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# Pulmonary pharmacokinetics of cyclosporin A liposomes

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**Abstract**

The development of liposomal formulations for aerosol delivery with jet nebulizers has expanded the possibilities for effective utilization of aerosol based therapies in the treatment of pulmonary diseases. The property of sustained release or depot effect of liposomes has been studied using different tracer molecules to monitor absorption and clearance of liposomes from the lung. With liposomal drug formulations, few studies have simultaneously monitored phamacokinetics of both the phospholipid carrier and the therapeutic agent. We have developed a cyclosporin A (CsA)-dilauroylphosphatidylcholine (DLPC) liposomal formulation for aerosol delivery to the lung. Recent studies of CsA-liposomes have reported that CsA displays a unique property of rapid bilayer membrane exchange with dissociation between CsA and its liposome carrier in vivo following intravenous delivery. The purpose of this study was to determine the pharmacokinetics of both CsA (determined by HPLC) and liposomal carrier (labeled with $^{99m}$technetium ($^{99m}$Tc)) to study potential dissociation after delivery to normal mouse lungs. Furthermore, the effects of pulmonary inflammation on the clearance of CsA-DLPC liposomes were compared with $^{99m}$Tc labeled human serum albumin (HSA). Results indicate that $^{99m}$Tc-DLPC liposome carrier is retained up to 16.9 times longer than the CsA half-life in normal lung and 7.5 times longer in inflamed lungs. Similar values were obtained for $^{99m}$Tc-labeled albumin (14.8 times for normal CsA half life (6.8 times in inflamed lungs)). These pharmacokinetic results help to delineate the most effective therapeutic regimens for pulmonary CsA-liposome aerosol delivery. © 1998 Elsevier Science B.V.

*Keywords:* Liposome; Cyclosporin A; $^{99m}$Technetium; Pharmacokinetics; Lung

## 1. Introduction

The development of liposomal formulations, compatible with aerosol delivery with jet nebulizers, has expanded the possibilities for more effective utilization of aerosol based therapies for the

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treatment of a variety of pulmonary diseases (Farr et al., 1985; Gilbert et al., 1988; Niven and Schreier, 1990; Schreier et al., 1993; Taylor and Farr, 1993). Such utilization of liposomes, as aerosol delivery vehicles, has many reported potential advantages for clinical development, including: aqueous compatibility, facilitated intra-cellular delivery particularly to alveolar macrophages and lymphocytes and sustained pulmonary release to maintain therapeutic drug levels within the lung (Schreier et al., 1993; Taylor and Farr, 1993; Hung et al., 1995). These factors, however, have not been extensively studied in the human lung.

The property of sustained release or depot effect of liposomes has been studied using different tracer molecules to monitor absorption and clearance of liposomes from the lung. Phosphatidylcholine liposomes (and/or associated radiolabel) are retained at 50–60% of delivered dose within the lungs of experimental animals for up to 24 h (Vidgren et al., 1994). Human studies of liposome aerosols (and/or associated radiolabel) have demonstrated similar retention within the normal lung, but, depending on the presence of inflammation (Vidgren et al., 1994; Waldrep et al., 1997c). With liposomal drug formulations, few studies have simultaneously monitored phamacokinetics of both the phospholipid carrier and the therapeutic agent. Some studies have demonstrated long term pulmonary retention of counts associated with both drug and liposome carrier (Suntres et al., 1993). However, pharmacokinetic studies of soluble drugs have demonstrated that each agent may behave differently in the lung, with lipid soluble drugs generally being absorbed and cleared more rapidly than water soluble drugs (Brown and Schanker, 1983; Schanker et al., 1985). These rates vary, however, from species to species and by the method of pulmonary delivery (Brown and Schanker, 1983; Schanker et al., 1985). The effect of liposomal formulation on the pulmonary pharmacokinetics of these drugs remains to be determined.

Factors affecting the clearance and/or elimination of exogenous materials from normal and diseased lung tissues are poorly understood. The mucociliary clearance system is impaired in the diseased lung (Schreier et al., 1993), including asthma (O'Riordan et al., 1992). In contrast, experimental studies in rats with infection and/or associated pulmonary inflammation there is enhanced clearance of instilled drug-liposomes and inhaled $^{99m}$Tc-HSA (Connelly and Peterson, 1993; Omri et al., 1994). Thus, multiple factors could also influence drug-liposome retention times within the lung.

