Association between paraoxonase activity and late saphenous vein graft occlusion in patients with coronary artery bypass grafting

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Abstract

Background: Coronary vein graft disease is an important contributor to the morbidity after coronary artery bypass grafting (CABG). Late occlusion of the graft is a serious complication that limits the use of the saphenous vein as a coronary bypass conduit. It is frequently encountered in old, degenerated vein grafts with advanced atherosclerotic plaque formation. Paraoxonase-1 (PON-1) is an HDL-bound enzyme which has anti-atherogenic properties and protects LDL cholesterol from oxidative modification.

Aim: To examine the association between PON-1 activity and late saphenous vein graft occlusion.

Methods: Thirty-eight patients who had at least one occluded saphenous vein graft (group 1; 12 females, 26 males) and 41 patients who had a patent saphenous vein graft (group 2; 7 females, 34 males) were enrolled in this study. Paraoxonase activity was measured spectrophotometrically.

Results: The mean PON-1 activity in group 1 was significantly lower than in group 2 (74.1 ± 52.1 vs. 114.4 ± 90.9 U/l, p = 0.02). The mean platelet volume was significantly higher in group 1 than group 2 (8.8 ± 1.6 vs. 8.2 ± 1.1 fl, p = 0.04). Multiple logistic regression analysis showed that only PON-1 activity (beta = 0.011, p = 0.042) was an independent predictor of late occlusion of a saphenous vein graft.

Conclusions: Our results show that PON-1 activity is lower in patients with late saphenous vein graft occlusion. Reduced PON-1 activity may lead to acceleration of saphenous vein graft occlusion.

Key words: paraoxonase, saphenous vein graft

Introduction

Coronary artery disease (CAD) still remains the major cause of mortality in developed countries. Essential treatment modalities include medical therapy, percutaneous transluminal coronary interventions and coronary artery bypass grafting (CABG). Surgical treatment still remains a valuable option despite the improvements in percutaneous transluminal coronary interventions, especially in three-vessel disease and left main CAD.

Although arterial graft conduits are intended to be used in bypass surgery most commonly, saphenous vein grafts are still frequently used. At the end of the first year, 15% of these saphenous grafts restenose, and by 10 years after surgery, approximately 50% of these grafts are occluded [1]. Saphenous vein graft occlusion is the main reason for the majority of reinterventions [2].

Studies have shown that typical atherosclerotic changes have not been observed up to 1 year and very rarely up to 2 years in vein grafts. After 2 years, graft atherosclerosis becomes a major source of mortality and morbidity. Atherosclerosis follows similar pathways in vein grafts as in native artery disease.

An increased level of high density lipoproteins (HDL) has been reported to be associated with decreased risk for
CAD. This protective effect of HDL against atherosclerosis is attributed to the enzymes associated with HDL metabolism. One of these enzymes is paraoxonase (PON).

There are three known PON genes: PON1, PON2 and PON3. The PON-1 is synthesised in the liver and transported to the HDL in the plasma [3]. It is a protein of 354 amino acids with a molecular weight of 43 kDa [4]. Studies have shown that HDL-associated PON-1 inhibits lipid peroxidation or degrades biologically active oxidised lipids in low density lipoprotein (LDL) [5]. The PON-1 is recruited with breakdown of lipid peroxides before they can accumulate on LDL [6]. The PON-1 over-expression protects mouse from atherosclerosis [7]. Its activity decreases in many diseases such as slow coronary flow [8], cardiac syndrome X [9], CAD [10, 11], diabetes mellitus, and myocardial infarction [12].

No studies have previously evaluated the effects of PON activity on late saphenous vein graft occlusion. Therefore, the goal of this study was to assess the association between PON activity and late saphenous vein graft occlusion.

Methods
Study population
The study was performed at the Ministry of Health, Diskapi Yıldırım Beyazıt Research and Educational Hospital, Cardiology Clinic and Yüksek İhtisas Education and Research Hospital, Cardiology Clinic in Turkey from June 2007 to May 2008.

