Plasma phospholipid transfer protein activity in patients with low HDL and cardiovascular disease treated with simvastatin and niacin

Marian C. Cheung a,*, Gertrud Wolfbauer a, Hal Kennedy a, B. Greg Brown b, John J. Albers a

a Northwest Lipid Research Laboratories, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, School of Medicine, 2121 North 35th Street, Seattle, WA 98103, USA
b Division of Cardiology, Department of Medicine, University of Washington, School of Medicine, Seattle, WA, USA

Received 11 January 2001; received in revised form 23 May 2001; accepted 30 May 2001

Abstract

Plasma phospholipid transfer protein (PLTP) is an important modulator of high-density lipoprotein (HDL) metabolism, regulating its particle size, composition, and mass. In patients with low HDL and cardiovascular disease (CVD), plasma PLTP activity is positively correlated with the concentration of HDL particles containing apo A-I but not apo A-II (Lp(A-I)). We recently completed a study to determine the effect of simvastatin and niacin (S^N) therapy on disease progression/regression in these patients, and found that this therapy selectively increased Lp(A-I). To determine if PLTP was also increased with this drug therapy, we measured the PLTP activity in the plasma of 30 of these patients obtained at baseline and after 12 months of therapy, and compared the changes to a similar group of 31 patients who received placebo for the drugs. No significant increase in PLTP activity was observed in either group of patients. However, changes in apo A-I and A-II between these two time points were correlated with the corresponding change in PLTP activity. The correlation coefficients were \( r = 0.57 \) (\( P = 0.001 \)) and \( r = 0.43 \) (\( P = 0.02 \)) for apo A-I, and \( r = 0.54 \) (\( P = 0.002 \)) and \( r = 0.41 \) (\( P = 0.02 \)) for apo A-II in the placebo and S^N group, respectively. At baseline, PLTP activity correlated positively with the percent of plasma apo A-I associated with Lp(A-I) (\( r = 0.38, P = 0.04 \)) and the amounts of apo A-I in these particles (\( r = 0.43, P = 0.02 \)). These relationships persisted in patients who took placebo for 12 months (\( r = 0.46, P = 0.009 \) and \( r = 0.37, P = 0.04 \), respectively), but was attenuated in those treated with S^N. These data indicate that S^N-induced increase in Lp(A-I) was PLTP-independent. It also confirms our previous observation that an interrelationship exists between PLTP and apo-specific HDL particle subclasses in CVD patients with low HDL, and that this relationship is altered by drug intervention. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Phospholipid transfer protein; Cardiovascular disease; Niacin; Simvastatin

1. Introduction

Human plasma phospholipid transfer protein is a hydrophobic glycoprotein containing 476 amino acids with a predicted molecular mass of 54.7 kDa [1]. In vitro, PLTP mediates the net mass transfer of phospholipid from very low density lipoproteins (VLDL) to high-density lipoproteins (HDL) [2,3], promotes the conversion of HDL into larger and smaller particles [4,5], and generates pre-β HDL [6].
PLTP can also enhance the removal of cholesterol from cholesterol-loaded human skin fibroblasts [7], facilitate the transfer of \( \alpha \)-tocopherol from HDL or albumin to oxidized low density lipoprotein (LDL) and endothelial cells, and prevent rabbit aortic endothelium dysfunction ex vivo [8]. These observations suggest that PLTP plays a crucial role in triglyceride-rich lipoprotein and HDL metabolism, and may be anti-atherogenic. Indeed, recent studies with PLTP gene knock-out mice reveal the accumulation of abnormal lipoproteins and hyper HDL catabolism in these animals [9]. Plasma from mice over-expressing human PLTP has also been shown to contain more pre-LDL and apoA-I, and is more efficient in preventing the accumulation of intracellular cholesterol in macrophages than plasma from wild-type mice [10].

