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Tissue Factor, Tissue Factor Pathway Inhibitor and Cytoadhesive Molecules in Patients with an Acute Coronary Syndrome

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Summary

The tissue factor plays a crucial role in initiating blood coagulation after plaque rupture in patients with acute coronary syndrome. It is abundant in atherosclerotic plaques. Moreover, P-selectin, some cytokines, endotoxin and immune complexes can stimulate monocytes and induce the tissue factor expression on their surface. The aim of the study was to compare plasma levels of the tissue factor, tissue factor pathway inhibitor, P-selectin, E-selectin and ICAM-1 in patients with acute myocardial infarction, unstable angina pectoris, stable coronary artery disease and normal control subjects. In addition, plasma levels of the tissue factor, tissue factor pathway inhibitor, P-selectin, E-selectin and ICAM-1 were measured in the blood withdrawn from the coronary sinus in a subgroup of patients with unstable angina pectoris and stable coronary artery disease in which the difference between concentrations in the coronary sinus and systemic blood was calculated. A significant increase in tissue factor pathway inhibitor plasma levels was detected in patients with acute myocardial infarction (373.3±135.1 ng/ml, $p<0.01$) and unstable angina pectoris (119.6±86.9 ng/ml, $p<0.05$) in contrast to the patients with stable coronary artery disease (46.3±37.5 ng/ml) and normal subjects (45.1±14.3 ng/ml). The plasma levels of tissue factor pathway inhibitor were significantly increased both in the coronary sinus and systemic blood in the patients with unstable angina pectoris. There was only a non-significant trend to higher plasma levels of the tissue factor in patients with acute myocardial infarction and unstable angina pectoris as compared to the patients with stable coronary artery disease and normal subjects, the values being 129.1±30.2 pg/ml, 130.5±57.8 pg/ml, 120.2±45.1 pg/ml and 124.9±31.8 pg/ml, respectively. Plasma levels of soluble P-selectin was only slightly, but non-significantly higher in patients with unstable angina pectoris and stable coronary artery disease (184.2±85.4 ng/ml and 201.6±67.9 ng/ml, respectively) than in patients with the acute myocardial infarction (157.4±88.4 ng/ml) or normal subjects (151.4±47.1 ng/ml). The difference in plasma levels of soluble ICAM-1 between the blood withdrawn from the coronary sinus and systemic circulation correlated significantly with the corresponding difference in plasma levels of soluble P-selectin and E-selectin. In conclusion**,** the tissue factor and the tissue factor pathway inhibitor play a crucial role in the initiation of arterial thrombosis. The tissue factor pathway inhibitor levels are increased both in the systemic blood and in the coronary sinus of patients with the acute coronary syndrome.

Key words
Tissue factor • Tissue factor pathway inhibitor • Cytoadhesive molecules • Acute coronary syndrome

Introduction

The tissue factor plays a crucial role in the initiation of blood coagulation after the plaque rupture in patients with acute coronary syndromes (Rapaport and Rao 1995). It was well documented that tissue factor is abundant in atherosclerotic plaques and its contact with circulating blood after the plaque rupture leads to activation of the coagulation cascade (Toschi *et al.* 1997). Moreover, P-selectin, some cytokines, endotoxin and immune complexes can stimulate monocytes and induce the tissue factor expression on their surface (Celi *et al.* 1994).

The aim of our study was to measure the plasma levels of the tissue factor, tissue factor pathway inhibitor, soluble P-selectin, soluble E-selectin and soluble ICAM-1 in patients with acute myocardial infarction and unstable angina pectoris and compare them with those found in a control groups of patients with stable coronary artery disease and normal healthy subjects.

Patients and Methods

Patients.

Patients who underwent coronary angiography as candidates for percutaneous transluminal coronary intervention or coronary bypass surgery were included in the study. There were patients with a ST segment elevation due to acute myocardial infarction lasting less than 12 h in the subgroup A. Subgroup B comprised patients with class IIIb unstable angina pectoris according to Braunwald classification (Braunwald 1989), whereas patients with chronic stable coronary artery disease were in subgroup C.

Moreover, a group of healthy young subjects without history or clinical findings suggesting a presence of heart disease served as controls (subgroup D). Blood sampling was performed in all patients from the peripheral or central vein in the fasting state after at least 30 min at rest.

In addition, samples in subgroup B and C were withdrawn from the coronary sinus by means of a catheter (right coronary Amplatz I, Goodal-Lubin or multipurpose catheter) introduced from the jugular or femoral vein. A blood sample from systemic circulation was obtained at the same time. No patient was treated by unfractionated heparin at the time of sampling.

