Atherosclerosis 231 (2013) 308–314

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Gender-specific correlation between plasma myeloperoxidase levels and serum high-density lipoprotein-associated paraoxonase-1 levels in patients with stable and unstable coronary artery disease

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ARTICLE INFO

Article history:
Received 20 February 2013
Received in revised form
3 July 2013
Accepted 27 August 2013
Available online 8 October 2013

Keywords:
Angina
Myeloperoxidase
Oxidative stress
Paraoxonase

ABSTRACT

Objective: Low high-density lipoprotein (HDL) cholesterol is well-established as a negative risk factor for coronary artery disease (CAD) and its anti-oxidant property has been attributed mainly to the HDL-bound enzyme paraoxonase-1 (PON-1). Recently, myeloperoxidase (MPO), a pro-oxidant enzyme released from activated neutrophils, has been shown to alter the atheroprotective function of HDL to a dysfunctional form. This study investigated the relationship between plasma MPO and serum PON-1 levels in patients with stable (SAP) and unstable angina pectoris (UAP).
Methods: Plasma MPO levels and serum PON-1 concentration/activity were measured in patients with SAP ($n = 226$), UAP ($n = 151$) and in control subjects ($n = 99$).
Results: Plasma MPO levels in UAP patients were significantly higher than those in SAP patients or in control subjects (UAP, 21.6[16.7–44.6]; SAP, 19.3[15.7–29.1]; control, 15.9[14.7–18.7] ng/mL; $P < 0.0001$). Serum PON-1 concentrations in UAP and SAP patients were significantly lower than those in control subjects (UAP, 55.6[45.9–69.7]; SAP, 55.0[46.9–64.9]; control, 62.5[51.1–78.8] μg/mL; $P = 0.0002$). Plasma MPO levels showed a weak inverse correlation with serum PON-1 concentrations in all subjects ($R = -0.163$, $P < 0.0005$). Moreover, in women, plasma MPO levels showed a significant inverse correlation with serum PON-1 concentrations and PON-arylesterase activity in SAP (concentration: $R = -0.537$, $P < 0.0001$; arylesterase-activity: $R = -0.469$, $P < 0.001$) and UAP (concentration: $R = -0.340$, $P < 0.05$; arylesterase-activity: $R = -0.350$, $P < 0.05$) patients, but not in men.
Conclusion: This study demonstrates that plasma MPO levels have a significant inverse correlation with PON-1 levels, especially in women, in SAP and UAP patients, and suggests that an imbalance between pro-oxidants and anti-oxidants may contribute to the progression of coronary plaque instability.

1. Introduction

Inflammation and oxidative stress play a key role in the progression of atherosclerosis and plaque instability. Recent studies have focused on an identification of biomarkers for risk stratification in patients with acute coronary syndrome (ACS) and for improved understanding of the pathophysiology of ACS [1].

Myeloperoxidase (MPO) is a hemeprotein stored in azurophilic granules of neutrophils and monocytes. Accumulating evidence suggests that MPO, released mainly from activated neutrophils, may play a key role in mediating destabilization of atherosclerotic plaques [2]. This enzyme has been implicated in the oxidation of lipids contained within low-density lipoprotein (LDL) and thereby promoted lipid-rich plaque formation [3]. We previously demonstrated that abundant MPO-positive neutrophils are infiltrated at sites of plaque rupture or erosion in coronary culprit lesions of ACS

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.08.037

patients [4]. Recently, mass assays based on an enzyme-linked immunoassay have been developed (Oxis Research and Assay Design). Using this method, several clinical studies showed the usefulness of plasma MPO levels for risk stratification in subjects presenting with chest pain [5] or with ACS [6]. Our recent studies also demonstrated that circulating MPO concentrations were higher in patients with ACS than those in patients with stable angina pectoris (SAP) [7,8] and that plasma MPO levels showed a positive correlation with plasma oxidized LDL levels in patients with acute ST-elevation myocardial infarction [9].

High-density lipoproteins (HDLs) have a well-established inverse relationship with the risk for coronary artery disease (CAD). The atheroprotective effects of HDL are mediated by its role in reverse cholesterol transport and its anti-inflammatory properties. HDLs have been shown to retard the oxidation of LDL, and this antioxidant property of HDL has been attributed largely to the HDL-bound enzyme paraoxonase-1 (PON-1) which catalyses the breakdown of oxidized phospholipids in LDL [10]. Recent studies have shown that PON-1 activity and concentration are significantly lower in subjects with CAD compared with controls and are associated with severity and extent of CAD [11]. Moreover, low serum PON-1 activity has been shown to be an independent risk factor for coronary events [12].

Recently, there is growing evidence that MPO converts the normally atheroprotective HDL molecules into a dysfunctional form [13]. MPO has been shown to oxidize apolipoprotein A-I (apoA-I), the major HDL protein, and this oxidized apoA-I inhibits cholesterol efflux by the ATP-binding cassette transporter A1 (ABCA1) pathway and impairs lecithin:cholesterol acyltransferase (LCAT), which rapidly converts free cholesterol to cholesterol ester, a critical step in HDL maturation [13]. Meanwhile, it has been shown that PON-1 enhances cholesterol efflux from macrophages by HDL binding mediated by ABCA1 [14].

