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Cholesteryl ester transfer protein (CETP) expression enhances HDL cholesteryl ester liver delivery, which is independent of scavenger receptor BI, LDL receptor related protein and possibly LDL receptor

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Abstract

Cholesteryl ester transfer protein (CETP) is a hydrophobic plasma glycoprotein that mediates the transfer and exchange of cholesteryl ester (CE) and triglyceride (TG) between plasma lipoproteins, and also plays an important role in HDL metabolism. Previous studies have indicated that, compared to wild type mice, human CETP transgenic mice had significantly lower plasma HDL CE levels, which was associated with enhancement of HDL CE uptake by the liver. However, the mechanism of this process is still unknown. To evaluate the possibility that this might be directly mediated by CETP, we utilized CETP transgenic (CETPTg) mice with liver scavenger receptor BI (SR-BI) deficiency [i.e., PDZK1 gene knockout (PDZK1O)], and with receptor associated protein (RAP) overexpression, to block LDL receptor-related protein (LRP) and LDL receptor (LDLR). We found that (1) CETPTg/PDZK1O mice have significantly lower HDL-C than that of PDZK1 KO mice (36%, $p<0.01$); (2) CETPTg and CETPTg/PDZK1O mice have same HDL-C levels; (3) CETPTg/PDZK1O/RAP mice had significant lower plasma HDL-C levels than that of PDZK1O/RAP ones (50%, $p<0.001$); (4) there is no incremental transfer of HDL CE radioactivity to the apoB-containing lipoprotein fraction in mice expressing CETP; and (5) CETPTg/PDZK1O/RAP mice had significant higher plasma and liver [^{3}H]CEt-HDL turnover rates than that of PDZK1O/RAP ones (50% and 53%, $p<0.01$, respectively). These results suggest that CETP expression in mouse increases direct removal of HDL CE in the liver and this process is independent of SR-BI, LRP, and possibly LDLR.

Keywords: Cholesteryl ester transfer protein (CETP); Scavenger receptor BI (SR-BI); LDL receptor; LDL related protein (LRP); PDZK1 gene knockout mice; Cholesterol; High density lipoprotein (HDL)

1. Introduction

Most prospective epidemiological studies have found an inverse correlation between levels of HDL and the incidence of atherosclerosis [1–3]. Some of these studies have indicated that HDL participates in the reverse cholesterol transport pathway, i.e., the centripetal movement of cholesterol from peripheral tissues, or from macrophage foam cells in the arterial wall to the liver, via the plasma compartment [4]. There are two steps in this process. First, excess cholesterol in peripheral tissues is removed by HDL, which in conjunction with LCAT action (converting free cholesterol into cholesteryl ester) provides the driving force for net cholesterol movement [5]. Second, HDL-cholesteryl esters (CE) are delivered to the liver. It is believed that the transport of HDL CE from plasma to the liver involves a direct uptake pathway, mediated by hepatic scavenger receptor B-I (SR-BI) [6], as well as an indirect pathway,

Abbreviations: CETP, cholesteryl ester transfer protein; Tg, transgene; WT, wild type; NFR, natural flanking region; SR-BI, scavenger receptor BI; RAP, receptor associate protein; LRP, LDL receptor related protein; LDLR, LDL receptor; HDL, high density lipoprotein; FCR, fractional catabolism rate; FPLC, fast protein liquid chromatography; TG, triglyceride

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involving transfer of HDL CE to triglyceride-rich lipoproteins (TRL) by stimulating CE–TG interchange, followed by the uptake of TRL remnants in the liver [7,8].

SR-BI is an HDL receptor that plays an essential role in the hepatic uptake of plasma HDL-derived cholesterol and CE into the liver for excretion into the bile [6,9]. SR-BI deficiency results in a significant increase in plasma HDL CE and increased atherosclerotic lesions in multiple mouse models [10]. The multi-PDZ domain-containing protein PDZK1 has been shown to interact with SR-BI from rat liver [11]. Recently, gene-targeted disruption of PDZK1 was found to result in the near absence of SR-BI in mouse liver, proving that PDZK1 is essential for SR-BI expression in the liver [12,13].

Two members of the LDL receptor (LDLR) gene family are highly expressed in the liver. One is LDLR, which regulates plasma cholesterol by mediating uptake and catabolism of plasma LDL [14,15]. The other is LDL receptor-related protein (LRP), which mediates removal of different ligands, including VLDL remnants and chylomicron remnants [16]. An important feature shared by all these receptors is the inhibition of ligand interaction by a 39-kDa receptor-associated protein (RAP). Studies have shown that RAP exhibits high-affinity (K_D 1–10 nM) for LRP [17], and low-affinity binding (K_D 250 nM) for the LDLR (18).

