Original Article

Impaired phosphate handling of renal allografts is aggravated under rapamycin-based immunosuppression

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Abstract

Background. Impaired phosphate handling of the renal allograft is a common problem and of multifactorial origin. The aim of the study was to elucidate whether a rapamycin- or a mycophenolate-based immunosuppressive therapy aggravates the renal phosphate leak in kidney transplant recipients.

Methods. Renal phosphate handling was determined in thirty-eight cadaveric allograft recipients, with good renal function at 8, 12, 20 and 28 weeks after transplantation. Nineteen patients (group 1) received triple immunosuppression with rapamycin, cyclosporine and prednisolone, nineteen other transplant recipients received mycophenolate mofetil, cyclosporine and prednisolone immunosuppression (group 2), and six healthy subjects (group 3) served as controls. After 12 weeks of stable graft function, group 1 patients were divided further into two subgroups. Ten patients were kept on their immunosuppressive regimen (group 1A), whereas the remaining nine randomly chosen subjects had their cyclosporine withdrawn; they were thus maintained on a dual immunosuppression regimen with prednisolone and a higher dosage of rapamycin (group 1B).

Results. Renal phosphate reabsorption was significantly lower in group 1 at 8 and 12 weeks after transplantation as compared with groups 2 and 3. At 20 weeks after transplantation, patients with rapamycin-based immunosuppression (groups 1A and 1B) continued to exhibit hypophosphataemia and impaired renal phosphate handling. Group 1B had the lowest TmP/GFR compared with all groups. At 28 weeks, renal phosphate reabsorption and plasma phosphate levels were no longer different between patient groups and controls.

Conclusion. These data suggest that rapamycin-based immunosuppression prolongs the phosphate leak of the allografted kidney, leading to low serum phosphate levels during the first weeks after transplantation.

Keywords: hypophosphataemia; kidney; mycophenolate; rapamycin; transplantation

Introduction

Kidney transplantation is the preferred treatment of end-stage renal failure, and hypophosphataemia is a well recognized problem during the first weeks after engraftment [1]. The kidney is the major arbiter of extracellular phosphate homeostasis. The majority of phosphate filtered through the glomerular capillaries is reabsorbed in the proximal tubule by the human sodium phosphate cotransporter (NaPi)-3 [2], which is located in the apical brush border membrane. This transepithelial transport in the proximal tubule is the rate limiting step in overall phosphate reabsorptive processes and the major site of its regulation [3]. The NaPi is mainly regulated by parathyroid hormone, thyroid hormone, calcitriol, serum phosphate levels, phosphate intake and glucocorticoids [4]. Parathyroid hormone excess due to secondary hyperparathyroidism directly suppresses the NaPi via its apical and basolateral receptors [5]. Additionally, during the first weeks after transplantation, the conversion of 25(OH)₂D to 1,25(OH)₂D in the proximal tubular epithelial cell is insufficient and it takes up to 3 months until calcitriol plasma levels normalize [6]. Besides these well known causes of impaired renal phosphate reabsorption, there is also evidence for a calcium- and parathyroid hormone-independent mechanism of decreased renal phosphate reabsorption in kidney transplant recipients [7]. Hypophosphataemia often persists weeks after transplantation, even when

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renal function is stable with a creatinine clearance >70 ml/min. This is caused by reduced renal reabsorption of filtered phosphate as assessed by a high fractional excretion of phosphate into the urine [1].

A contribution of immunosuppressive therapy to the impaired phosphate handling has been demonstrated. Glucocorticoids suppress NaPi cotransport activity \textit{in vivo} [8]. Furthermore, cyclosporine has been shown to cause a decrease in fractional tubular phosphate reabsorption [9]. The exact mechanism, however, has not been elucidated. Cyclosporine causes proximal tubular histological alterations such as the development of cytoplasmic vacuoles, dilation of endoplasmic reticulum, ribosome loss, as well as an increased number of lysosomes and cytosegresomes [10]. All of these are indicators of sublethal, but potentially reversible proximal tubular cell injury. To avoid these nephrotoxic effects of cyclosporine, new immunosuppressive drugs with less renal toxicity, such as mycophenolate or rapamycin, are currently being investigated and are already used in combination with lower doses of cyclosporine [11,12].

This study seeks to elucidate whether mycophenolate- or rapamycin-based immunosuppressive therapy is associated with a more severe defect of renal phosphate reabsorption during the first 28 weeks after cadaveric kidney transplantation.

