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An Increase of C-Reactive Protein Is Associated With Enhanced Activation of Endogenous Fibrinolysis at Baseline But an Impaired Endothelial Fibrinolytic Response After Venous Occlusion

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OBJECTIVES The goal of this study was to determine whether chronic inflammation of the vascular wall may be associated with an impaired activation of the fibrinolytic system.

BACKGROUND Inflammation plays an important role in the initiation and progression of atherosclerosis, and the fibrinolytic system may prevent local thrombus formation.

METHODS We included 50 patients six months after their first myocardial infarction. Plasma levels of the inflammatory marker C-reactive protein (CRP) were determined at basal conditions, and the fibrinolytic parameters tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type-1 (PAI-1) were measured at basal conditions and after a standardized venous occlusion (VO) of the forearm.

RESULTS Patients with high CRP levels (≥3 mg/l) showed a significantly higher t-PA activity at baseline compared with patients with medium (1 to 2.9 mg/l) and low (<1 mg/l) CRP levels ($p < 0.005$). In contrast, patients with low CRP levels showed a higher increase of t-PA activity ($p < 0.05$) and a higher reduction of PAI-1 activity during VO ($p < 0.05$) compared with patients with medium and high CRP levels. A multivariate analysis that included cardiovascular risk factors and medical treatment showed that CRP is an independent predictor of the t-PA response after a standardized VO.

CONCLUSIONS Chronic low-grade inflammation is associated with enhanced activation of endogenous fibrinolysis at baseline but a reduced fibrinolytic response to VO. This impaired endogenous fibrinolytic capacity might be an important contributor to the increased coronary event rate associated with elevated CRP levels. (J Am Coll Cardiol 2005;45:30–4) © 2005 by the American College of Cardiology Foundation

Inflammation within the vascular wall plays an important role in the pathogenesis of atherosclerosis (1). Recent studies have shown that elevation of proinflammatory cytokines like tumor necrosis factor (TNF)-alpha (2), soluble adhesion molecules (e.g., soluble intercellular cell adhesion molecule-1 [sICAM-1]) (3), as well as systemic inflammatory markers like the acute phase component C-reactive protein (CRP) (4,5) are associated with an unfavorable progression of disease and an increased risk for acute cardiovascular events. Thrombus formation after erosion or rupture of coronary plaques is the generally accepted cause of acute coronary syndromes including ST-segment elevation myocardial infarction (STEMI). Activation of the endogenous fibrinolytic system is a function of endothelial cells and a counterpart of local thrombus formation. It has been shown that inflammatory cytokines are able to induce procoagulant and antifibrinolytic activities in vascular cells (6,7). Venous occlusion (VO) of the forearm is a stimulator for the fibrinolytic system. The normal response to VO is an increase of tissue-type plasminogen activator (t-PA) and a concomitant decrease of plasminogen activator inhibitor type-1 (PAI-1) activity. This response of the fibrinolytic system to VO has been shown to be impaired in patients with atherothrombotic diseases (8). Acute systemic inflammation enhances the endothelial release of t-PA (9), but a relationship of chronic inflammation and endothelial fibrinolytic response has not been demonstrated so far. Therefore, we investigated a possible association between surrogate markers for chronic inflammation (e.g., plasma levels of CRP, sICAM, or TNF-alpha) and the fibrinolytic response of the vessel wall on a standardized VO test of the forearm in patients with proven stable coronary artery disease.

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Abbreviations and Acronyms

CRP = C-reactive protein
F1+2 = prothrombin fragment F1+2
PAI-1 = plasminogen activator inhibitor type-1
sICAM-1 = soluble intercellular cell adhesion molecule-1
STEMI = ST-segment elevation myocardial infarction
TNF = tumor necrosis factor
t-PA = tissue-type plasminogen activator
VO = venous occlusion

METHODS

Patients. We investigated 50 consecutive and clinically stable patients six months after they had been hospitalized for a first STEMI. Patients with accompanying diseases that are known to influence CRP-plasma levels were excluded (10). All patients gave their informed consent to participate in the trial that was approved by the local ethics committee and carried out according to the principles of the Declaration of Helsinki.

Blood sampling and VO. Blood for basal determination was drawn from an antecubital vein without or with only minimal VO at resting conditions. A standardized VO test of 15 min duration was performed on the contralateral forearm (11,12).

Laboratory analysis. Plasma levels of CRP were determined by use of a highly sensitive immunonephelometric assay (Dade Behring, Deerfield, Illinois). Plasma levels of sICAM (Bender MedSystems, Vienna, Austria), TNF-alpha (eBioscience, San Diego, California), prothrombin fragment F1+2 (Dade Behring), t-PA/PAI-1 complexes, and PAI-1 antigen (Technoclone, Vienna, Austria) were measured by specific ELISAs. Tissue-type plasminogen activator antigen and t-PA activity plasma levels were determined by a combined assay system (Technoclone); PAI-1 activity was measured by the Coatest PAI method (Chromogenix, Milano, Italy). Plasma concentration of fibrinolytic parameters after VO were corrected for hemoconcentration by use of the correction factor K = (1 − hematocrit after VO)/(1 − hematocrit before VO) (13). All determinations were performed in duplicate.

