Effect of treatment with simvastatin on serum cholesteryl ester transfer in patients on dialysis

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Abstract

Background. Plasma cholesteryl ester transfer activity is increased in patients with chronic renal failure on dialysis who have elevated levels of apolipoprotein B (apoB)-containing lipoproteins. Simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor, reduces levels of these lipoproteins but the effect of treatment on cholesteryl ester transfer activity in patients on dialysis remains to be determined.

Methods. We measured serum newly synthesized cholesteryl ester transfer (NCET) activity, lecithin:cholesterol acyltransferase (LCAT) activity and serum lipid, lipoprotein and apolipoprotein concentrations before and immediately after 6 months treatment with simvastatin (10 mg daily, \(n=24\)) or placebo (\(n=29\)) in 53 patients with chronic renal failure receiving haemodialysis or continuous ambulatory peritoneal dialysis (CAPD).

Results. Simvastatin therapy significantly reduced receptor numbers and reduce plasma levels of apoB-containing lipoproteins including very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and other remnants of triglyceride-rich lipoprotein metabolism, are frequently elevated and HDL levels are low [6–8]. We have previously documented increased transfer of newly synthesized cholesteryl esters from...
HDL to other lipoproteins in plasma from patients with chronic renal failure and hyperlipidaemia [9]. Furthermore, plasma newly synthesized cholesteryl ester transfer (NCET) activity is closely related to levels of apoB in patients with chronic renal failure [9]. The aim of the present study was, therefore, to determine whether treatment with an HMG CoA reductase inhibitor reduces apoB-lipoprotein acceptor levels and serum NCET activity in patients with chronic renal failure receiving dialysis. The study was part of a larger multicentre, double-blind, placebo-controlled trial which examined the effect of treatment with simvastatin or enalapril or both on left ventricular mass and serum lipids and lipoproteins in patients with chronic renal failure.

Patients and methods

Patients

The patients studied were drawn from a group of 107 patients (aged 18–75 years) with chronic renal failure treated by haemodialysis or continuous ambulatory peritoneal dialysis (CAPD) who were recruited into a multicentre study of the effect of therapy with simvastatin or enalapril or both on serum lipoprotein concentration and left ventricular mass. Exclusion criteria included definite indication for HMG CoA reductase inhibitor or angiotensin-converting enzyme (ACE) inhibitor therapy, known allergy to either drug, planned renal graft from living relative, or serious debilitating disease other than chronic renal failure, child-bearing potential without adequate contraception, and potential unreliability. Patients were screened, including a medical history and a physical examination with measurement of body weight and supine blood pressure. Patients who satisfied entry criteria were randomized in a factorial design to receive one of four treatments for 6 months: (a) simvastatin (10 mg daily) plus enalapril (2.5–5 mg daily) (b) simvastatin plus placebo enalapril (c) enalapril plus placebo simvastatin, and (d) placebo enalapril plus placebo simvastatin. The study was double-blinded and the code identifying the treatment received by individual patients was maintained by a person remote from the investigators. The patients (n = 53) in the present study were those for whom there was sufficient serum stored at −80 °C to perform measurements of plasma NCET activity and LCAT activity. Laboratory measurements of serum lipids, lipoproteins and apolipoprotein concentrations, NCET activity, and LCAT activity were performed at baseline and 6 months.

Laboratory measurements

Blood was collected in plain tubes after a 12-h fast. Serum HDL cholesterol was measured in the supernatant after precipitation of apoB-containing lipoproteins with phosphotungstic acid and magnesium chloride [10]. Serum was ultracentrifuged at densities 1.006 g/ml and 1.019 g/ml. VLDL was isolated in the d < 1.006 g/ml fraction and IDL triglycerides were obtained by subtracting lipid levels in this fraction from levels in the d < 1.019 g/ml fraction. Cholesterol, free cholesterol, and triglycerides in serum and serum fractions were measured enzymatically (kits from Boehringer Mannheim). LDL cholesterol was calculated using the Friedewald formula [11]. Serum apolipoproteins A1 and B concentrations were measured by an immunoturbidimetric method [12]. NCET activity in serum that had been stored at −80 °C was measured by an isotopic assay that uses endogenous lipoproteins [13]. Briefly, serum was incubated with a [3H] cholesterol-albumin emulsion for 3 h at 37 °C and the appearance of labelled cholesteryl esters in precipitated VLDL and LDL was measured. Using serum free cholesterol concentration, the NCET rate was calculated. The coefficient of variation for the assay was 10%. Previous studies from this laboratory have shown that NCET activity is similar in fresh or frozen (−80 °C) normolipidaemic or hyperlipidaemic plasma and is closely related to net mass cholesteryl ester transfer in plasma measured by chemical methods [9]. Also, serum NCET activity was measured in 10 fresh sera from one study centre (30.3 ± 12.8 mmol/ml/h, n = 10, mean ± SD) and the activities were not significantly different compared with values for the corresponding sera stored at −80 °C (28.9 ± 11.2 mmol/ml/h, n = 10, mean ± SD). Serum LCAT activity was measured by a modification of the Stokke and Norum method [13].

