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High dose cyclosporin A and budesonide-liposome aerosols.

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Abstract

Development of liposomal formulations for pulmonary delivery with jet nebulizers has vast potential for future aerosol therapies. Different variables determine the therapeutic efficacy of aerosols, including formulation. The purpose of this study was to develop concentrated, high dose liposomal formulations initially using immunosuppressive (cyclosporin A, CsA) and anti-inflammatory drugs (budesonide, Bud) for targeted pulmonary delivery with maximal aerosol output and particle size ranges within the optimal range of 1–3 μm mass median aerodynamic diameter (MMAD). Results indicate that with increasing drug-liposome concentrations there is reduced nebulized mass output but an inversely proportional increase in aerosol output of liposomes up to critical starting concentration ranges of 21.3 mg CsA:160 mg 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC)/ml and Bud 12.5 mg:187.5 mg DLPC/ml. Above these concentration ranges (which were unique for each formulation tested), there was a reduction in the liposome aerosol output. With the increased liposome concentrations, there was a linear increase in the apparent viscosity and reduction in apparent surface tension, however, there were no demonstrable correlations between these parameters and drug output rates. Aerosol particle size analysis demonstrated that the MMAD increased minimally with higher liposome concentrations. The size range of these high dose drug-liposome aerosols is optimal for penetration into the lung periphery. © 1997 Elsevier Science B.V.

Keywords: Liposome; Aerosol; Cyclosporin A; Budesonide; Nebulizer; Lung

1. Introduction

Many different diseases have been successfully treated through utilization of aerosol delivery systems to deposit drugs directly onto pulmonary surfaces. A variety of devices have been developed (i.e. metered dose inhalers and dry powder inhalers) for this purpose. Jet-nebulizers have been used clinically for aerosol delivery of water soluble drugs and micronized suspensions; however, their use with water insoluble, hydrophobic com-

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pounds has been limited (Arnon et al., 1992; Cameron et al., 1990; Lodrup-Carlsen et al., 1992; Nikander, 1994). The development of liposomal formulations compatible with aerosol delivery with jet nebulizers has expanded the possibilities for more effective utilization with additional drugs (Farr et al., 1985; Gilbert et al., 1988; Niven and Schreier, 1990; Schreier et al., 1993; Taylor and Farr, 1993; Waldrep et al., 1993). The utilization of liposomes for aerosol delivery has many potential advantages for clinical development including: aqueous compatibility, sustained pulmonary release to maintain therapeutic drug levels (Hung et al., 1995) and facilitated intra-cellular delivery (particularly to alveolar macrophages) (Schreier et al., 1993; Taylor and Farr, 1993). The efficacy of localized, topical therapy via aerosols is improved by drug levels delivered at the sites of disease within the lung.

There are several different parameters which determine the therapeutic efficacy of aerosol formulations. Nebulizer design and variation, operating conditions (e.g. flow rate) and ancillary equipment are important (Dalby and Tiano, 1993). Aerosol output efficiency can be increased through implementation of optimal jet nebulizers (Waldrep et al., 1994a). Inappropriate use of devices and/or procedures can affect aerosol particle sizes, inhaled dosages and thus influence therapeutic outcomes. The drug formulation is also a critical factor regulating aerosol output efficiency and aerodynamic properties of drug-liposomes (Waldrep et al., 1993). Drug-liposome output efficiency can be increased through the utilization of liposomes formulated with low gel to liquid phase transition temperatures (T_c) (Waldrep et al., 1993). An additional method to elevate aerosol drug-liposome output is to increase the drug and phospholipid reservoir concentrations. Nebulization of some drug-liposome formulations at greater than 50 mg/ml results in clogging of the nebulizer jets (Taylor et al., 1990). However, empty liposomal formulations up to 150 mg/ml have been successfully nebulized (Thomas et al., 1991). The aerosol performance (output and particle size) may be influenced in part by physicochemical properties, such as, viscosity and surface tension (McCallion et al., 1996a, 1995). Such variables affect the maximal drug-liposome concentrations compatible with aerosol delivery via a jet nebulizer.

