Molecular comparison of calcineurin inhibitor induced fibrogenic responses in protocol renal transplant biopsies


Departments of 1Pathology and 2Nephrology, Leiden University Medical Center, The Netherlands; Departments of 3Internal Medicine, Renal Transplant Unit and 4Pathology, Academic Medical Center, Amsterdam, The Netherlands.

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Abstract
The calcineurin inhibitor cyclosporine (CsA) induces a fibrogenic response that may lead to scarring of the renal allograft. We investigated whether tacrolimus (Tac), a novel calcineurin inhibitor, exerts fibrogenic effects to a similar extent. Sixty patients were enrolled in a randomized study: 29 received CsA and 31 received Tac. Patients were subjected to tailored exposure–controlled calcineurin inhibitor regimens. Protocol biopsies were obtained at the time of transplantation and 6 and 12 mo after transplantation. Cortical TGF–β, collagens α1(I) and α1(III) mRNA steady–state levels were determined with real–time PCR. The extent of protein deposition of TGF–β, α–smooth muscle actin (α–SMA), and interstitial collagens in the renal cortex were quantified with computer–assisted image analysis.
The extent of interstitial collagen deposition measured with Sirius red and the accumulation of α–SMA and TGF–β protein after 6 and 12 mo were similar for both immunosuppressive regimens. mRNA levels of TGF–β and collagens α1(I) and α1(III) were not significantly different in the treatment groups either.
We conclude that the fibrogenic response in renal allografts is similar in patients receiving CsA–based regimens and patients receiving Tac–based regimens.
Fibrogenic Effects of Calcineurin Inhibitors

Introduction
Allograft survival during the first year after kidney transplantation has improved significantly. This improvement is largely due to the introduction of potent immunosuppressive agents such as the calcineurin inhibitors cyclosporine (CsA) and tacrolimus (Tac). Unfortunately, long-term graft survival has not increased commensurately. The main cause of late graft loss is chronic allograft nephropathy (CAN), which is clinically characterized by a slow progressive decrease in renal function. Morphologically, interstitial fibrosis is a prominent feature. The histological alterations in CAN result from a complex interaction between immunologic and non-immunologic causes of injury including (sub)clinical acute rejections and nephrotoxicity due to chronic exposure to immunosuppressive agents.

CsA is known to have a fibrogenic effect in the renal allograft. There is no conclusive evidence that this effect is similar in patients treated with Tac. A large randomized study comparing the efficacy and safety of Tac– and CsA–based regimens showed that the adverse effect profile of CsA is less favorable than that of Tac. However, studies in rats have demonstrated that administration of both CsA and Tac cause nephrotoxicity and induce intrarenal transforming growth factor–β (TGF–β) expression. Application of anti–TGF–β antibodies in CsA–treated rats reversed the majority of CsA–associated renal lesions. This shows that TGF–β mediates the nephrotoxic effects of CsA, which is in accordance with its capacity to induce extracellular matrix (ECM) expression.

Studies of diagnostic renal biopsies have compared the fibrogenic effects of CsA and Tac. However, a disadvantage of these studies may be that differences in the extent of morphological damage between groups, rather than differences in the effects of the various types of medication themselves, account for any change in the level of expression of TGF–β and ECM-related components. Studies in protocol renal biopsies comparing the effects of CsA and Tac on intragraft TGF–β expression have shown discordant results. Discrepancies between findings in these studies may be explained by the fact that patients with acute rejection episodes before or during time of biopsy were included in some of the experiments, which may have resulted in biased TGF–β expression levels. Different compartments were studied in the various studies, either glomeruli or the whole cortex, and this may have led to different results. Furthermore, most studies have compared immunosuppressive regimens through a mere analysis of mRNA levels, which provides no answer to the question whether differences in mRNA expression in different types of medication would be accompanied by differences in expression of the corresponding proteins.

