Increase of proteinuria after conversion from calcineurin inhibitor to sirolimus-based treatment in kidney transplant patients with chronic allograft dysfunction

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Abstract

Background. Conversion from calcineurin inhibitor to sirolimus, rapamycin has become an option in patients with chronic allograft dysfunction (CAD). However, in many cases an increase of proteinuria has been observed. The aim was to characterize the course of this so far unexplained proteinuria after conversion.

Methods. In 149 renal transplant patients from various Spanish centres, proteinuria and renal function were analysed 6 months before until 6 months after conversion. Patients were divided into three groups according to mean proteinuria before conversion (1: \( \leq 300 \) mg/day; 2: \( >300 \)–\( 3500 \) mg/day; 3: \( >3.5 \) g/day).

Results. Generally patients showed an increase of proteinuria from \( 864 \)–\( 1441 \) (0–12125) to \( 1541 \)–\( 1878 \) (0–10976) mg/day after conversion; \( P < 0.001 \).

Group 1: \( 145 \) vs \( 92 \) mg/day, \( P < 0.001 \);

Group 2: \( 1041 \) vs \( 799 \) mg/day, \( P < 0.001 \);

Group 3: \( 6205 \) vs \( 3184 \) mg/day, \( P = \text{NS} \).

Patients with an increase of proteinuria of \( >500 \) mg/day \( (n = 60; 40\%) \) had a higher serum creatinine before conversion compared with patients with no or moderate increase \( (2.5 \pm 0.8 \text{ vs } 2.15 \pm 0.72 \text{ mg/dl}; P = 0.002) \). The group that experienced an increase \( >500 \) mg/day had a higher serum creatinine after conversion compared with the patients with no or moderate increase \( (2.8 \pm 1.0 \text{ vs } 2.1 \pm 1.2; P < 0.001) \). Of 64 patients, 19 in group 1 showed an increase \( >500 \) mg/day.

Conclusion. Conversion for CAD can be associated with an increase of proteinuria in patients with pre-existing renal damage; however, it preserves renal function in patients with better creatinine and proteinuria before conversion, and might not be of benefit if advanced loss of renal function and high proteinuria are already present before conversion.

Keywords: calcineurin inhibitor; chronic allograft dysfunction; conversion; mTOR inhibitor; proteinuria; sirolimus

Introduction

Conversion from calcineurin inhibitor (CNI)-based protocols to sirolimus, rapamycin (SRL) in maintenance immunosuppressive therapy for kidney transplantation has emerged as one possible therapeutic strategy mainly for chronic allograft nephropathy (CAN), but also for post-transplant malignancy, new onset diabetes, and other CNI-related complications [1–4]. Various studies showed that conversion could lead to stabilization or improvement of renal function in many of the patients. However, in a considerable number of patients with slowly deteriorating graft function, most of them with CAN, further deterioration of renal function has also been demonstrated [1,4]. In several studies and reports on conversion in patients with a certain extent of renal graft dysfunction, an increase of proteinuria has been observed—in some cases even to nephrotic range urinary protein excretion [4–6]. On the other hand, increased urinary protein excretion does not seem to be a problem in SRL-based treatment in de novo kidney transplantation with optimal organ quality [7].
The aim of this study was to examine if conversion from CNI-based protocols to SRL-based regimens in kidney transplant patients on maintenance immuno-suppressive therapy and with suboptimal graft function is associated with an increase of urinary protein excretion, and to possibly describe the type, the risk factors and evolution of this increase of proteinuria.

Methods

This is a descriptive, retrospective study performed in 5 Spanish transplant centres including patients who were converted to SRL for slowly deteriorating kidney graft function without any recent clinical or histopathological signs of acute rejection. Inclusion criteria were (i) transplantation more than 3 months before conversion, (ii) conversion from a CNI-based protocol [cyclosporine A or tacrolimus] to a CNI-free protocol based on Sirolimus according to local centre practice, (iii) withdrawal of CNI within 2 months of introduction of SRL and (iv) on therapy with Sirolimus with a functioning graft for at least 6 months after conversion. Patients with previous treatment with SRL were excluded. The conversion was performed according to the local centre guidelines consisting of a reduction of CNI on the day of introduction of SRL with or without a SRL loading dose. The CNI was then gradually withdrawn over a period of 1–6 weeks, although in some patients CNI was stopped on the day of conversion in combination with an SRL loading dose. Target SRL trough levels were 5–15 ng/ml.

Follow-up time after conversion was 6 months. Laboratory values, 6 months before and 6 months after introduction of SRL, were collected from patient medical records or electronic databases. These included creatinine, 24 h proteinuria and urinary protein electrophoresis; where available data on angiotensin converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB) treatment was collected.

