Effects of the Cholesteryl Ester Transfer Protein Inhibitor Torcetrapib on Apolipoprotein B100 Metabolism in Humans


Objective—Cholesteryl ester transfer protein (CETP) inhibition with torcetrapib not only increases high-density lipoprotein cholesterol levels but also significantly reduces plasma triglyceride, low-density lipoprotein (LDL) cholesterol, and apolipoprotein B (apoB) levels. The goal of the present study was to define the kinetic mechanism(s) by which CETP inhibition reduces levels of apoB-containing lipoproteins.

Methods and Results—Nineteen subjects, 9 of whom were pretreated with 20 mg atorvastatin, received placebo for 4 weeks, followed by 120 mg torcetrapib twice daily for an additional 4 weeks. After each phase, subjects underwent a primed-constant infusion of deuterated leucine to endogenously label newly synthesized apoB to determine very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL apoB100 production, and fractional catabolic rates (FCRs). Once-daily 120 mg torcetrapib significantly reduced VLDL, IDL, and LDL apoB100 pool sizes by enhancing the FCR of apoB100 within each fraction. On a background of atorvastatin, 120 mg torcetrapib significantly reduced VLDL, IDL, and LDL apoB100 pool sizes. The reduction in VLDL apoB100 was associated with an enhanced apoB100 FCR, whereas the decreases in IDL and LDL apoB100 were associated with reduced apoB100 production.

Conclusions—These data indicate that when used alone, torcetrapib reduces VLDL, IDL, and LDL apoB100 levels primarily by increasing the rate of apoB100 clearance. In contrast, when added to atorvastatin treatment, torcetrapib reduces apoB100 levels mainly by enhancing VLDL apoB100 clearance and reducing production of IDL and LDL apoB100. (Arterioscler Thromb Vasc Biol. 2006;26:1350-1356.)

Key Words: very low-density lipoproteins ■ triglyceride ■ low-density lipoproteins ■ cholesteryl ester transfer protein ■ CETP inhibition ■ lipoprotein kinetics

A n elevated level of plasma cholesterol is a major risk factor for the development of cardiovascular disease. Current treatments to reduce plasma cholesterol levels include diet, exercise, and treatment with cholesterol-lowering medications. Recently, a new class of drug designed to increase high-density lipoprotein (HDL) cholesterol levels through the inhibition of cholesteryl ester transfer protein (CETP) has been developed. CETP mediates the bidirectional exchange of cholesteryl ester and triglyceride between HDL and the apolipoprotein B (apoB)—containing lipoproteins, as well as among the different classes of apoB-containing lipoproteins. The main result of CETP activity is net transfer of cholesteryl ester from HDL to very low-density lipoproteins (VLDLs) and net transfer of triglyceride from VLDL to HDL. Thus, CETP provides a link between the metabolism of apoB-containing lipoproteins and HDL. Expression of CETP in mice, which normally lack CETP, or manipulation of CETP activity in other animals that express CETP changes the plasma concentration of apoB-containing lipoproteins in addition to expected changes in HDL cholesterol (HDL-C). In humans, CETP inhibitors not only raise HDL-C but also result in a significant reduction in triglyceride and low-density lipoprotein (LDL) cholesterol levels.

We reported previously the effects of a CETP inhibitor, torcetrapib, on plasma lipoproteins in subjects selected for low HDL-C (<40 mg · dl⁻¹). In these subjects, torcetrapib treatment resulted in a dose-dependent increase in HDL-C and reduction in triglyceride and LDL cholesterol (LDL-C) levels. We conducted kinetic studies to define the mechanism(s) by which torcetrapib, alone or on a background with the 3-hydroxy-3-methylglutaryl–coenzyme A reductase in-
hibitor atorvastatin, influences lipoprotein and apolipoprotein metabolism. We reported previously that torcetrapib slowed the rate of clearance of apoA-I in plasma. Here, we report the effects of torcetrapib on the metabolism of VLDL, intermediate-density lipoprotein (IDL), and LDL apoB100 in humans.

