Circulating Levels of Soluble P-Selectin in Patients in the Early and Recent Phases of Myocardial Infarction

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Background: Circulating soluble P-selectin (sP-selectin), a biomarker of platelet activation, is substantially increased in patients with acute myocardial infarction (AMI). However, the circulating level of sP-selectin in patients in the early (onset of AMI > 12 h but ≤ 7 d) or recent (onset of AMI ≥ 8 d but ≤ 21 d) phase after AMI remains unclear. The purpose of this study was to prospectively evaluate whether the circulating level of sP-selectin remains elevated in these two consecutive phases after an AMI.

Methods: Blood samples were collected in the catheterization room before coronary angiography to assess the circulating level of sP-selectin. A total of 53 consecutive patients, 34 with early MI (group 1) and 19 with recent MI (group 2), who had had no prior thrombolytic therapy were included. Circulating levels of sP-selectin were also measured in 30 risk control (stable angina) subjects undergoing elective percutaneous coronary intervention and in 20 healthy subjects who comprised the healthy control group.

Results: The circulating level of sP-selectin did not differ between patients with early AMI and those with recent MI (p = 0.632). However, the plasma level of sP-selectin was significantly higher in group 1 and 2 patients than in the risk control and healthy control subjects (all p values < 0.0001).

Conclusions: Circulating sP-selectin was elevated in patients 12 hours to 7 days after AMI and the elevation was maintained until 21 days after AMI. Therefore, investigation of longer utilization of anti-platelet and anti-inflammatory agents for patients following AMI might be worthwhile.

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Key words: Soluble P-selectin, early or recent myocardial infarction.

Numerous studies have indicated that the acute inflammatory response, as evoked by the cellular interaction of platelets, leukocytes and endothelium, plays a pivotal role in thrombus formation. This process subsequently leads to development of acute coronary syndromes. Other studies have demonstrated that endothelial adhesion molecules and human P-selectin mediate granulocyte binding to
blood vessels and the myocardium in the ischemic human heart\(^{(9)}\) to mediate further cardiomyocyte damage.

P-selectin, a cellular adhesion molecule of platelets and endothelial cells stored in both the \(\alpha\)-granules of the platelets and in the Weibel-Palade bodies of the endothelial cells,\(^{(4,5)}\) is rapidly expressed on the surface of activated platelets and endothelial cells.\(^{(6,7)}\) Furthermore, P-selectin binds to leukocytes under conditions of turbulent flow and modulates the initial tethering and rolling of these leukocytes.\(^{(3,8)}\) Moreover, P-selectin together with endothelial adhesion molecules, such as vascular cell adhesion molecule-1 and intercellular adhesion molecule-1, participates in the platelet-leukocyte-endothelial cell interaction.\(^{(3,4,7,9)}\)

Recent studies have demonstrated that the plasma level of soluble P-selectin (sP-selectin), a form of P-selectin, substantially increases in patients with acute myocardial infarction (AMI).\(^{(10,11)}\) However, no data is available regarding the circulating levels of sP-selectin in patients with early or recent MI. Therefore, the purpose of this investigation was to evaluate the circulating levels of sP-selectin in patients 12 hours to 3 weeks after an acute MI.

**METHODS**

**Study patients**

Patients who experienced post MI angina were considered eligible for percutaneous coronary intervention (PCI). For the purpose of this study, the plasma levels of sP-selectin of all patients undergoing elective PCI were prospectively evaluated. Blood samples were drawn after vascular puncture before coronary angiography was performed in the cardiac catheterization laboratory. Patients with a history of renal insufficiency (creatinine > 1.5 mg/dL), malignancy, febrile disorders, acute or chronic inflammatory disease at the beginning of the study, history of recent infection, gross hemolysis of the blood sample, or immunosuppressive therapy, were excluded to ensure that other variables would not influence the plasma levels of sP-selectin. Between November 2002 and September 2003, 65 consecutive adult patients with post infarction angina who were scheduled for an elective PCI in our hospital were prospectively investigated and recruited. Twelve (18.5%) of the 65 patients were subsequently excluded due to fever (three patients), infection (two patients), gross hemolysis (three patients), or impaired renal function (four patients). Therefore, the remaining 53 patients constituted the study population. Forty two patients (group 1) were diagnosed with AMI 12 hours to 8 days previously, and 23 (group 2) were diagnosed with AMI 8 to 21 days previously.