All patients gave their informed consent and the study was approved by the Hospital Ethical Committee.

Methods

The sampling of coronary sinus and systemic circulation blood preceded the coronary angiography. Blood was collected into a 3.8 % trisodium citrate anticoagulant solution in the proportion of 9:1. The sample was then centrifuged at 3000 rpm for 10 min and the plasma stored frozen.

The plasma level of the tissue factor was determined by means of the IMUBIND® Tissue Factor ELISA Kit (American Diagnostica). The kit employs a murine anti-human tissue factor monoclonal antibody for antigen capture. Plasma samples were incubated in microtest wells precoated with capture antibody. Once captured, the tissue factor was detected using a biotinylated antibody fragment that specifically recognizes the bound tissue factor. The subsequent binding of horseradish peroxidase conjugated streptavidin completes the formation of the antibody-enzyme detection complex. The reaction with added substrate (perborate/3, 3´, 5, 5´-tetramethyl-benzidine) turns blue. The sensitivity is increased by addition of 0.5 M sulfuric acid stop solution turning the color to yellow. Quantitative data were obtained by measuring the solution absorbance at 450 nm and relating it to the standard curve. All assays were performed in duplicate. The plasma levels of the tissue factor pathway inhibitor was measured using IMUBIND® Total Tissue Factor Pathway Inhibitor (TFPI) ELISA Kit (American Diagnostica). This sandwich immunoassay makes it possible to quantitate tissue factor pathway inhibitor in the plasma. It detects both intact and truncated forms of TFPI as well as complexes with the tissue factor (TF) and factor VII (TF/VIIa/TFPI). Binary complexes with factor Xa (TFPI/Xa) and quaternary complexes with tissue factor, factor VIIa and factor Xa (TF/VIIa/TFPI/Xa) are also recognized by this ELISA kit, but with a slightly lower sensitivity. The lower limit of detection for this assay is 0.360 ng TFPI/ml sample. This kit employs a rabbit anti-human tissue factor pathway inhibitor polyclonal antibody as the capture antibody. Its specificity for native complexes and truncated antibody was confirmed by Western blot analysis. Diluted plasma samples were incubated in micro-test wells precoated with the capture antibody. Tissue factor pathway inhibitor is detected using a biotinylated monoclonal antibody specific for the Kunitz domain 1. The subsequent steps were identical with those described above.

Human soluble P-selectin was assessed using Parameter® Human sP-selectin Immunoassay (R&D Systems). This 1.25 hour solid state ELISA measures

soluble P-selectin in cell culture supernatants, serum and plasma. Plasma was collected using citrate as an anticoagulant and within 30 min centrifuged at 1000 *x g*. The kit contains recombinant human soluble P-selectin and antibodies raised against the recombinant factor. This assay employs the quantitative sandwich immunoassay technique.

Similarly, the levels of soluble E-selectin were determined by means of the Parameter® Human sE-selectin Immunoassay (R&D Systems) and the levels of soluble ICAM-1 by means of Parameter® human sICAM-1 Immunoassay (R&D Systems). All assays were performed in duplicate.

Differences between the plasma concentrations in coronary sinus and systemic circulation were calculated for soluble ICAM-1, soluble P-selectin, soluble E-selectin, tissue factor and tissue factor pathway inhibitor. These differences were expressed as values relative to their original concentrations in the systemic blood *(CS – SYST)/SYST* where CS = plasma concentrations in the blood drawn from the coronary sinus and SYST = plasma concentrations in the peripheral blood.

Data were expressed as means ± SEM. Statistical evaluation included the analysis of variance and Student's t test for the assessment of differences of continuous variables. Correlations were analyzed using linear regression. A $p<0.05$ value was considered significant.

Results

Altogether 50 patients and 10 normal subjects were studied. Ten patients (6 males, 4 females, age 62.3±3.4 years) with acute myocardial infarction comprised group A. Group B included 23 patients with unstable angina pectoris (17 males, 6 females, mean age 63.6±8.1 years). Group C consisted of 17 subjects with chronic stable ischemic heart disease (13 males, 4 females, mean age 65.5±9.3 years). The demographic characteristics are shown in Table 1. There were 10 healthy subjects (2 males, 8 females, age 33.8±8.2 years) in the group D.

