Dry powder inhalation of biopharmaceuticals
Zijlstra, Gerrit

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
The efficacy of a new pulmonary cyclosporine

A powder formulation in the prevention

of transplant rejection in rats

Gerrit S. Zijlstra,1 Joske Wolting,1 Jochum Prop,2 Arjen H. Petersen,2 Wouter L.J. Hinrichs,1 Donald R.A. Uges,4 Huib A.M. Kerstjens,3 Wim van der Bij,3 Henderik W. Frijlink,1

1 Department of Pharmaceutical Technology and Biopharmacy,
University of Groningen, The Netherlands
2 Department of Pathology & Medical Biology,
University Medical Center Groningen, The Netherlands
3 Department of Respiratory Medicine,
University Medical Center Groningen, The Netherlands
4 Department of Hospital Pharmacy,
University Medical Center Groningen, The Netherlands

Published in: J Heart Lung Transplant. 2009 May;28(5):486-92. Epub 2009 Apr 5
ABSTRACT

**Background.** The aim of this pilot study was to determine the pharmacokinetics of cyclosporine A powder for inhalation (iCsA) and its efficacy in preventing rejection in experimental lung transplantation in rats.

**Methods.** Single dose pharmacokinetics (10 mg/kg) of pulmonary and orally administered cyclosporine A was determined in whole blood and in lung and kidney tissue. The efficacy of iCsA (2.5 and 5 mg/kg) in inhibiting rejection was determined in an orthotopic left lung transplantation rat model and compared with orally administered CsA (5 and 10 mg/kg). The ventilation score of lung allografts was assessed with roentgenograms. At day 10 post-operative, the rats were terminated and lungs were prepared for histological analysis.

**Results.** In the pharmacokinetic study, AUC$_{0-48}$ values in blood for iCsA and oral CsA were similar (47,790±1,739 and 46,987±2,439 ng·h·mL$^{-1}$, respectively). In contrast, iCsA levels in lung tissue were much higher than oral CsA levels (AUC: 9,152,977±698,920 versus 84,149±8,134 ng·h·g$^{-1}$, respectively), showing the effectiveness of the pulmonary administration. In the rejection study, non-treated animals showed complete rejection after 8 days on roentgenograms. Treatment with 5 mg/kg iCsA reduced rejection on day 10, while the 2.5 mg/kg dose did not inhibit rejection. Oral CsA 10 mg/kg strongly reduced rejection, while the 5 mg/kg dose showed hardly any effect on rejection.

**Conclusions.** We conclude that iCsA is an effective immunosuppressive formulation, which might become a valuable asset for clinical use in combination with systemic immunosuppression.
INTRODUCTION

Lung transplantation is an effective treatment for end-stage lung disease, since it improves quality of life and prolongs survival. Oral treatment with calcineurin inhibitors such as cyclosporine A (CsA) is a cornerstone of the current immunosuppressive strategies. To reduce the notorious adverse effect profile of CsA, newer strategies are employed such as exchanging CsA for Tacrolimus (1), improved pharmacokinetic monitoring and subsequent dosing based on the 2 hours post-dose CsA concentration (C2) instead of the trough (C0) concentration (2-5), delayed initiation of CsA therapy through induction therapy with Basiliximab (6), combination therapy with Everolimus (Certican®) to allow a lower CsA dose with similar efficacy and less adverse effects (7-12) or pulmonary delivery of CsA (13, 14). Pulmonary delivery of CsA by nebulization has shown to improve the therapeutic efficacy in lung transplantation in animal models (15-17) and humans (14, 18-20). In humans, aerosolized CsA resulted in lower rejection rates in patients with acute (13) and chronic (14, 20) rejection, resulting in improved survival.

In the clinical studies, a propylene glycol solution that contained CsA was used (13, 18, 20, 21). Unfortunately, inhalation of the CsA solution was accompanied by severe complications. First, patients had to be pretreated with aerosolized lidocaine and albuterol to reduce side effects like dyspnea, cough and pharyngeal soreness (14). Secondly, nebulization resulted in a lung deposition of only 5.4 - 11.2% of the metered dose in the lung (21). Since Corcoran showed that doses of at least 5 mg CsA are needed in the peripheral part of the lung to obtain a significant improvement in lung function (18), it is clear that with conventional administration, high nebulized doses are necessary for effective treatment. As detailed above, higher dosing is limited by local side effects.

