schreier et al., 1994) and spray-dried (Goldbach et al., 1993) liposomes can be successfully aerosolised using such devices.

The third type of inhalation device, the nebulisers, are the most suitable devices for delivering liposomes in the early stages of research, since formulation is relatively easy and large dose volumes can be administered. In jet nebulisers, compressed gas is used to convert a liquid into a spray. A small proportion of the generated, primary aerosol, has a size that is sufficiently small for inhalation and leaves the nebuliser directly. The remaining, large, non-respirable droplets impact on strategically positioned baffles, or the walls of the nebuliser chamber and are recycled into the reservoir fluid. The aerosol output from a jet nebuliser comprises aerosolised droplets and solvent vapour which saturates the outgoing air. This causes the solute concentration to increase and liquid temperature to decrease with time (Ferron et al., 1976). The suitability of such nebulisers for delivering liposomes has been demonstrated to be formulation dependent. Significant losses of entrapped drug may occur as liposomes and liposome aggregates are broken up during passage through the nebuliser (Taylor et al., 1990; Niven et al., 1991). Such losses are decreased when the initial size of liposomes is reduced prior to nebulisation (Taylor et al., 1990).

Ultrasonic nebulisers generate aerosols through high-frequency vibrations of a piezoelectric crystal, which generates cavitation bubbles and capillary waves in the fluid that subsequently yield aerosol droplets. Excess energy is converted to heat which may inactivate sensitive materials, such as proteins (Cipolla et al., 1994). Indeed, ultrasonic nebulisers are expressly not recommended for delivery of the commercially available enzyme Dornase Alfa (British National Formulary, 1996). Consequently, they have generally not been investigated for liposome delivery, since the increase in temperature may induce fusion of liposomes and loss of entrapped drug.

The aim of these studies was to investigate the potential of both jet and ultrasonic nebulisers for the aerosol delivery of liposomes.

2. Materials and methods

2.1. Materials

Unless otherwise stated, materials used in this study were AnalaR grade and obtained from BDH (UK). Polystyrene and styrene divinylbenzene latex spheres (0.605–11.90 μm) were purchased from Sigma (UK). Egg phosphatidylcholine (EggPC; about 90%, Merck, Germany) was purified chromatographically (Bangham et al., 1974). Cholesterol (Chol, 99 + %) was

obtained from Sigma, U.K. Water was deionised (Whatman WR50 RO/Deioniser, Whatman, UK).

Nebulisers were obtained from the following suppliers: Cirrus (Intersurgical, UK), Medix AII and Medix Electronic (Medix, UK), Pari LC (Pari Werk, Germany), PulmoNeb (DeVilbiss Health Care, UK), Respirgard II (Marquest, USA), Sidestream Durable (Medic-Aid, UK).

2.2. Preparation of liposomes

Multilamellar liposomes were prepared from EggPC alone, or with equimolar quantities of cholesterol (EggPC/Chol), by hydration of thin films as described previously (Leung et al., 1996). Final concentrations of 2.5, 10, 20, 40 and 80 mg lipid component per ml of aqueous phase were produced. The mean liposome size was reduced by repeated extrusion through polycarbonate membrane filters (Nucleopore, USA) held in 25-mm holders. The volume median size (VMD) and size distribution of liposomes was measured by laser diffraction analysis using a 63-mm lens (Malvern 2600c, Malvern Instruments, UK).

2.3. Production and characterisation of aerosols produced by nebulisers

For nebulised suspensions, jet nebulisers operated at a gas flow rate of 6 l/min, and the Medix Electronic ultrasonic nebuliser operated at high power setting, were employed to nebulise 4 ml of suspensions (0.01 and 0.1%, w/v). The proportion of latex spheres nebulised was determined by Coulter Counter analysis (Model TAII, Coulter Electronics, UK) of fluids prior to and following nebulisation. Aerosols were produced from liposome preparations (2.5–80 mg/ml) by jet nebulisers at flow rates between 5 and 8 l/min and from the Medix Electronic nebuliser operated at high-power setting.