\section*{Subjects and methods}

\subsection*{Patients}

Thirty-eight cadaveric renal transplant recipients transplanted at the University of Vienna Medical Center between September 1998 and February 1999 were included in the study. Nineteen patients with triple immunosuppression consisting of rapamycin (Wyeth, France), cyclosporine and prednisolone (group 1) were compared with 19 patients receiving triple immunosuppression with mycophenolate mofetil, cyclosporine and prednisolone (group 2). Group 1 patients were recruited from the Austrian centre of a worldwide multicentre trial of Sirolimus immunosuppression in renal transplant recipients (Wyeth, France). Patients in the two groups were matched for allograft function, parathyroid hormone, serum pH levels, cyclosporine trough levels, steroid doses, 1,25(OH)\textsubscript{2}D plasma levels, plasma calcium and proteinuria. Patients with levels of immunoreactive parathyroid hormone (iPTH) >400 pg/ml or <5 pg/ml before transplantation were not included in the study. There was no difference in the mean steroid dose between the patient groups. Six healthy individuals served as a control group (group 3). After 3 months of stable allograft function, patients of group 1 were randomized either to continuation of triple immunosuppression as before (group 1A, \(n=10\)) or to cyclosporine withdrawal and dual immunosuppression with prednisolone and higher doses of rapamycin (group 1B, \(n=9\)). There was no difference in allograft function, parathyroid hormone levels, proteinuria and steroid dose between groups 1A and 1B after randomization. None of the patients received calcium supplementation or phosphate binder therapy. Vitamin D or D\textsubscript{3} supplementation was stopped prior to study entry with the exception of five patients in group 1 who received calcitriol for 12–20 weeks post-transplant and in four group 2 patients who received calcitriol for 8 weeks. One patient in each group underwent parathyroidectomy with a well functioning antecubital autotransplantation 2 years before transplantation. In four patients of group 1 and in two patients of group 2, biopsy-proven rejections (Banff 1) were treated with steroid pulse therapy. In four patients of group 1 and five patients of group 2, the rejections (Banff 2) were treated with 7 or 10 days of anti-lymphocyte globulin infusions (ATG Thymoglobulin, Merieux, France). Two subjects in group 1 and two in group 2 did not differ in dietary habits from normophosphatemic patients, defined as anamnestic daily oral calorie intake. All participants eat a common central European diet.

\subsection*{Renal functional studies}

Serum and 24-hour urine collections for the calculation of renal excretory function and phosphate handling were performed at 8, 12, 20 and 28 weeks after transplantation. Serum and urine determination of creatinine, calcium, phosphate and pH were performed by standard techniques. iPTH was determined by a two-site immunoradiometric assay (CIS Bio International ELISA-PTH kit, Gif-Sur-Yvette Cedex, France). 25(OH)\textsubscript{2}D was quantitated by an equilibrium radioimmunoassay (reference range 30–85 nmol/l; Dia Sorin, Stillwater, USA). 1,25(OH)\textsubscript{2}D concentrations were measured by a competitive radioimmunoassay (reference range 15–65 pg/ml; Dia Sorin).

The best measure for the renal capacity of phosphate reabsorption is the maximum tubular capacity for phosphate (TmP) per unit volume of glomerular filtrate (TmP/GFR). This method has the advantage of being independent of the filtered load, and has the same units as plasma phosphate concentration. TmP/GFR was calculated at all given time points using the nomogram of Walton and Bijvoet [13]. As has been shown by Rosenbaum \textit{et al.} [7], there is a very good correlation in healthy and renal transplant recipients between the determination of the renal phosphate threshold TmP/GFR measured either by formal phosphate titration or determined by the nomogram of Walton and Bijvoet.

Cyclosporine trough plasma levels were determined by florescence polarization immunoassay using a TDx analyzer (Abbott Laboratories, Chicago, IL, USA), and rapamycin trough plasma levels were measured using a monoclonal antibody research kit and IMX detection (Abbott Laboratories).

\subsection*{Statistical analysis}

Statistical evaluation utilized ANOVA (analysis of variance) for repeated measurements and Scheffé \(F\)-test for multiple comparisons. Data are given as mean±SD and a \(P\)-value <0.05 was considered statistically significant. The study was approved by the local human subject committee and a written informed consent was obtained from every patient before the study.