Elevated plasma LDL cholesterol (LDL-C) and a reduced level of HDL cholesterol (HDL-C) are two of the risk factors of cardiovascular disease. Many intervention trials have shown that reducing LDL-C can decrease CVD events, slow progression of atherosclerosis, or induce atherosclerosis regression [11–13]. Thus, guidelines for the prevention and treatment of CVD based on LDL-C levels have been established [14]. However, the proper management of CVD in patients who do not have elevated LDL-C but have reduced HDL-C is less clear. Epidemiological evidence suggests that a 1 mg/dl (2–3%) increment in HDL cholesterol would be associated with a significant 2–3% decrement in CVD risk [15]. Thus, we hypothesize that in CVD patients with low HDL-C, raising the HDL-C level and the HDL-C/LDL-C ratio with either lifestyle modification or drug therapy would be a potentially effective treatment strategy. Niacin and statins are the most commonly used agents for effectively raising HDL-C and lowering LDL-C, respectively. We therefore performed a clinical trial to determine the effect of simvastatin and niacin on coronary disease progression and regression in this population of patients.

Since PLTP plays an important role in HDL metabolism, and may be anti-atherogenic, and since it is not known how niacin increases HDL, we measured the PLTP activity in these patients before and after 12 months of drug therapy to determine whether these drugs regulate PLTP. As simvastatin-niacin (S–N) significantly increased HDL particles containing apolipoprotein (apo) A-I but not A-II (Lp(A-I)) [16], and as we had previously observed a strong association between baseline PLTP and Lp(A-I) in these patients [17], we hypothesized that PLTP would also increase with S–N treatment. We did not find any consistent change in PLTP activity with this combination drug therapy. However, we obtained further evidence of an interrelationship between plasma PLTP activity and apo-specific HDL particle subclass distribution and concentration in this population of patients before drug treatment, and that this relationship persisted after placebo treatment, but disappeared during drug intervention.

2. Materials and methods

2.1. Study patients

This study focuses on a subset of CVD patients who participated in a clinical trial aimed at assessing the effects of different lipid-altering strategies on coronary disease progression/regression [18]. They include all the 61 patients in the Seattle cohort randomized to take either simvastatin (10–20 mg) plus niacin (2–4 g) (S–N, \( n = 30 \)) or placebo for these drugs (\( n = 31 \)). All patients had clinical coronary disease (prior myocardial infarction, angioplasty, or positive exercise tolerance test), and all demonstrated coronary stenosis in one or more vessels by angiography. All had low HDL-C (\( \leq 0.90 \text{ mmol/l or } 35 \text{ mg/dl for men and } \leq 1.03 \text{ mmol/l or } 40 \text{ mg/dl for women} \)) and normal LDL-C (\( \leq 3.75 \text{ mmol/l or } 145 \text{ mg/dl} \)) as averaged from two visits prior to randomization. All were taught a conventional healthy lifestyle approach to increase their HDL-C. This included counseling in weight reduction, smoking cessation, and dietary counseling to reduce saturated fatty acid intake. Also, professional training in moderate exercise was provided for four months at a rehabilitation facility. The study was approved by the Human Subjects Review Committee of the University of Washington, and informed consent was obtained from all subjects before entering the study. Venous blood was drawn into EDTA-containing Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) after a 12–14-h overnight fast. Plasma was promptly sepa-
rated by low-speed centrifugation at 4°C and immediately used for lipid and apolipoprotein analysis, and for HDL particle isolation, or frozen at −70°C for PLTP activity measurement.

