Association of Paraoxonase-1 Activity and Concentration With Angiographic Severity and Extent of Coronary Artery Disease

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OBJECTIVES The goal of this study was to examine the association between paraoxonase-1 (PON1) activity and concentration and the severity and extent of coronary artery disease (CAD).

BACKGROUND Paraoxonase-1, a high-density lipoprotein-associated enzyme, is proposed to have an antiatherogenic effect by protecting low-density lipoproteins against oxidation.

METHODS We studied PON1 activity and concentration in 107 patients with known or suspected CAD referred for cardiac catheterization. Based on visual estimation of coronary angiograms, subjects were classified as having no or mild CAD (<50% stenosis) and significant CAD (≥50% stenosis). Quantitative coronary angiography (QCA) was used to estimate the indexes of severity, extent, and overall atheroma burden of CAD.

RESULTS We found lower values of PON1 activity and concentration (p = 0.003 and p = 0.016, respectively) in the group with significant CAD as compared with the group with no or mild CAD. The PON1 activity was significantly inversely correlated with CAD severity (r = −0.364, p < 0.001), extent (r = −0.221, p = 0.022), and atheroma burden (r = −0.277, p = 0.004). Similarly, PON1 concentration correlated with CAD severity (r = −0.306, p = 0.001) and atheroma burden (r = −0.229, p = 0.017). In multiple regression analysis, gender and PON1 activity were significant determinants of the severity of CAD independently of age, hypertension, smoking, abnormal glucose regulation, and high-density lipoprotein cholesterol.

CONCLUSIONS Our results indicate that PON1 activity and concentration are lower in subjects with significant CAD, and that there is a significant relationship between PON1 activity and concentration and CAD assessed by QCA. (J Am Coll Cardiol 2006;47:2429–35) © 2006 by the American College of Cardiology Foundation

High-density lipoproteins (HDLs) have a well-established inverse relationship with the risk for coronary artery disease (CAD) (1,2). The oxidative modification of low-density lipoprotein (LDL) is a key event in the initiation and acceleration of atherosclerosis (3,4). As an antiatherogenic mediator, HDL, aside from playing an important role in the reverse cholesterol transport, protects LDL against oxidation (5,6). The antioxidant property of HDL has been attributed in part to the HDL-bound enzyme paraoxonase-1 (PON1) (7–9).

In vitro studies have shown the ability of PON1 to block the accumulation of lipid peroxides in LDL, and concurrently to prevent the activation of monocytes by oxidized LDL (7,8). The most convincing data to link PON1 with CAD come from animal studies. In knockout mice lacking the gene for PON1, atherosclerosis develops more rapidly than in wild-type mice, whereas mice that overexpress human PON1 are resistant to atherosclerosis (10,11).

Paraoxonase-1 has several genetic polymorphisms that modify its activity and mass concentration (12). Hypothesized differences in the ability of the polymorphic forms to protect oxidation of LDL have led to numerous studies attempting to determine the relationship between PON1 polymorphisms and CAD (13). These studies, irrespective of polymorphism, have yielded apparently conflicting results. Investigating only an association between PON1 polymorphism and vascular disease may not adequately assess the protective effect of the enzyme, which has led to the proposal that PON1 status (reflecting activity and genotype) rather than genotype alone may be more important in determining atherosclerosis risk (14).

To our knowledge there is only one prospective epidemiologic study, which has shown in middle-aged men that low PON1 activity toward paraoxon is an independent risk factor for coronary events (15). Similarly, few studies have examined the relationship between PON1 activity and concentration and angiographically proven CAD (14,16). In addition, no study has used rigorous computer-aided quantitative coronary angiographic (QCA) techniques to study this question. The present study was therefore undertaken to investigate, in a cohort of patients with known or suspected CAD, the association between PON1 activity and concentration and the severity and extent of CAD based on a computer-assisted analysis of coronary angiograms.
Abbreviations and Acronyms
- apoA-I = apolipoprotein A-I
- apoA-II = apolipoprotein A-II
- apoB = apolipoprotein B
- CAD = coronary artery disease
- HDL = high-density lipoprotein
- LDL = low-density lipoprotein
- LpA-I = lipoprotein A-I
- PON1 = paraoxonase-1
- QCA = quantitative coronary angiography
- VLDL = very-low-density lipoprotein

METHODS

A total of 108 patients who underwent coronary angiography for evaluation of known or suspected CAD were recruited in this study at Helsinki University Central Hospital. Subjects with a history of coronary artery bypass grafting, percutaneous coronary intervention, and those with insulin-dependent diabetes mellitus or significant renal failure (serum creatinine $>150$ μmol/l) were excluded. One patient was excluded because of severe hypertriglyceridemia (triglycerides $>14$ mmol/l). Written informed consent was obtained from all participants, and the study design was approved by the institutional ethics committee.

