Cyclosporin A Has Divergent Effects on Plasma LDL Cholesterol (LDL-C) and Lipoprotein(a) [Lp(a)] Levels in Renal Transplant Recipients

Evidence for Renal Involvement in the Maintenance of LDL-C and the Elevation of Lp(a) Concentrations in Hemodialysis Patients

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Abstract Cardiovascular disease is the major cause of mortality in renal transplant recipients. Plasma levels of low-density lipoprotein cholesterol (LDL-C) are often elevated following renal transplantation, and the immunosuppressant cyclosporin A has been implicated as a predisposing factor for posttransplantation hyperlipidemia. Lipoprotein(a) [Lp(a)] is an LDL-like lipoprotein particle; elevated levels of Lp(a) provide an independent and significant risk factor for cardiovascular disease. Plasma concentrations of Lp(a) vary greatly among individuals, and the mechanisms that govern changes in their levels in transplant patients are unknown. The effect(s) of cyclosporin A on Lp(a) was studied in two groups of renal transplantation patients. In group I plasma lipoproteins including Lp(a) were measured before and after successful renal transplantation; this group received both prednisone and cyclosporin A for immunosuppression. Group II patients were studied after renal transplantation and received prednisone alone for immunosuppression. Following surgery, group I patients demonstrated increased plasma concentrations of LDL-C (mean±SEM range, 11±2 to 14±2.7 mg/dL; P<.005). In contrast, plasma Lp(a) levels for this group were markedly decreased after renal transplantation (median, 34.3 to 19.7 mg/dL). Patients not treated with cyclosporin A (group II) exhibited mean LDL-C and median Lp(a) levels (118±42 and 33.1 mg/dL, respectively) that were remarkably similar to those observed before renal transplantation (group I). These data confirm that hyperlipidemia following renal transplantation is associated with cyclosporin A therapy and show that this drug has opposing effects on plasma Lp(a) and LDL-C accumulations. To elucidate the effect(s) of a failed or impaired kidney as a determinant of Lp(a) and other plasma lipoprotein concentrations in hemodialysis patients, five patients who had undergone bilateral nephrectomy were also studied. These anephric subjects had plasma levels of both Lp(a) and free apolipoprotein(a) that were similar to a control group. In contrast, plasma LDL-C levels were dramatically low in these anephric individuals (mean±SEM, 27±18 mg/dL). Together, these data suggest that a healthy or an impaired kidney is requisite for the maintenance of normal plasma LDL-C levels. In addition, these studies revealed that an impaired or failed kidney might play a role in the elevation of plasma Lp(a) concentrations seen in hemodialysis patients. (Arterioscler Thromb. 1994;14:1393-1398.)

Key Words • lipoprotein(a) • regulation • renal transplantation • cyclosporin A • LDL

Cardiovascular disease is the primary cause of mortality in renal transplant recipients. Hyperlipidemia involving elevated plasma LDL cholesterol (LDL-C) levels is common in these patients and is thought to be a major contributor to this postsurgical condition. Two immunosuppressant drugs commonly used after renal transplantation are cyclosporin A and prednisone. Of these, cyclosporin A is associated with posttransplantation hyperlipidemia. The purpose of this study was to investigate the role that the failed or impaired kidney plays in governing plasma Lp(a) and LDL-C levels in hemodialysis patients. In addition, we wanted to ascertain the effect of immunosuppressant therapy on plasma Lp(a) levels and the contribution of this potentially atherothrombotic lipoprotein to the hyperlipidemia that follows renal transplantation.

Methods

Subjects

Patients who underwent successful renal transplantation at the State University of New York (SUNY) Health Science...
Center at Brooklyn were selected for this study based on the immunosuppressant used for posttransplantation therapy. One group of patients (group I) was composed of 53 individuals who received both cyclosporin A (mean dose, 181 ± 65.7 mg twice a day; range, 75 to 350 mg/d) and prednisone (mean dose, 9.2 ± 2.7 mg once a day; range, 5 to 15 mg/d) for immunosuppression. This group was studied before and 6 months to 1 year after renal transplantation. The other group of renal transplantation patients studied (group II) consisted of 31 individuals who received prednisone (mean dose, 8.5 ± 1.8 mg once a day; range, 5 to 10 mg/d) alone for immunosuppression. This group underwent renal transplantation before cyclosporin A was widely employed; they were available for study only 6 months to 5 years after surgery. All patients studied were on hemodialysis due to end-stage renal failure prior to surgery, and all were stable transplant recipients. None of the patients admitted into the study were diabetic, nor were any of them treated for hyperlipidemia (during the study). Mean creatinine levels following surgery (at the same sampling time as lipoprotein analysis) were below 2 mg/dL for both groups (see Table 1).

