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Torcetrapib Does Not Reduce Atherosclerosis Beyond Atorvastatin and Induces More Proinflammatory Lesions Than Atorvastatin

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Background—Although cholesteryl ester transfer protein (CETP) inhibition is regarded as a promising strategy to reduce atherosclerosis by increasing high-density lipoprotein cholesterol, the CETP inhibitor torcetrapib given in addition to atorvastatin had no effect on atherosclerosis and even increased cardiovascular death in the recent Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events trial. Therefore, we evaluated the antiatherogenic potential and adverse effects of torcetrapib in humanized *APOE*3-Leiden.CETP* (*E3L.CETP*) mice.

Methods and Results—*E3L.CETP* mice were fed a cholesterol-rich diet without drugs or with torcetrapib (12 $mg \cdot kg^{-1} \cdot d^{-1}$), atorvastatin (2.8 $mg \cdot kg^{-1} \cdot d^{-1}$), or both for 14 weeks. Torcetrapib decreased CETP activity in both the absence and presence of atorvastatin (−74% and −73%, respectively; $P<0.001$). Torcetrapib decreased plasma cholesterol (−20%; $P<0.01$), albeit to a lesser extent than atorvastatin (−42%; $P<0.001$) or the combination of torcetrapib and atorvastatin (−40%; $P<0.001$). Torcetrapib increased high-density lipoprotein cholesterol in the absence (30%) and presence (34%) of atorvastatin. Torcetrapib and atorvastatin alone reduced atherosclerotic lesion size (−43% and −46%; $P<0.05$), but combination therapy did not reduce atherosclerosis compared with atorvastatin alone. Remarkably, compared with atorvastatin, torcetrapib enhanced monocyte recruitment and expression of monocyte chemoattractant protein-1 and resulted in lesions of a more inflammatory phenotype, as reflected by an increased macrophage content and reduced collagen content.

Conclusions—CETP inhibition by torcetrapib per se reduces atherosclerotic lesion size but does not enhance the antiatherogenic potential of atorvastatin. However, compared with atorvastatin, torcetrapib introduces lesions of a less stable phenotype. **(*Circulation*. 2008;117:2515-2522.)**

Key Words: atherosclerosis ■ cholesteryl ester transfer proteins ■ drugs ■ lipids ■ lipoproteins

The cholesteryl ester transfer protein (CETP) is an important regulator of the high-density lipoprotein cholesterol (HDL-C) level. CETP is secreted predominantly by the liver and associates mainly with HDL in plasma, where it transports cholesteryl esters from HDL to very low-density lipoprotein (VLDL) in exchange for triglycerides[1,2] and thus lowers HDL-C. HDL is atheroprotective in that it mediates reverse cholesterol transport (ie, transport of cholesterol from the vessel wall to the liver) and has antiinflammatory, antithrombotic, and antioxidative properties.[3,4] Therefore, CETP inhibition is regarded as a promising strategy to increase HDL-C levels and to reduce atherosclerosis.[2] However, the effect of CETP activity on atherosclerosis in humans has not been unequivocally determined. Mutations in the CETP gene that reduce CETP mass and activity—eg, D442G and Int14 G(+1)>A—lead to elevated HDL-C levels,[5,6] but the effects of these mutations on atherosclerosis are still in dispute.[7–10]

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Torcetrapib, which forms an inactive complex between CETP and HDL,[2] was the first CETP inhibitor tested in large human trials, in which it was shown to increase HDL-C levels by

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The online-only Data Supplement, which contains a table, can be found with this article at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.107.761965/DC1.

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≈60%.[11–13] The resulting HDL particles were able to mediate cellular cholesterol efflux more efficiently.[14] However, the large-scale Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial was stopped prematurely because of an excess of deaths in patients receiving torcetrapib with atorvastatin compared with those receiving atorvastatin alone, related mainly to cardiovascular events.[15] In addition, the Rating Atherosclerosis Disease Change by Imaging With a New CETP Inhibitor (RADIANCE) and Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trials revealed no therapeutic benefit of combining torcetrapib with atorvastatin with respect to atherosclerosis progression as assessed by coronary intima-media thickness and intravascular ultrasonography measurements.[11–13]

The effect of torcetrapib alone on atherosclerosis, however, has not yet been evaluated in humans, and the mechanism underlying the increased death rate associated with torcetrapib treatment has not yet been elucidated. Therefore, we examined the effect of torcetrapib with or without atorvastatin on atherosclerosis development in humanized *APOE*3-Leiden.CETP* (*E3L.CETP*) transgenic mice.[16] *E3L* mice show a human-like response to lipid-lowering therapies.[17] Crossbreeding with *CETP* transgenic mice, which express human CETP under control of its natural flanking regions, resulted in *E3L.CETP* mice that also respond to HDL-modulating intervention.[18,19]

