Inhibition of CETP by Torcetrapib Attenuates the Atherogenicity of Postprandial TG-Rich Lipoproteins in Type IIB Hyperlipidemia

Maryse Guerin, Wilfried Le Goff, Emilie Duchene, Zélie Julia, Tu Nguyen, Tom Thuren, Charles L. Shear, M. John Chapman

Objective—The purpose of this study was to evaluate the impact of torcetrapib on atherogenic TG-rich lipoprotein subfractions in the postprandial phase in Type IIB hyperlipidemia.

Methods and Results—The quantitative and qualitative features of the postprandial profile of TG-rich lipoproteins were determined at baseline, after treatment for 6 weeks with 10 mg/d atorvastatin, and subsequently with an atorvastatin/torcetrapib combination (10/60 mg/d) in Type IIB patients (n=18). After ingestion of a standardized mixed meal, TG-rich lipoprotein subfractions were evaluated over 8 hours after each experimental period. On a background of atorvastatin, torcetrapib significantly attenuated the incremental postprandial area under the curve (iAUC 0 to 8 hours) for VLDL-1 (−40%), and the AUC 0 to 8 hours for VLDL-2 (−53%), with minor effect on chylomicron iAUC (−24%); concomitantly, the CE/TG ratio in both VLDL-1 and VLDL-2 was significantly reduced (−27% to −42%). Such reduction was attributable to torcetrapib-mediated attenuation of postprandial CE transfer to Chylomicrons (−17%) and VLDL-1 (−33%). Marked reduction in postprandial VLDL-1 levels was associated with apoE enrichment.

Conclusions—On a background of atorvastatin, torcetrapib attenuated the quantitative and qualitative features of the atherogenic postprandial profile of chylomicrons, VLDL-1 and VLDL-2. Such changes reflect the sum of torcetrapib-mediated effects on TG-rich lipoprotein production, intravascular remodeling, and catabolism. (Arterioscler Thromb Vase Biol. 2008;28:148-154.)

Key Words: CETP ■ Torcetrapib ■ high-density lipoprotein ■ postprandial hypertriglyceridemia

Postprandial hypertriglyceridemia represents an independent risk factor for premature coronary artery disease (CAD). Indeed, it is now established that remnants of triglyceride-rich lipoproteins, principally chylomicrons and VLDL, may penetrate the arterial intima, with retention by the extracellular matrix and ensuing cholesterol accumulation. Postprandial lipoprotein metabolism is characterized by transient accumulation of intestinally-derived Chylomicrons (CM) and hepatically-derived very low density lipoproteins (VLDL) and their remnants. Hydrolysis of the triglyceride core of CM and VLDL by lipoprotein lipase facilitates the delivery of free fatty acids to muscle and adipose tissue, with formation of CM- and VLDL-remnants which are efficiently removed from the circulation by receptor-mediated pathways in the liver. During lipolysis of postprandial triglyceride-rich lipoproteins, an excess of surface components containing apolipoproteins, unesterified cholesterol, and phospholipids is generated and sequesters to HDL potentially via the action of hepatic lipase and PLTP, thereby increasing the total circulating HDL pool and enhancing the transformation of small HDL3 to large CE-rich HDL2 particles. Equally, CETP mediates heterotransfer of cholesteryl esters and triglycerides between HDL on the one hand, and apoB-containing lipoproteins on the other; such transfer is accelerated under postprandial conditions with CE enrichment of triglyceride-rich lipoprotein particles, and transient transformation of CE-enriched HDL into TG-enriched particles which become a substrate for hepatic lipase. In this way, HDL particle size is modulated. Dyslipidemic states associated with premature atherosclerotic disease and high cardiovascular risk are associated with elevated plasma CETP activity, which contributes significantly to elevation in the cholesterol burden of atherogenic apoB containing lipoproteins. Type IIB hyperlipidemia is characterized by elevated plasma levels of triglyceride-rich lipoproteins, LDL-cholesterol, and apoB, a small dense LDL phenotype, thereby leading to increased risk of CAD. In this atherogenic dyslipidemia, enhanced CETP-mediated CE transfer occurs during the postprandial phase as compared with normolipidemic subjects. Such accelerated CE transfer
results from elevation in acceptor particle numbers of both postprandial plasma chylomicrons and large VLDL-1, but also from postprandial targeting of VLDL-1 as the preferential TRL acceptor for CETP-mediated CE transfer from HDL. Thus the formation, accumulation and CE-enrichment of atherogenic remnant particles are markedly enhanced during the postprandial phase in type IIB hyperlipidemia.10

The 3-hydroxy-3-methylglutaryl (HMG) coenzyme A (CoA) (HMG-CoA) reductase inhibitor, atorvastatin, normalizes the abnormal intravascular remodeling of postprandial triglyceride-rich lipoprotein particles not only via marked reduction in the postprandial formation and accumulation of both chylomicrons and large VLDL-1, but also via a significant reduction in CETP-dependent CE transfer from HDL to these potentially atherogenic lipoprotein acceptors.11 Inhibition of CETP in the hyperlipidemic rabbit, which results in attenuated atherosclerosis, clearly indicates a proatherogenic role for CETP-mediated cholesteryl ester transfer in this model.12–14 These studies suggest therefore that CETP inhibition might constitute a potential therapeutic approach to delay atherogenesis in humans.