While there are no commercial liposomal formulations currently available for aerosol delivery to the lung, several intravenous formulations have been recently approved. In addition, our research group has developed a variety of liposomal drug formulations for aerosol lung delivery in preclinical (O'Riordan et al., 1997; Waldrep et al., 1994a,b, 1993) and clinical studies (Waldrep et al., 1997). Using gamma scintigraphy, nebulizer systems were evaluated and selected for optimal aerosol targeting of the peripheral lung regions (Vidgren et al., 1994). The purpose of this study was to develop concentrated, high dose-liposome formulations initially using immunosuppressive (cyclosporin A, CsA) and anti-inflammatory drugs (budesonide, Bud) for targeted pulmonary delivery with maximal aerosol output and particle size ranges within the optimal range of 1–3 μm mass median aerodynamic diameter (MMAD).

2. Materials and methods

2.1. Preparation of drug-liposomes

We have developed a lyophilization procedure for optimal formulation of various drug/liposomes. To determine the maximum concentration compatible with nebulization and aerosol delivery, formulations were produced at 10–300 mg CsA with 75–2250 mg 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC) at the optimal CsA to DLPC ratio of 1:7.5 by weight. For CsA-liposomes, CsA (Sandoz Research Institute, East Hanover, NJ or Chemwerth, Woodbridge, CT) was mixed with synthetic lecithin: DLPC (Avanti Polar Lipids, Alabaster, AL). CsA–DLPC liposomes were produced by lyophilization from t-butanol as previously described (Waldrep et al., 1993).

Multi-lamellar liposomes were produced by adding 10 ml ultra pure water above the DLPC phase transition temperature (−2°C) at 25°C, to deliver the desired final standard drug concentration of CsA 1–30 mg:DLPC 7.5–225 mg per ml. The mixture is incubated for 30 min at room temperature with intermittent mixing to produce multilamellar vesicular (MLV) liposomes. Aliquots are taken for determination of drug concentration by high pressure liquid chromatography (HPLC).

After swelling, the liposome preparations were checked by microscopy under polarized light and for the presence of drug aggregates, both before and after nebulization and by quasielastic light scattering with a Nicomp Model 370, Submicron Particle Sizer (see below). The drug-liposome association was determined using Percoll (Sigma, St. Louis, MO) gradient analysis as previously described (O'Riordan et al., 1997). Size reduction of the MLV high dose CsA–DLPC drug-liposome formulations was not needed prior to nebulization since the drug-liposomes (a heterogeneous starting mixture after swelling of 2.2–11.6 μm) are further reduced in size during nebulization (and continual reflux) by the shear forces generated by extrusion through the jet orifice (see below). After swelling, the formulations were used for nebulization within 1–2 h.

For formulation of high dose Bud–DLPC liposomes, the optimal drug to lipid ratio was determined by testing different formulations at Bud–DLPC ratios to be 1:15 (by weight) (Waldrep et al., 1994b). The formulations were produced by mixing 10–120.5 mg of Bud (Sigma, St. Louis, MO) and with 150–1870.5 mg of DLPC (as described above for CsA–DLPC). Multi-lamellar liposomes were produced by adding 10 ml ultra pure water as above to deliver a final standard drug concentration of 1–12.5 mg Bud:15–187.5 mg DLPC/ml of solution. The mixture is incubated for 30 min at room temperature with intermittent mixing to produce multilamellar vesicular (MLV) liposomes. The drug-liposome association was determined using Percoll gradient analysis. Aliquots were taken for determination of drug concentration by HPLC.

