High-density lipoprotein and C-reactive protein, friend and foe in cardiovascular disease
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Citation for published version (APA):
Chapter 6

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

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Abstract

Background Whereas current strategies to raise HDL-C are limited, novel cholesteryl ester transfer protein (CETP)-inhibitors are capable of mediating significant HDL-C elevation. Therefore, we evaluated the effects of CETP inhibition on lipid metabolism and markers of oxidation in subjects with familial hypoalphalipoproteinemia (FHA).

Methods Using a blinded cross-over design, 19 patients (mean HDL-C= 30.3 mg/dL) were treated with 600 mg JTT-705 or placebo for 4 weeks. Lipoprotein subclass concentration (NMR) and composition (FPLC), serum PON1-activity and circulating oxidized LDL markers were assessed at baseline and during JTT-705 treatment.

Results CETP-inhibition resulted in a 19% increase of HDL-C levels (p=0.01), as well as an enlargement (8.5 to 8.8 nm, p<0.001) and 24% cholesterol enrichment (30.2±10.6 to 37.4±16.6 mg/dL; p=0.01) of the HDL particle. Upon CETP inhibition the total number of LDL particles declined (1911.9±690.6 to 1609.9±468.4 nmol/L; p=0.01), particularly very small LDL particles. Concomitantly, serum PON1-activity increased (95.9±91.1 to 105.4±103.7 U/L; p=0.04), where OxLDL IgM antibodies showed a modest decrease of 11% (from 8148±3449 to 7293±3601 RLU/100 ms; P<0.001) during JTT-705, compared with baseline.

Conclusion In patients with FHA we provide evidence that even modest CETP inhibition confers beneficial effects beyond its well recognized HDL-C increasing action, including a reduced number of the smallest LDL particles as well as augmentation of the plasma antioxidant capacity. The results of ongoing trials evaluating the effect of CETP inhibition on hard cardiovascular end points are to be awaited to corroborate the impact of these beneficial changes on cardiovascular outcome.
Introduction

Statins have been proven to reduce clinical event rates, based predominantly on their LDL cholesterol (LDL-C) lowering capacity. However, the vast majority of cardiovascular events cannot be prevented with these drugs\(^1\) and, consequently, novel drug targets are sought to further improve the therapeutic arsenal. Strategies aimed at increasing high density lipoprotein cholesterol (HDL-C) hold great promise\(^2\).

The strong, inverse relationship between plasma HDL-C concentration and the risk of cardiovascular disease (CVD) has been a consistent finding throughout numerous epidemiological studies\(^3;4\). It has been recognized that the anti-atherogenic effect of HDL is not only confined to its established role in the reverse cholesterol transport pathway\(^5\), but also includes anti-inflammatory, anti-oxidative and direct vascular effects\(^6;7\). The impact of decreased HDL-C on CVD related morbidity and mortality has been illustrated in subjects with familial hypoalphalipoproteinemia (FHA). Carriers of mutations in the genes encoding apolipoprotein A-I (apoA-I), ATP binding cassette Al (ABCA1) and lecithin:cholesteryl acyltransferase (LCAT), all underlying FHA, have invariably been shown to be at increased risk for atherosclerosis\(^8\).

Current strategies to raise HDL-C are limited to fibrates and nicotinic acid derivatives. However, potential drug-drug interactions in combination regimes with statins as well as frequent side-effects, particularly with the use of niacin, have precluded large-scale prescription of these compounds\(^9\). Other strategies include the use of gene therapy and apoA-I mimetic peptides, but these are at early stages of development\(^2\). With this in mind, CETP-inhibitors, an emerging class of HDL-C increasing drugs that are currently being tested in phase III clinical trials, are of special interest. CETP, produced by the liver and adipose tissue, circulates in plasma in association with HDL particles. Its main function is considered facilitation of the exchange of cholesteryl esters (CE) and triglycerides (TG) between plasma lipoproteins\(^10\).