Statistics

Analyses were performed on the basis of intention-to-treat. Repeated measures ANOVA was used to compare the effects of treatments. Student’s t test was used to compare baseline values between patients treated by haemodialysis or CAPD. Pearson’s product moment correlation coefficients were used to test for relationships between variables. Partial correlation analysis was used to test relationships between two variables while a third variable was held constant. Two-tailed tests of significance were used and a P value of less than 0.05 was considered to be statistically significant.

Results

Mean (± SD) age, body mass index (BMI) and duration of dialysis were 50 ± 15 years, 25.3 ± 4.4 kg/m² and 30 ± 35 months respectively in the 53 subjects (32 men and 21 women) studied. The aetiology of renal failure in the patients was as follows: glomerulonephritis (n = 26), autosomal dominant polycystic kidney disease (n = 6), reflux nephropathy (n = 5), renal vascular disease (n = 6), diabetes (n = 3), and other causes (n = 7). Twenty-five patients were receiving haemodialysis therapy and 28 patients were being treated by CAPD. Simvastatin therapy was discontinued in five patients and enalapril therapy was discontinued in 16 patients before the end of the study. The study design allowed continuation of simvastatin or placebo treatment despite the withdrawal of enalapril or placebo enalapril.

The effect of simvastatin treatment on serum NCET activity, LCAT activity, and lipid, lipoprotein and apolipoprotein concentrations during a 6-month follow-up are shown in Table 1. Serum NCET activity and LCAT activity were reduced significantly with simvastatin treatment. The effect of simvastatin on
serum lipids lipoproteins, including decreases in total cholesterol, LDL cholesterol, and apoB concentrations were similar to the corresponding changes in the total study population (n = 107). Enalapril treatment did not significantly alter serum LCAT and LCAT activities and is not considered further in this report. Baseline values for variables were not significantly different between the placebo group and the simvastatin group. Mean (±SD) BMI and serum creatinine concentration were not significantly different between patients treated with placebo (25.2 ± 4.0 kg/m², 0.97 ± 0.27 mmol/l, n = 29 respectively) and those treated with simvastatin (25.4 ± 5.0 kg/m², 0.92 ± 0.22 mmol/l, n = 24 respectively) and did not change significantly during the study. There were no statistically significant effects of simvastatin on creatine kinase, ALT, urea, potassium, or haemoglobin.

Table 2 shows serum NCET activity in normolipidaemic dialysis patients and hyperlipidaemic patients during treatment with simvastatin or placebo. Simvastatin therapy reduced serum NCET activity significantly compared with placebo in hyperlipidaemic patients. In contrast, the decrease in plasma NCET activity in normolipidaemic dialysis patients treated with simvastatin was not significantly different compared with the change in NCET in the corresponding placebo group. The magnitude of the mean (±SD) decrease in serum NCET activity with simvastatin treatment was significantly (P = 0.036) greater in hyperlipidaemic patients (−5.2 ± 5.9 mmol/ml/h) compared with normolipidaemic patients (−12.4 ± 7.1 mmol/ml/h). Levels of plasma cholesterol and apoB concentrations paralleled levels of variables in Table 2 (data not shown).