The plasma levels of tissue factor and tissue factor pathway inhibitor in the studied groups are shown in Figures 1 and 2. The subgroups A and B displayed a significant rise in plasma levels of the tissue factor pathway inhibitor (Fig. 2). The tissue factor pathway inhibitor plasma levels was 373.3±135.1 ng/ml, ($p<0.01$) in patients with acute myocardial infarction, 119.6±86.9 ng/ml, ($p<0.05$) in unstable angina pectoris, 46.3±37.5 ng/ml in patients with stable coronary artery disease and 45.1±14.3 ng/ml in normal subjects. The plasma levels of tissue factor pathway inhibitor were significantly increased both in the coronary sinus and systemic blood in the patients with unstable angina pectoris.

Table 1. Demographic data about the present cohort

n =	Group A 10	Group B 23	Group C 17
Males	6	17	13
Females	4	6	4
Previous myocardial infarction	0	10	9
Angina pectoris			
CCS 1,2	-	-	9
CCS 3	-	-	1
CCS 4	-	-	0
Smokers	6	2	1
Diabetes	1	6	7
Hypertension	3	14	8
Hyperlipoproteinemia	4	6	8
Beta-blockers	3	15	6
Ca^{++} channel blockers	1	3	4
ACE inhibitors	3	6	8
Statins	2	4	2
Antiplatelet drugs	2	18	14
Low molecular weight heparins	0	7	1

There was only a non-significant trend to higher plasma levels of tissue factor in patients with acute myocardial infarction and unstable angina pectoris as compared to the patients with stable coronary artery disease and normal subjects, the values being 129.1±30.2 pg/ml, 130.5±57.8 pg/ml, 120.2±45.1 pg/ml and 124.9±31.8 pg/ml, respectively. Plasma levels of soluble P-selectin was only slightly, but non-significantly higher in patients with unstable angina pectoris and stable coronary artery disease (184.2±85.4 and 201.6±67.9 ng/ml) than in patients with the acute myocardial infarction (157.4±88.4 ng/ml) or normal subjects (151.4±47.1 ng/ml). The plasma levels of soluble E-selectin were 50.2±17.6, 33.2±12.9, 42.0±15.6 and 43.1±11.8 ng/ml, whereas the plasma concentrations of the soluble ICAM-1 were 308.6±68.7, 290.0±72.5, 247.8±54.1 and 303.7±38.6 ng/ml in the subgroup A, B, C and D, respectively.

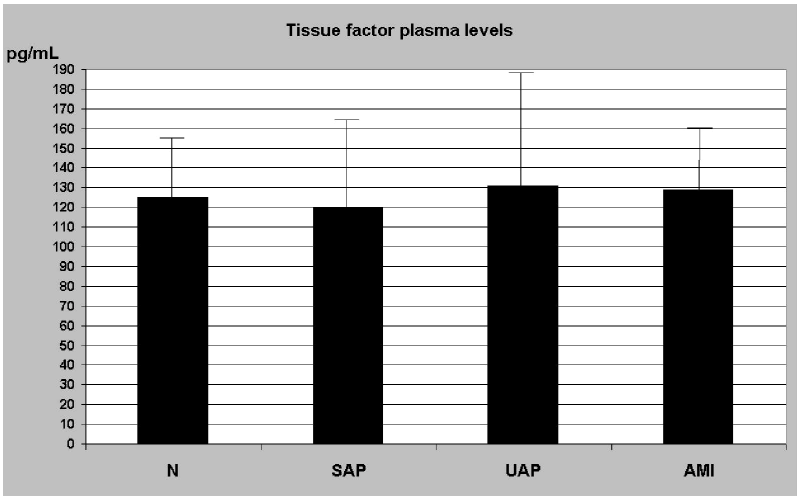

Fig. 1. *Plasma levels of the tissue factor in the studied subgroups (N = normal subjects, SAP = stable coronary artery disease, UAP = unstable angina pectoris, AMI = acute myocardial infarction). Mean values ± SEM..*

