Prognostic value of paraoxonase 1 in patients undergoing coronary artery bypass grafting surgery

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Statistical Analysis C
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Manuscript Preparation E
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Background: The aim of this study was to evaluate whether −108C/T polymorphism of the paraoxonase 1 (PON1) gene and the plasma enzyme activity are risk factors for adverse cardiac events after coronary artery bypass grafting (CABG).

Material/Methods: Seventy-one patients with coronary heart disease (CHD) undergoing CABG were enrolled in the study. Genomic DNA was extracted from the venous blood using the Gen Elute™ Blood Genomic DNA kit (Sigma) according to the manufacturer's instructions. PON1 activity was measured in 50 mM glycine/NaOH buffer (pH 10.5) containing 1.0 mM paraoxon, and 1.0mM $CaCl_2$.

Results: The mean PON1 activity toward paraoxon and toward phenyl acetate was equal (166.5±86.9 U/ml and 96.0±47.2 U/ml, respectively) in patients with CHD. The −108C/T polymorphism of PON1 gene was tested. In CABG patients, PON1 activities in dependence on genotypes were significantly different and equalled 266.2±117.9 U/ml for CC, 178.8±64.7 U/ml for CT, and 98.9±59.2 U/ml for TT genotype. Patients with PON1 activity lower than 193.5 U/ml exhibited significantly increased risk of a serious cardiac event in comparison with patients with PON1 activity higher or equal to this value (p=0.03). Additionally, TT genotype was significantly associated with shorter time of event-free survival in comparison with CT and CC genotypes (p=0.009).

Conclusions: The PON1 polymorphism and enzyme plasma activity are associated with CHD occurrence. High PON1 activity connected with the presence of CC and CT genotypes decreases the recurrence of symptoms of coronary heart disease and improve prognosis after CABG.

MeSH Keywords: **Coronary Artery Bypass • Aryldialkylphosphatase • Coronary Artery Disease**

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Background

Human paraoxonase/arylesterase (PON1: aryldialkylphosphatase; EC 3.1.8.1) is a serum enzyme closely associated with high-density lipoproteins (HDL). The enzyme is a 354 amino acid protein synthesized in the liver and secreted into the blood. PON 1 was first investigated for its ability to hydrolyze organophosphorus compounds, such as highly toxic oxon forms of the pesticide parathion or the nerve agents sarin and soman [1]. In recent years, PON1 has been believed to protect against low-density lipoprotein (LDL) oxidation, inhibiting initiation and progression of atherosclerosis. Several studies have shown that HDL can prevent accumulation of lipid peroxides in LDL and that the PON1 enzyme may be one of several components of HDL responsible for antioxidative activity of HDL particles. HDL obtained from PON1 'knockout' mice is unable to prevent oxidation of LDL in a model simulating the artery wall. Introducing PON1 to HDL restores its ability to retard LDL oxidation [2]. Several types of evidence suggest that low levels of the PON1 protein raise the risk of development of premature atherosclerosis and that the low activity of PON1 is a strong independent risk factor for coronary heart disease (CHD) [3–5]. Two polymorphisms (55L/M and 192 Q/R) within the coding region of the PON1 gene and several common polymorphisms in the promoter region of the PON1 gene have been identified. During experiments investigating the association between 2 polymorphic sequences in the coding region and the efficiency of HDL protection against LDL oxidative modifications, it was demonstrated that persons with 192QQ or 55MM genotype are better protected against LDL oxidation, while 192RR or 55LL homozygotes are less protected [4–6]. Involvement of the PON1 promoter region polymorphism in the development of cardiovascular disorders is not obvious, but it was suggested that variations in paraoxonase blood plasma activity and concentration are almost exclusively attributable to the −108 C/T polymorphism [7].

Coronary artery bypass grafting (CABG) is the most important intervention in the treatment of severe symptomatic coronary heart disease. Unfortunately, up to 30% of vein grafts become stenosed during the first year after surgery and almost 50% within 10 years. In the early period after surgery, thrombosis is the main process causing graft occlusion, and later intimal hyperplasia leads to graft failure 1–12 months after implantation. Beyond 1 year after surgery, grafts are generally stenosed as a result of atherosclerosis development [8]. It is interesting ask whether PON1 as a natural anti-atherosclerotic agent can be associated with maintaining graft-patency and with prognosis in patients after CABG. Nevertheless, there is still little information about the impact of PON1 activity and PON1 genotype in the clinical outcome of patients with CHD.

