International Journal of Pharmaceutics, 97 (1993) 205–212

IJP 03236

Cyclosporin A liposome aerosol: Particle size and calculated respiratory deposition

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(Received 5 August 1992)
(Modified version received 24 February 1993)
(Accepted 3 March 1993)

Key words: Cyclosporin A; Immunosuppression; Liposome; Aerosol; Nebulizer

Summary

Treatment of pulmonary diseases with the immunosuppressive drug cyclosporin A (CsA) is limited, in part, by poor penetration into the lung following oral or intravenous administration, and by the development of limiting renal, hepatic, and other toxicity following prolonged administration. CsA aerosol delivery may provide an alternate route of local administration that could improve treatment and reduce systemic toxicity. The total CsA dosage administered via aerosol would be less than the conventional oral or intravenous dosage routes. In the present study, lipophilic CsA was prepared with different phosphatidylcholine (PC) liposome formulations. CsA-liposome small particle aerosols generated using continuous jet nebulizers were compared according to particle size range, efficiency of drug delivery, and calculated amounts of respiratory tract deposition. Five different CsA-PC liposome formulations were tested in aerosol with varying degrees of efficiency of drug delivery. CsA-PC liposomes prepared using PC with phase transition temperatures (T_c) below 16–17°C nebulized more efficiently than those with higher T_c. Based on its size range and efficiency, CsA-DLPC was selected as the best formulation for aerosol delivery to the lung. Using the Puritan Bennett 1600, twin jet nebulizer modified to a single jet, the particle size, mass median aerodynamic diameter (MMAD), was 0.82 μm ± geometric standard deviation (GSD) = 1.7. A computer model of inhaled particle deposition in Weibel's lung generations 0–16 and 17–23 was utilized to predict the anatomical delivery of CsA. A calculated 11.6% of inhaled CsA-DLPC liposomes will deposit in the respiratory tract, almost exclusively in Weibel generations 17–23. The remainder, 88.4%, will be exhaled. A dosage of about 2 mg/h will deposit in the adult model respiratory tract used for calculations. Local delivery of CsA by aerosol may provide more efficient treatment of immunologically-mediated pulmonary diseases without systemic toxicity common with intravenous or oral therapy.

Introduction

The fungal metabolite, Cyclosporin A (CsA), is a neutral, lipophilic, cyclic endecapeptide of molecular weight 1203 and possessing potent immunosuppressive activities. CsA is currently one of the most effective immunosuppressive drugs utilized in man for the treatment of immunologically mediated diseases, including the prevention of allograft rejection episodes (Kahan, 1989). The prevailing modes of delivery to the patient are oral (dissolved in peanut oil vehicle) or intravenous administration (in Cremophor EL; polyoxyethylated castor oil). Some degree of nephro-

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toxicity has been attributed to the cremophor vehicle but no suitable substitute has been identified. CsA is widely utilized for the treatment of pulmonary allograft rejection episodes but the clinical results have often been unsatisfactory because of drug-related toxicity (Griffith et al., 1987). In a recent controlled study of pulmonary sarcoidosis, Martinet et al. (1988) reported no evidence of benefit to patients receiving up to 10 mg/kg/day oral CsA, despite profound immunosuppressive effects on their T-lymphocytes in vitro. The absence of clinical benefit may be due to failure of penetration of CsA into the lung despite the attainment of therapeutic blood levels of the drug. Moreover, CsA was not detected in pulmonary lavage fluids from these patients. In one study, oral CsA at 5 mg/day was reported to be effective for the treatment of asthma (Alexander et al., 1992). The pulmonary dosage with this treatment must have been quite low.

Direct drug delivery into the lung should increase the therapeutic availability of CsA for localized immunosuppression. Furthermore, such local immunotherapy could reduce the incidence of toxic complications associated with systemic administration of the drug. At present, there is no alternative method for direct CsA therapy for human pulmonary diseases. Recently, Dowling et al. (1990) in a canine lung allograft model and Keenan et al. (1992) in a rat lung allograft model showed a prophylactic effect of a CsA small particle aerosol generated via nebulization of a CsA-ethanol formulation. These results suggest that local pulmonary delivery of CsA via aerosols may provide therapeutic effects after deposition deep within the lung but with reduced dosage compared to requirements for oral or i.v. routes.