To date, the precise role of CETP in the course of atherogenesis remains poorly understood. Previous reports have demonstrated that CETP can be pro-atherogenic, since CETP may actively contribute to the formation of small, dense LDL and HDL particles through sequential activities of CETP and hepatic lipase (HL) under hypertriglyceridemic conditions\(^11;12\). Further, inhibition of CETP has been shown to result in increased HDL-C levels in normolipidemic subjects\(^13;14\). Ongoing trials with CETP inhibitors evaluating validated endpoints for cardiovascular disease will provide the final answer whether CETP is a foe or a friend\(^10\).

In the present study, we evaluated the effects of CETP inhibition on the lipid metabolism and markers of oxidation status in individuals with familial hypoalphalipoproteinemia.
Methods

Study population
Subjects were recruited from a Dutch population-based study to identify genes that control HDL-C levels, 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), 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. Lipids and (apo)lipoproteins in carriers of this defect have been described in detail previously. In the remainder (n=10) this genetic defect was excluded. Subjects were excluded in case of child-bearing potential, breast-feeding, significant co-morbidity (ie. renal, hepatic, gastro-intestinal, or endocrine disease), malignancy, diabetes mellitus, chronic inflammatory disease, alcohol abuse, body mass index ≥ 35 kg/m², or hepatic transaminases >1.5 ULN during screening. Lipid-lowering medication, if used, was discontinued at least 6 weeks before the study. Other medication, not affecting the lipid metabolism in any way, remained unaltered throughout the study. 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.

Study design
This study was designed as a single-center, randomized-sequence, double-blind, cross-over study. At study visit 1, all baseline parameters were evaluated. Subsequently, patients were randomized to receive either JTT-705 600 mg or placebo once daily for a period of 4-weeks. Subsequently, patients were crossed over to the alternate treatment for another 4 weeks without washout period. Safety evaluation was conducted on a monthly basis during both treatment modalities.

Biochemical analyses
Blood samples were obtained from the participants after an overnight fast at baseline, as well as 4 and 8 weeks after randomization. All samples were processed immediately by centrifugation at 1700g for 15 minutes at 20°C and stored at -80°C. Samples for safety monitoring, basic lipoprotein analysis and CETP measurements were shipped to the central laboratory of CRL Europe in Belgium. Total cholesterol, LDL-C and triglycerides were measured by established enzymatic methods (Reagents Boehringer Mannheim and Technicon USA). HDL cholesterol was determined as published previously. LDL cholesterol was calculated by the Friedewald formula. Serial ultracentrifugation was used to determine serum HDL subfractions. Apolipoproteins were measured using established immunonephelometric methods (reagents Dade Behring). CETP activity and concentrations were measured, as described previously.
Nuclear magnetic resonance spectroscopy
Lipoprotein subclass particle concentrations and particle diameters were determined by proton nuclear magnetic resonance spectroscopy, as previously described \(^\text{20}\). In the current study, subclasses of lipoproteins were grouped into the following categories: 3 VLDL subclasses (large: > 60 nm; intermediate, 35-60 nm; small, 27-35 nm), IDL (23-27 nm), 2 LDL subclasses (large LDL, 21.2-23 nm; small, 18-21.2 (medium small, 19.8-21.2 nm and very small, 18-19.8 nm), and 3 HDL subclasses (large, 8.8-13 nm; intermediate, 8.2-8.8 nm; small HDL, 7.3-8.2 nm).

