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High-density lipoprotein oxidation in type 2 diabetic patients and young patients with premature myocardial infarction

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Abstract *Background and aims:* ApoA-I can undergo oxidative changes that reduce anti-atherogenic role of HDL. The aim of this study was to seek any significant differences in methionine sulfoxide (MetO) content in the ApoA-I of HDL isolated from young patients with coronary heart disease (CHD), type 2 diabetics and healthy subjects.
Methods and results: We evaluated the lipid profile of 21 type 2 diabetic patients, 23 young patients with premature MI and 21 healthy volunteers; we determined in all patients the MetO content of ApoA-I in by MALDI/TOF/TOF technique. The typical MALDI spectra of the tryptic digest obtained from HDL plasma fractions all patients showed a relative abundance of peptides containing $Met^{112}O$ in ApoA-I in type 2 diabetic and CHD patients. This relative abundance is given as percentages of oxidized ApoA-I (OxApoA-I). OxApoA-I showed no significant correlations with lipoproteins in all patients studied, while a strong correlation emerged between the duration of diabetic disease and OxApoA-I levels in type 2 diabetic patients.
Conclusions: The most remarkable finding of our study lies in the evidence it produced of an increased HDL oxidation in patients highly susceptible to CHD. Levels of MetO residues in plasma ApoA-I, measured using an accurate, specific method, should be investigated and considered in prospective future studies to assess their role in CHD.

Introduction

No more than 25% of the risk of coronary heart disease (CHD) can be explained by known risk factors, despite their high prevalence [1].

High-density lipoprotein (HDL) protects artery wall from atherosclerosis, in particular they remove excess cholesterol from artery wall macrophages and carries it back to the liver for excretion in bile [2]. Apolipoprotein A-I (ApoA-I) is the main protein of HDL and it plays a crucial part in the first cholesterol transport reversal step by enhancing sterol efflux from macrophages [3].

Epidemiological studies have demonstrated that plasma HDL independently predict the risk of developing atherosclerosis and cardiovascular disease [4]. More recently, however, it has emerged that HDL quality also seems to be an important parameter in atheroprotection, though there is little data in the literature to support it [5].

An increasing body of evidence shows that HDL isolated from atheromas and the plasma of patients with established CHD lacks these anti-atherogenic properties [6]. HDL can be functionally deficient in populations at high

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risk of CHD, as in type 2 diabetes mellitus, due to glycation and oxidative changes in their HDL, apolipoproteins, and/or enzymes [7].

ApoA-I in particular can undergo oxidative changes that reduce its anti-atherogenic role [8]. Oxidation of the Tyr and Met residues in ApoA-I by myeloperoxidase drastically impairs the protein's ability to promote cholesterol efflux via the ABCA1 pathway [9]. Levine and colleagues [10] suggested that Met residues in protein serve as endogenous antioxidants, protecting functionally important amino acids against oxidation. In ApoA-I in particular, Met^{86} and Met^{112} are thought to be important for cholesterol efflux, and Met^{148} is believed to be involved in LCAT activation [11].

Brock et al. recently examined the extent and sites of methionine sulfoxide (MetO) formation in the ApoA-I of HDL isolated from the plasma of healthy controls and type 1 diabetic subjects, demonstrating that MetO formation was significantly greater in diabetic patients than in a control group at all three sites considered (Met^{86}, Met^{112}, and Met^{148}) [12].

Considering the relevant role of HDL oxidation in the onset of atherosclerotic processes, we ran a pilot study on a small group of type 2 diabetic patients and young people prematurely experiencing acute myocardial infarction (MI): in both these groups we found higher levels of $Met^{112}O$ than in healthy controls [13]. That investigation was carried out by microfluidic-LC/ESI-MS measurements. In a further study the determination of MetO content of ApoA-I in type 2 diabetic patients was performed by MALDI/MS [14] and the results obtained perfectly overlap those achieved in the previous LC/MS investigation. These results proved that possible oxidation phenomena, sometimes observed in MALDI conditions [15], are in this case absent.