Anephric individuals (n=5) had undergone bilateral nephrectomy surgery at the SUNY Health Science Center at Brooklyn. These patients were maintained on chronic hemodialysis and were not transplant recipients (prior to or during the study). Surgery was required due to either uncontrollable hypertension or infection; in each case, the condition was alleviated following the procedure.

Control individuals (n=104) were recruited from the Employee Health Cholesterol Screening Program at The Rockefeller University and were matched on a percentage basis for gender, race, and age with group I (74% male; 16.6% black, 40.0% Caucasian, and 43.4% Latino; mean age, 43.0 ± 12.9 years). All control individuals were normolipidemic and were free from renal, thyroid, and immunologic disorders by either history and/or laboratory screening.

### Plasma Lipid and Lipoprotein Determinations

Plasma lipoprotein level determinations were performed on fasting plasma at the Laboratory of Biochemical Genetics and Metabolism at The Rockefeller University. Total cholesterol, very-low-density lipoprotein cholesterol (VLDL-C), LDL-C, and high-density lipoprotein cholesterol (HDL-C) were determined. Lp(a) levels were determined by using a bi-site sandwich enzyme-linked immunosorbent assay (ELISA) system. A monospecific antibody against Lp(a) was employed as the capture reagent, and monospecific antibodies raised against apoB or apo(a) were used for detection. These assays are insensitive to the phenotypic variation in apo(a) size and are unaffected by plasminogen concentrations exceeding 250 mg/dL. Values given are in Lp(a) particle mass.

### Statistics

Values for all plasma lipid levels except for Lp(a) are presented as the mean ± SEM. Student’s paired t test was used to compare parameter means before and after renal transplantation (group I). The unpaired t test was used to compare parameter means between groups. Since the frequencies of Lp(a) concentrations were not normally distributed in all groups, median plasma Lp(a) concentrations were used for statistical analyses. The nonparametric Kruskal-Wallis test was employed for testing differences of plasma Lp(a) concentrations between groups. Significance is defined as P<.05.

### Results

Prior to renal transplantation, all patients were receiving regular hemodialysis due to chronic renal failure. The median plasma Lp(a) level measured at this time was significantly elevated relative to control subjects' levels (34.3 mg/dL for patients versus 19.7 mg/dL for control subjects, P<.005; mean, 27.6 ± 3.1 and 18.2 ± 2.7 mg/dL, respectively). Following surgery, mean creatinine levels were similar and approached normalcy for both transplantation groups (see Table 1). To determine the effects of both kidney transplantation and immunosuppressant therapy on plasma lipoproteins, patients who were treated with both prednisone and cyclosporin A (group I) were studied before and 6 months to 1 year after surgery. As shown in Fig 1, this group exhibited marked hyperlipidemia following kidney transplantation. Total cholesterol, VLDL-C, LDL-C, and HDL-C were each elevated in these patients relative to before surgery (249 ± 6, 63 ± 5, 142 ± 7, and 36 ± 4 mg/dL after surgery versus 189 ± 9, 41 ± 4, 111 ± 6, and 36 ± 2 mg/dL before surgery, respectively). There was a trend for an elevation in plasma triglyceride levels, but these were not significantly different before and after surgery (183 ± 21 and 210 ± 24 mg/dL, respectively). In contrast, following surgery, plasma Lp(a) levels were 42% lower (P<.01) in transplant recipients who received cyclosporin A together with prednisone for immunosuppression (Fig 2). Reduced plasma Lp(a)
concentrations were observed in 43 of the 53 patients studied in this group. If examined independently, this subset of 43 individuals exhibited an average 52% reduction (P<.001) of Lp(a) levels after renal transplantation relative to the same group before surgery (data not shown). Interestingly, as illustrated in Fig 2, median plasma Lp(a) concentrations were reduced to control levels with cyclosporin A treatment, whereas LDL-C levels were further elevated (see Fig 1). The opposing effects of cyclosporin A on LDL-C and Lp(a) levels support the notion that these two lipoprotein particles, although of similar size and lipid content, are regulated, at least in part, by independent mechanisms.

