Role of Cytokines and Adhesion Molecules in Ischemia and Reperfusion in Patients With Acute Myocardial Infarction

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Although acute myocardial infarction (AMI) may involve both plaque rupture and ischemia-reperfusion injury, the pathogenesis of these phenomena is unclear. To elucidate the pathogenesis of AMI, serial measurements of platelet activating factor (PAF), interleukin-6 and cell adhesion molecules were made in patients with AMI. The PAF levels were measured upon hospital admission and at 24 and 72 h in 8 patients with AMI. Serum levels of interleukin-6, soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 (sVCAM-1) were measured upon admission and at 24 h and 4 weeks in 30 patients with AMI and 15 patients with stable effort angina. PAF levels were higher in patients with AMI than in normal volunteers; the increased levels lasting at least 72 h. In contrast, interleukin-6 increased at 24 h. sE-selectin was elevated at admission and sVCAM-1 increased later. sE-selectin levels upon admission in patients with additional ST-segment elevation after reperfusion were significantly higher than those in patients without ST-elevation. In patients with AMI, the time-course of changes in blood levels of cytokines varied according to the individual substances. Although it is unclear what is the precise role of each of the cytokines in the pathophysiology of AMI, sE-selectin may be possibly related to the reperfusion injury in the infarcted myocardium. (Jpn Circ J 1999; 63: 362–366)

Key Words: Acute myocardial infarction; Platelet activating factor; Soluble E-selectin

Methods

Study Patients

The blood PAF levels were measured in 8 consecutive patients with AMI who were admitted within 12 h of the onset of symptoms and in 98 normal volunteers (Control). The study patients with AMI consisted of 6 men and 2 women, ranging in age from 47 to 79 years, with a mean age of 62.2±10.4 (mean ± SD) years (Study 1). We also measured the plasma levels of interleukin-6, soluble E-selectin (sE-selectin), soluble ICAM-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) in 30 patients with AMI who were admitted within 12 h of the onset of symptoms and in 15 patients with stable effort angina (SÉA). The patients with AMI consisted of 26 men and 4 women, ranging in age from 30 to 80 years, with a mean age of
Increased PAF and E-selectin in AMI

66.5±10.5 years. The age- and sex-matched patients with SEA consisted of 11 men and 4 women, ranging in age from 48 to 77 years, with a mean age of 65.1±8.7 years (Study 2). The diagnosis of AMI was made on the basis of chest pain persisting for at least 30 min, ST-segment elevation of at least 0.1 mV in at least 2 contiguous leads, and elevation of serum creatine kinase-MB isoenzyme (CK-MB) to more than twice the upper limit of the normal range. All patients with AMI were treated with aspirin and heparin after admission. Patients with renal failure (serum creatinine level >283 μmol/L) or severe inflammatory disease upon admission were excluded. Informed consent was obtained from each patient and/or his or her family in the early phase of AMI and after that from each patient. The study protocol was in agreement with the guidelines of the ethical committee of our institution.

Blood Sampling

Study 1: Platelet Activating Factor Blood samples (2 ml) were drawn immediately after admission, and then 24 and 72 h later to measure the plasma levels of PAF in 8 patients with AMI. Each sample was placed immediately in a polypropylene tube containing 3 volumes of ice-cold methanol and centrifuged at 3000×g for 20 min. The upper layer was stored at −70°C until analysis.

Study 2: Interleukin-6 and Cell Adhesion Molecules To measure plasma levels of interleukin-6 and cell adhesion molecules, blood samples (3 ml) were drawn upon admission from 30 patients with AMI. In 15 of these patients, samples were also taken 24 h and 4 weeks later. Blood sampling (3 ml) in the fourth week in patients with AMI and patients with SEA were done in the morning (08.00–09.00 h) in the supine position after fasting overnight. After collection by venepuncture, blood was allowed to clot at room temperature, and after centrifugation the serum was stored at −70°C until analysis.

Angiography and Hemodynamics

In all of the 38 patients (studies 1 and 2) with AMI, coronary angiography was performed immediately after admission, and was followed by left ventriculography in 27 patients. Six patients (75%) in study 1 and 14 patients (80%) in study 2 received thrombolytic therapy and/or urgent percutaneous transluminal coronary angioplasty and all of these patients except 2 were successfully reperfused. The remaining 8 patients were treated conservatively either because it had been already established that the infarct-related coronary artery had spontaneously reperfused at the time of the first coronary angiography, or because the admission time was over 6 h from the onset of symptoms. Four weeks after admission, coronary angiography was performed in 33 patients (7 patients in study 1 and 26 patients in study 2) and left ventriculography was performed in 26 patients (6 patients in study 1 and 20 patients in study 2). The left ventricular ejection fraction was calculated from the left ventriculogram in the right anterior oblique projection according to the area-length method. Improvements in the ejection fraction were calculated by subtracting the acute-phase values from the corresponding chronic-phase values. In all of the patients with SEA, coronary angiography was performed during hospitalization, and they had one or more severe (>75%) coronary stenosis.