Torcetrapib is a potent and selective inhibitor of CETP.15 In clinical trials, Torcetrapib has been shown to decrease plasma LDL-C and to increase plasma HDL-C levels in a dose-dependent manner in healthy subjects.16 Furthermore, the action of Torcetrapib favors enhanced formation of large HDL particles with reduction in those of small dense LDL in low HDL-C lipid phenotypes.17 When Torcetrapib was coadministered with atorvastatin, which alone reduces LDL-C cholesterol levels by up to 60% at maximal doses, an additional LDL-C lowering effect was observed.17 Moreover, on a background of atorvastatin, torcetrapib reduced VLDL particle levels by increasing their clearance and reduced IDL and LDL particle levels primarily by reducing their production.18 We therefore postulated that combination therapy with Torcetrapib/atorvastatin might be highly efficacious in attenuating the atherogenicity of the postprandial hyperlipemic phase. The current study was designed to examine the impact of a fixed combination of Torcetrapib/atorvastatin 60/10 mg/d on postprandial lipoprotein metabolism in subjects with Type IIB hyperlipidemia as compared with atorvastatin alone. In late 2006, the development program for Torcetrapib was suspended because the independent Data and Safety Monitoring Board for a large clinical trial (ILLUMINATE: the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events), involving therapeutic evaluation of Torcetrapib, recommended that the study be terminated because of a statistically significant imbalance in mortality among patients receiving torcetrapib/atorvastatin versus those receiving atorvastatin alone.19 The identification of the potentially beneficial therapeutic effects of Torcetrapib action on lipoprotein metabolism and on the vasculature, and their eventual distinction from deleterious direct or indirect effects on the cardiovascular system that may underlie the excess mortality in the Illuminate trial, are therefore especially relevant to future development of HDL-raising agents whose mechanisms of action target CETP. The present studies provide new insight into this question, and demonstrate that torcetrapib-mediated inhibition of CETP significantly attenuates the atherogenic profile of triglyceride-rich lipoproteins during the postprandial phase in Type IIB hyperlipidemia.

Methods

Study Design

Eighteen males aged between 36 and 59 years (mean: 46±7 years) and displaying a combined hyperlipidemia typical of the Type IIB lipid phenotype, ie, concomitant elevation of circulating levels of cholesterol and triglycerides, were selected for the study (please see the supplemental materials, available online at http://atvb.ahajournals.org, for more details). After screening for exclusion criteria, patients entered a 3-period fixed sequence study. In the first period, patients ceased taking lipid-lowering drugs and stabilized their diet (AHA Step one diet or equivalent) over a 6-week period immediately before active treatment. Measurements taken at the end of this period served as the baseline. After drug wash-out/diet run-in, a 6-week period of active treatment with atorvastatin only (10 mg/d) was initiated, followed directly by a 6-week period of active therapy with torcetrapib/atorvastatin (60/10 mg/d). All doses were administered orally, once daily immediately after the morning meal.

The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. The study protocol and amendment were reviewed and approved by an Ethics Committee and met national institutional requirements. Written informed consent was obtained from all patients.

Details of blood samples, postprandial time course, lipoprotein fractionation and characterization, determination of endogenous plasma CETP activity, and statistical analysis are available in the supplemental materials.

Results

Effects of Torcetrapib on Fasting Plasma Lipid and Apolipoprotein Levels

Under fasting conditions, torcetrapib/atorvastatin therapy induced a marked and significant increase (+61%; P<0.0001) in HDL-cholesterol levels as compared with baseline and as compared with atorvastatin therapy alone (+51%; P<0.0001; Table 1). Plasma levels of both apolipoprotein AI and AII were significantly increased by 30% (P<0.0001) and 21% (P=0.0001), respectively, after T/A treatment as compared with baseline, whereas only minor change was detected with atorvastatin alone. Torcetrapib/atorvastatin therapy equally decreased both LDL-cholesterol (~19%; P<0.0001) and apoB levels (~17%; P<0.0001), relative to treatment with atorvastatin alone. Relative to baseline, the TC/HDL-C ratio was significantly reduced in type IIB subjects treated with atorvastatin alone (~32%; P<0.0001), and in those receiving T/A therapy (~55%; P<0.0001). The TC/HDL-C ratio was reduced to values less than 5 (range 1.8 to 4.1) after T/A therapy in all 18 patients.

Effects of Torcetrapib on Postprandial Triglyceride Levels

Plasma triglyceride levels rose progressively to attain a maximum 4 hour postprandially in type IIB subjects both before and after atorvastatin treatment alone, and equally after combined T/A treatment (Figure 1). T/A therapy induced a significant reduction in plasma triglyceride levels 4 hours postprandially (220±22 mg/dL after T/A therapy; −32%; P=0.0005) as compared with baseline (323±27 mg/dL) or after atorvastatin therapy alone (266±28 mg/dL);
Table 1. Plasma Lipid and Apolipoprotein Levels in Type IIB patients Before and After Drug Phases

