Cholesteryl ester transfer protein, low density lipoprotein particle size and intima media thickness in patients with coronary heart disease

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Abstract

Cholesteryl ester transfer protein (CETP) plays a key role in reverse cholesterol transport and high density lipoprotein (HDL) metabolism. Predominance of small, dense LDL particles is associated with an increased risk of atherosclerosis and coronary heart disease (CHD).
The aim of the study was to determine the potential relationship between the CETP concentration and low density lipoprotein (LDL) particle size and their association with intima media thickness (IMT) in patients with CHD. Lipid parameters, CETP concentration and LDL particle size were determined in 100 healthy subjects (control group) and in 100 patients with CHD, aged 43 to 77 years. Plasma CETP concentrations were measured by an enzyme-linked immuno-sorbent assay with two different monoclonal antibodies. LDL subclasses were separated by nondenaturing polyacrilamide 3-31% gradient gel electrophoresis. CETP concentration was higher in patients compared to controls (2.02 ± 0.75 mg/ml vs. 1.74 ± 0.63 mg/ml, $p<0.01$). Mean LDL particle size (nm) was significantly smaller in patients than in controls (24.5 ± 1.1 vs. 26.1 ± 0.9; $p<0.001$). There was no relation between LDL particle size and CETP concentration (r=-0.1807, $p=0.072$). Age, diastolic blood pressure, CETP concentration and LDL particle size were independent factors for determing IMT by multiple linear regression analysis. They accounted for 35.2 % of the observed variability in IMT. CETP is not an independent contributor of LDL particle size. CETP might play a role in determining lipoprotein distributions, but did not seem to be the sole factor in the formation of small LDL particles.

KEY WORDS: cholesteryl ester transfer protein, coronary heart disease, intima media thickness, reverse cholesterol transport

INTRODUCTION

Number of studies have demonstrated the association of decreased levels of high density lipoprotein (HDL) cholesterol and elevated levels of low density lipoproteins (LDL) with coronary heart disease (CHD) [1-3]. LDL consists of a heterogeneous group of particles of varying size, density, electric charge, lipid and apolipoprotein composition [4, 5]. Nondenaturing gradient gel electrophoresis is commonly used for separation of LDL particles by size. LDL subclass phenotype A is characterized by a predominance of large LDL particles, whereas LDL subclass phenotype B is characterized by a predominance of small LDL particles [4, 5]. Griffin and colleagues showed that the predominance of small, dense LDL (sd LDL) particles is associated with an increased risk of atherosclerosis and coronary artery disease (CAD) [6]. The increased atherogenic potential of small LDL particles may result from their decreased binding to LDL receptors and increased binding to arterial wall proteoglycans [7] or their increased susceptibility to oxidative modification [8,9].

Increased plasma triglyceride (TG) levels and decreased levels of HDL cholesterol are strongly related to the prevalence of sd LDL particles [10-12]. Certain constituents of lipoprotein metabolism (lipoprotein lipase [LPL], hepatic lipase [HL], and cholesteryl ester transfer protein [CETP] participate in the modeling of LDL particles in the circulation and contribute to the formation of sd LDL. CETP is a crucial protein in reverse cholesterol transport (RCT) - a pathway that transports cholesterol from peripheral cells and tissues to the liver for metabolism and excretion in the bile [13,14]. CETP shuttles cholesteryl esters (CEs) from HDL particles to apolipoprotein B (apoB) – containing particles in exchange for triglycerides [15,16]. CETP is thought to facilitate the generation of sd LDL particles through an indirect mechanism of increased rate of triglyceride transfer from VLDL in exchange for CEs in LDL and HDL [15,16].

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The role of CETP in the development of atherosclerosis is controversial. Some studies indicate that CETP is atherogenic, as CETP decreases level of HDL. In contrast, other studies indicate that CETP has no effect on atherosclerosis development as CETP plays an important role in RCT by promoting the transport of CE to the liver by LDL or very low density lipoprotein (VLDL) [17-20]. Carotid IMT is considered to be a good surrogate marker of early atherosclerosis. Several studies have shown that carotid IMT, determined by B-mode ultrasound, correlates significantly with the presence of CAD and to predict coronary events [21-25]. IMT is increased in groups of patients with several cardiovascular risk factors and it has proved to be an independent risk factor for cardiac infarction and stroke. Since cholesterol ester transfer between lipoproteins plays a crucial role in intravascular LDL remodeling, we investigated the potential relationship between the CETP concentration and LDL particle size and association of plasma CETP concentration and LDL particle size with IMT in patients with CHD.