The aim of this study was to evaluate whether −108C/T PON1 gene promoter polymorphism and plasma PON1 activity are risk factors for adverse cardiac events after surgical myocardium revascularization.

Material and Methods

Study population

The study population consisted of 71 Caucasian patients (52 men, 19 women), with an age range of 43–70 years (mean ±SD: 60.9±9.1 years). All patients with CHD had been admitted to the Cardiosurgery Department of the Medical University of Lublin for CABG. CHD occurrence was confirmed by coronary angiogram. Patients with more than 50% stenosis of the lumen of at least 1 of the major coronary arteries were included into the study. The study protocol was approved by the local ethics committee (decision of Bioethics Committee of Medical University of Lublin Nr KE-0254/76/2002). Written informed consent was obtained from all of the participants. The investigation conforms to the principles outlined in the Declaration of Helsinki.

We regarded the level of total cholesterol equal to or higher than 5.0 mmol/l as hypercholesterolemia and the level of triacylglycerols equal to or higher than 1.7 mmol/l as hypertriglyceridemia. Obesity was defined as BMI (body mass index, calculated as weight in kg divided by height in meters squared) above 30 kg/m^2 and overweight as BMI above 25 kg/m^2. Hypertension was defined as systolic blood pressure greater than or equal to 140 mmHg and diastolic blood pressure greater than or equal to 90 mmHg and/or pharmacological antihypertensive treatment. Regular smoking was regarded as smoking of at least 1 cigarette per day, every day. Positive family history was defined as occurrence of coronary heart disease among first-degree relatives prior to age 60 years.

Patients were divided into 2 subgroups: patients with stable angina pectoris and patients with unstable angina pectoris. Symptoms of stable angina pectoris were assessed according to the Canadian Cardiovascular Society (CCS classes I – III) [9] and symptoms of unstable angina pectoris were assessed according to Braunwald classification [10]. Patients with acute myocardial infarction were excluded from the study. Fifty-eight patients were monitored postoperatively during a period of 21.1±12.0 months (mean ±SD) (range, 3–42 months). The occurrence of a serious cardiac event (sudden cardiac death, acute myocardial infarction or unstable angina pectoris) was an end point of the experiment for survival analysis. Patient age, lipid profile, left ventricle ejection fraction, and PON1 polymorphism and activity were analyzed as potential risk factors. For the purpose of survival analysis, patients were divided according to genotype at the −108C/T promoter region and according to PON1 activity. The cut-off value of PON1 activity towards paraoxon was calculated as 193.5 U/ml and

Table 1. Patients demographics at baseline.

Men (%)	52 (73.24)
Women (%)	19 (26.76)
Age (years ±SD)	60.91±9.13
Level of total cholesterol (mmol/l ±SD)	5.18±1.24
Level of LDL cholesterol (mmol/l ±SD)	3.21±1.04
Level of HDL cholesterol (mmol/l ±SD)	1.20±0.36
Triacylogliceroles (mmol/l ±SD)	1.77±1.21
Total cholesterol/ HDL cholesterol ratio	4.7±1.77
Body mass index (kg/m^2 ±SD)	27.96±4.39
Smokers (%)	19
Hypertensives (%)	46
History of myocardial infarction (%)	36
Family history of CAD (%)	10
Diabetes (%)	14

Table 2. Operative patients characteristic.