CETP is a hydrophobic plasma glycoprotein that promotes the transfer of CE from antiatherogenic HDLs to proatherogenic apoB-containing lipoproteins, including VLDLs, VLDL remnants, IDLs, and LDLs [7,8]. Mice lack CETP activity in plasma [19], and when a human CETP transgene is introduced, there is a lowering of plasma HDL levels associated with increased catabolism of HDL CE due to enhanced clearance by the liver [20,21]. However, the precise mechanism of this process is unknown.

To investigate the role of CETP in mediating HDL CE delivery to the liver, we utilized CETP transgenic mice with a liver deficiency of SR-BI (i.e., PDZK1 deficient), as well as RAP overexpression (blocking LRP and blocking or partially blocking LDLR pathways). We found that blocking SR-BI, LRP, and LDLR did not influence CETP-mediated HDL CE delivery to the liver. Moreover, our findings suggest that humans deficient in SR-BI would not exhibit high plasma HDL, as seen in SR-BI deficient mice, because of the actions of CETP in mediating hepatic clearance of HDL CE.

2. Materials and methods

2.1. Mice

All animals were between 8 and 12 weeks of age. Generation of PDZK1O mice has been described previously [13]. These PDZK1O animals were crossed with natural flanking region (NFR)-CETPTg transgenic mice [22] with a C57BL/6 genetic background. Fifty percent of the progeny had heterozygous PDZK1O and CETPTg genotypes, and these animals were further crossed with homozygous PDZK1O mice to generate CETPTg/PDZK1O mice and their littermates, PDZK1O. All were fed a chow diet.

2.2. RAP expression

Recombinant adenovirus containing either the RAP cDNA (AdCMV-RAP, 2×10^{11} viral particles) (a gift from Dr. Joachim Herz) or LacZ cDNA (AdCMV-LacZ, 2×10^{11} viral particles) (ViraQuest, Inc) were injected into mice. At 6 days after virus administration, samples (0.5 μl) were separated by SDS PAGE, and the proteins were transferred to nitrocellulose filters. Western blot analysis for RAP was performed, using polyclonal antimouse RAP antibody (also a gift from Dr. Joachim Herz). Horseradish peroxidase-conjugated rabbit polyclonal antibody to mouse IgG (Novus Biologicals) was used as a secondary antibody for RAP. The SuperSignal West detection kit (Pierce) was used for detection.

2.3. Plasma lipid and lipoprotein assays

Fasting plasma was collected for lipoprotein isolation and lipid measurement. The total cholesterol, phospholipids, and triglyceride in plasma were assayed by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan). Lipoprotein profiles were obtained by fast protein liquid chromatography (FPLC), using a Sepharose 6B column.

2.4. In vivo turnover studies

HDL was isolated by ultracentrifugation ($1.063<d<1.21$ g/ml). Mice were injected intravenously with their own HDL that had been labeled with [^{3}H] cholesteryl oleyl ether ([^{3}H]CEt) (2×10^6 cpm). After injection, blood (70 μl) was taken from the tail vein at 0.25, 0.5, 1, 2, 5, 9, and 24 h for determination of radioactivity. The clearance rate was calculated from the decay curves of [^{3}H] CEt according to the Matthews' method [23]. Finally, the animals were anesthetized and euthanized, the organs were harvested, the tissue content of [^{3}H]CEt was analyzed, and organ-clearance rates were determined as previously described [24]. To determine the CETP-mediated transfer of HDL CE to apoB-containing lipoproteins, VLDL+LDL fraction was isolated by ultracentrifugation of pooled plasma (70 μl) at $d<1.063$ and the amount of [^{3}H]CEt present in the fraction was determined at 0.25, 0.5, 1, 2 , 5 , and 9 h, after tracer injection in the different groups of mice.

2.5. Statistical analysis

Each experiment was conducted at least three times. Data are typically expressed as mean±SD. Differences between groups were tested by Mann–Whitney U test (non-parametric test) and among multiple groups by ANOVA followed by the Post-Hoc test.

3. Results

3.1. CETP expression masks the effect of SR-BI deficiency on HDL in mice

To evaluate the effect of liver SR-BI deficiency on plasma HDL levels under a "human-like" scenario with regards to HDL metabolism, we created a mouse model with human CETP expression (CETPTg) and PDZK1 deficient (PDZK1O). We utilized natural flanking region (NFR)-CETPTg mice, since they have human-like CETP concentration in the circulation [22]. We use PDZK1O mice but not SR-BI-deficient (SR-BIO) mice for our studies because PDZK1 deficiency results in the near absence of SR-BI in mouse liver. More importantly, PDZK1O mice do not have the abnormal HDL particles enriched in unesterified cholesterol that is found in SR-BIO mice [12,13]. As indicated in Table 1, plasma lipoprotein analysis by precipitation showed that PDZK1O mice had significantly higher total cholesterol (C), phospholipids (PL), HDL-C and HDL-PL levels (34%, 31%, 38% and 32%, $p<0.01$ respectively) than did wild type (WT) animals, confirming a previous report [12]. This increased HDL was shown to be due to the near absence of hepatic SR-BI [12,13]. Triglyceride and Non-HDL lipids were not significantly altered.