Methods

Subjects
Subjects were recruited at the University of Pennsylvania, Philadelphia, and Tufts-New England Medical Center, Boston, Mass. Subjects met the following eligibility criteria: age between 18 to 70 years, HDL-C of <40 mg·dL⁻¹, triglycerides <400 mg·dL⁻¹, LDL-C <160 mg·dL⁻¹, and a body mass index between 18 and 35 kg·m⁻². Subjects having an LDL-C of <160 mg·dL⁻¹ while stabilized on 20 mg atorvastatin for a minimum of 4 weeks were eligible to participate in the atorvastatin arm of this study. The study protocol was approved by the human investigation review committees of each institution, and informed, written consent was obtained from each participant.

Experimental Design

The study was a placebo-controlled, fixed-sequence crossover design study. A total of 19 subjects were enrolled, with 10 subjects in the nonatorvastatin group and 9 subjects in the atorvastatin group. A detailed description of the study design, depicted in Figure 1, has been reported previously. All subjects received placebo for 4 weeks, followed by 120 mg torcetrapib once daily for an additional 4 weeks. Six subjects from the nonatorvastatin group also participated in a third phase, in which they received 120 mg torcetrapib twice daily for 4 weeks.

At the end of each 4-week phase, subjects underwent a primed-constant infusion of deuterated leucine under constantly fed conditions to determine the kinetics of apoB100. At 11 AM, [5,5,5-²H₃]-L-leucine (10 μmol·kg body wt⁻¹) was injected intravenously as a bolus followed by a continuous infusion (10 μmol·kg body weight⁻¹·hr⁻¹), over a 15-hour period. Blood was collected at selected time points over the 15-hour infusion period as described.

Plasma Lipid and Lipoprotein Determinations

All laboratory personnel were blinded to the treatment phase. Blood samples for lipid and lipoprotein analyses were collected from subjects after a 12- to 14-hour fast into tubes containing EDTA. Plasma LDL-C levels were measured directly with the use of a reagent kit (Genzyme Diagnostics). Plasma HDL-C levels were determined after dextran sulfate-magnesium precipitation of apoB-containing lipoproteins. Total cholesterol, LDL-C, HDL-C, and plasma triglyceride levels were determined using enzymatic methods standardized through the Centers for Disease Control (Atlanta, Ga). Lipid composition on lipoprotein fractions isolated by sequential ultracentrifugation was measured using enzymatic reagents for total and free cholesterol, triglyceride, and phospholipids (Wako Chemicals USA).

Lipoprotein subclass concentrations were determined by proton nuclear magnetic resonance spectroscopy conducted on plasma samples (LipoScience). For the present study, LDL subclasses were defined as follows: large (21.3 to 23.0 nm); medium (19.8 to 21.2 nm), and small (18.3 to 19.7 nm).

Quantitative and Isolation of Apolipoproteins

VLDL, IDL, and LDL were isolated by sequential ultracentrifugation. Fasting plasma apoB levels were measured on a Cobas Fara II (Roche, Inc.) with an immunoturbidimetric assay using reagents and calibrators from Wako Diagnostics (Wako Chemicals USA). Nonfasting apoB levels were measured using an apoB ELISA. LDL apoB was calculated as the difference between plasma and VLDL plus IDL apoB. ApoB100 was isolated from VLDL, IDL, and LDL by SDS-PAGE using a Tris-glycine buffer system as described previously. The proportion of apoB within the triglyceride-rich lipoprotein fraction that was B100 and B48 was determined by densitometric scanning of Coomassie-stained gels.

Determinations of Isotopic Enrichment

ApoB100 bands were excised from polyacrylamide gels, hydrolyzed in 12N HCl at 110°C for 24 hours, and amino acids converted to N-heptafluorobutyramide derivatives and analyzed as described previously.

Kinetic Analysis

The kinetics of VLDL, IDL, and LDL apoB100 were assessed using a previously described multicompartmental model. The SAAM II program was used to fit the model to the observed tracer data and VLDL and IDL apoB100 concentration data using a weighted least-squares approach to determine the best fit. ApoB100 pool size (PS) in each fraction was computed as: PS (mg) = apoB100 concentration (mg·dL⁻¹) · plasma volume (0.45 dL·kg⁻¹ · body weight) · body weight (kg).

