Lung Deposition and Pharmacokinetics of Cyclosporine after Aerosolization in Lung Transplant Patients

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Purpose. Aerosolized cyclosporine (aCsA) has proven to be an effective therapy for refractory acute and chronic rejection in lung transplant (LTx) patients. The objective of this study is to evaluate the lung deposition and systemic absorption of aCsA after aerosolized cyclosporine administration in LTx patients in the immediate postoperative period.

Methods. Cyclosporine (CsA) was administered intravenously (1.0 mg/kg) to eight LTx patients, and multiple blood samples were collected over 24 h. At least 24 h later, aCsA (300 mg in propylene glycol) was administered to the same patients using nebulization and multiple blood samples were obtained again. Five patients had an additional inhalational gamma scintigraphy study with aCsA and 99MTc-labeled albumin to measure drug deposition.

Results. Peak blood concentrations of CsA after aerosol administration ranged from 119–402 ng/ml, and concentrations at 24 h ranged from 9–48 ng/ml. The rate of decline in drug concentration in blood in the apparent elimination phase was notably slower after administration of aCsA than after IV infusion. Terminal disposition half life ($t_{1/2}$ λz) values ranged from 4.1–9.9 h (mean 6.5 h) following IV administration and from 23.1 to 65.2 h (mean 40.7 h) following pulmonary administration, suggesting that drug absorption occurred throughout the 24-h sampling period following pulmonary administration. Deconvolution analysis indicated biphasic absorption of CsA from the lung in all patients, characterized by rapid initial absorption (absorption half-life 0.73 ± 0.38 h) over the first 4 to 6 h followed by slower, sustained absorption throughout the remainder of the sampling period (absorption half-life 16.2 ± 13.2 h). The absolute bioavailability of CsA after aerosol administration ranged from 5.4–11.2% (mean 8.2%) of the dose placed in the nebulizer. The total dose delivered to the lung estimated from scintigraphy ranged from 17.8–39.3 mg, and was in approximate agreement with the amount of drug absorbed, estimated using deconvolution. Essentially all drug deposited in the lungs was systemically absorbed.

Conclusions. This study documents that cyclosporine can be effectively delivered by aerosolization to the lung of transplant patients in the early postoperative period. Part of the cyclosporine deposited in the lung is absorbed rapidly into systemic circulation and a portion is absorbed slowly but completely over a prolonged period.

KEY WORDS: aerosol; cyclosporine; lung transplant; pharmacokinetics; deposition.

INTRODUCTION

Lung transplant (LTx) patients normally receive tacrolimus or cyclosporine, azathioprine, and prednisone orally to prevent organ rejection. A majority of LTx patients experience organ rejection, and many of the episodes respond to augmented immunosuppressive therapy consisting of pulsed corticosteroid or cytolytic agents. Some patients fail to respond to this therapy, and develop persistent acute rejection associated with bronchiolitis obliterans, which produces irreversible damage to airways. The inevitable outcome for most of these patients is chronic rejection or death (1–4).

Aerosolized cyclosporine (aCsA) has been used at the University of Pittsburgh Medical Center since 1993 as a rescue therapy for patients with lung rejection that was refractory to all other treatments (5–7). Aerosolized cyclosporine appears to be effective in reversing rejection, as evidenced by the changes in histologic and cytokine markers. Pulmonary function is stabilized or improved as a result of aCsA therapy, and the improvement is sustained for months to years (6–7).

The postoperative initiation of aCsA therapy with local deposition of cyclosporine could potentially reduce the number of acute rejection episodes. A reduction in the number of acute rejection episodes has a direct correlation to the development of chronic rejection and obliterative bronchiolitis (3). The first step in this direction is to assess the delivery efficiency, the lung disposition, lung distribution, systemic bioavailability and pharmacokinetics of nebulized cyclosporine in this patient population. The purpose of this study is to determine the pharmacokinetics of CsA following administration as an aerosol and to compare the estimates of amount absorbed with those obtained from pulmonary deposition studies using radionuclides.

