Clinical Research

Relationship between platelet P-selectin and severity of acute coronary syndromes

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Objective Recent studies reveal important roles of platelet P-selectin on progression of atherosclerosis. In the present study, we examine the relation between platelet P-selectin expression and severity of acute coronary syndromes.

Methods One hundred and eighty-four consecutive patients with proven or clinically suspected acute coronary syndromes (ACS) were enrolled in the study. Level of P-selectin expression was determined by flow cytometry. Platelet P-selectin level was expressed as the percentage of P-selectin positive platelet.

Results The level of P-selectin was higher in patients with a single diseased coronary artery or multiple diseased arteries compared to those with normal coronary arteries. P-selectin expression was significantly and positively correlated with angiographic Gensini score ($r=0.323$, $P=0.029$). Multiple regression analyses showed that the association of the percentage of P-selectin-positive platelets with ACS was independent of other clinical factors.

Conclusions Platelet P-selectin is associated with severity of acute coronary syndromes in patients with acute coronary syndromes. (J Geriatr Cardiol 2008; 5:146-149)

Key words platelets, P-selectin, acute coronary syndromes

Introduction

Coronary plaque disruption, with consequent platelet aggregation and thrombosis, is the most important mechanism by which atherosclerosis leads to the acute ischemic syndromes of unstable angina, acute myocardial infarction, and sudden cardiac death. Inflammation plays a pivotal role in the pathogenesis of atherosclerosis and its complications. In particular, atherosclerosis is an active process and the inflammatory component appears to be particularly correlated with the development of acute coronary syndromes (ACS). Accumulating data demonstrate that in ACS, elevated levels of circulating inflammatory markers, such as P-selectin, predict an unfavorable cardiovascular outcome.

P-selectin, which is a surface adhesion molecule, is deposited in the α-granules of thrombocytes and Weibel-Palade bodies of endothelial cells. Following the activation of these cells, P-selectin moves rapidly to the cellular surface and plays an important role in thrombosis by increasing the interaction between thrombocytes and coagulation factors.

In the present study, we examined the level of P-selectin in patients with ACS to further understand the relation of P-selectin to the severity of coronary artery disease.

Coronary angiography

Following clinical stabilization, all patients underwent...
selective coronary angiography. Coronary angiography in multiple views was performed according to the standard Judkins technique. At least five views, including two orthogonal views, were acquired for the left coronary artery and at least two orthogonal views for the right coronary artery. At least 50% obstruction in any of the major coronary arteries was evaluated as an evidence of ischemic heart disease.

**Determination of the severity of coronary atherosclerosis**

The severity of coronary stenosis in the patients was estimated by the number of affected vessels (> 50% of lumenal diameter) and the coronary score of Gensini. We used the Gensini score for this study to test the severity of coronary stenosis. The Gensini score system yields a qualitative and quantitative evaluation of the coronary angiogram which grades narrowing of the lumen of the coronary artery as 1 for 1–25% narrowing, 2 for 26–50% narrowing, 4 for 51–75% narrowing, 8 for 76–90% narrowing, 16 for 91–99% narrowing, and 32 for total occlusion. This score is then multiplied by a factor that takes into account the importance of the position of the lesion in the coronary arterial tree, e.g. 5 for the left main coronary artery, 2.5 for the proximal left anterior descending artery or proximal left circumflex artery, 1.5 for the mid-region and 1 for the distal left anterior descending artery and 1 for the mid-distal region of the left circumflex artery or right coronary artery. The Gensini scores of all patients were calculated after evaluating the degree of stenosis, eccentric or concentric lesion localization and multiplying with a constant. Gensini scores were based on the consensus opinion of two experienced angiographers.

**Blood sampling and measurement of the P-selectin**

Blood samples were obtained in the fasting, non-sedative state between 6:00 and 7:00 AM in the control and study groups. Blood was obtained under minimal tourniquet pressure from the antecubital vein using a sterile needle and syringe. We used a standard cytometric assay to measure P-selectin surface expression in whole blood without prior fixation with formaldehyde. Washed platelets (20 µL) stimulated with ADP, U46619, or thrombin were stained with 10 µL of FITC-conjugated anti-human CD62P and, after a 15-min incubation in the dark, were diluted with 1.5 mL of phosphate-buffered saline (PBS). All samples were analyzed at low flow rate on a Becton Dickinson FACSCalibur. The instrument settings were as follows: forward scatter, E00; side scatter, 337 V; fluorescence channel 1, 850 V. Platelets were differentiated from other cells on the basis of their scatter characteristics. Using CELLQuest software, we analyzed a total of 10 000 platelet events to obtain the mean fluorescence. Platelet P-selectin level was expressed as the percentage of P-selectin positive platelet.

**Statistical analysis**

Data were entered into SPSS 11.5 (Statistical Package for Social Sciences) for Windows program. Numeric values are reported as means ± SD or as proportions of the sample size. Comparisons between the study and control groups were made with the χ² test for categorical data and Student’s t test for continuous data. Multiple logistic regression analysis (backward stepwise) was applied to evaluate the association of presence of ACS with P-selectin and other coronary risk factors. To examine the correlation between the extent of P-selectin and Gensini scores, Pearson’s correlation coefficient was used. P value <0.05 was considered significant.

