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Paraoxonase-1 arylesterase activity is an independent predictor of myeloperoxidase levels in overweight patients with or without cardiovascular complications

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ABSTRACT

Objectives: Myeloperoxidase (MPO), matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) were shown to contribute to atherogenesis, while human paraoxonase-1 (PON1) protects against oxidative stress. Although several studies investigated these biomarkers, their associations have not been completely clarified yet. We aimed to investigate these parameters in overweight hyperlipidemic, lipid-lowering therapy-naive patients ($n = 167$) with and without vascular complications.

Design and methods: MPO, MMP-9 and TIMP-1 levels were measured by ELISA. PON1 activities were detected spectrophotometrically. PON1 phenotype was calculated by using a dual substrate method.

Results: Patients with vascular complications (VC) had significantly higher MPO and TIMP-1 levels compared to those without (patients with no vascular complications; NVC) (728 (367.25–1177.90) mg/ml vs. 315.9 (176.05–687.40) mg/ml; $p < 0.001$; and 172.7 (157.7–197.7) ng/ml vs. 152.6 (129.3–172.3) ng/ml; $p < 0.0001$; respectively). MPO levels showed a significant negative correlation with PON1 arylesterase activity (whole patient group (W): $r = 0.42$, $p < 0.0001$; VC: $r = 0.44$, $p = 0.01$; NVC: $r = 0.39$, $p < 0.0001$) and positive correlations with MMP-9 (W: $r = 0.37$, $p < 0.0001$; VC: $r = 0.29$, $p = 0.07$; NVC: $r = 0.42$, $p < 0.0001$) and TIMP-1 (W: $r = 0.42$, $p < 0.0001$; VC: $r = 0.33$, $p < 0.05$; NVC: $r = 0.41$, $p < 0.0001$), respectively. PON1 arylesterase activity was found to be an independent predictor of MPO levels in the whole patient group ($\beta = -0.350$, $p < 0.0001$) or when studied separately in the subgroups with or without cardiovascular complications (VC: $\beta = -0.57$, $p < 0.05$; NVC: $\beta = -0.33$, $p < 0.0001$).

Conclusions: Our results suggest that parallel investigation of MPO, MMP-9 and TIMP-1 levels and PON1 arylesterase activity may be a more accurate indicator of atherosclerosis, which may allow earlier treatment and therefore, improvement of treatment efficacy.

1. Introduction

Obesity, with its increasing worldwide tendency, represents a chronic, low-grade inflammatory state leading to accelerated atherosclerosis, therefore it is an established risk factor of cardiovascular diseases [1,2].

MPO is a free radical-generating heme peroxidase secreted by activated mononuclear cells including macrophages of atherosclerotic plaques [3]. MPO may play a role in the atherosclerotic process by oxidizing low-density lipoprotein (LDL) particles and promoting subendothelial foam cell formation [4]. MPO also binds to HDL (high-density lipoprotein) particles inhibiting their anti-atherogenic effects through oxidative and functional modifications [5]. Site-specific oxidation of apolipoprotein A1 (apoA1) in the HDL particle by MPO also impairs cholesterol efflux and anti-inflammatory and anti-apoptotic properties of HDL [6,7]. Previous investigations showed that increased MPO levels were associated with enhanced progression of atherosclerosis in diabetic patients [8].

PON1 is an HDL-associated enzyme that inhibits lipid oxidation and is considered to protect against the development of atherosclerosis. Recently, PON1 was shown to be associated primarily with the smaller HDL3 particle, which is a more potent antioxidant HDL particle [9]. PON1 activity was also found to be decreased in patients with enhanced

Abbreviations: ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; HsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; Lp(a), lipoprotein (a); MMP-9, matrix metalloproteinase-9; MPO, myeloperoxidase; NVC, without vascular complications; PON1, human paraoxonase-1; sTSH, supersensitive thyroid-stimulating hormone; TG, triglyceride; TIMPs, tissue inhibitors of metalloproteinases; VC, vascular complications.

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atherosclerosis including those with type 2 diabetes mellitus, cardiovascular complications [10] and renal diseases [11]. Correlating with low HDL-cholesterol (HDL-C) concentrations, decreased PON1 activity was measured in obese subjects compared to lean controls [12].

Since the physiological substrate of the PON1 enzyme is not yet known, its catalytic activity is often investigated using paraoxon and phenylacetate as substrates, reflecting the paraoxonase and arylesterase activities of the enzyme, respectively. PON1 arylesterase activity correlates strongly with the circulating amount of PON1 protein [13]. ApoA1, MPO, and PON1 were demonstrated to form a functional ternary complex within the HDL particle, wherein PON1 partially inhibits MPO activity and MPO inactivates PON1. MPO-mediated modification of apoA1 also leads to its inability to serve as an acceptor of cellular cholesterol [5]. A previous study reported serum MPO concentration to be an independent determinant of PON1 arylesterase and paraoxonase activities in a relatively small group of patients with and without type 2 diabetes [14].

MMP-9 is a member of the zinc-dependent endopeptidase family and plays a key role in the breakdown of the extracellular matrix. MMPs may also contribute to the formation and destabilization of vulnerable atherosclerotic plaques ultimately leading to cardiovascular events [15,16]. MMP-9 has recently been shown to form a complex with the HDL particle, especially HDL2; therefore MMP-9 promotes the formation of dysfunctional HDL [17]. TIMPs are a family of homologous proteins regulating the activity of MMPs via binding to them. Increasing number of studies showed that TIMP-1 level was elevated in patients with cardiovascular disease [18,19]. TIMP-1 was also proven to be an independent predictor of cardiovascular mortality [20]. It is suspected that elevated TIMP-1 level might reflect a compensatory response to elevated activity of MMP [21].