3. Aerosol studies

3.1. Drug-liposome aerosol particle size distribution

Aerodynamic particle sizing of the liposome aerosols was measured using an Andersen 1 ACFM non-viable ambient particle sizing sampler (Graseby Andersen Division, Smyrna, GA) equipped with an artificial throat as described in the US Pharmacopeia, Vol. 23 (Induction Port, MSP, Minneapolis, MN). Liposome aerosols generated from the Aerotech II nebulizer (ATII, CIS-US, Bedford, MA) which is a well characterized, high output, efficient nebulizer demonstrated to produce liposome aerosols in the optimal size range suitable for peripheral lung delivery (1–3 μm MMAD) (Smaldone et al., 1991; Vidgren et al., 1994). Aerosols were sampled directly from the nebulizer outlet mouthpiece port through a closed tube connector. Aerosol samples were collected by impaction within the throat and on the eight aluminum impactor stages at a standard sampling interval of 7–8 min after the start of nebulization. To eliminate the potential effects of diluting air on aerosol particle size during sampling at 1 ACFM, negative pressure compensation was added distal to the mouthpiece outlet port by the use of one-way intake valve connected through a Y-shaped T-tube.

Drug and/or DLPC concentrations within aerosol droplets between 0–10 μm sizes were collected on each stage and concentrations determined after elution from the throat and collection plates using 10 ml ethanol (Bud) or methanol (CsA and DLPC) as appropriate for maximum solubility, followed by HPLC analysis. After determination of the Bud, CsA or DLPC concentrations for each stage, the MMAD and geometric standard deviation (GSD) of the liposome aerosol was calculated (as described in the U.S. Pharmacopeia, Vol. 23) on log probability scale with the effective cutoff diameter as the ordinate and the cumulative percent less than the size range (by concentration) as the abscissa (Kaleida Graph 3.08, Synergy Software, Reading, PA). The MMAD and GSD were determined by the Bud, CsA and DLPC content distributed within the array of droplets comprising the aerosol. It is the aerosol droplet array rather than the liposome

size that determines the MMAD and the GSD (Waldrep et al., 1993).

3.2. Estimation of inhaled dosage

For the determination of the estimated inhaled dosage of drug-liposomes, nebulized samples were collected in a simulated human lung system (Smaldone et al., 1991). Using a respirator pump (Harvard Apparatus, Dover, MA), aerosol samples were collected onto 15 cm GF/F glass microfiber filters (Whatman International, Maidstone, UK) from the ATII nebulizer (10 l/min flow rate) using 15 breaths/min (BPM) with tidal volumes of 500 ml. Aerosol samples were collected over a 15 min nebulization interval. The amount of CsA or Bud deposited on the filters was determined after extraction (efficiency > 95%) with methanol or ethanol by HPLC analysis.

4. Drug analysis by HPLC

4.1. Bud analysis

The HPLC assay is utilized for multiple purposes to determine the Bud content of liposome formulations, the encapsulation efficiency and the Bud content of aerosol samples obtained with the lung simulator. Bud concentrations are determined by HPLC analysis using a WISP 717 autosampler and a Nova-Pak® C18 (60 Å, 4 μm, 3.9 × 150 mm) column (Waters, Milford, MA) at room temperature. Peak detection is performed at 238 nm using a 490 programmable multi-wavelength detector with quantification by a Millennium 2010 Chromatography Manager (Version 2.15; Waters, Milford, MA). The mobile phase utilized for these studies was ethanol:water (50:50 v/v) at a flow rate of 0.6 ml/min (Andersson and Ryrfeldt, 1984). Samples for Bud and DLPC analysis were dissolved directly into ethanol (to solubilize the liposomes).

4.2. CsA analysis

The CsA in liposomal formulations (to determine CsA content and encapsulation efficiency) and in aerosol samples was determined by HPLC. WISP 717 autosampler and a Supelcosil™ LC-1 (5.0 cm × 4.6 mm) column (Supelco, Bellefonte, PA) heated to 75°C was used in the assay. The mobile phase was acetonitrile:methanol:water (50:20:30 v/v/v) (Charles et al., 1988). Peaks were detected at 214 nm using the 490 programmable multi-wavelength detector and quantified with the Millennium 2010 Chromatography Manager. Samples for CsA and DLPC analysis are dissolved directly in methanol (to solubilize the liposomes).

