Consequences of Cholesteryl Ester Transfer Protein Inhibition in Patients With Familial Hypoalphalipoproteinemia

To the Editor:

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-creasing intervene in reverse cholesterol transport, comprising antiinflammatory, antioxidative, and direct vascular effects.2 Whereas current strategies to raise HDL-C are limited, novel CETP-inhibitors hold great promise. The impact of decreased HDL-C on cardiovas-

Subjects were recruited from a Dutch population-based study to identify genes that control HDL-C levels,1 meeting the following criteria: (1) plasma HDL-C level below 10th percentile for age and sex; (2) absence of secondary lipid disorders; and (3) high likelihood of inherited low HDL (defined as HDL-C below 10th 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,3 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, crossover 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 Fast protein liquid (FPLC) was used to separate VLDL, HDL, and LDL fractions. In individual fractions, total cholesterol (TC), free cholesterol (FC), triglyceride (TG), and phospholipids (PL) concentrations were measured. CETP activity and concentration, circulating oxidized LDL (oxLDL) antibodies,7 and serum paraoxonase (PON) activity8 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 ≤0.05 was considered significant.

At baseline, HDL-C and apoA-I levels in FHA patients (Table) were below 10th percentile. The number of HDL particles was also decreased compared with reference values derived from the Framingham Offspring study (FOS) (20.7±7.9 μmol/L versus mean reference values of 33±6 μmol/L).9 Whereas LDL-C levels were within the normal range, the number of LDL particles was increased (1912±691 nmol/L; reference values: men 1535±406, women 1370±427 nmol/L), particularly because of a higher number of very small LDL particles (978±717 nmol/L; reference values: men 479±391, women 267±261 nmol/L). Plasma triglyceride levels (Table) 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). JTT-705 increased the levels of HDL-C and apoA-I by 19% and 14%, respectively (Table). The increase in the HDL2 fraction (42%) clearly exceeded the increase in the HDL3 fraction (Table). 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-C did not change during JTT-705 treatment (Table). Using FPLC, JTT-705 was associated with a 165% decrease in TG 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 IgM antibodies showed a modest decrease of 7% (from 8148±3449 to 7293±3601 RLU/100 ms; P=0.001) during JTT-705, compared with baseline. Together with the HDL-C and apoA-I increase, JTT-705 also induced a slight but significant increase in serum PON-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.9 In the present study, CETP inhibition in FHA individuals is associated with favorable effects on the HDL and LDL subfractions with concomitantly enhanced plasma antioxidative capacity, involving reduced oxLDL-autoantibodies and enhanced serum PON-1 activity. In detail, JTT-705 preferentially increased the number of large HDL particles to a level comparable to normal young women.

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-</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-C, 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-C, 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 PON activity, U/L</td>
<td>95.9±91.1</td>
<td>71.6±55.3</td>
<td>105.4±103.7</td>
<td>0.4±14.5</td>
<td>0.04</td>
</tr>
<tr>
<td>OxLDL antibodies (lgM), RLU/100 msec</td>
<td>8148±3449</td>
<td>7601±2924</td>
<td>7293±3601</td>
<td>-11.2±19.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are given as Mean±SD.
P value based on baseline-JTT comparison.
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 cholesteryl content within the HDL particle on 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 TG levels. Apparently, in case of very low HDL-C levels, LDL-C takes over as the principle cholesteryl donor for triglyceride-rich particles. In line, particle concentration of small LDL diminishes significantly on JTT-705 treatment.

Regarding the oxidative status, JTT-705 resulted in a significant reduction of autoantibody levels against oxLDL. Because circulating oxLDL-autoantibodies are thought to reflect LDL oxidation, this may imply that the decreased number of oxidation-prone, very small LDL particles translates into reduced LDL oxidation rates. In parallel, JTT-705 was also associated with an increase in serum PON-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 because of 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 hypobetalipoproteinemia that even modest CETP inhibition confers beneficial effects beyond its well recognized HDL-C increasing action, including a reduced number of small LDL particles as well as augmentation of the plasma antioxidant capacity. Because these effects occurred at a JTT-705 dose associated with a modest 24% CETP inhibition, more profound changes can be expected on further optimization of the regimen. Taken together, these findings suggest an antatherogenic 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.

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