Recently, CsA has been incorporated into different liposomal formulations (Vadiei et al., 1989a; Stuhne-Sekalec and Stanacev, 1991a–c). These formulations were developed as alternative vehicles for intravenous delivery of CsA to reduce toxicity and to increase pharmacological activity. However, intravenous dosages of CsA-liposomes are rapidly removed by the reticuloendothelial system of the liver and spleen and do not readily accumulate in the lung. We have developed an alternate method of localized administration of CsA to the pulmonary tissues, namely by small particle aerosol generated by a continuous flow jet nebulizer with CsA-liposomal aqueous suspensions. Among several preparations tested, we selected the synthetic lecithin, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC) for formulation of CsA-liposomes as best suited for aerosol delivery. The basis for selection, particle size distribution, and a method for calculating deposition of inhaled CsA-liposomes in the human respiratory tract are described.

Materials and Methods

Preparation of CsA liposomes

A modification of the procedure of Vadiei et al. (1989b) was utilized to prepare CsA-liposomes. A variety of synthetic neutral, L-α-lecithins or phosphatidylcholines (PC) (chemically designated 1,2-diacyl-*sn*-glycero-3-phosphocholine or 1-acyl-2-acyl-*sn*-glycero-3-phosphocholine) (all from Avanti Polar Lipids, Alabaster, AL) species were tested for optimal aerosol formulations. These included: dipalmitoyl (DPPC), dimyristoyl (DMPC), egg yolk (EYPC), dioleoyl (DOPC), palmitoyloleoyl (POPC), and dilauroyl (DLPC). While vigorously stirring in *t*-butanol at 37°C, 100 mg of CsA powder (provided by Sandoz Research Institute, East Hanover, NJ) was mixed with 750 mg of synthetic L-α-lecithin powder. The CsA to PC ratio was typically 1:7.5 by weight. This was equivalent to a molar ratio of DLPC/CsA of 14.4. After mixing, the CsA/lipid mixture was pipeted into glass vials and rapidly frozen in dry ice-acetone and then lyophilized overnight to remove the *t*-butanol. Liposomes were produced by adding ultra-pure water to a final drug concentration of 1.0 mg/ml. The mixture was incubated for 20 min at 37°C to allow hydration of liposomes followed by a 10 min sonication at 100 W in a 37°C water bath before use. The freshly prepared liposomes were heterogeneous with sizes ranging from < 1 to about 50 μm in diameter. These were added to the nebulizer reservoir without further modification. With continued passage through the nebulizer jet, liposomes in the nebulizer reservoir were reduced in size. The explana-

tion for this appeared to result from the fact that > 99% of nebulized particles reflux to the reservoir of the nebulizer; < 1% is generated into aerosol (May, 1973). Constant recycling through the nebulizer jet shears the liposomes to smaller sizes (Gilbert et al., 1988). Output of CsA and aerosol droplet particle sizes were quite constant over time.

Liposome preparations were checked for size by microscopy using an eye-piece micrometer and for the presence of drug crystals, both before and after nebulization. The association between CsA and liposomes was determined using the Centrifree Micropartition System (Amicon, Beverly, MA) as previously described (Taniguchi et al., 1987). The total CsA concentration (CsA_T) and the soluble free CsA in the ultrafiltrate (CsA_W) were determined by HPLC. The CsA-liposome incorporation ratio, IR (%), was determined as follows: $IR\ (\%) = [(CsA_T - CsA_W)]/CsA_T) \times 100$.