Aerosol size was measured by laser diffraction (Malvern 2600c). The nebuliser mouthpiece was clamped 25 mm from the centre of the laser beam, and adjusted so that the emitted aerosol intersected the beam at a distance of 50 mm from the 63-mm lens. Nebulisation was continued for 5 or 10 min or until aerosol production ceased.

2.4. Characterisation of liposomes delivered in aerosols produced by Medix Electronic nebuliser

Liposomal aerosols produced with the Medix Electronic nebuliser were collected in a two-stage (twin) impinger (TI; Hallworth and Westmoreland, 1987). Aerosols were generated from 5 ml of EggPC or EggPC/Chol liposomes (lipid concentration, 10 mg/ml) and the aerosol drawn through the TI at 60 l $\min-1$ using a vacuum pump for 5 min ($t = 0–5$ min), or for 5 min subsequent to the nebuliser being operated for a previous 5 min ($t = 5–10$ min). The size distribution of liposomes collected in each stage of the TI was measured by laser diffraction analysis.

3. Results and discussion

3.1. Delivery of spheres by jet and ultrasonic nebulisers

The design and operating principles of the nebulisers are the predominant factors influencing the size of the aerosols generated from suspensions of latex spheres (McCallion et al., 1996). All of the nebulisers failed to deliver the largest spheres (Fig. 1). For each of the nebulisers studied there was an incremental increase in the particle output as the sphere size was reduced. Between 25 and 60% of the smallest spheres were delivered. Although the median aerosol sizes produced by all the nebulisers were less than 6.40 μm (Medix AII, 3.4–3.8 μm; Pari LC, 2.3–2.6 μm; PulmoNeb, 4.7–5.4 μm; and Medix Electronic, 4.3–4.6 μm), all four nebulisers were able to atomise a proportion of the 6.40-μm spheres, with between 7 and 40% of the spheres delivered. This was due to the polydispersed nature of the aerosols that are produced by such devices. The ultrasonic device gave less efficient and more erratic results than the jet nebulisers, and was unable to deliver any of the 1.6-μm latex spheres. This may result from the operating frequency being unable to atomise particles of a specific size and density (McCallion et al., 1996).

3.2. Delivery of liposomes by jet nebulisers

The studies with latex spheres indicate that even relatively large, rigid particles can be delivered from nebulisers. However, liposomes are more delicate structures and may be degraded by the shearing associated with droplet production from a bulk liquid (Taylor et al., 1990). Consequently, relatively large liposomes were nebulised to investigate differences in the shearing effects occurring within different nebulisers during use. In addition to increasing our knowledge about the generation of liposomal aerosols, such studies may also prove useful in predicting and minimising protein and peptide degradation when they are delivered from similar devices.

A direct relationship existed between droplet size and lipid concentration for all the formulations tested (Fig. 2), with increasing concentrations associated with an increase in emitted droplet size. This correlates with the increased viscosity of these liposome formulations at higher concentrations (Bridges et al., 1995a,b). The increase in droplet size, occurred with all the nebulisers investigated, but the magnitude was dependent on the particular nebuliser. Aerosols produced by the Sidestream were least affected by concentration changes, showing a mean increase in droplet size of about 8% over the concentration range studied. This may indicate that this nebuliser has a highly efficient baffling system which compensates for the increased size of the primary aerosol by filtering out an increased proportion of droplets. The Pari LC gave an average increase of about 30% and the Cirrus about 50%. For each nebuliser, the inclusion of equimolar cholesterol in liposomes had no significant effect on the mean droplet size produced nor, as was observed with nebulised latex spheres (McCallion et al., 1996), was there a clear relationship between the size of dispersed particles and the aerosol size.