The study population included 79 consecutive patients who underwent elective coronary angiography because of recurrence of postoperative symptoms. Two groups of patients were included in the study: 38 patients (12 females, 26 males, mean age 61.6 ± 8.9 years) who had at least one occluded saphenous vein graft on their late control coronary angiography after CABG, and 41 patients (7 females, 34 males, mean age of 61.0 ± 9.7 years) who had patent vein grafts on their last control coronary angiography.

Subjects who had acute or chronic inflammatory diseases, myeloproliferative diseases, malignancies, renal, hepatic and thyroid diseases, immunological diseases, haematocrit < 0.30 or > 0.52, platelet count < 100 000/mm³, patients with acute coronary syndromes during the last 48 h before hospitalisation, and those with severe valvular heart diseases were excluded from the study. Patients who underwent CABG within one year preceding the study were also excluded.

Transthoracic echocardiography was performed using the Vingmed Vivid 3 echocardiograph and a 2.5 MHz transducer to detect underlying structural heart disease. Written consent was obtained from all patients and our local ethical committee approved the study.

Coronary angiography
Coronary angiography was performed using standard angiographic techniques. All cineangiograms were reviewed by two interventional cardiologists who were blinded to the study protocol. The bypass grafts were examined in multiple projections and the degree of stenosis determined in the projection that showed the most severe narrowing. The stumps of occluded (100% stenosis) grafts were selectively injected or visualised on aortography in the appropriate projection.

Blood sample collection
Blood samples were obtained following an overnight fasting period before angiography. The serum was separated from the cells by centrifugation at 3000 r min⁻¹ for 10 min and stored at −78°C until measurement of PON activity.

Measurement of PON activity
The serum PON activity was measured using the synthetic substrate paraoxon (diethyl-p-nitrophenol, PS610, SUPELCO, USA). The rate of paraoxon hydrolysis was measured by monitoring the increase of absorbency at 415 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 16 900 M⁻¹cm⁻¹ [13]. Paraoxonase activity was expressed as U/l.

Measurement of lipids and lipoproteins
Measurement of lipids and lipoproteins was performed at the Ministry of Health, Diskapi Yıldırım Beyazıt Research and Educational Hospital, Cardiology Clinic and Yüksek İhtisas Education and Research Hospital, Cardiology Clinic in Turkey from June 2007 to May 2008.

Measurement of lipids
Fasting plasma glucose, total cholesterol, HDL cholesterol, and triglyceride levels were measured enzymatically by the autoanalyzer (Hitachi 911, Japan). The LDL cholesterol levels were determined with the Friedewald formula.

Other laboratory data
Blood samples were collected in an ethylene diamine tetra acetic acid (EDTA) tube for complete blood count, including platelet count and haemoglobin. Mean platelet volume was measured using a Coulter S+ resistive particle counting system.

Statistical analysis
Results are reported as mean ± standard deviation (SD) and percentages. Student’s t-test was used to compare normally distributed continuous variables and the Mann-Whitney U test was used for variables without normal distribution. Categorical variables were compared by using the χ² test. In order to determine independent predictors of late saphenous vein graft occlusion, multiple logistic regression analysis was performed by including parameters which were significantly different between group 1 and group 2. A p value of < 0.05 was considered statistically significant. The SPSS-15.0 for Windows statistical software package was used.

Results
The demographic and clinical characteristics of the groups are presented in Table I. There were no significant
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Differences in age, gender, body mass index (BMI) and presence of typical atherosclerotic risk factors between the groups. The mean period from CABG to last coronary angiogram was similar in both groups (71.6 ± 45.5 vs. 67.5 ± 56.3 months, NS). The two groups did not differ with regard to baseline medication taken before entry into the study including aspirin, beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, nitrates, and lipid-lowering drugs. However, statin use was significantly lower in patients with occluded grafts [13 (34.2%) vs. 24 (58.5%), p = 0.03].

The laboratory parameters of the groups are summarised in Table II. The values of total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, haemoglobin, fasting blood glucose, and fibrinogen levels were not different between the groups.