Puritan Bennett 1600 twin jet nebulizer modified to a single jet

In the present studies, small particle aerosol was generated with the Puritan Bennett 1600 twin jet nebulizer (PBtj) (Carlsbad, CA) which was modified in our laboratory by removing the liquid supply tubing from one of the two nebulizer internal ceramic jets (subsequently designated as the PBsj). Compressed dry air was supplied to the nebulizer at a flow rate of 15 l/min. This air supply was internally divided between the two jets. As modified, therefore, approx. 50% of the 15 l/min air supply passed through each nebulizer jet. One jet produced aerosol while the other without a liquid feed tube provided a jet of dry air within the nebulizer chamber. The addition of drying air internally was found to significantly reduce the particle size of the CsA-liposome aerosol produced by the PBsj compared to the standard PBtj. The amount of drug delivered in particles in the upper half of the size spectrum (3.3 μm to > 10 μm in diameter) was significantly less with the PBsj nebulizer than with the PBtj nebulizer (unpaired t-test, $n = 50, 30$; $P < 0.0005$). More drug was delivered by the PBsj in the smaller particle range (< 3.3 μm diameter) than the PBtj ($P = 0.025$). The single jet modification also produced about two-thirds of the amount of aerosol produced by the twin jet, thus a relative increase in efficiency. The PBsj nebulizer efficiently produces aerosol particles with the majority of particles in the size range of 0.5–5.0 μm. It is proposed that the reduction in particle size is principally due to the lowering of humidity in the head space of the nebulizer, but other factors may be operative. The PBsj has several sizes of reservoirs available, up to 250 ml volume, thus prolonged aerosol treatment is possible.

Particle size distribution of CsA liposome aerosol

The particle size distribution of CsA liposome aerosol was measured with the Andersen/ACFM non-viable ambient particle sizing sampler (Andersen Instruments, Inc., Atlanta, GA) (Andersen, 1958; Davies et al. 1976; Hallworth et al., 1976). Flowing aerosol was conducted for a sampling period of 5 min through the impactor at a rate of one CFM (28.3 l/min) by a vacuum pump. The input aerosol flowed at a rate of 15 l/min. The difference in volume was made up by passage of compensating air around the connection between the aerosol tubing and the impactor or by inserting a Y tube in the line from the nebulizer to the impactor so that one area of the Y tube was left open to ambient air. Results by two similar methods were compared with the Andersen sampler using slightly different connectors but with identical results.

Metal disks below sampling grids at each stage of the impactor were removed after sampling and agitated gently for 20 min in 10 ml of absolute methanol. This eluted material was assayed by high-pressure liquid chromatography (HPLC) for CsA (Charles et al., 1988). A Waters (Milford, MA) 710 B WISP automatic sample injector and a Waters Nova-Pak C18 column heated to 70°C was used in the assay. The mobile phase was 50% acetonitrile, 20% methanol and 30% water. Peaks were detected at 214 nm using a Waters 441 Detector and quantified with a Waters 746 Data Module integrator. After determination of the drug concentrations for each size range, the mass median aerodynamic diameter (MMAD) and geo-

metric standard deviation (GSD) of the CsA-liposomes were calculated using KaleidaGraph 2.0 (Synergy Software, Reading, PA) (Nilsson et al., 1977). We emphasize that particle size measurements in the Andersen sampler refer to the aerodynamic diameters of aqueous particles that contain one to several liposomes. The diameter of the liposomes is much smaller than the diameter of the aqueous particles in which they are carried. The validity of the Andersen sampler methodology for calculating the MMAD/GSD was independently verified using a Model 3300 TSI Laser Particle Sizer.

All-glass impinger (AGI) assay of total aerosol output

The AGI device (Ace Glass Co., Vineland, NJ) consists of a collecting flask containing 10 ml of water to which the aerosol is introduced at sonic velocity through a calibrated glass tube delivering the jet of aerosol 4 mm above the bottom of the flask (Tyler et al., 1959). The system is operated by a vacuum pump so that the flow rate through the impinger is 12.5 l/min (with pressure compensation as described above). The sampling period was 2 min. Paired samples were taken at the designated time intervals during the aerosol generation for analysis.