It was the objective of the current study to compare the fibrogenic effect of CsA and Tac at the mRNA and protein levels. Randomized patient groups were employed in a prospective fashion. Protocol biopsies were taken prior to transplantation and 6 and 12 mo after transplantation. Controlled target area–under–the–concentration–over–time–curve guided dosing of both calcineurin inhibitors was used to maintain efficacy and minimize toxicity. This was accomplished using a population–based pharmacokinetic model together with limited sampling combined with Bayesian estimation.
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Materials and Methods

Patient groups, drug monitoring, and study design
It has been estimated that a controlled trial regarding the effects of two different immunosuppressive regimens powered to demonstrate a 10% difference in renal allograft fibrosis at 6 mo post-transplant would require 23 patients to be randomized to each treatment arm. Therefore, sixty-five patients were enrolled in an open, prospective, randomized clinical trial at the Leiden University Medical Center in The Netherlands. Before transplantation, patients were randomized 1:1 to receive either a cyclosporine microemulsion, Neoral® (CsA)-based (n = 33) or tacrolimus, Prograf® (Tac)-based (n = 32) immunosuppressive regimen. CsA and Tac were started orally 3 hours before surgery (initial dose 4 mg/kg twice per day for CsA and 0.1 mg/kg for Tac). In the first week, target 12-hour trough levels were aimed at 225 ng/ml (range 200–250 ng/ml) and 12.5 ng/ml (range 10–15 ng/ml) for CsA and Tac respectively. CsA and Tac AUC0–12h were estimated at week 2, 4, 6, 8, 12, 17, 21, 26, 39, and 52 with a population based 2-compartmental pharmacokinetic model which was combined with Bayesian fitting and limited sampling. After each AUC-assessment, doses were adjusted to comply with predefined target AUCs: CsA AUC0–12h = 5400 ng.h/ml within the first 6 weeks (corresponding with a mean trough level of 225 ng/ml); and 3250 ng.h/ml after the initial six weeks (corresponding with a mean trough level of 125 ng/ml). Tac AUC0–12h = 210 ng.h/ml within the first 6 weeks (corresponding with a mean trough level of 12.5 ng/ml); after six weeks 125 ng.h/ml (corresponding with a mean trough level of 7.5 ng/ml).

Immunosuppressive co-medication consisted of prednisolone (in both groups 100 mg at days 1–3, 50 mg at day 4, 20 mg at days 5–14, 15 mg at days 15–21, 10 mg after day 22), mycophenolate mofetil (1000 mg twice per day in the CsA group and 500 mg twice per day in the Tac group), and basiliximab prophylaxis (20 mg iv at day 0 and day 4). Drugs that are known to alter concentrations of the calcineurin inhibitors were prohibited.

Protocol biopsies were taken prior to transplantation (t0) and 6 and 12 mo after transplantation. Biopsies were scored according to the Banff 97 classification scheme. Of all protocol biopsies obtained, two 6–mo biopsies from patients receiving CsA were not adequate for evaluation according to the Banff criteria. These were excluded from further study. Four patients from the CsA cohort and one patient from the Tac cohort showed morphologic signs of acute rejection in the protocol biopsies and were excluded. Thus, in the current study, 29 patients receiving CsA and 31 patients receiving Tac were included.

Control groups
mRNA transcripts from 16 kidneys with normal histology (12 samples from the unaffected part of a tumor nephrectomy kidney and 4 cadaver donor kidneys initially intended for transplantation purposes) and 28 acute rejection biopsies (20 Banff type Ia and 8 Banff type Ib) were studied.

