The puzzle of high-density lipoprotein in cardiovascular prevention

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Consequences of cholesteryl ester transfer protein inhibition in patients with familial hypoalphalipoproteinemia

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A large proportion of clinical events cannot be prevented during statin therapy, which calls for novel drug targets to further improve cardiovascular outcome. In particular, HDL-increasing strategies hold great promise. The impact of decreased HDL cholesterol on cardiovascular disease (CVD)-related morbidity and mortality has been sharply delineated in individuals affected by familial hypo-alphalipoproteinemia (FHA)\(^1\). HDL exerts multiple anti-atherogenic actions beyond its role in reverse cholesterol transport, comprising anti-inflammatory, anti-oxidative and direct vascular effects\(^2\). Whereas current strategies to raise HDL cholesterol are limited, novel CETP-inhibitors are capable of mediating significant HDL cholesterol elevation\(^3,4\). Therefore, we evaluated the effects of CETP inhibition on lipid metabolism and markers of oxidation in subjects with FHA.

Subjects were recruited from a Dutch population-based study to identify genes that control HDL cholesterol levels\(^1\), meeting the following criteria: (1) plasma HDL cholesterol level below 10\(^{th}\) percentile for age and sex; (2) absence of secondary lipid disorders; and (3) high likelihood of inherited low HDL (defined as HDL cholesterol below 10\(^{th}\) percentile in at least one first-degree family member). Nineteen FHA patients (13 men and 6 women; mean \(±\)SD age: 42.9 \(±\) 13.9 years), all free of overt macrovascular disease, were enrolled in the study. In 9 of these subjects the underlying defect was defined: heterozygosity for an apolipoprotein A-I (L178P) mutation\(^1\), whereas in the remainder this genetic defect was excluded. The study protocol was approved by the Institutional Review Board at the Academic Medical Center, University Hospital of Amsterdam. All subjects gave written informed consent. This study was designed as a single-center, randomized-sequence, double-blind, cross-over study with a 4-week treatment period of JTT-705 600 mg and placebo, respectively.

Lipid parameters were measured by routine methods. Serial ultracentrifugation was used to determine serum HDL subfractions. Lipoprotein profiling was performed by proton nuclear magnetic resonance spectroscopy\(^5\). FPLC was used to separate VLDL, HDL and LDL fractions. In individual fractions, total cholesterol (TC), free cholesterol (FC), triglyceride and phospholipids (PL) concentrations were measured. CETP activity and concentration\(^6\), circulating oxidized LDL (oxLDL) antibodies\(^7\) and serum paraoxonase activity\(^8\) were determined, as described previously. Data are expressed as mean \(±\) SD. A paired \(t\) test or Wilcoxon Signed rank test was used, depending on distribution of the tested parameter. A probability value of \(\leq 0.05\) was considered significant.

At baseline, HDL cholesterol and apoA-I levels in FHA patients (Table 1) were below 10\(^{th}\) percentile. The number of HDL particles was also decreased compared to reference values derived from the Framingham Offspring study (FOS) (20.7 \(±\) 7.9 \(\mu\)mol/L versus mean reference values of 33 \(±\) 6 \(\mu\)mol/L\(^5\)). Whereas LDL cholesterol levels were within the normal range, the number of LDL particles was increased (1912 \(±\) 691 nmol/L; reference values\(^5\): men 1535 \(±\) 406, women 1370 \(±\) 427 nmol/l), particularly due to a higher number of very small LDL particles (978 \(±\) 717 nmol/l; reference values\(^5\): men 479 \(±\) 391, women 267 \(±\) 261 nmol/l). Plasma triglyceride levels (Table 1) were within reference ranges. Treatment with JTT-705 was associated with a
24% decrease in CETP activity, with a concomitant 126% increase in CETP mass (Table 1). JTT-705 increased the levels of HDL cholesterol and apoA-I by 19% and 14%, respectively (Table 1). The increase in the HDL₂ fraction (42%) clearly exceeded the increase in the HDL₃ fraction (Table 1). Using FPLC, JTT-705 was shown to result in an elevation of HDL TC of 24% (p=0.01). NMR analysis showed a significant increase in the HDL particle number from 20.7 ± 7.9 to 23.6 ± 7.2 μmol/L (p=0.008). Particularly within the large HDL subclass, particle number rose from 2.2±1.4 to 3.5±1.9 μmol/L (p=0.004). Total LDL cholesterol did not change during JTT-705 treatment (Table 1). Using FPLC, JTT-705 was associated with a 19% decrease in triglycerides content in the LDL fraction (p=0.003). LDL subclass analysis with NMR showed that JTT-705 reduced the total number of LDL particles from 1912±691 to 1610±468 nmol/L (p=0.01) with a concomitant reduction in very small LDL particles (from 978±716 to 733±487, p=0.05).

OxLDL antibodies showed a modest decrease of 7% (from 10180±3584 to 9485±3576 RLU/msec; p=0.002) during JTT-705, compared to baseline. Together with the HDL cholesterol and apoA-I increase, JTT-705 also induced a slight, but significant increase in serum paraoxonase-1 activity (95.9±91.1 to 105.4±103.7 U/L; p=0.04).