Severity of coronary artery disease		
Patients with 1 – vessel disease (%)	5	(7.04)
Patients with 2 – vessel disease (%)	16	(22.54)
Patients with 3 – vessel disease (%)	45	(63.38)
Vessels involvement		
Patients with left main stenosis (%)	17	(23.94)
Patients with RCA stenosis (%)	51	(71.83)
Patients with LAD stenosis (%)	27	(38.03)
Patients with Cx stenosis (%)	26	(36.62)
Patients with EF<30%	2	(2.82)
Patients with 30%<EF<50%	17	(23.94)
Number of grafts/patient (+SD)	2.76±0.79	
Patients with implanted		
1 graft (%)	1	(1.41)
2 grafts (%)	25	(35.21)
3 grafts (%)	32	(45.07)
4 grafts or more (%)	8	(11.27)
Surgical time (min)	201.34	(±82.8)
Cross clamp time (min)	48.98	(±13.09)
Operation risk (Euroscore)	2.91	(±2.26)

98.6 U/ml towards phenyl acetate (values of lower bounds of 95% confidence intervals in the control group). PON1 activity and polymorphism were determined before surgery in blood samples collected through venipuncture in heparin- or EDTA-coated tubes, respectively.

DNA extraction and analysis

Genomic DNA was extracted from the venous blood using the Gen Elute™ Blood Genomic DNA kit (Sigma) according to the manufacturer's instructions. The genotype for –108C/T position PON1 promoter region was determined by PCR – RFLP method as previously described [11]. Briefly, the primers were used to amplify a 119-bp fragment of promoter polymorphic region. The PCR product was digested with Bsh126I restriction endonuclease (Fermentas) at 37°C for 12 h. The digested products were separated through agarose gel (2%) electrophoresis and identified by ethidium bromide staining. Allele C corresponded to the presence of a non-digested 119 bp fragment, while allele T corresponded to 2 digestion fragments of 52 and 67 bp.

Paraoxonase activity

Paraoxonase and arylesterase activities were determined according to Eckerson et al. [12]. Paraoxonase activity was measured in 50-mM glycine/NaOH buffer (pH 10.5) containing 1.0 mM paraoxon and 1.0mM CaCl$_2$. The absorbance was monitored spectrophotometrically by measuring absorption of plasma samples at 412 nm, with the use of a continuously recording spectrophotometer (DU 640; Beckman) at room temperature. One unit of paraoxonase activity produced 1 nmol of p-nitrophenol per minute. Arylesterase activity was measured in 20 mM TrisCl buffer (pH 8.0) containing 1 mM substrate and

1 mM CaCl$_2$. The absorbance was monitored spectrophotometrically at 270 nm at room temperature. One unit of arylesterase activity hydrolyzed 1 μmol of phenyl acetate per minute.

Lipid profile

Lipid profile was determined before surgery in blood samples collected through venipuncture in EDTA-coated tubes. Concentration of total cholesterol, triacylglycerols and high-density lipoprotein cholesterol were tested by specific enzymatic techniques. LDL cholesterol was calculated by the Friedewald formula [13].

Statistical analysis

Data was statistically analyzed using the software STATISTICA for Windows rel. 5.1 (Statsoft Inc.) and SPSS for Windows rel. 14 (SPSS Inc.). Continuous variables were compared using the nonparametric Mann-Whitney test. Discontinuous variables were analyzed by the Fisher exact test. Correlations were calculated using the Spearman test. The Kaplan-Meier method and the log-rank test were used to compare event-free survival between the groups of patients with different PON1 activities and PON1 genotypes. The Cox proportional hazard model was used to assess multivariate hazard ratio (HR) adjusted for potential risk factors. P values less than 0.05 were considered significant. Data are presented as means ±SDs.

Table 3. Paraoxonase 1 (paraoxonase and arylesterase) activities in plasma of patients with coronary heart disease.

	Whole group of patients (n=71)	Patients with unstable angina pectoris (n=25)	Patients with stable angina pectoris (n=46)	P value (Mann Whitney test)
PON1 paraoxonase activity U/ml (mean ±SD)	166.1 (±89.4)	160.5 (±86.9)	173.0 (±90.9)	p=0.61
PON1 arylesterase activity U/ml (mean ±SD)	97.2 (±48.7)	97.3 (±45.1)	97.2 (±50.9)	p=0.75

p – patients with unstable angina pectoris *vs.* patients with stable angina pectoris.

Table 4. Correlations between paraoxonase 1 activities in plasma and the presence of early postoperative complications in the group of patients with coronary heart disease after coronary artery bypass grafting.