Thirty subjects (group 3) matched for age, gender, hypertension, diabetes mellitus, current smoking and hypercholesterolemia served as risk control subjects. Twenty healthy age- and gender-matched volunteers (group 4) were also investigated at healthy clinics. Informed consent was obtained from all subjects. The Institutional Review Committee on Human Research in our institution approved the protocol.

**Procedure and protocol**

A transradial artery approach using a 6 F arterial sheath was routinely applied for AMI in our hospital unless Allen’s test was positive. A 6 F Kimny Miniradi (Boston Scientific, Scimed, Inc. Maple Grove, MN, USA) was used for both diagnosis and primary PCI.

Tirofiban therapy (loading dosage of 20 \(\mu\)g/kg of body weight) was administered to patients upon presentation in the emergency room, followed by a maintenance infusion of 0.15 \(\mu\)g/min for 18 to 24 hours after the procedure. Clopidogrel (300 mg loading dose after stenting then 75 mg/day) was given for at least four weeks to patients who underwent primary stenting and aspirin (100 mg orally once a day) was administered to each patient indefinitely.

**Blood sampling, laboratory investigations and assays**

Blood samples were obtained once in both healthy control subjects during clinic examinations and patients with stable angina (risk control subjects) in the cardiac catheterization room. White blood cell (WBC) counts and biochemical measurements were determined by standard laboratory methods.

The concentration of sP-selectin was measured using a standard enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (R and D Systems; Minneapolis, MN, USA). The method for the ELISA has been described in detail in our previous report.\(^{(12)}\) The assay was sensitive to
less than 0.5 ng/ml of P-selectin, according to the manufacturer of the assay kits. We assessed the intra-individual variability of sP-selectin levels in study patients, risk control subjects, and healthy control subjects. The mean intra-assay coefficients of variance were 4.93%, 4.71%, and 4.34%, respectively.

Definitions

Early AMI was defined as > 12 hrs to ≤ 7 days after AMI and recent MI was defined as ≥ 8 days ≤ 21 days after AMI. The diagnosis of AMI was based on following criteria: (1) a history of typical chest pain lasting for more than 30 minutes with either ST-segment elevation or a pathologic Q wave in two consecutive inferior, lateral or precordial leads; (2) typical chest pain lasting for more than 30 minutes with a new onset of complete left bundle branch block or (3) angiographic findings of an occluded coronary vessel with any one of the following criteria: elevated creatine kinase (CK) with CK-MB fraction > 4%, an elevated myoglobin or an elevated cardiac subtraction of Lactic Dehydrogenase levels on at least one occasion, new electrocardiogram changes suggestive of ischemia or new wall motion abnormalities on an echocardiogram.

Statistical analysis

Data were expressed as mean ± SD. Categorical variables were compared using the Chi-square test or Fischer’s exact test. Continuous variables among the 4 groups were compared using one-way ANOVA for parametric data and the Kruskal-Wallis test for non-parametric data. The Wilcoxon rank-sum test with Bonferroni’s correction was used for the post hoc multiple comparison procedure for non-parametric data. Statistical analysis was performed using SAS statistical software for Windows version 8.2 (SAS Institute, Cary, NC, USA). A probability value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of study patients, angina patients and normal control subjects (Table 1)

There were no age differences between groups. However, there were more men in group 1 and group 2 than in the risk control and healthy subject groups. The incidence of current smoking was significantly higher in groups 1 and 2 patients than in the risk control subjects. However, there were no significant differences in other coronary artery risk factors among group 1 patients, group 2 patients and risk control subjects. Additionally, group 1 and group 2 patients and the risk control subjects did not significantly differ in terms of previous MI or previous stroke. At the commencement of the study, aspirin was used more often and simvastatin less often by both group 1 and group 2 patients than in either the risk control or healthy control subjects.

