Elevated serum intercellular adhesion molecule-1 and vascular adhesion molecule-1 among patients with stable angina pectoris who suffer cardiovascular death or non-fatal myocardial infarction

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Aims Inflammatory mechanisms have been implicated in the pathogenesis of atherosclerosis. Cell adhesion molecules, expressed on endothelial cells and leukocytes, mediate transendothelial migration of leukocytes into the vessel wall, but also circulate in soluble forms. In the present study we related soluble cell adhesion molecules to the risk of suffering a cardiovascular death or a non-fatal myocardial infarction (cardiovascular death/myocardial infarction) in a substudy to the Angina Prognosis Study in Stockholm (APSIS).

Methods and Results Soluble intercellular adhesion molecule-1, vascular adhesion molecule-1 and E-selectin were measured in serum collected on inclusion in the APSIS study. During follow-up, seven patients suffered non-fatal myocardial infarction or cardiovascular death, whereas 86 patients were event-free. Cardiovascular death/myocardial infarction was associated with elevated intercellular adhesion molecule-1 (354 ± 142 vs 282 ± 62 ng . ml⁻¹; \( P < 0.01 \)) and vascular adhesion molecule-1 (538 ± 138 vs 433 ± 135 ng . ml⁻¹; \( P = 0.05 \)), and E-selectin levels tended to be higher (72 ± 54 vs 49 ± 20 ng . ml⁻¹). Clinical risk factors (history of hypertension, previous myocardial infarction, diabetes and smoking) were more abundant in the event group. Subgroup analyses showed that hypertension, smoking or male sex were associated with elevated intercellular adhesion molecule-1, whereas previous myocardial infarction or male sex were associated with elevated vascular adhesion molecule-1.

Conclusion Patients with stable angina pectoris who developed cardiovascular death/myocardial infarction had elevated serum levels of soluble cell adhesion molecules, indicating increased inflammatory activity. The value of soluble cell adhesion molecules as prognostic markers in patients with stable ischaemic heart disease merits further study.

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Introduction

Cell adhesion molecules are likely to play an important role in the pathogenesis of atherosclerosis\(^1\). Cell adhesion molecules mediate rolling and transendothelial migration of circulating leukocytes and may thus direct inflammatory cells into the intima\(^2\). Increased expression of cell adhesion molecules\(^3,4\) has been found in atherosclerotic plaques, and soluble cell adhesion molecules have been correlated to the surface expression of cell adhesion molecules\(^5\). Soluble forms of various cell adhesion molecules, such as E-selectin, intercellular adhesion molecule-1 and vascular adhesion molecule-1 may be related to disease activity in various diseases\(^6\). Elevated levels of intercellular adhesion molecule-1 have been reported in patients with ischaemic heart disease compared to healthy controls\(^7,9\), and very recently, soluble intercellular adhesion molecule-1 was shown to be an independent risk factor for myocardial infarction in healthy men\(^10\). To further study if soluble cell adhesion molecules are related to complications in stable coronary artery disease, we measured soluble intercellular adhesion molecule-1, vascular adhesion molecule-1...
and E-selectin in a subset of patients included in the Angina Prognosis Study in Stockholm (APSIS).

Methods

Subjects and study protocol

APSIS is a prospective, randomized, single centre trial, which comprises 809 patients with stable angina pectoris[11]. Briefly, patients with a typical history of stable angina pectoris below 70 years of age were examined before inclusion in the study. Exclusion criteria included: unstable or severe angina with an anticipated need for revascularization within a month, a history of acute myocardial infarction within the last 3 years, or a history of revascularization during the last year.

Sampling for inflammatory parameters including cell adhesion molecules was performed at inclusion in the study in 117 patients participating in a substudy of platelet function[12]. The study was approved by the Ethics Committee of the Karolinska Institute. All subjects gave their informed consent before participating.

Follow-up and definitions of end-points

Patients were followed with regular visits at 6 month intervals. Primary end-points for follow-up were death and non-fatal cardiovascular events, defined as: acute myocardial infarction, incapacitating or unstable angina (i.e. presumed need for revascularization), cerebrovascular events (including transitory ischaemic events) or peripheral vascular events (threatening or overt gangrene, or surgery for aortic aneurysm). The criteria for myocardial infarction were a typical clinical picture, a significant rise in cardiac enzymes, and/or development of Q waves on the ECG. Patients who developed a new significant Q-wave without hospitalization were classified as having had a myocardial infarction. In the present report we limit the analysis to relationships between soluble cell adhesion molecules, and cardiovascular death or non-fatal myocardial infarction (cardiovascular death/myocardial infarction).