In contrast to the results obtained with patients who received both prednisone and cyclosporin A, patients who did not receive cyclosporin A (ie, group II, which received only prednisone) exhibited mean plasma VLDL-C and LDL-C levels (48±3 and 118±42 mg/dL, respectively) that were similar to those observed prior to renal transplantation in group I (ie, during chronic renal failure) (Figs 1 and 2). The median plasma Lp(a) level in group II (33.1 mg/dL; mean, 54.1±10.0) was elevated relative to both control subjects (P<.01) and those patients who received cyclosporin A (group I; P<.01). However, unlike LDL-C, plasma HDL-C levels in group II (mean, 49±3 mg/dL) were significantly greater than in patients suffering from chronic renal failure (group I prior to surgery; 36±2 mg/dL; P<.001) and were similar to control subjects (mean, 54±3 mg/dL).

To more completely assess the relative contribution of renal metabolism to the determination of plasma Lp(a) and LDL-C levels, we studied five patients who had undergone bilateral nephrectomy and were maintained on regular hemodialysis. Plasma LDL-C levels in these individuals were markedly lower (mean, 27±13 mg/dL) (Table 2). Therefore, together with the data from group I patients prior to renal transplantation, these data indicate that the kidney or the presence of an impaired kidney might play a significant role in the maintenance of LDL-C concentrations.

The frequency distribution of Lp(a) in the five different study groups is shown in Fig 3. Before renal transplantation, 46% of the subjects in group I exhibited plasma Lp(a) concentrations below 30 mg/dL, and 37% of this group had very high Lp(a) levels (above 60 mg/dL). After successful transplantation and use of cyclosporin A, there was a dramatic change in the Lp(a) concentration profile, with over 68% of these patients exhibiting plasma Lp(a) levels below 30 mg/dL. In addition, no individual in the cyclosporin A–treated group displayed posttransplantation Lp(a) levels over 60 mg/dL. However, of those individuals who did not receive cyclosporin A for immunosuppression following surgery (group II), 50% had plasma Lp(a) levels below 30 mg/dL, with a trend towards higher values, and 36% had levels above 60 mg/dL. These data suggest that cyclosporin A can be a potent modulator of plasma Lp(a) levels in renal transplant recipients. It is noteworthy that in the control group the frequency of plasma Lp(a) concentrations above 60 mg/dL was high (33%). This might be due to the composition of this group with regard to race (17.3% black, 43.6% Latino, and 39.1% Caucasian) and gender (72% male). In dramatic contrast to individuals with either functional or impaired kidneys, none of the five patients who underwent bilateral nephrectomy had plasma Lp(a) concentrations above 30 mg/dL (Table 2 and Fig 3). The median plasma Lp(a) level in these anephric individuals was similar to the control group as measured by using either an anti-Lp(a) antibody [to quantify both Lp(a) and free apo(a), 20.7 mg/dL] or an anti-apoB antibody [to measure only Lp(a) levels, 19.0 mg/dL] as the detecting reagent (Fig 2). In addition, size-exclusion fast-protein liquid chromatography employing Superose-6 revealed low concentrations of free [non-Lp(a)-associated] apo(a) in the plasma of these anephric individuals as detected by ELISA (0.5 to 1.2 mg/dL). Therefore, in contrast to patients in group I prior to renal transplantation (ie, those suffering from chronic renal failure), plasma Lp(a) or free apo(a) levels in these patients were not elevated relative to control subjects. Interestingly, these anephric individuals exhibited severely reduced levels of the other major apoB-containing lipoprotein, LDL (Table 2). Thus, there appears to be

