Original Article

Cyclosporin does not inhibit the tubular secretion of creatinine


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Abstract

Background. The immunosuppressive drug cyclosporin is known to impair renal function. The degree of renal dysfunction is usually estimated from the clearance of creatinine (CCr). Theoretically, however, a fall in CCr can be caused by a decrease of GFR, an inhibition of the tubular secretion of creatinine, or the combination of both. CsA has convincingly been shown to decrease GFR, but detailed information on the effects of CsA on tubular secretion of creatinine is lacking.

Methods. We performed two studies to investigate the influence of CsA on tubular creatinine secretion. In study A we simultaneously measured CCr and GFR (using inulin) immediately before and 4 weeks after cessation of CsA therapy in 17 renal transplant patients. In study B, the rise in serum creatinine after administration of cimetidine, which blocks the tubular secretion of creatinine, was compared in renal transplant patients treated with either CsA (in whom secretion might already be inhibited) or azathioprine.

Results. Study A: After cessation of CsA there was an increase of GFR (54 ± 15 vs 63 ± 16 ml/min/1.73 m²; P < 0.01) and of CCr (71 ± 21 vs 82 ± 23 ml/min/1.73 m²; P < 0.01), but the ratio between CCr and GFR (a measure of the relative contribution of tubular secretion to the clearance of creatinine) did not change significantly (1.33 ± 0.21 vs 1.32 ± 0.30). Study B: In nine couples of patients matched for GFR the relative rise in serum creatinine after administration of cimetidine were 26 ± 21% and 22 ± 7% for the CsA and azathioprine treated patients respectively (NS).

Conclusion. CsA does not substantially inhibit the tubular secretion of creatinine. A rise in serum creatinine after administration of CsA can thus be attributed completely to a fall in GFR.

Key words: cimetidine; cyclosporin; creatinine clearance; glomerular filtration rate; renal transplantation; tubular creatinine secretion

Introduction

Impairment of renal function is a well-known side effect of the immunosuppressive drug cyclosporin (CsA). In clinical practice, as well as in many investigational circumstances, CsA-induced renal dysfunction is judged from a rise in serum creatinine or a fall in creatinine clearance (CCr) [1-3]. Since creatinine is not only filtered in the glomerulus but also actively secreted by the renal tubule, an increase in the serum creatinine level may result from (1) a decrease in GFR, (2) a decrease in tubular creatinine secretion, or (3) a combination of (1) and (2). Although CsA has repeatedly been demonstrated to reduce GFR [4], its effect on tubular secretion of creatinine is less clear. We had several reasons to suppose an inhibitory effect. First, CsA causes well-defined histopathological changes in the proximal tubule [4,5], the site of tubular secretion of creatinine [6]. Second, CsA has been demonstrated to inhibit the tubular secretion of N^methylnicotinamide [7], which also takes place in the proximal tubule [4,5]. Finally, it has been suggested that CsA inhibits urate excretion at the proximal tubular site [2]. The practical consequence of inhibition of tubular creatinine secretion by CsA would be that the effect of CsA on GFR is overestimated by measuring the change in serum creatinine or CCr. We have investigated the influence of CsA on creatinine secretion in recipients of renal allografts. The results of our studies indicate that CsA does not affect tubular secretion of creatinine. Hence, changes in serum creatinine or CCr during administration of CsA are likely to reflect merely changes in GFR.

Subjects and methods

We performed two separate studies in renal transplant patients to determine the effects of CsA on tubular secretion of creatinine. The study protocols were approved by the Hospital Ethics Committee and informed consent was obtained from all patients.

Study A

Eighteen renal transplant patients (15 M, 3 F; age 40 ± 12 years) were included for simultaneous measurements of GFR.
and \(C_{cr}\) immediately before and 4 weeks after conversion from CsA to azathioprine at 3 months after renal transplantation. The conversion formed part of another study protocol and was elective in all cases. Patients were eligible for this study if renal function was stable (variation in serum creatinine of less than 15% in 2 weeks prior to the study). Patients who were using diuretics or drugs known to interfere with tubular creatinine secretion were excluded. Furthermore one patient was withdrawn during the study period because an acute rejection episode was diagnosed. During the first 3 months after transplantation, immunosuppressive treatment consisted of CsA (initial oral dose of 12 mg/kg per day, tapered to approximately 4 mg/kg per day in two divided doses) and prednisone (20 mg/day at the time of the study) in all patients. In the conversion group, CsA was replaced by azathioprine in a dosage of 3 mg/kg per day. The prednisone dosage was temporarily increased from 20 to 25 mg/day during the first 2 weeks after conversion but was back on 20 mg/day during the following 2 weeks.