Lipoprotein composition
FPLC was used to separate VLDL, HDL and LDL fractions. In these fractions, we determined total cholesterol (TC), free cholesterol (FC), triglyceride (TG) and phospholipids (PL) concentrations. FC was converted to delta-cholestenone by cholesterol oxidase, releasing hydrogen peroxides that dose dependently convert homovanilinic-acid in a metabolite, which can be measured fluorescently on line (Exitation wavelength 320 nm/ Emission wavelength 450 nm). All reagents were from Boehringer (Ingelheim, Germany) and Roche (Nutley, NJ, USA) except for the homovanilinic acid (Sigma Aldrich, Zwijndrecht, The Netherlands). The TC content was converted by an oxidase and peroxidase reaction to delta-cholestenone, whereas the released hydrogen peroxides caused phenol and 4-aminoantipyrine to undergo quantitatively an oxidative condensation in the presence of peroxidase. The resulting metabolite was measured colorimetrically at 505 nm. Here, reagents were from Biomerieux (Marcy-l’Etoile, France). Using the Triglycerides Enzymatic PAP 100 kit (Biomerieux), TG were converted in a 4-step enzymatic reaction into a quinoneimine compound which were measured colorimetrically at 505 nm. PL (lecithin, lyssolecithin and sphingomyelin) were hydrolyzed by phospholipase D and the liberated choline is measured by the TRINDER reaction. Subsequently, the generated quinoneimine compounds are quantified colorimetrically at 505 nm. Reagents used were derived from the Phospholipids B kit (WAKO Chemicals, Germany). CE concentrations were calculated by subtraction of free cholesterol from the total cholesterol concentration.

OxLDL autoantibody titers and Paraoxonase-1 activity
Circulating markers of oxidized LDL (oxLDL) in plasma were determined as described previously \(^\text{21}\). Paraoxonase-1 activity was assessed as the rate of hydrolysis of paraoxon, as previously described \(^\text{22}\). Paraoxonase activity is expressed as units per liter of serum, where 1 unit equals 1 μmol of paraoxon hydrolyzed per minute.

Statistical analysis
Data are expressed as mean ± SD. Statistical analysis (SAS System for Windows, Version 8.2, SAS institute) of the data was performed to compare the treatment to baseline period or placebo to baseline period. A paired \(t\) test or Wilcoxon Signed rank test was used, depending on distribution of the tested parameter. Because of the relatively small number of participants,
the Shapiro-Wilk goodness-of-fit test was used to assess normal distribution of the values of the measured parameters. A probability value of ≤0.05 was considered significant.

Results

Baseline characteristics
At baseline, HDL-C and apoA-I levels in FHA patients were below 10th percentile (table 1). The number of HDL particles was also decreased compared to references values derived from the Framingham Offspring study (FOS) (20.7 ± 7.9 μmol/L; reference values 33 ± 6 μmol/L). LDL-C levels were within the normal range (table 1). However, the number of LDL particles was increased compared to reference values (1911.9 ± 690.6 nmol/L; reference values: men 1535 ± 406, women 1370 ± 427 nmol/l), particularly due to a higher number of very small LDL particles (978.4 ± 716.5 nmol/l; reference values: men 479 ± 391, women 267 ± 261 nmol/l). Plasma triglyceride levels (table 1) as well as VLDL particle distribution (table 2) were within reference ranges.

Effects of CETP inhibition on clinical findings and CETP mass & activity
Throughout the study, including the follow up period, no serious adverse events or early withdrawals for adverse events were observed. Treatment with JTT-705 had no effect on vital signs, body mass index or blood pressure (data not shown). JTT-705 was associated with a 24% decrease in CETP activity, with a concomitant 126% increase in CETP mass (table 1).

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</th>
<th>PValue</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 (IgM), 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.
Effects of CETP inhibition on the HDL particle

Compared to baseline, JTT-705 increased the levels of HDL-C and apoA-I by 19% and 14%, respectively (table 1). The increase in HDL$_2$ fraction (42%) clearly exceeded the increase in HDL$_3$ fraction (18%; table 1). Using FPLC, JTT-705 resulted in an elevation of HDL TC of 24% (p=0.01). Concomitantly, NMR analysis (table 2) 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, the particle number rose from 2.2±1.4 to 3.5±1.9 μmol/L (p=0.004)(table 2). In line, mean particle size of HDL increased from 8.5±0.3 to 8.8±0.4 nm (p<0.001) during JTT-705 treatment (table 2).