The mean PON-1 activity in patients with graft occlusion was significantly lower than in patients with patent grafts [13 (34.2%) vs. 24 (58.5%), p = 0.03].

Table I. Demographic and clinical characteristics of the study patients

<table>
<thead>
<tr>
<th></th>
<th>Patients with graft occlusion (n = 38)</th>
<th>Patients with patent grafts (n = 41)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>61.6 ± 8.9</td>
<td>61.0 ± 9.7</td>
<td>0.75</td>
</tr>
<tr>
<td>Male/female</td>
<td>26/12</td>
<td>34/7</td>
<td>0.13</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>28.9 ± 4.0</td>
<td>28.1 ± 3.4</td>
<td>0.31</td>
</tr>
<tr>
<td>Hypertension [%]</td>
<td>17 (44.7)</td>
<td>20 (48.8)</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetes mellitus [%]</td>
<td>13 (34.2)</td>
<td>12 (29.3)</td>
<td>0.64</td>
</tr>
<tr>
<td>Smoking [%]</td>
<td>11 (28.9)</td>
<td>9 (22.0)</td>
<td>0.48</td>
</tr>
<tr>
<td>Family history of CAD [%]</td>
<td>7 (18.4)</td>
<td>9 (22.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>Ejection fraction [%]</td>
<td>49.3 ± 10.0</td>
<td>48.0 ± 11.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Aspirin, n (%)</td>
<td>32 (84.2)</td>
<td>34 (82.9)</td>
<td>0.88</td>
</tr>
<tr>
<td>Beta-blockers, n (%)</td>
<td>29 (76.3)</td>
<td>34 (82.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>ACE inhibitors, n (%)</td>
<td>17 (44.7)</td>
<td>14 (34.1)</td>
<td>0.34</td>
</tr>
<tr>
<td>ARB, n (%)</td>
<td>4 (10.5)</td>
<td>4 (9.8)</td>
<td>0.91</td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>13 (34.2)</td>
<td>24 (58.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Nitrates, n (%)</td>
<td>5 (13.2)</td>
<td>9 (22)</td>
<td>0.30</td>
</tr>
<tr>
<td>Time from surgery [months]</td>
<td>71.6 ± 45.5</td>
<td>67.5 ± 56.3</td>
<td>0.72</td>
</tr>
<tr>
<td>SBP [mmHg]</td>
<td>132.6 ± 10.4</td>
<td>133.8 ± 11.1</td>
<td>0.64</td>
</tr>
<tr>
<td>DBP [mmHg]</td>
<td>83.9 ± 7.4</td>
<td>85.2 ± 9.6</td>
<td>0.52</td>
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</table>

Table II. Comparison of laboratory parameters between patients with occluded and patent grafts

<table>
<thead>
<tr>
<th></th>
<th>Patients with graft occlusion (n = 38)</th>
<th>Patients with patent grafts (n = 41)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Haemoglobin [g/dl]</td>
<td>14.2 ± 2.1</td>
<td>13.9 ± 1.9</td>
<td>0.55</td>
</tr>
<tr>
<td>Platelets [x 10³/mm³]</td>
<td>253.7 ± 75.3</td>
<td>249.9 ± 85.6</td>
<td>0.83</td>
</tr>
<tr>
<td>MPV [fl]</td>
<td>8.8 ± 1.6</td>
<td>8.2 ± 1.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Fibrinogen [mg/dl]</td>
<td>4.0 ± 1.1</td>
<td>3.8 ± 1.0</td>
<td>0.28</td>
</tr>
<tr>
<td>Total cholesterol [mg/dl]</td>
<td>189.4 ± 44.3</td>
<td>174.1 ± 36.1</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL cholesterol [mg/dl]</td>
<td>111.2 ± 37.6</td>
<td>97.6 ± 32.1</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL cholesterol [mg/dl]</td>
<td>38.8 ± 9.6</td>
<td>38.6 ± 11.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Triglyceride [mg/dl]</td>
<td>189.9 ± 111.6</td>
<td>170.8 ± 94.2</td>
<td>0.41</td>
</tr>
<tr>
<td>Fasting glucose [mg/dl]</td>
<td>96.3 ± 12.8</td>
<td>92.1 ± 11.8</td>
<td>0.14</td>
</tr>
<tr>
<td>PON-1 activity [U/l]</td>
<td>74.1 ± 52.1</td>
<td>114.4 ± 90.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Abbreviations: BMI – body mass index, ACE – angiotensin-converting enzyme, ARB – angiotensin receptor blocker, SBP – systolic blood pressure, DBP – diastolic blood pressure