Methods

Animals

Human CETP transgenic mice that express CETP under control of its natural flanking regions (strain 5203)[20] were obtained from The Jackson Laboratory (Bar Harbor, Me) and crossbred with *E3L* mice[21] to obtain *E3L.CETP* mice.[16] All mice used in this study were heterozygous *E3L.CETP* transgenic females on a C57Bl/6 background. Mice were housed under standard conditions with a 12-hour light/dark cycle and had free access to food and water unless indicated otherwise. Mice were fed regular chow (Ssniff, Soest, Germany) or a diet with 15% (wt/wt) cacao butter (diet T, Hope Farms, Woerden, the Netherlands) supplemented with 0.1% or 0.25% (wt/wt) cholesterol (Sigma-Aldrich, Zwijndrecht, the Netherlands) with or without torcetrapib (2R,4S)-4-[[[3,5bis(trifluoromethyl) phenyl]methyl]-(methoxycarbonyl)amino]-2-ethyl-3,4-dihydro-6-trifluoromethyl)-3-phenyl-1(2H)-quinolinecarboxylic acid, ethyl ester ($C_{26}H_{25}N_2O_4F_9$) (kindly provided by Roche, Basel, Switzerland) and/or atorvastatin ([R-(R*,R*)]-2-(4-fluorophenyl)-β,Δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid ($C_{33}H_{24}FN_2O_5$) (Lipitor, Pfizer, Capelle a/d IJssel, the Netherlands). Unless indicated otherwise, blood was drawn after 4 hours of fasting in EDTA-containing cups by tail bleeding, and plasma was isolated. All animal experiments were approved by the institutional ethics committee on animal care and experimentation.

Single Torcetrapib Treatment

To verify that torcetrapib inhibits CETP activity in *E3L.CETP* mice in vivo, mice on a chow diet were given a single intragastric gavage of torcetrapib (0, 1, 3, and 10 mg/kg) in ≈200 μL ethanol:solutol:saline 10:10:80 (vol:vol:vol). Blood was drawn before gavage and at 1, 2, 4, 6, 8, and 24 hours after gavage. During the first 8 hours after gavage, mice were fasted. Plasma was assayed for total CETP activity as described below. Alternatively, mice were fed a diet containing 15% cacao butter with 0.1% or 0.25% cholesterol, and the effect of 10 mg/kg torcetrapib was determined on plasma CETP activity at 2 hours after gavage.

Total Plasma CETP Activity, Endogenous CETP Activity, and CETP Mass

Total plasma CETP activity was measured as the transfer of [^{3}H]cholesteryl oleate from LDL to HDL.[16] Briefly, 5 μL (diluted) mouse plasma was incubated with human [^{3}H][^{3}H]cholesteryl oleate–labeled LDL and HDL in sodium phosphate buffer containing 5,5′-dithio-bis(2-nitrobenzoic acid) to inhibit lecithin-cholesterol acyltransferase activity. After overnight incubation, LDL was precipitated. The supernatant containing [^{3}H][^{3}H]cholesteryl oleate–HDL was counted for ^{3}H activity. CETP activity was calculated as nanomoles of cholesteryl ester transfer per 1 mL plasma per 1 hour. Endogenous CETP activity was determined by a fluorescent method using donor liposomes enriched with nitrobenzoxadiazole-labeled cholesteryl esters (RB-CETP, Roar Biomedical, New York, NY) as described.[22] CETP mass was determined with the Daiichi CETP ELISA kit according to manufacturer's instructions (Daiichi, Tokyo, Japan).

Long-Term Torcetrapib Treatment

To determine the effect of torcetrapib without and with atorvastatin on atherosclerosis development and plasma cholesterol, *E3L.CETP* mice were fed a diet containing 0.25% cholesterol to increase plasma cholesterol levels to ≈16 mmol/L. After 4 weeks, mice were randomized into 4 groups according to their plasma cholesterol levels. Mice were fed a control diet or a diet with atorvastatin (0.0023% at 2.8 mg · kg^{-1} · d^{-1}), torcetrapib (0.01% at 12 mg · kg^{-1} · d^{-1}), or both. Blood was drawn 1 week before randomization and at weeks 6, 9, and 14 of drug treatment and was assayed for lipids, CETP mass, and activity. After 14 weeks, mice were euthanized, and atherosclerosis development was assessed as described below.

Plasma Lipids and Lipoprotein Profiles

Plasma was assayed for cholesterol and phospholipids with commercially available enzymatic kits according to the manufacturer's protocols (236691, Roche Molecular Biochemicals, Indianapolis, Ind; and phospholipids B, Wako Chemicals, Neuss, Germany, respectively). To determine the lipid distribution over plasma lipoproteins, lipoproteins were separated by fast protein liquid chromatography. Plasma was pooled per group, and 50 μL of each pool was injected onto a Superose 6 HR 10/30 column (Äkta System, Amersham Pharmacia Biotech, Piscataway, NJ) and eluted at a constant flow rate of 50 μL/min in PBS and 1 mmol/L EDTA, pH 7.4. Fractions of 50 μL were collected and assayed for cholesterol and phospholipids as described above.