ApoB100 production rate (PR) in each fraction was calculated using the formula: PR (mg·kg⁻¹·day⁻¹) = [fractional catabolic rate (FCR; pools·day⁻¹) · PS (mg·pool⁻¹)] · body weight (kg⁻¹).

One subject in the nonatorvastatin group that also participated in the 120 mg twice daily phase of the study had Unsatisfactory tracer enrichment data. The tracer data for this subject were not modeled, and all data for this subject were excluded from the kinetic data analysis.

Statistical Analyses

Because of the relatively small sample sizes used in the study, a nonparametric approach to data analysis was chosen. The Wilcoxon matched-pair signed-rank test (single paired comparisons), conducted with the Intercooled Stata 8.2 for Windows program (Stata-Corp), was used to assess differences between the placebo and 120 mg once daily drug phase within a given group. The Friedman test (multiple paired comparisons), conducted with GraphPad Prism version 3.00 for Windows (GraphPad Software), was used to assess differences among drug phases in subjects enrolled in both the 120 mg once daily group and 120 mg twice daily group followed by Dunn’s test to detect individual differences post hoc. A 2-sided P value of <0.05 was considered statistically significant. The percentage change between torcetrapib and placebo phases was computed for individual subjects. Multiple regression was conducted with Intercooled Stata 8.2 for Windows using a backward stepwise approach. All data in the text and tables are presented as median (25th percentile to 75th percentile). Analyses were conducted on the full set of subjects in each group unless otherwise indicated.

Results

Plasma Lipids

Changes in plasma lipids in these subjects treated with 120 mg torcetrapib once daily have been reported previously and

Figure 1. Flow chart depicting the design of the study.
are shown in the supplemental Table (available online at http://atvb.ahajournals.org). After the placebo phase, the nonatorvastatin group had median total cholesterol and LDL-C levels of 196 and 131 mg \( \cdot \) dL\(^{-1} \), respectively, and median triglyceride and total apoB levels of 157 and 93 mg \( \cdot \) dL\(^{-1} \), respectively. After treatment with 120 mg torcetrapib once daily, these values decreased to 172 and 106 mg \( \cdot \) dL\(^{-1} \) for total cholesterol and LDL-C, respectively, and to 138 and 79 mg \( \cdot \) dL\(^{-1} \) for triglyceride and apoB, respectively. The decrease in total apoB \((P=0.02)\) in response to torcetrapib was statistically significant.

Changes in plasma lipids in response to treatment with 120 mg torcetrapib once daily on a background of atorvastatin have been reported previously\(^a\) and are shown in the supplemental Table. Under treatment with 20 mg atorvastatin plus placebo, these subjects had median total cholesterol and LDL-C levels of 138 and 82 mg \( \cdot \) dL\(^{-1} \), respectively, and total triglyceride and plasma apoB levels of 103 and 73 mg \( \cdot \) dL\(^{-1} \). Adding 120 mg torcetrapib to the existing atorvastatin treatment resulted in changes in the median total cholesterol and LDL-C levels to 140 and 80 mg \( \cdot \) dL\(^{-1} \) and total triglyceride and apoB levels changed to 87 and 67 mg \( \cdot \) dL\(^{-1} \), respectively. The decreases in LDL-C \((P=0.03)\) and total apoB \((P=0.01)\) were statistically significant.

**Lipid Composition**

In both the nonatorvastatin and atorvastatin groups, treatment with 120 mg torcetrapib once daily significantly reduced the relative amount of cholesterol ester and significantly increased the relative amount of triglyceride in VLDL (Table 1). The triglyceride:cholesterol ester molar ratio in VLDL increased from 5.7 (5.1 to 6.4) to 16.4 (11.8 to 26.4; \(P=0.008; n=9\)) in the nonatorvastatin group and from 7.0 (5.6 to 9.6) to 18.4 (14.7 to 28.7; \(P=0.008; n=9\)) in the atorvastatin group. There was also a significant decrease in the percentage of VLDL phospholipids and an increase in the percentage of VLDL free cholesterol, the latter only observed in the nonatorvastatin group (Table 1).