There was a time-dependent reduction in the size of 5 μm liposomes in the reservoir of the four jet nebulisers during use (Fig. 3), which was great-

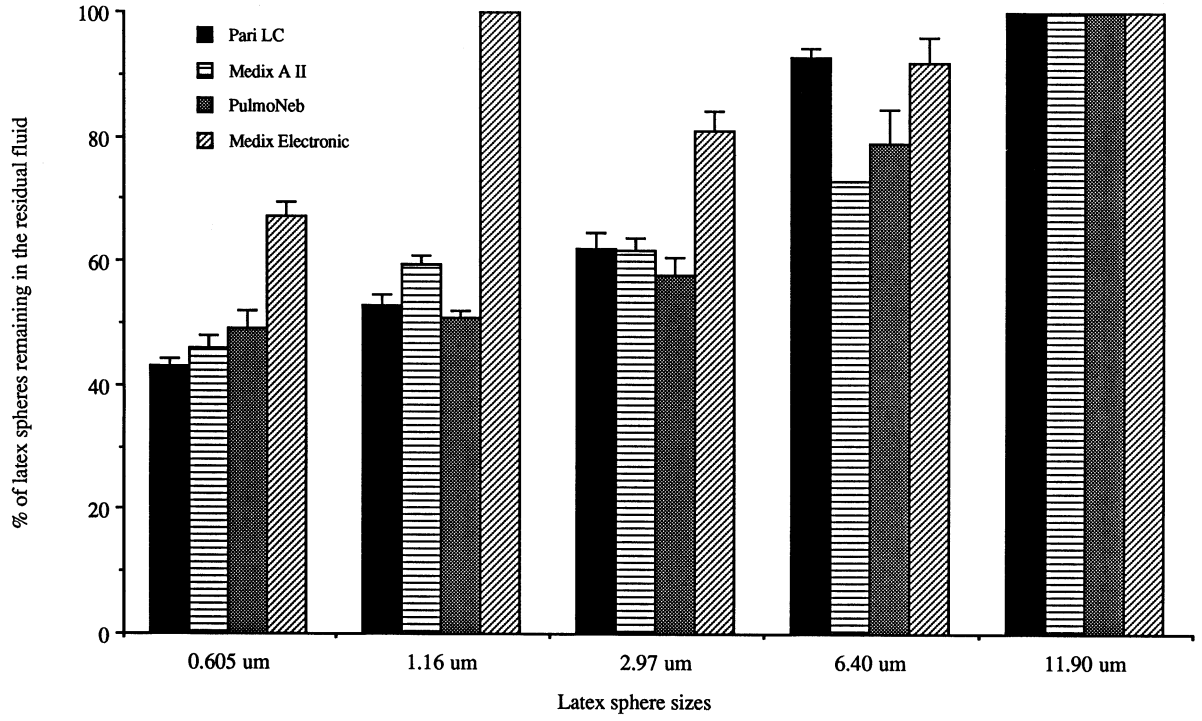

Fig. 1. Mean percentage ($\pm$ S.E.) of latex spheres (0.1%, w/v) remaining in nebuliser reservoir after 10 min nebulisation in Pari LC, Medix AII, PulmoNeb and Medix Electronic nebulisers (reproduced with permission from McCallion et al. (1996)).