2.2. Lipoprotein and apolipoprotein measurements

Standard enzymatic methodologies performed on an Abbott Spectrum Bichromatic Analyzer (Irving, TX) were used to determine cholesterol, triglyceride, and phospholipid in plasma and/or fractionated lipoproteins [19,20]. Apolipoproteins (apo) A-I, A-II, and B were measured with a Behring nephelometer using Behring reagents (Behring Diagnostic, Somerville, NJ) and calibrated with the Northwest Lipid Research Laboratories calibrator. The HDL particles containing both apo A-I and A-II (Lp(A-I, A-II)) and those containing apo A-I but no A-II (Lp(A-I)) were isolated from fresh plasma samples of 29 baseline and 60 treatment samples by established sequential dextran sulfate, anti-A-II, and anti-A-I chromatography [21,22]. The distribution of apo A-I between Lp(A-I) and Lp(A-I, A-II), and the lipid composition in these particles were determined by quantifying the apo A-I, cholesterol, triglyceride, and phospholipid in these particles. HDL subpopulations were separated by size using non-denaturing gradient gel electrophoresis [23]. Based on clustering of particle sizes of healthy normolipidemic subjects, the percentage of particles within four size intervals (7.0–8.2 nm, 8.2–9.2 nm, 9.2–11.2 nm, and 11.2–17.0 nm) were used to describe the particle size profiles of Lp(A-I) and Lp(A-I, A-II) [24].

2.3. Phospholipid transfer protein activity

The plasma phospholipid transfer activity mediated by PLTP was determined by measuring the transfer of [14C]phosphatidylcholine from phospholipid liposomes to HDL using an established radio-assay [25]. Three separate dilutions of each plasma sample obtained from each patient at baseline and 12 months after drug or placebo intervention were assayed. Baseline and treatment samples from the same patient were analyzed within an assay. Aliquots from three frozen plasma samples with different PLTP activities were included in each assay to control for inter-assay variation.

2.4. Arteriography

At baseline catheterization, eight views of the left and right arteries were filmed after nitroglycerin and repeated in the follow-up angiogram 36 months after the baseline catheterization. The fully-blinded assessment of stenosis change has been described [13]. The primary arteriographic patient end-point was the mean change, from initial to final arteriogram, in percent stenosis for the worst lesion in each of the nine proximal coronary segments.

2.5. Statistical analysis

Statistical differences between patient groups were assessed using the Mann–Whitney U-test. Lipid, apolipoprotein, and PLTP activity levels of each patient at baseline and after 12 months of intervention were compared using Wilcoxon matched pair signed rank test. Spearman rank order correlation analyses were used to determine association between PLTP activity and lipoproteins. All analyses were carried out using statistical software (Stat Soft, Tulsa, OK), and P values of less than 0.05 (two-tailed) were considered significant.

3. Results

The 61 patients in this study consisted of 59 men and two women in the age range of 38–71 years (mean age 52 years). Age was not significantly correlated to PLTP activity among these patients. Also, PLTP activity did not differ significantly between diabetics and non-diabetics, smokers and non-smokers, obese (body mass index (BMI) > 27 kg/m²) and non-obese patients, and those with and without high blood pressure, although the sample size for diabetics (n = 7) and smokers (n = 14) was relatively small. In contrast, the majority of the patients (41 out of 60) were overweight with a BMI over 27 kg/m². Neither baseline BMI nor mean proximal percent stenosis was significantly correlated with PLTP activity in these patients. Also, neither baseline PLTP activity nor PLTP activity measured at 12 months after treatment was correlated with the change in atherosclerotic disease progression/regression. Changes in PLTP activity also did not correlate with disease progres-
sion/regression. Baseline characteristics, including coronary risk factors, lipids, lipoproteins and PLTP activity, did not differ significantly between the patient group taking placebo and that taking simvastatin and niacin (Tables 1 and 2).

Combination S-N treatment significantly decreased total cholesterol and triglyceride and LDL-C by 24–39%, and increased both HDL2-C (42%) and HDL3-C (27%). Consistent with these lipid changes, plasma apo B decreased 33% while plasma apo A-I increased 14%. When the HDL particles in a subset of these patients in each group were isolated and analyzed, only the apo A-I in Lp(A-I) but not Lp(A-I, A-II) was increased (Table 2). Despite these changes in plasma lipoproteins, no consistent change in PLTP activity was detected in either the placebo group or the treatment group. Thus S-N selectively increased Lp(A-I) without affecting PLTP. However, when changes in PLTP and plasma lipid and apolipoproteins between the baseline and treatment samples of each patient were analyzed, changes in apo A-I and A-II between these two time points were correlated with the corresponding difference in PLTP activity (Fig. 1). The correlation coefficients were \( r = 0.570, P = 0.001 \) and \( r = 0.428, P = 0.018 \) between PLTP and apo A-I, and \( r = 0.535, P = 0.002 \) and \( r = 0.411, P = 0.024 \) between PLTP and apo A-II in the placebo and S-N group, respectively.