Treatment with 120 mg torcetrapib once daily resulted in a significant reduction in cholesterol ester and an increase in triglyceride in IDL in the nonatorvastatin group (Table 1). In the atorvastatin group, torcetrapib treatment resulted in a nonsignificant reduction in the IDL cholesteryl ester content, whereas the IDL triglyceride was significantly increased. Both groups had significant reductions in the percentage of free cholesterol and phospholipids in IDL after once-daily 120 mg torcetrapib treatment.

There were modest changes in the LDL lipid composition in both the nonatorvastatin and atorvastatin groups in response to once-daily 120 mg torcetrapib treatment (Table 1). Treatment of the nonatorvastatin group with torcetrapib significantly reduced the percentage of free cholesterol in LDL compared with the placebo phase. In the atorvastatin group, torcetrapib treatment resulted in a small but significant reduction in the LDL percentage of cholesteryl ester.

**Kinetics of VLDL ApoB100**

In the nonatorvastatin group, there was a significant decrease \((-34\%; P=0.02)\) in VLDL apoB100 PS in response to 120 mg torcetrapib once daily (Table 2). The mechanism responsible for this reduction in VLDL apoB100 was a significant increase \((59\%; P=0.02)\) in the VLDL apoB100 FCR. This increase was partially offset by a small but significant increase in the VLDL apoB100 PR \((11\%; P=0.04)\). The subjects enrolled in the 120 mg torcetrapib twice daily arm of the study had a 34% reduction in the VLDL apoB100 PS at the 120 mg torcetrapib twice daily dose compared with the placebo phase resulting from a 53% increase in the VLDL apoB100 FCR \((P=0.12)\). Although there was a significant increase in the VLDL apoB100 PR compared with placebo at the 120 mg once daily dose, the VLDL apoB100 PR was similar to placebo at the 120 mg twice daily dose (Table 3).

The addition of 120 mg torcetrapib once daily to the atorvastatin treatment regimen further significantly decreased the VLDL apoB100 PS by 29% \((P=0.01; Table 4)\). The apparent mechanism responsible for this reduction in the VLDL apoB100 PS was a nonsignificant 38% increase in the VLDL apoB100 FCR. This change was accompanied by a nonsignificant increase of 12% in the VLDL apoB100 PR.

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**TABLE 1. Percentage Lipid Composition of VLDL, IDL, and LDL Fractions During the Placebo and 120 mg Torcetrapib Once-Daily Phases**

<table>
<thead>
<tr>
<th></th>
<th>VLDL*</th>
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<tr>
<td></td>
<td>% FC</td>
<td>% CE</td>
<td>% PL</td>
<td>% TG</td>
<td>% FC</td>
<td>% CE</td>
<td>% PL</td>
<td>% TG</td>
<td>% FC</td>
<td>% CE</td>
<td>% PL</td>
<td>% TG</td>
<td>% FC</td>
<td>% CE</td>
</tr>
<tr>
<td>Placebo</td>
<td>5.7</td>
<td>8.9</td>
<td>17.4</td>
<td>68.2</td>
<td>7.2</td>
<td>23.6</td>
<td>15.6</td>
<td>51.5</td>
<td>11.6</td>
<td>48.9</td>
<td>27.3</td>
<td>11.8</td>
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<tr>
<td></td>
<td>(5.2–6.1)</td>
<td>(8.0–9.9)</td>
<td>(16.5–18.1)</td>
<td>(67.2–69.1)</td>
<td>(6.2–9.4)</td>
<td>(15.3–32.1)</td>
<td>(13.5–21.2)</td>
<td>(41.0–59.7)</td>
<td>(10.3–12.4)</td>
<td>(48.4–50.3)</td>
<td>(26.5–28.2)</td>
<td>(11.2–13.3)</td>
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<tr>
<td>Torcetrapib</td>
<td>5.9</td>
<td>3.4</td>
<td>16.4</td>
<td>73.3</td>
<td>4.9</td>
<td>11.6</td>
<td>9.3</td>
<td>73.8</td>
<td>10.6</td>
<td>50.9</td>
<td>26.4</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5.6–6.6)</td>
<td>(2.3–4.8)</td>
<td>(15.9–17.3)</td>
<td>(72.6–75.7)</td>
<td>(4.2–6.2)</td>
<td>(7.8–16.7)</td>
<td>(5.9–16.2)</td>
<td>(60.0–78.5)</td>
<td>(10.1–11.3)</td>
<td>(49.5–53.0)</td>
<td>(24.9–28.2)</td>
<td>(9.3–14.3)</td>
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</table>