MATERIAL AND METHODS

Patients and Procedures

Blood samples were obtained from 100 patients with CHD (58 men and 42 women) aged 43 to 77 years. CHD included the presence of myocardial infarction, angina pectoris and coronary insufficiency. The diagnosis of CHD was based on the subject's medical history, clinical signs and symptoms, characteristic electrocardiogram changes, increased concentrations of cardiac enzymes and echocardiography assessment of left ventricular systolic and diastolic function. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, or use of antihypertensive medications. Patients treated with lipid-lowering drugs were excluded from the study. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m^2). For comparative analysis, 100 healthy individuals (60 men and 40 women) aged 45 to 73 years were selected as a control group. Control subjects were apparently healthy study participants, with a negative history of cardio-vascular, endocrine, renal and liver disease or dyslipidemia. The study was approved by the local medical Ethics Committee, and all subjects gave informed consent to participate. Fasting venous blood was collected into EDTA-containing glass tubes. The samples were stored at -80°C until analysis. Plasma total cholesterol (TC) and TG concentrations were determined by enzymatic methods (Cholesterol PAP, Triglyceride PAP -Roche Diagnostics, Mannheim, Germany). HDL cholesterol was determined using a precipitation procedure with dextran sulfate and magnesium chloride. Apolipoprotein A-1 (apoA-1) and apolipoprotein B (apoB) were determined by immunoturbidimetry. Plasma LDL-cholesterol was calculated according to the equation of Friedewald [26]. Non-denaturing polyacrylamide 3–31% gradient gel electrophoresis (PAGE) was performed to separate LDL subclasses and estimates their size. The gradient gel characteristics and all details of the method have been presented in previous publications [27-30]. In summary, plasma samples and commercial human plasma standard were prestained with Sudan Black B for 18 h. After separation, gels were scanned with a Pharmacia Biotech laser scanner at wavelength of 632 nm, using Image Master Software. Peak particle sizes were calculated from standard curve. Plasma CETP concentrations were measured by an enzyme-linked immuno-sorbent assay (ELISA) with two different monoclonal antibodies (AplcoDiagnostics, Salem, NH, USA). Briefly, test wells were coated with anti-CETP MoAb (3-11D). CETP in the sample was captured by the antibody in the 1st incubation. After the 1st incubation and washing to remove all of the unbound material, HRP-labeled anti-CETP MoAb (14-8F) was added. After the 2nd incubation and subsequent washing, substrate solution was added. Next, stop reagent was added. The intensity of color that develops was read by a microplate reader. The absorbance was proportional to the concentration of CETP in the sample. The lower detection limit was 0.2 μg/ml. Intima media thickness of the bilateral common right and left carotid arteries were measured with high resolution B-mode ultrasonography (SSA-770A; Toshiba, Tokyo, Japan) with a linear array probe (7.5 MHz), according to a standardized protocol.

Statistical analysis

Data is expressed as mean ± SD. Comparison of means between two groups was made by using two-sample *t* tests. χ^2 analysis was used to compare frequencies between groups. Pearson's correlation coefficient *r* was used to show the degree of linear association between the different variables. Multiple linear regression analysis was used to reveal independent relationships between IMT and other variables. $p < 0.05$ was considered significant.

RESULTS

Table 1 shows clinical and laboratory characteristics of control group and CHD patients. The concentrations of TG and apoB were increased whereas HDL-C and apoA1 were lower in CHD patients than in control group. SBP and DBP were higher in CHD patients compared to control group. The range of plasma CETP concentration was 0.55-3.01 mg/ml in control group and 0.65-3.09 mg/ml in patient group.