4.3. DLPC analysis

A modification of the HPLC protocol of Grit and Crommelin has been utilized to measure DLPC (Grit and Crommelin, 1992). The 717 WISP autosampler and a Nova-Pak® Silica (3.9 × 150 mm) column (Waters, Milford, MA) was used with acetonitrile:methanol:10 mM ammonium/trifluoroacetic acid, pH 4.8 (64:28:8 v/v/v) mobile phase. Peaks were detected with a SEDEX 55 mass evaporative detector (Sedere, Alfortville, France) and quantified with the Millennium 2010 Chromatography Manager. Samples for analysis were dissolved directly in ethanol or methanol (to solubilize the liposomes).

5. Physicochemical analyses

5.1. Apparent surface tension and apparent viscosity

The surface tension on 10 ml of each pre-nebulized liposomal formulation was measured using a Surface Tensiomat® 21 (Fisher Scientific, Indiana, PA). A platinum iridium ring of known dimension is raised from the surface of the liquid to be tested under precisely controlled conditions. Apparent values read from the machine were multiplied by a correction factor, F incorporating dimensions and size of the measuring ring, interfacial tension and density of the two phases. In rheograms generated using a rotational viscosimeter, it was determined that the liposomal formulations demonstrated non-Newtonian characteristics

with shear rate dependent viscosity. For this study, apparent viscosity measurements were performed on 10 ml of pre-nebulized liposomal formulation under constant conditions using a falling ball viscometer (stainless steel ball, tube 2; Gilmont Instruments, Barrington, IL). The apparent viscosity in centipoises (cP) and the apparent surface tension in dynes/cm (dyn/cm) were determined at ambient room temperature (23°C).

5.2. Size measurements of drug-liposomes

The particle size of drug liposomal solutions was measured by quasielastic light scattering in a laser beam with the Nicomp Model 370, Submicron Particle Sizer (Program Version 5.0; Nicomp, Santa Barbara, CA). Drug-liposome samples diluted into 6mm glass tubes in distilled water and were analyzed by Nicomp Distribution Analysis and the data expressed as intensity weighted vesicle size (relative intensity of scattered light vs. diameter). Drug-liposome mean particle diameters were measured from reservoir samples initially, after 10 min of nebulization and on aerosol samples collected in water using an All Glass Impinger (AGI-4, Ace Glass, Vineland, NJ) as previously described (Waldrep et al., 1993). The AGI-4 impinger device was utilized with the collecting flask containing 10 ml of water to which the aerosol was introduced through a calibrated glass tube and critical orifice delivering the jet of aerosol 4 mm above the bottom of the flask. The system was operated by a vacuum pump (through connector tubes with negative pressure differential compensation as described) using a sampling period of 1 min.

6. Results

The goal of this study was to improve the efficiency of drug-liposome aerosol delivery of CsA and Bud-liposomes for clinical utilization by increasing aerosol drug concentrations to reduce therapeutic inhalation intervals. Studies were designed to delineate the maximum concentration ranges compatible with efficient nebulized drug output of CsA and Bud with a well characterized, continuous-flow jet nebulizer (ATII), while maintaining the optimal aerosol particle size ranges suitable for peripheral lung delivery 1–3 μm MMAD (Vidgren et al., 1994). Drug-liposome experiments were designed to test the relationships between drug-liposome starting concentration, aerosol drug particle size range and output rate, as well as, the formulation effects on apparent viscosity and apparent surface tension (McCallion et al., 1995, 1996b).

Analyses were designed initially to determine the effects of increasing liposomal concentration on aerosol mass output rate as an indicator of nebulizer efficiency (Fig. 1). There was a similar inverse relationship demonstrated between increasing starting liposome concentration and mass output rate (decreasing) for DLPC (37.5–200 mg/ml) (Fig. 1(a)), Bud–DLPC (1–15 mg Bud; 15–225 mg DLPC/ml) (Fig. 1(b)) and CsA–DLPC (10–24 mg CsA; 75–180 mg DLPC/ml) (Fig. 1(c)). Since the thickness of each liposomal formulations increased with concentration to resemble an emulsion, apparent viscosity measurements were performed for correlation nebulizer efficiency to determine the maximum allowable range for nebulization (Fig. 1). There was a linear relationship demonstrated between the increasing starting liposome concentration and the apparent viscosity for all three formulations tested (Fig. 1(a–c)). As the apparent viscosity increased with higher liposomal concentrations, there was reduced aerosol mass output.

