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Improved plasma cholesterol efflux capacity from human macrophages in patients with hyperalphalipoproteinemia

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ABSTRACT

Objectives: CETP or HL deficiencies lead to a marked increase in HDL-C levels however the atheroprotective effect of this phenotype, in particular the ability of HDL particles to remove cholesterol from human macrophages, remains to be determined.
Methods: We measured cholesterol efflux from human THP-1 macrophages to total plasma or to isolated HDL subfractions in patients with HALP carrying molecular defect in either the CETP or LIPC gene.
Results: We demonstrate that HALP is associated with an increased plasma cholesterol efflux capacity from human macrophages. This observation is primarily related to a stimulation of both SR-BI and ABCA1 dependent efflux pathways as a result of quantitative elevation in HDL2 and enhanced intrinsic capacity of HDL3 subspecies, respectively.
Conclusion: HDL particles from HALP patients with molecular defect within either CETP or LIPC gene are not dysfunctional and are efficient to stimulate cholesterol efflux from human macrophages.

1. Introduction

Epidemiological studies identified low circulating levels of HDL-cholesterol (HDL-C) as a strong, independent risk factor for premature atherosclerosis and coronary heart disease [1]. These findings reflect the capacity of plasma HDL particles to exert potent anti-atherogenic effects, including reverse cholesterol transport (RCT), antioxidative, anti-inflammatory or anti-thrombotic activities. Interestingly, the RCT pathway which allows the removal of cholesterol from peripheral tissues, including the macrophages within the vessel wall, to the liver represents a major atheroprotective function of HDL particles [2]. To date only few studies have evaluated the association between cardiovascular risk and cholesterol efflux capacity. An inverse relationship between HDL mediated cholesterol efflux, as evaluated by measuring efflux capacity of apoB-depleted serum using mouse macrophage, with the prevalence of cardiovascular disease independently of HDL-C levels has been reported by Khera al [3]. More recently, Li et al. [4] revealed that cholesterol efflux capacity into apoB-depleted serum, using the same experimental approach, was paradoxically associated with incidence of risk myocardial infarction or adverse cardiovascular events [4]. These apparent conflicting observations might result from differences regarding specific outcome between these latter studies, prevalence or incidence of cardiovascular disease, suggesting that HDL mediated macrophage cholesterol efflux capacity might represent a marker of atherosclerosis progression rather than a marker of cardiovascular events or mortality. Taken together these observations highlight the importance of measuring HDL functionality rather than HDL-C levels in order to assess its level of protection against cardiovascular disease.

Genome Wide Association Studies have identified CETP and hepatic lipase as two candidates of major importance in HDL metabolism because they influence the size, composition and quantity of HDL [5]. Indeed human genetic CETP and HL deficiency lead to a striking increase in HDL-C levels. However, this observation was associated with controversial results regarding its athero-

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protective effect [6,7]. In addition, evaluation of HDL functionality, a better marker than HDL-C levels, in CETP or HL deficient patients showed controversial results regarding the capacity of HDL to stimulate cholesterol efflux from cells. It is likely that this discrepancy mainly results from the variety of cellular models used in each of these studies, i.e. skin fibroblast [8], mouse [9–11] or rat [12] macrophages. In this context, pharmacological inhibition of CETP revealed an enhanced capacity of large HDL2 particles to stimulate cholesterol efflux through SR-BI and ABCG1 pathways [13]. In addition, HDL particles isolated following CETP inhibition therapy with torcetrapib or anacetrapib displayed an increased capacity to stimulate cholesterol efflux from mouse macrophages [14,15]. The stimulation of HDL mediated macrophage efflux following CETP inhibition was attributed to an enrichment of HDL particles in both apoE and LCAT. In order to decipher mechanisms underlying the relationship between HALP phenotype and cardiovascular risk, we evaluate the overall plasma and HDL efflux capacities of patients carrying molecular defect in either the *CETP* or the *LIPC* gene using cholesterol loaded human macrophage, the main cell involved in the regulation of cholesterol homeostasis in the atherosclerotic plaque. This latter cellular model represents to our knowledge a more relevant model than mouse macrophage as the relative contribution of the ABCA1, ABCG1, and SR-BI/Cla-1 pathways to cholesterol export is species specific [16].