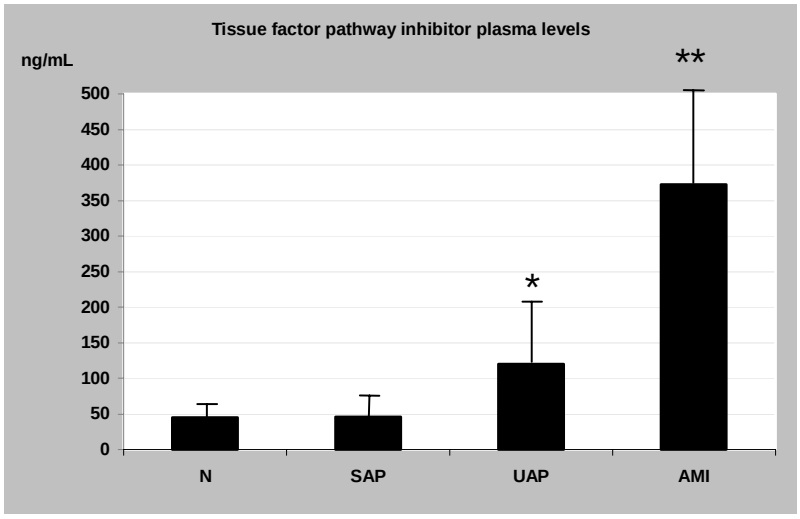

Fig. 2. *Plasma levels of the tissue factor pathway inhibitor in the studied subgroups (N = normal subjects, SAP = stable coronary artery disease, UAP = unstable angina pectoris, AMI = acute myocardial infarction). Mean values ± SEM. * p<0.05; ** p<0.01*

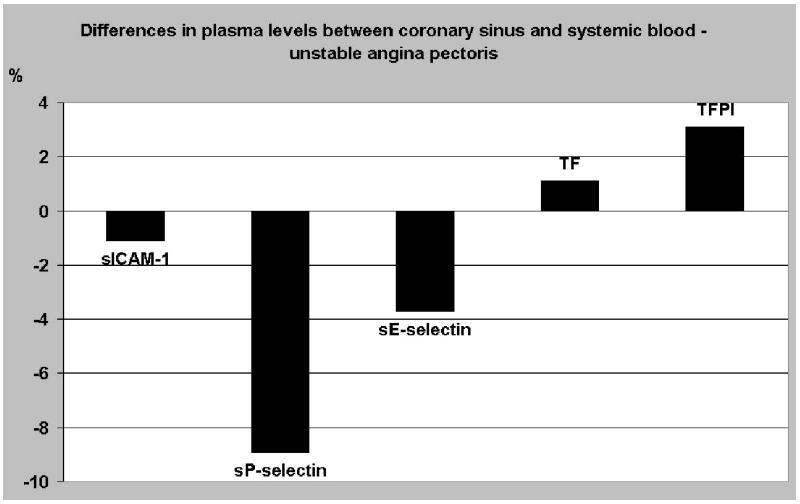

Fig. 3. *Relative differences in the soluble ICAM-1, soluble P-selectin, soluble E-selectin, tissue factor and tissue factor pathway inhibitor between coronary sinus and systemic circulation plasma concentrations in patients with unstable angina pectoris (group B). [TF = tissue factor; TFPI = tissue factor pathway inhibitor; ICAM-1 = intercellular adhesion molecule-1, s = soluble].*

The relative differences in the plasma levels of tissue factor, tissue factor pathway inhibitor, soluble P-selectin, soluble E-selectin and soluble ICAM-1 between coronary sinus and systemic blood plasma concentrations in patients with unstable angina pectoris and stable coronary artery disease are shown in Figures 3 and 4. Non-significant tendency to increase plasma levels of tissue factor, tissue factor pathway inhibitor and

soluble ICAM-1 were noticed in subgroup B, whereas the trend to non-significant decrease in coronary sinus plasma concentration of soluble P-selectin was present in subgroup C. The coronary sinus – systemic blood differences in the plasma levels of the soluble ICAM-1 were positively related to those of soluble E- and P-selectin (Figs. 5 and 6). The tissue factor levels in coronary sinus displayed a trend to an inverse relation to the levels of soluble P-selectin in coronary sinus (Fig. 7). The correlation was, however, non-significant.

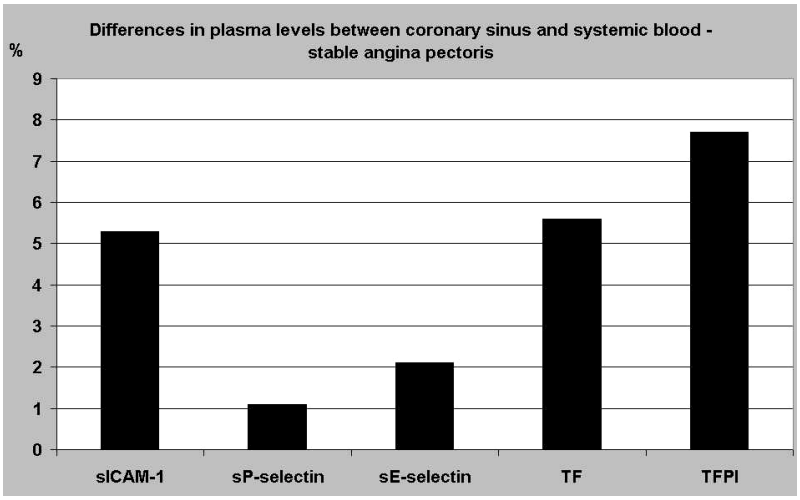

Fig. 4. *Relative differences in the soluble ICAM-1, soluble P-selectin, soluble E-selectin, tissue factor and tissue factor pathway inhibitor between coronary sinus and systemic circulation plasma concentrations in patients with stable coronary artery disease (group C).*

