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Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL

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ABSTRACT

Objective: Low high-density lipoprotein (HDL) cholesterol and loss of atheroprotective functions of HDL are associated with coronary artery disease (CAD). Here, we investigated the associations of HDL phospholipids with acute and stable CAD as well as with the anti-apoptotic activity of HDL.
Methods: 49 species of phosphatidylcholines (PCs), lysophosphatidylcholines and sphingomyelins (SMs) as well as three species of sphingosine-1-phosphate (S1P) were quantified by liquid chromatography - mass spectrometry in HDL isolated from 22 healthy subjects as well as 23 and 22 patients with stable CAD and acute coronary syndrome (ACS), respectively. Native HDL and artificially reconstituted HDL (rHDL) were tested for their capacity to inhibit apoptosis of endothelial cells (ECs) induced by serum deprivation.
Results: HDL of CAD or ACS patients differed from HDL of healthy controls by the content in nine of the 52 quantified phospholipid species as well as reduced anti-apoptotic activity. The capacity of HDL to inhibit EC apoptosis correlated significantly with five of eleven odd-chain PC's (= plasmalogens), two S1P's, SM42:2, PC34:2, and PC32:0. An orthogonal partial least square - discriminant analysis revealed independent associations of stable CAD with HDL-associated PC34:2, PC33:3 and PC35:2 as well as anti-apoptotic activity of HDL and of ACS with HDL-associated PC33:3, PC35:2, SM42:1, PC34:2 and PC36:2. rHDL reconstituted with apoA-I, PC34:1, and PC35:2 inhibited apoptosis of EC's more effectively than rHDL containing only apoA-I and PC34:1.
Conclusions: The inverse association of HDL-plasmalogen levels with both stable and acute CAD may reflect direct anti-apoptotic effects of plasmologens on ECs.

1. Introduction

High-density lipoprotein (HDL) particles are believed to play an important role in the pathogenesis of coronary artery disease (CAD). Indeed, a low plasma level of HDL cholesterol (HDL-C) represents a strong independent risk factor for CAD [1]. Moreover, HDLs isolated from healthy blood donors exert multiple atheroprotective functions that are attenuated or lost in HDLs of CAD patients [2,3]. Recent proteomic studies related the loss of atheroprotective functions to alterations in the protein composition of HDL [4,5] as well as to structural modifications of HDL proteins [6]. However, not only proteins but also lipids contribute to the structural and functional heterogeneity of HDL.

The HDL lipidome is complex and contains more than 200 different molecular lipid species. Among them phospholipids (PLs)

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comprise the major part of the HDL lipidome, followed by cholesteryl esters, triglycerides and free cholesterol [7]. Phosphatidylcholines (PCs), sphingomyelins (SMs), and free cholesterol are key structural molecules of the surface monolayer of HDL particles [7]. The length and desaturation degree of acyl chains of PC species as well as the content of SMs and free cholesterol define the fluidity of the lipid monolayer [8,9]. Through modulation of the fluidity, the surface PCs directly affect HDL's ability to accept cholesterol from peripheral tissues [8] as well as PL hydroperoxides from low-density lipoproteins (LDLs) [10]. Moreover, the fatty acid composition of PCs defines the capacity of artificially reconstituted HDL (rHDL) to inhibit the expression of inflammatory markers in activated endothelial cells (ECs) [11]. HDL PLs also have impact on HDL-associated proteins and enzymes. Indeed, a reduced abundance of PLs on the surface of HDL facilitates CETP-mediated dissociation of lipid-free apoA-I from HDL particles [12]. Furthermore, changes in the molecular composition of PCs in rHDL lead to conformational changes of apoA-I and changes in the activity of lecithin:cholesterol acyltransferase (LCAT) [13]. In contrary, enrichment of HDL with SMs inhibits the unfolding of apoA-I and cholesterol esterification catalyzed by LCAT [9,14]. In previous studies, total content of PCs and SMs in HDL was found to correlate with the severity of coronary atherosclerosis [15,16].

In addition to PCs and SMs, of which 10 to 100 molecules are present on each HDL particle, HDLs contain low abundant lipids at concentrations which are below particle concentration [2]. For example, plasmalogens constitute a sub-class of PCs and phosphatidylethanolamines (PEs) [17] which scavenge oxygen radicals and thereby inhibit the oxidation of cholesterol and polyunsaturated fatty acids in plasma membranes and LDL [18–20]. Other low abundant lipids exert biological activities through direct interaction with G-protein coupled receptors. Among them sphingosine-1-phosphate (S1P) is the best characterised lipid agonist carried by HDL. HDL-associated S1P mediates several atheroprotective functions of HDL via interaction with at least five different S1P receptors [21,22]. Specifically, S1P modulates the capacity of HDL to stimulate nitric oxide production, promote EC growth and survival, inhibit EC apoptosis and migration of smooth muscle cells [21–23]. However, S1P is not a distinct molecule but, in contrast to the general notion, differs by the length and desaturation degree of its sphingoid base [24,25]. The heterogeneity in length results from the substrate promiscuity of serine:palmitoyltransferase [26], the rate limiting enzyme in sphingolipid biosynthesis. However, the functional importance of S1P's structural heterogeneity is as yet unknown.