To avoid the difficulties faced with propylene glycol nebulization, we developed CsA as a powder for inhalation (iCsA) using an inulin-based solid dispersion formulation technique (22). A major advantage of dry powder inhalation over fluid nebulization is that no solvent is needed, which could make the premedication redundant. Other advantages are the higher pulmonary deposition efficiency and ease of administration (23).

Since this is the first study of iCsA in animals, the purpose of this study was to investigate 1) single dose pharmacokinetics for dose determination
in the efficacy part of this study and 2) the effectiveness of iCsA in preventing rejection in orthotopic left-lung transplantation in rats.

**MATERIALS AND METHODS**

**Materials**

Neoral® was obtained from Novartis, Basel, Switzerland. Cyclosporine as a powder for inhalation (iCsA) was compounded at the Hospital Pharmacy of the University Medical Center Groningen (UMCG) as described previously (22).

**Animal experiments**

Brown-Norway (BN) and Albino-Oxford (AO) male rats (200-260 gram) were purchased from Harlan (Horst, the Netherlands). Our studies conformed to the Dutch Law on Experimental Animal Care and were approved by the Institutional Animal Care and Use Committee of the University of Groningen. During all experimental procedures, rats were under isoflurane anesthesia.

Single dose (10 mg/kg) pharmacokinetics (iCsA) and oral CsA (Neoral®, Novartis, Basel, Switzerland) were determined in BN rats (n=18 per treatment). iCsA was administered by insufflation (model DP-4, Penn-Century, Philadelphia, PA, USA) after orotracheal intubation. Oral CsA was administered undiluted by oral gavage. Blood was collected at t= 0, 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 hours after administration; kidneys and lungs were harvested at t= 0, 2, 6, 12, 24 and 48 hours.

Orthotopic, left lung transplantation was performed across a major histocompatibility barrier with AO (RT1<sup>u</sup>) as donor and BN (RT1<sup>n</sup>) as recipient (24, 25). In total, twenty-one transplantations were performed (table 1). Rats were treated with 2.5 or 5 mg/kg iCsA on day 0, 2, 4, 6 and 8. Reference treatment was oral CsA in a dose of 5 or 10 mg/kg. Isogenic and allogenic transplantation served as untreated transplantation controls. Non-transplanted BN rats served as treatment controls and received 5 mg/kg iCsA or 10 mg/kg oral CsA. Determination of systemic CsA trough levels and ventilation score was performed at day 2, 4, 6, 8 and 10. At day 10, lungs were collected for histological analyses.
In vivo efficacy of iCsA

Table 1. Experimental groups in lung allograft rejection study.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Transplantation (Donor-recipient)</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ischemia time (min ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5*</td>
<td>AO-BN</td>
<td>iCsA</td>
<td>2.5</td>
<td>34.2 ± 5.2</td>
</tr>
<tr>
<td>2</td>
<td>4*</td>
<td>AO-BN</td>
<td>iCsA</td>
<td>5</td>
<td>32.7 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>AO-BN</td>
<td>Oral CsA</td>
<td>5</td>
<td>33.0 ± 3.5</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>AO-BN</td>
<td>Oral CsA</td>
<td>10</td>
<td>34.0 ± 1.7</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>BN-BN</td>
<td>-</td>
<td>-</td>
<td>37.0 ± 7.9</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>AO-BN</td>
<td>-</td>
<td>-</td>
<td>37.3 ± 4.0</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>BN</td>
<td>iCsA</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>BN</td>
<td>Neoral, orally</td>
<td>10</td>
<td>-</td>
</tr>
</tbody>
</table>

* In group 1, 2 animals died during iCsA treatment and 1 animal died during the transplantation procedure. In group 2, 1 animal died during iCsA treatment. Autopsy revealed that the three deaths during treatment of iCsA were caused by destruction of the larynx as a consequence of the intubation procedure.