Determinations were performed in parallel with the experiments represented in Fig. 1 using analytical HPLC assays to measure the nebulized drug-liposome output rate (Fig. 2). Up to a point, there was linear relationship noted between the starting liposome concentration and the DLPC (Fig. 2(a)), Bud (Fig. 2(b)) and CsA (Fig. 2(c)) aerosol output rate. This was inversely related to the total aerosol mass output rate. For each formulation series tested in this study, there was a maximum starting concentration range identified in which the aerosol drug-liposome output rate fell precipitously. This concentration range was near 170 mg/ml for empty DLPC liposomes (Fig. 2(a)); 12.5 mg Bud:187.5 mg DLPC/ml for Bud–DLPC (Fig. 2(b)) and 21.3 mg CsA:160 mg DLPC/ml for

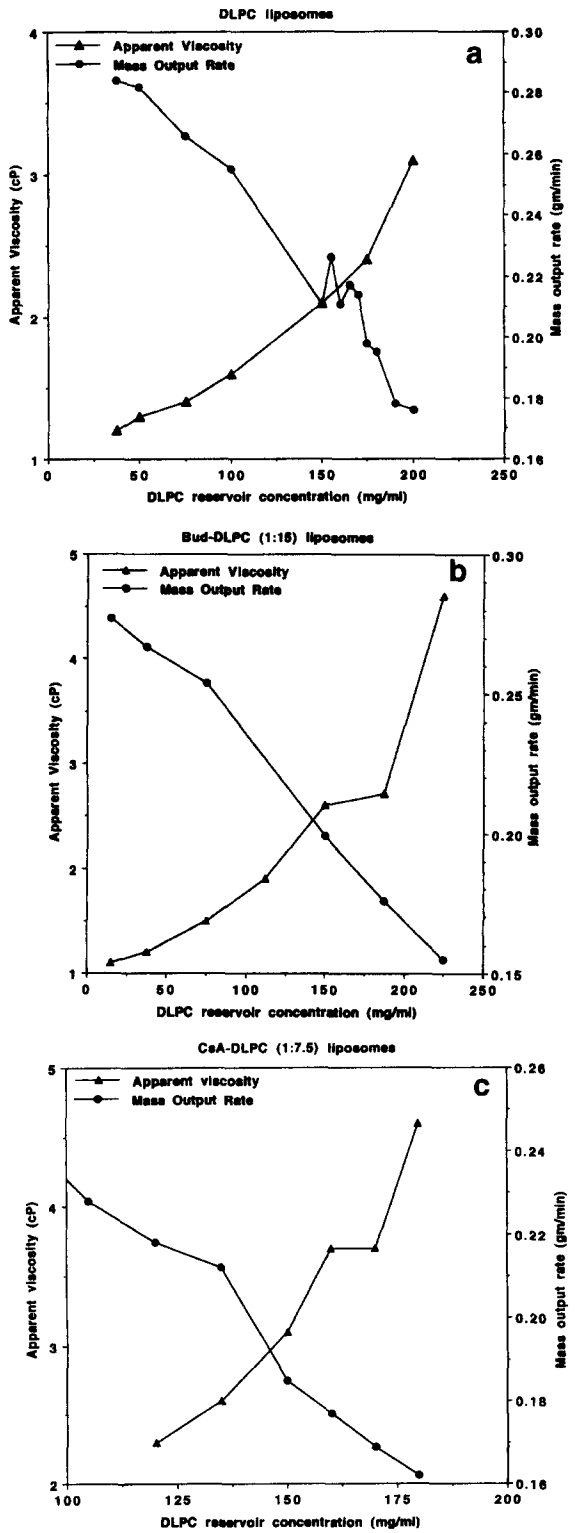

Fig. 1.