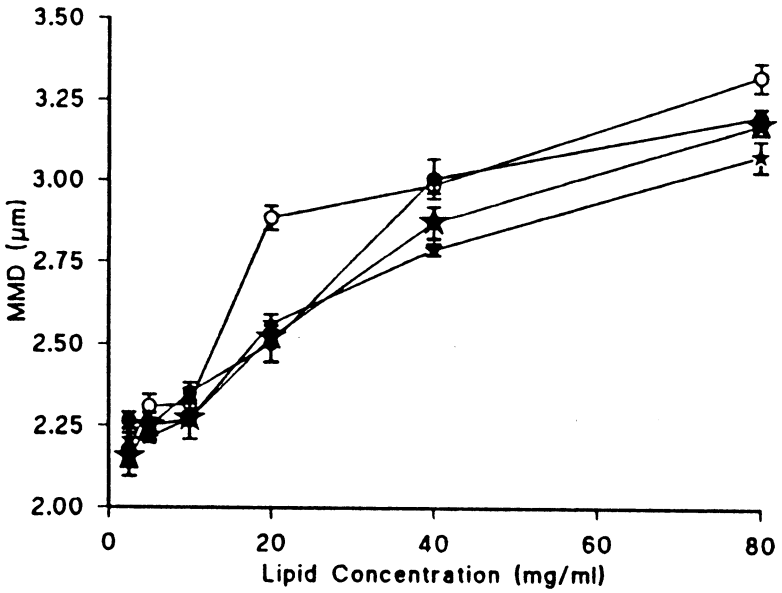

Fig. 2. Mean (± S.E.) aerosol size as a function of concentration for Egg PC liposomes having median size 1 (✱) and 5 μm (○) EggPC, and EggPC/Chol having median size 1 (☆) and 5 μm (•) for the Cirrus jet nebuliser (adapted with permission from Bridges et al. (1995a)).

est for EggPC liposomes. The ambient temperature exceeded the main phospholipid phase transition temperature of EggPC. As a result, EggPC bilayers are relatively fluid, which makes them more susceptible to disruption by the shearing action of the driving gas flow, than liposomes with more rigid DPPC/cholesterol bilayers. This is in agreement with previous studies which demonstrated that loss of entrapped carboxyfluorescein during nebulisation was dependent on bilayer composition (Niven and Schreier, 1990). Over 5 min, the extent of size reduction was determined by the nebuliser used, with the Respirgard II producing greatest size reduction. Depending on the design of the nebuliser, a major fraction of the primary aerosol mass is recycled into the nebuliser reservoir (Newman et al., 1987). With certain nebulisers, notably the Respirgard II, 5 min is not sufficient for complete aerosol generation. When such nebulisers are operated to 'dryness' the profile of vesicle degradation, and thus the rate of liposome damage, may be altered.

There was some relationship between vesicle size reduction and the size of the aerosol emitted from a nebuliser, as the Respirgard II nebuliser, which produced the smallest aerosol droplets (VMD = 1.5 μm), also gave the greatest vesicle instability. This might be predicted, since the production of smaller droplets is generally associated with higher shear forces and increased droplet recycling. Recycling of liposomes too large to be included in the aerosol output of the nebuliser is likely to continue until they are processed into a size small enough to be included in the secondary aerosol emitted from the nebuliser. However, for the Sidestream, Pari LC and Cirrus there was no clear relationship between aerosol size and vesicle disruption. Thus the size of the aerosol is not the sole factor determining vesicle disruption.

The design features of particular nebulisers govern the droplet size selectivity. Thus, the efficiency of aerosol output and the extent of droplet recycling, likely to be important factors determining liposome stability, depend on the design of nebuliser used. For instance, in the Respirgard II, air is forced through a nozzle with a small orifice (Fig. 4). Fluid is drawn up from the nebuliser reservoir surrounding the base of nozzle, air and fluid mix, and the aerosol is forced through a larger orifice in a baffle which fits over the air jet. In the