Table 2. NMR analysis of lipoprotein subclasses 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>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL Subclass (nmol/L)</td>
<td>80.8 ± 40.3 (78.9)</td>
<td>70.7 ± 37.3 (68.2)</td>
<td>81.3 ± 46.8 (78.2)</td>
<td>0.29</td>
</tr>
<tr>
<td>Large</td>
<td>4.5 ± 7.0 (1.8)</td>
<td>5.6 ± 8.9 (0.9)</td>
<td>5.0 ± 7.3 (2.5)</td>
<td>0.92</td>
</tr>
<tr>
<td>Medium</td>
<td>41.3 ± 36.4 (30.0)</td>
<td>33.6 ± 25.4 (34.9)</td>
<td>36.3 ± 32.1 (27.8)</td>
<td>0.12</td>
</tr>
<tr>
<td>Small</td>
<td>35.0 ± 19.5 (36.4)</td>
<td>31.4 ± 19.1 (35.4)</td>
<td>40.0 ± 22.2 (34.0)</td>
<td>0.18</td>
</tr>
<tr>
<td>LDL Subclass (nmol/L)</td>
<td>1911.9 ± 690.6 (1946.2)</td>
<td>1884.9 ± 700.0 (1884.4)</td>
<td>1609.9 ± 468.4 (1593.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>IDL</td>
<td>24.7 ± 36.0 (10.9)</td>
<td>19.8 ± 22.1 (12.2)</td>
<td>23.5 ± 32.6 (18.4)</td>
<td>0.98</td>
</tr>
<tr>
<td>Large</td>
<td>680.2 ± 440.5 (646.8)</td>
<td>746.5 ± 502.0 (719.4)</td>
<td>665.0 ± 388.6 (611.4)</td>
<td>0.81</td>
</tr>
<tr>
<td>Small</td>
<td>1206.9 ± 876.0 (1263.2)</td>
<td>1118.5 ± 750.9 (1259.7)</td>
<td>921.4 ± 609.2 (915.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Medium Small</td>
<td>228.5 ± 160.3 (229.9)</td>
<td>225.2 ± 139.6 (222.6)</td>
<td>188.1 ± 124.0 (178.7)</td>
<td>0.12</td>
</tr>
<tr>
<td>Very Small</td>
<td>978.4 ± 716.5 (1032.5)</td>
<td>893.4 ± 614.5 (1037.1)</td>
<td>733.3 ± 487.7 (736.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>HDL Subclass (μmol/L)</td>
<td>20.7 ± 7.9 (23.1)</td>
<td>20.8 ± 8.2 (23.3)</td>
<td>23.6 ± 7.2 (25.9)</td>
<td>0.008</td>
</tr>
<tr>
<td>Large</td>
<td>2.2 ± 1.4 (2.0)</td>
<td>2.2 ± 1.1 (2.2)</td>
<td>3.5 ± 1.9 (3.5)</td>
<td>0.004</td>
</tr>
<tr>
<td>Medium</td>
<td>4.3 ± 4.0 (4.7)</td>
<td>4.1 ± 4.5 (1.5)</td>
<td>4.2 ± 3.8 (3.1)</td>
<td>0.65</td>
</tr>
<tr>
<td>Small</td>
<td>14.2 ± 7.8 (14.3)</td>
<td>14.5 ± 8.0 (14.4)</td>
<td>15.9 ± 7.5 (15.9)</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean Particle Diameter (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL Subclass</td>
<td>52.1 ± 12.9 (48.9)</td>
<td>56.0 ± 16.5 (53.7)</td>
<td>50.5 ± 11.4 (49.6)</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL Subclass</td>
<td>21.0 ± 1.2 (20.7)</td>
<td>21.1 ± 1.1 (20.5)</td>
<td>21.2 ± 0.9 (21.0)</td>
<td>0.28</td>
</tr>
<tr>
<td>HDL Subclass</td>
<td>8.5 ± 0.3 (8.5)</td>
<td>8.5 ± 0.3 (8.5)</td>
<td>8.8 ± 0.4 (8.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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

Effects of CETP inhibition on the LDL particle

Total LDL-C did not change during JTT-705 treatment (table 1). Using FPLC, JTT-705 was associated with a 16.5% 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 1911.9±690.6 to 1609.9±468.4 nmol/L (p=0.01) (table 2) with a concomitant reduction in the very small LDL particles (from 978.4±716.5 to 733.3±487.7, p=0.05).
Effects of CETP inhibition on the VLDL particle
Triglyceride levels (table 1) and VLDL particle concentration (table 2) were not significantly changed upon JTT-705 treatment.