Atherosclerosis Quantification

After 14 weeks of drug intervention, mice were killed by CO_2 inhalation. Blood was drawn via cardiac puncture, and hearts were isolated. Hearts were fixed in phosphate-buffered 4% formaldehyde, dehydrated, embedded in paraffin, and cross-sectioned (5 μm) throughout the aortic root area. Four sections per mouse with 50-μm intervals were used for atherosclerosis measurements. Sections were stained with hematoxylin-phloxin-saffron for histological analysis. Lesions were categorized for severity according to the guidelines of the American Heart Association, adapted for mice.[23,24] Various types of lesions were discerned: type 0 (no lesions), types 1 through 3 (early fatty streak-like lesions containing foam cells), and type 4 to 5 (advanced lesions containing foam cells in the media, presence of fibrosis, cholesterol clefts, mineralization, and/or necrosis). Lesion area was determined with Leica Qwin image analysis software (EIS, Asbury, NJ). AIA 31240 antiserum (1:3000, Accurate Chemical and Scientific, Westbury, NY) was used to quantify the macrophage area and the number of monocytes adhering to the endothelium. Sirius Red was used to quantify the collagen area, and the antibody M0851 (1:800, Dako) against smooth muscle cell actin to quantify the smooth muscle cell area. Monocyte chemoattractant protein-1 (MCP-1) was detected with goat anti-mouse MCP-1 (M18, 1:300, Santa Cruz Biotechnology, Santa Cruz, Calif).

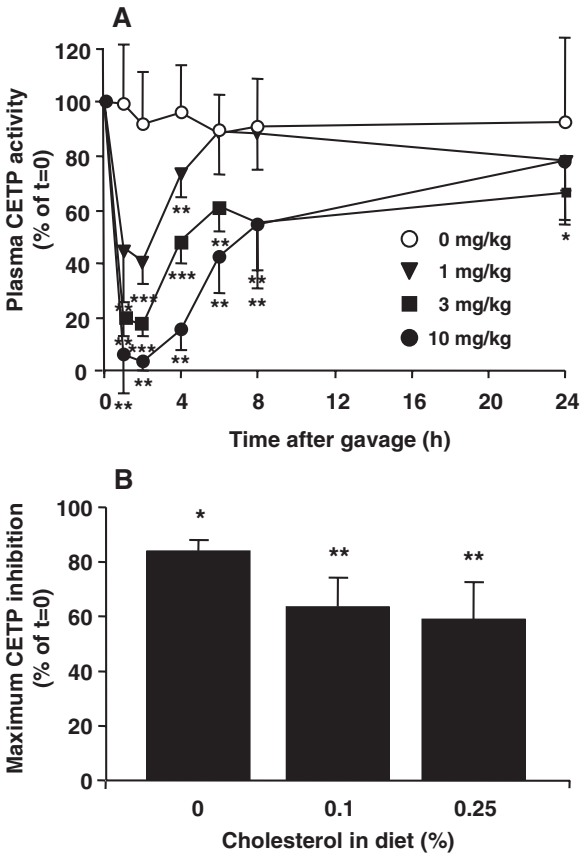

Figure 1. A single dose of torcetrapib inhibits CETP in vivo. *E3L.CETP* mice fed a chow diet received the indicated amounts of torcetrapib via intragastric gavage. Blood was drawn at the indicated time points, and plasma was assayed for CETP activity (A). *E3L.CETP* mice, fed a chow diet or a diet containing 0.1% and 0.25% cholesterol, received torcetrapib (10 mg/kg) by intragastric gavage, and total CETP activity was measured 2 hours after gavage (B). Values are mean±SD (n=4 to 6). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs the control group.

Statistical Analysis

Data are presented as mean±SD unless indicated otherwise. Statistical differences were assessed with the Mann–Whitney *U* test. For lesion typing, differences were assessed by the χ^2 test. SPSS 14.0 (SPSS Inc, Chicago, Ill) was used for statistical analysis. Values of $P<0.05$ were regarded as statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Torcetrapib Inhibits CETP Activity in E3L.CETP Mice

To verify that *E3L.CETP* mice appropriately respond to CETP inhibition, *E3L.CETP* mice on a chow diet received an oral garage of torcetrapib (1, 3, and 10 mg/kg) or vehicle. As expected, torcetrapib time and dose dependently reduced plasma CETP activity, reaching a minimum at 2 hours after gavage (−59±8%, −83±4%, and −96±4%; $P<0.01$). At 3 and 10 mg/kg, significant reductions were still observed after 8 hours (−45±25% and −45±17%, respectively; $P<0.01$; Figure 1A). Because feeding *E3L.CETP* mice cholesterol increases plasma CETP mass and activity,[16] we next measured the inhibitory capacity of torcetrapib on plasma CETP activity in mice fed a diet without or with 0.1% (wt/wt) or 0.25% (wt/wt) cholesterol, which increased plasma CETP activities (3.4- and 4.3-fold, respectively). Despite the increase in plasma CETP activity, an oral gavage of torcetrapib (10 mg/kg) still profoundly decreased CETP activity in the presence of 0.1% (−64±11%; $P<0.05$) and 0.25% (−59±13%; $P<0.05$) cholesterol in the diet (Figure 1B).