We have developed a variety of liposomal drug formulations, including CsA-liposomes, for aerosol lung delivery in preclinical (Gilbert et al., 1993; Waldrep et al., 1993; Vidgren et al., 1994; Waldrep et al., 1994a,b; Gilbert et al., 1997; O'Riordan et al., 1997; Waldrep et al., 1997a); and clinical studies (Waldrep et al., 1997b). Using gamma scintigraphy, nebulizer systems were evaluated and selected for optimal aerosol targeting of drug-liposomes to the peripheral lung regions (Vidgren et al., 1994). However, recent studies of CsA-liposomes in other experimental systems have reported that CsA displays a unique property of rapid bilayer membrane exchange (Choice et al., 1995; Fahr et al., 1995; Ouyang et al., 1995). This dissociation between CsA and its liposome carrier was also demonstrated in vivo following intravenous delivery (Choice et al., 1995; Fahr et al., 1995; Ouyang et al., 1995). The purpose of this study was to determine the pharmacokinetics of both CsA (determined by HPLC) and liposomal carrier (labeled with $^{99m}$technetium) to study potential dissociation after delivery to normal and/or inflamed mouse lungs.

## 2. Experimental

### *2.1. Preparation of CsA-DLPC liposomes*

Formulations were produced at an optimal CsA to DLPC ratio of 1:7.5 (w/w). CsA (Chemwerth, Woodbridge, CT) was mixed with synthetic lecithin: 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC; Avanti Polar Lipids, Alabaster, AL). CsA-DLPC liposomes were produced by lyophilization from t-butanol (Waldrep et al., 1993). Multi-lamellar liposomes were produced by adding 5 ml of ultra pure water above the DLPC

phase transition temperature to deliver the desired final standard drug concentration of CsA 5 mg:DLPC 37.5 mg per ml. The mixture was incubated for 30 min at room temperature with intermittent mixing to produce multilamellar vesicular (MLV) liposomes. Aliquots were taken for determination of drug concentration by HPLC. After swelling, the liposome preparations were examined by micropartition centrifugation, Percoll gradient analysis and microscopy under polarized light for the presence of drug aggregates and after sizing by quasielastic light scattering with a Nicomp Model 370, Submicron Particle Sizer, as previously described (O'Riordan et al., 1997; Waldrep et al., 1997a). The MLV CsA-DLPC drug-liposomes (a heterogeneous starting mixture after swelling of 2.2–11.6 $\mu$m) were sized by extrusion and reflux for 1 min through the orifice of an Aerotech II jet nebulizer (ATII, CIS-US, Bedford, MA). Aerodynamic particle sizing of the liposome aerosols was employed to determine the stable association between $^{99m}$Tc and CsA-DLPC, as measured using an Andersen 1 ACFM non-viable ambient particle sizing sampler (Graseby Andersen Division, Smyrna, GA) equipped with an artificial throat. Liposome aerosols generated from the ATII nebulizer, 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 $\mu$m MMAD) (Smaldone et al., 1991; Vidgren et al., 1994). The MMAD and GSD of the liposome aerosol was calculated as previously described. The MMAD and GSD were determined by the CsA content distributed within the array of droplets comprising the aerosol (Waldrep et al., 1993).

### 2.2. CsA analysis by HPLC

The CsA in liposomal formulations (to determine CsA content in liposome samples) was determined by HPLC. WISP 717 autosampler (Waters, Millford, MA) 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) (13,18). Peaks were detected at 214 nm using the 490 programmable multi-wavelength detector and quantified with the Millennium 2010 Chromatography Manager (Waters, Millford, MA). Samples for CsA and DLPC analysis are dissolved directly in methanol (to solubilize the liposomes). The limits of detection were 10 ng CsA.

### 2.3. Radioactive labeling of CsA liposomes.

$^{99m}$Tc was attached to liposomes by using stannous chloride (3 mM solution) as a reducing agent (Vidgren et al., 1994). The stannous chloride solution was made by bubbling sterile oxygen-free nitrogen and helium through 50 ml of sterile water for 30 min each, to expel free oxygen. A total of 33.5 mg of stannous chloride ($SnCl_2 \times 2H_2O$, Merck, Darmstadt, Germany) was added to the water and nitrogen bubbling was continued for 30 min. One ml of pre-formed CsA-DLPC liposome suspension, containing 5 mg of CsA, was mixed with 0.5 ml of 3 mM $SnCl_2$ solution. Following this, 1 ml of pertechnetate ($^{99m}TcO_4^-$) in sterile saline with radioactivity of 4 mCi was added. The mixture was shaken vigorously for 30 s and left to stand at room temperature for 30 min. Final CsA concentration in the labeled liposome suspension was 2 mg/ml.