### Table 1
Baseline characteristics of study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (n = 31)</th>
<th>Simvastatin+niac (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>51.9</td>
<td>52.3</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>100.0</td>
<td>93.3</td>
</tr>
<tr>
<td>High blood pressure (%)</td>
<td>54.8</td>
<td>36.7</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>22.6</td>
<td>23.3</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>17.4</td>
<td>18.6</td>
</tr>
<tr>
<td>Diabetes (Type II) (%)</td>
<td>16.1(^{a})</td>
<td>6.7(^{b})</td>
</tr>
<tr>
<td>Body mass index (kg/cm(^2))</td>
<td>29.9</td>
<td>29.4</td>
</tr>
<tr>
<td>Previous myocardial infarction (%)</td>
<td>48.4</td>
<td>66.7</td>
</tr>
<tr>
<td>Mean proximal % stenosis</td>
<td>34.2</td>
<td>33.6</td>
</tr>
</tbody>
</table>

\(^{a}\)Four of the five diabetics had elevated triglycerides (\( > 1.7 \text{ mmol/l} \)).
\(^{b}\)One of the two diabetics had elevated triglycerides.

### Table 2
Baseline lipoprotein and PLTP activity level and 12-month treatment changes

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 31)</th>
<th>S-N (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.D. (mg/dl)</td>
<td>Median (% change)</td>
</tr>
<tr>
<td>Plasma CH</td>
<td>198 ± 35</td>
<td>−4.9</td>
</tr>
<tr>
<td>Plasma TG</td>
<td>209 ± 112</td>
<td>−3.0</td>
</tr>
<tr>
<td>LDL CH</td>
<td>126 ± 28</td>
<td>−4.9</td>
</tr>
<tr>
<td>HDL CH</td>
<td>32 ± 6</td>
<td>6.7*</td>
</tr>
<tr>
<td>HDL2 CH</td>
<td>4 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>HDL3 CH</td>
<td>28 ± 5</td>
<td>10.0**</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>109 ± 14</td>
<td>4.0</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>29 ± 5</td>
<td>1.1</td>
</tr>
<tr>
<td>Apo B</td>
<td>118 ± 24</td>
<td>−8.2</td>
</tr>
<tr>
<td>PLTP activity(^{a})</td>
<td>15 ± 2</td>
<td>0.3</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I)(^{b})</td>
<td>23 ± 5</td>
<td>0.4</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I, A-II)(^{b})</td>
<td>90 ± 6</td>
<td>4.0</td>
</tr>
</tbody>
</table>

\(^{a}\)*, **, *** denote changes between treatment and baseline statistically significant at \( P \leq 0.05, 0.01, \) and 0.001, respectively.
\(^{b}\)PLTP activity expressed in \( \mu \text{mol/ml} \) per hour.
\(^{b}\)Values based on \( n \) of 13 in the placebo group and \( n \) of 16 in the S-N group.
At baseline, the HDL particles with and without apo A-II were separated and quantified in a consecutive subset of 13 plasma samples from the placebo group and 16 from the S–N-treated group. There was a positive correlation between PLTP activity and the percent of plasma apo A-I associated with Lp(A-I) ($r = 0.381, P = 0.041$), and the concentration of apo A-I, cholesterol, and phospholipid in these particles ($r = 0.429, P = 0.020$; $r = 0.488, P = 0.007$; and $r = 0.438, P = 0.018$, respectively) (Table 3). Corresponding relationships were not seen with Lp(A-I, A-II). When a similar analysis was performed on

![Fig. 1. Correlation between change in plasma PLTP activity and changes in plasma apo A-I (A,B) and apo A-II (C,D) in CVD patients treated with placebo (A,C), and combination simvastatin-niacin (B,D).](image)