TABLE 1. Clinical characteristics, plasma lipids, lipoproteins, apolipoproteins and CETP in control group and CHD patients

Parameter	Control group (n=100)	CHD patients (n=100)	*p* value
Age,y	58.05 ± 8.32	57.50 ± 6.80	>0.05
Sex (men/women)	60/40	58/42	>0.05
Systolic blood pressure (mmHg)	110.00 ± 11.02	144.20±18.03	**<0.0001**
Diaistolic blood pressure (mmHg)	75.86 ± 11.34	80.78 ± 12.47	**<0.01**
BMI (kg/m^2)	27.50 ± 3.21	27.23 ± 3.78	>0.05
Total cholesterol (mmol/l)	4.91 ± 0.82	4.76 ± 1.23	>0.05
Triglyceride (mmol/l)	1.32 ± 0.43	1.57 ± 0.76	**<0.01**
HDL-cholesterol (mmol/l)	1.34 ± 0.21	1.00 ± 0.31	**<0.0001**
LDL–cholesterol (mmol/l)	3.10 ± 0.72	3.03 ± 1.16	>0.05
ApoA1 (g/L)	1.38 ± 0.24	1.27 ± 0.22	**<0.05**
ApoB (g/L)	0.89 ± 0.21	1.06 ± 0.38	**<0.0001**
CETP concentration (mg/ml)	1.74 ± 0.63	2.02 ± 0.75	**<0.01**
LDL particle size (nm)	26.1 ± 0.9	24.5 ± 1.1	**<0.0001**

Data are summarized as mean ± SD

The mean value of CETP in patient group was significantly higher compared to control group (2.02 ± 0.75 mg/ml vs. 1.74 ± 0.63 mg/ml, p=0.0047). Using CETP as dependent variable, no relation was found between the CETP concentration and any clinical, lipid or lipoprotein parameter by correlation analysis (data not shown). LDL subclass distribution of the 200 subjects is presented in Table 2. A majority (89%) of control group had LDL subclass phenotype A. The LDL 3 subclass (24.2-25.5 nm), which belongs to LDL subclass phenotype B, comprised 58.0% of total LDL subclasses in patient group. Mean LDL size (nm) was significantly smaller in patients than in controls (24.5 ± 1.1 vs.26.1 ± 0.9; p<0.0001). To identify the determinants of LDL particle size, the interaction between different parameters of lipoprotein metabolism were evaluated using correlation analysis. LDL size correlated inversely with TG (r=-0.3997, p=0.000), TC (r = - 0.2567, p=0.010) and apoB (r=-0.2236, p=0.025) (Table 3). Since a role of CETP for the LDL size was considered, the relation between LDL size and CETP concentration was investigated. There was no relation between LDL particle size and CETP plasma concentration (r=-0.1807, p=0.072). The mean IMT of both common carotid arteries was 0.83 ± 0.11 mm (range 0.600-1.10) in patients with CHD. Positive association of carotid IMT with age, SBP, DBP and TG was found. CETP plasma concentration was directly associated with carotid IMT (r= 0.2990, p=0.003). We found a strong inverse correlation between LDL particle size and carotid IMT (r=-0.3039, p=0.002) (Table 4). Finally, multiple regression analysis was performed to identify the independent determinants of IMT of carotid arteries. Variables that were significant in univariate analyses were used as independent variables in multiple linear regression analysis.

TABLE 2. Distribution of dominant LDL subclasses in control group (n=100) and CHD patients (n=100)

Dominant LDL subclass	Controls (%)	Patients (%)
Phenotype A		
LDL 1	43.0	2.0
LDL 2	46.0	38.0
Phenotype B		
LDL 3	10.0	58.0
LDL 4	1.0	2.0

TABLE 3. Correlation of LDL particle size with clinical, lipid parameters and CETP concentration in patients with CHD

Parameter	LDL size	
	r	*p* value
Age, y	0.0626	0.536
BMI (kg/m^2)	0.0304	0.764
Total cholesterol (mmol/L)	**- 0.2567**	**0.010**
HDL cholesterol (mmol/L)	0.1826	0.069
LDL cholesterol (mmol/L)	-0.1803	0.073
Triglycerides (mmol/L)	**-0.3997**	***p*<0.0001**
ApoA-1 (g/L)	-0.0358	0.724
ApoB (g/L)	**-0.2236**	**0.025**
Systolic pressure (mm Hg)	-0.1373	0.173
Diastolic pressure (mm Hg)	-0.0806	0.425
CETP (mg/ml)	-0.1807	0.072

TABLE 4. Correlation of IMT (0.83 ± 0.11 mm) with age, blood pressure, lipid parameters, LDL size and CETP concentration in patients with CHD