### 2.4. Radioactive labeling of human serum albumin

Human serum albumin (HSA, Sigma, St. Louis, MO) was labeled with $^{99m}TcO_4^-$ using stannous chloride as a reductant (De Ligny et al., 1976), 0.5 ml of $SnCl_2$ (0.04 mM $SnCl_2$ in 4 mM HCl prepared immediately before use) was mixed with 1.0 ml of HSA in water (2 mg/ml) and the pH adjusted to 1.5 with 0.1 M HCl. To this mixture, ~1.0 ml of the $^{99m}TcO_4^-$ in saline, was added. The reaction mixture was shaken vigorously for 30 s. After 5 min the labeling efficiency was tested with Centrifree™ Micropartition System (Amicon, Beverly, MA): 150 $\mu$l of the labeled mixture was filled into sample reservoir and the ultrafiltration was made by 10 min centrifugation at $1000 \times g$. The activities of the sample reservoir and filtrate containing free $^{99m}TcO_4^-$ were measured with a gamma counter (2200 Scaler

Ratemeter, Ludlum Measurements, Sweetwater, TX). The labeling efficiency was found to be $\sim 95\%$.

### 2.5. Pulmonary delivery of CsA-DLPC and HSA

Administration of CsA/DLPC liposomes or HSA was made by intranasal (i.n.) instillation of the liposomal preparation into Balb/c mice (Harlan Sprague–Dawley, Indianapolis, IN). In order to investigate the effect of the inflammation in the airways, some groups of mice received 35 $\mu$l 1:1 mixture of pertussis vaccine (Michigan Department of Public Health, Lansing, MI) plus alum precipitated ovalbumin (AP-OVA), 1 week before the study. The labeled suspension was pre-sized with an ATII nebulizer by recycling the suspension for 1 min by airflow of 10 l/min through the nebulizer. A total of 35 $\mu$l of the labeled preparation was instilled into the mice under brief methoxyflurane (Pitman-Moore, Mundelain, IL) anesthesia. The mice were then sacrificed at various time points (1, 30, 60 and 120 min) after instillation and the lungs, intestine and oropharynx were removed. The distribution of the radiotracer in different tissues was detected with the gamma counter and the amount of CsA remaining in mice lungs was measured with HPLC after solid phase extraction of lung samples.

### 2.6. Solid phase extraction and drug analysis of lung tissues

After pulmonary administration of the $^{99m}$Tc-labeled CsA-DLPC liposomes, the mice were sacrificed by methoxyflurane anesthesia followed by cervical dislocation and $^{99m}$Tc counts determined. Tissues were then rapidly frozen at $-20$°C. After sufficient time for radioisotopic decay ($\approx 12$ half-lives), the CsA content of the lung tissues was determined. For HPLC analysis, 10 $\mu$g of cyclosporin D (CsD, Sandoz Research, East Hanover, NJ) was added to the weighed tissues samples as an internal standard and the tissues were homogenized for 1 min in 1 ml of sterile water with a Wig-L-Bug (Crescent Dental, Lyons, IL) in polypropylene tubes containing 5 glass beads (4 mm borosilicate) per tube. The homogenized slurry was then mixed with 2 ml of 98% acetonitrile/2% methanol solution and this volume was centrifuged ($g = 1000\times$, 20 min) to obtain a clear supernatant. Next, 5 ml of sterile water was added to dilute the tissue extraction. Meanwhile, Sep-Pak™ Plus C18 cartridges for solid phase extraction (Waters, Milford, MA) were prepared for use. First, these columns were activated with 5 ml of 95% ethanol and then washed with 5 ml of sterile water before layering the extracted supernatant onto the column. The sample was applied slowly to the column and all fluid was allowed to drain from the resin bed. The column was washed again with 5 ml of sterile water and then with 5 ml of 50% acetonitrile. The sample was finally eluted with 1.5 ml of methanol and 0.5 ml of sterile water. The eluted material was evaporated and then reconstituted in CsA HPLC mobile phase, described above. These samples were then analyzed by HPLC to determine the concentration and extraction efficiency of the CsA. Data was expressed as $\mu$g CsA/gram tissue analyzed.