Results

CsA-liposome aerosol particle sizes with different phosphatidylcholine derivatives

The purpose of our study was to develop a simple aerosol delivery system for the treatment of immunologically mediated, human pulmonary diseases with CsA-liposomes. Formulations were developed using a lyophilization method which can readily be scaled up for clinical use. Lyophilization was selected to limit the use of toxic, organic solvents, such as chloroform and methanol. Selection of the appropriate nebulizer was also critical. Many different nebulizers (originally designed for soluble, aqueous drug administration) were tested for both quantity and quality of CsA-liposome aerosol output. Our basis for selection was production of particles in the 0.5–5 μm size range with few particles larger than 5 μm. The PBsj nebulizer was selected since it

TABLE 1

Recovery of CsA from Andersen cascade impactor according to particle size[a,b]

Range (μm)	CsA DLPC 0.82 ± 1.7 [c]	CsA DOPC 1.35 ± 2.3 [c]	CsA POPC 1.38 ± 2.8 [c]	CsA EYPC 1.29 ± 2.2 [c]	CsA DMPC 1.23 ± 2.7 [c]	CsA DPPC 1.25 ± 3.6 [c]
Presep.	0	9	8	9	8	10
9.0–10.0	0	4	19	0	0	0
5.8–9.0	0	13	31	0	19	2
4.7–5.8	0	27	9	0	45	3
3.3–4.7	0	23	26	6	3	10
2.1–3.3	19	59	22	50	18	16
1.1–2.1	179	232	142	168	67	30
0.7–1.1	171	178	112	66	84	14
0.4–0.7	133	66	89	43	103	16
0–0.4	79	43	45	29	32	25
Total [d]	581	654	503	371	379	126

[a] Aerosol flow rate 15 l/min, sampling interval for 5 min at 30–35 min after initiation.
[b] Mean CsA recovery per stage (μg) of two different preparations.
[c] MMAD ± GSD.
[d] Total μg CsA recovered.

came with different reservoirs of 10 up to 250 ml capacity and had the highest sustained aerosol drug output with the appropriate size characteristics.

In initial studies (Table 1), it was found that CsA-liposomes prepared with DPPC (the predominant lung PC) and DMPC (previously designed for intravenous use) did not nebulize efficiently. This was thought to be due to the rigidity of the DPPC and DMPC containing liposomes at the operating temperature of the nebulizer (16–17°C). The phase transition temperatures (T_c) of DPPC and DMPC (respectively 41 and 23°C) are well above the operating temperature of the nebulizer. The T_c effect is shown in Table 1 with CsA-DPPC liposome aerosol of MMAD of 1.25 $\pm$ GSD 3.57 μm, but with only 126 μg total output/5 min. The CsA-DMPC aerosol was similar with an MMAD of 1.23 $\pm$ GSD 2.7 μm, but higher output of 379 μg/5 min; however, 19% of the CsA-DMPC aerosol particles were greater than 4.7 μm diameter. CsA-EYPC was similar to CsA-DMPC. We observed lot-to-lot variation in the performance of EYPC liposomes and subsequently chose to utilize only synthetic PC.

PCs of low T_c, DOPC and POPC, were better producing aerosols with, respectively, MMAD/GSD of 1.35 $\pm$ 2.3 and 1.38 $\pm$ 2.8 and higher outputs of 654 and 503 μg CsA delivered in 5 min. Both CsA-DOPC and CsA-POPC aerosols also contained some particles in the ranges greater than 4.7 μm. CsA-DOPC produced an oil upon lyophilization from *t*-butanol; both DOPC and POPC powders were very hygroscopic and difficult to work with. Furthermore, the presence of unsaturated double bonds, uncertain long-term stability, and specialized storage questions led us to abandon these PC. Aerosol experiments with CsA-DLPC produced the most consistent results. Incorporation ratios determined using the Amicon Centrifree Micropartition System indicated that 94–99% of the CsA was associated with liposomes, with less than 1–6% in the aqueous filtrate. CsA-DLPC liposomes suspended in water were stable for up to 2 weeks at 4°C. The aerosol generated from CsA-DLPC had an MMAD of 0.82 $\pm$ 1.7 μm with all aerosol particles less than 3.3 μm and a peak in the range of 1.1–2.1 μm. Total CsA output was 581 μg in 5 min. For the above reasons, CsA-DLPC liposomes were selected for use.