The aim of this study was to assess larger study groups to seek any significant differences in MetO between patients with premature MI, type 2 diabetics and healthy subjects, and to identify any correlations with these individuals' lipoproteins. A secondary aim was to see whether the duration of the diabetic patients' disease correlated with HDL oxidation.

Methods

The study involved 21 consecutive type 2 diabetic patients (10 men and 11 women) attending our outpatient clinic from July 2012 to December 2012, with no history of coronary artery or valve disease. We also studied 23 young patients (22 men and 1 woman) diagnosed with acute MI at <45 years of age, with no diagnosis of diabetes, attending the cardiology outpatient clinic for rehabilitation from July 2012 to December 2012. The access to the clinic occurred within 10–15 days after MI. The diagnosis of acute MI was based on finding ≥ 2 of the following 3 criteria: (1) characteristic chest pain lasting >30 min; (2) ST elevation >0.1 mV on at least 2 adjacent electrocardiographic leads; and (3) an increase in troponine I levels to at least the upper limit of normal values. Twenty-one healthy volunteers with no cardiovascular disease and no personal or family history of illness, were also recruited as controls. The study complied with the ethical guidelines of the 1975 Declaration of Helsinki and met with the approval of the local institutional review boards, and informed consent was obtained from each patient.

In the study protocol assessment, the sample size was determined *a priori*, taking into account previous data from a pilot investigation [13] in which peptides related to ApoA-I oxidation resulted about 2.3- to 2.5-fold higher in diabetic and CHD patients. Considering a resulting effect size expressed as Cohen's f of 0.59, for a one-way ANOVA test, at an alpha error level of 0.05 to achieve a power of 0.95, the estimated total sample size, including the three groups, was 60 patients, which has been respected with the performed patient recruitment procedure.

All patients were assessed in terms of body mass index (BMI), diastolic and systolic pressure, any hypertension and/or use of antihypertensive drugs and lipid-lowering drugs. Blood was obtained from fasting venous samples for biochemical analysis.

Fasting plasma glucose (FPG) was measured using a glucose-oxidase method [16]. HbA1c was measured by liquid chromatography aligned with IFCC standardization [17] (Adams HA-8180, Arkrary, Kyoto, Japan). Total cholesterol, low-denisty lipoprotein (LDL) and HDL cholesterol were measured using enzymatic analytical chemistry [18] (CHOP-PAP method; Roche, Milan, Italy), as were triglycerides [19] (GPO-PAP colorimetric enzyme test; Roche Diagnostic System).

HDL fraction preparation

Blood samples were collected in vacutainers after 12–14 h of overnight fasting. To protect methionine residues from oxidation, serum was prepared immediately by low-speed centrifugation at 2500 rpm for 15 min at 10 °C, adding Na_2-EDTA (0.04% w/v) and HDLs were isolated from the serum by sequential ultracentrifugation on the day when the blood was collected, using the Beckman 50 Ti fixed-angle rotor in an Optima XL90 ultracentrifuge (Beckman Instruments, Palo Alto, California, USA) [20]. The density of 6 mL of serum was adjusted to 1.21 kg/L by adding solid KBr (Carlo Erba reagents, Milan, Italy). The resulting solution was placed in polyallomer quick-seal centrifuge tubes (Beckman Instruments, Palo Alto, California, USA) and over-layered with a density solution of KBr, d = 1.21 kg/L, pH 7.4. Densities were adjusted and checked on a Densito 30P density meter (Mettler Toledo, Switzerland). Centrifugation was done at 40,000 rpm, at 6 °C for 30 h. All the lipoproteins were removed in 4 mL of supernatant by tube slicing. The density of 4 mL of supernatant was adjusted to 1.063 (kg/L). Centrifugation was repeated as described above, at 40,000 rpm and a temperature of 6 °C, for 24 h. HDLs (d = 1.063–1.210 kg/L) were removed in 4 ml of bottom fraction and used in the subsequent analysis. Recovery of the 3 fractions of cholesterol was 90%. The HDL fraction was exhaustively dialyzed and concentrated with an Amicon Ultracel 10K centrifugal filter (Millipore Corporation, Billerica USA). Apo-AI and cholesterol concentrations were measured in serum and in the fractions