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Serum myeloperoxidase/paraoxonase 1 ratio as potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease

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A B S T R A C T

Objective: Granular leukocyte-derived myeloperoxidase (MPO) promotes oxidation of lipoproteins, while paraoxonase 1 (PON1) has antioxidant properties for high-density lipoprotein (HDL). We evaluated their effects on coronary risk stratification and function of lipoproteins.
Methods and results: A total 158 patients who had previously undergone percutaneous coronary intervention and who had been hospitalized for coronary re-angiography were enrolled. Coronary lesions (restenosis or de novo lesion) were observed in 84 patients but not associated with conventional lipid profile. In contrast, serum MPO levels and PON1 activities were significantly associated with the prevalence of coronary lesions. The high MPO/PON1 ratio, when cutoff values were set at 1.59, was independently correlated with restenosis (odds ratio 6.4, 95% CI 2.2–19.3, $P = 0.001$) and de novo lesions (odds ratio 3.5, 95% CI 1.3–9.4, $P = 0.014$). We isolated HDL from patients with high or low MPO/PON1 ratio, and compared anti-inflammatory properties of HDL. Human umbilical vein endothelial cells were stimulated with inflammatory cytokine, and the expression of vascular cell adhesion molecule-1 (VCAM-1) was evaluated. HDL isolated from patients with low serum MPO/PON1 ratio inhibited VCAM-1 expression significantly greater than that with high MPO/PON1 ratio. We also demonstrated that the cholesterol efflux capacity of apolipoprotein B-depleted serum from patients with high MPO/PON1 ratio was significantly decreased than that with low MPO/PON1 ratio.
Conclusions: MPO/PON1 ratio could be a useful marker for secondary prevention of coronary artery disease through modulation of HDL function.

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1. Introduction

Although high plasma level of low-density lipoprotein cholesterol (LDL-C) is an established risk factor for coronary artery diseases (CAD), the adequate LDL-lowering therapy with statins has been shown to reduce the prevalence of CAD by 30–40% at most [1,2]. LDL displays several phenotypes such as oxidized LDL and small dense LDL, which accelerate atherosclerosis much greater than native LDL, therefore the modulation of LDL size and subclasses may be a next therapeutic target for the residual cardiovascular risk [3]. On the other hand, high-density lipoprotein cholesterol (HDL-C) is a negative risk factor for CAD [4]. HDL exhibits a variety of anti-atherogenic functions including anti-inflammatory and anti-oxidative as well as promoting reverse cholesterol transport [5]. However, it has been reported that HDL may lose its anti-atherogenic properties and become pro-atherogenic (dysfunctional) under conditions such as inflammation, diabetes, and oxidative stress [5]. These lines of evidence suggest that the function of lipoproteins may significantly modulate and predict the progression of CAD in addition to the quantity of lipoproteins.

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Myeloperoxidase (MPO) is a heme peroxidase, which is produced from granular leukocytes. Besides its antibacterial effects, MPO causes oxidative- or chemical-modification against circulating lipoproteins [6]. In addition, MPO generates reactive oxygen and nitrogen species which facilitate lipid peroxidation, protein nitration, and protein carbamylation [6]. LDL which undergoes these modifications promotes foam cell formation. On the other hand, the modified HDL loses its cholesterol efflux activity and anti-inflammatory properties [7]. Through these functional changes, MPO may promote the progression of atherosclerosis.

Paraoxonase 1 (PON1) is one of the major HDL-associated proteins. PON1 is strongly lipophilic and co-exists with apolipoprotein (apo)A-I and apo-J in HDL particles. PON1 hydrolyzes organophosphates and its activity is stabilized in the presence of the apolipoprotein [8]. PON1 inhibits oxidation of LDL, and reduce oxidative stress in blood vessels [8]. In fact, previous studies have shown that the activities of PON1 are negatively associated with the prevalence of cardiovascular events [9]. Given the function of lipoproteins is impaired by MPO and improved by PON1, the plasma level or activity of MPO and PON1 is a marker and modulator of not only lipoprotein functions but also initiation and progression of CAD. However, it has not been fully elucidated whether these molecules, alone or in combination, predict the prevalence of CAD. In the present study, therefore, we evaluated serum MPO level and PON1 activity in patients with chronic CAD, and validated a hypothesis that the MPO/PON1 ratio could be a useful marker for secondary prevention of CAD through modulation of HDL functions.