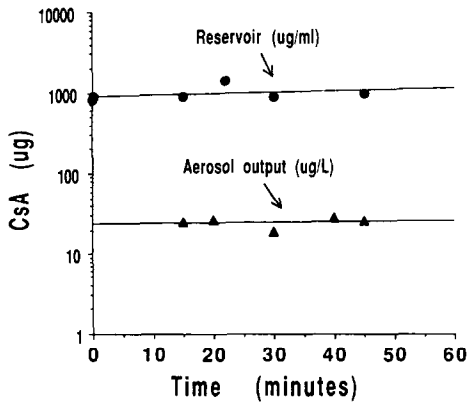

Fig. 1. Total aerosol output of the PBsj with CsA-DLPC liposomes analyzed by AGI. Paired 2-min samples were taken from the aerosol at the designated intervals. Initial reservoir concentrations were 1 mg CsA/ml. Curves represent the mean of two separate analyses using different CsA-DLPC formulation lots.

AGI measurements of DLPC-CsA aerosol

Fig. 1 shows typical measurements of the total drug concentration as determined by AGI analysis of CsA-DLPC liposome aerosol generated over a 45 min interval with the PBsj nebulizer. Using a 30 ml starting reservoir volume, there was a slight trend of increase in the CsA concentration in the reservoir with time of nebulization due to evaporation of water in excess of aerosol generation as previously reported in other systems. The fluid consumption of the PBsj nebulizer is 15 ml of liquid per h. Fig. 1 also shows a parallel increase in the concentrations of CsA in the aerosol output and in the nebulizer reservoir. The mean reservoir concentration for the nebulizer was 0.99 mg/ml with a mean of 25.3 μg/l of CsA contained in the aerosol over a 45 min period of operation. The calculated yield of CsA (μg/l of aerosol) by Andersen sampler was consistently only 31% of the AGI values. This discrepancy may be due to differences in the efficiency of collection of impacted aerosol particles on the eight stages of the Andersen sampler vs collection in liquid within the AGI. Also, an unknown

amount of CsA is lost from the Andersen sampler during collection, extraction, and analysis.

Respiratory tract deposition of CsA-DLPC liposome aerosol

A model of an adult lung with a tidal volume of 750 cm^3, respiratory rate of 15 breaths per min (bpm), mouth breathing, log normal particle distribution, functional residual volume of 3300 cm^3, respiratory cycle of 2 s inspiration/2 s expiration, without breath holding, and an aerosol osmolality of 77 mM was chosen for calculating the anatomical distribution of inhaled particles. Data for these calculations were derived from a computer model modified for this study by P.W. Scherer using the MMAD of the CsA-DLPC liposome aerosol (0.82 $\pm$ 1.7 GSD) (Persons et al., 1987). Deposition in the mouth and pharynx was calculated to be 0.03%, for Weibel generations 0–16, 1.36%, and for Weibel generations 17–23, 10.2% (Weibel, 1963). A total of 11.6% of the inhaled CsA-DLPC was retained. The remaining 88.4% would be exhaled.

An estimate of CsA deposition in the respiratory tract of CsA-DLPC aerosol can be made as follows: 1 l of inhaled CsA-DLPC aerosol contains 25.3 μg of CsA (Fig. 1). With a lung minute volume of 11.25 l (15 bpm $\times$ 750 cm^3) pulmonary deposition would be as follows: in the mouth and pharynx, 0.1 μg/min, Weibel generations 0–16, 3.9 μg/min, and Weibel generations 17–23, 29.0 μg/min, for a total deposition of 33 μg/min. 250 μg would be exhaled per min. Thus, in 1 h, approx. 2 mg of CsA would deposit almost exclusively on the lung periphery of the selected model. Note that these data were derived from a model employing mouth breathing only. Nasal deposition of CsA-DLPC aerosol would exceed that by mouth causing some reduction in lower respiratory tract deposition. However, due to the small size of particles of CsA-DLPC, the resultant changes would be small.