**Coronary angiography.** Coronary angiography was performed using standard angiographic techniques. Angiographic scoring was carried out by interventional cardiologists who were blinded to the study protocol. Mild CAD on visual interpretation was defined as lumen diameter reduction $<50\%$, and significant CAD as the presence of any luminal stenosis $\geq50\%$. Based on visual angiographic results, patients were divided into two subgroups such that those without any or with mild CAD comprised one group and those with $\geq1$ significantly affected coronary artery comprised the other.

**Frame selection for quantitative coronary angiography.** Coronary segments were analyzed in one angiographic view. The frames for analysis were selected by one of the investigators (M. G.). We classified coronary segments into four categories based on their location. The left main coronary artery was analyzed separately. Proximal parts of the anterior descending, the left circumflex, and the right coronary arteries were considered proximal segments. Mid segments comprised the mid parts of the three main coronary arteries. All segments distal to the mid segments and $\geq1.5$ mm in diameter were regarded as distal segments.

**Quantitative analysis of coronary angiograms.** The coronary angiograms were analyzed using Cardiovascular Measurement System version 3.0 (Medis, Nuenen, the Netherlands) (17). All QCA analyses were carried out by one of the investigators (M. G.).

**Measures of angiographic disease severity and extent.** The QCA-derived parameters were integrated into indexes, as reported elsewhere in detail (18). Briefly, the severity index was defined as the average of the most severe stenoses in the left main, the left anterior descending, the left circumflex, and the right coronary arteries. Extent index was calculated as the longitudinal percentage of coronary segments involved in stenosis ($100 \times \sum$ [stenosis lengths]/$\sum$ [segment lengths]). Atheroma burden index was based on plaque area, reflecting both severity and extent, calculated as: $100 \times \sum$ (plaque areas)/$\sum$ (segment lengths). One global severity, extent, and atheroma burden index was determined for each patient.

**Biochemical investigations.** Blood samples were collected 1 month after coronary angiography after overnight fasting. To avoid bias caused by medications, the patients were requested to withhold any lipid-lowering drugs between the time of angiography and blood sampling; only highly cardioselective and metabolically inert beta-blockers (mainly bisoprolol) were permitted.

Serum and ethylenediaminetetraacetic acid plasma were separated by centrifugation and stored at $-80^\circ$C until analyzed. Cholesterol and triglyceride levels were measured by automated enzymatic procedures (Hoffman-La Roche, Basel, Switzerland). The LDL, very low-density lipoprotein (VLDL), HDL, HDL2, and HDL3 cholesterol levels were determined after separating the lipoprotein fractions from fresh fasting sera by sequential ultracentrifugation (19). Concentrations of apolipoproteins A-I, A-II, and B (apoA-I, apoA-II, and apoB) were measured by immunoturbidimetric methods using commercial kits (Boehringer-Mannheim, Mannheim, Germany). Lipoprotein A-I (LpA-I) particles were quantified using a differential electroimmunoassay (Sebia, Issy-les-Moulineaux, France) (20). The concentration of LpA-I/A-II particles was calculated by subtracting the concentration of LpA-I from the total concentration of apoA-I in serum.

All subjects underwent a 75-g standard oral glucose tolerance test, and blood specimens were collected 0 and 120 min after loading for determination of plasma glucose. Fasting and postload glucose were measured by the hexokinase method (Gluco-quant, Roche Diagnostic, Basel, Switzerland) using either a Hitachi 917 or a Modular analyzer (Hitachi Ltd., Tokyo, Japan).

**Paraoxonase enzyme activity and serum concentration.** Enzyme activity, using phenylacetate as the substrate, was analyzed in serum samples as described previously (21,22). The serum concentration of paraoxonase-1 was assayed using a competitive enzyme-linked immunoassay (21).

**Definition of risk factors.** Body mass index was calculated by dividing weight in kilograms by height in meters squared ($\text{kg/m}^2$). Presence of hypertension was defined as current use of antihypertensive drugs. Smoking status was recorded as those who had never smoked and those who smoked one or more cigarettes per day or had quit smoking. Abnormal glucose regulation was defined as a history of known diabetes or as a fasting plasma glucose $\geq6.1$ mmol/l or as 2-h plasma glucose $\geq7.8$ mmol/l (23).