Abbreviations: MPV – mean platelet volume, LDL – low-density lipoprotein, HDL – high-density lipoprotein, PON – paraoxonase
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was an independent predictor of late occlusion

parameters with significant differences between the two
groups showed that only PON-1 activity ($\beta = 0.011, p = 0.042$) was an independent predictor of late occlusion of a saphenous vein graft (Table III).

Discussion

To our knowledge, this is the first study to evaluate the effects of PON-1 activity on late saphenous vein graft occlusion. In our study, we found that mean PON-1 activity was significantly lower in patients with occluded saphenous vein graft than in the patent saphenous vein graft group. Also, mean platelet volume was significantly higher in the occluded saphenous vein group than in the patent saphenous vein group. In multiple logistic regression analysis, only PON-1 activity was an independent predictor of late occlusion of a saphenous vein graft.

Coronary artery bypass grafting is one of the commonly used revascularisation techniques and it improves the patient’s quality of life unless graft occlusion occurs. Graft occlusion remains the major problem of this procedure. Three consecutive phases of venous graft disease have been described: a phase of thrombotic occlusion (especially during the first month), an intermediate phase of neointimal hyperplasia (up to 1 year), and a phase of atherosclerotic disease (after 1 year). Late vein graft occlusion is considered to be a part of the atherosclerotic process [14-16]. Therefore, this study was performed in patients with a long-term follow-up of a saphenous vein graft. The mean time from surgery was $71.6 \pm 45.5$ months in the occluded saphenous vein group and $67.5 \pm 56.3$ months in the patent saphenous vein group.

Increased levels of HDL have been reported to be associated with decreased risk for CAD. This protective effect of HDL is largely related to the PON-1 enzyme which is located on the HDL molecule [17]. Several studies have shown that HDL protects LDL from oxidative modification, which is thought to be the principle step of atherosclerosis. The PON-1 can hydrolyse lipid peroxides in oxidised lipoproteins [18]. It has been recently observed that PON-1 hydrolyses and reduces lipid peroxides not only in LDL but also in human coronary and carotid lesions (Michael Aviram, unpublished). Several studies have demonstrated that there is a significant relationship between PON-1 activity and concentration and severity of CAD [19-21]. The reduced PON-1 activity in the occluded saphenous vein graft group revealed in our study supports these studies.

Previous studies have shown that changes of PON-1 activity may occur independently of changes in the HDL cholesterol [22, 23]. Navab et al. demonstrated in patients with CAD that oxidation of LDL was not protected by HDL due to low serum PON activity despite relatively normal HDL concentrations [24]. Similarly, in our study, there was no difference in HDL cholesterol between the groups. The PON activity decreases in many diseases such as slow coronary flow [8], cardiac syndrome X [9], CAD [10, 11], diabetes mellitus, myocardial infarction [12], and acute phase response [25]. The PON-1 activity is determined genetically; however, several factors can influence its activity. Van Der Gaag et al. have reported that PON activity was increased in subjects with daily moderate alcohol intake [26]. James et al. have shown that smoking decreased serum PON activity [27]. Degraded cooking oil has been reported to lower serum PON-1 levels in humans [28]. In addition, previous studies demonstrated that PON-1 activity was increased with statin use [29-31]. In the present study, statin use was lower in the occluded saphenous vein group than in the patent saphenous vein group. This finding might explain the reduced PON-1 activity of the occluded saphenous vein group. However, this increased statin use did not cause a significant difference in the HDL, LDL and total cholesterol values between the two groups. In addition, in the multiple logistic regression analysis, only PON-1 activity was an independent predictor of late occlusion of a saphenous vein graft.