RNA extraction
Frozen biopsy tissue was available for 34 t0 biopsies, 53 6–mo biopsies, and
48 12–mo biopsies. A 2–μm section from each biopsy was analyzed with light microscopy to demonstrate the presence of the renal cortex according to a procedure which has been described in a previous study 27. Ten to fifteen 10–μm sections of renal cortex were cut in a Leica CM3050 S cryostat, collected in an Eppendorf tube, and stored at –70°C until usage. RNA extraction was performed with RNeasy spin columns (Qiagen, Westburg, The Netherlands). RNA concentration was determined with photospectometry. The mean A260 to A280 ratio of the samples was between 1.8 and 2.0. A maximum amount of 1 mg of RNA was used as input for cDNA synthesis (mean input 0.75 ± 0.30 mg of RNA). The cDNA reactions (24 ml total volume) consisted of the following reagents: 0.5 mM dNTP, 100 ng oligo dT (Roche, Mannheim, Germany), 500 ng random hexamer primers (Invitrogen, Breda, The Netherlands), 5 U of RT–AMV (Roche), 20 U of rRNasin (Promega Benelux BV, Leiden, The Netherlands), and 1 x RT buffer (Roche).

Real–time polymerase chain reaction
A real–time polymerase chain reaction (PCR) with the ABI Prism 7700 Sequence Detector System (Perkin Elmer Biosystems, Foster City, CA, USA) was performed. This procedure has been described in detail in a prior publication 28. cDNA samples were diluted at a ratio of 1:50, and 5 ml of the dilution was used for PCR. mRNA levels of TGF–β, collagen α1(I), and α1(III) were quantified and normalized for the mean of mRNA levels of the household genes glyceraldehyde–3–phosphate dehydrogenase (GAPDH) and hypoxanthine guanine phosphoribosyltransferase–1 (HPRT1). A previous study has shown that there is a significant correlation between these household genes 29. In the current study, levels of GAPDH significantly correlated with those of HPRT1 (r = 0.61, P < 0.001). Primer and probe sequences and PCR conditions have been described in a prior publication 29. To allow comparison of samples from different PCR plates, a 1:5 dilution range of a reference sample was included in each PCR run.

Immunohistochemistry and quantification of stainings
Paraffin–embedded tissue was available for 54 6–mo biopsies and 54 12–mo biopsies. Four μm sections were cut and used for immunohistochemistry. To detect deposition of TGF–β, slides were heated in a 0.01M citrate buffer solution for 10 minutes and subsequently cooled for 20 minutes. Slides were then incubated for 1 hour at room temperature with polyclonal antibodies against TGF–β prior to 30 minutes of incubation with anti–rabbit EnVision™ (DAKO, Glostrup, Denmark) as the secondary antibody. The rabbit polyclonal anti–human TGF–β antibody raised against a synthetic peptide corresponding to the C–terminal amino acids 371–390 of human TGF–β had been synthesized in our laboratory 10,31. Antibody specificity was further confirmed by Western blotting and immunoabsorption assays. To detect alpha–smooth muscle actin (α–SMA)–positive cells, slides were stained for 1 hour with monoclonal antibodies against α–SMA (Promega Benelux BV) and thereafter incubated for 30 minutes with anti–mouse EnVision™ (Dako). NovaRed™ (Vector®, Burlingame, USA) was used to visualize the immunohistochemical signal in both stainings. Further details on staining protocols have been described in a previous report 32. For each
immunohistochemical assessment, all sections were stained simultaneously in one session. The extent of interstitial collagen accumulation was visualized in both paraffin sections of 6–mo and 12–mo biopsies and frozen sections of t0 biopsies with Sirius red staining, a process which has been described in a previous study. Slides from frozen biopsies were first fixated in formalin for 10 minutes before Sirius red staining. Digital analysis was performed on each biopsy with a Zeiss microscope equipped with a full–color 3CCD camera (Sony DXC 950p) and KS–400 image analysis software version 3.0 (Zeiss–Kontron, Eching, Germany) to quantitate the extent of staining. An average of ten microscopic sections were examined from each slide, commencing at the capsular side and following a linear fashion. Using the 20X objective, an average of 63.2% ± 19.5% of the total cortex was analyzed in each biopsy. For both Sirius red and the α-SMA, staining blood vessels larger than adjacent tubules were excluded. The procedure has been described previously.