**Statistical analyses.** All statistical analyses were performed using SPSS for Windows 11.5 (SPSS Inc., Chicago, Illinois).
Data are presented as frequencies or percentages for categorical variables and as means ± SEM for continuous variables, unless otherwise noted. Correlations were calculated by the univariate Spearman correlation coefficients. Between-group differences were assessed by the Mann-Whitney U test. Furthermore, we performed a Kruskal-Wallis test to compute means of severity and extent of CAD for categories of PON1 activity and concentration, respectively. For this endeavor, values of PON1 activity and concentration were categorized into tertiles (cutoff values for PON1 activity were 74 and 97 U/ml, and for PON1 concentration 84 and 108 µg/ml). Multivariate linear regression analysis was performed with the global percent diameter stenosis index as dependent variable. A value of p < 0.05 was considered statistically significant.

RESULTS

Patient population. Demographic and clinical characteristics are presented in Table 1. Typical atherosclerotic risk factors and symptoms of CAD were prevalent. A high percentage of participants was taking aspirin, beta-blockers, and lipid-lowering drugs at baseline. The biochemical characteristics are summarized in Table 2.

Associations between PON1 activity and concentration and clinical and lipid variables. In univariate correlation analyses, PON1 activity and concentration were significantly correlated with HDL cholesterol (p = 0.005 for both), apoA-I (p < 0.001 and p = 0.006, respectively), apoA-II (p < 0.001 for both), and LpA-I/A-II (p = 0.001 and p = 0.016, respectively). The HDL3 cholesterol level was associated with borderline significance to PON1 activity and concentration (p = 0.050 and p = 0.065, respectively). In addition, PON1 activity was associated with gender (p = 0.035), abnormal glucose regulation (p = 0.045), and LpA-I (p = 0.013). Gender (p = 0.058), hypertension (p = 0.088), total cholesterol (p = 0.097), and LpA-I (p = 0.067) were associated with borderline significance to PON1 concentration (Table 3).

Relationship between PON1 activity and concentration and the severity and extent of CAD. As outlined in Table 3, PON1 activity was significantly associated with the QCA-derived global indexes for the severity (p < 0.001), extent (p = 0.022), and global atheroma burden (p = 0.004). Similarly, PON1 concentration correlated significantly with global percent diameter stenosis index (p = 0.001) and global atheroma burden index (p = 0.017). The correlation with global extent index was of borderline significance (p = 0.097).

We found lower values of PON1 activity (84 ± 2 U/ml vs. 100 ± 5 U/ml, p = 0.003) and PON1 concentration (94 ± 3 µg/ml vs. 110 ± 6 µg/ml, p = 0.016), respectively, in the group with significant CAD as compared with the group with no or mild CAD (Fig. 1).

Furthermore, mean values of QCA-derived global indexes for the severity, extent, and atheroma burden of CAD decreased in a stepwise manner across the tertiles of PON1 activity (Fig. 2). Similar associations were found when tertiles of PON1 concentration instead of tertiles of PON1 activity were used (data not shown).

Table 2. Biochemical Characteristics and Other Study Variables (n = 107)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>218 ± 5</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>51 ± 1</td>
</tr>
<tr>
<td>HDL2 cholesterol (mg/dl)</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>HDL3 cholesterol (mg/dl)</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>134 ± 4</td>
</tr>
<tr>
<td>VLDL cholesterol (mg/dl)</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>ApoA-I (mg/dl)</td>
<td>135 ± 2</td>
</tr>
<tr>
<td>ApoA-II (mg/dl)</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>123 ± 3</td>
</tr>
<tr>
<td>LpA-I (mg/dl)</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>LpA-I/A-II (mg/dl)</td>
<td>85 ± 2</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>214 ± 4</td>
</tr>
<tr>
<td>2-h glucose (mg/dl)</td>
<td>298 ± 12</td>
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<td>PON1 activity (U/ml)</td>
<td>87 ± 2</td>
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<tr>
<td>PON1 concentration (µg/ml)</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>Global PDS index</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>Global extent index</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>Global atheroma burden index</td>
<td>7 ± 0</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. ApoA = apolipoprotein A; ApoB = apolipoprotein B; HDL = high-density lipoprotein; LDL = low-density lipoprotein; LpA = lipoprotein A; PDS = percent diameter stenosis index; PON1 = paraoxonase-1; VLDL = very low-density lipoprotein.
Multivariate regression analyses. To establish independent determinants of global percent diameter stenosis index, we performed a linear regression analysis controlled for age, gender, hypertension, abnormal glucose regulation, smoking status, HDL cholesterol, and PON1 activity. In the final model that explained 25.1% of variation of global percent diameter stenosis index, the most important determinants were gender \((p < 0.001)\) and PON1 activity \((p = 0.016)\) (Table 4). The outcome was similar when replacing HDL cholesterol with HDL2 cholesterol, HDL3 cholesterol, LDL cholesterol, or VLDL cholesterol (data not shown).