\( P \) value vs placebo

| Placebo + atorvastatin | 0.02  | 0.005 | 0.02 | 0.005 |
| Placebo + atorvastatin | 0.07% | 0.008 | 0.009 | 0.008 |

Note: FC indicates free cholesterol; CE, cholesteryl ester; PL, phospholipids; TG, triglyceride.

*Nonatorvastatin group \( n=10 \), atorvastatin group \( n=9 \); †Nonatorvastatin group \( n=9 \), atorvastatin group \( n=7 \); ‡Nonatorvastatin group \( n=8 \), atorvastatin group \( n=9 \);

\( %F C \) indicates % free cholesterol for placebo and % free cholesterol for the 120 mg torcetrapib twice daily dose compared with the 120 mg once daily dose. The VLDL apoB100 PS at the 120 mg once daily dose, the VLDL apoB100 PR was similar to placebo at the 120 mg twice daily dose (Table 3).

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The LDL apoB100 PR was at the 120 mg twice daily dose of torcetrapib, there was a significant 17% decrease in the LDL apoB100 PS ($P=0.009$) when compared with placebo. This was associated with a nonsignificant 22% increase in the LDL apoB100 FCR and essentially no change in the LDL apoB100 PR (Table 3).

The LDL apoB100 PS was significantly decreased ($-12%$; $P=0.01$) when 120 mg torcetrapib once daily was added to atorvastatin treatment (Table 4). The LDL apoB100 FCR, which was already relatively high at 0.44 pools·day$^{-1}$ likely as a result of atorvastatin treatment, did not change significantly in response to the addition of torcetrapib. There was a nonsignificant 8% reduction in the LDL apoB100 PR that apparently accounted for the decrease in the LDL apoB100 PS.

**LDL ApoB100 Response to Torcetrapib**

In both the nonatorvastatin and atorvastatin groups, there was considerable interindividual variability in the response of LDL apoB100 metabolism to torcetrapib. To establish whether there were any features that allowed prediction of changes in LDL apoB100 levels, correlations were conducted using lipid and apolipoprotein markers measured during the placebo phase as independent variables and the percentage change in LDL apoB100 after 120 mg torcetrapib once-daily treatment as the dependent variable. The plasma triglyceride level and the relative amount (%) of small LDL-C (as

**Kinetics of LDL ApoB100**

In the nonatorvastatin group, 120 mg torcetrapib once daily significantly reduced LDL apoB100 PS by 5% ($P=0.04$; Table 2). This reduction was associated with a nonsignificant 18% increase in the LDL apoB100 FCR with no change in the LDL apoB100 PR. The LDL apoB100 PS was significantly decreased ($-25%$; $P=0.008$; Table 4). The primary mechanism responsible for the reduction in LDL apoB100 PS was a 31% decrease in the LDL apoB100 PR. Although this change was not reflected in the median values of the LDL apoB100 PRs, which were the same during the placebo and torcetrapib phases, 6 of 9 subjects decreased the LDL apoB PR ($P=0.05$) in response to torcetrapib.