Clearance measurements were performed from 9.00 a.m. till noon. A sufficient diuresis was attained by an initial oral water load of 10 ml per kg body weight, followed by i.v. infusion of a solution of 0.25% NaCl (to compensate for expected sodium losses in the urine) in 3.3% glucose at a rate of 400 ml/h. Urinary fluid losses in excess of the infused volume were replaced orally by tap water. Except for spontaneous voiding in upright position, patients remained supine. GFR and renal plasma flow (RPF) were measured using a continuous infusion technique. Renal clearances of inulin (polyfructosan, Inutest8, Laevosan-Gesellschaft, Linz, Austria) and para-aminohippuric acid (PAH) were used as markers of GFR and RPF respectively. After an equilibrium period of 90 min, urine was collected at three 30-min intervals. Blood samples were drawn at the midpoint of each urinary collection period. Clearances of inulin, PAH, and creatinine were calculated for each of the three 30-min intervals and these values were averaged subsequently. All clearance values were adjusted to a standard body surface area of 1.73 m². The filtration fraction (FF) was defined by \(GFR / RPF \times 100\%\). The tubular clearance of creatinine was calculated as 

\[C_{	ext{cr}} - \text{GFR.}\]

**Study B**

In this study, cimetidine was used as a tool to examine the tubular handling of creatinine. Cimetidine has been shown to be able to completely block the tubular secretion of creatinine [9,10]. Therefore, changes in serum creatinine after administration of cimetidine reflect the level of tubular secretion. The rise in serum creatinine after administration of cimetidine was measured in 36 renal allograft recipients at 403±56 (range 325–615) days after transplantation. Renal function had to be stable and patients who already used \(H_{2}\)-receptor antagonists or drugs known to interfere with creatinine secretion were excluded. Immunosuppression consisted of CsA with or without prednisone in 21 patients (13 M, 8 F; age 44±13 years) and of the combination of azathioprine and prednisone in 15 patients (10 M, 5 F; age 47±11 years). The participants of this study visited the outpatient clinic on two separate occasions. During the interval between these visits they used oral cimetidine in a dosage adjusted to renal function as estimated by the formula of Cockcroft and Gault [11] (daily dose of 2000 mg in case of estimated \(C_{cr}\) > 75 ml/min), according to a dosage scheme described previously [9]. The minimum interval required to achieve a new steady state after a rise in serum creatinine was calculated from estimated \(C_{cr}\) and body weight [9].

Serum creatinine and urea levels, as well as CsA trough levels in CsA-treated patients, were measured on both study days. During the second visit, patients received an oral water load of 10 ml/kg and we subsequently measured the cimetidine-aided 1.5-h \(C_{cr}\), which we have demonstrated to be a quite accurate measure of GFR [9].

**Analytical procedures**

Inulin and PAH concentrations in serum or urine samples were measured by semi-automated techniques. For study A, creatinine concentrations were determined by a modified Jaffé technique using an autoanalyser, while for study B an enzymatic method was used (Boehringer Mannheim GmbH, Mannheim, Germany). With the use of these creatinine assays, the measurement of non-creatinine chromogens is minimized (modified Jaffe technique) or absent (enzymatic method). Whole-blood CsA levels were measured with a monoclonal antibody against the CsA parent molecule using Abbott TDx (Abbott Laboratories, North Chicago, IL, USA).

**Statistical analysis**

Data are reported as means±SD. Results were analysed with Wilcoxon's test for paired or unpaired observations when appropriate. Correlations were assessed by calculating Spearman's correlation coefficient. A probability value less than 0.05 was considered statistically significant.

**Results**

**Study A**

At the time of replacement of CsA by azathioprine, the blood CsA level was 179±75 ng/ml. Renal function parameters before and after conversion are given in Table 1. As expected, conversion from CsA to azathioprine resulted in a rise in GFR (20±21%, \(P<0.001\)) and a decrease in serum creatinine (−15±9%, \(P<0.001\)). During treatment with CsA, \(C_{cr}\) overestimated GFR on average by 33%, reflecting the presence of tubular secretion of creatinine. Neither the ratio between \(C_{cr}\) and GFR, nor the tubular clearance of creatinine showed any change after withdrawal of CsA. In this patient population there was no significant correlation between \(C_{cr}/GFR\) on the one hand and the CsA level, GFR, or FF on the other.