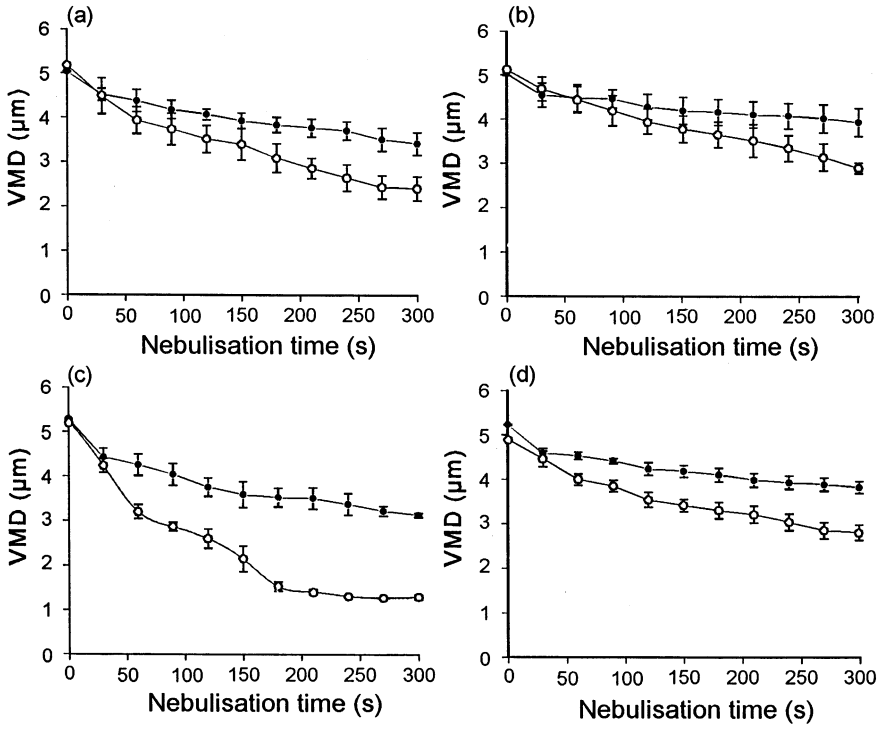

Fig. 3. Mean (± S.E.) vesicle size of (○) EggPC and (●) DPPC/Chol liposomes during nebulisation in (a) Pari LC, (b) Sidestream, (c) Respirgard II and (d) Cirrus jet nebulisers (adapted with permission from Bridges et al. (1995a)).

Sidestream nebuliser, which produces aerosols only slightly larger than the Respirgard II (VMD = 2.0 μm), air is forced up a central jet and mixes with fluid drawn up two capillaries (Fig. 5). The air jet is larger than that of the Respirgard II, and thus for a given airflow rate the pressure drop across the nozzle will be less. The droplets produced are carried out of the

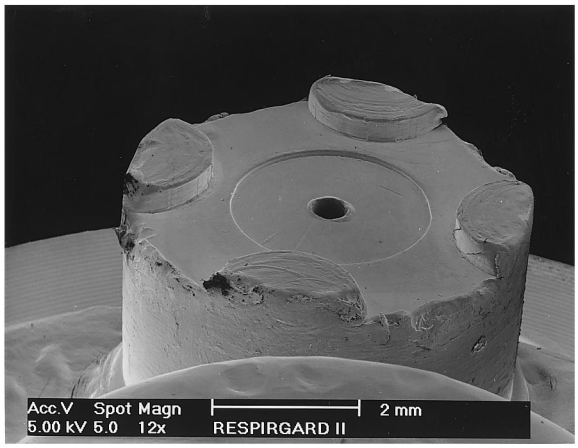

Fig. 4. Scanning electron micrograph of the air jet of a Respirgard II air jet nebuliser.

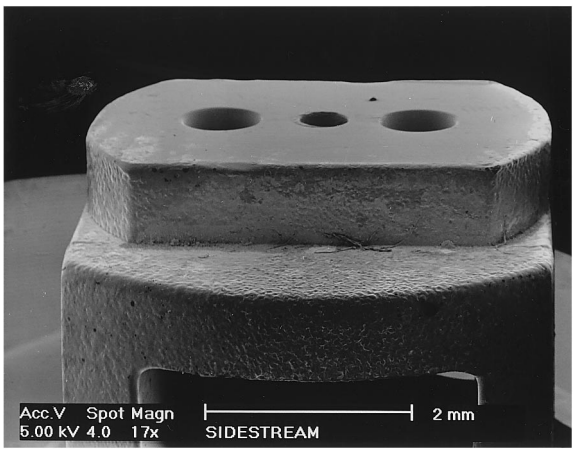

Fig. 5. Scanning electron micrograph of the air jet and capillaries of a Sidestream air jet nebuliser.

device through a complex combination of baffles and air passages, which are efficient at large droplet removal, without the need to expose the droplets, and contained liposomes, to as harsh an environment as occurs in the Respirgard II. This may, in part, explain why, although producing aerosols of comparable size, the Sidestream produces less liposome damage than the Respirgard II. However, with the wide variety of nebuliser designs and the interplay between the various component part geometry and dimensions, it is not possible to say for each nebuliser which features of their design are critical in determining liposome stability.