Fig 1. (Panels A–D) Plot of the time-course of the serum levels of the soluble form of adhesion molecules [(A) Soluble E-selectin (sE-selectin), (B) soluble intercellular adhesion molecule-1 (sICAM-1), (C) soluble vascular cell adhesion molecule-1 (sVCAM-1)] or (D) interleukin-6 (IL-6) in patients with acute myocardial infarction (n=30) and rest serum levels in patients with stable effort angina (SEA) (n=15). (Panel E) Plot of the time-course of blood levels of platelet activating factor (PAF) in patients with acute myocardial infarction (n=8) and rest values in normal control subjects (Control). Values are expressed as mean±SEM. *p<0.05, **p<0.01 compared with values of SEA or Control. § p<0.01 compared with values upon admission. *p<0.01, significant change using analysis of variance with repeated measures.
Assays for Blood PAF, Serum Adhesion Molecules and Serum Interleukin-6 Concentration

The blood samples, which had been stored at −70°C, were extracted before being assayed. The blood PAF concentration was determined by a gas-chromatography/mass spectrometry method, which was very accurate, rather than by an immunological assay, as reported previously. The spectra were obtained either by scanning m/z (mass to charge ratio) 50 to 800 or by monitoring selected ions. When the selected-ion monitoring mode was used, the molecular anions at m/z 552 for the PAF (1-O-(2,3-tetradeuterio)hexadecyl-2-acetyl-sn-glycerophosphocholine) derivative and m/z molecular anions at m/z 552 for the PAF (1-O-hexadecyl-ions. When the selected-ion monitoring mode was used, the (mass to charge ratio) 50 to 800 or by monitoring selected ions. When the selected-ion monitoring mode was used, the molecular anions at m/z 552 for the PAF (1-O-(2,3-tetradeuterio)hexadecyl-2-acetyl-sn-glycerophosphocholine) derivative and m/z 556 for [2H4] PAF (1-O-(2,3-tetradeuterio)hexadecyl-2-acetyl-sn-glycerophosphocholine) were monitored as an internal standard. The minimum detectable amount of PAF was 5 pg by this method. The intra-experimental coefficient of variation was 3.8% at 50 pg/ml. Soluble E-selectin, sICAM-1 and sVCAM-1 were evaluated in serum using monoclonal antibody based enzyme linked immunosorbent assay kits (R&D Systems, Minneapolis, MN, USA). Briefly, 100 ml of streptavidin conjugated to horseradish peroxidase were added to each well, which was coated with a murine antibody to human E-selectin or ICAM-1 or VCAM-1. One hundred milliliters of the diluted samples was added to each well. The wells were then incubated for 1.5 h and, after washing with buffer and decanting, 100 l of tetramethylbenzidine were added to each well. The wells were then incubated for 30 min at room temperature and, finally, 100 l of an acid solution were added to each well. The optical density of each well was determined within 30 min using a microtiter plate reader set at 450 nm with a correction wavelength of 620 nm. The concentration of circulating interleukin-6 was determined with a one-step ‘sandwich’ enzyme immunoassay (Immunotech S.A., France). This assay is similar to that for the adhesion molecules, except that it used a 96-well anti-interleukin-6 monoclonal antibody coated microtiter plate. The lower limits of detection of interleukin-6, E-selectin, sICAM-1 and sVCAM-1 were 3 pg/ml, 0.1, 0.35, 2.0 ng/ml, respectively.

Statistical Analysis

Data are expressed as mean values ± SD unless otherwise indicated. Statistical analyses were performed using a commercially available statistical software program (Statistica for Windows 5.0, Statsoft Inc, Tulsa, OK). The levels of PAF, sE-selectin, sICAM-1, sVCAM-1 and interleukin-6 were compared over time using analysis of variance with repeated measures. Correlation coefficients were calculated by linear regression analysis. Categorical indices (sex, risk factors) were compared using the Chi-squared test. Comparison of parameters and those levels between 2 groups were performed with unpaired Student’s t test. A p value <0.05 was considered significant.

Results

Patient Characteristics

Table 1 shows the patient characteristics in each study group. Both groups were comparable regarding age, sex, number of stenosed arteries, percentage having risk factors, serum lipid levels and previous myocardial infarction. Coronary interventions and the method of reperfusion, peak CK levels and values of left ventricular ejection fraction at admission were not different between the AMI groups. Of all the 38 patients with AMI, only 1 patient died during the study and that was on the second day because of cardiac pump failure.