MATERIALS AND METHODS

Subjects

The protocol for the intravenous and aerosol studies was approved by the University of Pittsburgh Institutional Review Board, and the gamma scintigraphy study was approved by the Institutional Review Board of the State University of New York at Stony Brook. This study was performed according to the Declaration of Helsinki, and informed consent was obtained from each patient. All pharmacokinetic studies were conducted in the General Clinical Research Center of the University of Pittsburgh Medical Center. Ten LTx recipients were recruited for the trial. These patients were receiving the standard oral immunosuppressive regimen of tacrolimus,

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ABBREVIATIONS: LTx , lung transplant recipients; CsA, cyclosporine; aCsA, aerosolized cyclosporine; FRC, functional residual capacity.

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prednisone and azathioprine that was held constant during the study. One patient voluntarily withdrew from the trial at one week after initiation of the aCsA treatment and no pharmacokinetic studies were performed in this patient. One of the remaining nine subjects was not able to complete the pharmacokinetic study due to medical and scheduling problems.

Drug Preparation and Therapy

The commercially available intravenous solution of cyclosporine (Sandimmune for Infusion, Novartis Pharmaceuticals, East Hanover, NJ) was used for the intravenous study. For the aerosol solution, cyclosporine powder provided by Novartis Pharmaceuticals was dissolved in propylene glycol to achieve a final concentration of 62.5 mg/ml. The cyclosporine solution was filtered through a 0.45-micron filter, and then aliquoted into patient dosing vials. The concentration of cyclosporine in propylene glycol was verified by HPLC analysis. This same cyclosporine solution was used for the gamma scintigraphy studies.

Aerosolized cyclosporine therapy was initiated 7 to 14 days post lung transplantation in conjunction with the standard oral immunosuppressive regimen. Patients were premedicated by inhalation of 2% lidocaine (5 ml) and 5 mg albuterol via a conventional nebulizer to minimize any possible irritation from the aCsA treatment. An Aerotech II nebulizer (Cis-Us, Bedford, MA) was used to administer the aCsA. A 100 mg nebulizer dose of aCsA the first day, followed by a 200 mg nebulizer dose the second day, and finally a 300 mg nebulizer dose was administered daily for ten consecutive days. Subsequently, a 300-mg nebulizer dose was administered three times weekly as chronic therapy.

Pharmacokinetic Studies

Pharmacokinetic studies were performed in these patients after intravenous and aerosol dosing of cyclosporine to determine the extent of cyclosporine lung deposition and subsequent systemic absorption during the postoperative period. These studies were scheduled as soon after the initial 10 days of therapy as possible. An intravenous dose of cyclosporine (1 mg/kg) was administered as a 4-h infusion to each patient at least 24 h after the last aCsA dose. Blood samples were collected predose and approximately 2.0, 4.0, 5.0, 6.0, 12.0, 18.0, and 24.0 h after initiation of the infusion. The next day after obtaining a predose sample, the patient inhaled a 300-mg nebulizer dose of aCsA and the duration of nebulization was recorded. Additional blood samples were collected at 0.25, 0.5, 0.75, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0, and 24.0 h after administration of aCsA.

Cyclosporine concentrations in whole blood were measured using a monoclonal antibody immunoassay (Abbott TDX®, Abbott Park, IL), and concentrations down to 10 ng/ml were used in the analysis. The CV for the assay at 10 ng/ml is less than 25%. Whole blood cyclosporine concentration-time data following intravenous and pulmonary administration to each patient were analyzed using established pharmacokinetic methods. Concentration-time data following infusion of cyclosporine were fit with a 2-compartment IV infusion model using the program WinNonMix Version 1.0 (Pharsight Corp, Mountain View, CA). Compartment model-independent pharmacokinetic analysis of data following pulmonary administration was performed using WinNonlin Pro 3.1 (Pharsight Corp, Mountain View, CA) to take advantage of the ability to model intersubject variability offered by mixed-effect modeling. The rate of drug absorption and cumulative amount absorbed following pulmonary administration were estimated using deconvolution implemented with the program WinNonlin Pro 3.1.

Drug Deposition Using Radionuclide Aerosolization

Deposition studies were performed by two investigators (GCS and ATI), and separate consent was obtained as required by the Institutional Review Board of the State University of New York at Stony Brook.

