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A simple approach to predict the stability of phospholipid vesicles to nebulization without performing aerosolization studies

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ABSTRACT

Membrane extrusion was investigated for predicting the stability of soya phosphatidylcholine liposomes and surfactosomes (Tween 80-enriched liposomes) to nebulization. Formulations were prepared with or without cholesterol, and salbutamol sulfate (SBS) or beclometasone dipropionate (BDP) were incorporated as model hydrophilic or hydrophobic drugs respectively. Formulations were extruded through 5, 2, 1 and 0.4 μm polycarbonate membrane filters to study the influence of membrane pore size on drug retention by the vesicles. Surfactosomes were found to be very leaky to SBS, such that even without extrusion greater than 50% of the originally entrapped drug was lost; these losses were minimized by the inclusion of cholesterol. The smaller the membrane pore size, the greater the leakage of SBS; hence only around 10% were retained in cholesterol-free surfactosomes extruded through 0.4 μm filters. To study the influence of vesicle size on SBS retained entrapment, an excessive extrusion protocol was proposed (51 extrusion cycles through 1 μm filters) to compare the stability of freshly prepared vesicles (i.e. unextruded; median size approx. 4.5–6.5 μm) with those previously extruded through 1 μm pores. Cholesterol was essential for minimizing losses from liposomes, whilst for surfactosomes size reduction prior to extrusion was the only way to minimize SBS losses which reached up to 93.40% of the originally entrapped drug when no cholesterol was included. When extrusion was applied to BDP-loaded vesicles, greater proportions of the drug were retained in the vesicles compared to SBS. Even with extrusion through 0.4 μm, BDP retention was around 50–60% with little effect of formulation. Excessive extrusion showed BDP retention using small liposomes (1 μm) to be as high as 71–87%, compared to 50–66% for freshly prepared vesicles. The findings, based on extrusion, were compared to studies of vesicle stability to nebulization, published by a range of investigators. It was concluded that extrusion is a valid method for predicting the stability of liposomes to nebulization.

1. Introduction

Inhalation of liposomal drug formulations via nebulization can prolong drug residence within the lung, potentially maximizing therapeutic benefit, whilst reducing systemic adverse effects (Taylor et al., 1989; Saari et al., 1999; Fauvel et al., 2012; Gaspar et al., 2012; Cipolla et al., 2013; Clancy et al., 2013). However, damage of the liposome structures during air-jet nebulization can cause loss of the originally entrapped material; thus liposome stability to nebulization-induced damage should be considered during formulation and manufacturing development (Taylor et al., 1990b; Niven et al., 1991; Elhissi et al., 2006a, 2007; Chadha et al., 2012; Nasr et al., 2013). Loss of entrapped hydrophilic agent can be minimized by reducing liposome size before jet-nebulization to 1 μm or less (Taylor et al., 1990b; Niven et al., 1991), using high phase transition phospholipids, such as hydrogenated soya phosphatidylcholine (HSPC) (Niven and Schreier, 1990), or by inclusion of cholesterol (Taylor et al., 1990b; Tseng et al., 2007; Chadha et al., 2012). High phase phospholipids or cholesterol may make liposomes more rigid and less “leaky” to the entrapped hydrophilic material (Kirby and Gregoriadis, 1999).

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Vibrating-mesh nebulizers have revolutionized pulmonary delivery of conventional solutions (Dhand, 2002) and advanced drug delivery systems, such as liposomes (Elhissi and Taylor, 2005; Kleemann et al., 2007; Nasr et al., 2013; Cipolla et al., 2014; Lehofer et al., 2014). Compared to air-jet nebulizers, vibrating-mesh devices may cause less damage to liposomal structures and hence higher proportions of the originally entrapped hydrophilic drug can be retained in the vesicles during aerosolization (Elhissi et al., 2006a, 2007), especially when the vesicles are extruded to the size of 1 μm prior to nebulization, and/or by using devices with large mesh apertures (Elhissi et al., 2007). Arikace® (Insmed, NJ, USA), a novel nebulizable liposome formulation of the hydrophilic anti-pseudomonal antibiotic amikacin is currently in phase III trials, and has been reported to be well tolerated by cystic fibrosis patients, with prolonged drug residence in the lung and enhanced penetration through *Pseudomonas aeruginosa* biofilms. The success of this formulation has been attributed to the use of an appropriate formulation (cholesterol-enriched dipalmitoylphosphatidylcholine with vesicle size around 300 nm) and a suitable inhalation device (Pari e-Flow mesh nebulizer) (Clancy et al., 2013; Ehsan et al., 2014; Waters and Ratjen, 2014).

Unlike hydrophilic drugs, the loss of hydrophobic materials (e.g. steroids) from liposomes during nebulization is dependent on lipid bilayer composition and mode of drug interaction with the bilayers (Darwis and Kellaway, 2001; Elhissi et al., 2006b). For example, beclometasone dipropionate (BDP) inhaled in liposomes showed prolonged retention in the respiratory tract of human volunteers, although liposomes underwent marked size reduction during jet-nebulization from 3.49 to 0.83 μm and from 5.07 to 0.91 μm for dilauroylphosphatidylcholine (DLPC; a low T_m phospholipid) and dipalmitoylphosphatidylcholine liposomes (DPPC; a high T_m phospholipid) respectively (Saari et al., 1999). Hence, size reduction (i.e. massive disruption) of the liposomes during nebulization did not cause marked losses of the entrapped BDP. These findings are consistent with *in vitro* studies using BDP (Elhissi et al., 2011) and other hydrophobic drugs, such as ciprofloxacin (Desai et al., 2002). Clinical trials have been conducted with Pulmaquin™ (Aradigm Corp., CA, USA), a nebulizable liposomal formulation of ciprofloxacin for inhalation by non-cystic fibrosis bronchiectasis patients (Cipolla et al., 2013; Serisier et al., 2013).