The duration of time since AMI (from onset of chest pain to blood sampling) was noticeably longer in group 2 than in group 1 patients. No significant differences appeared in terms of anterior wall infarction, advanced congestive heart failure (defined as congestive heart failure ≥ class 3 of the New York Heart Association Functional Classification), multi-vessel disease, or pre-interventional thrombolysis in myocardial infarction flow ≤ 1.

Comparison of sP-selectin between study patients and control subjects

Circulating levels of sP-selectin in study patients and control subjects are shown in the Figure. The plasma level of sP-selectin did not differ between group 1 and group 2 patients (47.4 ± 21.5 vs. 48.8 ± 15.2, p = 0.832). Additionally, the plasma level of sP-selectin also did not differ between risk control and normal control subjects (33.7 ± 15.6 vs. 29.5 ± 6.3, p = 0.607). However, the plasma level of sP-selectin in group 1 and group 2 was significantly higher than in the risk control and healthy control subjects (p < 0.0001) (Figure).

DISCUSSION

In vitro study has shown that sP-selectin can be upregulated during AMI. Other studies have found that circulating levels of sP-selectin markedly increase in patients following AMI. However, there appears to be no available data regarding circulating levels of sP-selectin in patients with early or recent MI. To our knowledge, this study was the first concerning the circulating level of sP-selectin and its course from the early to late stages of MI.
The crucial finding in the present study was that the circulating levels of sP-selectin were substantially higher in patients with early or recent MI than in either healthy control subjects or risk control subjects. A second important discovery was that the circulating levels of sP-selectin did not vary between patients with early MI and patients with recent MI. Therefore, our findings extend those of previous studies (10,11) because the circulating level of sP-selectin was not only significantly elevated in the acute stage of MI (10,11) but was also persistently elevated from the acute stage through the late stages of MI.

This investigation has two striking clinical implications. First, platelet activation is persistently enhanced after myocardial infarction. This suggestion is based on a previous study which claimed that sP-selectin is a new marker of platelet activation (13). Our contention is further corroborated by a recent study (14) demonstrating that platelet activation remains significantly higher in patients 90 days after an ischemic stroke than in healthy control subjects. We hypothesize that patients with AMI due to plaque rupture from coronary atherosclerosis may be clinically similar to patients with ischemic stroke due to sudden occlusion from cerebrovascular atherosclerotic disease. Therefore, the persistently enhanced platelet activation which has been found in stroke patients either due to diffuse atherosclerotic lesions (15) or the vascular damage due to the stroke (16) was also present in our patients, as reflected by persistently elevated circulating sP-selectin. Accordingly, we conclude that our finding is of clinical importance because it provides additional useful information for daily clinical practice in regard to the longer-term use of effective and potent anti-platelet agents in patients in the clinical setting of AMI.