Discussion

Initial experiments with liposome formulations developed for intravenous administration revealed properties unsuitable for efficient aerosol delivery. In contrast to other CsA-liposomal formulations (Vadiei et al., 1989a; Stuhne-Sekalec and Stanacev, 1991a–c), we employed a higher CsA : PC molar ratio of 1 : 14.4 with DLPC (with low T_c) in water rather than buffers to reduce particle size and maximize aerosol drug output. There was a stable association of CsA within the DLPC liposomes produced in this study, even though there is continual passage and extrusion of liposomes through the nebulizer jet. In an earlier study using electron microscopy, liposome particles were found to be reduced in size from 63% of particles, 250 nm diameter or greater at initiation of nebulization, to only 9%, 250 nm in diameter or greater, after 1 h of nebulization (Gilbert et al., 1988). Our results indicate that the CsA-DLPC liposomes are similarly processed to a smaller size during nebulization. To further examine the effect of nebulization on the stability of liposomes, we re-nebulized CsA-DLPC that had been previously nebulized and captured in the AGI. The MMAD and GSD from the first and second nebulizations were nearly identical. The first sample was MMAD 0.82 $\pm$ GSD 1.7 μm, and the second was MMAD 0.8 $\pm$ GSD 0.6 μm. The only difference was the reduction in the GSD, which may represent the further reduction in numbers of larger particles after nebulization.

Treatment of many immunologically mediated human pulmonary diseases by systemically administered CsA has proven to be ineffective, perhaps due to poor penetration into the lung as well as limitations of treatment by toxicity (Atkinson et al., 1983; Martinet et al., 1988; Kahan, 1989). The goal of this work was to develop a simple aerosol delivery system for localized, topical CsA therapy of the lung. These studies demonstrate CsA liposomal preparations which seem suitable for treatment of human pulmonary diseases when administered by small particle aerosol based on particle size and drug content. The particle size of the aerosol studied, generated with the Puritan Bennett 1600 nebulizer, modified to a single jet, configuration was quite small (0.82 μm MMAD $\pm$ 1.7 GSD). This value was less than the aerosol particle ranges generated with CsA and the other PC (Table 1). The finer particles generated with CsA-DLPC (and the absence of particles greater

than 3.3 μm) were predicted by our computer model to deposit principally in the peripheral lung areas (Weibel's generations 17–23), a property that is important for treatment of parenchymal lung diseases. Very little CsA would deposit in the mouth and pharynx.

A major question to be answered with respect to these studies is whether topically delivered CsA lung dosage would be sufficient to provide adequate immunomodulatory therapy. The amount of CsA that enters the lung of patients given systemic treatment is uncertain. Alexander et al. (1992) reported clinical benefit of asthmatics given CsA, 5 mg/kg per day, orally, but Martinet et al. (1988) in a controlled study showed no clinical benefit in patients with sarcoidosis treated over a long period with daily oral doses of 10 mg/kg per day. Trough serum levels in the latter patients ranged from 150 to 250 ng/ml, but no CsA could be detected in their pulmonary lavage fluids. Pulmonary lavage cells from these patients were, however, responsive to low doses of CsA in vitro.

Experimental studies have demonstrated that topical CsA therapy of the lung is effective. Dowling et al. (1990) performed CsA aerosol studies to control rejection of lung allografts. CsA was administered in an ethanol vehicle using a Collison small-particle aerosol generator connected directly via an endotracheal tube into the transplanted lung. The reservoir contained 200 mg CsA in 5 ml of ethanol with therapy given daily for 7 days (291 ng CsA/l aerosol). An estimated 2.8 mg CsA was deposited in the transplanted lung with each daily treatment. However, only two of 44 blood samples showed therapeutic concentrations of CsA. Lung concentrations were variable, but when values of 6–51 μg per g were achieved (based on tissue assay), lung transplant rejection was much less than in controls (or in animals with lower CsA lung tissue levels). Using rats with pulmonary allografts, Keenan et al. (1992) administered CsA in ethanolic small particle aerosol compared with conventional systemic CsA therapy. In this study, the highest aerosol CsA dose (3.6 mg/kg per day for 7 days) gave significantly greater protection from graft rejection than the lower aerosol dosages. The protective effect was also greater than a dose of 25 mg/kg per day given intramuscularly for 1 or 4 days. The lung transplants utilized were selected to cross major histocompatibility barriers and thus, the protective effects of CsA aerosol therapy are highly significant.