Levels of Adhesion Molecules and Interleukin-6 in Patients With AMI Upon Admission

The level of sE-selectin upon admission (59.9 ± 32.7 ng/ml) was higher in patients with AMI compared with the mean level (40.3 ± 16.7 ng/ml) in patients with SEA (p < 0.05). The levels of sICAM-1, sVCAM-1 and interleukin-6 at admission in patients with AMI did not differ from those in patients with SEA.

Time-Course of Serum Levels of Adhesion Molecules, Interleukin-6 and PAF

The serum levels of sE-selectin, which was elevated upon admission, decreased gradually thereafter (Fig 1A). The serum levels of sVCAM-1 increased first at 24 h from the onset of symptoms and were still elevated 4 weeks later (Fig 1C). The serum levels of sICAM-1 showed no changes with time and were not different from those in patients with SEA (Fig 1B). In contrast, interleukin-6 levels were normal upon admission but increased at 24 h and were significantly higher (38.8 ± 45.5 pg/ml) than levels in SEA patients (0.4 ± 1.1 pg/ml) (Fig 1D, p < 0.01).

Table 1  Baseline Characteristics of Patients on Admission to the Study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study 1 AMI (n=8)</th>
<th>Study 2 AMI (n=30)</th>
<th>SEA (n=15)</th>
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<tr>
<td>Age (years)</td>
<td>67±11</td>
<td>62±10</td>
<td>65±5</td>
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<td>Men/women</td>
<td>6/2</td>
<td>26/4</td>
<td>11/4</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>173±15</td>
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<td>186±28</td>
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<td>Triglyceride (mg/dl)</td>
<td>85±23</td>
<td>92±38</td>
<td>115±43</td>
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<td>49±15</td>
<td>45±16</td>
<td>43±8</td>
</tr>
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<td>Smokers (%)</td>
<td>5 (63)</td>
<td>20 (67)</td>
<td>10 (67)</td>
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<tr>
<td>Hypertension (%)</td>
<td>5 (63)</td>
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<td>10 (67)</td>
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<tr>
<td>Diabetes mellitus (%)</td>
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<td>2 (13)</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>1 (13)</td>
<td>4 (13)</td>
<td>3 (20)</td>
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<tr>
<td>MultivesSEL disease (%)</td>
<td>3 (38)</td>
<td>11 (37)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>Peak CK, IU/L</td>
<td>2327±1204</td>
<td>3590±2329</td>
<td>–</td>
</tr>
<tr>
<td>LVEF on admission (%)</td>
<td>48±16</td>
<td>44±9</td>
<td>–</td>
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</table>

Data presented are mean ± SD or number (%) of patients. AMI, acute myocardial infarction; SEA, stable effort angina; MI, myocardial infarction; CK, creatine kinase; LVEF, left ventricular ejection fraction.
The blood PAF levels upon admission (201.6±157.9 pg/ml) were higher in patients with AMI than in the normal volunteers (36.1±23.8 pg/ml); the increased level moderated, but continued for at least 72 h (Fig 1E, p<0.01).

Serum Levels of Adhesion Molecules in Patients With Reperfusion Phenomenon

In 30 patients with AMI, 24 patients received reperfusion therapy and successful recanalization was achieved in all but one of these patients. The 23 patients with successful reperfusion were divided into 2 subgroups: the ‘ST-elevated group’ consisted of 11 patients with additional ST-segment elevation (>0.2 mV) immediately after reperfusion, and the ‘no additional ST change’ group consisted of the other 12 patients. In the ST-elevated group, the serum sE-selectin levels (70.3±33.8 ng/ml) were significantly higher than the levels (45.4±20.2 ng/ml) in the no ST change group (Fig 2, p<0.05), but there was no significant relation between sE-selectin levels and reperfusion time from onset of chest pain (r=−0.11, p=NS). The levels of sVCAM-1 and sICAM-1 in the ST-elevated group did not significantly differ from the patients in the no additional ST change group.

Correlation Between Serum Levels of Adhesion Molecules and Clinical Parameters

We examined the relation between the serum levels of the adhesion molecules and the hemodynamics at admission and peak CK levels. There were no significant correlation between the serum adhesion molecule levels and pulmonary capillary wedge pressure, cardiac index, left ventricular ejection fraction at the time of admission or peak CK levels.

Discussion

The present study is the first clinical report to demonstrate by the highly sensitive gas-chromatography/mass-spectrometry method that the blood PAF levels increase in patients with AMI. Additionally, in patients with AMI, (1) serum sE-selectin levels and blood PAF levels were already elevated upon admission and the PAF levels remained increased for at least 3 days, (2) serum interleukin-6 levels increased transiently at 24 h after the onset of symptoms, sVCAM-1 levels increased at later time points and sICAM-1 levels did not elevate, and (3) the serum sE-selectin levels upon admission were significantly higher in patients with additional ST-segment elevation immediately after reperfusion.