CsA–DLPC liposomes (Fig. 2(c)). Formulations above these critical ranges demonstrated reduced drug-liposome aerosol content but without noticeable changes in aerosol mass output rate or apparent viscosity (Fig. 1). There was a concentration dependent reduction of the apparent surface tension of the liposomal formulations up to 100 mg DLPC/ml (data not presented). The addition of CsA and Bud to DLPC liposomes caused a reduction in the apparent surface tension, there was no clear association between changes in drug-liposome aerosol output rates and apparent surface tension or apparent viscosity values (data not presented).

Drug-liposomes consisting of 10 mg Bud:150 mg DLPC and CsA 20 mg:DLPC 150 mg were selected as 'high dose' formulations for further studies. Analysis of drug-liposome formulations prior to nebulization by quasielastic light scattering demonstrated a heterogeneous starting size range of approximately 2.2–11.6 μm (this is at or near the upper accuracy limit of the Nicomp 370). After nebulization, there were minimal differences detected among the formulations. The size range of liposomes within the nebulizer reservoir were 294–502 nm and aerosol samples collected by the AGI-4 impinger ranged from 271 to 555 nm. Analysis with the Andersen Cascade Impactor demonstrated values of 2.0 μm MMAD/1.5 GSD for Bud–DLPC and 2.0 μm/1.8 for CsA–DLPC (Table 1). Analysis of these formulations in a simulated human lung model at 15 BPM and 500 ml tidal volume demonstrates that a 3 min inhala-

Fig. 1. Aerosol mass output rates of nebulized DLPC (a), Bud–DLPC (b), and CsA–DLPC (c) liposomal formulations of increasing concentration with corresponding apparent viscosity (centipoises). Aerosols were generated with water tested and standardized Aerotech II nebulizers (initial starting volume of 5 ml; 10 l/min flow rate) and the mass output determined using an analytical balance after 10 min of nebulization. Aerosol mass output rates data is representative paired analyses each indicated concentration (measurement variations < 10%) and plotted by initial liposomal DLPC content (mg/ml). Apparent viscosity data presented is the mean of ten observations for each of the formulations tested at each indicated concentration (measurement variations < 5%) and plotted by initial liposomal DLPC content (mg/ml). Initial starting volume 10 ml at 23°C.

tion interval would be required to inhaled a 1000 μg daily dose of Bud in liposomes, up to 5000 μg could be inhaled in 12 min (Table 1). The results of CsA–DLPC inhalation in the simulated lung model demonstrated that with high dose CsA-DLPC, 4 min would be required to inhale 5000 μg nebulized CsA in liposomes; 11.5 min would be required to inhale 15 000 μg of CsA (Table 1). These results demonstrate the high capacity of liposomes for aerosol drug delivery.

7. Discussion

There are multiple variables which influence nebulization of drug formulations, including drug-liposomes (Schreier et al., 1993). Nebulizer designs, flow rate, time, operating volumes and the formulation characteristics are paramount for optimal aerosol drug delivery (Waldrep et al., 1994a). For suspensions, such as drug-liposomes, incompatibilities between these factors can lead to segregation of the formulation and preferential aerosol output of water or buffer vehicle and retention of drug-liposomes within the nebulizer reservoir. Thus, aerosol output measurements, such as in this study, should be based on chemical measurements rather than on mass output determinations or laser diffraction analyses. Under incompatible conditions the latter techniques can underestimate the nebulized drug output. The goal of this study was to improve the efficiency of drug-liposome aerosol delivery of CsA and Bud-liposomes by implementation of high dose formulations compatible with nebulized drug output in continuous-flow jet nebulizers. The increased aerosol drug concentration would require a