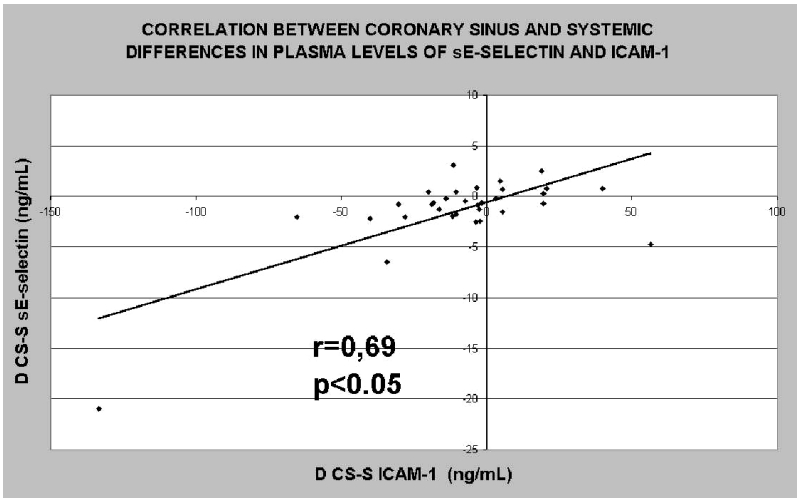

Fig. 5. *Correlation between soluble ICAM-1 and soluble E-selectin differences between coronary sinus and systemic blood concentrations. [D CS – S = differences in plasma levels between coronary sinus and systemic blood in ng/ml].*

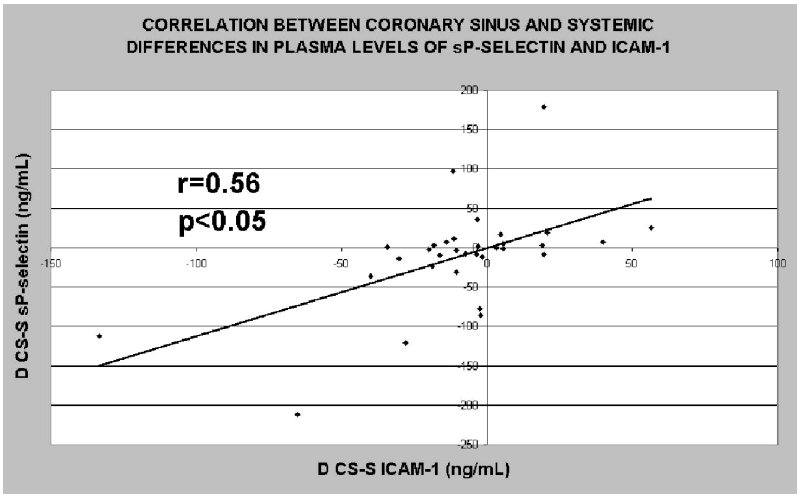

Fig. 6. *Correlation between soluble ICAM-1 and soluble P-selectin differences between coronary sinus and systemic blood concentrations.*

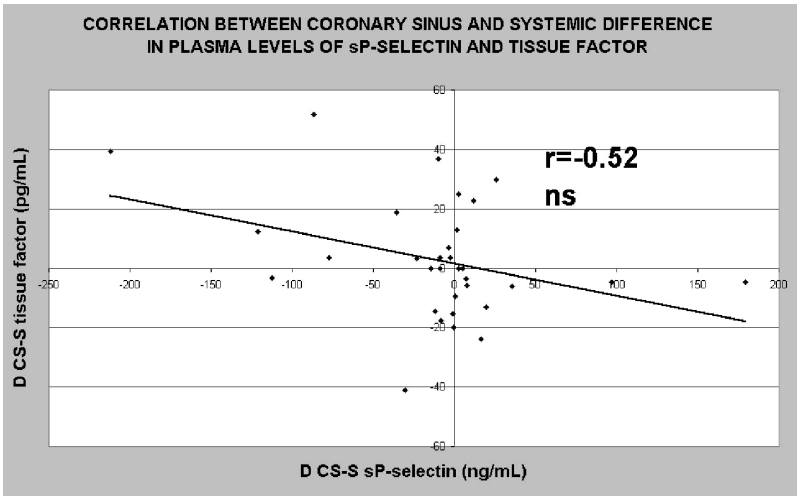

Fig. 7. *Correlation between tissue factor and soluble P-selectin differences between coronary sinus and systemic blood concentrations. [D CS – S tissue factor = difference in plasma levels of the tissue factor between coronary sinus and systemic blood in pg/ml; D CS – S sP-selectin = difference in plasma levels of the soluble P-selectin between coronary sinus and systemic blood in ng/ml; ns = non-significant].*