Platelet Activating Factor

Inflammation is postulated to contribute to the pathogenesis of AMI, based on histologic studies of thrombosed coronary atherosclerotic plaques. Accumulation of activated leukocytes and evidence for the systemic release of inflammatory cytokines PAF is a potent mediator of several types of inflammation and is produced by a wide variety of cells with target cells in many organs. Recent studies in a prolonged ischemia/reperfusion model demonstrated that PAF antagonists reduce both myocardial infarct size and the incidence of ischemia-induced arrhythmia. PAF is released not only from injured vascular endothelial cells and activated neutrophils, but also from the ischemic myocardium where it increases cardiac permeability, stimulates neutrophil accumulation and activation and causes coronary vasoconstriction. Thus, PAF and neutrophils have important interactions during myocardial infarction and may have a cooperative effect via a positive feedback mechanism. These factors may explain why the blood PAF levels increased throughout the early phase of AMI.

Time-Course and Role of Adhesion Molecules

Patients with AMI showed similar levels of sICAM-1 to those observed in subjects with SEA. In contrast, sE-selectin levels increased upon admission in patients with AMI, and returned to normal values at 24 h and 4 weeks. Levels of sVCAM-1 were still elevated at 4 weeks post-MI. Interestingly, the results of recent in vitro studies showed that E-selectin was upregulated only transiently during the first hours after cytokine or thrombin stimulation, whereas immunoglobulin-type adhesion molecules were persistently expressed at cellular surfaces for at least 72 h. Several of these adhesion molecules have been examined in patients with AMI. Shyu et al reported that the serum levels of ICAM-1 were higher in patients with AMI than in control subjects, but there was no difference in the levels of patients with stable angina which was comparable to our results. For the levels of sE-selectin, they reported that there was no significant difference between the AMI group and the stable angina group. This discrepancy might be due to the fact that their sampling point was later in AMI, because their entry criteria was within 24 h of the onset of symptoms. In fact, another report has shown that the sE-selectin levels increased in patients with AMI who were admitted early compared with the levels of patients with angina. These adhesion molecules mediate the adhesion, activation, and subsequent passage of leukocytes into injured tissue. Selectins are thought to mediate leukocyte margination and the initial light attachment of circulating leukocytes to activated microvascular endothelium. The immunoglobulin-type adhesion molecules are particularly important for the subsequent firm attachment and transendothelial migration into the surrounding tissue. The accumulation of leukocytes plays a key role in ischemic myocardial damage through microvascular obstruction and release of deleterious substances such as inflammatory cytokines, oxygen free radicals and PAF.

Increasing E-selectin in Reperfusion Injury

Adhesion of leukocytes to the ischemia-reperfused coronary endothelium is thought to be a prerequisite for leukocyte-mediated myocardial reperfusion injury and involves the interaction of a number of adhesion molecules acting in concert. The initial interaction between leukocytes and endothelial cells is characterized by leukocytes ‘rolling’ along the endothelium and is believed to be mediated by the selectin family of glycoprotein adhesion molecules, including E-selectin. Previous animal studies have demonstrated that inhibition of adhesion molecules, such as intercellular adhesion molecule-1 and CD18, with monoclonal antibodies significantly reduces the degree of myocardial injury that results from coronary artery occlusion and reperfusion. Another study has shown that E-selectin is related to the pathogenesis of experimental myocardial ischemia-reperfusion injury. However, the effect of this inhibition of adhesion molecules in myocardial reperfusion injury in humans is not yet understood.

Rapid resolution of ST-segment elevation is one of the reperfusion-associated electrocardiographic changes associated with AMI. Several studies suggested that patients...
with additional ST-segment elevation upon reperfusion had a higher peak CK activity, lower ejection fraction and poorer regional wall motion in the chronic phase than those without the additional ST-segment elevation. 29, 30 The present study shows that E-selectin levels in patients with additional ST-segment elevation were significantly higher upon admission than those without it, suggesting that E-selectin may be related to the myocardial ischemia reperfusion injury in patients with AMI.

Study Limitation

Although an increase in PAF in patients with AMI was evident in this study, we could not perform serial measurement of PAF in more patients and at more sampling points because of technical problems. We showed that in AMI the time-course of changes in the blood levels of PAF, interleukin-6 and adhesion molecules varied according to the individual substance, but there was no significant correlation between the blood levels of these factors and clinical parameters (pulmonary capillary wedge pressure, cardiac index, left ventricular ejection fraction at the time of admission and peak CK levels). Further study is necessary to clarify the precise role of these factors in patients with AMI.

Conclusion

In patients with AMI, the time-course of changes in the blood levels of PAF, interleukin-6 and adhesion molecules varied according to the individual substance. Although the precise role of each of these factors in the pathophysiology of AMI is unclear, sE-selectin may possibly be related to the reperfusion injury in the infarcted myocardium.

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