Effects of CETP inhibition on LDL oxidation parameters
OxLDL IgM antibodies showed a modest decrease of 11% (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).

Discussion
In the present study we show that in subjects with familial hypoalphalipoproteinemia inhibition of CETP-activity induces a significant increase in HDL-C levels, due to an increase of larger HDL particles. In addition, LDL particle number also declines, predominantly due to a decrease in the smallest LDL subfraction. These beneficial changes of the lipoprotein profile during JTT-705 treatment are accompanied by enhanced plasma anti-oxidant capacity, as illustrated by reduced IgM autoantibody levels against oxLDL and an increase in serum PON-1 activity.

Effects of CETP inhibition on the HDL particle
The 24% decrease in CETP activity upon JTT-705 treatment in the present study is comparable to 30%, observed in subjects with mild combined hyperlipidemia. The decreased activity upon CETP inhibition was accompanied by a two-fold increase in CETP mass, which is in line with previous findings. Whereas the exact mechanism for this increase remains to be elucidated, plausible explanations include decreased clearance of inactive CETP, tightly bound to the HDL particle, as well as an increased CETP expression. In our cohort, the 24% decrease of CETP activity translated into a 19% HDL-C increase. Whereas this appears to be slightly less than the 28% HDL-C increase in patients with combined hyperlipidemia upon treatment with 600 mg JTT705, it does concur with predictions based on the 'CETP-inhibition – HDL-C-increase' dose-response curves in healthy volunteers receiving torcetrapib.

JTT-705 preferentially increased the amount 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 the fact that particularly larger, CE-rich HDL particles show a strong, inverse relationship with CVD risk. To further characterize the compositional changes of the lipoprotein profile, we also quantified the individual lipid components within the HDL particle.
In line with earlier reports by Brousseau et al.\textsuperscript{13,14}, we observe an increase in cholesterol content within the HDL particle upon JTT-705 treatment, compatible with decreased cholesterol ester exchange from HDL by CETP. Cholesterol enrichment contributes to decreased HDL catabolism\textsuperscript{26} by HL, resulting in increased plasma residence time of the HDL particle.\textsuperscript{27,28} In contrast, cholesterol ester depletion would deteriorate HDL particle stability\textsuperscript{29}, and substitution of cholesteryl esters by TG would have enhanced renal apoA-I clearance.\textsuperscript{30} Thus, the observed compositional changes of HDL during CETP inhibition are likely to stabilize the HDL particle. Indeed, Brousseau and colleagues elegantly demonstrated that CETP inhibition with torcetrapib was associated with a reduced fractional catabolic rate for apoAI without affecting apoAI production rate.\textsuperscript{31}

**Effects of CETP inhibition on the LDL particle**

At baseline, familial hypoalphalipoproteinemia patients were characterized by an increased number of LDL particles, whereas plasma LDL-C levels were within the normal range. More precisely, FHA subjects showed higher levels of very small LDL particles compared to normolipidemic subjects of the Framingham Offspring Study.\textsuperscript{20} Very small LDL particles are considered to confer greater atherogenic risk, which is likely to be related to prolonged plasma LDL residence time,\textsuperscript{32} increased susceptibility to oxidation,\textsuperscript{33} as well as more facilitated extravasation followed by subendothelial retention \textit{in vitro}.\textsuperscript{34} The clinical significance of increased levels of small, dense LDL-C for atherosclerosis progression has been underscored by several large observational studies.\textsuperscript{35,36} In these cohorts, the presence of small LDL is usually confined to subjects with elevated triglyceride levels, reflecting the intensified exchange of LDL-derived CE by TG from triglyceride-rich lipoproteins (TRLs).\textsuperscript{20,32} In the present study, we find high levels of very small LDL particles in FHA subjects, in the absence of elevated TG levels. In this respect, it is worth noting that CETP has the capacity to exchange CE from both HDL-C and LDL-C towards TRLs. Thus, it is likely that, in case of very low HDL-C levels, LDL-C may take over as the principle cholesterol ester donor for TRLs. The latter concept is supported by the fact that the particle concentration of small LDL, and particularly that of the very small LDL particles, diminishes significantly upon JTT-705 treatment.

