Original Article

Elevated plasminogen activator inhibitor levels in cyclosporin-treated renal allograft recipients

G. A. Verpooten1, F. J. Cools1, M. G. Van der Planken2, L. C. Bedert1, R. Claes1, L. F. Van Gaal3 and M. E. De Broe1

1Department of Nephrology, 2Laboratory of Haematology, and 3Department of Endocrinology, University Hospital Antwerpen, Edegem-Antwerpen, Belgium

Abstract Atherosclerosis and thrombosis, two major causes of morbidity and mortality in renal transplant recipients, share the same clinical risk factors including decreased fibrinolysis and lipid disturbances. In a cross-sectional study we have determined parameters of fibrinolysis in control subjects (n = 23) and stable renal allograft recipients without cyclosporin (CsA) (n = 10) and with CsA (n = 87) in their immunosuppressive treatment. In CsA-treated patients, tissue-type plasminogen activator was moderately increased compared to patients without CsA (8.4 ± 3.3 vs 5.5 ± 2.8 ng/ml). The plasminogen activator inhibitor (PAI) activity in plasma was clearly increased in CsA-treated patients: 14.5 ± 8.8 vs 7.2 ± 3.2 in normal controls and 8.5 ± 2.4 AU/ml in patients without CsA. Total cholesterol and LDL cholesterol levels were higher in CsA-treated patients (256 ± 62 and 169 ± 60 mg/dl) than in patients without CsA (209 ± 45 and 136 ± 44 mg/dl). The two groups did not differ in HDL cholesterol, triglycerides, and lipoprotein(a). Hypercholesterolaemia, obesity, and steroid-induced diabetes could be identified as risk factors for elevated plasma PAI activity in CsA-treated patients. Hypofibrinolysis induced by elevated PAI levels and increased LDL cholesterol may contribute to the increased thrombogenicity and accelerated atherosclerosis observed in cyclosporin-treated patients.

Key words: cholesterol; cyclosporin; fibrinolysis; lipids; plasminogen activator inhibitor; renal allograft recipients

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in renal transplant recipients.

Accelerated atherosclerosis may be one of the contributing factors. Some of the cardiovascular events in transplant patients are caused by arterial thrombosis, the incidence of which is increased in cyclosporin-treated patients [1]. Atherosclerosis and thrombosis are currently considered to have similar physiopathological mechanisms [2]. There is increasing evidence that fibrin deposition in the vessel wall plays an initiating role in the development of atherosclerotic lesions, in addition to its role in the late complications of vascular disease [3]. It is therefore not surprising that both disorders share the same clinical risk factors including lipid disturbances [4] and decreased fibrinolysis [5,6].

The fibrinolytic system is a serine protease system composed of the enzyme plasmin, its precursor plasminogen, and the plasminogen activators (PA) of the urokinase type (uPA) and of the tissue type (tPA). The catalytic activity of the PA/plasmin system is regulated by the plasmin inhibitors and the PA inhibitors (PAI) [7]. Deficient fibrinolysis is most frequently the result of the presence of excess plasminogen activator inhibitor (PAI) and less frequently due to decreased plasminogen activator release (tPA) or the presence of plasmin inhibitors [3].

A recent study [8] in renal allograft recipients demonstrated impaired fibrinolysis in the subgroup of patients treated with cyclosporin. In diabetes and obesity an association of impaired fibrinolysis with lipid disorders, particularly hypertriglyceridaemia, has been found [9]. There is clinical evidence that cyclosporin may induce dyslipidaemia, through an increase in levels of low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL) [10–14].

In the present cross-sectional study, parameters of the fibrinolytic system in renal allograft recipients were studied and a relationship between increased plasma levels of PAI, cyclosporin therapy, and dyslipidaemia was found.
Subjects and methods

Subjects

Cadaveric renal allograft recipients with stable renal function were included in the study. The patients were divided into two groups according to the presence of cyclosporin in their immunosuppressive regimen. Patients not receiving cyclosporin, and thus treated with azathioprine/prednisolone, had either been transplanted before the cyclosporin era, or had stopped cyclosporin because of toxicity (for more than 3 months). In patients not on cyclosporin the prednisolone dose was significantly higher than in patients on cyclosporin: 10.5 ± 2.0 mg/day vs 6.6 ± 3.1 mg/day; P<0.001. Patients on lipid-lowering drugs and patients with proteinuria >2 g/day or creatinaemia >2.5 mg/dl were excluded from the analysis. Patients on cyclosporin received either a dose of cyclosporin with target blood levels of 100-150 ng/ml or a low dose of cyclosporin together with azathioprine with target cyclosporin blood levels of 50 ng/ml. Initially the cyclosporin subgroups were analysed separately. Since the observed differences concerning blood lipids and fibrinolytic parameters were only marginal, the cyclosporin subgroups were later analysed together. Normal volunteers were studied to determine the reference value of plasma PAI activity.

Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Creatinine clearance was calculated by means of the Cockroft–Gault equation. Patient characteristics are shown in Table 1.

Blood sampling

Blood samples were collected after an overnight fast. For the determination of fibrinolytic parameters blood was drawn into citrate-containing tubes without venous occlusion.

Laboratory measurements

PAI activity and α2-antiplasmin were measured with a chromogenic substrate method (Coatest PAI and Coatest Antiplasmin, Chromogenix, Mölndal, Sweden). The CV of PAI activity determination was 4% for low and 2% for high values. tPA antigen was measured with an enzyme immunoassay (Asserachrom tPA, Diagnostica Stago, Asnières-sur-Seine, France).

Cholesterol and triglycerides were measured with a Boehringer Mannheim Kft. HDL cholesterol was determined in whole plasma after precipitation of LDL and VLDL with 4% sodium tungstenate in the presence of 2M MgCl₂. LDL cholesterol was determined after precipitation of VLDL and HDL fractions using dextran sulphate. Apolipoproteins A1 and B and lipoprotein(a) were measured by means of a Behring nephelometer, using the appropriate rabbit anti-human antibodies (Behring Werke, Germany).

Statistical analysis

Statistical analysis was performed by SPSS for MS Windows, release 6.0. Data shown are mean ± SD. For comparisons of continuous data between three groups analysis of variance was performed, followed by the Student–Newman–Keuls test for multiple comparisons. For the comparison of the mean of two groups the Student test was used with the appropriate correction for the inequality of variances. For lipoprotein(a) values geometric mean and range are reported and the Mann–Whitney U test was used for comparisons. Categorical data were compared by the χ² test. Relative risk was calculated as the ratio of two incidence rates, namely the incidence of the event in the cohort that was exposed to the risk factor and the incidence rate in the cohort that was not exposed. The confidence interval of the risk estimate was constructed after logarithmic transformation [15].

Results

The mean plasma levels of fibrinolytic parameters in normal volunteers and renal allograft recipients with and without cyclosporin included in their therapy are shown in Table 2. We observed a moderately higher tPA level in cyclosporin-treated patients. The plasminogen activity was not different between normal volunteers and renal allograft recipients.

Table 2. Plasma fibrinolytic parameters (mean ±SD)

<table>
<thead>
<tr>
<th></th>
<th>Normal volunteers</th>
<th>Renal allograft recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without CsA</td>
<td>With CsA</td>
</tr>
<tr>
<td>PAI (AU/ml)</td>
<td>7.2 ± 3.2</td>
<td>8.5 ± 2.4</td>
</tr>
<tr>
<td>tPA (ng/ml)</td>
<td>ND</td>
<td>5.5 ± 2.8</td>
</tr>
<tr>
<td>α2-antiplasmin (%)</td>
<td>ND</td>
<td>97.6 ± 25.1</td>
</tr>
</tbody>
</table>

*p Significantly (P<0.05) different from two other groups.

Table 1. Subject characteristics (mean ±SD)

<table>
<thead>
<tr>
<th></th>
<th>Normal volunteers (n=23)</th>
<th>Renal allograft recipients (n=10)</th>
<th>With CsA (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50.5 ± 15.4</td>
<td>46.9 ± 15.5</td>
<td>46.5 ± 13.5</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>8/15</td>
<td>4/6</td>
<td>41/46</td>
</tr>
<tr>
<td>Body mass index</td>
<td>24.4 ± 1.8</td>
<td>23.0 ± 2.4</td>
<td>25.8 ± 4.0</td>
</tr>
<tr>
<td>Type I diabetes (%)</td>
<td>0</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Steroid-induced diabetes (%)</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Duration of transplantation (months)</td>
<td>ND</td>
<td>92.6 ± 49.0</td>
<td>43.2 ± 29.8</td>
</tr>
<tr>
<td>Ccr (ml min)</td>
<td>ND</td>
<td>64.2 ± 24.3</td>
<td>64.2 ± 20.8</td>
</tr>
</tbody>
</table>

*p<0.05
Increased plasma PAI in renal transplantation.