For statistical comparative analysis, values of proteinuria were assigned to a time period: 180–91 days before conversion: ‘< 6 months’; 90–31 days before conversion: ‘< 3 months’; 30 days before day of introduction of SRL: ‘0 months’; 1–30 days after conversion: ‘1 month’; 31–90 days after conversion: ‘3 months’; 91–180 days after conversion: ‘6 months’. The mean of all available values for one time period was used. For the pre–post-analysis, the mean value of the last available time period before introduction of SRL was compared with the last available time period after conversion. Statistical analysis was performed using non-parametric tests for related values (Wilcoxon), or for unrelated values (Mann–Whitney U and Kruskal–Wallis). P ≤ 0.05 was considered to be significant.

The patients were divided into three groups according to their level of urinary protein excretion just before the conversion. Patients with proteinuria of 300 mg/day or less were assigned to group 1. Patients with proteinuria >300 mg/day, but ≤3.5 g/day were assigned to group 2. Patients with pre-existing nephrotic range proteinuria were assigned to group 3. In a subsequent analysis, parameters were also evaluated for quartiles of pre-conversion proteinuria.

Results

Data of 149 patients were available. A total of 913 24 h proteinuria evaluations were available in 52 female and 97 male patients. The mean age at conversion was 51.5 ± 13.3 years, and the mean time after transplantation was 75.4 ± 56.3 months.

Sixty-four patients (43%) showed proteinuria ≥0.3 g/day pre-conversion (group 1). Seventy-nine patients (53%) had proteinuria levels > 0.3 g/day and ≥3.5 g/day (group 2), whereas six patients (4%) had nephrotic range proteinuria before conversion (group 3). Mean urinary protein excretion increased significantly from 864 (0–12125 mg/day) to 1541 (0–10976) mg/day, P = 0.001 (Figure 1A).

Compared with the last available value before conversion, 46 patients (31%) experienced unchanged or decreased urinary protein excretion after conversion. Of the patients, 103 (69%) had an increase of proteinuria. In 64 of these 103 patients, this increase was higher than 100%. The number of patients with nephrotic range proteinuria increased from six before conversion to 19 at the last available value.

Changes of proteinuric levels leading to assignment of patients to a different group than before conversion are shown in Table 1.

The increase of proteinuria in the different groups is shown in Figure 1B–D. The highest nominal increase is observed in the group of patients who already had proteinuria before conversion [1041 (306–3205) to 1995 (94–10976) mg/day, P < 0.001]. However, also the group of patients without prior proteinuria experienced a significant mean increase of urinary protein excretion [145 (0–300) mg/day vs 669 (0–4500) mg/day, P < 0.001]. Figure 2 shows the increase of proteinuria over time in the different groups.

In order to characterize the origin of urinary protein excretion, we were able to obtain results on urinary protein electrophoresis in 16 patients. The mean albumin content was 93 ± 13% suggesting that the protein excreted is mainly of glomerular origin. In accordance with the latter result, serum albumin levels—available in all patients at various time points—decreased from 4.1 ± 0.37 to 3.9 ± 0.41 g/dl, P < 0.01.

The analysis of serum creatinine concentrations showed no significant differences in a comparison of all patients, however, a slight improvement in group 1, no change in group 2 and an increase in group 3 could be observed (Table 2).

Overall, patients who showed an increase of proteinuria after conversion of more than 500 mg/day (n = 60) had a higher serum creatinine at conversion (2.5 ± 0.8 vs 2.15 ± 0.72; P = 0.002) and a higher baseline proteinuria (956 ± 1123 vs 794 ± 1624; P = 0.014). Moreover, in the patients with an increase of more than 500 mg/day, the last available serum creatinine was significantly higher (2.8 ± 1.0 vs 2.1 ± 1.2; P < 0.001). This holds also true in the
group of patients who did not have any pre-
conversional proteinuria [serum creatinine 2.35 ±
0.69 mg/dl (n = 19 with increase >500 mg/day) vs
1.8 ± 0.5 mg/dl (n = 45, ≤500 mg/day increase),
P = 0.002] (Figure 3).

Although our data failed to demonstrate a signi-
cificant linear correlation between either baseline protein-
uria and the increase of proteinuria, or between
baseline creatinine and the increase of proteinuria,
a chi-square analysis revealed a significant association
of baseline proteinuria <300 mg/day (group 1 patients)
and not developing an increase of proteinuria
>500 mg/day during the first 6 months after conver-
sion (P = 0.038).

Furthermore, there was no correlation between
baseline creatinine and the increase of creatinine. How-
ever, baseline proteinuria correlated inversely
with an increase of creatinine in a univariate analysis
as well as in a linear regression including baseline
proteinuria and baseline creatinine (P < 0.001).