The standard dosing solution of cyclosporine in propylene glycol was mixed with 0.3 ml of normal saline containing the radioisotope tracer 99mTc bound to human serum albumin (99mTc-HSA, Medi Physics, Paramus, NJ). In preliminary experiments, we have demonstrated that this system generated an aerosol with a mass median aerodynamic diameter (MMAD) of 1.2 μm and a geometric standard deviation (σg) of 2.1 measured by cascade impaction. Analysis of the captured radiolabeled particles indicated that the ratio of radioactivity to drug activity was 1.2 / 1 (8,9).

Total aerosol deposition in each patient was measured using a mass balance technique, with the amount of radioactivity inhaled and exhaled being measured after capture on filters. The measured difference determined the amount deposited in the patient (8). To determine the deposited mass of cyclosporine specifically in the transplanted lungs, regional analysis of deposition was performed using gamma camera imaging. The patient was seated in front of a posteriorly positioned gamma camera (Picker Dyna Camera, Northford, CT) initially set for xenon (133Xe). After spirometry and immediately prior to aerosol deposition, an equilibrium 133Xe scan was performed with lung images at functional residual capacity (FRC) obtained via computer (Advanced Medical Computer, Sunnyvale, CA). After 133Xe equilibrium and washout, the camera was switched to 99mTc and the patient inhaled the full dose of aerosolized cyclosporine labeled with 99mTc-HSA. Immediately following inhalation and after a drink of water to wash out the pharynx, a deposition image was obtained. The regions of interest based on lung outlines delineated in the 133Xe equilibrium image were drawn. The amount of 99mTc-HSA aerosol deposited in the transplanted lung was expressed as a percentage of the sum of the 99mTc-HSA activity in both lungs and in the stomach.

RESULTS

Pharmacokinetic studies were performed in six patients at 2 to 3 weeks post transplantation, and in two patients at 3 to 4 months post transplantation. Radionuclide deposition studies were conducted on five of these patients. Table I lists the intravenous CsA dose, time of infusion, and duration of nebulization of aCsA for each patient. The initial blood sample from all intravenous and aerosol studies was below the detectable limits of the assay and was assumed to be zero. Peak blood concentrations of cyclosporine after aerosol administration ranged from 119–402 ng/ml, and concentrations at 24 h ranged from 9–48 ng/ml. Figure 1 shows individual

Table I. Cyclosporine Dose and Duration of Administration by IV and Pulmonary Routes

Patient	Infusion dose[a] (mg)	Infusion duration (hr)	Nebulizer duration[b] (h)
1	62.7	4.00	0.45
2	66.6	3.75	0.43
3	90.5	4.60	0.75
4	73.0	4.60	0.75
5	78.1	4.10	0.38
6	72.0	4.00	0.28
7	84.6	4.00	0.50
8	71.8	4.00	0.50

[a] IV dose: 1.0 mg/kg.
[b] Nebulizer dose: 300 mg.

patient and mean CsA concentration-time profiles following administration of intravenous and aerosolized cyclosporine. Mean pharmacokinetic parameter estimates following infusion are listed in Table II. Pharmacokinetic parameter values from compartment model-independent analysis of the concentration-time data following pulmonary administration are listed in Table III. Inspection of concentration-time profiles for both treatments within patients revealed differences in the rate of decline in drug concentration in the period from 8 to 24 h, with concentrations declining at a slower rate after administration of aCsA than after IV infusion. This pattern occurred in all patients, similar to that shown in Fig. 2 for Patient 1, and was also reflected in $t_{1/2}$ λz values that ranged from 4.1 to 9.9 h (mean 6.5 h) following IV administration and from 23.1 to 65.2 h (mean 40.7 h) following pulmonary administration. These findings suggested that drug absorption was occurring throughout the 24-h sampling period following pulmonary administration rather than only during the period of rapid rise and decline in concentration associated with the occurrence of the peak concentration as would be expected. This was further investigated using deconvolution to characterize the rate of absorption and estimate the cumulative amount of drug absorbed during the sampling period.