<table>
<thead>
<tr>
<th>Variables</th>
<th>Before Treatment (Baseline, n=18)</th>
<th>Atorvastatin Alone (A, 10 mg/day, n=18)</th>
<th>Torcetrapib/Atorvastatin (T/A, 60/10 mg/day, n=18)</th>
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</thead>
<tbody>
<tr>
<td><strong>Cholesterol, mg/dL</strong></td>
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</tr>
<tr>
<td>Total</td>
<td>258 ± 7</td>
<td>198 ± 6*</td>
<td>181 ± 8*</td>
</tr>
<tr>
<td>LDL</td>
<td>177 ± 6</td>
<td>113 ± 5*</td>
<td>80 ± 6‡</td>
</tr>
<tr>
<td>HDL</td>
<td>44 ± 2</td>
<td>47 ± 2*</td>
<td>71 ± 5‡</td>
</tr>
<tr>
<td>HDL2</td>
<td>24.3 ± 1.7</td>
<td>25.6 ± 2.0</td>
<td>43.1 ± 4.1‡</td>
</tr>
<tr>
<td>HDL3</td>
<td>19.8 ± 1.0</td>
<td>20.0 ± 0.8</td>
<td>24.3 ± 1.0‡</td>
</tr>
<tr>
<td>TC/HDL-C ratio</td>
<td>6.0 ± 0.2</td>
<td>4.1 ± 0.2*</td>
<td>2.7 ± 0.2‡</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>A-I</td>
<td>127 ± 4</td>
<td>132 ± 5§</td>
<td>165 ± 8‡</td>
</tr>
<tr>
<td>A-II</td>
<td>34 ± 0.9</td>
<td>35 ± 1.3</td>
<td>41 ± 1.4‡</td>
</tr>
<tr>
<td>B</td>
<td>134 ± 4</td>
<td>94 ± 4*</td>
<td>71 ± 4‡</td>
</tr>
<tr>
<td>E</td>
<td>4.0 ± 0.2</td>
<td>3.0 ± 0.2*</td>
<td>2.8 ± 0.1*</td>
</tr>
<tr>
<td>CII</td>
<td>5.6 ± 0.4</td>
<td>5.9 ± 0.6</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>CIII</td>
<td>17.9 ± 0.8</td>
<td>17.3 ± 1.0</td>
<td>18.4 ± 0.9</td>
</tr>
<tr>
<td>HDL-apoE</td>
<td>0.91 ± 0.13</td>
<td>0.55 ± 0.08*</td>
<td>0.43 ± 0.04§</td>
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<tr>
<td><strong>Apolipoprotein, mg/dL</strong></td>
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<tr>
<td>A-I</td>
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<tr>
<td>HDL-apoE</td>
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</table>

Values are mean ± SE. *P<0.001, †P<0.001, and $#P<0.05 vs Baseline ‡P<0.001 vs therapy with atorvastatin alone.

−18%; P=0.038). More strikingly, the postprandial iAUC for plasma triglyceride from 0 to 8 hours was significantly reduced from 690±81 at baseline to 628±95 after atorvastatin only (−9%) and further reduced to 439±74 after T/A treatment (−36%; P=0.0005).

Effects of Torcetrapib on Postprandial Triglyceride-Rich Lipoprotein Subfractions

T/A therapy induced marked and differential effects on postprandial plasma triglyceride-rich lipoprotein subfraction levels (Table 2). T/A induced a significant reduction (−43%; P=0.0004) in the absolute variation in plasma chylomicron levels 4 hours postprandially (80±17 mg/dL after T/A therapy) as compared with baseline (141±21 mg/dL; Figure 1 and supplemental Figure I). Moreover, after T/A therapy, the iAUC for plasma chylomicron levels from 0 to 8 hours was significantly reduced (−37%; P=0.002) as compared with baseline.

Combined T/A therapy significantly decreased the absolute change in plasma VLDL-1 levels 4 hours postprandially (29±4 mg/dL; −44%; P<0.006) relative to that at baseline.

Table 2. Postprandial AUC for Triglyceride-Rich Lipoprotein Subfractions in Type IIB Patients Before and After Drug Phases

<table>
<thead>
<tr>
<th></th>
<th>AUC 0–8h</th>
<th>% Change from (B)</th>
<th>iAUC 0–8h</th>
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<tbody>
<tr>
<td><strong>Chylomicrons</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Before treatment</td>
<td>1046±114</td>
<td>637±86</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>753±107*</td>
<td>−28%</td>
<td>528±92</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin</td>
<td>671±85#</td>
<td>−36%</td>
<td>400±74#</td>
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<tr>
<td><strong>VLDL-1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>1272±112</td>
<td>252±41</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>1021±93$</td>
<td>−20%</td>
<td>263±43</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin</td>
<td>893±72#</td>
<td>−30%</td>
<td>157±31$§</td>
</tr>
<tr>
<td><strong>VLDL-2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>538±32</td>
<td>−84±16</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>380±23*</td>
<td>−29%</td>
<td>−65±18</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin</td>
<td>250±18‡</td>
<td>−53%</td>
<td>−37±13$</td>
</tr>
<tr>
<td><strong>IDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td>931±84</td>
<td>2±21</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>628±45*</td>
<td>−33%</td>
<td>−47±21</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin</td>
<td>612±40*</td>
<td>−34%</td>
<td>−33±14</td>
</tr>
</tbody>
</table>

AUC indicates area under the curve; iAUC, incremental AUC. Values are mean ± SE.

*P<0.001, †P<0.001, and $#P<0.05 vs Baseline. ‡P<0.001 and $P<0.05 vs therapy with atorvastatin alone.
(51±7 mg/dL), and that after atorvastatin alone (52±7 mg/dL; Figure 3). After T/A therapy, the iAUC (0 to 8 hours) for plasma VLDL-1 was significantly reduced (−38%; P<0.001) as compared with baseline. Interestingly, postprandial VLDL-1–apoE levels were significantly increased by 2- to 3-fold, 2 hours and 4 hours after meal intake, respectively, after T/A therapy relative to nontreated type IIB patients, indicating that VLDL-1 particles became apoE-enriched during the postprandial phase under conditions of CETP inhibition (supplemental Figure II). In parallel, we observed a slight but nonsignificant reduction in HDL–apoE levels throughout the postprandial phase (supplemental Table I).