Torcetrapib Reduces Plasma Cholesterol Levels to a Lesser Extent Than Atorvastatin

To determine the effect of torcetrapib on plasma lipid levels in the absence or presence of atorvastatin, *E3L.CETP* mice were fed a diet containing 0.25% (wt/wt) cholesterol without or with torcetrapib and/or atorvastatin. Addition of torcetrapib, atorvastatin, or both to the diet did not affect food intake or body weights of *E3L.CETP* mice (not shown). The cholesterol-rich diet resulted in a plasma cholesterol level of 16.1±3.5 mmol/L in the control group. Torcetrapib decreased plasma cholesterol (−20%; $P<0.01$) to a lesser extent than atorvastatin (−42%; $P<0.001$). The combination of torcetrapib and atorvastatin did not decrease plasma cholesterol further compared with atorvastatin alone (−40% versus −42%; Figure 2A). Because torcetrapib and atorvastatin consistently lowered plasma cholesterol throughout the study (see Table in the online-only Data Supplement), they similarly decreased total cholesterol exposure (Figure 2B). Thus, torcetrapib alone reduced total cholesterol exposure to a lesser extent than atorvastatin and combination therapy (Figure 2B).

To determine the distribution of lipids over lipoproteins, lipoproteins were fractionated by fast protein liquid chromatography, and cholesterol and phospholipids were measured in the individual fractions (Figure 3). Torcetrapib reduced VLDL-C (−26%; Figure 3A) to a lesser extent than atorvastatin (−42%; Figure 3A and 3B), and torcetrapib did not enhance the VLDL-C–reducing effect of atorvastatin (Figure 3B). In addition, torcetrapib increased plasma HDL-C levels by 30% in the absence of atorvastatin (Figure 3A) and by 34% in the presence of atorvastatin as judged from the cholesterol content of the fast protein liquid chromatography fractions 17 to 22 (Figure 3B). This torcetrapib-induced increase in HDL-C was paralleled by an increase in phospholipids in the HDL fractions (Figure 3C and 3D). Despite these increased HDL-C levels, apolipoprotein A-I levels were not altered by torcetrapib treatment (not shown).

Torcetrapib Reduces CETP Activity and Increases CETP Mass, Whereas Atorvastatin Decreases Both CETP Activity and Mass

Torcetrapib decreased CETP activity efficiently in both the absence (−73%; $P<0.001$) and presence (−74%; $P<0.001$) of atorvastatin (Figure 4A). Atorvastatin alone also decreased CETP activity but to a lesser extent (−32%; $P<0.001$). Despite the decreased CETP activity, torcetrapib treatment increased CETP mass (33%; $P<0.001$). On the contrary, atorvastatin decreased CETP mass (−24%; $P<0.001$), whereas the combination therapy did not significantly affect CETP mass compared with untreated mice (Figure 4B).

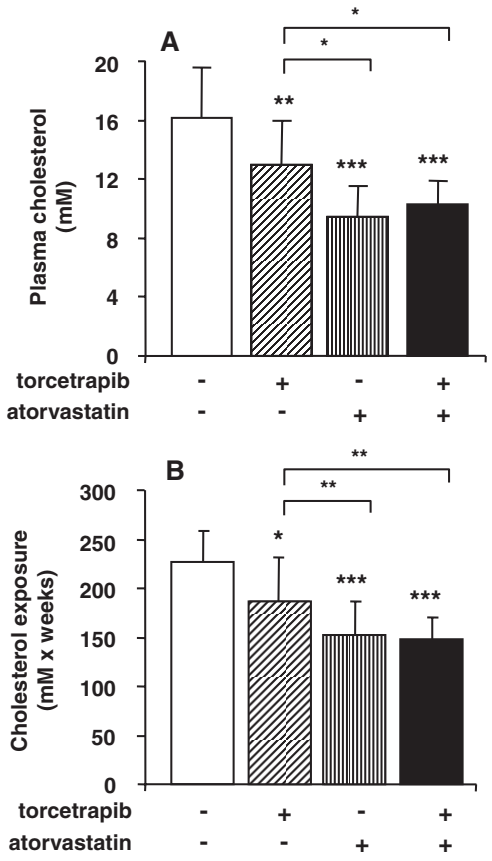

Figure 2. Torcetrapib reduces plasma cholesterol to a lesser extent than atorvastatin. Mice were fed a diet containing 0.25% cholesterol without or with torcetrapib (0.01%), atorvastatin (0.0023%), or both. After 9 weeks of drug intervention, blood was drawn, and plasma was assayed for cholesterol (A). Blood was drawn at additional time points (0, 6, 9, and 14 weeks), and total cholesterol was measured. Total cholesterol exposure during the study was calculated (B). Values are mean±SD (n=14 to 15). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs the control group.