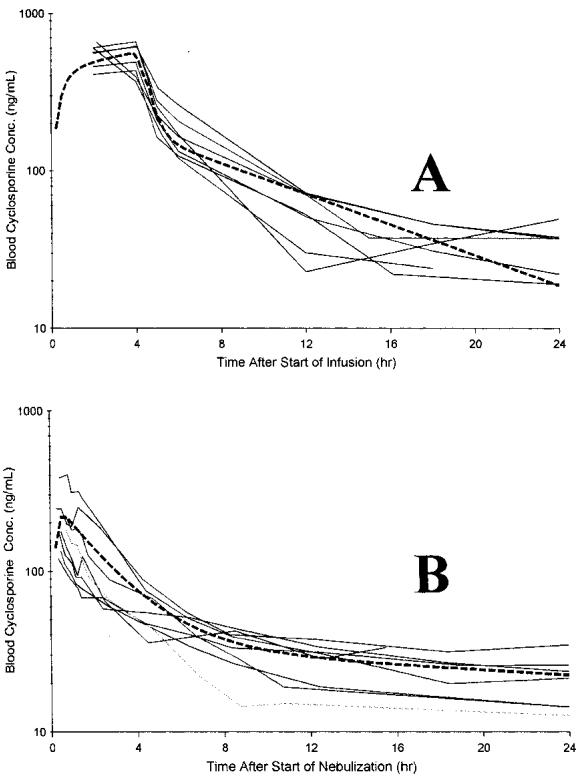

Fig. 1. Whole blood cyclosporine concentration-time profiles following administration of IV (panel A) and aerosolized (panel B) cyclosporine. The dashed line in each plot shows mean concentration-time data; solid lines are observed concentration-time data in individual patients. The log-linear plots provide the best demonstration of changes in elimination rate after intravenous (panel A) and aerosolized (panel B) cyclosporine.

Table II. Mean Parameter Estimates from the Fit of a 2-Compartment IV-Infusion Pharmacokinetic Model to IV Data from Seven Lung Transplant Patients (All Except Patient 2)

Parameter	Mean	Std dev	CV%
V1 (L)	18.2	4.8	26.5
K10 (1/hr)	1.168	0.329	28.2
K12 (1/hr)	1.089	0.438	40.2
K21 (1/hr)	0.227	0.081	35.5
t1/2 λz (hr)	6.5	2.3	35.4
AUC (0-INF) (ng/mL*hr)	3580.2	154.2	4.3

The mean cumulative amount of drug absorbed vs. time derived from the deconvolution analysis indicated biphasic absorption of cyclosporine from the lung (Fig. 3), characterized by rapid initial absorption over the first 4 to 6 h followed by slower, sustained drug absorption throughout the remainder of the sampling period. Neither the individual nor the mean cumulative absorption curves become asymptotic during the 24-h sampling period, further indicating the existence prolonged drug absorption. Half-life estimates for the fast and slow absorption phases were 0.73 ± 0.38 h and 16.2 ± 13.2 h, respectively. The absolute bioavailability of CsA after aerosol administration, calculated using the amount of drug absorbed estimated from deconvolution, ranged from 5.4–11.2% (mean 8.2%) of the dose placed in the nebulizer (Table III).

Five patients participated in the aerosol deposition studies with aCsA and 99mTc-labeled albumin. The intravenous study results for Patient 2 were incomplete because of an analytical problem and could not be used for deconvolution. That patient's deconvolution-based amount absorbed was interpolated using mean IV data from the other seven patients. Table IV lists estimates of the amounts deposited in body and lung calculated from the radionuclide deposition studies and from the pharmacokinetic analyses. Figure 4 shows the relationship between amount absorbed based on deconvolution analysis and scintigraphy-estimated lung dose. This relationship appears to follow the line of identity, although the limited number of patients precludes meaningful statistical analysis. The observed similarity of the scintigraphy and pharmacokinetic estimates of amount absorbed indicates that essentially all drug deposited in the lungs was systemically absorbed.