Over the postprandial time course, significant reductions occurred in plasma VLDL-2 levels both before and after atorvastatin treatment alone, and equally after combined T/A treatment. An approximately 2-fold reduction in the absolute change in plasma VLDL-2 levels was seen 4 hours postprandially in subjects receiving T/A therapy (−8±2 mg/dL; P<0.05) as compared with the phase involving atorvastatin alone (−14±2 mg/dL) or to baseline (−17±2 mg/dL). Equally, the AUC for VLDL-2 levels was significantly reduced after atorvastatin treatment alone (−29%; P<0.001), with an additional decrement of 24% (P<0.001) on T/A treatment (Table 2).

Analysis of triglyceride-rich lipoprotein subfractions revealed a major effect of T/A therapy on the neutral lipid core content of VLDL-1 and VLDL-2 (supplemental Table II). Indeed, under CETP inhibition, the relative proportion of triglyceride increased significantly, whereas a marked reduction in cholesterol ester content occurred concomitantly in both VLDL-1 and VLDL-2 subfractions, thereby resulting in a significant reduction in the CE/TG ratio in these particles as compared with baseline (−50% and −60% in VLDL-1 and VLDL-2, respectively; P<0.0001) and as compared with atorvastatin therapy alone (−27% and −42% in VLDL-1 and VLDL-2, respectively; P<0.0001). By contrast, T/A therapy induced no significant alteration in the chemical composition of both Chylomicrons and IDL as compared with baseline or to atorvastatin therapy alone.

Effects of Torcetrapib on Postprandial LDL and HDL Subfraction Profile
T/A therapy induced marked and differential effects on the postprandial profile of both LDL and HDL subfractions (supplemental Table III). Compared with baseline and to atorvastatin treatment alone, the postprandial AUC for total HDL levels was significantly increased by T/A (+29%; P<0.001) as a result of a marked increment (+60%; P<0.001) in the AUC for large CE-rich HDL2 levels. In addition, T/A therapy induced a marked reduction in plasma HDL3 levels 4 hours (−22±4 mg/dL, P<0.03) and 8 hours (−24±4 mg/dL, P<0.02) postprandially as compared with baseline (−12±4 mg/dL and −9±4 mg/dL at 4 hours and 8 hours, respectively), and as compared with atorvastatin treatment alone (−11±3 mg/dL at 4 hours and 10±3 mg/dL at 8 hours, respectively).

T/A therapy equally induced significant modification in LDL particle profile. Indeed, the AUC for total plasma LDL levels was reduced (−10%; P=0.003) among patients receiving T/A therapy as compared with those receiving atorvastatin alone (supplemental Table III). Moreover, the decrement observed in plasma LDL levels after T/A therapy resulted primarily from a significant and specific additional reduction in plasma dense LDL concentrations (−16%; P<0.001).

Effects of Torcetrapib on Postprandial Endogenous CETP activity
In the fasting state, dyslipidemic subjects receiving atorvastatin alone displayed attenuated plasma CETP activity (−17%; P=0.001); this decrement was considerably greater (+61%; P<0.001) in patients who received combined T/A therapy (41±3% before treatment, 34±3% after atorvastatin alone and 16±1% after T/A therapy) (Figure 4).

Plasma CETP activity was significantly increased 4 hours postprandially in Type IIB patients before treatment (+17% at baseline; P<0.0001) and after each drug phase (+20% after atorvastatin alone and +55% after T/A therapy) as compared with CE transfer activities before meal intake. Interestingly, the marked increment in CETP activity after
CETP activity was associated with marked elevation in postprandial CE mass transferred from HDL to Chylomicrons (+124%; *P*<0.001) and VLDL-1 (+35%; *P*<0.01), whereas the rate of CE transfer from HDL to VLDL-2 and IDL remained essentially unchanged over the postprandial phase (supplemental Figure III). In addition, the evolution of the CE/TG ratio in both chylomicron and VLDL-1 subfractions over the postprandial phase (2 to 8 hours) revealed progressive enrichment of the hydrophobic core of these particles in CE at the expense of triglyceride before treatment and after each drug phase (supplemental Table II, and supplemental Figures I and II). These findings are entirely consistent with an enhanced CETP-mediated CE transfer from HDL to triglyceride-rich lipoproteins, notably to chylomicrons and VLDL-1, during postprandial lipemia in type IIB patients. However, the postprandial CE transfer from HDL to both Chylomicrons and VLDL-1 were significantly reduced after T/A therapy as compared with atorvastatin therapy alone.

**Discussion**

For the first time, we presently demonstrate that CETP inhibition mediated by torcetrapib on a background of statin treatment constitutes a highly efficacious pharmacological approach to attenuate the proatherogenic quantitative and qualitative features of postprandial intravascular lipoprotein metabolism in hyperlipidemic patients at elevated risk of premature CAD. Robust changes attributable to the primary pharmacological action of T/A relative to that of atorvastatin alone included marked and significant reduction in the increment in postprandial triglyceride levels (−30%) with a concomitant increase in the AUC for HDL2 (+58%). Major reductions in circulating levels of postprandial chylomicrons and of large VLDL-1 particles after combined T/A therapy, as well as significant reductions in the CE/TG ratio in these particles relative to those under statin treatment alone, are consistent with marked CETP inhibition and reduced CE mass transfer from HDL to triglyceride-rich lipoproteins during alimentary lipemia. Equally, CETP inhibition was associated with enrichment of postprandial VLDL-1 particles in apoE and depletion in CE, potentially facilitating their lipolysis with rapid receptor-mediated clearance from the circulation. These findings are entirely consistent with a recent study showing that triglyceride response is reduced after an oral fat load in CETP-deficient subjects.20