**Statistical analysis**

Differences between groups were assessed with independent–samples t-tests. Correlations between expression levels were analyzed with Pearson correlation tests. Analyses were performed with SPSS software (version 10.0). P < 0.05 was considered statistically significant.

**Results**

**Patient population**

Twenty–nine participants received CsA and 31 received Tac. Demographic characteristics in the treatment groups were similar, as depicted in Table 1. There were no significant differences between treatment groups regarding the donor’s and the recipient’s age and gender, cold ischemia time, occurrence of delayed graft function, donor source, blood pressure, anti hypertensive medication use, total HLA mismatches, incidence of CMV infection, blood pressure, and glomerular filtration rate (GFR) 6 mo and 12 mo after transplantation. There was no significant difference in the number of patients showing borderline subclinical rejection after 6 and 12 mo between treatment groups. In these groups, GFR was not different for the patients with borderline subclinical rejection and for those without. During the first 12 mo after transplantation, ten biopsies were taken on clinical indication in addition to the protocolized biopsies (5 in each treatment group). These were taken within the first 2.5 mo after transplantation. In the CsA group four biopsies showed signs of acute rejection and 1 was taken because of delayed graft function (no morphological abnormalities). In the Tac group three biopsies were taken because of delayed graft function and one because of a slight increase in creatinine (all demonstrating no morphological abnormalities). The fifth biopsy taken showed signs of acute transplant glomerulopathy. Calcineurin–inhibitor nephrotoxicity (CIN) was regarded as the presence of nodular arteriolar hyalnosis and/or striped fibrosis and tubular microcalcification. We found CIN to be present in 13% of the biopsies obtained
### Table 1. Clinical parameters of cyclosporine- and tacrolimus-based regimens

<table>
<thead>
<tr>
<th>Medication</th>
<th>Cyclosporine</th>
<th>Tacrolimus</th>
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<tbody>
<tr>
<td>Patients included</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Donor age (yr)</td>
<td>43.0 ± 14.7</td>
<td>46.6 ± 13.5</td>
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<tr>
<td>Recipient age (yr)</td>
<td>47.4 ± 12.7</td>
<td>44.7 ± 12.7</td>
</tr>
<tr>
<td>Donor gender (m/f)</td>
<td>12 / 17</td>
<td>19 / 12</td>
</tr>
<tr>
<td>Recipient gender (m/f)</td>
<td>23 / 6</td>
<td>23 / 8</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
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<td></td>
</tr>
<tr>
<td>6 mo</td>
<td>65.5 ± 14.5</td>
<td>67.3 ± 20.7</td>
</tr>
<tr>
<td>12 mo</td>
<td>67.8 ± 15.1</td>
<td>64.5 ± 22.4</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
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<td></td>
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<tr>
<td>t0</td>
<td>140 / 80 ± 18 / 13</td>
<td>139 / 83 ± 21 / 11</td>
</tr>
<tr>
<td>6 mo</td>
<td>135 / 80 ± 12 / 8</td>
<td>136 / 80 ± 11 / 8</td>
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<tr>
<td>12 mo</td>
<td>136 / 80 ± 15 / 9</td>
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<td>Anti-hypertensive medication</td>
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<tr>
<td>6 mo</td>
<td>2.00 ± 1.16</td>
<td>1.68 ± 1.11</td>
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<tr>
<td>12 mo</td>
<td>2.17 ± 1.17</td>
<td>1.94 ± 1.06</td>
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<tr>
<td>Cold ischemia time (h)</td>
<td>20.0 ± 11.2</td>
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<tr>
<td>Delayed graft function (%)</td>
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<td>19</td>
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<tr>
<td>Donor source</td>
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<td>Cadaveric</td>
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<td>13</td>
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<tr>
<td>Non-heart-beating</td>
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<td>4</td>
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<tr>
<td>HLA-A, B, DR mismatches</td>
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<tr>
<td>CMV infection (%)</td>
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<td>55</td>
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<tr>
<td>Borderline subclinical rejection</td>
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</tr>
<tr>
<td>12 mo</td>
<td>2 / 29</td>
<td>6 / 31</td>
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</tbody>
</table>