Blood sampling and laboratory measurements

Blood samples for determination of adhesion molecules, tissue type plasminogen activator antigen and fibrinogen levels were obtained between 0800 h and 1100 h after 30 min of rest in the supine position. Samples for determination of serum lipids, orosomucoid and leucocyte counts were collected the following day after an overnight fast. The samples were stored at −80 °C until analysed.

Concentrations of the soluble cell adhesion molecules intercellular adhesion molecule-1, vascular adhesion molecule-1 and E-selectin were determined in serum using commercially available enzyme immuno assays (R&D systems, Abingdon, U.K.); measurements were carried out according to the instructions from the manufacturer. The levels of tissue type plasminogen activator antigen in plasma were determined on citrated plasma by enzyme immuno assay (Tintelise, Biopool AB, Umeå, Sweden). Fibrinogen in plasma was determined from a modified thrombin time[13]. Orosomucoid in serum was determined quantitatively by an immunoturbidimetric assay, using a Cobas Fara multichannel analyser (Roche AG, Switzerland). Leucocyte counts were determined in an automated blood cell counter (Technicon H1 Hematology system, Technicon Instruments Corp, Tarrytown, New York, U.S.A.). Serum cholesterol and triglyceride concentrations were determined by standard enzymatic techniques (Boehringer-Mannheim, Germany). Immunonephelometric analyses (Behring Diagnostics, Germany) were used to determine apolipoprotein A-I and apolipoprotein-B levels.

Statistics

Data are presented as means and standard deviations, if not otherwise stated. Unpaired t-tests were used for between-group comparisons; for variables with skewness < −1 or >1, Mann–Whitney U-tests were used. Relationships between variables were tested by calculating Pearson correlation coefficients. Analyses were carried out using Statistica®, statistical package version 5·0 (Statsoft, Tulsa, OK, U.S.A.).

Results

Altogether, cell adhesion molecules were measured in 117 patients. During follow-up, six patients died a cardiovascular death, one patient had a non-fatal myocardial infarction, and 86 patients had no event. The remaining 24 patients had other non-fatal cardiovascular events, as defined in the APSIS study[11]. From Table 1 it can be seen that basal characteristics of patients included in the present substudy were very similar to patients included in the main study. As displayed in Table 2, patients who suffered cardiovascular death/myocardial infarction were more likely to have clinical risk factors at inclusion than patients with other cardiovascular events or patients without events.

Cell adhesion molecules

Serum levels of cell adhesion molecules in patients with cardiovascular death/myocardial infarction and event-free patients are shown in Fig. 1. Patients who subsequently developed cardiovascular death/myocardial death/myocardial infarction were more likely to have clinical risk factors at inclusion than patients with other cardiovascular events or patients without events.
in patients with stable angina pectoris[14]. We did not measure C-reactive protein, but failed to find an

**Table 1** Basal characteristics of patients in the present substudy of cell adhesion molecules and in the main study[11]

<table>
<thead>
<tr>
<th></th>
<th>Substudy (n=117)</th>
<th>Main study (n=692)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 ± 7</td>
<td>59 ± 7</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>79</td>
<td>68</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Smoker (%)</td>
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<td>22</td>
</tr>
</tbody>
</table>

CV=cardiovascular; MI=myocardial infarction.

Table 2 Basal characteristics of event free patients, patients who suffered a non-fatal myocardial infarction or cardiovascular death, and patients who suffered an event other than cardiovascular death+myocardial infarction

<table>
<thead>
<tr>
<th>Event free (n=86)</th>
<th>CV death+MI (n=7)</th>
<th>Other CV event† (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 ± 7</td>
<td>61 ± 3</td>
</tr>
<tr>
<td>Sex (males/Females)</td>
<td>64/22</td>
<td>7/0</td>
</tr>
<tr>
<td>Previous MI (%)</td>
<td>8</td>
<td>29</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7</td>
<td>29</td>
</tr>
<tr>
<td>Smoker (%)</td>
<td>21</td>
<td>57</td>
</tr>
</tbody>
</table>

†Events included revascularization (coronary bypass surgery or PTCA; n=11), coronary angiography without revascularization (n=7), cerebrovascular events (n=3), unstable angina pectoris (n=1), aortic abdominal aneurysm (n=1), malignancy (n=1).