\subsection*{Results}

Demographic as well as renal functional data at 8 and 20 weeks after transplantation can be seen in Tables 1 and 2. The mean cyclosporine trough plasma levels
Table 1. Demographic and renal function data of the allograft recipients 8 weeks after transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Sex (m/f)</th>
<th>GFR (ml/min)</th>
<th>1,25(OH)2D (pg/ml)</th>
<th>pH</th>
<th>Serum albumin (g/l)</th>
<th>UproV (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 19)</td>
<td>48 ± 13</td>
<td>9:10</td>
<td>57 ± 39</td>
<td>24 ± 24</td>
<td>7.36 ± 0.04</td>
<td>40 ± 1</td>
<td>355 ± 308</td>
</tr>
<tr>
<td>2 (n = 19)</td>
<td>49 ± 11</td>
<td>13:6</td>
<td>64 ± 28</td>
<td>20 ± 26</td>
<td>7.36 ± 0.02</td>
<td>37 ± 3</td>
<td>534 ± 511</td>
</tr>
<tr>
<td>3 (n = 6)</td>
<td>46 ± 20</td>
<td>5:1</td>
<td>115 ± 33*</td>
<td>n.d.</td>
<td>7.44 ± 0.02</td>
<td>46 ± 6</td>
<td>36 ± 80*</td>
</tr>
</tbody>
</table>

GFR, creatinine clearance; UproV, proteinuria; n.d., not determined.
*P < 0.05 group 1 vs group 3 and group 2 vs group 3.

Table 2. Demographic and renal function data of the allograft recipients 20 weeks after transplantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Sex (m/f)</th>
<th>GFR (ml/min)</th>
<th>1,25(OH)2D (pg/ml)</th>
<th>pH</th>
<th>Serum albumin (g/l)</th>
<th>UNaV (mmol/day)</th>
<th>UproV (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A (n = 10)</td>
<td>50 ± 8</td>
<td>6:4</td>
<td>61 ± 29</td>
<td>21 ± 10</td>
<td>7.36 ± 0.02</td>
<td>46 ± 1</td>
<td>250 ± 112</td>
<td>283 ± 148</td>
</tr>
<tr>
<td>1B (n = 9)</td>
<td>46 ± 17</td>
<td>3:6</td>
<td>62 ± 34</td>
<td>35 ± 18</td>
<td>7.37 ± 0.03</td>
<td>39 ± 7</td>
<td>256 ± 108</td>
<td>408 ± 258</td>
</tr>
<tr>
<td>2 (n = 19)</td>
<td>49 ± 11</td>
<td>13:6</td>
<td>76 ± 25</td>
<td>29 ± 13</td>
<td>7.37 ± 0.04</td>
<td>41 ± 6</td>
<td>270 ± 103</td>
<td>213 ± 284</td>
</tr>
</tbody>
</table>

GFR, creatinine clearance; UNaV, 24 h sodium excretion; UproV, proteinuria.

were 183 ± 64 and 197 ± 47 ng/ml in groups 1 and 2 at 8 weeks, and 173 ± 67 and 149 ± 32 ng/ml in group 1A and 2 at 20 weeks after transplantation. The mean rapamycin trough level was 8 ± 1 ng/ml in group 1 at 8 weeks, and 9 ± 1 in group 1A and 24 ± 6 in group 1B at 20 weeks after transplantation. The mean dose of mycophenolate mofetil in group 2 was 1.8 ± 0.2 g/day at 8 as well as 20 weeks post-transplant. The daily oral maintenance steroid dose was the same in all patient groups (20 mg at 8 weeks and 10 mg at 20 weeks post-transplant).

The concentration of serum calcium and phosphate and iPTH, as well as the TmP/GFR over time is depicted in Figure 1. Both groups of transplant patients exhibited a significantly reduced renal threshold phosphate concentration in the first weeks after transplantation when compared with healthy control subjects. Patients with rapamycin immunosuppression had significantly lower TmP/GFR values compared with patients receiving cyclosporine, mycophenolate mofetil and prednisolone. This pattern persisted until month 3 when group 1 patients were randomized to cyclosporine withdrawal and rapamycin increase (group 1B) or continued on triple immunosuppression with cyclosporine and lower rapamycin (group 1A). At 20 weeks, both group 1A and 1B patients still showed significantly lower TmP/GFR values than group 2 patients, who also exhibited subnormal TmP/GFR measures. At 28 weeks after transplantation, group 1A and 1B patients' TmP/GFR values were not different to group 2, but still lower statistically. GFR was stable and not different between groups 1A, 1B and 2 over time. iPTH plasma levels decreased from values twice to three times the normal range (10–60 pg/ml) to slightly supranormal values 5–7 months after transplantation. This reduction correlated with the increase in serum calcium levels in all three groups ($r^2 = 0.77$, $P < 0.05$). Serum albumin concentrations as well as proteinuria were not different between the groups at any time (data for 8 and 20 weeks post-transplant are given in Tables 1 and 2).