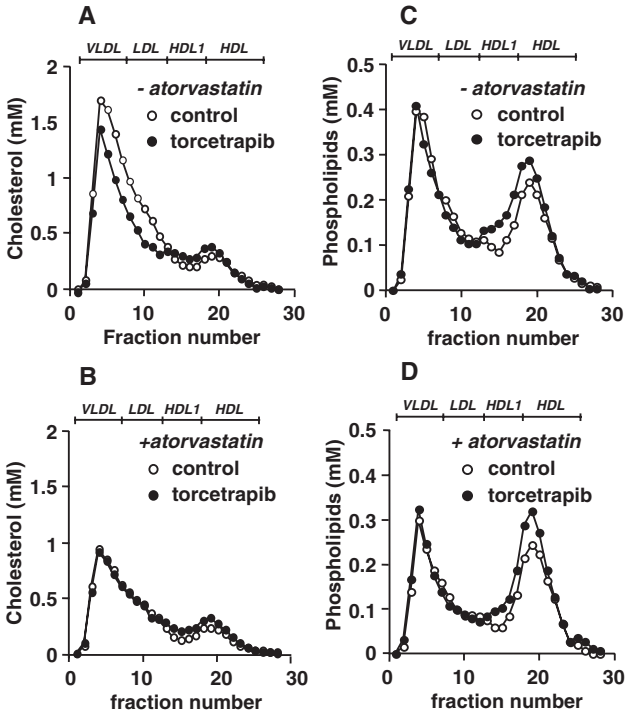

Figure 3. Torcetrapib reduces plasma VLDL and increases HDL levels. Mice were fed a diet containing 0.25% cholesterol without or with torcetrapib (0.01%), atorvastatin (0.0023%), or both. After 14 weeks of drug intervention, blood was drawn, and plasma was pooled per treatment group (n=14 to 15). Pooled plasma was fractionated using fast protein liquid chromatography on a Superose 6 column, and the individual fractions were assayed for total cholesterol (A and B) and phospholipids (C and D).

These data are in line with previous observations that torcetrapib increases CETP mass in humans despite the decrease in CETP activity[25] and that atorvastatin decreases CETP levels[26,27] by decreasing CETP expression.[19]

Torcetrapib Reduces Atherosclerotic Lesion Severity and Lesion Area but Does Not Enhance the Antiatherogenic Effect of Atorvastatin

To determine the effect of torcetrapib on atherosclerosis development in the absence or presence of atorvastatin, the 4 groups of mice were euthanized after 14 weeks, and atherosclerosis severity and lesion size were measured in the aortic root. Representative pictures of each group are shown in Figure 5A. Compared with the control group, mice treated with torcetrapib, atorvastatin, or both had more lesion-free sections and fewer severe lesions of type 4 to 5. Thus, torcetrapib, atorvastatin, and their combination reduced lesion severity similarly (Figure 5B). Accordingly, torcetrapib and atorvastatin alone induced a similar reduction in lesion area (−43% and −46%, respectively; $P<0.05$). Combination treatment also reduced atherosclerosis compared with the control group (−60%; $P<0.001$) but did not significantly enhance the atherosclerosis-reducing potency of atorvastatin alone (Figure 5C).

Torcetrapib Induces Monocyte Recruitment and Results in a More Proinflammatory Lesion Phenotype Compared With Atorvastatin

We next evaluated the effect of torcetrapib, atorvastatin, and their combination on monocyte recruitment and lesion composition with respect to the macrophage area, smooth muscle cell area, and collagen area. Torcetrapib alone and in combination with atorvastatin increased the adherence of monocytes to the vessel wall compared with the control- and atorvastatin-treated groups (Figure 6A). Although torcetrapib did not significantly raise MCP-1 compared with the control group, torcetrapib significantly increased MCP-1 compared with atorvastatin (99%; $P<0.05$; Figure 6B). The increase in adhering monocytes induced by torcetrapib was accompanied by an increased area of macrophages in the intima (Figure 6C). Although torcetrapib did not appear to affect the smooth muscle cell content (Figure 6D), torcetrapib alone and in combination with atorvastatin tended to decrease the area of collagen ($P=0.14$ and $P=0.13$, respectively; Figure 6E). Thus, although atorvastatin reduces lesion size without affecting lesion composition compared with untreated mice, torcetrapib reduces lesion size accompanied by a more proinflammatory lesion phenotype, reflected by an increased macrophage-to-collagen ratio, compared with control-treated mice (75%) and atorvastatin-treated mice (67%).

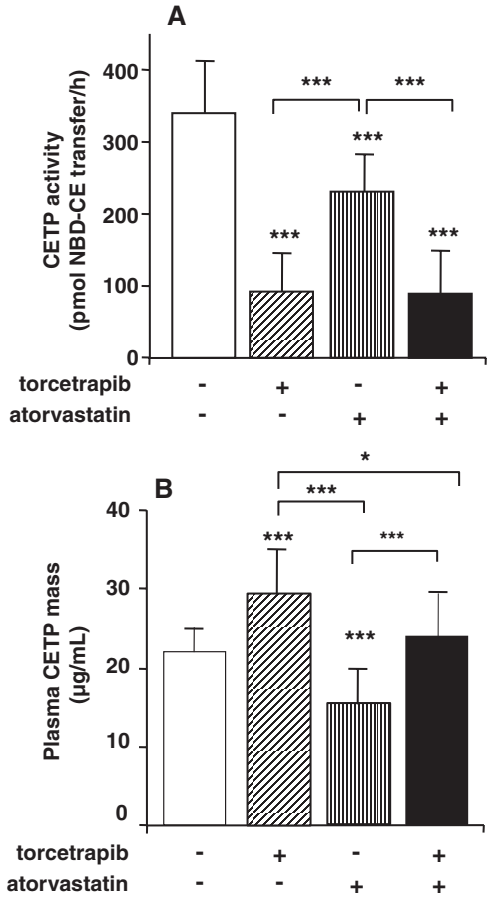

Figure 4. Torcetrapib reduces plasma CETP activity and increases CETP mass. Mice were fed a diet containing 0.25% cholesterol without or with torcetrapib (0.01%), atorvastatin (0.0023%), or both. After 9 weeks of drug intervention, blood was drawn, and plasma was assayed for endogenous CETP activity (A) and CETP mass (B). Values are mean±SD (n=14 to 15). *$P<0.05$, ***$P<0.001$ vs the control group.