Parameter	IMT	
	r	*p* value
Age, y	**0.2298**	**0.021**
BMI (kg/m^2)	0.177	0.219
Total cholesterol (mmol/L)	0.0184	0.856
HDL cholesterol (mmol/L)	-0.1554	0.123
LDL cholesterol (mmol/L)	0.0649	0.521
Triglycerides (mmol/L)	**0.2019**	**0.044**
ApoA-1 (g/L)	-0.0478	0.637
ApoB (g/L)	0.0974	0.335
Systolic pressure (mm Hg)	**0.4593**	***p*<0.0001**
Diastolic pressure (mm Hg)	**0.4894**	***p*<0.0001**
CETP (mg/ml)	**0.2990**	**0.003**
LDL size (nm)	**-0.3039**	**0.002**

IMT of carotid arteries is expressed as mean ± SD

TABLE 5. Determinants of carotid artery IMT in 100 patients with coronary heart disease by multiple linear regression analysis

CHD patients (N=100)	R^2 = 0.39181739 Adjusted R^2 = 0.35257980	
	beta	*p*
Intercept		0.003
Age (years)	0.195	**0.020**
Diastolic pressure (mm Hg)	0.378	**0.002**
Systolic pressure (mm Hg)	0.059	0.640
CETP concentration (mg/ml)	0.180	**0.042**
LDL size (nm)	-0.211	**0.021**
Triglycerides (mmol/L)	0.086	0.335

The present data showed that age, DBP, CETP and LDL particle size were independent determinants of IMT (Table 5). They accounted for 35.2 % of the observed variability in IMT.

DISCUSSION

In the present study we provide novel data on the association of CETP with LDL particle size and their association with carotid artery IMT in CHD patients. Hypertrigliceridemia, low HDL concentration and prevalence of sd LDL particles are often present together in CHD patients. Our results are in agreement with other case-control studies reporting an increased prevalence of small LDL particles in patients with CHD [6, 11, 31, 32]. Mean LDL size is significantly smaller in patients than in controls (24.5 ± 1.1 nm vs.26.1 ± 0.9 nm; $p<0.001$). In accordance with Skoglund-Andersson et al. [33], our data show that plasma TG concentration is the major determinant of the LDL size distribution. LDL particle size was inversely related to the IMT of the carotid arteries suggesting that a predominance of small LDL particles is associated with an increased IMT, independent of total cholesterol or LDL cholesterol. The potential impact of CETP on cardiovascular disease is still debated [34-39]. In the present study, the CETP concentration in CHD patients was significantly higher compared to control group. In line with our observations, Zhuang et al. [40] reported that CETP plasma levels were higher in a group of patients who had myocardial infarction compared to healthy controls. In agreement with earlier study of Föger et al. [41], we observed that CETP plasma concentrations are directly associated with intima media thickness of carotid arteries. Klerx et al. [34] demonstrated that CETP concentration is positively associated with the progression of atherosclerosis as assessed by coronary angiography. In the Epic-Norfolk study [36], the detrimental effect of plasma CETP on cardiovascular risk was limited in those in whom plasma triglycerides were greater than median value of 1.7 mmol/l [37]. As expected, the concentrations of triglycerides (median triglyceride level of 1.5 mmol/l) and apoB were increased while the concentrations of HDL cholesterol and apoA1 were decreased in our patients. It is likely that the higher CETP concentration results in an atherogenic pattern of lipoproteins, affect the reverse transport of cholesterol, and accelerates development of atherosclerosis. But, we have not found any relationship between CETP concentrations and any lipid parameter or LDL particle size. Our results show that CETP is not an independent contributor of LDL particle size. CETP might play a role in determining lipoprotein particle distribution, but did not seem to be the only factor in the formation of small LDL particles. LDL particles are remodeled in the circulation as a result of many interrelated processes. The role of LPL and HL should be additionally investigated as determinants of LDL size heterogeneity.

CONCLUSION

Small dense LDL particles and CETP concentration are independently associated with carotid intima media thickness in Macedonian patients with CHD. Our data suggest that plasma triglyceride level is the strongest contributor to the LDL particle size whereas CETP did not correlate with LDL size in this cohort of patients. These data support the idea that LDL particle size is regulated by several contributing factors. They should be further investigated.

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DECLARATION OF INTEREST

Authors declare no conflict of interest.

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