Table 3 shows measured variables at baseline in patients treated by haemodialysis or CAPD. Serum NCET activity, IDL cholesterol and apoB concentrations were significantly higher in the CAPD patients and serum LCAT activity was higher and HDL cholesterol significantly higher in the CAPD patients. In contrast, the decrease in plasma NCET activity in normolipidaemic dialysis patients treated with simvastatin was not significantly different compared with the change in NCET in the corresponding placebo group. The magnitude of the mean (±SD) decrease in serum NCET activity with simvastatin treatment was significantly (P = 0.036) greater in hyperlipidaemic patients (−5.2 ± 5.9 mmol/ml/h) compared with normolipidaemic patients (−12.4 ± 7.1 mmol/ml/h). Levels of plasma cholesterol and apoB concentrations paralleled levels of variables in Table 2 (data not shown).

Table 3. Serum newly synthesized cholesteryl ester transfer activity, lecithin:cholesterol acyltransferase activity, and lipid, lipoprotein, and apolipoprotein concentrations at baseline in patients treated by haemodialysis or continuous ambulatory peritoneal dialysis. Means ± SD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Haemodialysis (n = 32)</th>
<th>CAPD (n = 21)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCET (mmol/ml/h)</td>
<td>24.4 ± 9.1</td>
<td>32.9 ± 10.4</td>
<td>0.003</td>
</tr>
<tr>
<td>LCAT (mmol/ml/h)</td>
<td>69.3 ± 17.2</td>
<td>78.7 ± 22.2</td>
<td>0.09</td>
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<td>TC (mmol/l)</td>
<td>6.16 ± 3.14</td>
<td>6.71 ± 1.29</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL-C (mmol/l)</td>
<td>1.02 ± 0.66</td>
<td>1.15 ± 0.74</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>0.49 ± 0.22</td>
<td>0.64 ± 0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>4.17 ± 0.98</td>
<td>4.64 ± 1.15</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL-TG (mmol/l)</td>
<td>1.12 ± 0.37</td>
<td>0.96 ± 1.15</td>
<td>0.09</td>
</tr>
<tr>
<td>IDL-TG (mmol/l)</td>
<td>2.09 ± 0.92</td>
<td>2.28 ± 0.93</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA1 (g/l)</td>
<td>1.12 ± 0.19</td>
<td>1.07 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1.41 ± 0.28</td>
<td>1.59 ± 0.29</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Abbreviations as shown in Table 1.
terol was lower at a marginal level of significance. Mean (±SD) plasma NCET activity was significantly (P < 0.001) higher in hyperlipidaemic haemodialysis patients (29.5 ± 7.6 nmol/ml/h, n = 19) and hyperlipidaemic CAPD patients (36.8 ± 8.4 nmol/ml/h, n = 16) compared with the corresponding normolipidaemic patients (haemodialysis; 17.0 ± 5.2 nmol/ml/h, n = 13; CAPD; 20.1 ± 2.9 nmol/ml/h, n = 5).

Table 4 shows the effect of simvastatin treatment on serum NCET activity and LCAT activity in patients receiving haemodialysis or CAPD. The decrease in these activities with simvastatin treatment was not significantly different between patients on haemodialysis compared with those on CAPD.

At baseline, serum NCET activity was correlated significantly with serum LCAT activity (r = 0.605, n = 53, P < 0.001) and serum concentrations of apoB (r = 0.842, n = 53, P < 0.001), cholesterol (r = 0.559, n = 53, P < 0.001) and triglycerides (r = 0.724, n = 53, P < 0.001). The decrease in serum NCET activity in hyperlipidaemic haemodialysis patients (n = 23) was correlated significantly with the concomitant decreases in LCAT activity (r = 0.715, P < 0.001) and apoB (r = 0.715, P < 0.001). The decrease in LCAT activity during this therapy was not correlated significantly with the decrease in apoB (r = 0.331). The decrease in serum NCET activity during treatment with simvastatin was not significant when serum LCAT activity (P = 0.07) or apoB concentration (P = 0.14) or both (P = 0.64) were taken into account in two-way ANOVA. This decrease in serum NCET activity was not correlated significantly with duration of dialysis (r = −0.301, P = 0.15).

Discussion

The results of the present study show that treatment with simvastatin substantially reduces serum NCET activity and to a lesser extent serum LCAT activity, in association with a decrease in serum apoB-containing lipoproteins levels in patients with chronic renal failure on dialysis. The magnitude of the decrease in serum NCET activity with simvastatin treatment was not influenced by the type of dialysis treatment used in the management of the patients but was greater in hyperlipidaemic patients compared with those with lower lipid levels.