With respect to the postprandial decrease in VLDL-2, which contrasted with the increase in VLDL-1 levels, it is relevant that subjects with elevated plasma triglyceride levels produce an excess of VLDL-1 apoB, whereas those with raised plasma cholesterol levels overproduce VLDL-2 apoB.21 In plasma, large VLDL particles (VLDL-1 Sf 60 to 400) are eliminated as such or catabolized to smaller VLDL (VLDL-2 Sf 20 to 60) remnants through lipolysis of triglycerides by lipoprotein lipase. Concomitant postprandial elevation of plasma levels of large VLDL-1 and reduction of those of small VLDL-2 mainly result therefore from delayed lipolysis of VLDL-1 particles due to competition between chylomicrons and VLDL-1 for the same lipolytic pathway.22

Torcetrapib has been shown to block the major lipid transfer functions of plasma CETP including cholesteryl ester, triglyceride, and phospholipid transfer.15 In addition, Torcetrapib is without effect on either plasma LCAT or PLTP activities, or on hepatic or lipoprotein lipases.15 In this context, it is of special relevance that significant increase in plasma HDL2 levels and reduction in those of HDL3 occurred throughout the postprandial phase in type IIB patients; this effect was most marked with T/A therapy. Indeed, after Torcetrapib therapy, the decrement in plasma HDL3 concentrations was enhanced, thereby suggesting that remodeling of HDL3 to HDL2 is amplified during postprandial lipemia under conditions of CETP inhibition.6 It is well established that variation in plasma levels of HDL subspecies during postprandial lipemia is associated with enhanced transfer of polar surface components from triglyceride-rich lipoproteins to the HDL pool. Moreover, it has been previously shown that individuals with elevated plasma concentrations of HDL2 particles catabolize chylomicrons at a faster rate than individuals with lower levels.23 In agreement with earlier studies,23–24 we presently observed a significant inverse relationship between the degree of postprandial hypertriglycerideremia and plasma concentrations of HDL2 particles (*P*<0.02). We therefore conclude that elevation in plasma HDL2 levels mediated by CETP inhibition represents a major feature of the action of torcetrapib on intravascular lipoprotein metabolism during postprandial phase in type IIB patients. Clearly then CETP inhibition potentiates enhanced remodeling of small, CE-poor HDL3-like particles to large, CE-rich HDL2.

In human plasma, apoE is mostly associated with apoB or apoAI-containing lipoproteins depending on metabolic state.25 However, several studies have demonstrated the existence of a minor lipoprotein subfraction of HDL size with apoE as the major protein component.26–27 Such an HDL-apoE particle subpopulation has been shown to be active in the transfer of apoE to triglyceride-rich lipoprotein particles during postprandial lipemia.27–28 Furthermore, several studies have reported increased levels of apoE-rich HDL particles in CETP-deficient patients.20,28,29 Therefore it is probable that apoE enrichment of VLDL-1 particles during CETP inhibitor therapy results from enhanced formation of large HDL-apoE.
of triglyceride-rich lipoproteins within the arterial intima, we speculate that the torcetrapib-mediated changes and notably reduction in the postprandial cholesterol burden in triglyceride-rich lipoproteins attenuate the potential atherogenicity of the postprandial phase in type IIB patients. It is equally noteworthy that such patients typically exhibit endothelial dysfunction, hence enhancing such arterial triglyceride-rich lipoprotein deposition.

Our present studies of the impact of Torcetrapib action on postprandial metabolism of apo-B-containing lipoprotein particles, and potentially on atherogenesis at the arterial wall, did not encompass functional evaluation of the biological activities of HDL particles formed under conditions of CETP inhibition, nor of cholesteryl ester flux from HDL to the liver in the reverse cholesterol transport pathway. It is however relevant in this context that Tall and colleagues have demonstrated that torcetrapib-mediated inhibition of CETP modestly increases cholesterol efflux from macrophages to HDL isolated from Torcetrapib-treated patients. Moreover, in the rabbit, a species deficient in hepatic lipase, in vivo transport of cholesteryl esters from HDL to the liver was not perturbed by Torcetrapib-mediated inhibition of CETP. Nonetheless, we cannot exclude the possibility that attenuated CETP-mediated transfer of cholesteryl esters from HDL to apoB-containing lipoprotein acceptors, with ensuing accumulation of large cholesteryl ester–rich HDL particles may have attenuated flux of cholesteryl esters to the liver. Whether such a potential effect may have contributed to the failure of Torcetrapib to reduce atherosclerosis progression per se in recent clinical trials remains indeterminate. Further metabolic studies of these questions are critically required.

In conclusion, we demonstrate for the first time that Torcetrapib, a potent CETP inhibitor, in association with atorvastatin, not only significantly reduces the postprandial formation and accumulation of atherogenic triglyceride-rich subspecies including chylomicrons and VLDL-1 in male subjects displaying type IIB hyperlipidemia, but equally attenuates their atherogenicity by reducing core CE content. In this way, pharmacological inhibition of CETP by Torcetrapib, in association with atorvastatin-mediated upregulation of cellular LDL receptors, acts in concert to reduce cardiovascular risk in this mixed dyslipidemic phenotype typical of common metabolic diseases such as Type 2 diabetes and metabolic syndrome.

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Disclosures
T.N., T.T., and C.L.S. were employees of Pfizer during this study.