3.3. Delivery of liposomes by ultrasonic nebulisers

Ultrasonic nebulisers are inefficient at delivering high viscosity fluids (McCallion et al., 1995). Liposome preparations, particularly those containing cholesterol, with high phospholipid concentrations were relatively viscous (Bridges et al., 1995a,b). As a result they were atomised only sporadically, with concentrations of 80 mg/ml showing such high variability that they were not further investigated.

The ultrasonic nebuliser produced aerosols having larger droplet size than the jet nebulisers. The mean diameters of aerosols produced over 10 min from all liposome preparations were between 4.3 and 5.1 μm, with an increase in aerosol size with time (Leung et al., 1996). For the EggPC formulations, the median diameter of the aerosol was independent of lipid concentration, whilst the aerosol size for cholesterol-containing liposomes was significantly decreased when concentrations greater than 10 mg/ml were employed.

There was a marked reduction in the size of EggPC liposomes throughout nebulisation (Fig. 6). Initially, the mean size was 5.2 μm, with a broad size distribution. After 5 min this was reduced to 1.2 μm as the complete distribution of sizes shifted to smaller sizes. There was a further reduction to 1 μm after 10 min of nebulisation. At these latter times, many vesicles were too small for accurate measurement by laser diffraction, thus the values for mean diameter should be treated with caution, although the shift to smaller

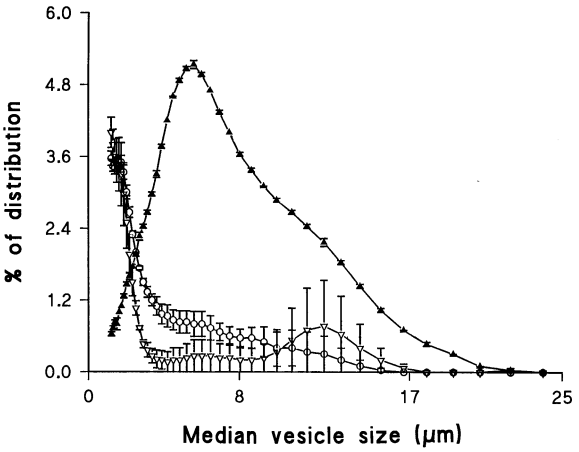

Fig. 6. Changes in the size distribution of EggPC liposomes remaining in the nebuliser reservoir at $t = 0$ s (▲), $t = 300$ s (○) and $t = 600$ s (▽). Each point is the mean ($\pm$ S.E.) (reproduced with permission from Leung et al. (1996)).

sizes is clear. At 10 min there was a small subpopulation of vesicles with a mean size of approximately 13 μm, which suggests that, after a prolonged time, when the reservoir temperature is at its highest, some aggregation and/or fusion of liposomes may occur.

The reduction in size of the more rigid EggPC/Chol liposomes during nebulisation was less marked (Fig. 7). However, a shift in the size distribution to a smaller mean size was still apparent. With these liposomes, aggregation may also be induced with time as the distribution moves to larger sizes in the latter stages of nebulisation.

Aerosols collected in the throat and Stage 1 of the TI have a mass median aerodynamic diameter (MMAD) greater than 6.4 μm and are considered 'non-respirable' (Hallworth and Westmoreland, 1987). Any aerosol droplets penetrating to stage 2 of the TI are considered the 'useful' or 'respirable' aerosol, having an MMAD less than 6.4 μm. Such aerosols are predicted to deposit in the peripheral regions of the lung.

