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Small, dense high-density lipoprotein 3 particles exhibit defective antioxidative and anti-inflammatory function in familial hypercholesterolemia: Partial correction by low-density lipoprotein apheresis

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BACKGROUND: Familial hypercholesterolemia (FH) features elevated oxidative stress and accelerated atherosclerosis driven by elevated levels of atherogenic lipoproteins relative to subnormal levels of atheroprotective high-density lipoprotein (HDL). Small, dense HDL3 potently protects low-density lipoprotein (LDL) against proinflammatory oxidative damage.

OBJECTIVE: To determine whether antioxidative and/or anti-inflammatory activities of HDL are defective in FH and whether such defects are corrected by LDL apheresis.

METHODS: Antioxidative and antiinflammatory activities of HDL were evaluated as protection of reference LDL from oxidative stress and capacity to prevent accumulation of proinflammatory oxidised lipids, respectively. Lipid surface rigidity of HDL was assessed using a fluorescent probe. HDL components were measured by analytical approaches. Systemic oxidative stress was characterized as plasma 8-isoprostanes.

RESULTS: Pre-LDL-apheresis, FH patients (n = 10) exhibited elevated systemic oxidative stress (3.3-fold, $P < 0.001$) vs. sex- and age-matched normolipidemic controls (n = 10). Both antioxidative and antiinflammatory activity of HDL3 were impaired (up to −91%, $P < 0.01$) in FH. Sphingomyelin and saturated fatty acid contents were elevated in FH HDL3, resulting in enhanced lipid surface rigidity. The surface lipid content (phospholipids, free cholesterol) was reduced in FH (up to −15%, $P < 0.001$), whereas content of core lipids (cholesteryl esters, triglycerides) was elevated (up to +17%, $P < 0.001$). Molar apolipoprotein A-I content of HDL3 was subnormal in FH. A single

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LDL-apheresis session partially corrected (by up to 76%) deficient HDL antiatherogenic activities, attenuated systemic oxidative stress and partially normalised both the lipid composition and surface rigidity of HDL particles.

CONCLUSIONS: FH features elevated oxidative stress and deficient antioxidative and anti-inflammatory activities of small, dense HDL3; such functional deficiency is intimately linked to anomalies in lipid and protein composition, which may impair the capacity of HDL to acquire and inactivate oxidized lipids.

Mutations in the gene coding for the low-density lipoprotein (LDL) receptor underlie most cases of familial hypercholesterolemia (FH), an autosomal dominant disorder whose phenotype in both heterozygous and homozygous states is characterized by markedly elevated plasma levels of LDL cholesterol (LDL-C), tendinous xanthomas, accelerated atherosclerosis, and premature coronary heart disease.[1–3] FH is equally associated with subnormal concentrations of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein A-I (apoA-I),[4,5] which represent strong predictive and independent risk factors for ischemic heart disease.[6,7]

Plasma HDL particles display multiple atheroprotective and vasculoprotective activities, including cellular cholesterol efflux capacity and antioxidative and anti-inflammatory properties.[8,9] HDL particles are however heterogeneous in their structure, metabolism, and biological functions.[10] Among plasma HDL subpopulations in healthy, normolipidemic subjects, it is small, dense, protein-rich HDL3 particles, which display potent capacity to protect LDL from free radical–induced oxidative damage.[10] Furthermore, the distinct proteome[11] and lipidome[12,13] of HDL3 in such subjects are intimately associated with their antioxidative activity. Indeed, both the surface phospholipid (PL) monolayer and apoA-I content represent major determinants of the antioxidative function of HDL particles, ensuring transfer of phospholipid hydroperoxides (PLOOH) from oxidized LDL to HDL; such transfer is modulated by the rigidity of the HDL surface lipid monolayer, with subsequent reduction of PLOOH to redox-inactive hydroxides by Met residues of apoA-I.[14]

By contrast, HDL particles in FH display qualitative anomalies, including triglyceride (TG) and sphingomyelin enrichment, attenuated capacity to promote cholesterol efflux from macrophages, and altered anti-inflammatory activity.[15–18] In this latter context, it is relevant that oxidative stress, a key factor implicated in formation of LDL-derived proinflammatory oxidized PLs in the arterial intima, is elevated in FH.[19,20]

In FH patients exhibiting a severe clinical and biological phenotype, which is inadequately normalized by classic LDL-lowering pharmacotherapies, extracorporeal LDL apheresis constitutes an effective therapeutic approach to attenuation of the highly atherogenic lipid profile.[21] In this way, plasma levels of atherogenic apoB-containing lipoproteins may be reduced by more than 50% in a single apheresis session.[21] In successive sessions maintained over a period of 1 year or more, cumulative reductions in baseline, preapheresis levels of LDL-C of more than 3-fold may be achieved.[21] Concomitantly with LDL reduction, LDL apheresis may selectively bind and remove HDL particles containing apoE,[22,23] but may equally decrease proinflammatory HDL in FH.[24]

In light of the previously mentioned data and equally available evidence for functional deficiency of HDL particles in metabolic disease featuring dyslipidemia and elevated cardiovascular risk,[9] we hypothesize that (1) antioxidative and anti-inflammatory activities of HDL may be deficient in FH as a result of an altered lipid and/or protein composition and (2) LDL apheresis may partially correct such deficiency. Our data indicate that the antioxidative and anti-inflammatory activities of small, dense HDL3 are indeed defective in FH relative to normolipidemic controls and concomitant with elevated systemic oxidative stress and altered lipidome of these particles. Furthermore, we observe that despite decrement in HDL-C levels during LDL apheresis, such extracorporeal therapy attenuates oxidative stress and partially normalizes defective HDL3 function in FH.

Methods

Subjects

Ten patients aged between 25 and 60 years (4 women and 6 men) displaying a severe FH phenotype typical of type IIa dyslipidemia and 10 age- and sex-matched healthy controls were recruited at the LDL-Apheresis Unit (La Pitié-Salpetrière Hospital, Paris, France). The control group was well comparable, in terms of plasma lipid profile and properties of HDL particles, with healthy normolipidemic controls from the French population studied by us earlier[13,14,25–27] and was therefore representative of this population. All patients were homozygous or compound heterozygous for mutated allele(s) of the LDL receptor gene and were receiving intensive statin treatment, together with bi-weekly LDL-apheresis treatment, which was initiated in each subject at least 24 months before the study. Blood was first drawn immediately before initiation of