References


Inhibition of CETP by Torcetrapib Attenuates the Atherogenicity of Postprandial TG-Rich Lipoproteins in Type IIB Hyperlipidemia
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SUPPLEMENTAL METHODS

SUBJECTS

Plasma cholesterol (ranged from 205 mg/dl to 316 mg/dl) and triglycerides (ranged from 117 mg/dl to 366 mg/dl) concentrations were verified 15 days before inclusion in the study and on a second occasion 7 days before the initial blood sampling at inclusion. We cannot exclude the possibility that a patient(s) with type IIB phenotype may in fact have displayed Familial Hypercholesterolemia (FCH). Patients were excluded if they displayed dysbetalipoproteinemia, diabetes mellitus, secondary causes of hyperlipidemia such as uncontrolled hypothyroidism, renal impairment or nephrotic syndrome, or known liver or muscle disease. Other exclusion criteria included uncontrolled hypertension, or history of a major cardiovascular event. Subjects were on average overweight (Body Mass Index: 27±2 kg/m²) but none was obese nor displayed the apolipoprotein E2/E2 genotype.

In this single-blind fixed combination study, torcetrapib/atorvastatin was well tolerated and safety data observed were consistent with previous treatment experience with torcetrapib/atorvastatin as well as with the study population of subjects with Type IIb mixed dyslipidemia. There was no significant change in mean BP at the end of the study. There were no serious adverse events or discontinuations from therapy. Three subjects (16.7%) experienced adverse events during the atorvastatin phase of the study; 2 of these experienced myalgia which was considered related to treatment by the investigator. One subject experienced 1 adverse event (creatinine kinase elevation :697 U/L; UPN: 130 U/L) during the T/A phase of the study, which the investigator considered unrelated to treatment. Mean SBP (sitting) at baseline was 124.0±2.3 mm Hg. Treatment with atorvastatin alone for 6 weeks was associated with a decrease in SBP as compared to baseline (-2.1 mm Hg). Treatment with
atorvastatin alone (10mg/d) for 6 weeks followed by 6 weeks of treatment with T/A (60/10mg/d) resulted in an increase of +5.5 mm Hg in SBP as compared to baseline. Mean DBP (sitting) at baseline was 79.1±1.5 mm Hg. Mean DBP was 77.2±1.9 mm Hg following atorvastatin alone and 81.7±1.1 following T/A therapy. No subject displayed an adverse event related to blood pressure, and none discontinued the study due to a blood pressure increase or became hypertensive. Mean heart rate at baseline was 68.5±2.8 bpm and 70.1±3.1 and 68.8±2.7 following atorvastatin alone and following T/A therapy, respectively.

**STUDY DESIGN AND POSTPRANDIAL TIME COURSE**

After an overnight fast, blood samples were obtained: (i) after the drug wash-out/diet run-in period (B: baseline), (ii) after six weeks of atorvastatin treatment at 10mg/d (A) and (iii) after an additional six weeks of combination torcetrapib/atorvastatin treatment 60/10 mg (T/A). For each subject, a postprandial time course was performed as follows: a standardized breakfast (300 kcal) was given at 8:30 am at which time study drug was taken. A test meal containing a total of 1200 kcal was then consumed at 11:30 am. Subjects were asked to abstain from alcohol, smoking and vigorous exercise for 24 hours before the day of the test. Blood samples were obtained immediately before the test meal and at 1h, 2h, 4h, 6h and 8h after ingestion. Blood was collected by venipuncture from the antecubital vein into sterile EDTA-containing tubes (final concentration of EDTA, 1mg/ml), and plasma separated immediately by low-speed centrifugation (2500 rpm) for 20 min at 4°C.

**BIOCHEMICAL ANALYSIS**
The lipid content of plasma and isolated lipoprotein fractions, total protein and apolipoproteins was quantified with a calibrated AutoAnalyzer (Konelab 20). Total cholesterol and triglyceride levels were determined with reagent kits from Roche Diagnostics and ThermoElectron, respectively. The levels of unesterified cholesterol and choline-containing phospholipids (lecithin, lysolecithin and sphingomyelin) which represent >90% of lipoprotein phospholipids were determined with reagent kits (Wako Diagnostics). Cholesteryl ester (CE) mass was calculated as (TC-FC) x 1.67 and thus represents the sum of the esterified cholesterol and fatty acid moieties.\(^4\) Bicinchoninic acid assay reagent (Pierce) was utilized for protein quantification. Lipoprotein mass was calculated as the sum of the mass of the individual lipid and protein components for each lipoprotein fraction. Fasting plasma LDL-cholesterol was calculated using the Friedewald formula. HDL-cholesterol levels were determined after dextran sulfate-magnesium precipitation of apolipoprotein B-containing lipoproteins. ApoAI and apoB concentrations were determined using immunoturbidimetric assays (ThermoElectron reagents and calibrators). ApoAI, apoE, apoCII and apoCIII levels were determined using immunoturbidimetric assays (Wako Diagnostics reagents and calibrators). The coefficients of variation, intra-assay and inter-assay, for chemical analysis of lipids and apolipoproteins were <5%.

**Lipoprotein Fractionation**

Chylomicrons (CM; Sf>400) were isolated by centrifugation at 20,000 rpm for 45 min at 15°C using a SW41 Ti rotor in a Beckman XL70 ultracentrifuge.\(^1\) Subfractions of triglyceride-rich lipoproteins, i.e. VLDL1 (Sf 60-400), VLDL2 (Sf 20-60) and IDL (Sf 12-20) were isolated from chylomicron free-plasma by nonequilibrium density gradient ultracentrifugation as previously described.\(^5\) LDL and HDL subfractions
were isolated from chylomicron free-plasma by isopycnic density gradient ultracentrifugation.