Our data suggest that reduced levels of apoB-containing lipoprotein acceptor particles and reduced serum LCAT activity may be mainly responsible for the decrease in serum NCET activity. In patients on dialysis and treated with simvastatin. The decrease in serum NCET activity was closely and independently linked with the concomitant decreases in serum apoB concentration and LCAT activity during simvastatin therapy and was no longer evident when these changes were taken into account. The decrease in serum apoB levels appeared to be more influential in this context because serum NCET activity still tended to decrease when LCAT activity but not when serum apoB concentration was taken into account. The present findings seem to be consistent with a recent study which has reported a decrease in plasma cholesteryl ester mass transfer as a result of a decrease in LDL particle numbers in patients with familial hypercholesterolemia treated with pravastatin [2].

The simvastatin-induced decrease in serum NCET activity in the present study is also consistent with the reported fall in plasma NCET activity in patients with NIDDM treated with an HMG CoA reductase inhibitor [4]. However, in NIDDM patients, the decrease in plasma NCET activity was attributed to a decrease in the free cholesterol content of triglyceride-rich lipoproteins [4]. On the other hand, increased free cholesterol content of VLDL and LDL from patients with end-stage renal failure treated by haemodialysis has been previously associated with low rates of cholesteryl ester transfer [14]. Thus it seems unlikely that altered lipoprotein free cholesterol content contributed to the present decrease in plasma NCET activity in patients on dialysis treated with simvastatin.

Theoretically a decrease in serum NCET activity could be due to a decrease in CETP mass. In a previous study, therapy with an HMG CoA reductase inhibitor did not reduce plasma CETP mass in patients with familial hypercholesterolemia [2]. Thus, it is conceivable that in the present study, CETP mass was also unaffected by simvastatin treatment and did not mediate the simvastatin-induced decrease in serum NCET activity which we observed. Nevertheless, the effect of simvastatin therapy on circulating levels of CETP in patients with renal failure requires investigation.

Table 4. Serum newly synthesized cholesteryl ester transfer activity and lecithin:cholesterol acyltransferase activity in patients treated by haemodialysis or continuous ambulatory peritoneal dialysis before and after treatment with simvastatin. Means ± SD

<table>
<thead>
<tr>
<th></th>
<th>Haemodialysis</th>
<th>CAPD</th>
<th></th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 16)</td>
<td>Simvastatin (n = 16)</td>
<td>Placebo (n = 13)</td>
<td>Simvastatin (n = 8)</td>
</tr>
<tr>
<td>NCET (nmol/ml/h)</td>
<td>22.9 ± 8.2</td>
<td>25.9 ± 10.0</td>
<td>32.9 ± 9.4</td>
<td>32.7 ± 12.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>19.0 ± 8.4</td>
<td>16.8 ± 8.5</td>
<td>28.2 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>65.7 ± 17.4</td>
<td>73.3 ± 16.7</td>
<td>77.0 ± 23.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>61.0 ± 19.5</td>
<td>58.3 ± 19.9</td>
<td>73.4 ± 21.1</td>
</tr>
</tbody>
</table>

Abbreviations are as shown in Table 1.

*Significance associated with haemodialysis vs CAPD in two factor (simvastatin, type of dialysis) repeated measures analysis of variance.
The fall in serum LCAT activity that we observed in patients on dialysis treated with simvastatin conflicts with findings in a recent report [15]. Treatment with pravastatin, an HMG CoA reductase inhibitor with similar properties to simvastatin, did not affect plasma LCAT activity in patients with chronic renal failure receiving haemodialysis or CAPD [15]. The differing findings between the two studies may relate to the method used to determine LCAT activity. Nishizawa and co-workers [15] used a 'common substrate' method [16] and this activity is independent of endogenous LCAT substrate and probably reflects plasma LCAT concentration. In contrast, we used a 'self substrate' method which is influenced by the effects of both endogenous substrate levels and concentration of LCAT in serum. Thus, simvastatin therapy in patients with chronic renal failure may reduce LCAT activity by altering plasma lipoprotein levels and composition but not the concentration of the enzyme.