Inflammatory and endothelial markers, lipids and correlations

Data on inflammatory and endothelial markers and lipid parameters are shown in Table 3. Intercellular adhesion molecule-1 levels were correlated to fibrinogen levels (r=0.28, P<0.01) and leukocyte counts (r=0.23, P<0.05). E-selectin levels were correlated to plasma levels of tissue type plasminogen activator antigen (r=0.24, P<0.05), and leukocyte counts (r=0.25, P<0.05). No significant correlations were found between cell adhesion molecules and the lipid parameters measured. Vascular adhesion molecule-1 did not show significant correlations to any of the parameters measured (P>0.2).

Discussion

The present study shows that patients with stable angina pectoris, who subsequently suffer cardiovascular death/myocardial infarction, have elevated serum levels of intercellular adhesion molecule-1 and vascular adhesion molecule-1, and possibly also E-selectin. Very recent data show that soluble intercellular adhesion molecule-1 is an independent risk factor for myocardial infarction in healthy males[10]. The ECAT study (European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study) showed that plasma levels of the liver-derived acute phase reactant C-reactive protein were positively associated with the risk of coronary events in patients with stable angina pectoris[14]. We did not measure C-reactive protein, but failed to find an
elevation of orosomucoid (another acute phase reactant) among patients with cardiovascular death/myocardial infarction. From a mechanistic point of view – and in contrast to C-reactive protein or orosomucoid – cell adhesion molecules like intercellular adhesion molecule-1 and vascular adhesion molecule-1 may have a direct role in the pathogenesis of atherosclerosis.[2].

Taken together with previous studies, our data strongly support the view that increased inflammatory activity is associated with increased risk of cardiovascular complications in patients with stable ischaemic heart disease.

The cellular origins of soluble intercellular adhesion molecule-1 and vascular adhesion molecule-1 are uncertain, but probably include various vascular cells, such as endothelial cells and smooth muscle cells, and leukocytes. In contrast, E-selectin has been shown to be produced exclusively by the endothelium.[2]. The physiological or pathophysiological role of soluble cell adhesion molecules is unclear. It has been suggested that they may reflect chronic inflammation[6] or serve as immunomodulators of subclinical inflammation[19]. In the setting of stable ischaemic heart disease, they may indicate a low grade inflammatory response in the vascular walls of arteries.

Apart from intercellular adhesion molecule-1 and vascular adhesion molecule-1, plasma fibrinogen was the only laboratory marker that differed significantly between patients with and without events (i.e. cardiovascular death/myocardial infarction; Table 3); neither leukocyte counts nor orosomucoid – both general markers of inflammation – differed between the groups.

In patients with end-points other than cardiovascular death/myocardial infarction soluble cell adhesion molecules were not significantly elevated compared to patients without events. This suggests that soluble cell adhesion molecules may be more sensitive markers of disease activity and future severe cardiovascular complications than other – and less specific – markers of inflammatory activity in ischaemic heart disease.

Clinical risk factors for cardiovascular complications were more abundant among patients with cardiovascular death/myocardial infarction (Table 2). Subgroup analyses showed that several of these risk factors were associated with elevated cell adhesion molecules. It cannot be excluded that the associations between cardiovascular complications and elevated cell adhesion molecules were due to confounding by the risk factors. However, the increased risk associated with these factors...
may also be mediated, in part, by inflammatory mechanisms. Larger studies are needed to resolve whether soluble cell adhesion molecules are independent risk factors for cardiovascular complications. Regardless of the issue of causality, the observations that serum levels of cell adhesion molecules are elevated in patients with stable angina who later suffer from cardiovascular death or myocardial infarction are of interest, not the least from a mechanistic point of view.

In conclusion, the present study suggests that patients with stable angina pectoris and elevated levels of circulating intercellular adhesion molecule-1, and perhaps also vascular adhesion molecule-1 and E-selectin, have an increased risk of non-fatal myocardial infarction and cardiovascular death. Data from the present study should encourage further studies of soluble cell adhesion molecules in relation to coronary artery disease and its outcome.

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References