DISCUSSION

Aerosolized cyclosporine has proven to be a valuable adjunctive therapy for refractory acute and chronic rejection

Table III. Pharmacokinetic Parameter Values from Compartment Model-Independent Analysis of aCsA Concentration-time Data following Pulmonary Administration in Lung Transplant Patients

Patient	Cmax (ng/mL)	Tmax (hr)	AUC(0–24) (ng/mL*hr)	Vz/F (L)	$t_{1/2}$ λz (hr)	Amt absorbed[b] (mg)	F[c] (%)
1	401.6	0.83	1565.2	4582	23.8	33.7	11.2
2	171.7	0.43	1030.6	5554	23.1	21.1[a]	7.0
3	179.6	0.75	715.6	14927	65.2	19.4	6.5
4	220.0	0.75	970.5	8528	31.8	25.4	8.5
5	119.4	0.38	950.3	7553	45.1	21.4	7.1
6	250.6	1.30	1551.8	6150	67.6	31.3	10.4
7	132.1	0.50	731.9	9263	27.7	16.3	5.4
8	175.4	0.50	755.3	6763	41.5	27.7	9.2
Mean	206.3	0.68	1033.9	7915	40.7	24.5	8.2
SD	89.6	0.30	344.7	3221	17.7	6.1	2.0

[a] IV data from Patient 2 not available; Amt Absorbed estimated using mean of data for other 7 patients.
[b] Cumulative amount of drug absorbed based on deconvolution.
[c] F = Amt Absorbed/300 mg nebulizer dose * 100.

in LTx patients at the University of Pittsburgh. The history of the development of this therapy from animal models and to its early use in humans in Pittsburgh has been previously described (10). The pharmacokinetic results of this study support the concept of adequate deposition of cyclosporine in the lung using nebulization, with the initial rapid systemic absorption followed by prolonged systemic absorption.

A recent report by Rohatagi *et al.* (11) evaluated the pharmacokinetics of an aerosolized form of cyclosporine (ADI628) in healthy subjects and asthmatic patients in aerosolized doses of 1–20 mg. They observed a rapid peak of blood ADI628 at 0.5–0.75 h after the dose. Repeated doses of ADI628 given twice daily resulted in drug accumulation (ratios for Cmax and $AUC_{0\text{-}12}$ of 1.1–2.0) and an observed longer apparent half-life with higher doses.

One of the potential difficulties in interpreting pharmacokinetics following pulmonary drug administration is that drug can be deposited in the oropharynx and upper airways and then swallowed. The swallowed drug potentially contributes to the overall amount absorbed systemically. If a sufficient quantity were swallowed, oral absorption of cyclosporine (12) might give the false impression that prolonged pulmonary absorption was taking place. However, the gamma scintigraphy results conclusively demonstrate that little aerosolized drug was deposited outside of the lung (Table IV; total body dose minus lung dose). Based on these scintigraphy results and the highly respirable characteristics of the aerosol created by the Aerotech II nebulizer (MMAD = 1.2 μm; σg = 2.1), we assumed that absorption from the gastrointestinal tract was negligible.

Previous investigations by other researchers provide hypotheses to explain why this prolonged retention of nebulized cyclosporine occurs in the lung . Kawai *et al.* (13) reported on pulmonary absorption and accumulation after inhalation of a cyclosporine derivative, IMM 125 in a symposium in 1997. An *in vitro* portion of their study demonstrated that alveolar macrophages avidly take up the powdered drug that is deposited within the lungs. This uptake was then modeled in rats and

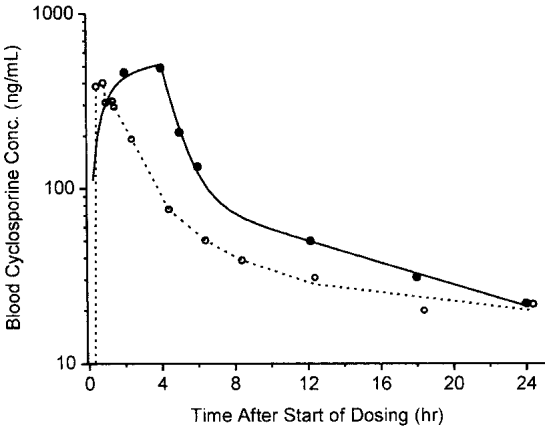

Fig. 2. Blood cyclosporine concentration-time profiles from Patient 1. Solid circles and line show observed concentrations and model-fit line after IV; open circles and dashed line show observed concentrations and compartment model-independent analysis line after nebulization. Note differences in terminal phases; IV t1/2 λz = 5.3 h, aCsA t1/2 λz = 23.8 h