### 2.7. Statistical analysis

The effects of inflammation on lung clearance rates were analyzed by statistical evaluation using InStat 2.00 (Macintosh), GraphPad, San Diego, CA. The Spearman correlation coefficient was utilized as an indication of statistical significance.

## 3. Results

### 3.1. In vitro assessment of $^{99m}$Tc-labeled CsA-DLPC liposomes

The CsA-DLPC liposome formulation developed was stable in vitro with greater than 99% of the CsA associated with DLPC. In the presence of 10% normal mouse or fetal bovine serum there was minimal increased release of CsA at 24 h of incubation, detected at 8.8 $\mu$g/ml from the initial 5 mg/ml starting concentration. No aggregates were detected by gradient analysis The initial goal of this study was thus, to develop a system suitable for radioimaging of pulmonary delivered

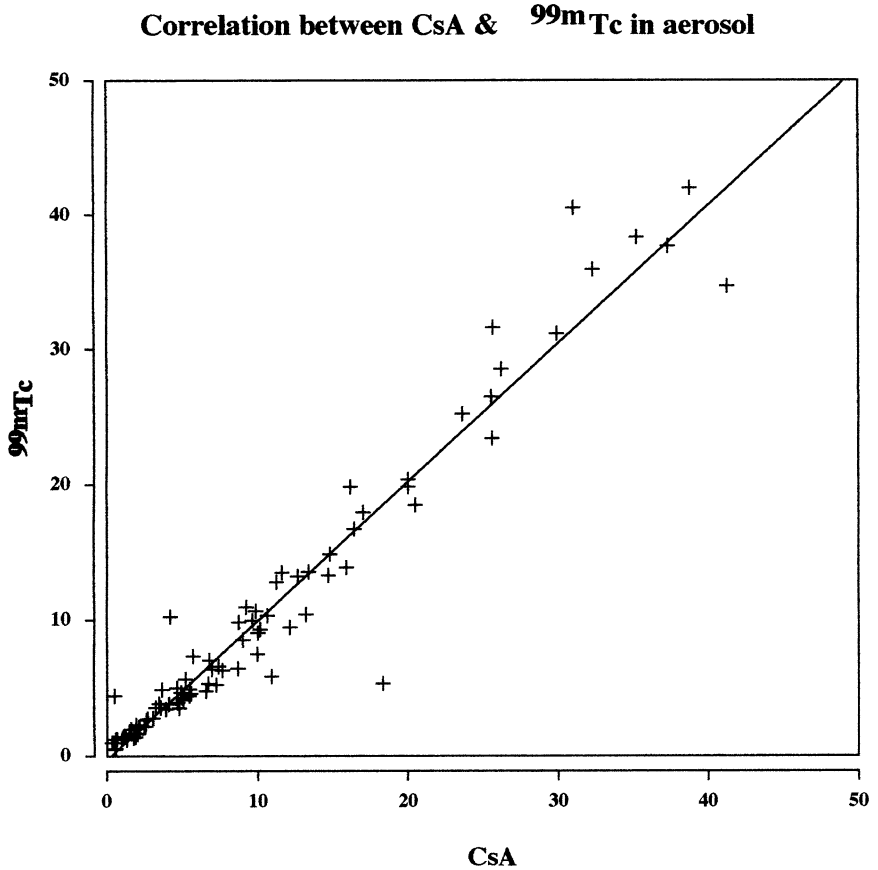


Fig. 1. Association between the $^{99m}$Tc cpm and CsA in CsA-DLPC liposomes processed through the jet of an ATII nebulizer to produce small MLV (500–600 nm). The distribution between both the $^{99m}$Tc counts and CsA in the liposome aerosol droplets captured in the Andersen Cascade Impactor was essentially a linear distribution, with a correlation coefficient of 0.944. The aerosol particle sizes of these labeled liposomes were 1.54 (1.64) $\mu$m MMAD and 2.62 (2.65) GSD by CsA and $^{99m}$Tc distribution, respectively.

$^{99m}$Tc-CsA-DLPC liposomes. A previously developed protocol for efficient $^{99m}$Tc labeling of drug-liposomes was employed (Vidgren et al., 1994). There was a stable association in vitro between the $^{99m}$Tc and the CsA-DLPC liposomes. This was demonstrated after nebulization of the labeled formulation as demonstrated in Fig. 1. The distribution between both the $^{99m}$Tc counts and CsA in the liposome aerosol droplets, captured in the Andersen Cascade Impactor, was essentially a linear distribution with a correlation coefficient of 0.944. The aerosol particle sizes of these labeled liposomes was 1.54 (1.64) $\mu$m MMAD and 2.62 (2.65) GSD by CsA and $^{99m}$Tc distribution, respectively. The mean particle size of the liposomes in solution by Nicomp analysis was 564 nm. These results indicate that this labeled CsA-DLPC formulation would be suitable for lung deposition and clearance studies.