	Presence of postoperative complications	
	R value	p value
PON1 paraoxonase activity (U/ml)	R=−0.32	p=0.002
PON1 arylesterase activity (U/ml)	R=−0.03	p=0.75

R and p values – Spearman test; PON1 – human paraoxonase/arylesterase (aryldialkylphosphatase; EC 3.1.8.1).

Results

PON1 activity in patients with CHD

Baseline and operative patient characteristics are summarized in Tables 1 and 2. Paraoxonase 1 (paraoxonase and arylesterase) activities in plasma of patients with coronary heart disease undergoing CABG are presented in Table 3. Our data indicate that paraoxonase activity of the PON1 enzyme was lower in patients with unstable angina pectoris in comparison with patients with stable angina pectoris qualified to surgical procedure, but the difference was not significant (p=0.61). Any differences regarding to arylesterase PON1 activity was found in particular subgroups of patients undergoing CABG.

Genetic polymorphism at –108C/T promoter region of the PON1 gene

In the group of patients with coronary heart disease, the frequency of allele C occurrence was 0.43 and allele T frequency was 0.57. The frequency of occurrence CC, CT, and TT genotypes was 14.1%, 57.8%, and 28.2%, respectively. Statistical analysis revealed significant differences in paraoxonase activity among genotypes in patients. CC homozygotes exhibited the highest paraoxonase activity (mean 266.2±117.9 U/ml), CT heterozygotes had intermediate paraoxonase activity (mean 178.8±64.7 U/ml), and TT homozygotes had the lowest paraoxonase activity (mean 98.9±59.2). Comparing paraoxonase activity in heterozygous patients with CT genotype and homozygous patients with CC and TT genotypes, calcalculated p values were equal 0.002. The difference in paraoxonase activity

in patients with CC genotype and TT genotype was even more significant (p<0.001).

Prognosis in patients after cardiac surgery as a function of paraoxonase activity and –108C/T promoter region polymorphism

The most commonly occurring complications in the early postoperative period (until 30 days after surgery) in the study group were: 3 patients (4.2%) died, 2 patients (2.8%) survived periprocedural myocardial infarction, and 6 patients (8.5%) developed acute left ventricle insufficiency. A significant negative correlation between the presence of postoperative complications and paraoxonase activity (p=0.002) was found (Table 4). During a 42-month follow-up period, occurrence of serious cardiac event was monitored in 58 patients who survived cardiac surgery and the early postoperative period and who agreed to the observation. During the follow-up time, 4 patients (6.9%) survived nonfatal myocardial infarction and 5 patients (8.6%) developed symptoms of unstable angina pectoris. In all patients with serious cardiac events, occlusion of at least 1 venous graft was confirmed by coronary angiogram. Kaplan-Meier analysis demonstrated (Figure 1) that in patients with PON1 paraoxonase activity below 193.5 U/ml, the acute coronary syndrome appeared significantly earlier in comparison with patients with PON1 activity ≥193.5 U/ml (p=0.032). When the PON1 gene promoter region polymorphism was considered (Figure 2), TT genotype was significantly associated with decreased time of event-free survival in comparison with CT and TT genotypes (p=0.004). Previously reported clinical prognostic factors for adverse cardiac events and PON1 activity and genotype were
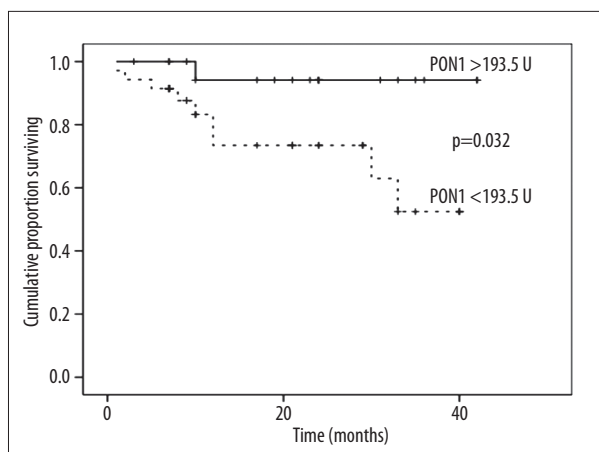
Figure 1. Cumulative Kaplan-Meier curves of event-free survival in patients after coronary artery bypass grafting according to PON1 paraoxonase activity.
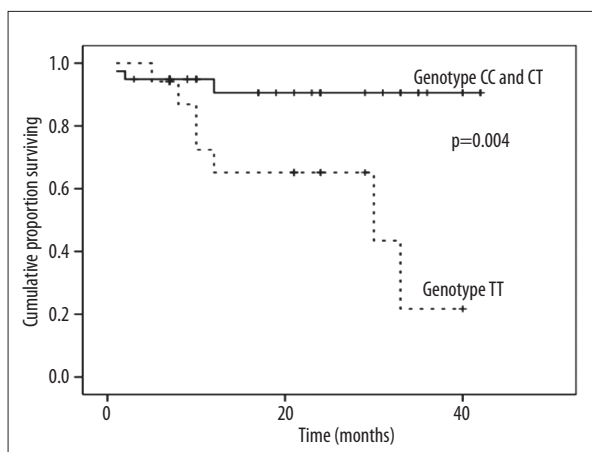