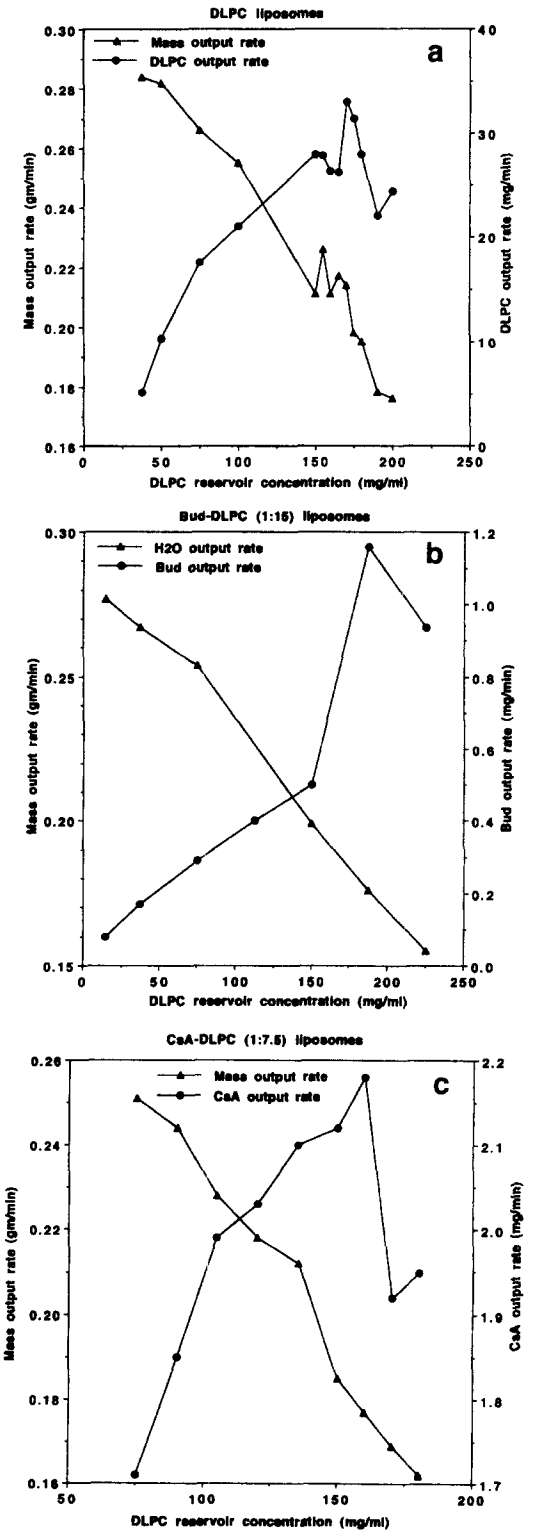

Fig. 2.

Fig. 2. Aerosol drug output rates of nebulized DLPC (a), Bud–DLPC (b), and CsA–DLPC (c) liposomal formulations of increasing concentration with corresponding aerosol mass output rates. Aerosols were generated with water tested and standardized Aerotech II nebulizers (initial starting volume of 5 ml; 10 l/min flow rate) and paired samples were collected in AGI-4 impingers at 4–5 and 6–7 min of nebulization. Bud, CsA and DLPC concentrations were determined by HPLC analysis. The mass output determined as described in Fig. 1. The data is plotted by the initial liposomal DLPC content (mg/ml).

shorter inhalation interval to deliver a therapeutic dose. The study involved the use of flexible, low transition temperature liposomes produced using the synthetic phospholipid, DLPC, which is optimal for nebulized delivery at reduced operating temperatures of the nebulizer (Waldrep et al., 1993). Chemical analysis of DLPC, Bud and CsA by HPLC was employed.