### *3.2. Pulmonary pharmacokinetics of $^{99m}$Tc-labeled CsA-DLPC liposomes*

In order to study the potential differential lung clearance of $^{99m}$Tc-labeled CsA-DLPC liposomes, a model system was developed to simulate the murine inhalation pattern. In this system, $^{99m}$Tc-labeled CsA-DLPC liposomes were delivered to

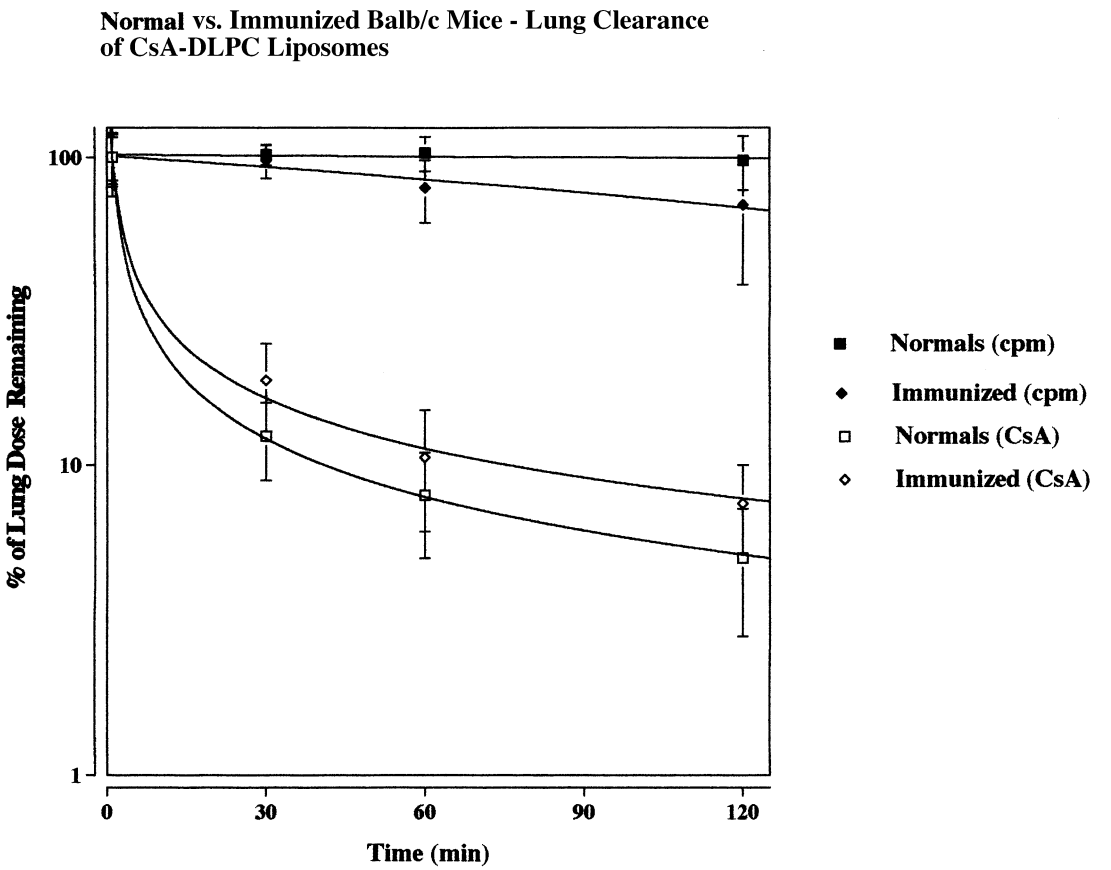


Fig. 2. Pulmonary pharmacokinetics of $^{99m}$Tc-labeled CsA-DLPC liposomes delivered to the lungs via intranasal instillation (35 $\mu$l containing $\approx$ 60 000 cpm) to the lungs of Balb/c mice under methoxyflurane anesthesia. Lungs were removed at 1, 30, 60 and 120 min post-instillation. Radioactive counts were determined immediately after sacrifice and stored at $-20$°C and tissue CsA levels were determined 3 days later after $^{99m}$Tc decay. Values at each time point represent the mean $\pm$ SD of six mice from two separate experiments. $^{99m}$Tc values were corrected for time decay. Pulmonary inflammation was induced following instillation of alum precipitated ovalbumin supplemented (1:1 v/v) with *Bordetella pertussis* adsorbed vaccine. Mice were utilized at day 7 post-immunization.