**Study B**

In three CsA-treated patients the creatinine excretion during the 90 min collection interval at the second study day differed more than 50% from the value predicted by the formula of Cockcroft and Gault. We assumed that voiding errors were made in these patients and excluded them from further analysis. The data of the remaining 33 patients are given in Table 2. The time interval between the two measurements was exactly the same in both groups: 9±5 days. Compared to azathioprine-treated patients, CsA-treated patients...
Table 1. Parameters of renal function before and 4 weeks after conversion from CsA to azathioprine in 17 renal transplant patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before conversion</th>
<th>After conversion</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>54 ± 15</td>
<td>63 ± 16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RPF (ml/min per 1.73 m²)</td>
<td>252 ± 101</td>
<td>292 ± 89</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FF (%)</td>
<td>23 ± 4</td>
<td>22 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>CCr (ml/min per 1.73 m²)</td>
<td>71 ± 21</td>
<td>82 ± 23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serum creatinine (umol/l)</td>
<td>1.32 ± 0.21</td>
<td>1.32 ± 0.30</td>
<td>NS</td>
</tr>
<tr>
<td>Tubular creatinine clearance (ml/min per 1.73 m²)</td>
<td>17 ± 12</td>
<td>19 ± 18</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2. Renal function parameters and cyclosporin level before and after administration of cimetidine in renal transplant patients treated with cyclosporin or azathioprine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cyclosporin (n = 18)</th>
<th>Azathioprine (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before cimetidine</td>
<td>After cimetidine</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>163 ± 38</td>
<td>200 ± 53*</td>
</tr>
<tr>
<td>Serum urea (mmol/l)</td>
<td>10.2 ± 2.5</td>
<td>10.3 ± 2.7</td>
</tr>
<tr>
<td>Blood cyclosporin (ng/ml)</td>
<td>153 ± 43</td>
<td>179 ± 53</td>
</tr>
<tr>
<td>1.5-h CCr (ml/min per 1.73 m²)</td>
<td>—</td>
<td>41 ± 17</td>
</tr>
</tbody>
</table>

* P < 0.001 for difference with pre-cimetidine value.

Discussion

Our studies indicate that CsA at levels that are currently considered therapeutic does not affect the tubular secretion of creatinine to a clinical relevant degree. This conclusion is based on two lines of evidence. In study A, cessation of CsA at 3 months after renal transplantation did not result in an increase in the ratio of CCr to GFR, as would have been expected if creatinine secretion was inhibited by CsA. In study B we used cimetidine, a drug that completely abolishes the tubular secretion of creatinine when a sufficient dose is given [9,10]. Increases in serum creatinine after administration of cimetidine quantitatively reflect the tubular secretion process. We observed similar increases in serum creatinine in CsA-treated as compared to azathioprine-treated renal transplant patients, while a smaller increase in the CsA group would have been expected if creatinine secretion already was impeded by CsA.

It can be argued that a correct interpretation of the data is hampered by the fact that tubular secretion of creatinine is influenced by changes in renal haemo-
dynamics. Several investigators have provided evidence that the ratio of \( C_C \) to GFR increases with declining GFR [12–14]. In the absence of any influence of CsA on tubular creatinine secretion, one might therefore have expected a decrease of \( C_C \)/GFR after cessation of CsA treatment (and a concurrent increase in GFR) in study A. Similarly, after cimetidine in study B a more pronounced increase of serum creatinine could have been expected in the CsA group (with lower GFR as compared to the azathioprine group). Since in our hands \( C_C/GFR \) remained unchanged (study A) and the increase of serum creatinine was similar in patients on CsA or azathioprine (study B), a slight effect of CsA on tubular secretion of creatinine might have been present. However, in both studies the differences in GFR were small and consequently the effects, if any, on tubular creatinine secretion would have been rather limited. Furthermore, in study B we analysed the results again after matching the patients receiving CsA or azathioprine for GFR. In these matched groups we also observed no difference in the increase of serum creatinine. Finally, it has been suggested that tubular secretion of creatinine (i.e. the \( C_C/GFR \)) ratio is more closely associated with the filtration fraction than with GFR per se [15]. In study A we measured GFR and RPF and observed no change in FF after discontinuation of CsA. Taken together our data are in strong support for the conclusion that CsA does not inhibit the tubular secretion of creatinine.

Our results differ from those of Versluis [16] who observed a significant increase of \( C_C/GFR \) by 28% after conversion from CsA to azathioprine in 17 renal transplant recipients. However, the mean value of \( C_C/GFR \) measured in these patients during use of CsA (1.04) seems far too low for the degree of renal dysfunction (mean GFR as measured by the clearance of \( ^{125} \)I)iothalamate, 46 ml/min). It seems most likely that in these patients \( ^{125} \)Iiothalamate is not an ideal marker of GFR, since iothalamate itself may be subject to tubular secretion [17]. Although the topic is not specifically addressed, information on the effect of CsA on the tubular secretion of creatinine can be found in some other clinical studies. In agreement with our findings, the degree of overestimation of GFR by creatinine clearance was reported to be similar in renal transplant patients treated with CsA or azathioprine [18]. In that study however, patients in both groups were not matched for GFR. In diabetics treated with CsA, a reversible increase in the \( C_C \) to GFR ratio has been observed, suggesting an increase rather than a reduction of tubular creatinine secretion, which may have been related to a concurrent decrease in GFR [19].

In conclusion, we have no evidence for an inhibitory effect of usual dosages of CsA on the tubular secretion of creatinine. An increase in serum creatinine or a decrease in creatinine clearance during treatment with CsA most probably merely reflect a decrease in GFR.

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References


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