1 Patients with acute rejection (AR), as defined by the Banff 97 classification, in the 6- or 12-mo protocol biopsies were excluded from the study. Data are means ± SD. CMV, cytomegalovirus.
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at 6 mo and in 18% of the biopsies obtained at one year. No biopsies showed signs of intimal arteritis. During the course of the study, an increase in vascular fibrous intimal thickening was found in twelve patients (nine in the Tac group and three in the CsA group).

mRNA assessments

The mRNA levels of TGF–β, collagen α1(I), and α1(III) at t0, after 6 mo, and after 12 mo for the study groups are summarized in Table 2.

In both treatment groups the mean TGF–β mRNA levels 6 mo and 12 mo after transplantation were significantly higher than those at the time of transplantation (P < 0.005). No significant difference was found in TGF–β mRNA levels between treatment regimens at t0 (CsA, 0.14 ± 0.09 versus Tac, 0.11 ± 0.08), at 6 mo (CsA, 0.54 ± 0.30 versus Tac, 0.53 ± 0.37), and at 12 mo (CsA, 1.03 ± 0.60 versus Tac, 0.91 ± 0.62) (Figure 1A). There were no significant differences in collagen α1(I) and α1(III) mRNA expression levels at t0, after 6 mo, and after 12 mo in the treatment groups either (Figure 1, C and D).

The Tac recipients showed significant correlations at each time point between mRNA expression levels of TGF–β with collagen α1(I) and α1(III) (6 mo: r = 0.80 and 0.77, and 12 mo r = 0.71 and 0.55). There was a significant correlation between collagen α1(I) and α1(III) at 6 mo (r = 0.90, P < 0.01) and at 12 mo (r = 0.87, P < 0.01) in the patient population treated with Tac. Significant correlations were found at 6 mo of TGF–β with both collagen α1(I) and α1(III) (r = 0.46 and r = 0.68, P < 0.05) for the patients treated with CsA. This significance was also found at 12 mo (r = 0.45 and r = 0.72, P < 0.05). Collagen α1(I) and α1(III) correlated significantly in the CsA treatment group after both 6 and 12 mo (r = 0.86 and r = 0.80, respectively).

Interstitial protein deposition

The extent of protein deposition of TGF–β and α-SMA and the extent of Sirius red staining for both treatment groups are summarized in Table 2. Consistent with the findings for TGF–β mRNA expression, no significant difference in TGF–β protein expression levels was seen between treatment groups after 6 mo (CsA, 5.61 ± 6.12 % versus Tac, 5.48 ± 4.04 %) and after 12 mo (CsA, 4.48 ± 3.08 % versus Tac 4.64 ± 4.46 %) (Figure 1B).