2. Materials and methods

2.1. Study subjects

From April 2008 to March 2010, we enrolled 158 consecutive patients who had previously undergone successful percutaneous coronary intervention (PCI) with stenting one or more times and who had been admitted to Kobe University Hospital for the purpose of coronary angiography (CAG) because of 6-month follow-up or stable angina or inducible ischemia (CAD group). On the other hand, 174 patients without past history of CAD were enrolled as a non-CAD group in the same period. In addition, the CAD group was classified according to the following definition: patients who showed restenosis in the original stented segment (in-stent restenosis; ISR group); patients with occurrence of other non-target coronary atherosclerotic lesions (de novo lesion group); and patients with neither ISR nor de novo lesion (no lesion group). ISR and de novo lesions were defined as displaying luminal stenosis $\geq$75% and demonstrating ischemia in the perfusion area of narrowed coronary by stress myocardial scintigram. Weight and height were measured and body mass index (BMI) was obtained by dividing a patient's weight by their height squared. Patients who had acute coronary syndrome, renal failure (creatinine $>$3.0 mg/dL), and a history of cancer in the previous 5 years were excluded. To exclude inflammatory diseases (e.g., infections, malignancies, autoimmune diseases), patients whose serum C-reactive protein (CRP) was more than 1.0 mg/dL were also not assigned because MPO is produced from granular leukocytes, and serum MPO levels might be influenced by inflammatory conditions. All patients gave written informed consent, and the clinical study was approved by the Institutional Review Board of Kobe University Graduate School of Medicine. The investigation conforms to the principles outlined in the Declaration of Helsinki.

2.2. Blood chemistry

Serum was obtained after overnight fast. LDL-C, HDL-C, apoA-I, apoA-II, apoB, apoC-II, apoC-III, apoE, glucose, hemoglobin (Hb) A1c, high sensitivity C-reactive protein (hsCRP), and triglyceride (TG) were measured using standard methods at Kobe University Hospital. PON1 paraoxonase activity was measured using paraoxon as the substrate by SRL (Kobe, Japan). The rate of generation of p-nitrophenol was determined spectrophotometrically. Residual serum was stored at -80 °C for assays of MPO and PON1 arylesterase activity. Arylesterase activity was measured as follows; Serum was preincubated with 10 μM eserine (Sigma, St. Louis, MO, USA) for 10 min at room temperature for the inhibition of butyrylcholinesterase activity. After adding 10 mM Tris–HCl buffer (pH 8.0) containing 1 mM $CaCl_2$ and 1 mM phenyl acetate (Wako Chemicals, Richmond, VA, USA), the rate of hydrolysis of phenyl acetate was determined spectrophotometrically at 270 nm in an automated Shimadzu UV-1600, UV-visible Spectrophotometer (Shimadzu, Kyoto, Japan) [10]. Serum MPO levels were measured by human MPO ELISA kit (Hycult Biotechnology, Uden, Netherland) as per manufacturer's protocol.

2.3. Inhibition of tumor necrosis factor-α induced vascular cell adhesion molecule-1 expression by HDL

HDL fraction was obtained from the serum with MPO/PON1 ratio $<$1 or $>$3 by ultracentrifugation as described elsewhere [11]. Human umbilical vein endothelial cells (HUVEC) were purchased from the American Type Culture Collection (Manassas, VA, USA). Subconfluent HUVECs were seeded in a 24 well-plate at 1.2×10^5 cells/well. After 16 h, the media was replaced with new one containing human HDL (100 μg protein/well) which was purified from serum with MPO/PON1 ratio $<$1 or $>$3, or bovine serum albumin (control, 100 μg protein/well). HUVECs were incubated for 16 h, then stimulated with or without tumor necrosis factor (TNF)-α (10 ng/ml) for 6 h. Experiments were terminated by aspiration of the medium and washing cells twice with 500 μl of cold phosphate buffered saline. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) followed by cDNA preparation using reverse transcript agent, then quantitative real-time polymerase chain reaction (PCR) was performed. PCR primers for human vascular cell adhesion molecule-1 (VCAM-1) and glyceraldehyde 3-phosphate dehydrogenase were purchased from Takara-Bio Perfect Real Time Support System (Takara, Shiga, Japan).

2.4. Cholesterol efflux assays

To quantify HDL efflux capacity, apolipoprotein B-depleted serum was prepared from patients with MPO/PON1 ratio $>$3 or $<$1 as previously reported [12]. THP-1 human monocyte cells were seeded into 12 well plates at density of 3×10^6 cells per well. Cells were differentiated into macrophages with phorbol 12-myristate 13-acetate (Sigma, final concentration 100 ng/ml) for 60 h. Cellular cholesterol was labeled with 1 μCi of [^{3}H]-cholesterol for 12 h. Then cells were washed with PBS and incubated for 18 h with medium containing 0.2% BSA and TO-901317 (Cayman, final concentration 10 μM/l) to up-regulate ABC transporters. Cells were washed again with PBS and incubated for 4 h with medium containing 2% apolipoproteinB-depleted serum. After 4 h, medium was collected and centrifuged at 15,000 rpm for 5 min and then aliquots of supernatant were counted in liquid scintillation. The cell layer was washed twice with PBS and incubated with isopropanol for 1 h at room temperature, and radioactivity was measured as for the supernatant. Cholesterol efflux was given as the following; the proportion of [^{3}H]-cholesterol counts in the medium to the total

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