**DETERMINATION OF ENDOGENOUS CETP ACTIVITY**

Determination of endogenous CE transfer from HDL to apolipoprotein B-containing lipoproteins was assayed by modification of the method of Guérin et al. as previously described. Radiolabeled $^3$H-HDL were isolated from the d>1.063 g/ml plasma fraction (1 ml) as previously described. Cholesteryl ester transfer was determined after incubation of whole plasma from individual subjects at 37°C or 0°C for 3 hours in the presence of radiolabeled HDL (less than 5% of the total HDL-CE mass present in 1 ml of subject’s plasma) and iodoacetamide (final concentration 1.5 mmol/l) for inhibition of Lecithin Cholesterol Acyltransferase (LCAT). Then, for determination of plasma CETP activity, apolipoprotein B-containing lipoproteins were precipitated using the dextran sulfate-magnesium procedure. The radioactive content of the supernatant was quantified by liquid scintillation spectrometry with a Trilux 1450 (Perkin Elmer). CETP activity (expressed as the percentage of CE transferred from HDL to total apoB-containing lipoproteins), was calculated as the amount of label recovered in the supernatant after incubation and divided by the amount of label present in the supernatant before incubation. CETP-dependent CE transfer was calculated from the difference between the radioactivity transferred at 37°C and 0°C.

For determination of CE transfer from HDL to individual triglyceride-rich lipoproteins, CM, VLDL1, VLDL2 and IDL were isolated as described above. The radioactive CE content of each isolated lipoprotein fraction was quantified and the rate of CE transfer was calculated from the known specific radioactivity of radiolabelled HDL-CE and expressed as $\mu$g CE transferred/h/ml plasma.
**STATISTICAL ANALYSIS**

Experimental data were analyzed using the SAS Package software (SAS/STAT User’s Guide, Version 8 by SAS Institute Inc., Cary, NC, 1999). The paired t-test was used to evaluate changes observed from baseline (B: Before Treatment) in plasma lipid, apolipoprotein or lipoprotein subfraction levels and CETP activity after atorvastatin (A) or torcetrapib/atorvastatin (T/A) therapy. Postprandial lipemia was quantified by calculating the area under the curve (AUC) and the incremental AUC (iAUC) for plasma triglyceride and lipoprotein subfractions. The incremental AUC represents the increase in area in response to the test meal relative to lipid or lipoprotein concentrations determined before meal intake. Repeated-measure analysis of variance was performed to assess changes in plasma lipid levels, in lipoprotein concentrations and in CETP activity during the postprandial phase. Results were considered statistically significant at p<0.05. Values are given as means±SE.

**REFERENCES**


**Supplemental Table I**

**Postprandial variations in plasma apoE and in HDL-apoE in Type IIB patients Before and After Drug Phases**

<table>
<thead>
<tr>
<th></th>
<th>Hours</th>
<th>Before Treatment</th>
<th>Atorvastatin Alone</th>
<th>Torcetrapib/Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma apoE</strong></td>
<td>0</td>
<td>4.0±0.2</td>
<td>3.0±0.2</td>
<td>2.8±0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.7±0.3</td>
<td>2.9±0.2</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.8±0.2</td>
<td>3.4±0.3</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>3.8±0.2</td>
<td>3.7±0.3</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td><strong>HDL-apoE</strong></td>
<td>0</td>
<td>0.91±0.13</td>
<td>0.55±0.08</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.81±0.10</td>
<td>0.42±0.05</td>
<td>0.38±0.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.82±0.08</td>
<td>0.41±0.06</td>
<td>0.34±0.04</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.94±0.07</td>
<td>0.51±0.09</td>
<td>0.36±0.05</td>
</tr>
</tbody>
</table>

Values are expressed in mean±SE.
Supplemental Table II: Modification of Neutral lipid content of Triglyceride-Rich Lipoprotein Subspecies during Postprandial State Before and After Drug Phases

<table>
<thead>
<tr>
<th>Hours</th>
<th>Before Treatment (Baseline, n=18)</th>
<th>Atorvastatin Alone (A, 10mg/day, n=18)</th>
<th>Torcetrapib/Atorvastatin (T/A, 60/10 mg/day, n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%CE</td>
<td>%TG</td>
<td>CE/TG</td>
</tr>
<tr>
<td>CM (Sf&gt;400)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16.5±1.4</td>
<td>60.6±2.0</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>8.4±0.7§</td>
<td>74.7±1.1§</td>
<td>0.11§</td>
</tr>
<tr>
<td>4</td>
<td>6.7±0.9§</td>
<td>76.5±0.9§</td>
<td>0.09§</td>
</tr>
<tr>
<td>8</td>
<td>14.5±1.2</td>
<td>63.7±1.6</td>
<td>0.24</td>
</tr>
<tr>
<td>VLDL-1 (Sf 60-400)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>12.2±0.5</td>
<td>56.9±0.9</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>11.4±0.4§</td>
<td>59.1±0.8§</td>
<td>0.19§</td>
</tr>
<tr>
<td>4</td>
<td>11.2±0.4§</td>
<td>59.0±0.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>13.0±0.5</td>
<td>56.3±0.8</td>
<td>0.23</td>
</tr>
<tr>
<td>VLDL-2 (Sf 20-60)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.6±1.0</td>
<td>26.9±1.0</td>
<td>1.05</td>
</tr>
<tr>
<td>2</td>
<td>27.2±0.8</td>
<td>28.5±1.4</td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td>27.1±0.9</td>
<td>27.5±1.0</td>
<td>1.02</td>
</tr>
<tr>
<td>8</td>
<td>26.9±0.8</td>
<td>28.3±1.0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are mean±SE and are expressed as percent mean weight. CE: Cholesteryl Esters. TG: Triglyceride.
* p<0.001 and ‡ p<0.05 versus Baseline. † p<0.001 versus therapy with atorvastatin alone. § p<0.005 and || p<0.05 versus 8 hours.
Supplemental Table III: Postprandial areas under the curve for LDL and HDL subfractions in Type IIB patients Before and After Drug Phases