the lungs via intranasal instillation to mice under methoxyflurane anesthesia (as previously described, Vidgren et al., 1994) and to minimize environmental contamination with radioactive aerosols. After labeling, the CsA-DLPC, liposomes were processed though the ATII nebulizer jet to produce a small particle size range similar to that contained in aerosol droplets. The $^{99m}$Tc remained associated with the CsA-DLPC liposomes as demonstrated in Fig. 1. The clearance of $^{99m}$Tc-labeled CsA-DLPC liposomes delivered to the lungs of normal, Balb/c mice is demonstrated in Fig. 2. As previously described, for intravenous liposomal CsA (Choice et al., 1995; Fahr et al., 1995; Ouyang et al., 1995)., in the Balb/c lung, there is an apparent dissociation between CsA and the $^{99m}$Tc-labeled DLPC molecule independent of inflammation. At 30 min post-instillation, $\approx$ 12% of the CsA remained with little loss of the $^{99m}$Tc-labeled DLPC. Similarly, 18.8% of the CsA remained at 30 min in inflamed lungs with little loss of the $^{99m}$Tc-labeled DLPC. This trend continued at 60 and 120 min, progressing to only $5 \pm 2$ and $8 \pm 3$% of the instilled CsA remaining at 120 min. CsA clearance from the lung was unaffected by inflammation. In contrast, $98 \pm$