### Table 2. Plasma Lipoprotein Levels in Anephric Individuals

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total Chol</th>
<th>Trig</th>
<th>VLDL-C</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.R.</td>
<td>109</td>
<td>152</td>
<td>57</td>
<td>21</td>
<td>31</td>
<td>21.4</td>
</tr>
<tr>
<td>J.W.</td>
<td>120</td>
<td>222</td>
<td>20</td>
<td>46</td>
<td>54</td>
<td>15.3</td>
</tr>
<tr>
<td>K.R.</td>
<td>141</td>
<td>121</td>
<td>79</td>
<td>32</td>
<td>30</td>
<td>26.2</td>
</tr>
<tr>
<td>G.D.</td>
<td>110</td>
<td>84</td>
<td>60</td>
<td>18</td>
<td>32</td>
<td>19.0</td>
</tr>
<tr>
<td>C.R.</td>
<td>149</td>
<td>106</td>
<td>90</td>
<td>16</td>
<td>43</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Mean±SEM 126±18 137±54 61±27 27±13 38±10 18.4±2.7

Chol indicates cholesterol; Trig, triglycerides; VLDL-C, very-low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and Lp(a), lipoprotein(a). Plasma lipid levels are reported as milligrams per deciliter; Lp(a) concentrations, as Lp(a) particle mass. Median Lp(a) level was 19.0.
enough LDL produced by the liver to support the Lp(a) concentrations observed, but steady-state LDL-C levels themselves are dramatically reduced in the absence of either a functional or impaired kidney.

**Discussion**

Elevated plasma Lp(a) levels are associated with an increased risk for cardiovascular disease. It is therefore important to determine the factors and/or mechanisms that control these levels. There are at least two independent processes involved in the governing of individual plasma Lp(a) concentrations. These include the size of the apo(a) moiety present on the Lp(a) particle and another mechanism that determines the steady-state level of apo(a) mRNA. This latter process appears to be confined to the liver, since this is the major site of apo(a) gene expression. Recently Lackner et al. and Cohen et al. have shown, using apo(a) genotyping by pulsed-field gel electrophoresis and single-strand conformational polymorphism analysis, respectively, that the apo(a) locus is remarkably polymorphic. This heterogeneity involves both protein coding regions as well as noncoding promoter and intronic sequences. In addition, by using sibling-pair analysis it was revealed that greater than 90% of the variability of individual plasma Lp(a) levels can be explained by the heterogeneity at this locus. However, the relative contribution(s) of the hepatic and extrahepatic processes that might determine Lp(a) levels, probably as a function of the specific apo(a) allele involved, are yet to be defined.

It is well documented that patients suffering from chronic renal failure or with nephrotic syndrome exhibit elevated plasma Lp(a) levels. Interestingly, apo(a) protein fragments have been found in urine; the concentration of these peptides in the urine was decreased in patients with renal failure. These studies prompted us to wonder whether an impaired kidney might play a role in governing serum Lp(a) concentrations.

In an effort to elucidate determinants of plasma Lp(a) concentration, we studied the effects of renal transplantation on individual lipoprotein profiles. In addition, these studies also enabled us to determine the relative contribution that plasma Lp(a) provides in postsurgery hyperlipidemia. We found that plasma Lp(a) concentrations prior to renal transplantation surgery, ie, during chronic renal failure, were elevated relative to control subjects' levels. This corroborates findings that plasma Lp(a) levels are elevated during renal failure. Elevated plasma Lp(a) levels might be the result of either an enhanced hepatic secretion of Lp(a) or a decrease in the fractional plasma catabolic rate. Since Lp(a) has no significant affinity for the LDL receptor, a decreased fractional catabolic rate would probably be the result of a modified clearance by an LDL receptor–independent pathway by either the liver or another tissue. Especially in the case of renal failure, it might be reasonable to consider an altered renal clearance as a possible contributor to the elevation of

![Graph showing frequency distribution of lipoprotein(a) concentrations in renal transplant (RTX) recipients, anephric patients, and control subjects.](http://atvb.ahajournals.org/)
Lp(a) levels. We found that renal transplant recipients who did not receive cyclosporin A (group II) had elevated plasma Lp(a) concentrations that were similar to those observed in patients prior to surgery (group I). Since renal function was similar and tended to approach normalcy in both groups following renal transplantation (as shown by serum creatinine values), the elevated plasma Lp(a) concentrations found in group II are probably not due to an altered renal clearance but might be a result of either enhanced hepatic Lp(a) or apo(a) synthesis and/or secretion. The effects of glucocorticoids on human plasma Lp(a) metabolism have not been extensively studied. It is therefore possible that the elevated levels of Lp(a) seen in these patients are associated with either the prednisone treatment (in the absence of cyclosporin A) or an inherent feature of this slightly older group of patients (Table 1). It was recently demonstrated that another glucocorticoid, prednisolone, can effectively decrease plasma Lp(a) levels in patients suffering from rheumatic disease either with or without nephrotic syndrome. The specific effect(s) that either prednisolone or prednisone therapy has on Lp(a) metabolism in renal transplant patients, however, remains to be determined. The mechanism(s) for the modulation of plasma Lp(a) levels by pharmacological agents and in patients suffering from renal disease is currently a focus of research efforts in our laboratory.