Discussion

Torcetrapib has been shown to markedly raise HDL-C and therefore was expected to reduce atherosclerosis in humans. Despite this, the recent RADIANCE, ILLUSTRATE, and ILLUMINATE trials have concluded that torcetrapib was ineffective in reducing atherosclerosis[11–13] and increased clinical event rate.[15] However, it should be realized that the effectiveness of torcetrapib has been assessed only in dyslipidemic patients who also received atorvastatin. Therefore, in the present study, we examined the effect of torcetrapib per se on atherosclerosis development. In our study, we show that torcetrapib alone reduces the progression of atherosclerosis but does not enhance the antiatherosclerotic potency of atorvastatin and that torcetrapib results in a more proinflammatory lesion phenotype compared with atorvastatin.

Torcetrapib reduced total cholesterol exposure to a lesser extent (−17%) than atorvastatin (−41%), whereas torcetrapib and atorvastatin equally reduced atherosclerotic lesion size (both ≈−45%). Previous diet-induced atherosclerosis studies in mice have consistently demonstrated that atherosclerotic lesion area generally could be reliably predicted from cholesterol exposure (H.M.G.P. and P.C.N.R., unpublished data, 2007). Therefore,

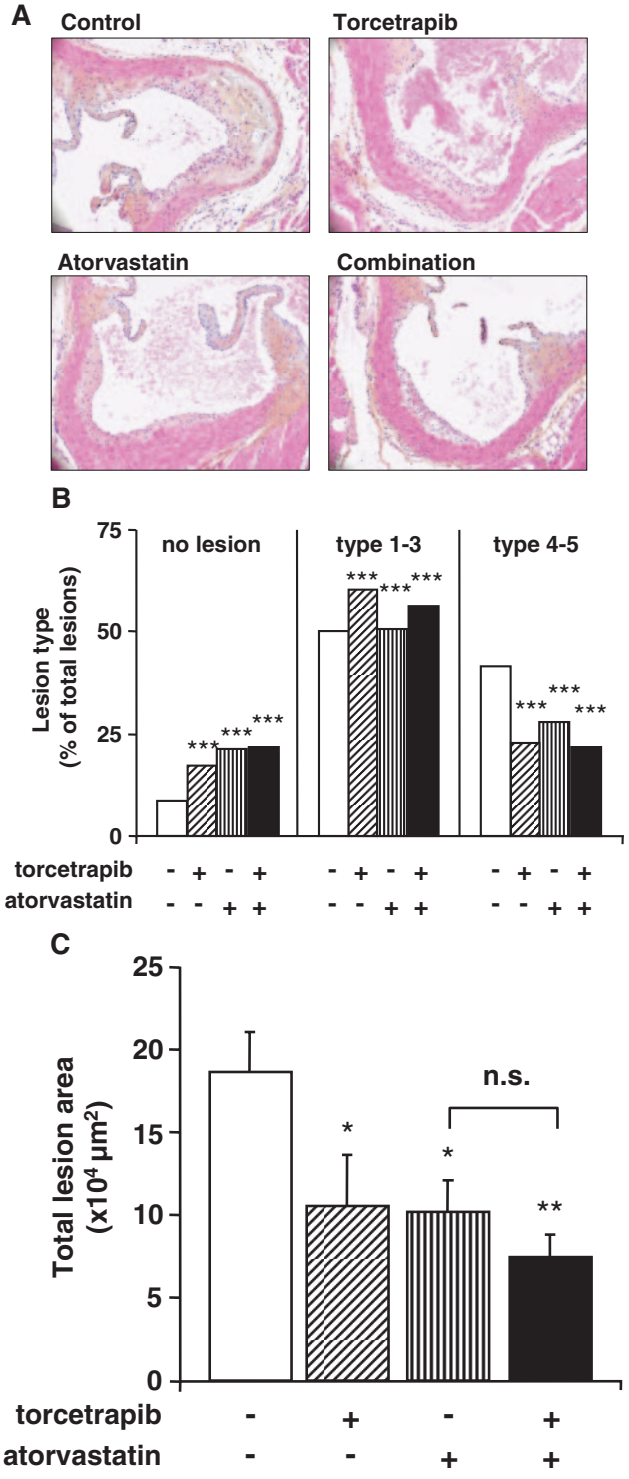

Figure 5. Torcetrapib reduces atherosclerosis development but does not enhance the atherosclerosis-reducing effect of atorvastatin. Mice were fed a diet containing 0.25% cholesterol without or with torcetrapib (0.01%), atorvastatin (0.0023%), or both. After 14 weeks of drug intervention, hearts were isolated, fixed, dehydrated, and embedded in paraffin; representative hematoxylin-phloxin-saffron–stained pictures of each group are shown (A). Four sections per mouse with 50-μm intervals were typed and categorized according to lesion severity (B), and total lesion area was calculated (C). Values are mean±SEM (n=14 to 15). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs the control group.