F**igure 2.** Cumulative Kaplan-Meier curves of event-free survival in patients after coronary artery bypass grafting according to PON1 gene polymorphism.

Table 5. Multivariate analysis of the prognostic factors for serious cardiac events in patients after coronary artery bypass grafting.

Prognostic factor	HR	95% CI		p
PON1 activity ≥193.5 U/ml	0.22	0.02	2.71	0.235
Genotype CC and CT	0.24	0.04	1.51	0.128
LCA trunk stenosis	5.28	0.83	33.43	0.077
Total cholesterol ≥5.0 mmol/l	0.98	0.92	1.05	0.599
HDL ≤1.0 mmol/l	0.99	0.91	1.09	0.860
LDL ≥3.0 mmol/l	0.99	0.92	1.06	0.758

HDL – high density lipoproteins; HR – hazard ratio; LCA – left coronary artery; LDL – low density lipoproteins; PON1 – human paraoxonase/arylesterase (aryldialkylphosphatase; EC 3.1.8.1); 95% CI – 95% confidence intervals; p values (Cox proportional hazard model).

entered into Cox regression analysis. None of he investigated parameters were found to be independent prognostic factors preventing serious cardiac events (Table 5).

Discussion

Several studies have suggested an association between PON1–108C/T promoter region polymorphism, as well as PON1 activity and the susceptibility to cardiovascular disease. The present study demonstrates that TT genotype is associated with the lowest plasma activity, CT is associated with intermediate plasma activity, and CC with the highest PON1. The association of PON1 promoter region polymorphism with the development of cardiovascular disorders is not obvious. A recently published meta-analysis of studies did not prove the relationship between PON1 promoter region polymorphism and the occurrence of coronary heart disease [14]. Authors of another meta-analysis [15], providing the same conclusion, underlined

a strong linkage disequilibrium between −108 C/T promoter region polymorphism and 55 L/M or 192 Q/R coding region polymorphisms, which additionally complicates the understanding of the influence of genetic factors in the development of coronary heart disease. It would appear that the −108 C/T polymorphism may be related to CHD occurrence by virtue of its association with enzyme paraoxonase activity. It was previously shown that the −108 C/T polymorphism is the main contributor to plasma PON1 activity variation, accounting for 23–24% of the total variation [7]. The polymorphism is located in a binding site for the transcription factors Sp1 and Sp3. This site is abolished by the presence of the −108T form and binding of Sp1 to this site is weaker in comparison with the presence of the −108C variant. As the result of this process, the expression of the PON1 gene is lower, so the effect of the polymorphism can cause lower PON1 enzyme activity in the event of allele T.

The frequencies of the PON1–108C/T promoter region polymorphism estimated by us in Polish patients with CHD undergoing

CABG are similar to that of previously reported [14] genotype frequencies characteristic for non-Polish European populations.