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assessed by nuclear magnetic resonance) during the placebo phase were significantly correlated with percentage change in the LDL apoB100 PS after torcetrapib treatment (data not shown). Multiple regression analysis showed that of these 2 significant correlates of the percentage change in the LDL apoB100 PS after 120 mg torcetrapib once-daily treatment, the percentage of small LDL-C during the placebo phase was the only statistically significant independent predictor of the percentage change in the LDL apoB100 PS after torcetrapib treatment. The significantly positive correlation between the percentage of small LDL-C during the placebo phase and the change in the LDL apoB100 PS after torcetrapib treatment is shown in Figure 2A. Overall, subjects with the highest percentage of small LDL-C had little change in the LDL apoB100 FCR and thus little change in the LDL apoB100 PS in response to 120 mg torcetrapib once daily (Figure 2B). This relationship was only statistically significant for subjects in the nonatorvastatin group ($R^2=0.64, P=0.01$), the relationship in the atorvastatin group being nonsignificant ($R^2=0.00, P=0.99$).

**Discussion**

CETP mediates the net transport of cholesteryl ester from HDL to apoB-containing lipoproteins and, in turn, the net transfer of triglyceride from apoB-containing lipoproteins to HDL. Inhibition of CETP increases HDL-C by preventing cholesteryl ester transfer to apoB-containing lipoproteins. Two small molecule CETP inhibitors, JTT-705 and torcetrapib, have advanced into clinical trials. Studies with torcetrapib demonstrated a dose-dependent increase in HDL-C from $\approx 50\%$ at the 120 mg once-daily dose to $\approx 100\%$ at the 120 mg twice-daily dose. In addition to the HDL-raising effects of these drugs, significant reductions in plasma triglyceride, LDL-C, and apoB levels have been reported.

In the current study with torcetrapib, we found that in addition to the increase in HDL-C, CETP inhibition significantly reduced plasma concentration of LDL-C and all apoB-containing lipoproteins in these subjects. In subjects treated with torcetrapib alone, these reductions were associated with increases in the VLDL apoB100 FCR and a trend toward increased IDL and LDL apoB FCR. In subjects who added torcetrapib to their background atorvastatin, treatment trended toward a nonsignificant increase in the VLDL apoB100 FCR and reduced IDL and LDL apoB100 production.

Because CETP directly links the metabolism of HDL with that of apoB-containing lipoproteins, it is not surprising that manipulation of CETP activity is associated with changes in the concentrations of apoB-containing lipoproteins. Mice,

**TABLE 4. VLDL, IDL, and LDL ApoB100 Kinetic Parameters From Subjects (n=9) Who Underwent Torcetrapib Plus Atorvastatin Treatment**

<table>
<thead>
<tr>
<th>Study Phase</th>
<th>VLDL ApoB100 PS (mg)</th>
<th>VLDL ApoB100 FCR (pools d⁻¹)</th>
<th>VLDL ApoB100 PR (mg kg⁻¹ d⁻¹)</th>
<th>IDL ApoB100 PS (mg)</th>
<th>IDL ApoB100 FCR (pools d⁻¹)</th>
<th>IDL ApoB100 PR (mg kg⁻¹ d⁻¹)</th>
<th>LDL ApoB100 PS (mg)</th>
<th>LDL ApoB100 FCR (pools d⁻¹)</th>
<th>LDL ApoB100 PR (mg kg⁻¹ d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo + atorvastatin</td>
<td>(215–330)</td>
<td>(5.8–9.2)</td>
<td>(16.8–26.3)</td>
<td>(54–92)</td>
<td>(6.4–9.0)</td>
<td>(3.8–9.0)</td>
<td>(2048–2940)</td>
<td>(0.35–0.53)</td>
<td>(12.1–14.9)</td>
</tr>
<tr>
<td>Torcetrapib + atorvastatin</td>
<td>(14–29)</td>
<td>(1.7–14.8)</td>
<td>(17.7–31.5)</td>
<td>(55–86)</td>
<td>(7.6–9.5)</td>
<td>(3.3–4.4)</td>
<td>(1688–2588)</td>
<td>(0.35–0.69)</td>
<td>(10.3–14.4)</td>
</tr>
</tbody>
</table>