**Results**

The baseline clinical characteristics of these patients are summarized in Table 1. There were statistically significant differences between the 2 groups in history of diabetes, smoking and LDL-cholesterol (P<0.05). The level of P-selectin of ACS group was significantly higher than the control (P=0.001).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=71)</th>
<th>ACS (n=113)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(year)</td>
<td>57 ± 10</td>
<td>61 ± 12</td>
<td>0.182</td>
</tr>
<tr>
<td>Male(%)</td>
<td>58 (81.7%)</td>
<td>89 (78.8%)</td>
<td>0.629</td>
</tr>
<tr>
<td>Diabetes(%)</td>
<td>13 (18.3%)</td>
<td>38 (33.6%)</td>
<td>0.024</td>
</tr>
<tr>
<td>Hypertension(%)</td>
<td>22 (31.0%)</td>
<td>31 (27.4%)</td>
<td>0.604</td>
</tr>
<tr>
<td>Smoking(%)</td>
<td>39 (54.9%)</td>
<td>79 (69.9%)</td>
<td>0.039</td>
</tr>
<tr>
<td>Total cholesterol(mmol/L)</td>
<td>3.90 ± 1.03</td>
<td>4.48 ± 1.01</td>
<td>0.684</td>
</tr>
<tr>
<td>HDL-C(mmol/L)</td>
<td>1.11 ± 0.20</td>
<td>1.05 ± 0.19</td>
<td>0.710</td>
</tr>
<tr>
<td>LDL-C(mmol/L)</td>
<td>2.78 ± 0.46</td>
<td>3.14 ± 0.31</td>
<td>0.037</td>
</tr>
<tr>
<td>Triglyceride(mmol/L)</td>
<td>2.96 ± 1.06</td>
<td>3.25 ± 1.33</td>
<td>0.485</td>
</tr>
<tr>
<td>P-selectin(%)</td>
<td>2.29 ± 1.05</td>
<td>4.72 ± 1.76</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ACS, acute coronary syndromes; HDL-C, high-density lipoprotein-cholesterol; LDL-C, lower-density lipoprotein-cholesterol. Data are mean ± SD and number (%).
A multiple logistic regression analysis was performed with the presence/absence of ACS as the dependent variable and risk factors included in Table 1 as the predictive variables. The risk factors included age, gender, history of diabetes, hypertension, smoking, total cholesterol, HDL-C, LDL-C, triglyceride and P-selectin. Analysis showed that diabetes (odds ratio [OR] 10.62, 95% confidence interval [CI] 0.95–146.32, P=0.041), LDL-C (OR 3.03, 95% CI 1.47–6.81, P=0.001) and P-selectin (OR 0.65, 95% CI 0.961–147.950, P=0.044) were significant predictors of ACS (Table 2).

Table 2 Multivariate predictors of the presence of acute coronary syndrome

<table>
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<tr>
<th>Risk factors</th>
<th>OR</th>
<th>95% confidence interval</th>
<th>P value</th>
</tr>
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<td>Diabetes</td>
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<td>0.65</td>
<td>0.47 - 0.89</td>
<td>0.001</td>
</tr>
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</table>

When patients were classified according to the number of angiographically diseased coronary arteries, the level of P-selectin was significantly higher in patients with a single diseased artery (n=39, P-selectin level 3.62±1.37%, P<0.05 vs. control group) and with multiple diseased arteries (n=74, P-selectin level 4.49±1.41%, P<0.05 vs. control group; P<0.05 vs. single vessel disease group) than in patients with control group (n=71, P-selectin level 2.31±1.02%) (Figure 1).

The correlation between the P-selectin level and the severity of coronary artery stenosis, as diagnosed by coronary arteriography, was studied. A simple regression analysis revealed that the P-selectin level was positively and significantly correlated with the Gensini score. The higher the P-selectin level, the more severe the coronary artery stenosis (r=0.323, P=0.029) (Figure 2).

Discussion

Platelet activation resulting from plaque disruption is important in the pathogenesis and clinical outcome of acute coronary syndromes. There is therefore of great interest in the measurement of in vivo platelet activation. P-selectin is a component of the α-granules membrane of resting platelets that is expressed only on the platelet surface during and after platelet degranulation and secretion. Platelet surface P-selectin is considered to be the "gold standard" marker of platelet activation.

In the present study we measured the P-selectin expression on platelets in patients with ACS by using flow cytometry. Flow cytometry has the advantage of directly analyzing individual platelets with a high degree of sensitivity in their native milieu and with minimal artificial platelet activation. In this study, we showed that the level of platelet P-selectin was higher in patients with ACS than in those without vessel disease. We also showed that P-selectin level was found to be an independent factor associated with occurrence of ACS. Some studies have show that the increase levels of P-selectin in patients with unstable angina may be due to plaque rupture and thrombus formation, as well as to an interaction of platelets by activated leukocytes before plaque disruption, and P-selectin level could be used as a marker of plaque destabilization in unstable angina.

It is becoming clear that P-selectin is essential for progression of atherosclerosis. P-selectin deficiency reduces atherosclerotic lesion formation in mice. Deficiency of P-selectin inhibits neointimal formation after arterial injury. Besides the role of endothelial P-selectin in monocyte recruitment to the atherosclerotic lesion, recent observations unveil involvement of platelet P-selectin on progression of atherosclerosis. Fang et al reported that the level of sP-selectin in patients with multivessel disease could be higher than in those with single-vessel disease. In our study, the severity of coronary stenosis was assessed by the number of diseased coronary arteries or by angiographic Gensini score, and both of the results demonstrated correlations between the P-selectin level and coronary stenosis. This is in good agreement with experimental observations in genetically modified mice study, with platelet P-selectin expression closely associated with atherosclerosis.

In summary, the level of platelet P-selectin may help us to understand the pathophysiology of ACS, and may indirectly reflect clinical condition of patients with ACS.
with potential diagnostic and therapeutic modalities, and may be useful to predict the severity of ACS.

References