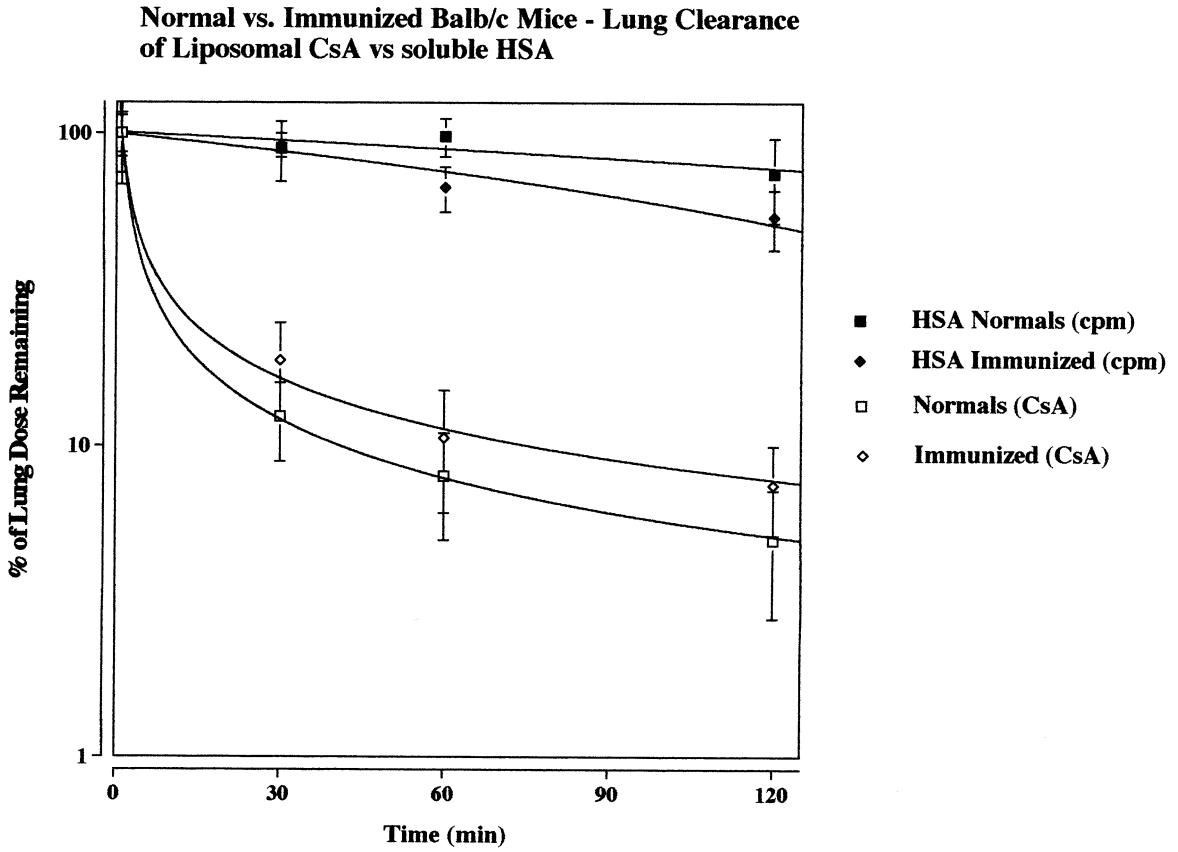


Fig. 3. Pulmonary pharmacokinetics of $^{99m}$Tc-labeled HSA delivered to the lungs via intranasal instillation (35$\mu$l containing $\approx$80 000 cpm) to the lungs of Balb/c mice under methoxyflurane anesthesia. Lungs were removed at 1, 30, 60 and 120 min post-instillation. Radioactive counts were determined immediately after sacrifice and were corrected for time decay. Values at each time point represent the mean $\pm$ SD of six mice from two separate experiments. Pulmonary inflammation was induced as described in Fig. 2.

20% of the $^{99m}$Tc cpm remained in the normal lung and 70 $\pm$ 31% of the $^{99m}$Tc cpm remained in the inflamed lung at 120 min post-instillation. A negative Spearman correlation coefficient ($r = -0.0195$) indicates that there is a significant difference between the DLPC clearance rate in inflamed and normal lungs.

### 3.3. Pulmonary pharmacokinetics of $^{99m}$Tc-labeled HSA

For comparative analysis with previous data and to determine whether there was altered tracer clearance in inflamed mouse lungs, the clearance of $^{99m}$Tc-labeled HSA was determined in this system. The results demonstrated in Fig. 3 show that soluble, less lipophilic, protein HSA was cleared from the lung more slowly than liposomal CsA. At 30 min there was 11 and 10% loss of the $^{99m}$Tc-labeled HSA, respectively from the normal and inflamed lung. There was a trend toward more rapid clearance from the inflamed lung, although the differences noted were not statistically significant.

### 3.4. Pulmonary half-lives of CsA, $^{99m}$Tc-DLPC and $^{99m}$Tc-HSA

The first order rate of elimination of CsA and the DLPC liposomal carrier is presented in Table

1. In the lung, the T1/2$\alpha$ for CsA was equivalent at $17 \pm 3.8$ min in normal and $17.6 \pm 7.3$ min in inflamed lung. In contrast to CsA, the T1/2$\alpha$ for the liposomal carrier was much different at $4.8 \pm 0.1$ h for $^{99m}$Tc-DLPC in normal lung, with a faster clearance rate at $2.2 \pm 0.9$ h from inflamed lung tissue. As compared with CsA, the T1/2$\alpha$ for the soluble, protein $^{99m}$Tc-HSA (MW 67K) was $4.2 \pm 2.4$ h in normal lung and $2.0 \pm 0.3$ h in inflamed lung and very similar to the results obtained for the liposomal carrier.

## 4. Discussion

The wide-spread utilization of aerosol technologies clearly demonstrates its effectiveness for therapeutic application for certain pulmonary diseases. In the lung, many different infectious, inflammatory and allergic diseases have been successfully treated employing aerosol delivery systems to deposit drugs directly onto the pulmonary surfaces (Kohler and Fleischer, 1991; Reed, 1991; Szefler, 1991; O'Doherty and Miller, 1993). The rationale for the development of aerosol drug delivery systems is based on the generalized, basic pharmacological principal that localized drug delivery (to the affected target organ) is often preferable to systemic treatment. This is due primarily to the secondary development of systemic toxic complications which are often prevalent with many drugs. Localized therapy of the target organ generally requires smaller total doses to achieve clinically effective results. A suitable vehicle is, however, an absolute prerequisite for aerosol delivery of hydrophobic, lipophilic drugs like CsA. Our development of CsA-liposomes as an aerosol delivery vehicle has been previously reported (Waldrep et al., 1993, 1997a). Chemically and biologically active CsA levels can be selectively delivered to the lung by liposome aerosol technology (Waldrep et al., 1993, 1997a).