In an analysis of quartiles of pre-conversional
proteinuria 75% of patients have proteinuria of
≤792 mg/day (Table 3). These patients showed at
least a stabilization of creatinine after conversion.

**Table 1.** Proteinuria groups before conversion and after conversion

<table>
<thead>
<tr>
<th>Before conversion</th>
<th>After conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change from group 1 To group 2</td>
<td>30/64 (47%)</td>
</tr>
<tr>
<td>Change from group 1 To group 3</td>
<td>2/64 (3%)</td>
</tr>
<tr>
<td>Change from group 2 To group 3</td>
<td>13/79 (17%)</td>
</tr>
<tr>
<td>Change from group 2 To group 1</td>
<td>6/79 (8%)</td>
</tr>
<tr>
<td>Change from group 3 To group 2</td>
<td>2/6 (33%)</td>
</tr>
</tbody>
</table>

Fig. 1. (A) Values of 24 h urinary protein excretion at conversion and the last available value after conversion for all 149 patients; P < 0.001. (B–D) Values of 24 h urinary protein excretion for the patient groups 1–3 at conversion and the last available value after conversion. B: Group 1: ≤0.3 g/day at conversion; P < 0.001. C: Group 2: >0.3 g up to 3.5 g/day at conversion; P < 0.001. D: Group 3: >3.5 g/day at conversion; P = NS.

**Drug levels and safety**

At the end of the study period mean SRL whole blood
concentration was measured to be 10.7 ± 4.8 ng/ml
at a mean dose of 3.7 ± 1.8 mg/day. Neither SRL concentrations nor doses correlated with the increase of proteinuria.

After the conversion, oedema occurred in 24 cases, anaemia in 36, skin rashes in nine and mouth ulcers in six cases.

**Discussion**

This is to our knowledge the largest study so far describing the evolution of urinary protein excretion after conversion from a CNI-based to an SRL-based protocol in patients with slowly declining kidney graft function. Keeping in mind that the observed patients had some degree of pre-existing kidney damage and that 60% of the patients had proteinuria before the conversion, it can be stated that conversion to SRL is associated with an increase of mean urinary protein excretion in this patient population. The increase appeared mainly within the first 3 months after conversion and was observed in two-thirds of the patients. Again, in two-thirds of the patients with increase, that increase was >100%. Remarkably, even 30% of patients without proteinuria before conversion experienced an increase of urinary protein excretion after conversion of more than 500 mg/day. However, in our study patients with no baseline proteinuria appear to be less likely to develop an increase of proteinuria >500 mg/day. Increase of proteinuria >500 mg/day was associated with a worse outcome in terms of post-conversional creatinine compared with the patients who did not show this increase (Figure 3). The analysis of development of creatinine after conversion in terms of quartiles of pre-conversional proteinuria revealed that the quartile of patients with a baseline proteinuria above 792 mg/day showed a deterioration of serum creatinine within the first 6 months after conversion. This is confirmed by a significant inverse correlation of baseline proteinuria and increase of creatinine after 6 months. These findings are in accordance with results from a previous study of 59 conversion patients where baseline proteinuria predicts positive outcome after 1 year [4].

Our findings are in accordance with several other studies, which also demonstrated an increase of proteinuria after conversion from CNI to SRL [6,8,9]. Recently, Letavernier and co-workers [9] published a study of 68 conversion patients. Proteinuria increased from 0.39 to 1.44 g/day at 3 months and remained elevated thereafter. In addition to that, Letavernier and colleagues identified a baseline proteinuria of more than 0.3 g/day to be associated with a decline of renal function after the conversion. Some of the studied patients were re-converted from SRL to CNI for various reasons, which led to a decrease of proteinuria from 1.95 to 0.9 g/day. Therefore, the authors suggest that haemodynamic changes induced by withdrawal and re-introduction of CNI might play a role in these patients.

Indeed one possible explanation could be a change of intraglomerular haemodynamics after withdrawal of the CNI that itself has a vasoconstrictive influence on the afferent arteriole and thus reduces intraglomerular pressure [10]. One study showed that
conversion of heart transplant patients from CNI to azathioprin-based treatment for chronic kidney dysfunction also led to an increase of proteinuria [11]. The authors offer the haemodynamic changes in the glomerulus as a possible pathophysiological explanation. However, another study of conversion from CNI-based to CNI-free mycophenolate mofetil-based treatment for CAN showed a decrease of proteinuria after conversion [12]. Therefore, a mere increase of intraglomerular pressure after CNI withdrawal might not be sufficient to completely explain proteinuria in all patients, and another SRL-associated pathomechanism might also play a role at least in patients who develop a large increase of proteinuria. Dittrich and co-workers [5] identified de novo glomerulopathies in four conversion patients to be responsible for an increase of proteinuria.