The Sirius red staining was performed in duplicate on all sections. A significant correlation was found between duplicate measurements (6 mo: r = 0.75; and 12 mo: r = 0.80, P < 0.001). For each patient the mean of the duplicate measurements was calculated. There was no significant difference in the extent of Sirius red staining between CsA and Tac treatment regimens (t0: CsA, 14.2 ± 5.8 % versus Tac, 14.8 ± 6.4 %; 6 mo: CsA, 24.3 ± 5.0 % versus Tac, 23.4 ± 4.0 %; 12 mo: CsA, 23.4 ± 5.1 % versus Tac, 23.5 ± 4.5 %) (Fig. 2A). In accordance with the results found for Sirius red, the extent of α-SMA staining after 6 mo (CsA, 7.08 ± 2.60 % versus Tac, 6.88 ± 2.39 %) and after 12 mo (CsA, 7.75 ± 2.78 % versus Tac, 7.01 ± 2.49 %) was not significantly different between the treatment groups (Figure 2B).
<table>
<thead>
<tr>
<th></th>
<th>Cyclosporine</th>
<th>Tacrolimus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
<td>6 mo</td>
</tr>
<tr>
<td>TGF-β1 mRNA</td>
<td>0.14 ± 0.09</td>
<td>0.54 ± 0.30 †</td>
</tr>
<tr>
<td>Collagen α1(II) mRNA</td>
<td>0.16 ± 0.21</td>
<td>2.84 ± 4.48 †</td>
</tr>
<tr>
<td>Collagen α1(III) mRNA</td>
<td>0.18 ± 0.30</td>
<td>1.38 ± 1.82 †</td>
</tr>
<tr>
<td>TGF-β staining</td>
<td>-</td>
<td>5.61 ± 6.12</td>
</tr>
<tr>
<td>α-SMA deposition</td>
<td>-</td>
<td>7.08 ± 2.60</td>
</tr>
<tr>
<td>Sirius red (%)</td>
<td>14.2 ± 5.8</td>
<td>24.3 ± 5.0 †</td>
</tr>
</tbody>
</table>

Data are means ± SD. α-SMA, α-smooth muscle actin. †, P < 0.005 versus t0; ‡, P < 0.05 versus t0.
Correlations between demographics and fibrogenic markers
Two correlations were found between demographics, as depicted in Table 1, and the results found in mRNA and protein evaluation. At 12 mo, donor age and mRNA levels for both collagen α1(I) and α1(III) at 12 mo correlated with each other (collagen α1(I): \( r = -0.351, p < 0.05 \) and α1(III): \( r = -0.447, p < 0.005 \)).

Figure 1. Fibrogenic mRNA molecules and TGF-β protein expression in protocol biopsies of CsA- and Tac-treated patients. mRNA expression and protein deposition was determined using real-time PCR and immunohistochemistry, respectively. (A) TGF-β mRNA levels in protocol biopsies at t0, after 6 mo, and after 12 mo did not significantly differ between treatment regimens. As a comparison, expression was measured in 28 biopsy samples with acute rejection (AR); TGF-β mRNA levels were significantly higher in the clinical rejection biopsies than in the protocol biopsies. (B) TGF-β protein staining in protocol biopsies after both 6 and 12 mo was not significantly different in the immunosuppressive medication groups either. (C,D) No significant difference was found between medication regimens for both collagen α1(I) and collagen α1(III) mRNA levels at any time.
Figure 2. The extent of interstitial collagen deposition and the accumulation of α-SMA protein. (A) Sirius red staining was performed to assess the extent of interstitial collagen accumulation. No difference in interstitial staining was found between CsA- and Tac treatment regimens. (B) α-SMA staining was performed as a marker for interstitial myofibroblasts. There was no significant difference in the extent of α-SMA staining between treatment groups after 6 and 12 mo.
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Discussion

Chronic graft damage can be partly attributed to the toxic effect of immunosuppressive agents. CsA has been shown to cause fibrosis, but it is controversial if Tac induces a similar effect on renal allografts. The objective of the current study was to compare the fibrogenic effect of CsA and Tac at the mRNA and the protein level in whole cortical tissue of protocol transplant biopsies from rejection-free patient cohorts who were exposed to tailored regimens of these drugs. With both CsA and Tac a progressive increase in expression of fibrogenic molecules was observed over time. We found no significant difference in expression of fibrosis-related components between treatment groups.