<table>
<thead>
<tr>
<th></th>
<th>AUC 0-8h</th>
<th>% Change from (B)</th>
<th>iAUC 0-8h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>3432±163</td>
<td></td>
<td>-12±44</td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>2327±83*</td>
<td>-32%</td>
<td>-87±24</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>1994±106*†</td>
<td>-42%</td>
<td>-69±29</td>
</tr>
<tr>
<td><strong>Light LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>509±28</td>
<td></td>
<td>-7±14</td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>317±20*</td>
<td>-38%</td>
<td>-17±11</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>293±20*</td>
<td>-43%</td>
<td>-5±15</td>
</tr>
<tr>
<td><strong>Intermediate LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>1062±97</td>
<td>40±31</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>702±54*</td>
<td>-34%</td>
<td>-20±21$</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>703±46#</td>
<td>-34%</td>
<td>-5±21</td>
</tr>
<tr>
<td><strong>Dense LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>1861±107</td>
<td>-45±43</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>1309±58*</td>
<td>-30%</td>
<td>-50±19</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>998±63‡</td>
<td>-46%</td>
<td>-60±16</td>
</tr>
<tr>
<td><strong>Total HDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>2365±95</td>
<td>15±23</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>2363±102</td>
<td>0%</td>
<td>-28±22</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>3060±148‡</td>
<td>+29%</td>
<td>-59±40</td>
</tr>
<tr>
<td><strong>HDL2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>1129±77</td>
<td>83±21</td>
<td></td>
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<tr>
<td>Atorvastatin (A)</td>
<td>1182±75</td>
<td>+2%</td>
<td>86±20</td>
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<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>1801±132‡</td>
<td>+60%</td>
<td>85±35</td>
</tr>
<tr>
<td><strong>HDL3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before Treatment (B)</td>
<td>1237±38</td>
<td></td>
<td>-68±22</td>
</tr>
<tr>
<td>Atorvastatin (A)</td>
<td>1213±40</td>
<td>-2%</td>
<td>-74±18</td>
</tr>
<tr>
<td>Torcetrapib/Atorvastatin (T/A)</td>
<td>1260±37</td>
<td>+2%</td>
<td>-148±23$§</td>
</tr>
</tbody>
</table>

Values at B correspond to lipoprotein levels at baseline. Values at A correspond to lipoprotein levels after 6 weeks of atorvastatin treatment (10 mg/day) and values at T/A correspond to lipoprotein levels after 6 weeks of therapy with torcetrapib/atorvastatin (60/10 mg/d). AUC: Area under the curve. iAUC: incremental AUC. Light LDL subfraction (d=1.019 to 1.029 g/ml), Intermediate LDL subfraction (d=1.023 to 1.039 g/ml) and dense LDL subfraction (d=1.039 to 1.063 g/ml). Values are mean±SE.

* p<0.001, † p<0.01 and ‡ p<0.05 versus Baseline.
$p<0.001, ^p<0.01$ and $^p<0.05$ versus therapy with atorvastatin alone.
**LEGEND**

**Supplemental Figure I:** Postprandial time course of plasma Chylomicron: CE, TG, protein, apoE, apoCII, apoCIII levels in type IIb subjects before (open circles), after atorvastatin (closed circles) and after combination Torcetrapib/atorvastatin (closed triangles). *p<0.001, #p<0.01 and $ p<0.05 versus Baseline. ‡ p<0.001 and †p<0.01 versus therapy with atorvastatin alone.
LEGEND

Supplemental Figure II: Postprandial time course of plasma VLDL-1: CE, TG, apoB, apoE, apoCII and apoCIII levels in type IIb subjects before (open circles), after atorvastatin (closed circles) and after combination Torcetrapib/atorvastatin (closed triangles). *p<0.001, #p<0.01 and $ p<0.05 versus Baseline. ‡ p<0.001, †p<0.01 and §p<0.05 versus therapy with atorvastatin alone.
Supplemental Figure III

A

CE Transfer to CM
(μg CE Transferred/h/ml)

+161%

+149%

+124%

B A T/A

B A T/A

B A T/A

B A T/A

B A T/A

B A T/A

B A T/A

Transfer to VLDL-1
(μg CE Transferred/h/ml)

+174%

+52%

+35%

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Supplemental Figure III: Bar graph showing CETP-mediated CE mass transferred from HDL to triglyceride-rich lipoproteins: Chylomicron (Panel A), VLDL-1 (Panel B) determined before (open bar) and 4 hours after meal intake (closed bar) in type IIB subjects before, after atorvastatin and after combination Torcetrapib/atorvastatin. Inserts: dashed bar graph showing the absolute change in CE mass transferred from HDL to chylomicron and VLDL-1 in response to ingestion of a typical Western meal in type IIB subjects. #p<0.01 and $ p<0.05 versus Baseline. †p<0.01 and §p<0.05 versus therapy with atorvastatin alone.