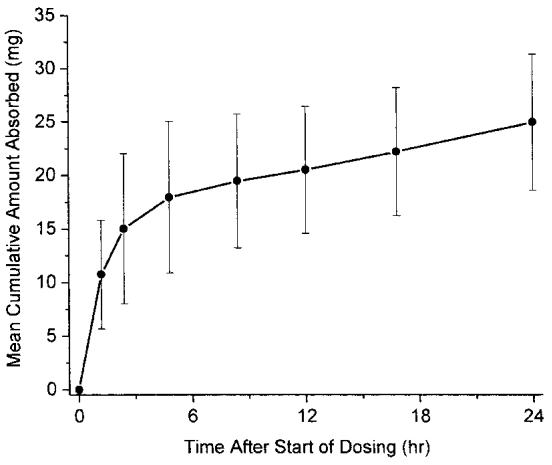

Fig. 3. Mean (+/– SD) cumulative amount of cyclosporine absorbed vs. time following pulmonary administration of 300 mg aCsA in eight lung transplant patients.

Table IV. Amount of Cyclosporine Deposited as Determined by Radionuclide Deposition Studies and Pharmacokinetic Studies

Patient	Total body dose based on scintigraphy (mg)	Total lung dose based on scintigraphy (mg)	Amt absorbed in 24 hr based on deconvolution (mg)
1	30.2	28.3	33.7
2	18.4	16.2	21.1[a]
3	19.5	17.8	19.4
4	31.4	29.1	25.4
8	49.3	39.3	27.7

[a] Interpolated result.

found to have a half-life of 3 days for macrophage-retained drug. Kawai also studied one human subject who inhaled 10 mg per day of IMM 125. The percentage of the drug concentration in the lung that was accounted for by lung storage of drug was predicted by the investigator's analysis to approach 60%. Therefore, the uptake and slow release of drug from alveolar macrophages, which make up the majority of cells found in bronchoalveolar lavage fluid, could account for a portion of the slow release of cyclosporine from the lung that is observed in the current study.

Another possible reason for the prolonged retention of cyclosporine in the lung is suggested by McAllister *et al.* (14). The investigators used immobilized artificial membranes to make a quantitative comparison of polypeptide binding. They concluded that two of the polypeptides that they examined, one of which was cyclosporine, should exhibit prolonged pulmonary retention. They hypothesized that the lipophilicity of cyclosporine and its ability to interact with phospholipids may allow it to bind to pulmonary surfactant or the alveolar epithelial cell membrane.

Our earlier studies of nebulized cyclosporine have demonstrated a positive clinical response in patients with refractory rejection who had deposition of at least 20 mg of drug in their lungs (9). Six of the eight early postoperative LTx patients in this study exceeded this target lung dose (Table III), with the other 2 patients achieving lung doses of 16.3 and 19.4 mg. This indicates that the deposition of CsA using the dosing methods described in this study is of sufficient magnitude for immunosuppressive treatment even in the early postoperative period, assuming that adequate deposition will occur in the transplanted lung(s). Since previous studies have demonstrated the heterogeneity of deposition in LTx patients, further deposition studies are warranted to more fully assess aCsA therapy in a larger group of lung transplant recipients.

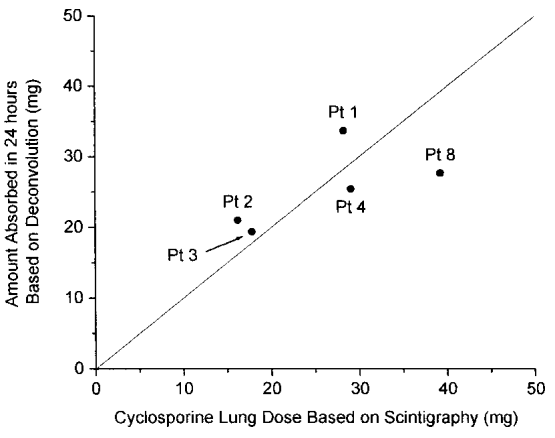

Fig. 4. Relationship between amount of drug absorbed in 24 h based on deconvolution analysis vs. the lung dose estimated by scintigraphy. The line of identity is shown for reference.

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