Opposing results have been found in recently published studies of renal protocol transplant biopsies with respect to the influence of CsA and Tac on fibrogenesis and intragraft expression levels of TGF-β and ECM molecules. Most of these studies included biopsies taken within one year after transplantation and demonstrated lower TGF-β expression levels in Tac-treated recipients. This may suggest that fibrosis develops slower in Tac-treated patients than in recipients treated with CsA. However, the majority of these studies included renal biopsies taken for diagnostic purposes. Thus, acquired results may be distorted through the presence of infiltrating cells due to acute rejection or through the presence of chronic damage resulting from long-term medication use. Furthermore, previous studies in protocol biopsies have not examined mRNA and protein levels simultaneously and have not conclusively answered the question if increments in mRNA levels of fibrogenic molecules result in increased deposition of the corresponding proteins.

Previous studies have shown that the extent of fibrosis, demonstrated by Sirius red staining, in 6-mo protocol biopsies predicts renal function deterioration. We found that the extent of fibrosis, measured with digital analysis of Sirius red staining, did not significantly differ between CsA and Tac in our cohort at any moment. The development of interstitial fibrosis is for a large part mediated by the action of interstitial myofibroblasts, which are positive for alpha-smooth muscle actin (α-SMA). We performed immunohistochemical staining for α-SMA in our patient cohorts and did not find a significant difference between medication groups.

We found higher mRNA expression levels of TGF-β and collagen α1(I) in acute rejection biopsies than in the protocol biopsies without rejection. This is another indication that the expression of fibrogenic molecules can be affected by the presence of such rejection episodes. Recipients showing clinical or morphologic signs of acute rejection in their protocol biopsies were therefore excluded from the current study. No significant differences were found for TGF-β mRNA expression levels and TGF-β protein deposition between patients treated with either CsA or Tac. This finding is in accordance with previous findings for TGF-β mRNA expression in glomeruli. No significant correlation between TGF-β mRNA levels and TGF-β protein levels was found, which might be due to the fact that the antibodies against TGF-β visualize both its active and its latent and inactivated forms. Since TGF-β mRNA levels significantly correlated with collagen α1(I) and α1(III) mRNA levels in the current study, we
suppose that mRNA assessment gives a good impression of the extent of TGF–β activity in this case. In both Tac and CsA treatment groups TGF–β mRNA levels progressively rose from their time of transplantation until 12 mo later. Since the patients who had been treated with Tac and CsA showed no differences with respect to TGF–β mRNA expression levels at any moment, these findings suggest that the two drugs have a similar effect on TGF–β mRNA synthesis. Similarly, mRNA levels of interstitial collagens α1(I) and α1(III) rose over time in both treatment groups. CsA has been known to target the promoter fragment of collagen III 37 and in the process specifically affects the rate of synthesis of the mRNA transcript. A similar mechanism may be involved in the case of collagen α1(I). It is not clear whether tacrolimus also has the capacity to interact with response elements in the interstitial collagen genes.

The present study puts forward results that appear to be at variance with the results in previous studies in the literature, because no significant difference in fibrogenic response of the kidney graft between Tac and CsA was observed in this study. This difference may be due to the fact that, in this study, patients received tailored calcineurin–inhibitor regimens, while only protocol biopsies with no subclinical rejection were included. The calcineurin inhibitor regimens follow a population based 2–compartmental pharmacokinetic model, which require only limited sampling and are combined with Bayesian estimation. As previous studies have shown, this method gives an accurate and precise estimation of systemic exposure while being very flexible in allowing non rigid sampling times as long as dosing and sampling times are recorded accurately. 38,39

It appears that this study is the first to have compared predefined area–under–the–concentration–over–time–curve guided dosing regimens of Tac and CsA through an assessment of the expression of TGF–β and ECM molecules in protocol renal allograft biopsies that are free of rejection. CsA and Tac have a similar inducing effect on intragraft TGF–β, collagen α1(I), and α1(III) mRNA levels within the first 12 mo after transplantation. The extent of deposition of TGF–β protein and interstitial collagens did not significantly differ between recipients of either CsA or Tac, which shows that the fibrogenic response of renal allografts is similar for either calcineurin inhibitor.

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References