Study limitations

The most important limitation of our study is the small number of patients. In addition, the PON-1 genotype was not determined in the study population. However, serum PON-1 concentration and activity have been shown to be better predictors of the risk for cardiovascular diseases than PON-1 genotype [32, 33]. In addition, the levels of ox-LDL, homocysteine as well as hs-CRP or IL-6 have not been measured in our study. Therefore, it cannot be ruled out that the basic pathomechanisms of late saphenous vein graft occlusion include the activation of monocytes/macrophages caused by high homocysteine level and the associated oxidative stress. Lastly, although multivariate analysis showed that PON-1 activity was an independent predictor of graft occlusion, this association was of almost borderline significance ($p = 0.042$) and differences in statin use were striking. Thus, some uncertainty concerning the independent value of PON-1 exists.

Conclusion

Our results showed that PON-1 activity is lower in patients with late saphenous vein graft occlusion than in

<table>
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<th>Table III. Multivariate analysis of parameters predicting saphenous vein graft occlusion</th>
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<tr>
<td>Parameter</td>
</tr>
<tr>
<td>PON-1 activity [U/l]</td>
</tr>
<tr>
<td>MPV [fl]</td>
</tr>
<tr>
<td>Statin use</td>
</tr>
</tbody>
</table>

Abbreviations: PON – paraoxonase, MPV – mean platelet volume

(74.1 ± 52.1 vs. 114.4 ± 90.9 U/l, $p = 0.02$). The mean platelet volume was significantly higher in patients with occluded grafts ($8.8 \pm 1.6$ vs. $8.2 \pm 1.1$ fl, $p = 0.04$).

Multiple logistic regression analysis including parameters with significant differences between the two groups showed that only PON-1 activity ($\beta = 0.011, p = 0.042$) was an independent predictor of late occlusion of a saphenous vein graft (Table III).
those with patent grafts. Reduced PON-1 activity may lead to the acceleration of saphenous vein graft occlusion. These patients require more aggressive medical treatment in order to prevent the atherosclerotic process.

References

Związek pomiędzy aktywnością paraoksonazy a późną niedrożnością pomostów żylnych u chorych poddawanych chirurgicznej rewaskularyzacji wieńcowej

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Streszczenie


Cel: Zbadanie związku pomiędzy aktywnością PON-1 a późnym zamknięciem pomostu żylnego.

Metody: Do badania włączono 38 chorych z co najmniej jednym zamkniętym pomostem żylnym (grupa 1 – 12 kobiet, 26 mężczyzn) i 41 chorych z drożnym pomostem żylnym (grupa 2 – 7 kobiet, 34 mężczyzn). Aktywność PON-1 mierzono metodą spektrofotometryczną.

 Wyniki: Średnia aktywność PON-1 w grupie 1 była istotnie niższa niż w grupie 2 (74,1 ± 52,1 vs 114,4 ± 90,9 U/l, p = 0,02), natomiast średnia objętość płytek krwi była istotnie wyższa w grupie 1 niż 2 (8,8 ± 1,6 vs 8,2 ± 1,1 fl, p = 0,04). Wieloczynnikowa analiza regresji wykazała, że aktywność PON-1 była jedynym niezależnym czynnikiem związanym z późnym zamknięciem żylnego pomostu aortalno-wieńcowego ($\beta = 0,011$, $p = 0,042$).

Wnioski: Aktywność PON-1 jest mniejsza u chorych z zamkniętym pomostem żylnym, co może wskazywać, że zmniejszona aktywność tego enzymu przyspiesza zamknięcie żylnych pomostów aortalno-wieńcowych.

Słowa kluczowe: paraoksonaza, żylny pomost aortalno-wieńcowy, okluzja

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