In this cross-sectional study we used both hypothesis-free and hypothesis driven approaches to identify PLs which are associated with both CAD and correlate with the capacity of HDL to inhibit the apoptosis of ECs. 49 PL species of unknown relevance were measured by liquid chromatography – tandem mass spectrometry (LC-MS/MS). Three species of S1P which is a known mediator of HDL's anti-apoptotic activity and has been previously associated with CAD were quantified by a targeted LC-MS/MS method.

2. Materials and methods

Materials and Methods are available in the online-only Supplement.

3. Results

3.1. Characteristics of the study population

HDL samples were isolated from 23 to 22 patients with stable coronary artery disease (CAD) and acute coronary syndrome (ACS), respectively, as well as from 22 healthy subjects. Their clinical and biochemical characteristics are shown in Table 1. All CAD patients but only 86% of the healthy controls and the ACS patients were male. Furthermore, 91% of CAD patients but only 41% of ACS patients and no healthy subject received statin medication. Patients with CAD were older (adj. $p = 0.049$; adj. $p = 0.019$) and had higher systolic BP (adj. $p = 0.008$; adj. $p = 0.004$) than healthy subjects and ACS patients, respectively. Both CAD and ACS patients had higher glucose levels (adj. $p = 0.022$ and adj. $p = 0.017$, respectively) than healthy subjects. The ACS patients had lower HDL-C (adj. $p < 0.001$; adj. $p = 0.027$) than both healthy subjects and CAD patients. Probably as the consequence of more prevalent statin treatment, the ACS patients had lower levels of total cholesterol (adj. $p = 0.022$), while CAD patients had lower levels of both total cholesterol (adj. $p = 0.002$) and LDL-C (adj. $p = 0.002$) as compared to healthy subjects. As expected, troponin T levels (adj. $p < 0.001$) and leukocyte counts (adj. $p < 0.001$) were higher in blood samples of ACS patients as compared to healthy individuals or CAD patients.

3.2. Characteristics of the HDL lipidome

The LC-MS/MS analysis of HDL-associated PLs resulted in the quantification of 29 PC species, 4 lysophosphatidylcholine species (LPC) and 16 SM species (Suppl. Table 1). Several PE species and ceramide/hexosylceramide (Cer/HexCer) species were also recovered in HDL, however at levels below the limit of quantification. Phosphatidylglycerols (PGs) and phosphatidic acids (PAs) were below the level of detection.

HDL-associated PC lipids comprise species with even or odd numbers of carbon atoms in the acyl chains. We hypothesized that odd-chain PC species are either PCs with an odd number of carbon atoms in one of esterified fatty acids, or PC plasmalogens with a vinyl-ether bond at the *sn-1* position of the glycerol backbone. *Structural analysis of the plasma odd-chain PC35:3 using collision-induced dissociation of* $(M\text{-}CH_3)^-$ *ions showed* similar fragmentation patterns as a commercially available plasmalogen standard PC(P-18:0/18:1) (PC35:2). Specifically, the fragmentation of PC(P-18:0/18:1) and plasma PC35:3 ions resulted in one predominant ion which corresponds to the fatty acid at the sn-2 position of the glycerol backbone (Suppl. Figures 1A and 1B). In contrast, the fragmentation of PC(16:0/18:1) showed two ions that correspond to the fatty acids at the sn-1 and sn-2 positions, see Suppl. Figure 1C. A similar fragmentation pattern for PC lipids and PC-based plasmalogens was previously described by Berdeaux O. et al. [27]. This structural analysis of PC35:3 revealed that odd-chain PC species represent PC-derived plasmalogens rather than a PCs esterified with an odd chain fatty acid.

Our second LC-MS/MS method identified three S1P species in HDL which differ by length or desaturation of the sphingoid base: 18:1-S1P, corresponding to the canonical S1P, 16:1-S1P and 18:2-S1P. These lipids were previously discovered and characterized in human plasma and fissues [24,25]. The retention times of 16:1-S1P, 18:2-S1P and 18:1-S1P are 3.82 min, 4.41 min and 5.63 min, respectively. Mass spectrometry (MS) analysis of S1P and its structural analogues was performed by selective reaction monitoring (SRM) in positive ionization mode. MS analysis with SRM provide highly selective and very specific determination of S1P species, since only specific transition from parent ion to daughter ion can be detected.

3.3. Associations of HDL phospholipids with CAD

We compared the protein normalized content of PL species in HDL isolated from healthy subjects with those in HDL isolated from CAD or ACS patients. The entire data set is shown in the