Abnormally low rates of plasma cholesteryl ester transfer and LCAT activity in patients with chronic renal failure treated by haemodialysis and normal levels of these variables in those treated by CAPD have been reported previously [14]. In contrast, baseline serum NCET activity in the present haemodialysis patients was not appreciably different from the activity in healthy subjects with comparable plasma lipid levels (22.1 ± 7.9, n = 27) and was higher than levels in a subgroup of these healthy subjects with plasma cholesterol < 6.5 mmol/l and plasma triglycerides < 2.0 mmol/l that we have reported in an earlier study [9]. This apparent discrepancy might be explained by differences in plasma levels of lipids and apoB-containing lipoproteins in particular, which are probably due to differences in dietary intake in patients between the studies. In the study reported by Dieplinger and co-workers [14], plasma lipid levels were normal in the CAPD patients and plasma cholesterol concentration was low (mean, 3.42 mmol/l) in the patients treated by haemodialysis. Also, body weight was low which suggests that dietary intake was restricted in these dialysis patients. In contrast, body weight and serum lipid levels and undoubtedly serum levels of apoB-containing lipoproteins were substantially higher in the corresponding groups of patients in the present study. Furthermore, serum NCET activity was markedly higher in patients with hyperlipidaemia compared with those who were normolipidaemic and serum NCET was correlated strongly with serum lipids and apoB concentrations. These data suggest that the higher rates of cholesteryl ester transfer in the dialysis patients we studied may be due to higher levels of apoB-containing lipoproteins compared with patients in the previous study [14]. ApoB-containing lipoproteins are acceptors of cholesteryl esters transferred from HDL and are an important determinant of plasma cholesteryl ester transfer rates [2,17]. The strong correlation between serum NCET activity and apoB concentration and elevated levels of these variables in patients with CAPD in the current study are similar to our previous findings [9] and suggest that accelerated cholesteryl ester transfer may be linked with hyperapobetalipoproteinaemia in patients treated by CAPD [18].

Plasma LCAT activities in the haemodialysis patients at baseline were comparable with the corresponding activities in haemodialysed uremic patients and healthy controls reported by Bories and co-workers [19] but were markedly higher than values in haemodialysed patients reported by Dieplinger and co-workers [14]. Again, restricted dietary intake and low plasma lipid levels may underlie the low plasma LCAT levels in haemodialysis patients in one of the previous studies [14]. In that study, plasma free cholesterol levels were low (mean, 1.0 mmol/l), which in the light of the close correlation between molar LCAT rate and plasma free cholesterol levels in the literature [20], may contribute to the low plasma LCAT activity in the haemodialysis patients. In addition, a low-calorie diet is reported as reducing plasma LCAT activity [21].

There are limitations to the present findings. The availability of suitably stored serum samples may have introduced bias. However, given that the serum lipid values in Table 1 are similar to those for the total study population, it is unlikely that this limitation affected the interpretation of our data. There were downward trends in serum cholesterol, LDL cholesterol, NCET activity, and LCAT activity in the placebo group, which may be due to changes in life-style and in particular diet during the study. Whether or not dialysis treatment contributed to these changes is uncertain. A previous study has reported that dialysis does not alter plasma cholesterol levels but reduces plasma apoB levels during a 2-year period of therapy [22].

Increased plasma NCET activity independent of plasma lipids and LCAT activity in patients with angiographic evidence of coronary artery disease has been documented previously [23]. Thus the reduced plasma NCET activity that we observed in dialysis patients during simvastatin therapy might conceivably be associated with reduced risk of coronary artery disease. Slow transfer of cholesteryl esters from HDL to atherogenic apoB-containing lipoproteins as well as decreased levels of these lipoproteins during simvastatin therapy may delay the premature onset of atherosclerotic disease. On the other hand it is possible that decreased movement of cholesteryl esters into apoB-containing lipoproteins that are taken up by hepatic receptors may reduce the efficiency of reverse cholesterol transport from peripheral tissues including the artery wall. There is evidence that reverse cholesterol transport may be inefficient in dialysis patients. Reversal of the normal cell to plasma cholesterol efflux between cultured fibroblasts and plasma from haemodialysis patients has been documented [14]. Also, we have reported previously that plasma levels of HDL, an effective acceptor of cell cholesterol in vitro [24], are low and are of abnormal composition in patients with chronic renal failure [25]. Furthermore, the net transfer rate of radiolabelled cholesterol from red blood cells to HDL, in vitro is abnormally low in these patients nearly all of whom were treated by dialysis...
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