**Discussion**

The aim of this study was to investigate whether rapamycin- and/or mycophenolate-based immunosuppression aggravate the renal phosphate leak occurring early after renal transplantation. TmP/GFR was taken as a measure of renal phosphate handling in patients with good and stable functioning kidney allografts. The renal threshold for phosphate of patients with rapamycin immunosuppression was compared with patients under triple immunosuppression with mycophenolate, cyclosporine and prednisolone who were matched for transplant function. Cyclosporine is well known to be tubulotoxic, but so far it has not been shown that this is the reason for renal phosphate loss after renal transplantation. It has been shown in patients with healthy kidneys taking cyclosporine for autoimmune uveitis that cyclosporine does not cause a decrease in TmP/GFR [14]. However, patients with a GFR < 75 ml/min were excluded from this study. In renal transplant recipients, a GFR > 75 ml/min is the exception.

In animal studies, rapamycin exhibited less acute tubular toxicity than cyclosporine [15], and when used in combination with cyclosporine or as a primary agent it is intrinsically non-nephrotoxic [11]. To compare the immunosuppressive effect of a dual therapy with rapamycin and prednisolone with a triple therapy with additional cyclosporine and to have an adequate control group, this study was designed as a triple immunosuppression trial for 3 months with randomization of half of the stable patients to cyclosporine withdrawal within 4–6 weeks (Wyeth, Protocol).
During the first 3 months, patients receiving rapamycin and cyclosporine immunosuppression had significantly lower TmP/GFR values compared with patients treated with the cyclosporine and mycophenolate regimen. Both groups had significantly impaired renal phosphate handling when compared with healthy controls. TmP/GFR increased continuously over time, but group 1B patients always tend to have lower values, although the difference between groups 1A and 1B at 20 weeks failed to reach statistical significance. One reason might be the low number of patients in each group at 20 and 28 weeks. This finding might be an indication for a dose-dependent suppression of the renal phosphate reabsorption by rapamycin. Low TmP/GFR values independent of hyperparathyroidism for hypophosphatemia patients after kidney transplantation has been described by Rosenbaum et al. [7], although these patients had been transplanted considerably longer. No effect of steroid on renal phosphate excretion, at least in healthy subjects receiving 20 mg prednisolone daily for 2 weeks, could be observed, but this does not rule out an effect of long-term steroid administration in the phosphaturia of hypophosphatemic renal transplant recipients. In our study, plasma phosphate levels were $0.7 \pm 0.2$ and $0.82 \pm 0.2$ mmol/l, the values for TRP were $40 \pm 25\%$ and $61 \pm 15\%$, and TmP/GFR values were $0.31 \pm 0.23$ and $0.52 \pm 0.27$ mmol/l in transplant patient groups 1 and 2, respectively, 8 weeks after transplantation. TmP/GFR values were lower in group 1B than in group 1A and group 2 at 20 weeks, although IPTh, calcium, 25(OH)2D and vitamin D plasma levels were not different. The phosphate leak gradually improved in all patient groups but did not reach normal values for TmP/GFR. Plasma phosphate concentration in patients with rapamycin immunosuppression remained low. Of note is that serum IPTh levels still remained slightly elevated at that time. Some of the observed effect of renal phosphate loss in patients after kidney transplantation might be mediated by the persisting secondary hyperparathyroidism. Saha et al. [16] showed that 1 year after successful renal transplantation less than half of their patients exhibited complete resolution of hyperparathyroidism. Oral phosphate supplementation can usually not restore a normal plasma phosphate concentration and should be avoided because of parathyroid stimulation and 1,25(OH)2D suppression, as suggested by Caravaca et al. [17]. Interestingly, not only the renal phosphate transporter is suppressed; the intestinal absorption of phosphate is also reduced in renal transplant recipients [18]. A type IIb sodium phosphate cotransporter was
recently cloned in mouse small intestine and is highly (57–75%) homologues to non-mammalian and mammalian type IIa renal sodium phosphate cotransporters [19]. It might be possible that the immunosuppressive drugs not only effect the renal but also the intestinal sodium phosphate cotransporter; however this theory has not yet been proven. Nevertheless, current evidence suggests that urinary phosphate loss is the major reason for hypophosphataemia in renal transplant recipients [7].

Greger and co-workers [20] reported a parathyroid hormone-independent influence of calcium on renal phosphate transport. Since serum calcium was not different in our patient groups, it is likely that the immunosuppressive medication contributed to the renal loss of phosphate in the setting of true hypophosphataemia.

In summary, our data suggest that rapamycin compared with mycophenolate-based immunosuppression significantly prolongs a temporary renal phosphate leak occurring in the first months after kidney transplantation, leading to low serum phosphate levels.

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References


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