% Difference (median) | −29 | 12 | −25 | 8 | −31 | −12 | 0 | −8 |
% Difference (Mean) | −22 | 16 | −29 | 8 | −25 | −14 | 5 | −10 |
P value vs placebo | 0.01 | 0.11 | 0.44 | 0.008 | 0.59 | 0.05 | 0.01 | 0.72 | 0.17 |

*Table 4. VLDL, IDL, and LDL ApoB100 kinetic parameters from subjects (n=9) who underwent torcetrapib plus atorvastatin treatment.*

$d$ indicates day.
which do not normally express CETP, increase their LDL-C levels in response to CETP expression. The increase in LDL-C resulting from CETP expression in mice is associated with an increased cholesteryl ester content in VLDL and LDL and downregulation of hepatic LDL receptor, presumably as a result of increased cholesteryl ester delivery to the liver.

Studies in CETP-deficient subjects have shed some light on possible mechanisms responsible for the reduced levels of apoB100-containing lipoproteins in CETP deficiency. CETP-deficient subjects have IDL and LDL apoB100 levels that are ≈50% of normal. Kinetic studies revealed that these reduced levels were the result of increased fractional catabolism of IDL and LDL apoB100, probably because of hepatic LDL receptor upregulation. We observed a nonsignificant trend toward an increase in VLDL, IDL, and LDL apoB100 fractional catabolism with CETP inhibition in subjects in the nonatorvastatin group. Although it is likely that the trend toward an increase in the IDL and LDL apoB100 FCR was the result of LDL receptor upregulation, the LDL receptor has been shown to have minimal impact on the clearance of VLDL apoB100 in humans. Instead, we suggest that the enhanced VLDL apoB100 fractional catabolism may be the result of compositional changes in VLDL lipids in response to CETP inhibition. The relative enrichment with triglyceride, as indicated by the increased molar ratio of triglyceride to cholesteryl ester in the core of the VLDL, may make VLDL a better substrate for lipoprotein lipase in vivo. Alternatively, the change in VLDL composition may have increased lipoprotein lipase activity by altering the exchangeable apolipoprotein content of VLDL. Such a change could also increase receptor-mediated clearance of VLDL remnants from plasma and conversion of VLDL to LDL.

The decrease in VLDL and LDL apoB100 levels in response to atorvastatin treatment has been reported to be attributable to increased fractional catabolism, although reduced VLDL production has also been reported in some subjects in response to statin treatment. Reduced CETP expression has been reported in subjects treated with atorvastatin and may have contributed to the significantly increased IDL FCR in these subjects. The addition of torcetrapib to atorvastatin treatment resulted in further apoB100 decrease in all lipoprotein fractions. The magnitude of the decrease was similar to what was observed with torcetrapib alone, indicating that there was an additive effect of combining torcetrapib with atorvastatin treatment on apoB100 kinetics. The VLDL apoB100 PS was reduced as a result of a trend toward increased VLDL apoB100 clearance, similar to what was seen with torcetrapib alone. However, the changes in IDL and LDL apoB100 PS resulted from a different mechanism than what was found during treatment with torcetrapib alone. The subjects in the atorvastatin group had a relatively high IDL and LDL apoB100 FCR during treatment with atorvastatin alone when compared with the nonatorvastatin group likely attributable to upregulation of LDL receptors with statin therapy. The addition of torcetrapib provided no further increase in the IDL and LDL apoB100 FCRs, perhaps indicating maximal upregulation of apoB100 clearance by this pathway had already been achieved before the addition of torcetrapib. Instead, there was reduced IDL apoB100 production and a trend toward reduced LDL apoB100 production. The mechanism responsible for this decrease in IDL and LDL apoB100 production is not clear but may relate to changes in lipoprotein lipid and apolipoprotein composition in response to torcetrapib in these subjects.