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Table 1. Baseline Characteristics of Study Patients, Risk Control Subjects and Healthy Control Subjects*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (n = 34)</th>
<th>Group 2 (n = 19)</th>
<th>Risk control (n = 30)</th>
<th>Normal control (n = 20)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>62.1 ± 9.5</td>
<td>61.9 ± 11.5</td>
<td>65.3 ± 8.9</td>
<td>59.1 ± 8.0</td>
<td>0.163</td>
</tr>
<tr>
<td>Men</td>
<td>88.2% (30)</td>
<td>89.5% (17)</td>
<td>56.7% (17)</td>
<td>50.0% (10)</td>
<td>0.001</td>
</tr>
<tr>
<td>Current smoking</td>
<td>61.8% (21)</td>
<td>57.9% (11)</td>
<td>30.0% (9)</td>
<td>—</td>
<td>0.028</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58.8% (20)</td>
<td>52.6% (10)</td>
<td>46.7% (14)</td>
<td>—</td>
<td>0.623</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>29.4% (10)</td>
<td>31.6% (6)</td>
<td>30.0% (9)</td>
<td>—</td>
<td>0.986</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>47.1% (16)</td>
<td>42.1% (8)</td>
<td>43.3% (13)</td>
<td>—</td>
<td>0.928</td>
</tr>
<tr>
<td>Previous MI</td>
<td>11.8% (4)</td>
<td>10.5% (2)</td>
<td>16.7% (5)</td>
<td>—</td>
<td>0.843</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>11.8% (4)</td>
<td>10.5% (2)</td>
<td>3.3% (1)</td>
<td>—</td>
<td>0.525</td>
</tr>
<tr>
<td>Current medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>55.9% (19)</td>
<td>63.2% (12)</td>
<td>26.7% (8)</td>
<td>—</td>
<td>0.018</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>14.7% (5)</td>
<td>21.1% (4)</td>
<td>13.3% (4)</td>
<td>—</td>
<td>0.798</td>
</tr>
<tr>
<td>Statins</td>
<td>23.5% (8)</td>
<td>31.6% (6)</td>
<td>56.7% (17)</td>
<td>—</td>
<td>0.020</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>20.6% (7)</td>
<td>42.1% (8)</td>
<td>40.0% (12)</td>
<td>—</td>
<td>0.152</td>
</tr>
<tr>
<td>WBC counts†</td>
<td>8.9 ± 3.1 a</td>
<td>9.4 ± 3.0 b</td>
<td>6.1 ± 1.5 c</td>
<td>5.0 ± 1.0 d</td>
<td>0.0169</td>
</tr>
<tr>
<td>† AMI duration (day)</td>
<td>3.3 ± 1.7 a</td>
<td>12.7 ± 4.0 c</td>
<td>—</td>
<td>—</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Anterior infarction</td>
<td>50.0% (17)</td>
<td>47.4% (9)</td>
<td>—</td>
<td>—</td>
<td>0.854</td>
</tr>
<tr>
<td>† Advanced CHF</td>
<td>14.7% (5)</td>
<td>15.8% (3)</td>
<td>—</td>
<td>—</td>
<td>1.000</td>
</tr>
<tr>
<td>Multi-vessel disease</td>
<td>52.9% (18)</td>
<td>52.6% (10)</td>
<td>—</td>
<td>—</td>
<td>0.983</td>
</tr>
<tr>
<td>Pre-PCI TIMI flow ≤ 1</td>
<td>70.6% (24)</td>
<td>68.4% (13)</td>
<td>—</td>
<td>—</td>
<td>0.869</td>
</tr>
</tbody>
</table>

Abbreviations: AMI: acute myocardial infarction; ACE: angiotensin converting enzyme; CHF: congestive heart failure; PCI: percutaneous coronary intervention; TIMI: thrombolysis in myocardial infarction; WBC: white blood cell.

* Values are given as mean value ± SD or No. (%), unless otherwise indicated.
† Means with different letters (a, b) indicate significant difference (at 0.05 level) by Wilcoxon Rank-Sum test with Bonferroni’s correction.
‡ AMI duration was defined as the time from chest pain to blood sample collection.
§ Advanced congestive heart failure (CHF) was defined as New York Association classification ≥ 3.
after a PCI than patients with low platelet reactivity.\(^{(17)}\) The second striking clinical implication of this investigation is that an inflammatory process was maintained through the recent phase of myocardial infarction in our patients. This implication is supported by the fact that the circulating WBC count was found to be significantly higher in patients in the early and recent phases of myocardial infarction than in either healthy control or risk control subjects. A previous study has suggested that the formation of sP-selectin on platelet activation may have biological implications in mediating leukocyte recruitment.\(^{(18)}\)

This evidence could explain why the WBC count remained substantially higher in our patients three weeks after AMI than in the control subjects. Additionally, another study has shown that leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets.\(^{(19)}\)

Accordingly, we contend that the persistently elevated circulating WBC counts found in this investigation could directly participate in enhancement of intracoronary thrombus formation, maintain an occluded pattern in the infarct-related artery and further damage the myocardial cells, as well as maintain an inflammatory reaction in patients. Our findings, based on previous reports,\(^{(19)}\) laboratory investigation and clinical observation, that more than 68% of our patients had totally occluded infarct-related arteries following an early and/or recent MI were further supported by a recent study which showed that P-selectin mediates granulocyte binding to myocardium.\(^{(20)}\)