Since lipid lowering agents have been shown to modify MPO and MMP-9 concentrations and PON1 activities [22–24], their associations are worth evaluating in untreated individuals. Increased levels of MPO, MMP-9, and TIMP-1 and decreased PON1 activity have been reported in obese and overweight patients [12,25,26]; however, associations between these proteins, as well as their role in atherogenesis in overweight hyperlipidemic patients have not been completely clarified. Therefore, we aimed to investigate the correlations between MPO concentration, PON1 arylesterase and paraoxonase activities, MMP-9 and TIMP-1 levels in an untreated hyperlipidemic patient population with and without vascular complications.

2. Patients and methods

2.1. Study population

167 overweight adult Caucasian patients with Fredrickson type IIa and IIb hyperlipidemia were enrolled. Participants were recruited from our lipidology outpatient clinic at Institute of Medicine, University of Debrecen. Overweight was defined according to the subjects' body mass index (BMI ≥ 25 kg/m^2).

Physical examination and carotid ultrasound were performed regularly. Other imaging techniques (Doppler ultrasound and computer tomography) were performed in case of complaints or abnormal physical and electrocardiography (ECG) examinations. We determined the presence of hypertension, type 2 diabetes mellitus and smoking habits in all patients. Hypertension was defined as the recurrent use of antihypertensive drugs or systolic blood pressure ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg. The diagnosis of type 2 diabetes mellitus was made by recurrent use of antidiabetic drugs or insulin or a fasting blood glucose level ≥ 7 mmol/l. Smoking was defined as previous (in the last 10 years and longer-than-six-months-lasting) and current smoking habits. Study subjects were divided into two gender-matched subgroups as patients with pre-existing vascular complications (VC) and patients without vascular complications (NVC). Vascular complications were defined as known ischemic heart disease (myocardial infarction or coronary sclerosis), ischemic cerebrovascular disease (ischemic stroke, transient ischemic attack, carotid artery stenosis/occlusion) and peripheral arterial disease. Vascular complications were established by the history data of patients or the results of imaging techniques. Patients were divided into the "patients with vascular complications" group if they had at least one complication. At the time of the enrollment, patients were free of acute complaints. Exclusion criteria included previous and ongoing lipid lowering therapy, autoimmune disease, chronic inflammatory states, active liver or endocrine disease including type 1 diabetes mellitus, malignancy and end-stage kidney failure. The study was carried out according to the Declaration of Helsinki and informed consent was obtained from all patients after approval of the local ethics committee.

2.2. Biochemical assays

Venous blood samples were taken between 08:00 and 10:00 a.m. after an overnight fast and sera were prepared immediately. Routine laboratory analyses (high-sensitivity C-reactive protein [hsCRP], triglyceride [TG], total cholesterol, LDL-cholesterol [LDL-C], ApoA1, apolipoprotein B [ApoB], lipoprotein (a) [Lp(a)], hemoglobin A1C [HbA1C], uric acid and sTSH ultrasensitive thyroid-stimulating hormone] levels) were performed from fresh sera with Cobas c501 and e602 autoanalyzers (Roche Ltd., Mannheim, Germany) in the Department of Laboratory Medicine, University of Debrecen. Reagents were purchased from the same vendor and the tests were performed according to the recommendations of the manufacturer. The sera for MPO, MMP-9, TIMP-1 and PON1 paraoxonase and arylesterase measurements were kept at −70 °C until analysis.

2.3. Paraoxonase-1 measurements

PON1 paraoxonase activity was analyzed by a kinetic, semiautomated method. Briefly, paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate, Sigma, Hungary) was used as a substrate, and the generation of 4-nitrophenol was measured on a microtiter plate (Greiner Bio-One GmbH, Germany). 15 μl serum was mixed with 285 μl Tris–HCl buffer (100 mmol/l, pH = 8.0) containing 2 mmol/l $CaCl_2$ and 5.5 mmol/l paraoxon. The absorbance was monitored at 405 nm (25 °C), every minute for 6 min by a Beckman Coulter DTX880 Plate Reader (Beckman Coulter, California, USA) equipped with multimode detector. Enzyme activity was calculated using the molar extinction coefficient 17,600 M^{-1} cm^{-1}. PON1 paraoxonase activity was expressed as units per liter of serum, where 1 unit equals 1 μmol of substrate hydrolyzed per minute [27].

Arylesterase activity was also measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris/HCl pH 8.0. The reaction was started by the addition of the serum and the increase in absorbance was detected at 270 nm as already described [28,29]. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate and enzyme activity was calculated using a molar extinction coefficient of 1310 M^{-1} cm^{-1}. 1 unit of arylesterase activity is defined as 1 μmol phenylacetate hydrolyzed per minute.

PON1 phenotype was calculated by the dual substrate method [30]. The genetic polymorphism at codon 192 Q → R (Arg/Gln at position 192) has the most significant impact on the enzyme activity as hydrolysis of paraoxon is faster by the R allele than by the Q allele. The allozyme determined by the R allele was designated type B, while the allozyme identified by the Q allele was nominated type A. In contrast, both R and Q alleles had similar arylesterase activity. The ratio of the hydrolysis of paraoxon in the presence of 1 mol/l NaCl (salt-stimulated paraoxonase) to the hydrolysis of phenylacetate was used to assign individuals to one of the three possible PON1 phenotypes: AA (low activity), AB (intermediate activity) and BB (high activity). Cut-off values between phenotypes were as follows: ratio below 3.0 for AA, ratio between 3.0 and 7.0 for AB and ratio over 7.0 for BB phenotype.