The precise role of CETP activity in the course of atherogenesis has been a matter of debate. In the present study, CETP inhibition in FHA individuals is associated with favourable effects on the HDL and LDL subfractions with concomitantly enhanced plasma anti-oxidant capacity, involving reduced oxLDL-autoantibodies and enhanced serum paraoxonase-1 activity. In detail, JTT-705 preferentially increased the number of large HDL particles to a level comparable to that reported in healthy, normolipidemic controls in the Framingham Offspring study. The clinical relevance of this finding is underscored by observations that particularly larger, CE-rich HDL particles show a strong, inverse relationship with CVD risk. In line with Brousseau, we observe an increase in cholesterol content within the HDL particle upon JTT-705 treatment, which is likely to contribute to increased plasma residence time of the HDL particle.

With respect to LDL, we find increased levels of atherogenic, very small LDL particles in FHA subjects, in the absence of elevated triglycerides levels. Apparently, in case of very low HDL cholesterol levels, LDL cholesterol takes over as the principle cholesterol ester donor for triglyceride rich particles. In line, particle concentration of small LDL diminishes significantly upon JTT-705 treatment.

Regarding the oxidative status, JTT-705 resulted in a significant reduction of autoantibody levels against oxLDL. Since circulating oxLDL-autoantibodies are thought to reflect LDL oxidation, this may imply that the decreased number of oxidation-prone, very small LDL particles translate into reduced LDL oxidation rates. In parallel, JTT-705 was also associated with an increase in serum paraoxonase-1 activity, most likely reflecting delayed HDL apoA-I catabolism, thus further enabling HDL to attenuate oxidative modification of the LDL particle.

A limitation of the current study was the presence of a carry-over phenomenon for several parameters of interest, likely due to the lack of a washout between treatment arms. Therefore,
we only compared the JTT-705 period to the baseline observations, rather than with the placebo period.

In summary, we provide evidence in patients with familial hypoalphalipoproteinemia, that even modest CETP inhibition confers beneficial effects beyond its well recognized HDL cholesterol increasing action, including a reduced number of small LDL particles as well as augmentation of the plasma anti-oxidant capacity. Since these effects occurred at a JTT-705 dose associated with a modest 24% CETP inhibition, more profound changes can be expected upon further optimization of the regimen\textsuperscript{3,4}. Taken together, these findings suggest an anti-atherogenic effect of CETP-inhibition in FHA patients. The results of ongoing trials evaluating the effect of CETP inhibition on hard cardiovascular endpoints are to be awaited to corroborate the impact of these beneficial changes on cardiovascular outcome.

**Table 1.** CETP mass and activity, lipoproteins and apolipoproteins A-I and B at baseline and during placebo and JTT-705 treatment periods

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Placebo</th>
<th>JTT-705</th>
<th>% change Baseline-JTT-705</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CETP Mass, μg/mL</td>
<td>1.9 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>4.3 ± 1.5</td>
<td>126.4±47.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CETP Activity, % of control</td>
<td>83.9 ± 20.5</td>
<td>88.3 ± 18.9</td>
<td>61.6 ± 17.7</td>
<td>-24.4±24.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total Cholesterol, mg/dL</td>
<td>204.3 ± 35.5</td>
<td>204.8 ± 37.5</td>
<td>203.3 ± 38.2</td>
<td>0.3±9.9</td>
<td>0.90</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>30.3 ± 8.5</td>
<td>28.7 ± 8.4</td>
<td>35.5 ± 11.0</td>
<td>19.3±29.6</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>139.1 ± 26.4</td>
<td>138.8 ± 27.7</td>
<td>136.8 ± 33.8</td>
<td>-1.1±15.1</td>
<td>0.77</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>177.8 ± 110.0</td>
<td>184.5 ± 135.9</td>
<td>159.8 ± 134.3</td>
<td>-13.4±16.3</td>
<td>0.74</td>
</tr>
<tr>
<td>HDL Subfraction 2, mg/dL</td>
<td>10.5 ± 3.4</td>
<td>9.5 ± 4.2</td>
<td>13.0 ± 6.6</td>
<td>42.0±61.6</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL Subfraction 3, mg/dL</td>
<td>21.2 ± 5.3</td>
<td>19.0 ± 5.7</td>
<td>22.5 ± 6.2</td>
<td>17.6±27.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Apolipoprotein B, mg/dL</td>
<td>143.1 ± 34.1</td>
<td>146.8 ± 36.1</td>
<td>135.9 ± 34.3</td>
<td>-4.6±12.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Apolipoprotein A-I, mg/dL</td>
<td>103.9 ± 32.8</td>
<td>100.3 ± 30.9</td>
<td>113.3 ± 32.2</td>
<td>14.3±24.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum PARAOXONASE activity, U/L</td>
<td>95.9 ± 91.1</td>
<td>71.6 ± 55.3</td>
<td>105.4 ± 103.7</td>
<td>6.4±14.5</td>
<td>0.04</td>
</tr>
<tr>
<td>OxLDL antibodies (IgG + IgM), RLU/ msec</td>
<td>10180 ± 3584</td>
<td>9663 ± 2932</td>
<td>9185 ± 3576</td>
<td>-9.7±16.0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

All values are given as Mean ± SD.
P-value based on baseline-JTT comparison
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