• 专题报告 •

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CETP as a Target in HDL-raising Therapy: lessons from APOE* 3-Leiden. CETP Transgenic Mice

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Background Cardiovascular disease (CVD) is mainly caused by atherosclerosis, for which dyslipidemia (i.e. high (V)LDL-cholesterol (C), high triglycerides and low HDLC is a major risk factor. To reduce the risk to develop CVD, patients with dyslipidemia are usually treated with lipid-lowering drugs including statins and fibrates. These drugs efficiently lower (V)LDLC (up to ~ 40%) and generally result in a modest increase in HDLC, but they only prevent a fraction of all cardiovascular events (~ 30%). Therefore new therapeutic strategies to reduce cardiovascular events more efficiently are necessary. HDL is clearly inversely correlated with CVD risk, and has been attributed multiple protective effects in atherosclerosis by its role in reverse cholesterol transport and its anti-inflammatory and anti-oxidative properties. The first clinical trials that specifically aimed at increasing HDLC by CETP inhibition using torcetrapib, did not show a protective effect of torcetrapib on atherosclerosis progression. Despite this recent failure, HDL-raising therapy is still generally considered as a promising strategy to further reduce CVD risk. **Objective** Our overall aim was to evaluate the significance of CETP as a therapeutic target for future experimental anti-atherosclerotic strategies. Specifically, we aimed at (1) elucidating the mechanism underlying the adverse effects of CETP inhibition by torcetrapib on atherosclerosis development, and (2) determining the contribution of CETP in the HDL-raising effects of classical lipid-lowering drugs. **Results** The CETP inhibitor torcetrapib effectively raised HDLC in humans, but did not reduce atherosclerosis in humans treated with the combination of torcetrapib and atorvastatin compared to humans treated with atorvastatin only, and even increased cardiovascular death rate. Therefore, we evaluated the anti-atherogenic potential and adverse effects of torcetrapib in APOE* 3-Leiden. CETP (E3L.CETP) transgenic mice, a unique mouse model with human-like lipoprotein metabolism. Mice were fed a cholesterol-rich diet with or without torcetrapib (0.01% in diet), atorvastatin (0.0023%) or both. Torcetrapib decreased CETP activity in the absence (-73%) and presence (-74%) of atorvastatin and increased CETP mass. Torcetrapib decreased plasma cholesterol (-20%), albeit to a lesser extent than atorvastatin (-42%), and increased HDLC in the absence (+30%) and presence (+34%) of atorvastatin. After 14 weeks of drug treatment, atherosclerotic lesion area was assessed in the aortic root. Torcetrapib and atorvastatin alone resulted in a similar reduction in atherosclerotic lesion severity, as reflected by reduced lesion size (-43% and -46%, respectively). Combination therapy did not enhance the atherosclerosis-reducing effect of atorvastatin alone. Remarkably, as compared to atorvastatin, torcetrapib increased plasma aldosterone (+15%), enhanced monocyte recruitment to the vascular wall (+45%) and resulted in lesions of a more unstable phenotype, as reflected by an increased macrophage-to-collagen ratio (+70%). CETP inhibition by torcetrapib per se thus reduces atherosclerotic lesion size but does not enhance the anti-atherogenic potential of atorvastatin. However, as compared to atorvastatin, torcetrapib introduces unstable lesions1.

Next, we evaluated the role of CETP in the modest HDL-raising effects of the classical lipid-lowering dugs statins, fibrates, and niacin, which raise HDLC levels in humans up to +10%, +15% and +30%, respectively. Interestingly, normolipidemic or conventional hyperlipidemic (LDL receptor-knockout and apoE-knockout) mice fail to respond to these drugs with respect to lowering of (V)LDL and raising of HDL. In contrast, E3L mice do respond to these drugs with respect to dose-dependent decreases of (V)LDL-C and triglycerides. However, they generally fail to respond to these drugs by raising HDL-C2. As mice naturally lack CETP, which is an important determinant of HDL metabolism in humans, we reasoned that the HDL-raising effect of the classical lipid-lowering drugs may thus relate to the presence of CETP.