Plasminogen activator inhibitor level, however, was almost doubled in this group of patients (Figure 1) in comparison with normal volunteers and patients not on cyclosporin. No difference was found for the plasmin inhibitor α2-antiplasmin.

The mean serum lipid levels in renal allograft recipients with and without cyclosporin included in their therapy are shown in Table 3. The distribution of the serum cholesterol levels in patients not on cyclosporin was comparable to that found in the general Belgian population [16]. The significant increase in total cholesterol levels observed in cyclosporin-treated renal allograft recipients was the result of increased LDL cholesterol levels. Apolipoprotein B levels were concomitantly increased. In cyclosporin-treated subjects, their was a tendency (P = 0.19) towards a higher LDL cholesterol in females (178 ± 48 mg/dl) than in males (162 ± 68 mg/dl). HDL cholesterol and apolipoprotein A1 levels were not different between patients with and without cyclosporin. In both groups, HDL cholesterol was significantly (P = 0.001) higher in females (63 ± 16 mg/dl) than in males (46 ± 15 mg/dl). When the diabetic patients were analysed separately they followed the same trends in serum cholesterol. Lipoprotein(a) levels were not increased in this population with normal or mildly impaired renal function.

In the cohort of cyclosporin-treated patients we analysed the risk factors for an increased level of PAI activity. Increased PAI activity is defined as a value above the 95th percentile of normal controls, i.e. 13.7 AU/ml. In cyclosporin-treated patients this value represents the 62nd percentile. Male sex, type 1 diabetes, hypertriglyceridaemia, and a low HDL cholesterol level were not associated with an increased risk for an elevated PAI plasma level. On the other hand, in the case of hypercholesterolaemia, obesity, and steroid-induced diabetes the risk rate for an increased PAI level was 1.5 to 2 times that observed in patients not having the risk factor (Figure 2).

**Table 3. Serum lipids (mg/dl) (mean ± SD, except * geometric mean and range)

<table>
<thead>
<tr>
<th>Renal allograft recipients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without CsA</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>209 ± 45</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>50 ± 17</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>136 ± 44</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>152 ± 33</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>157 ± 48</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>153 ± 100</td>
</tr>
<tr>
<td>Lipoprotein (a)*</td>
<td>17 (5–60)</td>
</tr>
<tr>
<td>With CsA</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>256 ± 62</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>55 ± 17</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>169 ± 60</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>164 ± 42</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>186 ± 57</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>199 ± 161</td>
</tr>
<tr>
<td>Lipoprotein (a)*</td>
<td>17 (5–156)</td>
</tr>
</tbody>
</table>

**Fig. 1. Plasminogen activator inhibitor distributions in normal volunteers and renal allograft recipients with and without cyclosporin (CsA) included in their treatment. Data are represented as box plots (see legend).

**Fig. 2. Risk factors for increased plasma activity of plasminogen activator inhibitor in cyclosporin-treated renal allograft recipients. Increased PAI activity is defined as a value above P95 of normal controls (13.7 AU/ml). Relative risks are represented with 95% confidence interval.

**Discussion**

In this cross-sectional study in renal allograft recipients, we found an elevated plasma activity of the plasminogen activator inhibitor in CsA-treated patients. In the same patients, an increase in total and LDL cholesterol levels were associated with an increased risk for an elevated PAI level.

The activation of plasminogen is the critical step in the regulation of fibrinolysis, and PAI is considered to be the primary modulator of plasminogen activation in vivo [3]. Consequently, elevated PAI levels should entail a decreased fibrinolytic activity in these patients. Our findings are partially in agreement with those of Levi et al. [8]. These authors found a decreased fibrinolytic activity in cyclosporin-treated patients compared to azathioprine-treated patients, but their
basal tPA and PAI levels did not differ. Their results suggest a defective tPA release as the reason for hypofibrinolysis. Our study, however, suggests that not defective tPA release, but upregulation of PAI is responsible for hypofibrinolysis in cyclosporin-treated patients.