The results of this study indicate that increased drug-liposome reservoir concentrations are associated with reduced nebulized output mass. However, there was an inversely proportional increased aerosol output of liposomes to a range at about 175 mg/ml for DLPC liposomes, 160 mg DLPC/ml for CsA liposomes and 187.5 mg DLPC/ml for Bud-liposomes. Above these concentrations, there was a reduction in the liposome aerosol output. There was a concentration dependent increase in the apparent viscosities but no clear correlation with apparent surface tension. Analysis of liposome formulations by quasielastic light scattering demonstrated that there were minimal differences between the starting MLV size ranges and after processing by extrusion and reflux through the nebulizer jet orifice. It was noted that nebulization reduced the size of liposomes in the reservoir of the nebulizer from several mm in diameter initially to about 300–500 nm and that nebulized liposomes recovered from aerosol samples were also about 300–500 nm. Aerosol particle size analysis demonstrated that the MMAD increased minimally with higher liposome concentrations. The size range of these liposome aerosols generated in our system is optimal for penetration into the lung periphery (Vidgren et al., 1994).

In previous studies of nebulized solutions, the effects of lowering surface tension with surfactants was associated with an increased aerosol output without affecting the aerosol particle size (McCallion et al., 1995, 1996b). Conversely, increased viscosity was inversely related to particle size (McCallion and Patel, 1996; McCallion et al., 1995). The results of our study with nebulized drug-liposomes do not correlate with published results, in part due to differences between solutions and drug-liposome suspensions developed for therapeutic purposes. The previous studies employed ideal solutions displaying Newtonian properties in contrast to the non-Newtonian behavior of liposomes. In a study of jet-nebulized latex suspensions, it was noted that there was a size related retention of spheres remaining within the device partially attributable to concentration effects due to loss of diluent by evaporation or blockage of the nebulizer intake orifice (McCallion et al., 1996c). Our results suggest that a similar phenomenon may occur with highly concentrated liposomes. However, the noted effects of particle size on apparent viscosity may also contribute. Increased viscosity with reduced particle size has been reported (Carstensen, 1973). Similar increases in apparent viscosity were noted between starting formulations and drug-liposome samples from the nebulizer reservoir (data not presented). Multiple factors thus seem to govern nebulization efficiency.

Liposomes demonstrate a large capacity as aerosol drug carriers. The results of this study clearly demonstrate that high dose Bud–DLPC and CsA–DLPC-liposomes can be formulated for aerosol delivery to the lung using continuous flow jet nebulizers with MMAD within the optimal size range for peripheral lung deposition. The high dose Bud–DLPC at 10 mg/ml is 40 times more concentrated than currently available commercial micronized Bud suspensions for jet nebulizers (Arnon et al., 1992; Cameron et al., 1990; Lodrup-Carlsen et al., 1992; Nikander, 1994). High dose Bud–DLPC liposome aerosol could be clinically useful for targeted lung therapy for diseases, such as, asthma, interstitial lung disease, pulmonary fibrosis, or bronchiolitis obliterans (Waldrep et al., 1993, 1994b, 1997; Knight and Waldrep, 1996). Similarly, the high-dose CsA–DLPC could prove effective for topical therapy of refractory chronic lung allograft rejection, as recently described for inhaled CsA in ethanol (O'Riordan et al., 1995). This CsA–ethanol aerosol therapy proved to be effective but was limited due to extreme irritation (Iacono et al., 1996). The high dose Bud and CsA-liposome therapy could offer increased efficacy, sustained delivery and prolonged duration of action, and reduced localized toxicity (Waldrep et al., 1993, 1994a,b, 1997; Knight and Waldrep, 1996). Further clinical investigation of these possibilities is warranted.

Table 1
Aerosol analysis and inhaled concentrations of nebulized high dose Bud–DLPC and CsA–DPLC liposomal formulations

	1000 μg dose	5000 μg dose	15 000 μg dose
Bud 10 mg–DPLC 150 mg 2.0 μm MMA[a] 1.5 GSD	3 min inhalation[b]	12 min inhalation[b]	
CsA 20 mg–DPLC 150 mg 2.0 μm MMAD[a] 1.8 GSD		4 min inhalation[b]	11.5 min inhalation[b]

[a] Anderson cascade impactor (mean of three determinations).
[b] Human lung simulation model (15BPM/500 ml TV) dosage calculated by linear regression analysis (Bud–DPLC ($n = 3$); CsA–DPLC ($n = 2$) analyses).

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