Discussion

The tissue factor is a membrane protein spontaneously expressed in many cells. Its release into circulating blood is responsible for the activation of coagulation cascade (Rapaport and Rao 1995). The tissue factor is present in the subendothelium, and any damage to the endothelial layer in experimental animal models triggers thrombus formation (Weiss *et al.* 1994). Although monocytes and endothelial cells do not produce tissue factor under normal conditions, expression of the tissue factor on the surface of these cells can be upregulated under certain circumstances. P-selectin, some cytokines, endotoxin and immune complexes can stimulate monocytes and induce tissue factor expression on their surface (Celi *et al.* 1994). Thus, the systemic activation of blood coagulation in patients suffering from disseminated intravascular coagulation is almost entirely attributable to the elevated production of tissue factor (Levi and Ten Cate 1999). Peripheral blood monocytes express tissue factor when stimulated, and together with the tissue factor of monocyte-derived macrophages in arterial plaques (Wilcox *et al.* 1989) may provide the primary source of tissue factor in patients with coronary artery disease. Fibroblasts and smooth muscle cells within the vessel wall constitutively express tissue factor and may serve as an additional source (Iakhiaev *et al.* 1999). Atherosclerotic plaques contain a substantial amount of tissue factor. When exposed to circulating blood after the plaque rupture it binds to factor VIIa, a vitamin K-dependent enzyme, which circulates in low concentration in the blood (Toschi *et al.* 1997). The catalytic subunit – factor VIIa is a soluble plasma protease that forms the first step in the blood clotting cascade together with the regulatory subunit cell – surface integral membrane protein – tissue factor (Morrissey *et al.* 1997). This complex of factor VIIa and tissue factor subsequently activates factor IX and factor X and thus initiates the fibrin formation pathway.

The tissue factor/factor VIIa complex catalyzed activation of factor IX can be inhibited by a complex formed by tissue factor pathway inhibitor and factor Xa (Lindhout *et al.* 1995, Camici and Sagripanti 1999). Successive steps involved in this process are the following: 1) formation of the factor VIIa/tissue factor complex; 2) activation of factor Xa; 3) formation of the TFPI/factor Xa complex; 4) inactivation of tissue factor/ factor VIIa complex by the TFPI-factor Xa complex; 5) formation of the reversible quaternary complex consisting of tissue factor, factor VIIa, TFPI and factor Xa (Camici and Sagripanti 1999).

Tissue factor production by activated circulating blood particles (Giesen *et al.* 1999) promotes thrombi formation in microcirculation of patients with acute coronary syndrome. Tissue factor is the key initiator of monocyte-mediated coagulation and monocyte-platelet interaction. Monocytes stimulated with lipopolysaccharide express tissue factor and elicit a pronounced fibrin deposition and platelet-thrombus formation (Barstad *et al.* 1995). Plasma markers of coagulation (fibrinopeptide A) and platelet activation (β-thromboglobulin) showed a significant increase after lipopolysaccharide stimulation. This can be almost completely blocked by anti-tissue factor monoclonal antibody (Barstad *et al.* 1995). Elevated plasma levels of tissue factor and large amounts of monocyte procoagulant

activity have been documented in patients with unstable angina pectoris (Gori *et al.* 1999). Heparin is able to blunt the production of tissue factor by monocyte *in vitro*. Heparin treatment is associated with a decrease in tissue factor plasma levels as well as monocyte procoagulant activity in patients with unstable angina pectoris (Gori *et al.* 1999).

Factor X activation that occurs in response to tissue factor/factor VIIa complex is abolished in the presence of heparin and this effect requires both antithrombin and tissue factor pathway inhibitor. Antithrombin in the presence of heparin blocks the activation of the tissue factor/factor VII complex, whereas tissue factor pathway inhibitor inhibits the tissue factor/factor VIIa complex that is generated (Jesty *et al.* 1996). Several tissue factor/factor VIIa inhibitors are being developed, including the protein-based inhibitors such as NAPc2, Corsevin M, FFR-FVIIa, and Tifacogin (Girard and Nicholson 2001).