Analyses

Cyclosporine concentrations were determined using a fluorescence polarization immunoassay (AxSym®, Abbott Laboratories, Chicago, USA) (26). CsA blood levels were fitted with first-order kinetics (equation 1), followed by calculation of pharmacokinetic parameters:

\[ C(t) = \left( \frac{C_0 k_a}{k_a - k_{el}} \right) e^{-k_{el}t} - \left( \frac{C_0 k_a}{k_a - k_{el}} \right) e^{-k_at} \]

where \( C(t) \) is the concentration in the compartment (ng/mL), \( C_0 \) a constant (ng/mL) representing the maximum compartment concentration obtained by only absorption at \( t=0 \) and \( k_a \) and \( k_{el} \) the absorption and elimination rate constants, respectively (h⁻¹). Bailer’s method was used to calculate the area under the curve (AUC) and standard error from 0 to 48 hours (27).

CsA tissue levels were determined by mixing an aliquot EDTA blood with a known amount of tissue sample extract and processed similarly to a whole blood sample.

Rejection was determined by two blinded and independent observers on basis of roentgenograms and histology (24, 25). Ventilation scores of roentgenograms ranged from 6 (normal looking lungs) to 0 (opaque, fully
rejected lungs). Histological scores ranged from 0 (normal looking lungs) to 4 (completely rejected lungs).

**Statistical analysis**

All results are expressed as mean ± standard error of the mean (SEM), unless mentioned otherwise. Statistical comparisons between groups were performed by the Wilcoxon-test. Statistical significance was taken as $p<0.05$.

![Cyclosporine whole blood levels after single 10 mg/kg dose. (A) iCsA 10 mg/kg and (B) oral CsA 10 mg/kg. Each circle represents an individual data point and lines represent the fit of the data points.](image)

**Figure 1.** Cyclosporine whole blood levels after single 10 mg/kg dose. (A) iCsA 10 mg/kg and (B) oral CsA 10 mg/kg. Each circle represents an individual data point and lines represent the fit of the data points.
RESULTS

Pharmacokinetic study

Inhalation and oral administration of CsA resulted in substantial systemic blood levels (figure 1). Insufflation of CsA (figure 1A) led to a lower variation of individual whole blood levels compared to orally administered CsA (figure 1B).

Table 2. Summary of fitting parameters, calculated \( C_{\text{max}} \), \( t_{\text{max}} \) and \( t_{1/2\text{el}} \) by using eq. 1 and Calculated area under the curve in whole blood, kidney and lung tissue. Targeting index was calculated by dividing tissue AUC’s by whole blood AUC. \( C_0 \) is a constant (ng/mL) representing the maximum compartment concentration obtained by only absorption at \( t=0 \), \( k_a \) the absorption rate constant and \( k_{\text{el}} \) the elimination rate constant.

<table>
<thead>
<tr>
<th>Administration</th>
<th>iCsA Insufflation</th>
<th>Neoral Oral gavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Results of fit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_1 ) (ng·ml(^{-1}))</td>
<td>1,201.59</td>
<td>1,727.05</td>
</tr>
<tr>
<td>( k_a ) (h(^{-1}))</td>
<td>1.474</td>
<td>0.653</td>
</tr>
<tr>
<td>( L_1 ) (h(^{-1}))</td>
<td>0.00968</td>
<td>0.03309</td>
</tr>
<tr>
<td>Pharmacokinetic parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\text{max}} ) (ng·ml(^{-1}))</td>
<td>1,162.32</td>
<td>1,472.78</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (h)</td>
<td>3.4</td>
<td>4.8</td>
</tr>
<tr>
<td>( t_{1/2\text{el}} ) (h)</td>
<td>71.6</td>
<td>20.9</td>
</tr>
<tr>
<td>AUC(_{0-48})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole blood (ng·h·ml(^{-1}) ± SD)</td>
<td>47,790 ± 1,739</td>
<td>46,987 ± 2,439</td>
</tr>
<tr>
<td>Kidney (ng·h·g(^{-1}) ± SD) *</td>
<td>166,679 ± 8,371</td>
<td>130,962 ± 8,541</td>
</tr>
<tr>
<td>Lung (ng·h·g(^{-1}) ± SD) *</td>
<td>9,152,977 ± 698,920</td>
<td>84,149 ± 8,134</td>
</tr>
<tr>
<td>Targeting index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney (-)</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Lung (-)</td>
<td>191.5</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Significant difference, P<0.05