**DISCUSSION**

In this cohort of patients undergoing elective coronary angiography, there was a significant relationship between PON1 activity and concentration and coronary atherosclerosis expressed as quantitative angiographic indexes of severity, extent, and overall atheroma burden of CAD.

Multiple studies have examined the association between PON1 polymorphisms and CAD. A recent meta-analysis using all 43 available studies of the PON1 polymorphisms involving 11,212 CAD cases and 12,786 controls suggested that the link between PON1 polymorphism and CAD is at best weak (13). However, the vast majority of the studies have not assessed the quality of PON1, i.e., its activity and concentration in the serum.

There is, so far, only limited information available about the association between directly measured PON1 activity and concentration and angiographically proven CAD. Although the difference did not reach statistical significance, Azarsiz et al. (16) found that PON1 activity of patients with CAD \((n = 68)\) was lower than that of patients without CAD \((n = 33)\) and control subjects \((n = 24)\). Further, Mackness et al. (14) showed that PON1 activity and PON1 concentrations were lower in subjects with CAD than in control subjects. Notably, the result was independent of the PON1 genotype, and the investigators concluded that the quality of the PON1 enzyme is a more important factor in CAD than is the PON1 gene.

The QCA was developed to overcome the limitations of visual interpretation of coronary angiograms, but QCA has been applied in only one study comparing PON1 polymorphisms and the angiographic severity and extent of CAD (24). In a report from the WISE study including 711 women, Chen et al. (24) did not find any significant association between the PON polymorphisms and stenosis severity in either white or black women. However, when

**Table 3.** Correlation Coefficients Between PON1 Activity and Concentration and Selected Variables

<table>
<thead>
<tr>
<th></th>
<th>PON1 Activity (U/ml)</th>
<th></th>
<th>PON1 Concentration (μg/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>-0.150</td>
<td>0.123</td>
<td>-0.132</td>
<td>0.175</td>
</tr>
<tr>
<td>Gender</td>
<td>0.204</td>
<td>0.035</td>
<td>0.184</td>
<td>0.058</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-0.100</td>
<td>0.307</td>
<td>-0.165</td>
<td>0.088</td>
</tr>
<tr>
<td>Current or former smoker</td>
<td>-0.112</td>
<td>0.250</td>
<td>-0.143</td>
<td>0.142</td>
</tr>
<tr>
<td>Abnormal glucose regulation</td>
<td>-0.194</td>
<td>0.045</td>
<td>-0.115</td>
<td>0.237</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.075</td>
<td>0.444</td>
<td>0.161</td>
<td>0.097</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.268</td>
<td>0.005</td>
<td>0.270</td>
<td>0.005</td>
</tr>
<tr>
<td>HDL2 cholesterol</td>
<td>0.150</td>
<td>0.124</td>
<td>0.170</td>
<td>0.080</td>
</tr>
<tr>
<td>HDL3 cholesterol</td>
<td>0.190</td>
<td>0.050</td>
<td>0.179</td>
<td>0.065</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.034</td>
<td>0.728</td>
<td>0.044</td>
<td>0.653</td>
</tr>
<tr>
<td>VLDL cholesterol</td>
<td>0.049</td>
<td>0.618</td>
<td>0.056</td>
<td>0.565</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.084</td>
<td>0.387</td>
<td>0.107</td>
<td>0.272</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>0.355</td>
<td>&lt;0.001</td>
<td>0.263</td>
<td>0.006</td>
</tr>
<tr>
<td>ApoA-II</td>
<td>0.368</td>
<td>&lt;0.001</td>
<td>0.386</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>-0.034</td>
<td>0.725</td>
<td>0.080</td>
<td>0.412</td>
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<tr>
<td>LpA-I</td>
<td>0.240</td>
<td>0.013</td>
<td>0.178</td>
<td>0.067</td>
</tr>
<tr>
<td>LpA-I/A-II</td>
<td>0.304</td>
<td>0.001</td>
<td>0.232</td>
<td>0.016</td>
</tr>
<tr>
<td>Global PDS index</td>
<td>-0.364</td>
<td>&lt;0.001</td>
<td>-0.306</td>
<td>0.001</td>
</tr>
<tr>
<td>Global extent index</td>
<td>-0.221</td>
<td>0.022</td>
<td>-0.161</td>
<td>0.097</td>
</tr>
<tr>
<td>Global atheroma burden index</td>
<td>-0.277</td>
<td>0.004</td>
<td>-0.229</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 2.
patients with significant CAD (≥50% stenosis) were stratified into groups with one-, two-, or three-vessel CAD, significant associations were noted between PON polymorphisms and the number of diseased vessels in white but not in black subjects. In contrast to our study, the study of Chen et al. (24) included only women, and data of PON1 activity and concentration were not available.