**Effects of CETP inhibition on OXLDL autoantibody titers and serum PON-1 activity**

Treatment with JTT-705 during 4 weeks resulted in a significant reduction of IgM antibodies levels against oxLDL. Cumulating evidence suggest that oxidative modification of LDL (oxLDL) plays a pivotal role in the progression of atherosclerosis.\textsuperscript{37} Circulating autoantibodies against oxLDL are thought to reflect LDL oxidation\textsuperscript{38} and correlate with subclinical atherosclerosis and with the severity of acute coronary syndromes.\textsuperscript{37,39} It is tempting to speculate that the decreased number of very small LDL particles, susceptible to oxidation, combined with the presence of larger HDL particles translate into reduced LDL oxidation rates.
CETP inhibition in familial hypoalphalipoproteinemia patients was also associated with a modest, but significant increase in serum PON-1 activity. Serum PON activity has been shown to be reduced in subjects with established CVD, in patients with familial hypercholesterolemia and in diabetic individuals. PON-1, LCAT and PAF-AH as well as apolipoproteins including apoA-I enable HDL to attenuate oxidative modification of the LDL particle. This particularly applies to small LDL subfractions, in which structural and compositional factors contribute to increased oxidizability. It is conceivable that the collective findings of larger LDL, HDL-C elevation and enhanced serum PON-1 activity may diminish the formation and accumulation of oxLDL within the arterial wall. Overall, the present data suggest that the increased serum PON-1 activity in familial hypoalphalipoproteinemia patients upon CETP inhibition may contribute to reduced oxidation of LDL in the circulation, which is compatible with the observed decrease in oxLDL autoantibody titers.

Study limitations
In our study, the design did not include a washout between both treatment modalities, since the 4-week treatment period was considered to be sufficient to exclude significant carry-over effects. However, the carry-over effect was statistically significant for paraoxonase, one of our main parameters of interest. In the group of patients who received first JTT-705 and then placebo the mean log(paraoxonase) values were 4.31 (SD 0.73) and 4.34 (SD 0.67), and hence the mean difference is 0.03 (SE 0.02). In contrast, in the group of patients who received first placebo, and then JTT-705 the mean log(paraoxonase) values were 4.51 (SD 0.69) after placebo, and 4.61 (SD 0.69) after JTT-705, and hence the mean difference is 0.10 (SE 0.04). The difference between these two mean-differences is clearly significant (0.13 (SE 0.05); p=0.006). Trends towards similar carry-over effects of JTT-705 into the placebo-period was observed with other parameters. Therefore, we compared the JTT-705 period to the baseline observations, rather than the placebo period. This approach has the advantage of preserving the methodological characteristics of the cross-over design in that each patient serves as its own control. Since this study included only 19 patients, this methodology also leaves us with more power than the potential alternative approach of using only the data of the first period comparing the effect of 4 weeks of JTT-705 treatment with the effect of 4 weeks of placebo treatment.

Clinical implications
We provide evidence in patients with familial hypoalphalipoproteinemia that CETP inhibition is associated with beneficial effects beyond its well recognized HDL-C increase. Thus, inhibition of CETP-activity results in a reduced number of small LDL particles. These changes in lipid profile are accompanied by a modest, but significant improvement in the plasma antioxidant capacity, as illustrated by increased serum PON1 activity and decreased IgM oxLDL autoantibodies. These favourable changes upon CETP inhibition in patients with familial hypoalphalipoproteinemia can be expected to be associated with an atheroprotective effect.
However, it should be taken into account that we still have to wait for the results of ongoing trials with surrogate or hard cardiovascular endpoints using CETP inhibition.

Reference List