Table 3  
Inter-relationship between PLTP activity and apo-specific HDL particles at baseline and after 12 months of treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($n=29$)</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r_s$</td>
<td>$P$</td>
</tr>
<tr>
<td>% Plasma A-I in Lp(A-I)</td>
<td>0.38</td>
<td>0.041</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I)</td>
<td>0.43</td>
<td>0.020</td>
</tr>
<tr>
<td>Cholesterol in Lp(A-I)</td>
<td>0.49</td>
<td>0.007</td>
</tr>
<tr>
<td>Phospholipid in Lp(A-I)</td>
<td>0.44</td>
<td>0.018</td>
</tr>
<tr>
<td>Triglyceride in Lp(A-I)</td>
<td>0.07</td>
<td>0.726</td>
</tr>
<tr>
<td>Apo A-I in Lp(A-I, A-II)</td>
<td>0.02</td>
<td>0.917</td>
</tr>
<tr>
<td>Cholesterol in Lp(A-I, A-II)</td>
<td>0.32</td>
<td>0.085</td>
</tr>
<tr>
<td>Phospholipid in Lp(A-I, A-II)</td>
<td>0.09</td>
<td>0.642</td>
</tr>
<tr>
<td>Triglyceride in Lp(A-I, A-II)</td>
<td>−0.16</td>
<td>0.423</td>
</tr>
</tbody>
</table>

*Baseline values based on $n$ of 13 in the placebo group and $n$ of 16 in the S–N group.*
the HDL particles isolated from the 12-month samples from the 31 placebo-treated patients and 29 S-N-treated patients, a comparable significant correlation was seen in the placebo group but not in the S-N group (Table 3). Hence, under normal physiological conditions, plasma Lp(A-I) level and PLTP activity in CVD patients with low HDL are interrelated. This relationship was attenuated by S-N treatment.

As PLTP promotes the conversion of HDL into larger and smaller particles, the correlation between PLTP activity and HDL size distribution was also studied. PLTP activity at baseline was not correlated with the proportion of Lp(A-I) or Lp(A-I, A-II) particles in any of the four HDL size intervals (7.0–8.2 nm, 8.2–9.2 nm, 9.2–11.2 nm, or 11.2–17.0 nm, respectively). The percentage of Lp(A-I) particles in the 7.0–8.2 nm range (30.3 ± 7.1%) and the 11.2–17.0 nm range (16.9 ± 4.6%) decreased significantly to 24.7 ± 7.7% (P = 0.027) and 14.8 ± 5.2% (P = 0.036), respectively, after 12 months on simvastatin and niacin treatment. In contrast, the proportion of particles in the 9.2–11.2 nm range increased substantially from 21.5 ± 5.2% to 28.8 ± 9.8% (P = 0.003), while the percentage of particles in the 8.2–9.2 nm range did not change. For Lp(A-I, A-II), the percentage of particles in the 7.0–8.2 nm range decreased from 42.8 ± 8% to 34.7 ± 6.6% (P = 0.001), whereas the particles in the 8.2–9.2 nm (42.5 ± 6.4%) and particularly those in the 9.2–11.2 nm (10.2 ± 2.1%) ranges increased to 46.7 ± 5.2% (P = 0.036) and 13.4 ± 3.8% (P = 0.001), respectively. However, the change in the proportion of Lp(A-I) or Lp(A-I, A-II) particles in any of the four HDL size intervals was not significantly correlated with the change in PLTP activity.