The individual response of LDL apoB100 PS to 120 mg torcetrapib once daily in subjects in both the atorvastatin and nonatorvastatin groups was variable, ranging from a 33% decrease in the LDL apoB100 PS to a 15% increase (data not shown). We found that the best predictor of the percentage change in the LDL apoB100 PS response to torcetrapib treatment was the relative amount (%) of small LDL-C during the placebo phase. Small LDL has been shown to bind poorly to the LDL receptor and is associated with elevated triglyceride levels. Interestingly, the percentage of small LDL-C during the placebo phase was a significant predictor of the change in the LDL apoB100 FCR in response to torcetrapib in subjects in the nonatorvastatin group. However, this inverse relationship between the percentage of small LDL-C and the percentage of LDL apoB100 PS response to torcetrapib was not observed in subjects in the atorvastatin group. This is consistent with the finding that a change in the LDL apoB100 PR appeared to be primarily responsible for lowering the LDL apoB100 PS in these subjects. Regardless of the mechanism for the decrease in LDL apoB100 PS, subjects with a relatively high percentage of small LDL-C might have less of an LDL apoB100 PS change in response to torcetrapib treatment, something that should be examined in future studies.

In conclusion, these data suggest that treatment with torcetrapib alone lowers VLDL, IDL, and LDL apoB100 PS by increasing VLDL, IDL, and LDL apoB100 clearance. On a background of atorvastatin, where LDL apoB100 FCR is increased, the addition of torcetrapib to treatment does not further increase LDL apoB100 clearance but appears to lower the LDL apoB100 PS by enhancing VLDL apoB100 clearance and reducing LDL apoB100 production. In both cases, the overall effect of torcetrapib on lowering apoB100 PSs could contribute to a beneficial effect on atherosclerosis despite different kinetic mechanisms.

Acknowledgments

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References


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Supplementary table I. Fasting plasma lipid and apoB levels (mg • dL⁻¹) at the end of the placebo and torcetrapib 120 mg once daily treatment phases. Data are expressed as median (25th-75th percentile). Non–atorvastatin group n=10; atorvastatin group n=9.

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>apoB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo</strong></td>
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<td>157</td>
<td>131</td>
<td>32</td>
<td>93</td>
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<tr>
<td><strong>Torcetrapib</strong></td>
<td>172</td>
<td>138</td>
<td>106</td>
<td>44</td>
<td>79</td>
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<tr>
<td></td>
<td>(159–215)</td>
<td>(93–198)</td>
<td>(92–155)</td>
<td>(40–58)</td>
<td>(75–100)</td>
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<tr>
<td><strong>P value</strong></td>
<td>0.84</td>
<td>0.44</td>
<td>0.15</td>
<td><strong>0.005</strong></td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>vs. placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td>138</td>
<td>103</td>
<td>82</td>
<td>28</td>
<td>73</td>
</tr>
<tr>
<td><strong>Torcetrapib</strong></td>
<td>140</td>
<td>87</td>
<td>80</td>
<td>44</td>
<td>67</td>
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<tr>
<td><strong>P value</strong></td>
<td>0.34</td>
<td>0.10</td>
<td><strong>0.03</strong></td>
<td><strong>0.008</strong></td>
<td><strong>0.01</strong></td>
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<tr>
<td>vs. placebo</td>
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</table>
**Supplementary table II.** Fasting plasma lipid and apoB levels (mg • dL\(^{-1}\)) at the end of the placebo, torcetrapib 120 mg once daily and torcetrapib 120 mg twice daily treatment phases. Data are expressed as median (25\(^{th}\)-75\(^{th}\) percentile); n=6.

<table>
<thead>
<tr>
<th></th>
<th>TC</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>apoB</th>
</tr>
</thead>
<tbody>
<tr>
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<td>152</td>
<td>137</td>
<td>33</td>
<td>113</td>
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<tr>
<td><strong>Torcetrapib</strong></td>
<td>211</td>
<td>112</td>
<td>137</td>
<td>51</td>
<td>89</td>
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<tr>
<td>120 mg once</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>daily</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Torcetrapib</strong></td>
<td>200</td>
<td>96</td>
<td>121</td>
<td>74*</td>
<td>83*</td>
</tr>
<tr>
<td>120 mg twice</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>daily</td>
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<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td>0.96</td>
<td>0.25</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>0.002</td>
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<td>(Friedman)</td>
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*\(p<0.05\) vs. placebo