Studies investigating the physical stability of liposomes during nebulization are usually conducted using nebulizers linked with appropriate aerosol collection systems (e.g. impingers or impactors), followed by analysis to determine drug losses from aerosolized liposomes (Taylor et al., 1990b; Desai et al., 2002; Elhissi et al., 2007; Kamalaporn et al., 2014). This approach is laborious, time-consuming and poorly sensitive due to the need to assay very low concentrations of liposome associated and free drug Moreover, loss of aerosol to the surrounding environment during nebulization experiments is a potential hazard, even with standard aerosol-collection models. It has been reported that aerosolized particles delivered to impingers may bounce from the bottom of the collection compartment, or be re-aerosolized with liquid bubbles created by the air flowing into the impinger, causing particles to escape with the effluent air, resulting in reduced aerosol collection efficiency (Grinshpun et al., 1997). Furthermore, hydrophobic particles might be poorly collected by the impinger's liquid owing to poor particle wettability. The particle "bouncing" phenomenon and reduced collection efficiency is even more significant with dry collection models such as impactors (Xu et al., 1993). The "bouncing" effect has also been reported to be dependent on formulation, impactor design (Mitchell et al., 2003) and angle of particle deposition (Xu et al., 1993).

In this study, we have proposed a convenient, economical, and environment-friendly approach to predict the stability of liposomes during nebulization without conducting aerosolization studies. This was achieved by performing excessive extrusion of the formulations through polycarbonate membrane filters. The repetitive shearing provided by extrusion (51 cycles through 1 μm pore filters) aimed to simulate that occurring during nebulization. The extrusion approach was evaluated using salbutamol sulfate (SBS; also known as albuterol sulfate) and beclometasone dipropionate (BDP) as vesicle-entrapped hydrophilic and hydrophobic drugs respectively. Drug retention by liposomes upon extrusion was assessed as a stability indicator, using cholesterol as "rigidity" enhancer (Kirby et al., 1980) and Tween 80 as "fluidity" promoter (Young et al., 1983) of the vesicles. Vesicles made using Tween 80 were referred to as "surfactosomes" and were compared to conventional liposomes. The findings of extrusion studies were appraised in relation to a range of liposome nebulization studies available in the literature.

2. Materials and methods

2.1. Materials

Soya phosphatidylcholine (SPC; Lipoid S-100) was a gift from Lipoid, Switzerland. Cholesterol, beclometasone dipropionate (BDP) and Tween 80 were purchased from Sigma Aldrich, UK. Sodium chloride (ACS, 99.0%), salbutamol sulfate (SBS, 99%), sodium 1-hexane sulfonate monohydrate (99%) and Triton X-100 were all purchased from Alfa Aesar, UK. Glacial acetic acid, chloroform (stabilized with ethanol), water (HPLC-grade) and methanol (HPLC-grade) were all supplied by Fisher Scientific, UK. Ferric chloride and Ammonium thiocyanate were purchased from VWR, UK, and Deuterium oxide (D_2O; NMR-grade) was purchased from Acros Organics, UK.

2.2. Methods

2.2.1. Preparation of liposomes and surfactosomes

SPC alone or with cholesterol (1:1 mol ratio) was used to prepare liposomes by dissolving the lipids in chloroform (7.5 mL; 20 mg/mL) in a round bottomed flask. The organic solvent was removed using a rotary evaporator (Büchi Rotavapor R-215, Büchi, Switzerland) under vacuum for 1 h in a water bath at 37 °C using the maximum rotation speed (280 rpm). The resultant thin lipid film was hydrated by adding SBS dissolved in 1 mL NaCl (0.9%) solution followed by manual shaking. The dispersion was left to anneal for 15 min before further dilution with drug-free NaCl (0.9%) solution, followed by vigorous hand shaking to give a lipid concentration of 15 mg/mL, and further annealing was allowed for 2 h at room temperature. In other batches, the same procedure was repeated by inclusion of BDP in the lipid phase (2.5 mol% of lipid) and the same hydration procedure was followed, using D_2O for hydration. Surfactosomes were prepared by using SPC, with or without cholesterol (1:1 mol ratio) with Tween 80 (15% w/w of total lipid). The mole ratio between SPC, cholesterol and Tween 80 was 7:7:1. The lipid and surfactant were dissolved in chloroform in a round-bottomed flask to give a lipid concentration of 20 mg/mL. Following organic solvent removal, the thin film was hydrated as described above using either SBS (15 mg/mL; added with the aqueous phase) or BDP (2.5 mol% incorporated into the lipid phase).

2.2.2. Extrusion of formulations

Avestin Liposofast Mini-extruder (GC technologies, UK) was employed to extrude liposomes or surfactosomes using Nucleopore Track-etched polycarbonate membrane filters with pore sizes: 5 μm (11 cycles), 2 μm (11 cycles), 1 μm (7 cycles) and 0.4 μm (7 cycles) (Nucleopore, UK). The number of cycles was