Thus, MPO and HDL-associated PON-1 are potentially associated with each other and considered to be biomarkers which could reflect the pathophisiology of ACS, i.e. acute inflammatory responses, and oxidative and anti-oxidative stress. However, no head-to-head comparisons between those markers have been carried out in patients with CAD. In this study, we measured blood levels of MPO and PON-1 activity and mass concentration in patients with SAP and unstable angina pectoris (UAP), and evaluated those relationships.

2. Methods

2.1. Study populations

Study population contained 377 patients with either SAP or UAP, who admitted to our hospital (Osaka City General Hospital). SAP was diagnosed in 226 patients and defined as chest pain typical of cardiac ischemia on exertion [15]. UAP was diagnosed in 151 patients, who were defined as new-onset angina within 2 months after a previous bout; angina with a progressive crescendo pattern, with the anginal episodes increasing in frequency and/or duration; angina that occurred at rest [16]. The UAP patients were further divided into class I ($n = 58$), class II ($n = 9$), and class III ($n = 84$), according to Braunwald's criteria [16]. All patients had primary unstable angina, corresponding to subclass "B". All patients had undergone coronary angiography and had angiographically documented narrowing of at least 70% of the luminal diameter of a major coronary artery. We excluded patients with variant angina, concomitant inflammatory diseases, or malignant tumors, and patients undergoing dialysis. Of all 377 patients, only 3 patients with SAP were treated with antioxidant drugs (3 patients with probucol); the remaining 374 patients did not receive any antioxidant drugs. A total of 99 age- and gender-matched healthy volunteers served as controls (71 men, aged 64 ± 10 years). Among the control subjects, none had diabetes mellitus, 25 had a history of hypertension, 40 met the diagnostic criteria for hypercholesterolemia and 34 were smokers. All 25 hypertensives were in stage I according to the criteria established by the Joint National Committee VII [17]; none used anti-hypertensive medication. Antioxidants were not administered to any controls.

Serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride (TG), and creatinine, plasma levels of MPO, serum PON-1 concentration, PON activity, and PON-arylesterase activity were measured in the 2 groups of patients and in the control subjects. Serum high-sensitivity C-reactive protein (hs-CRP) levels, a leukocyte count, and a neutrophil count were also measured in the 2 groups of patients.

The following data were also obtained: age, gender, body mass index, medications on admission and the presence of risk factors (cigarette smoking, hypertension as defined by the Joint National Committee VII [17], diabetes mellitus (DM) as defined by the WHO Study Group [18], and hypercholesterolemia defined by Japan Atherosclerotic Society Guideline 2002) [19], multivessel disease (≥2 the number of disease vessels narrowed >70% detected angiographically in the major coronary artery), arteriosclerosis obliterans, and old cerebral infarction.

All patients provided written informed consent and the study was approved by the hospital ethics committee.

2.2. Biochemical analysis

Venous blood samples from all patients were obtained on admission to the hospital, prior to heparin administration. For measuring total cholesterol, HDL cholesterol, LDL cholesterol and TG levels, blood samples were obtained after an overnight fast. The concentration of hs-CRP was measured by the latex agglutination photometric immunoassay with an automated immunochemistry analyzer (LXz-6000; Eiken Chemical Co., Tokyo, Japan) with normal values <0.3 mg/dL. The serum troponin T (TnT) level was determined by an enzyme-linked immunosorbent assay (ELISA) using an ES-300 immunoassay analyzer (Boehringer-Mannheim, Mannheim, Germany) with normal values <0.1 μg/L.

2.3. Measurements of MPO, PON-1 concentration, and PON-1 enzyme activities

Plasma MPO levels were measured with an ELISA method (Oxis) according to procedures previously reported [5].

PON-1 activity and mass concentration were measured at the clinical reference laboratory of BML Inc. (Saitama, Japan). PON-1 concentration was determined by sandwich ELISA using two different monoclonal antibodies, as previously described by Kujiraoka et al. [20]. The inter- and intra-assay coefficients of variation were <3.8% and <8.7%, respectively. PON-1 enzyme activities were analyzed according to Eckerson et al. [21], using both paraoxon (PON activity) and phenylacetate (PON-arylesterase activity) as substrates with a modification by adapting the procedure to a Prestage autoanalyzer (TOKYO BOEKI LTD., Tokyo, Japan). PON activity was defined as 1 nmol of *p*-nitrophenol liberated by the hydrolysis of paraoxon per minute at 412 nm at 37 °C at pH 10.5 [22]. The ε_{412} for *p*-nitrophenol was 18,290 M^{-1} cm^{-1}. PON-arylesterase activity was defined as 1 μmol of phenol liberated by the hydrolysis of phenylacetate per minute at 37 °C at pH 8.0 [22]. The liberated phenol by hydrolysis was converted to the colored compound of 4–(*p*-benzoqunone-monoimino)-phenazone2,6-dichloro-4-acetylphenol with 4-aminoantipyrine and measured the absorbance at $\lambda = 510$ nm [22]. The inter- and intra-assay coefficients of