Pharmacokinetic parameters (table 2) were calculated using equation 1, and curve fitting resulted in the levels given in figure 1. Inhalation of iCsA resulted in similar whole blood AUC\(_{0-48}\) as oral CsA (table 2), despite a lower peak level (\( C_{\text{max}} \) of 1,162 vs. 1,472 ng/mL), which was reached
earlier ($t_{\text{max}}$ of 3.4 vs. 4.8 hours) (table 2). Furthermore, the elimination half-time in blood ($t_{1/2\text{el}}$) of iCsA was substantially longer than of oral CsA (72 vs. 21 hours, respectively). The low $t_{\text{max}}$ demonstrates that CsA after inhalation is rapidly absorbed into the systemic circulation, while the long $t_{1/2\text{el}}$ suggests that CsA is slowly released from the lungs.

Figure 2. (A) CsA tissue levels after administration of 10 mg/kg iCsA (lung: ———; kidney: ••••••••) and oral CsA (lung: — — —; kidney: — • • •). (B) AUC0-48 of CsA in tissue (lung: black bars; kidney: grey bars) and blood (white bar). Error bars represent standard deviation calculated according to Bailer’s method.
In vivo efficacy of iCsA

The small difference of the AUC$_{0-48}$ in blood for iCsA and oral CsA suggests that the absolute bioavailability in the systemic compartment was similar over a 48-hour period. In contrast, iCsA resulted in a 109-fold higher lung availability (AUC$_{0-48}$) compared to oral CsA (table 2 and figure 2). During the complete period, the lung tissue level after insufflation of iCsA was much higher than after oral CsA (figure 2A). In kidney tissue, the AUC$_{0-48}$ was 27% higher for iCsA than for oral CsA, demonstrating that the lung:kidney ratio was much higher for iCsA than for oral CsA. The increased availability of iCsA in the lung after insufflation is clearly seen in figure 2B. The lung targeting index, calculated by dividing lung tissue AUC by whole blood AUC, was 191.5 and 1.8 for iCsA and oral CsA, respectively, again demonstrating that insufflation of iCsA has higher availability in the lung compared to oral CsA administration.

**Allograft rejection study**

**Ventilation**

Treatment of transplanted animals with iCsA as monotherapy (groups 1 and 2) prevented rejection up to 10 days, but only at a 5 mg/kg dose (figure 3A). Up to 6 days post transplant, no difference between the 2.5 and 5 mg/kg dose was observed while after 6 days a substantial decrease in ventilation score was observed in the 2.5 mg/kg group, leading to full loss of ventilation at day 10. Compared to allogenic transplantation without treatment (group 6), inhalation of 5 mg/kg iCsA was effective in maintaining ventilation (p=0.026), indicating that monotherapy with iCsA inhibits the underlying rejection. Inhalation of 2.5 mg/kg did not differ significantly from allogenic transplantation without treatment. Treatment with 5 or 10 mg/kg oral CsA (groups 3 and 4) prevented rejection compared to allogenic transplantation (p=0.01 and p=0.002, respectively) (figure 3B). The higher dose (10 mg/kg) was more effective than 5 mg/kg in preventing rejection (p=0.01). Treatment with 5 or 10 mg/kg oral CsA did not differ from treatment with 5 mg/kg iCsA (group 2) (p=0.352 and p=0.937, respectively).