2. Materials and methods

2.1. Patients and control subjects

Clinical and biological parameters of patients with hyperalphaproteinemia and of 10 normolipidemic control subjects (4 men and 6 women) are presented in Table 1. Patients with Hyperalphaproteinemia recruited in the present study have been previously described for their molecular defect in the CETP promoter and in the LIPC gene [17–19]. Patient no. 1 displays a heterozygous 18 bp deletion mutation from position −25 to −42 (relative to the transcription start site). Earlier *in vitro* analyses of the transcriptional activity of the CETP promoter revealed that this deletion is associated with a marked decrease (12% of wild type) in the transcriptional activity of the CETP promoter [18]. Patient no. 2 display a 5-bp duplication in exon 13, c.1226-1230dupAGACT that causes a frameshift mutation resulting in a premature stop codon 6 amino acids downstream (p.Val1411ArgfsX6) and leads to the synthesis of a truncated CETP protein lacking the lipid binding C terminus. Patient no. 2 is heterozygous for this mutation and display low plasma CETP activity (20% of normal CETP protein) [17]. Subjects no. 3—no. 6 equally display heterozygous rare point mutations in the CETP which were associated with a marked reduction in the transcriptional activity of the CETP promoter (5% for −49G > T; 8% for −70C > T, and 30% for −372C > T CETP promoter variants as compared to that of the normal CETP promoter activity [19]. Patients no. 7 and no. 8, were heterozygous rare variants causing point mutation in the LIPC. Recent *in vitro* analyses revealed that these two mutations are associated with a marked reduced activity of the secreted HL activity. In addition, postheparin plasma HL activity was reduced by 90% in subject no. 7 and by 40% in subject no. 8 [19]. The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all subjects.

2.2. Biochemical analyses

Lipids and apolipoproteins of plasma and in isolated lipoprotein fractions were determined by using an Autoanalyzer (Konelab 20) and commercial reagent kit from Roche Diagnostics for Total cholesterol (TC), from Thermo-Electron for Triglycerides (TG), HDL-C, and apolipoprotein AI and B, from Diasys for Phospholipids (PL), free cholesterol (FC) and apolipoprotein AII and E, from Pierce for total protein quantification (Bicinchoninic acid assay reagent). Plasma LDL-C was calculated using the Friedewald formula.

2.3. Plasma lipoprotein fractionation

Individual lipoprotein subfractions were isolated from plasma by isopycnic density gradient ultracentrifugation for 48 h at 40,000 rpm using a Beckman XL70 centrifuge and a SW41 rotor as previously described [20]. After centrifugation, gradients were collected from the top of the tubes with an Eppendorf precision pipette in 23 fractions of 0.4 ml corresponding to VLDL (density <1.006 g/ml, fraction number 1), IDL (density from 1.006 g/ml to 1.019 g/ml, fraction number 2), 10 LDL subfractions (density from 1.019 g/ml to 1.063 g/ml, fraction number 3–12) and 11 HDL subfractions (density from 1.063 g/ml to 1.179 g/ml, fraction number 13–23). All lipoprotein subfractions were analyzed for their lipid and protein contents. Total lipoprotein mass was calculated as the sum of the mass of individual lipid and protein component.

2.4. Free cholesterol efflux assays

Efflux assays were performed using human THP-1 macrophages and several cellular models Fu5AH, CHO-K1, CHO-hABCG1 and CHO-hABCA1 as previously described [21], using the release of labeled cell cholesterol to quantify efflux. Although cholesterol efflux can be mediated by a number of different mechanisms, the

Table 1
Major clinical and biological characteristic of subjects with HALP.

Subject	Age (y)	Sex	Gene	Genetic variant	Plasma lipid and apolipoprotein levels (mg/dl)						
					TC	TG	HDL-c	Apo B	Apo AI	Apo AII	Apo E
no. 1	57	F	*CETP*	g.4989-5006 delGGGCGGACATTATACACA	278	67	121	97	248	38	7.2
no. 2	50	M	*CETP*	g.1226-1230dup AGACT	184	26	131	38	199	41	2.2
no. 3	61	F	*CETP*	*g.4659C > T (−372C > T)*	244	61	105	81	168	24	5.5
no. 4	71	M	*CETP*	*g.4961C > T (−70C > T)*	232	147	116	60	202	45	5.4
no. 5	71	F	*CETP*	*g.4961C > T (−70C > T)*	309	38	108	63	197	39	4.5
no. 6	64	F	*CETP*	*g.4982G > T (−49G > T)*	247	87	112	90	185	37	5.2
no. 7	38	F	*LIPC*	*c.517G > A (pV173M)*	351	65	151	130	216	33	6.7
no. 8	72	F	*LIPC*	*c.421A > G (p.G141S)*	270	64	137	79	209	38	6.4
Ctrl ($n = 10$) mean ± SD	33.7 ± 17.8	4M/6F		–	191.4 ± 34.4	58.7 ± 16.6	52.2 ± 8.5	77.9 ± 18.1	124.7 ± 17.0	30.4 ± 4.7	3.0 ± 0.6
Range of reference values					150–260	35–135	60–90	70–135	130–185	NA	NA

TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein-cholesterol; HDL, high density lipoprotein-cholesterol; Ctrl, control subjects; NA, not applicable.