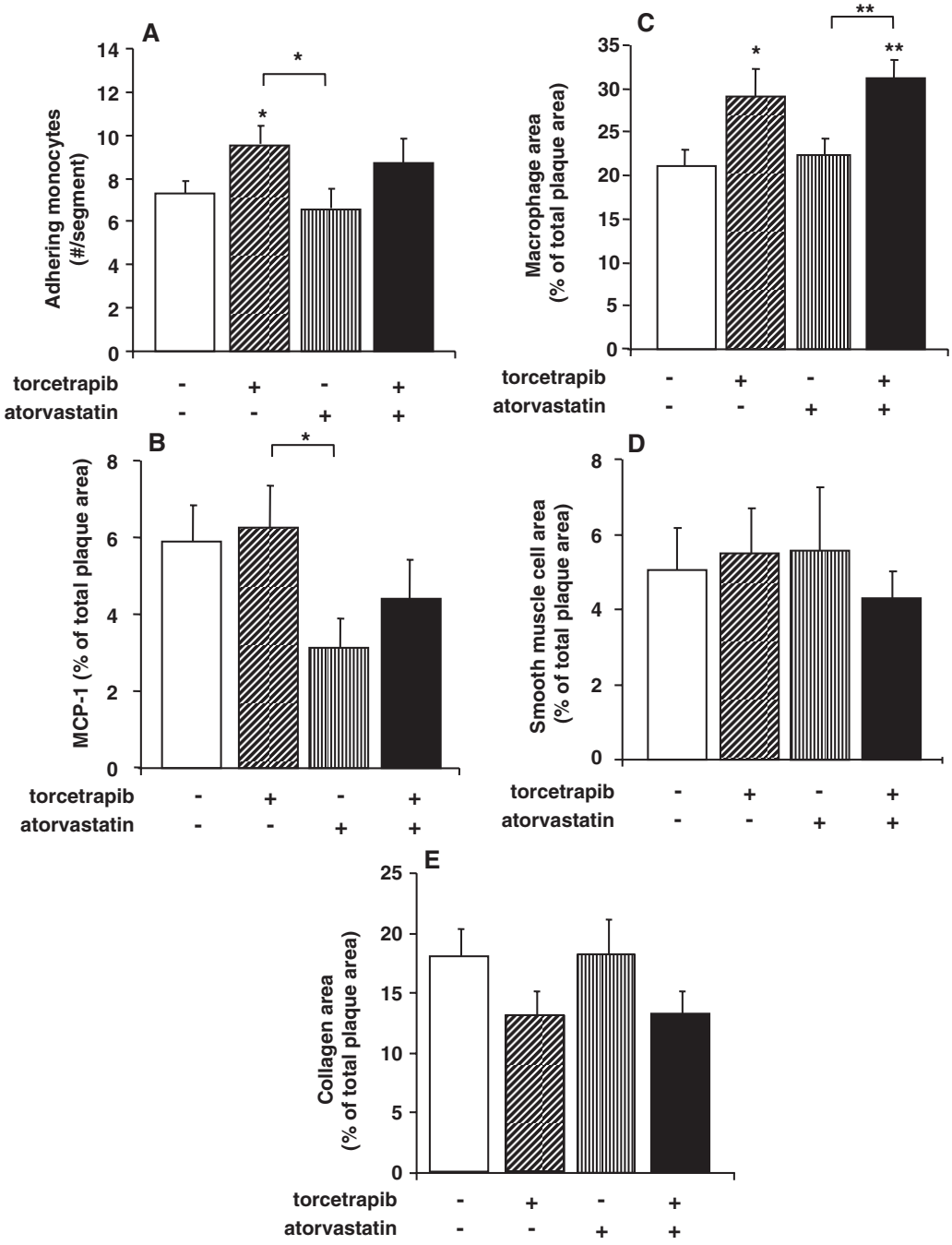

Figure 6. Torcetrapib unfavorably alters plaque composition compared with atorvastatin. In the sections obtained as described in Figure 5, the adhesion of monocytes to the lesions was determined (A), as were the MCP-1 content (B) and macrophage content (C) of the lesions. In addition, the SMC content (D) and collagen content (E) of the lesions were quantified. Values are mean±SEM (n=14 to 15). *$P<0.05$, **$P<0.01$ vs the control group.

torcetrapib decreased atherosclerosis development more drastically than could be expected merely on the basis of the observed reduction in cholesterol exposure. Because torcetrapib treatment results in increased HDL levels, it is likely that HDL is involved in the atheroprotective effect of torcetrapib. In line with this hypothesis, we have observed previously that *E3L.CETP* mice showed a 7-fold increased atherosclerotic lesion area compared with *E3L*-only mice, which was much more than could be expected on the basis of a modest increase in total plasma cholesterol per se. In fact, we showed that plasma from *E3L.CETP* mice was less effective in mediating SR-BI–dependent cholesterol efflux than plasma from *E3L* mice, which is in line with a large reduction in HDL-1.[16] In the present study, we did not detect an effect of torcetrapib on either SR-BI– or ABCA1-mediated cholesterol efflux (not shown), possibly related to the relatively mild effect of torcetrapib on the HDL level compared with total CETP deficiency. We therefore speculate that effects of torcetrapib on other properties of HDL, including its antiinflammatory, antioxidative, and/or antithrombotic properties, may have resulted in a more prominent reduction in

atherosclerotic lesion size than could be expected merely on the basis of a reduction in total cholesterol.