In isogenic control animals (group 5), orthotopic left lung transplantation resulted in a reversible ischemia-induced decrease in the ventilation score at post-operative day 2, which surprisingly was especially seen in the untreated isogenic control group (figure 3C).
Figure 3. Rejection of the left lung as measured with ventilation scores (6: normal looking lungs; 0: opaque, fully rejected lungs) and histologic rejection score (0: normal looking lungs; 4: fully rejected lungs). (A) Ventilation score of animals that received allogenic transplantation and were treated with iCsA (black squares: 5 mg/kg; open squares: 2.5 mg/kg). (B) Ventilation score of animals that received allogenic transplantation and were treated with oral CsA (black triangles: 10 mg/kg; open triangles: 5 mg/kg). (C) Control animals that did not receive cyclosporine (black circles: isogenic transplantation BN-BN; open circles: allogenic transplantation AO-BN). (D) Histologic rejection score at day 10 post-operative. Error bars represent SEM (n=3). Statistically significant difference: allogenic control versus 5 mg/kg iCsA (*; P=0.026), allogenic versus 5 mg/kg (**; P=0.01) and 10 mg/kg oral CsA (***; P=0.002), isogenic versus 2.5 mg/kg iCsA (†; P=0.01) and isogenic versus 5 mg/kg oral CsA (††; P=0.01).
After 10 days, the ventilation score normalized. In allogenic left lung transplantation without treatment (group 6), the ventilation score decreased to 0 at day 8 after transplantation, which indicated complete rejection after 8 days. Despite full loss of ventilation of the left lung, the rat functioned normally due to the unaffected right lung. The non-transplanted groups treated with 5 mg/kg iCsA (group 7) or 10 mg/kg oral CsA (group 8) did not show any loss of ventilation (data not shown), proving that the treatment itself did not have an influence on the ventilation score.

The ventilation score of isogenic transplants didn't differ significantly from allogenic transplants treated with 5 mg/kg iCsA or 10 mg/kg oral CsA (p=0.589 and p=0.699, respectively). In contrast, isogenic transplantation was significantly different compared to 2.5 mg/kg iCsA (p=0.01) and 5 mg/kg oral CsA (p=0.01). These results show that monotherapy with 5 mg/kg iCsA or 10 mg/kg oral CsA effectively prevents rejection as measured by the ventilation score.

**Histology**

After 10 days the animals were sacrificed and the histology of the lungs was analyzed (figure 4). After treatment with 5 mg/kg iCsA, the allografts exhibited some perivascular, peribronchial and interstitial infiltrates, while the pulmonary architecture and alveolar integrity were preserved. On average, administration of 5 mg/kg iCsA resulted in a grade 2.7 rejection (figure 3D). The lower dose of 2.5 mg/kg iCsA was not effective in suppression of rejection (grade 4, figure 3D).

Oral administration of CsA in a 5 mg/kg dose resulted in almost complete rejection similar to 2.5 mg/kg iCsA but with edematous alveoli and more infiltrates. These allografts showed grade 3.5 rejection on average (figure 3D). At a 10 mg/kg oral dose, the allografts exhibited some perivascular inflammation and had a mild grade 1.3 rejection score.

Control recipients with isogenically transplanted lungs (group 5) showed no signs of rejection. In contrast, allografts from non-treated control recipients exhibited full rejection grade 4 rejection. The alveoli of the allografts were obliterated and contained necrotic debris. In addition, interstitial destruction and vascular thrombosis was observed. Histological analysis revealed that inhalation of 5 mg/kg iCsA in non-transplanted animals (group 7) resulted in some alveolar, perivascular and interstitial
reaction which differed from reaction typically seen with rejection. Focal infiltrates of macrophages, lymphocytes and polymorphous nuclear cells were visible (data not shown). However, this reaction was not visible on roentgenograms. The control animals that received 10 mg/kg oral CsA (group 8) did not show such signs of reaction in the lungs.

**Systemic trough CsA blood levels**

Trough CsA blood levels were determined in rats that received lung transplantation and were treated with iCsA or oral CsA systemic (figure 4). Generally, rats treated with 2.5 mg/kg iCsA had relatively lower trough levels (average 93±26 ng/mL) than 5 mg/kg iCsA (average 243±54 ng/mL). In line with the findings in the pharmacokinetic study, treatment with 5 mg/kg iCsA resulted in lower trough blood levels than treatment with 5 mg/kg oral CsA (average 439±207 ng/mL). Treatment with 10 mg/kg oral CsA led to highest systemic trough levels (average 687±258 ng/mL). Furthermore, treatment with iCsA led to more constant trough levels compared to oral treatment, a finding that was also observed in the pharmacokinetic study.