The present study provides an important and novel observation concerning PON1 prognostic value. Our data reveal that prognosis in patients after CABG in the early postoperative period and during long-term follow-up is associated with PON1 promoter region polymorphism and PON1 activity towards paraoxon. A significant negative correlation between decreased PON1 activity and early postoperative complications was also observed. Moreover, our results indicate that allele C is associated with decreased risk of serious cardiac event due to venous graft occlusion. PON1–108 CT and CC genotypes were associated with longer survival without serious cardiac event in patients after CABG. Considering the impact of the PON1 activity on susceptibility to CHD, we found significantly longer survival without serious cardiac events in patients with higher PON1 activity towards paraoxon. The Caerphilly Prospective Study [16] proved that in patients who survive an acute cardiovascular event, PON1 activity at the beginning of the experiment was lower in comparison with patients who did not develop cardiac disorders. That study revealed that PON1 activity is an independent risk factor for acute coronary syndrome occurrence. The association between PON1 activity and prognosis in patients after CABG has not yet been investigated. Another study [17] observed that PON1 paraoxonase and arylesterase activities were significantly reduced in the first hour after CABG in patients receiving atorvastatin therapy before surgery and after the first and sixth hour in patients not previously treated with statins. Because of a physical connection of the PON1 enzyme to HDL particles, it seems to be reasonable to examine the association of lipid profile with patient outcome after cardiac surgery. It was previously revealed [18] that the decreased concentration of apo A-I is associated with an increased mortality and with more frequent occurrence of myocardial infarction. On the other hand, it was found that levels of total cholesterol, LDL, HDL and triacylglycerols were not related to total and cardiac mortality. In the present study, we found a lack of association between the PON1 enzyme activity and the plasma lipid profile, with the exception of the positive correlation between PON1 activity towards phenyl acetate and HDL concentration. These findings suggest that the risk of CHD is not mediated only through conventional risk factors. In view of the accumulating evidence for PON1-mediated protection of LDL against oxidative modifications, genetic polymorphism of the PON1 gene promoter seems to be involved in the determination of an individual's susceptibility to LDL oxidation.

The postoperative complications observed in patients undergoing cardiac surgery are generally caused by thrombosis within coronary artery bypass grafts. Oxidatively modified lipoproteins stimulate the development of atherosclerosis as a result of prothrombotic abilities, platelet activation, and fibrinolysis system dysfunction. PON1 activity may protect against oxidative modifications of plasma lipoproteins and against thrombotic occlusions of aorto-coronary grafts. De Rijke et al. [19] suggested that oxidatively modified lipoproteins formed during the perioperative period stimulate smooth muscle proliferation and may cause early thrombotic occlusions of venous grafts and atherosclerotic occlusions months or years after surgery and the recurrence of angina pectoris symptoms. It is possible that at the time of surgery a series of events begins, finally followed by the development of atherosclerosis and graft occlusion. Increased PON1 activity plays a protective role due to its anti-oxidative function.

PON1 is a negative acute-phase protein: PON1 plasma concentration rapidly decreases as a response to systemic inflammatory reaction. Enzyme synthesis is regulated by changes in mRNA level during the acute-phase reaction. In human hepatocytes it was observed that the level of PON1 mRNA decreases after incubation with oxidatively modified phospholipids or with cytokines stimulating acute-phase reaction, such as Il-1β and TNF-α [20]. Acute-phase activation during CABG is triggered by several agents such as surgical trauma itself, blood contact with extracorporeal surface, endotoxemia, and tissue ischemia. Luyten et al. [21] observed that when using extracorporeal circulation, total plasma antioxidative ability is decreased. Under the circumstances of intensified oxidative stress, PON1 plasma supply may become exhausted as a result of PON1 gene expression inhibition and decreased protein synthesis in the liver, which may influence prognosis after surgery.

Results of the present study have several important implications regarding the role of paraoxonase in patients with CHD undergoing CABG. We proved that PON1 CC and CT genotypes at −108C/T promoter region were associated with significantly higher paraoxonase activity. High PON1 activity connected with the presence of CC and CT genotypes decreased the recurrence of symptoms of coronary heart disease and improved prognosis after CABG.

Conclusions

High paraoxonase 1 activity connected with the presence of CC and CT genotypes decrease the recurrence of symptoms of coronary heart disease and improve prognosis after CABG.

Conflict of interest

None declared.

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