In preliminary studies of mice treated with CsA-PC liposome aerosol formulations, we have recovered up to 4 μg of CsA per g of lung tissue following 2 h of inhalation therapy. Minimal levels have been detected in the liver, kidney or blood. In contrast, 2 h after an equivalent dosage of oral CsA, CsA lung concentrations are negligible. These preliminary studies suggest that topical delivery to the lung by aerosol may produce higher local concentrations than systemic administration. This pattern was suggested in a study of patients who died from complications following organ transplantation but had received therapeutic dosages of intravenous and oral CsA prior to death (Reid et al., 1988). Among 29 sites sampled, CsA concentrations in lung tissue averaged 434 ng/g. Several-fold higher levels were noted in most other tissues. Efficient targeting of CsA to the lung by aerosol may produce lung concentrations within a therapeutic range. An added potential benefit of localized aerosol CsA therapy may be reduced systemic toxicity. Smeesters et al. (1988) have reported reduced toxicity of CsA-liposomes.

While the use of DLPC for liposome formulations has been limited, its biophysical properties make it well-suited for aerosol use. In animal studies, we noted no acute toxicity from inhalation of DLPC liposome aerosol. The present studies are preliminary to clinical studies of CsA aerosol treatment of obliterative bronchiolitis in pulmonary allograft recipients. This progressive and fatal lung disease is a prime candidate for early aerosol CsA therapy since much of the damage is irreversible once clinical symptoms appear (Griffith et al., 1988). Studies will be performed when required approvals are obtained.

Acknowledgments

Supported by The Clayton Foundation for Research, Houston, TX, and The State of Texas

Advanced Technology Program, no. 004949-054. The authors thank Melanie Black, B.S. and Kelly Glass, B.S. for expert technical assistance in this study.

References

Alexander, A.G., Barnes, N.C. and Kay, A.B., Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet,* 339 (1992) 324–328.

Andersen, A.A., New sampler for the collection, sizing, and enumeration of viable airborne particles. *J. Bacteriol.,* 76 (1958) 471–84.

Atkinson, K., Boland, J., Britton, K. and Biggs, J., Blood and tissue distribution of cyclosporin in humans and mice. *Transplant. Proc.,* 15 (1983) 2430–33.

Charles, B.G., Norris, R.L.G. and Ravenscroft, P.J., A modified assay for cyclosporin in blood using solid-phase extraction with high-performance liquid chromatography. *Ther. Drug Monitor.,* 10 (1988) 97–100.

Davies, P.J., Hanlon, G.W. and Molyneux, A.J., An investigation into the deposition of inhalation aerosol particles as a function of air flow rate in modified 'Kirk Lung'. *J. Pharm. Pharmacol.,* 28 (1976) 908–911.

Dowling, R.D., Zenati, M., Burckard, G.J., Yousem, S.A., Schaper, M., Simmons, R.L., Hardesty, R.L. and Griffith, B.P., Aerosolized cyclosporin as single-agent immunotherapy in canine lung allografts. *Surgery,* 108 (1990) 198–205.

Gilbert, B.E., Six, H.R., Wilson, S.Z., Wyde, P.R. and Knight, V., Small-particle aerosol of enviroxime-containing liposomes. *Antiviral Res.,* 9 (1988) 355–56.

Griffith, B.P., Hardesty, R.L. and Trento, A., Heart-lung transplantation-lessons learned and future hopes. *Ann. Thorac. Surg.,* 43 (1987) 6–17.

Griffith, B.P., Paradis, I.L., Zeevi, A., Rabinowich, H., Yousem, S.A., Dequesnoy, R.J., Dauber, J.H., Hardesty, R.L. Immunologically mediated disease of the airways after pulmonary transplantation. *Ann. Surg.,* 208 (1988) 371–378.