Although there was a mean increase of proteinuria, 31% of our patients did not show an increase of proteinuria. This suggests that the increase of proteinuria depends not on SRL treatment alone, but requires at least one other accompanying circumstance, possibly some already pre-existing structural damage, or a multi-factorial event including an increase of intraglomerular pressure in an already damaged kidney leading to hyperfiltration.

This retrospective study has various limitations. It can only be a descriptive study and is not at all designed to explore a possible pathomechanism of SRL-associated proteinuria. An additional limitation is the absence of data on renal biopsies that would certainly give more insight in terms of a possible explanation. Furthermore, our study lacks a control group of patients remaining on CNI treatment. It is to be assumed that a certain increase of proteinuria.

Table 3. Parameters of groups according to quartiles before and after conversion

<table>
<thead>
<tr>
<th>Quartile proteinuria at conversion (mg/day)</th>
<th>Age at conversiona</th>
<th>Time after TX at conversionb</th>
<th>Proteinuria after 6 monthsd</th>
<th>Creatinine at conversionc</th>
<th>Creatinine after 6 monthsf</th>
<th>Serum albumin at conversiong</th>
<th>Serum albumin after 6 monthsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;156</td>
<td>53.1 ± 15</td>
<td>76.6 ± 58</td>
<td>79 ± 52</td>
<td>485 ± 771</td>
<td>1.9 ± 0.6*</td>
<td>4.2 ± 0.4</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>156–398</td>
<td>50.5 ± 13</td>
<td>72.4 ± 53</td>
<td>267 ± 63</td>
<td>915 ± 915</td>
<td>2.3 ± 0.6**</td>
<td>4.1 ± 0.4</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>&gt;398–792</td>
<td>50.8 ± 14</td>
<td>71.0 ± 59</td>
<td>568 ± 124</td>
<td>1126 ± 681</td>
<td>2.4 ± 0.9</td>
<td>4.1 ± 0.3</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>&gt;792–12125</td>
<td>51.6 ± 12</td>
<td>81.5 ± 58</td>
<td>2558 ± 2115</td>
<td>3655 ± 2500</td>
<td>2.5 ± 0.9</td>
<td>4.1 ± 0.3</td>
<td>3.8 ± 0.4##</td>
</tr>
</tbody>
</table>

*aNo significant differences.
*bNo significant differences.
*cP < 0.05 between groups.
*dKruskal–Wallis P < 0.001; Mann–Whitney P < 0.05 between groups.
*eKruskal–Wallis P < 0.008; Mann–Whitney *P < 0.02 compared with quartiles 2–4.
*fKruskal–Wallis P < 0.001; Mann–Whitney **P < 0.02 compared with quartiles 2–4, †P < 0.04 compared with quartiles 2 and 3.
*gNo significant differences.
*hKruskal–Wallis P < 0.001; Mann–Whitney ##P < 0.001 compared with quartiles 1 and 3.
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Moreover, since the objective of the study was to evaluate proteinuria 6 months after conversion, only patients on sirolimus therapy with a functioning graft 6 months after conversion were included. This could imply a bias in terms of analysis of graft outcome, because those patients were not included who were not on SRL therapy during the whole observation period or in whom proteinuria was not available due to graft loss before 6 months. However, overall outcome of conversion has been evaluated in several other studies [4,14,15]. This study design does not allow the evaluation of the interventional value of treatment with ACE-I of ARB, since these were not given in a controlled, randomized manner in our patients. However, Letavernier et al. [9] demonstrated in a subgroup of 11 patients that introduction of ARB or ACE-I treatment after the conversion to SRL led to a decrease of proteinuria from 2.29 to 1.26 g/day suggesting this treatment has a positive influence on post-conversion proteinuria. This was also confirmed by other groups [6,16].

Today there is little doubt that conversion to mTOR inhibitors leads to short and mid-term stabilization of renal function in many patients, also in the setting of CAD [14,16,17]. Our findings confirm that conversion to mTOR inhibitors preserves renal function in those patients with better functional parameters such as creatinine and proteinuria and might not be of benefit for patients with advanced damage. This should shift our attention of mTOR inhibitors away from a rescue drug for advanced CAD towards a drug that is used for prevention of chronic loss of renal function.

In conclusion, in this study first we demonstrate that conversion from CNI to SRL in patients with some degree of kidney graft dysfunction can be associated with a mean increase of proteinuria or with new onset of proteinuria. Second, baseline proteinuria as well as an increase of proteinuria of more than 500 mg/day after conversion were associated with deterioration of kidney function. Patients with low pre-conversational serum creatinine and no proteinuria show lower post-conversational proteinuria, and they benefit from conversion in terms of better creatinine 6 months after conversion. Conversion in patients with already existing nephrotic range proteinuria is associated with decline of renal function and therefore not advisable.

Conflict of interest statement. None declared.

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