The fact that torcetrapib alone reduced atherosclerosis development is in line with a previous study showing that torcetrapib treatment alone reduces atherosclerosis in rabbits.[28] However, we also show that torcetrapib did not significantly enhance the antiatherogenic potential of atorvastatin. We have evaluated the effects of torcetrapib and atorvastatin in *E3L.CETP* mice with a relatively high plasma cholesterol level of ≈16 mmol/L to avoid the possibility that the combined cholesterol-lowering actions of atorvastatin and torcetrapib would result in a plasma cholesterol level below that required for atherosclerosis development in *E3L.CETP* mice (ie, 6 to 8 mmol/L). Despite this limitation, torcetrapib per se (ie, without concomitant administration of atorvastatin) may thus have an antiatherosclerotic effect in humans as well.

From recent clinical trials, it has become clear that torcetrapib has several adverse effects. The ILLUMINATE trial showed that torcetrapib elevated blood pressure, increased cardiovascular events, and increased death rate, related mainly to cardiovascular causes.[15] However, the mechanisms underlying these unexpected adverse effects have not yet been completely elucidated. In the present study, we did not detect a significant effect of torcetrapib on blood pressure, probably because of small experimental groups (data not shown). However, compared with atorvastatin, torcetrapib enhanced monocyte adherence to the vessel wall, enhanced vascular MCP-1 expression, and increased the macrophage area within the lesions. Torcetrapib thus appears to enhance the recruitment of monocytes to the endothelium and transmigration of the monocytes into the intima, resulting in an enhanced macrophage content of the plaque, compared with similarly sized lesions resulting from atorvastatin treatment. The observation that torcetrapib tended to reduce the collagen content of the plaque independently of the smooth muscle cell content can be explained by induction of collagen breakdown by macrophages (eg, via secretion of metalloproteinases). Although plaque rupture is a rare phenomenon in mice, such inflammatory lesions with a high ratio of macrophage to collagen are more unstable and may well have caused an increased incidence of plaque rupture in humans, thereby explaining increased cardiovascular death. It would be interesting to evaluate in future studies whether these effects of torcetrapib are compound specific or are related to its effect on lipoprotein metabolism by comparison with other CETP inhibitors that are currently under development (eg, JTT-705 and anacetrapib).

Interestingly, recent data from the ILLUMINATE trial indicate that torcetrapib increased plasma aldosterone levels via an as-yet unknown mechanism.[15] In addition to increasing blood pressure,[29] aldosterone increases atherosclerosis development in mice.[30–32] This is related to its proinflammatory properties, including increased MCP-1 expression, increased monocyte infiltration into the coronary artery, increased lipid loading of macrophages, and increased expression of matrix metalloproteinases.[29,30] Preliminary data on aldosterone levels in pooled plasma of the various mouse groups indicated that the average aldosterone level is higher in the torcetrapib-treated group (15%) and combination-treated group (48%) than in the atorvastatin-treated group. This suggests that the torcetrapib-induced increase in aldosterone levels may causally increase the inflammatory plaque phenotype in mice.

Conclusions

Torcetrapib inhibits the progression of atherosclerosis but does not enhance the antiatherosclerotic potency of atorvastatin. In addition, compared with atorvastatin, torcetrapib causes a more proinflammatory and unstable lesion phenotype.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Inhibition of cholesteryl ester transfer protein (CETP) activity is regarded as a promising strategy to increase high-density lipoprotein cholesterol and to reduce atherosclerosis. Torcetrapib was the first CETP inhibitor tested in large human trials and was shown to increase high-density lipoprotein cholesterol by ≈60%. However, the Rating Atherosclerosis Disease Change by Imaging With a New CETP Inhibitor (RADIANCE) and the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trials revealed no therapeutic benefit of combining torcetrapib with atorvastatin with respect to atherosclerosis progression. Moreover, the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial was stopped prematurely because of an excess of (cardiovascular) deaths in patients receiving torcetrapib. By using hyperlipidemic *APOE*3-Leiden.CETP* transgenic mice, which respond in a human-like manner to the lipid-lowering effect of atorvastatin and the high-density lipoprotein cholesterol–raising effect of torcetrapib, we demonstrated that torcetrapib did not increase the antiatherogenic potency of atorvastatin, but torcetrapib alone did reduce atherosclerosis progression to a higher extent than expected on the basis of the cholesterol reduction. On the other hand, torcetrapib resulted in lesions of a more proinflammatory phenotype than atorvastatin. Although plaque rupture is a rare phenomenon in mice, such lesions with a high ratio of macrophage to collagen are more unstable and may well have caused an increased incidence of plaque rupture in humans, thereby partially explaining the increased cardiovascular death in the of trial. Whether the effects of torcetrapib on cardiovascular events and death are compound specific or related to CETP inhibition remains to be evaluated. Taken together, these results suggest that CETP inhibition per se is still a valid strategy for reducing cardiovascular risk, given that (novel) CETP inhibitors (eg, JTT-705 and anacetrapib) do not adversely affect plaque composition.