Hallworth, G.N. and Andrews, U.G., Size analysis of suspension inhalation by inertial separation methods. *J. Pharm. Pharmacol.,* 28 (1976) 898–907.

Kahan, B.D., Cyclosporin. *New Engl. J. Med.,* 321 (1989) 1725–1738.

Keenan, R.J., Duncan, A.J., Yousem, S.A., Zenati, M., Schaper, M., Dowling, R.D., Alarie, Y., Burckart, G.J. and Griffith, B.P., Improved immunosuppression with aerosolized cyclosporin in experimental pulmonary transplantation. *Transpl. Proc.,* 53 (1992) 20–25.

Martinet, Y., Pinkston, P., Saltini, C., Spurzem, J., Muller-Quernheim, J. and Crystal, R.G., Evaluation of the in vitro and in vivo effects of cyclosporin on the lung T-lymphocyte alveolitis of active pulmonary sarcoidosis. *Am. Rev. Respir. Dis.,* 138 (1988) 1242–48.

May, K.R., The Collison nebulizer: description, performance and application. *Aerosol Sci.,* 4 (1973) 235–243.

Nilsson, G. and Brunzell, A., Estimation of particle size in medical aerosols with a cascade impactor. *Acta Pharm.,* 14 (1977) 95–104.

Persons, D.D., Persons, G., Hess, G.D., Muller, W.J. and Scherer, P.W., Airway deposition of hygroscopic heterodispersed aerosols: Results of a computer calculation. *J. Appl. Physiol.,* 63 (1987) 1195–1204.

Reid, M., Gibbons, S., Kwok, D., Van Buren, C.T., Flechner, S. and Kahan, B.D., Cyclosporin levels in human tissues of patients treated for one week to one year. *Transpl. Proc.,* 15 (1988) 2434–37.

Smeesters, C., Giroux, L., Vinet, B., Arnoux, R., Chaland, P., Corman, J., St.-Louis, G. and Daloze, P., Reduced nephrotoxicity of cyclosporin A after incorporation into liposomes. *Transpl. Proc.,* 20 (1988) 831–832.

Stuhne-Sekalec, L. and Stanacev, N.Z., Liposomes as cyclosporin A carriers: the influence of ordering of hydrocarbon chains of phosphotidylglycerol liposomes on the association with and the topography of cyclosporin A. *J. Microencapsulation,* 8 (1991a) 283–294.

Stuhne-Sekalec, L. and Stanacev, N.Z., Liposomes as carriers of cyclosporin A. *J. Microencapsulation,* 8 (1991b) 441–446.

Stuhne-Sekalec, L. and Stanacev, N.Z., Liposomes as cyclosporin A carriers: ESR study of cyclosporin A interaction with 1,2-dimyristoyl-sn-3-phosphatidylglycerol liposomes. *J. Microencapsulation,* 8 (1991c) 455.

Taniguchi, K., Yamazawa, N., Itakura, K., Morisaki, K., Hayashi, S. Partition characteristics of anti-inflammatory steroids in liposomal ophthalmic preparations. *Chem. Pharm. Bull.,* 35 (1987) 1214–1222.

Tyler, M.E. and Shipe, E.L., Bacterial aerosol samplers: I. Development and evaluation of the all-glass impinger. *Proc. Exp. Med. Biol.,* 7 (1959) 337–348.

Vadiei, K., Perez-Soler, R., Lopez-Bernstein, G., and Luke, D.R., Pharmacokinetic and pharmacodynamic evaluation of liposomal cyclosporin. *Int. J. Pharm.,* 57 (1989a) 125–131.

Vadiei, K., Lopez-Bernstein, G., Perez-Soler, R. and Luke, D.R., In vitro evaluation of liposomal cyclosporin. *Int. J. Pharm.,* 57 (1989b)133–138.

Weibel, E.R., *Morphometry of Human Lung,* Academic Press, New York, 1963, pp. 110–111.