Webb et al reported elevated plasma Lp(a) levels in renal transplant recipients treated with cyclosporin A. The discrepancy between this study and ours might stem from a difference in doses (these authors do not report the dose of cyclosporin A used) or in possible differences in racial composition between these two studies. Interestingly, both renal15-21 and cardiac22 transplant recipients who received cyclosporin A show a reduction of Lp(a) levels of similar magnitude as we report here.

Cyclosporin A had opposing effects on Lp(a) and LDL-C levels in our renal transplantation recipients (this was also seen in cardiac transplantation recipients). Thus there is an apparent divergence in the mechanisms that modulate the levels of these two very similar lipoprotein particles by cyclosporin A. These differences might be manifest as a differential clearance of Lp(a) and the other apoB-containing particles, namely LDL and VLDL, by the LDL receptor, which might be downregulated by this immunosuppressant. Since Lp(a) is not an avid ligand for this receptor, then it would be relatively unaffected by any modulation in its activity. Cyclosporin A is metabolized primarily by the liver via the cytochrome P-450 system and has an inhibitory effect on the bile acid synthesis pathway. Therefore, the reduction of plasma Lp(a) (and possibly the enhancement of HDL-C) levels by cyclosporin A might be manifest as a modulation of hepatic metabolism involving synthetic, secretory, and/or degradative pathways. Alternatively, the effect of cyclosporin A on plasma Lp(a) levels might be the result of an enhanced plasma clearance by an LDL receptor-independent pathway. Cyclosporin A has a high affinity for both LDL and HDL, with over 80% of plasma levels found associated with these lipoprotein particles. Unfortunately, no data are currently available on the interaction(s) of this immunosuppressant with Lp(a), but it is easy to envision such an association. Two different receptors that appear to be involved in the binding, uptake, and degradation of Lp(a) have been localized on fibroblasts and macrophages. The effect(s) of cyclosporin A on the activity of these receptor systems remains to be determined.

Treatment with cyclosporin A following surgery was associated with a marked hyperlipidemia involving predominantly LDL-C and VLDL-C. Plasma HDL-C levels, although similar to control subjects' levels, were elevated relative to the pretransplant condition (ie, during chronic renal failure). Therefore the concomitant elevation of both LDL-C and HDL-C as a result of cyclosporin A therapy maintained a relatively constant LDL/HDL ratio.

In an attempt to further elucidate the role of the kidney in determining individual plasma Lp(a) concentrations, we studied lipoprotein parameters in patients who underwent bilateral nephrectomy. Since all anephric patients must undergo regular hemodialysis, this study enabled us to determine the effect of hemodialysis on patients without any possible effect(s) of failed kidneys. Several studies have shown that hemodialysis is associated with elevated plasma Lp(a) levels in patients suffering from chronic renal failure. We found that plasma Lp(a) concentrations in these anephric individuals were similar to those in control subjects. Thus these data provide evidence that the elevation of plasma Lp(a) concentrations often observed in hemodialysis patients suffering from chronic renal failure is probably not a direct result of the dialysis procedure. In addition, since bilateral nephrectomy does not result in elevated Lp(a) levels relative to control subjects, these data suggest that significantly impaired or failed native kidneys might play a role in elevating plasma Lp(a) levels.

In summary, an impaired or failed kidney might play a role in the elevation of plasma Lp(a) concentrations seen in hemodialysis patients. This might occur as a factor that is secreted by the kidney remnant. In addition, we found that cyclosporin A has potent and divergent effects on plasma LDL-C and Lp(a) concentrations. The mechanisms whereby Lp(a) levels are modulated remain to be explored. Once known, these data might eventually enable us to develop drugs and/or therapies designed to reduce plasma Lp(a) levels, especially in patients such as those suffering from renal disease, in whom the risk for atherosclerotic and thrombotic events is increased.

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