The tissue factor pathway inhibitor is now recognized as a major physiological anticoagulant that acts as a natural tissue factor/factor VIIa inhibitor (Lindahl 1997). Immunodepletion of tissue factor pathway inhibitor lowers the threshold by which tissue factor induces disseminated intravascular coagulation (Sandset 1996). Infusion of a recombinant tissue factor pathway inhibitor was found to protect against thrombosis and disseminated intravascular coagulation in different experimental models (Sandset 1996).

Human tissue factor pathway inhibitor is a plasma protease inhibitor that consists of three tandem Kunitz-type inhibitor domains. The first and second Kunitz-type domains are involved in the inhibition of factor VIIa/tissue factor complex and factor Xa on the cell surface, respectively (Hamamoto *et al.* 1993). Tissue factor pathway inhibitor has a dual inhibitory function it inhibits the complex factor VIIa/ tissue factor and directly inhibits factor Xa by binding at or near its active serine site. Tissue factor pathway inhibitor promotes the binding of Xa to monocytes (Li *et al.* 2001). The recovery of Xa activity from Xa/TFPI complex may be related to the cleavage of tissue factor pathway inhibitor by monocyte proteases. This process may add to monocytes procoagulant activity, apart from tissue factor expression on their surface (Salemink *et al.* 1998).

Tissue factor pathway inhibitor circulates in blood in several forms, mostly as a complex with LDL-HDL-VLDL. Approximately 10 % of tissue factor pathway inhibitor are carried by platelets, and are released once the platelets are activated by thrombin. Consequently, an elevated level of tissue factor pathway inhibitor is present at the site of platelet aggregation. Heterozygous deficiency of tissue factor pathway inhibitor in mice was associated with a greater atherosclerotic burden involving the carotid and common iliac arteries and shortened the time before an occlusive thrombosis occurred after photochemical atherosclerotic plaque injury (Westrick *et al.* 2001). Continuous intravenous infusion of unfractionated heparin increased the levels of tissue factor pathway inhibitor more than twofold (Brown and Kuter 2001). Its level remained high during the infusion, but returned to baseline soon after the infusion had been stopped. On the other hand, therapeutic doses of low molecular weight heparin resulted in significantly weaker tissue factor pathway inhibitor induction. Tissue factor pathway inhibitor is released from the vascular endothelium after an injection of heparin and concentrates at sites of tissue damage and ongoing thrombosis. The cessation of a treatment with unfractionated heparin, but not low molecular weight heparin, given in therapeutic doses was associated with a progressive depletion of tissue factor pathway inhibitor (Sandset *et al.* 2000). This phenomenon might be responsible for the observed rebound activation of coagulation after withdrawal of unfractionated heparin. Partial depletion of intravascular pools of tissue factor pathway inhibitor during repeated or continuous intravenous infusion of heparin in man has been reported (Hansen *et al.* 1996). This might explain the attenuation of the tissue factor pathway inhibitor contribution to the antithrombotic effect of heparin. Subnormal levels of tissue factor pathway inhibitor increase the risk of disseminated intravascular coagulation in septic patients, and the risk of occlusive thrombi over damaged vascular intima or fissured atherosclerotic plaques (Abilgaard 1995). Elevated plasma levels of tissue factor pathway inhibitor have been documented in patients with unstable angina pectoris (Gori *et al.* 1999).

In the present study, the tissue factor plasma level increased only marginally in patients with acute coronary syndrome and there was no further rise even in the blood withdrawn from coronary sinus. Nevertheless, the increased plasma levels of another marker of ongoing thrombosis, the tissue factor pathway inhibitor, were found both in patients with the acute myocardial infarction and unstable angina pectoris. The increase was detected in both the peripheral blood and the blood withdrawn from the coronary sinus. Plaque thrombosis is the cause of the acute coronary syndrome, and presumably blood-borne rather than plaque-derived tissue

factor is responsible for the detected elevation in our patients. On the other hand, while no changes in tissue factor coronary sinus plasma level were noticed in patients with stable ischemic heart disease, slightly elevated values of soluble P-selectin were detected in this subgroup. This suggests that some platelet activation takes place in both stable and unstable coronary artery disease, but tissue factor pathway inhibitor upregulation in coronary vascular bed only dominates in patients with acute coronary syndrome. The changes in the level of soluble ICAM-1 were related to the changes of soluble P- and E-selectin, but the changes of soluble P-selectin were only marginally and inversely related to the changes in tissue factor. Detectable circulating soluble form represents only a portion of native P-selectin. Because it lacks the transmembrane anchoring domain, it is conceivable that a consumption of the soluble form of P-selection takes place while the tissue factor on monocytes is being upregulated in patients with an acute coronary syndrome.