Atorvastatin (0.01% in diet) reduced plasma cholesterol in both E3L and E3L.CETP mice (-26% and -33%) mainly in (V)LDL, but increased HDLC only in E3L.CETP mice (+52%). Hepatic mRNA expression levels of genes involved in HDL metabolism such as Pltp, Abca1, Sr-b1 and Apoa1 were not differently affected by atorvastatin treatment between E3L and E3L.CETP mice. However, atorvastatin reduced the hepatic cholesterol content and down-regulated the hepatic CETP mRNA expression (-57%) as well as the total plasma CETP level (-29%) and CE transfer activity (-36%) in E3L.CETP mice3. Fenofibrate (0.004-0.04% in diet) dose-dependently decreased plasma TG, both in E3L and E3L.CETP mice (-59% and -60%), which was also caused by a strong reduction in (V)LDL, whereas it increased HDLC only in E3L.CETP mice (up to +91%). Similarly to atorvastatin, fenofibrate did

not differentially affect the main genes in HDL metabolism between E3L and E3L. CETP mice, but reduced the hepatic cholesterol content as well as the hepatic CETP mRNA expression (- 72%) and the plasma CE transfer activity (- 73%) in E3L. CETP mice[4]. Niacin (0.03-1.0% in diet) dose-dependently decreased plasma TG (- 77%) and cholesterol (- 66%), accompanied by a dose-dependent increase in HDLC (+ 87%) and apoAI (+ 72%) in E3L. CETP mice. Similar to atorvastatin and fenofibrate, niacin decreased the hepatic cholesterol content as well as the hepatic CETP mRNA expression (- 88%) as well as plasma CETP mass (- 45%) and activity (- 52%)[5].

Albeit that the primary mechanism underlying the lipid-lowering effect is different between atorvastatin (i.e. inhibition of de novo cholesterol synthesis), fenofibrate (i.e. stimulation of VLDL clearance) and niacin (i.e. inhibition of FA release from adipose tissue), the mechanism underlying their HDL-raising effect is thus very similar. They all decrease the hepatic cholesterol content, which presumably results in a reduction of LXR-dependent CETP expression. The HDL-increasing effect of these drugs can thus be explained by the combined effect of a reduction in CETP expression and a reduction in (V)LDL, which is the acceptor of CETP-mediated HDL-CE transfer.

Based on these data obtained in E3L. CETP mice, we have just initiated translational studies in humans to evaluate whether the hepatic lipid content also regulates HDL levels by affecting CETP expression. Initial data showed that treatment of patients with type 2 diabetes mellitus with metformin had no effect on either the liver lipid content, plasma CETP mass or plasma HDLC. In contrast, pioglitazone treatment markedly reduced the liver lipid content (- 37%), which translates into a reduction in plasma CETP mass (- 12%) and an increase in HDLC (+ 10%) (Jonker et al, unpublished observations). **Conclusions** We have developed the unique E3L. CETP transgenic mouse as a valuable model for human-like lipoprotein metabolism. By using this mouse model, we have been able to show that inhibition of plasma CETP activity by torcetrapib resulted in (presumably compound-specific) adverse effects including induction of unstable lesions, which may explain the observed increased cardiovascular events and death rate in the prematurely terminated ILLUMINATE trial. However, we also demonstrated that CETP inhibition per se did in fact reduce atherosclerosis progression. Furthermore, our studies in E3L. CETP mice have shown that classical lipid-lowering drugs reduce the lipid content of the liver, thereby decreasing hepatic CETP expression and increasing HDL, which may add to their therapeutic benefit. Based on our data, we anticipate that reducing CETP activity in plasma may still be relevant strategy to combat CVD, either by CETP inhibition (provided that new CETP inhibitors do not adversely affect the lesion phenotype) or reduction of hepatic CETP expression.

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