![Graph showing systemic CsA trough levels](image)

**Figure 4.** Systemic CsA trough levels of rats that received allogenic lung transplantation and were treated with 2.5 mg/kg iCsA (black bars), 5 mg/kg iCsA (dark grey bars), 5 mg/kg oral CsA (grey bars) or 10 mg/kg oral CsA (white bars). Time point “Average” represents the average systemic CsA trough level per group and calculated over all time points. Error bars represent SEM.
DISCUSSION
In this study, a new cyclosporine powder formulation suitable for pulmonary administration (iCsA) was effective in preventing rejection after lung transplantation in rats as single therapy. A trend was observed between suppression of rejection and CsA dose, irrespective of the administration route, with 10 mg/kg being most effective, followed by 5 mg/kg. A dose of 2.5 mg/kg was not effective in suppression of rejection. In equal doses (5 mg/kg), iCsA showed suppression of rejection at lower trough blood levels than orally administered CsA. Therefore, the efficacy of iCsA appears to be related to both systemic and pulmonary concentrations.

Inhalation of iCsA resulted in much higher lung tissue levels than oral CsA. The high lung tissue levels after insufflations may have led to local pulmonary reaction as observed in the 5 mg/kg iCsA control group. Such reaction was not reported previously and may be the consequence of the extremely high tissue levels. This reaction requires further investigation. In the literature (15-17, 28) lower doses for effective suppression of rejection have been found, albeit in different animal models. The 10 mg/kg oral CsA group (group 8) displayed normal lung histology without signs of pulmonary reaction. Therefore, less, or even no pulmonary reaction is expected when iCsA would be given in lower doses.

The difference in lung tissue levels after inhalation and oral administration (lung targeting index) were about 7- to 14-fold higher in this study than Blot et al. (15) and Mitruka et al. (29) previously reported, which may have been due to the different administration method used in this study. Both Blot et al. and Mitruka et al. used nose-only exposure to the aerosol, while in this study insufflation was used. With nose-only exposure, a small part of the dose is inhaled; of the inhaled dose a portion is possibly swallowed, leading to systemic absorption. In contrast, the complete dose is given to the lungs with insufflation, which would result in much higher deposition and lower systemic exposure. As a consequence, the different administration method may have contributed to the occurrence of the local reaction by enabling much higher local doses.

The pharmacokinetics show that iCsA was for the largest part absorbed in the lung. After $t_{\text{max}}$ has been reached, the lung tissue level declines very slowly, as well as the corresponding blood levels as shown by the high $t_{\text{1/2el}}$. This indicates that CsA is only gradually released into systemic
compartment. The systemic blood levels after administration iCsA are more reproducible than after oral administration of CsA, which is the result of the less complicated route of entrance into the systemic compartment: directly from the lungs. In contrast, oral administration of CsA is influenced by gastrointestinal transit, food and first-pass metabolism, resulting in a reported bioavailability of 27% (30). Consequently, the availability in the target organ is much higher for iCsA. The side effects of iCsA are limited. The kidney tissue level, was not different for iCsA and oral CsA and indicates comparable safety of both products. On the other hand, the ratio of lung to kidney tissue level (in fact an efficacy/safety parameter) is much higher for iCsA than for oral CsA. Therefore, iCsA as add-on therapy over conventional oral therapy would not drastically increase the systemic exposure and thereby the risk of nephrotoxicity.

To conclude, our study indicates that results of lung transplantation may be improved by combination therapy of iCsA with standard oral immunosuppressive therapy. In addition to other approaches to improve the efficacy/safety balance of immunosuppressive therapy with calcineurin inhibitors, iCsA may be an option. The different route of administration of iCsA in lung transplantation in combination with oral therapy may lead to more effective local immune suppression than can be reached by oral therapy only. Lower doses of iCsA would only have a limited effect on the systemic CsA levels, which would not increase the risk of side effects. Future studies should focus on establishing the safety of iCsA in pre-clinical studies and establishing the appropriate dose in combination with oral treatment.

ACKNOWLEDGEMENTS

Henk Moorlach (Department of Pathology & Medical Biology,—Endothelial Biomedicine & Vascular Drug Targeting Research, University Medical Center Groningen) is gratefully acknowledged for the histological stainings. Wim Timens (Department of Pathology, University Medical Center Groningen) is acknowledged for evaluation of the histology. Erwin Jongedijk (Hospital Pharmacy of University Medical Center Groningen) is thanked for setting-up and validation of the extraction procedure of CsA from tissue and CsA determinations in whole blood and tissue.
REFERENCES