Table 1
Pulmonary half-lives of CsA, $^{99m}$Tc-DLPC and $^{99m}$Tc-HSA

| Component | T1/2$\alpha$ |
|---|---|
| CsA-normal lung* | $17.0 \pm 3.8$ min |
| CsA-inflamed lung* | $17.6 \pm 7.3$ min |
| $^{99m}$Tc-DLPC-normal lung* | $4.8 \pm 0.1$ h |
| $^{99m}$Tc-DLPC-inflamed lung* | $2.2 \pm 0.9$ h |
| HSA-normal lung # | $4.2 \pm 2.4$ h |
| HSA-inflamed lung # | $2.0 \pm 0.3$ h |

* Calculated from the data presented in Fig. 2.
# Calculated from the data presented in Fig. 3.

The utilization of liposomal drug formulations for aerosol delivery has many potential advantages, including: aqueous compatibility, sustained pulmonary release to maintain therapeutic drug levels and facilitated intra-cellular delivery particularly to alveolar macrophages (Schreier et al., 1993). Furthermore, drug-liposomes may prevent local irritation and reduce toxicity both locally and systematically (Schreier et al., 1993; Gonzalez-Rothi and Schreier, 1995). Increased potency with reduced toxicity is characteristic of many drug-liposomal formulations (Cullis et al., 1989). Liposomal aerosols (including CsA) have proven to be non-toxic in acute human and animal studies (Thomas et al., 1991; Myers et al., 1993; Waldrep et al., 1997b). These results suggest that drug-liposome aerosols should be more effective for delivery, deposition and retention of water-insoluble, hydrophobic, lipophilic compounds in contrast to water soluble compounds (Niven and Schreier, 1990; Taylor et al., 1990; Taylor and Farr, 1993).

The goal of targeted drug-liposomal aerosol therapy is to maximize delivery and retention while minimizing clearance. Studies of liposomal CsA have demonstrated a propensity for bilayer membrane exchange both in vitro and in vivo (Choice et al., 1995; Fahr et al., 1995; Ouyang et al., 1995). Following intravenous injection of liposomal CsA, there is dissociation with CsA being eliminated from the circulation more rapidly than the liposomal carrier (Choice et al., 1995; Fahr et al., 1995; Ouyang et al., 1995). These results are comparable to the present study for liposomal CsA delivered to the both normal and inflamed lungs by instillation (in this study) or by aerosol (JCW, unpublished). Our studies demonstrate that the $^{99m}$Tc-DLPC is retained up to 16.9 times longer than the CsA T1/2$\alpha$ in normal lung (7.5 times in inflamed lung) (Table 1). The CsA T1/2$\alpha$

in this system was unaffected by pulmonary inflammation with concurrent lymphocytic infiltration and cyclophilin binding proteins. While the CsA-DLPC liposomes used in this study were extremely stable in vitro, the results suggest that there is dissociation mediated by multiple uncharacterized factors in the lung following pulmonary delivery of CsA-DLPC. This in vivo dissociation phenomenon does not, however, preclude the delivery of immunosuppressive activities to the lung (JCW, manuscript in preparation).

The retention times for both $^{99m}$Tc-DLPC and $^{99m}$Tc-HSA were similar in both normal and inflamed lungs with the latter being of a similar and shorter duration. The enhanced pulmonary clearance associated with inflammation is similar to that described for liposomes and HSA in other systems (Connelly and Peterson, 1993; Omri et al., 1994). Inflammation-mediated inhibition of mucociliary clearance (O'Riordan et al., 1992; Schreier et al., 1993) does not apparently play a major role in this system. Whether the differences between CsA and HSA are related to partition coefficients or molecular weights is unknown. Pulmonary clearance of lipid insoluble drugs is reportedly slower than for lipophilic compounds (Brown and Schanker, 1983; Schanker et al., 1985). Other factors affecting lung clearance rates also include whether the drug was delivered by instillation or aerosol (Brown and Schanker, 1983; Schanker et al., 1985).

The biophysical properties attributed to liposomal CsA may require unique therapeutic regimens to produce the desired immunosuppressive effects in the lung. However, the potential significance of liposome aerosol CsA therapies has been demonstrated by clinical benefit of systemic CsA in chronic severe asthma (Alexander et al., 1992; Lock et al., 1996). CsA-aerosol may prove to be effective in inhibiting T-lymphocyte alloreactive responses preceding the development of bronchiolitis obliterans of lung allografts (Iacono et al., 1996). Thus, CsA-liposome aerosol therapy may be utilized in the treatment of chronic bronchiolar asthma and a variety of pulmonary diseases, such as pulmonary sarcoidosis and allergic hypersensitivity.

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