Other regulators of the fibrinolytic system were also studied. α2-antiplasmin is a plasma inhibitor of activated plasmin and was found not to be influenced by cyclosporin therapy. Lipoprotein(a) interferes with the binding of plasminogen to fibrin and is considered to be an independent risk factor for cardiovascular disease [17]. Conflicting data are published concerning lipoprotein(a) levels in transplant patients. Irish et al. [13] did not find any difference between renal transplant recipients and controls. Black and Wilcken [18] found a decrease in lipoprotein(a) immediately after renal transplantation, following the improvement of renal function. Webb et al. [14] found significantly higher lipoprotein(a) levels in cyclosporin-treated renal allograft recipients compared to azathioprine-treated recipients, the levels in the latter group already differing from normal controls. The results in this study, however, may be influenced by the presence in the study population of a number of patients with renal failure and proteinuria, clinical conditions known to increase lipoprotein(a) concentrations [19,20]. In the present study, in which we excluded patients with severe renal failure and nephrotic-range proteinuria, we did not find a significant increase in lipoprotein(a).

After introduction of cyclosporin in immunosuppressive regimens, it became apparent that this drug caused a significant increase in LDL cholesterol [21]. This was a constant finding in all subsequent studies on lipid disturbances after organ transplantation [10–12]. The mechanism of the rise in LDL plasma levels is not known. Circulating cyclosporin is bound to plasma lipoproteins, mainly HDL and LDL [22]. The presence of cyclosporin on the surface of the lipoprotein particle may interfere with its metabolism. López-Miranda et al. [23], in normal rats and CsA-treated rats, studied the disappearance rate of exogenous homologous LDL from normal donor rats and donor rats treated with cyclosporin. They found a decrease in LDL disappearance rate in cyclosporin-treated rats independent of the donor LDL source. This suggests that the elevated concentrations of LDL cholesterol associated with cyclosporin treatment do not result from a cyclosporin-induced modification of the LDL molecule, but possibly from a cyclosporin-induced reduction in hepatic or endothelial clearance.

The clinical finding of an association between increased PAI levels and hypercholesterolaemia may have a physiopathological basis. Although untested by the present study, the following hypotheses may be put forward. Excess LDL cholesterol can be modified to ox-LDL by endothelial cells [24]. A recent study suggests that LDL from women with renal transplants is abnormally susceptible to oxidation [25]. In vitro, ox-LDL is a potent stimulator of PAI secretion by endothelial cells [26]. Recently, evidence has been provided that the LDL receptor-related protein, a member of the LDL receptor gene family, mediates the internalization and cellular degradation of complexes of plasminogen activator and its inhibitor [27]. Thus, another possibility is that cyclosporin induces malfunction or decreased expression of this receptor, leading to a diminished clearance of both LDL and PA/PAI complexes. In the blood, 90% of the circulating PAI is present in platelets. PAI may also be released by platelets chronically activated by cyclosporin [28]. Finally, cyclosporin might be able to directly stimulate PAI synthesis in vascular endothelial cells or hepatocytes.

Clinical studies have established significant correlations between PAI levels and obesity, non-insulin-dependent diabetes [9], and hypertriglyceridaemia [29]. In the present study we found a moderately increased risk for elevated PAI levels in obese cyclosporin-treated patients. The risk in patients with steroid-induced diabetes was clearly increased. Steroid-induced diabetes may be considered metabolically as the equivalent of type II diabetes.

It is likely that the increased LDL cholesterol and PAI levels contribute to the increased thrombogenicity and accelerated atherosclerosis observed in cyclosporin-treated patients. Furthermore, cyclosporin nephrotoxicity might be related to this impaired activity of the fibrinolytic system. The renal interstitial fibrosis induced by cyclosporin may result from defective tissue remodelling as a result of the decreased activity of the PA/plasmin protease system.

In conclusion, in this cross-sectional study we found increased plasma PAI levels in cyclosporin-treated renal allograft recipients. Patients with steroid-induced diabetes, obesity, or hypercholesterolaemia are particularly at risk for this alteration of the fibrinolytic system.

Acknowledgements. The authors wish to thank Francine Vertessen, senior technician of the Laboratory of Haematology, and the nursing team of the Transplantation Clinic for their careful performance of the study. In addition the help of Dirk De Weerdt and Erik Snelders in the preparation of this manuscript is gratefully acknowledged.

References
Increased plasma PAI in renal transplantation.


*Received for publication: 31.3.95
Accepted in revised form: 28.9.95*