In the first 5 min of nebulisation the mean sizes of EggPC liposomes collected in stages 1 and 2 were 6.7 and 3.9 μm, respectively, compared with an initial mean liposome size of 5.2 μm. The fractionation of the aerosol in the impinger is thus reflected in the size of vesicles deposited on the

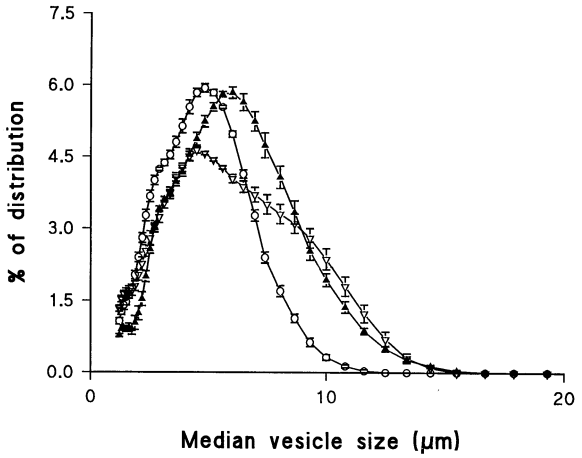

Fig. 7. Changes in the size distribution of EggPC/Chol liposomes remaining in the nebuliser reservoir at $t=0$ s (▲), $t=300$ s (○) and $t=600$ s (▽). Each point is the mean (±S.E.) (reproduced with permission from Leung et al. (1996)).

stages, with the smallest vesicles delivered in the aerosol fraction containing the smaller droplets. The same can be seen with liposomes collected between 5 and 10 min. However, the size of deposited vesicles on each stage was increased in the latter stages of nebulisation, reflecting a time-dependent increase in the aerosol droplet size, rather than exhibiting the demonstrated reduction in the residual liposome size.

In the first 5 min of nebulisation the mean sizes of EggPC/Chol liposomes collected in stages 1 and 2 were, respectively, 4.6 and 8.2 μm, compared with the initial mean liposome size of 4.7 μm. However, the size distribution data suggested vesicle aggregation in stage 2, and the mean figure consequently represents the size of these aggregates. The rapid evaporation of warm solute in the aerosol droplets as they pass through the impinger offers an opportunity for such aggregation to occur. Between 5 and 10 min, the mean size of EggPC/Chol liposomes deposited in both stages was about 3.4 μm, which compares to the size of liposomes in the nebuliser reservoir of 3.8 and 4.1 μm at 5 and 10 min, respectively. Thus, for these less 'fluid' liposomes, changes in residual liposome size appears more important than time-dependent changes aerosol size. The regional distribution of liposomes, within the impinger, is governed by the droplet size of the aerosols produced by the nebuliser.

4. Summary

The droplet size of aerosols was dependent on the phospholipid concentration of the liposome preparations, particularly for jet nebulisers. This is probably a function of the changing fluid viscosity. Other formulation factors, such as bilayer composition and vesicle size, had no significant impact on the aerosol size. Studies with suspended spheres indicated that even relatively large particles can be delivered from nebulisers. However, the smaller the size of the dispersed solid in a suspension, the more efficiently it was delivered from the nebulisers. With the jet nebulisers, the Respirgard II, which produced the smallest sized aerosols caused greatest liposome damage. However, specific design features of nebulisers seem to be as important as the size of aerosol generated in contributing to liposome instability during use.

Ultrasonic and jet nebulisers operate on different principles and produce aerosol droplets of different sizes. However, when liposome preparations were nebulised in either of these devices they were processed in a similar manner, with the structure of relatively fluid EggPC liposomes most likely to be disrupted during nebulisation. Rigidity imparted to the bilayers by the inclusion of cholesterol, or the use of phospholipids with elevated phase-transition temperatures, such as DPPC, made the liposomes more resistant to the disruptive forces to which they are exposed during either jet or ultrasonic nebulisation.

Acknowledgements

We thank K. Leung and O. McCallion for their contributions to this work whilst at the School of Pharmacy, and D. McCarthy, School of Pharmacy, University of London, for assistance with scanning electron microscopy. We also thank The Royal Pharmaceutical Society of Great Britain, Glaxo Wellcome and RP Sherer for their financial support.

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