This, in turn causes damage to the cardiomyocyte. Furthermore, growing numbers of studies report that inflammation plays a crucial role in the cell biology of atherosclerosis.\(^{(21-23)}\) Yet, another study has emphasized the important role of P-selectin in facilitating atherosclerosis lesion development.\(^{(21)}\)

The results from this and other investigations further imply that circulating sP-selectin may not only be a biomarker of platelet activation,\(^{(24)}\) but also directly

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**Figure** Comparison of circulating levels of soluble P-selectin (sP-selectin) among the control subjects and study patients. White, shadowed, gray, and black bars represent the plasma levels of sP-selectin in healthy control subjects, risk control subjects, group 1 and group 2 patients, respectively. * *\(p = 0.607\) vs. healthy control; † *\(p = 0.832\) vs. group 2 patients; ‡ *\(p < 0.0001\); § *\(p < 0.0001\) compared with both the risk control subjects and healthy control subjects.
participate in an ongoing inflammatory process in atherosclerosis in the coronary artery. This encourages us to investigate the possibility of using this biomarker for risk stratification of patients for future development of atherosclerotic lesions.

The present study has two limitations. First, as this study was only designed to investigate plasma levels of sP-selectin in patients following early or recent MI, it could not provide a long-term value of plasma levels of sP-selectin in patients following myocardial infarction. Second, although sP-selectin has been suggested as an index of platelet activation, another study has emphasized that flow cytometry could more accurately assess the serial changes in platelet activation. Without comparing these two methods, it is uncertain which method is superior for accurate measurement of platelet activity during long-term follow-up. Therefore, caution should be used when extrapolating the results of this study to management of patients in a similar clinical setting.

In conclusion, the plasma level of circulating sP-selectin, an index of platelet activation and an inflammatory biomarker, was markedly increased after AMI, and thereafter, was maintained without a significant change in level throughout the recent phase of MI. The findings from this investigation encourage the long-term use of potent anti-platelet and anti-inflammatory agents for patients after AMI.

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早期或近期心肌梗塞循環中可溶性黏著因子 sP-Selectin 的血中濃度

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洪尉欽 洪志凌 吳炯仁 葉漢根

背 景：循環中可溶性黏著因子 (soluble P-Selectin) 是血小板活化的生物標靶，已經被廣泛了
解在急性心肌梗塞後的患者中會顯著增加。然而，早期心肌梗塞 (early myocardial
infarction)（定義為：心肌梗塞的發作時間大於 12 小時且小於 7 天）或近期心肌梗塞
(recent myocardial infarction)（定義為：心肌梗塞的發作時間大於 8 天但小於 21 天）
的病人中的可溶性黏著因子值依然未明瞭。這個研究的目的是去評估是否在這兩個
連續心肌梗塞時期的可溶性黏著因子的值仍會持續升高。

方 法：血液樣本分別在導管室做血管攝影前收集來評估可溶性黏著因子的值。其中早期心
肌梗塞 (第一組) 有 34 位患者，近期心肌梗塞（第二組）有 19 位患者。這些病患
發生心肌梗塞後都是延誤到院求診，而且未事先接受血栓溶解劑的治療。同時，30
位穩定性心絞痛的患者 (risk-control group) 進行選擇性冠狀動脈整形術 (elective
coronary angioplasty) 及 20 位健康對照者 (healthy control group) 接受可溶性黏著因子
的測量。

結 果：可溶性黏著因子的值在早期心肌梗塞和近期心肌梗塞的病人間比較並沒有統計上顯
著差異 (p 值 = 0.632)。然而，血中可溶性黏著因子的值在第一組和第二組的病人比
危險因子及健康對照組有明顯的升高 (所有 p 值 < 0.0001)。

結 論：循環中的可溶性黏著因子的值在早期心肌梗塞的病人顯著的升高而且持續至心肌梗
塞近期。這些發現鼓勵我們進行前瞻性研究對於急性心肌梗塞的病人更長時間使
用更有效的抗血小板和抗發炎藥物的可行性。

(長庚醫誌 2005;28:613-20)

關鍵字：循環的可溶性黏著因子 (soluble P-Selectin)，早期或近期的心肌梗塞。