This study is, to our knowledge, the first to present data on PON1 activity and concentration in patients with CAD measured both by visual interpretation and by refined computer-assisted scoring of coronary angiograms. Our results show that PON1 activity and concentration were lower in patients with significant CAD. Moreover, PON1 activity and concentration were significantly associated with the indexes for global severity, extent, and atheroma burden of CAD. We found a stepwise decrease in these QCA-derived indexes across the tertiles of PON1 activity and concentration. Subjects in the lowest tertile of PON1 activity and concentration, respectively, had a more severe and extensive disease than subjects in the highest tertile.

Activity and concentration of PON1 can vary up to 40-fold in human populations (25,26). Part of this variability is explained by the polymorphism of PON1 gene because of an amino acid substitution at 192 (27). The R allele (arginine at position 192) displays several-fold higher activity toward paraoxon hydrolysis than the Q allele (glutamine at position 192), and may have a greater capacity to protect against copper ion-induced LDL oxidation (28). In contrast, phenylacetate hydrolysis is largely unaffected by the polymorphism, and has been used as a surrogate for protein concentrations (29). Thus the use of phenylacetate, as in our study, provides a better overall indication of PON1 activity levels. However, which PON1 substrates should be used in correlative studies with cardiovascular disease is unresolved (30).

Although PON1 activity and concentration are determined genetically, various factors, such as diet, lifestyle, and environmental factors, can influence PON1 activity and/or concentration. Degraded cooking oil has been reported to lower serum PON1 levels in humans (31). Dietary polyphenols increase PON1 activity, as does moderate alcohol intake (32,33). Smoking is known to decrease serum PON1 activity (34). Recent evidence shows that exposure to environmental chemicals can inhibit PON1 activity (35,36). Furthermore, low serum PON1 activity independent of genotype has been reported in diseases associated with accelerated atherogenesis, such as diabetes mellitus, hypercholesterolemia, and renal failure (37–39).

In human serum, most if not all of the paraoxonase-1 (PON1) activity tertile by global percent diameter stenosis (PDS) index (A), global extent index (B), and global atheroma burden index (C), respectively. For definition of the indexes, see the Methods section.
line with population studies that have shown a statistical association of PON1 activity with HDL cholesterol, apoA-I, and apoA-II (38,41,42).

Interestingly, our results in the multivariable analyses indicate that PON1 activity is a significant determinant of the severity of CAD independently of HDL cholesterol, HDL2 cholesterol, HDL3 cholesterol, apoA-I, and apoA-II. Recently, Rozek et al. (43) reported that in 91 male subjects with severe carotid artery disease and 184 control subjects, PON1 activity in the case group was strongly correlated with LDL and VLDL cholesterol in the absence of the expected HDL3 and apoA-I associations. In the present study, however, we could not confirm their findings, possibly in part because of the different study design and population. Furthermore, in our multivariate analyses, the outcome was similar when LDL and/or VLDL cholesterol were added in the model (data not shown), suggesting that PON1 activity indeed has an independent and important role in the pathogenesis of coronary atherosclerosis.

In summary, we have shown that PON1 activity toward phenylacetate and PON1 concentration are lower in subjects with significant CAD, and there is a significant relationship between activity and concentration of PON1 and the severity and extent of coronary atherosclerosis. The result show the relevance of PON1 activity and concentration for describing associations between atherosclerotic disease and the enzyme. More importantly, the study shows how the protective role of HDL could be modulated by its components such that equivalent serum concentrations of HDL cholesterol may not equate with an equivalent potential protective capacity.

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