The role of adhesion molecules is to mediate interactions of cells with extracellular matrix or with other cells. The immunoglobulin superfamily of proteins contains a large class of adhesion molecules with multiple immunoglobulin-like domains. Adhesion of monocytes and neutrophils to endothelial cells is probably one of the first steps in the pathway leading to plaque rupture and acute coronary thrombosis with clinical manifestation of an acute coronary syndrome. Apart from plaque rupture, an interaction between activated platelets, monocytes and neutrophil leukocytes is responsible for the growth of the thrombus and for possible propagation of the thrombus into the microvasculature. Soluble isoforms of these adhesion molecules believed to be shed from the surface of activated cells can now be quantified in peripheral blood (Gearing and Newman 1993).

P-selectin is a cell surface glycoprotein that plays a critical role in the migration of lymphocytes into tissues. It is found constitutively in a preformed state in the Weibel-Palade bodies of endothelial cells and in α-granules of platelets. This stored P-selectin is mobilized to the cells surface within minutes in response to a variety of inflammatory and trombogenic agents. The mobilized P-selectin is apparently present on the cell surface for only a few minutes after which it is recycled to intracellular space. P-selectin also binds monocytes and neutrophils to activated platelets and is responsible for incorporation of leukocytes into the growing thrombus. Circulating soluble P-selectin has a smaller molecule than native P-selectin, because it lacks the transmembrane anchoring domain. ICAM-1 also appears either in the form of a transmembrane protein (mICAM-1) or in circulating soluble form (sICAM-1). Up-regulation of ICAM-1 expression is initiated by inflammatory cytokines (TNF-α, IFN-γ, IL-1), whilst down-regulation is mediated by anti-inflammatory agents. The soluble form arises either from proteolytic cleavage of mICAM-1 or may be synthesized *de novo* from alternatively spliced mRNA. E-selectin (Endothelial Leukocyte Adhesion Molecule-1) is a transmembrane glycoprotein expressed on endothelial cells after activation by some inflammatory cytokines (IL-1β, TNF-α) and by endotoxin. The expression is reaching a transitory maximum within 6 h after stimulation. The decline coincides with the shedding of its soluble form. The transmembranous form of E-selectin is a mediator of rolling movements of leukocytes and their attachment to the endothelium leading to a migration of leukocytes to the site of inflammation.

Platelet activation with elevated levels of P-selectin has been documented in patients with unstable angina pectoris (Singh *et al.* 1995). Plasma levels of soluble ICAM-1 and soluble P-selectin were also significantly higher in blood withdrawn from coronary sinus immediately after coronary angioplasty (Inoue *et al.* 1999). The increase persisted for 48 h with maxima at 48 h for ICAM-1 and at 24 h for P-selectin. None of these changes was observed in peripheral blood samples. The plasma levels of the soluble E-selectin did not change during 48 h after coronary angioplasty in blood samples taken from coronary sinus. Increased levels of some inflammatory markers including sICAM have been identified as risk factors for the development of the acute coronary syndrome (O'Malley *et al.* 2001). Soluble ICAM-1 monoclonal antibody has been shown to limit infarct size and reduce reperfusion injury (Simpson *et al.* 1988, Yamazaki *et al.* 1993).

In our study, no significant changes in soluble P-selectin, E-selectin or soluble ICAM-1 levels were found in patients with acute coronary syndrome. There were no changes of plasma levels of soluble E-selectin and ICAM-1 in the coronary sinus and only slight decrease of P-selectin in coronary sinus was detected. The decrease in soluble P-selectin in the coronary sinus showed a non-significant correlation with the increase of coronary sinus tissue factor plasma levels.

Tissue factor and its inhibitor play an important role in the pathogenesis of the acute coronary syndrome. Our results suggest that they act not only

locally but also as circulating elements. There were no significant differences in the local concentrations of tissue factor, soluble P-selectin, E-selectin or ICAM-1 in the coronary sinus and systemic blood in our patients. However, the plasma levels of tissue factor pathway inhibitor were significantly increased both in the coronary sinus and systemic blood in the patients with the acute coronary syndrome. As tissue factor/factor VIIa blockers are being developed and the role of the tissue factor pathway inhibitor in the therapeutic effects of heparin was recently recognized, the crucial role of the tissue factor in the development of arterial thrombosis might be not only theoretical in the near future, but we can expect also some therapeutic consequences in this respect.

Acknowledgements

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