4. Discussion

In an earlier study, we found that among all plasma lipid and apolipoprotein parameters, plasma PLTP activity is most strongly correlated with the percent of total plasma apo A-I associated with Lp(A-I), and to the concentration of these particles in a population of CVD patients with low HDL-C [17]. The patients in that study were the first 52 consecutive patients randomized to participate in a clinical trial aimed at assessing the effects of different lipid-altering strategies on CVD progression/regression. A total of 153 patients (129 from Seattle, USA and 24 from Vancouver, Canada) in four treatment groups have completed the study. As part of the protocol of that study, apo-specific HDL particles were isolated and characterized in the first 60 baseline blood samples, and all samples obtained at 12 months after randomization. Of the four treatment groups, we found that only those who received simvastatin and niacin demonstrated a significant selective increase in Lp(A-I) particles. Since PLTP activity was strongly associated with Lp(A-I) in the baseline samples of these patients, and since existing evidence suggests that PLTP plays an important role in regulating HDL particle concentration, size, and composition [4,5,9,26–32], we hypothesized that S-N treatment also increases PLTP. This study focused therefore only on the group of patients who received S-N treatment and those who received placebo for the drugs. Our data show that despite a median increase of 64% in the apo A-I in Lp(A-I), no consistent change in PLTP was detected in response to S-N treatment. This suggests that the S-N-induced increase of Lp(A-I) was not related to PLTP. Our data are consistent with an earlier report which showed that simvastatin at 20 mg/day did not change PLTP activity in patients with type IIb hyperlipidemia [33]. We now show that the combination of simvastatin and niacin also has no effect on PLTP activity.

Although no consistent change in PLTP activity was observed in either the S-N- or the placebo-treated groups, variation in PLTP activity was seen between the baseline and treatment samples. When changes in PLTP activity were analyzed with the corresponding lipid and apolipoprotein changes in each patient between these two time points, we found that changes in PLTP activity were highly correlated with changes in apo A-I and A-II. This suggests that although PLTP is not a major contributor to the increase of Lp(A-I) in patients treated with S-N, it does play a modulating role in apo A-I and A-II metabolism in vivo, as between 17% and 32% of variation in plasma apo A-I and A-II could be explained by the variation in PLTP activity in this patient population.

Our previous report shows that PLTP activity was
positively correlated with plasma Lp(A-I) in 52 baseline samples from the patients who participated in this clinical trial. The baseline data in Table 3 is a subset of that data, and is consistent with the original data set. Of particular interest is the new observation that this relationship persisted after 12 months of placebo treatment, but was attenuated in those treated with S-N, confirming not only our previous study, but providing further evidence for an interrelationship between PLTP and Lp(A-I) under steady metabolic state. Also, such a relationship was perturbed by S-N. The observation that PLTP activity is associated with the partition of plasma apo A-I between HDL particles with and without apo A-II suggests a role of PLTP in regulating apo-specific HDL subclass distribution. It is not clear why plasma PLTP is positively correlated with Lp(A-I) and its components but not with Lp(A-I, A-II). Even though we have previously shown that PLTP promotes the conversion of both Lp(A-I) and Lp(A-I, A-II) to larger and smaller particles in vitro [26], it is still possible that PLTP preferentially interacts with Lp(A-I) in vivo. It should be noted that Tahvanainen et al. [34] reported that serum PLTP activity correlated negatively with the concentration of apo A-I in Lp(A-I) particles in healthy Finns. The discrepancy between our observation and Tahvanainen’s report is unclear, but may be related in part to the differences in the study populations.

PLTP mass concentration and plasma phospholipid transfer activity have been demonstrated to be elevated in patients with non-insulin-dependent diabetes mellitus [35]. Our failure to detect significant differences in phospholipid transfer activity between diabetics and non-diabetics most likely relates to the relatively small number of diabetics (n = 7) in our study population. Furthermore, the proportion of non-diabetic patients with elevated triglyceride (38 of 54 or 70%) was similar to that observed for the diabetic group (5 of 7 or 71%).

In conclusion, we have demonstrated that in CVD patients with low HDL-C, simvastatin and niacin significantly raise Lp(A-I) levels without any consistent effect on PLTP activity. We have also provided evidence of the role of PLTP in regulating apo-specific HDL particle subclass distribution, and that under normal physiological conditions, an inter-relationship exists between plasma PLTP activity and the concentration of Lp(A-I). Furthermore, drug intervention can attenuate this relationship.

Acknowledgements

This study was supported by Grants HL30086 and HL49546 from the National Institutes of Health.

References