Statistical analysis. Data are represented as median values and interquartile range or as mean values ± SD. The significance of any differences in proportions was tested by using the chi-square test. Statistical differences between groups in continuous variables were determined by analysis of variance or, if the Kolmogorov-Smirnov test for deviation from normal distribution was significant, by the Kruskal-Wallis test. In either case, post-hoc pairwise comparisons were done according to the Bonferroni-Holm method (14). Statistical differences before and after VO were determined by the Wilcoxon signed rank test for paired samples. Spearman's correlation was used to compare inflammatory markers with VO-induced response of t-PA activity. Multivariate analysis was performed with the linear regression model after log-transformation of t-PA activity. All p values were two-tailed, values lower than 0.05 were considered statistically significant, and confidence intervals were calculated at the 95% level. All calculations were performed using a computer program (SPSS for Windows 10.0.1, SPSS Inc., Chicago, Illinois).

RESULTS

Patients with low CRP plasma levels (CRP <1 mg/l), medium CRP plasma levels (CRP 1 to 2.9 mg/l), and high

Table 1. Characteristics of the Study Participants—Inflammatory and Coagulatory Parameters

	Total	Low Risk (CRP <1.0 mg/l)	Medium Risk (CRP 1.0–2.9 mg/l)	High Risk (CRP ≥3.0 mg/l)	p Value
n	50	20	17	13	
CRP (mg/l)	1.36 (0.51–3.13)	0.42 (0.29–0.59)	2.00 (1.32–2.51)	6.17 (4.66–7.85)	
Age (yrs)	55.4 ± 12.2	55.9 ± 11.7	56.8 ± 13.9	52.6 ± 10.9	0.638*
Male gender (%)	90.0	95.0	94.1	76.9	0.188
Smoker (%)	50.0	50.0	47.1	53.8	0.934
Systolic blood pressure (mm Hg)	124.8 ± 19.6	127.0 ± 21.3	125.0 ± 20.3	121.2 ± 16.7	0.712*
Diastolic blood pressure (mm Hg)	79.2 ± 8.4	79.4 ± 9.2	78.8 ± 7.0	79.6 ± 9.5	0.967*
Body mass index (kg/m^2)	26.9 ± 4.0	25.7 ± 3.2	26.5 ± 2.5	29.2 ± 5.7	0.045*
Total cholesterol (mg/dl)	187.0 ± 26.3	182.2 ± 24.7	188.5 ± 23.9	192.4 ± 31.8	0.538*
LDL cholesterol (mg/dl)	114.6 ± 25.1	110.9 ± 21.4	115.1 ± 25.6	119.9 ± 30.5	0.606*
HDL cholesterol (mg/dl)	45.8 ± 10.2	48.6 ± 11.4	45.3 ± 10.1	42.3 ± 7.6	0.220*
Triglycerides (mg/dl)	137.5 ± 75.9	114.5 ± 51.1	154.8 ± 108.8	150.15 ± 46.3	0.219*
Peak creatine kinase (U/l)	900.1 ± 848.2	847.2 ± 596.5	1,144.18 ± 1,136.0	689.5 ± 679.3	0.342*
Diabetes (%)	20.0	10.0	29.4	23.1	0.322
Family history of CAD (%)	42.0	55.0	29.4	38.5	0.278
Statins (%)	54.0	60.0	52.9	46.2	0.734
Beta-blockers (%)	66.0	60.0	76.5	61.5	0.531
TNF-alpha (ng/ml)	6.0 (5.0–9.4)	7.8 (5.3–11.5)	6.0 (5.0–10.4)	6.0 (5.0–7.0)	0.194†
sICAM (ng/ml)	75.8 (60.65–88.95)	66.9 (57.0–78.7)	71.5 (61.6–85.8)	92.2 (87.2–112.6)	< 0.001†
F1+2 (nmol/l)	0.85 (0.70–1.20)	0.80 (0.60–0.85)	0.9 (0.70–1.70)	1.20 (0.75–1.40)	0.025†

Values are mean ± SD, median (interquartile range), or percentage. Statistical differences were determined by analysis of variance (*) or by the Kruskal-Wallis test (†). All patients were treated with aspirin and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.

CAD = coronary artery disease; CRP = C-reactive protein; F1+2 = prothrombin fragment F1+2; HDL = high-density lipoprotein; LDL = low-density lipoprotein; sICAM = soluble intercellular cell adhesion molecule; TNF = tumor necrosis factor.

Table 2. Plasma Levels of Fibrinolytic Parameters at Baseline, After VO, and x-Fold Increase

	Total	Low Risk (CRP <1.0 mg/l)	Medium Risk (CRP 1.0–2.9 mg/l)	High Risk (CRP ≥3.0 mg/l)	p Value
Before VO					
t-PA activity (IU/ml)	0.30 (0.10–0.70)	0.20 (0.10–0.58)	0.20 (0.10–0.35)	0.80 (0.35–1.50)	0.005
t-PA antigen (ng/ml)	5.8 (3.7–7.1)	5.8 (4.0–8.3)	5.8 (4.0–8.3)	6.8 (3.8–9.4)	0.679
PAI-1 activity (IU/ml)	8.3 (2.9–16.3)	5.3 (2.9–14.1)	8.8 (2.8–22.9)	11.7 (2.1–17.6)	0.588
PAI-1 antigen (ng/ml)	108.1 (57.6–193.8)	66.2 (44.8–107.8)	160.1 (118.0–210.1)	101.7 (58.0–206.9)	0.012
t-PA/PAI-1 complexes (ng/ml)	26.9 (18.0–36.6)	22.6 (15.5–30.9)	28.9 (16.8–45.2)	33.3 (19.4–44.0)	0.203
Ratio of t-PA/PAI-1 activity	0.046 (0.012–0.12)	0.044 (0.006–0.19)	0.017 (0.006–0.091)	0.085 (0.028–4.10)	0.199
After VO					
t-PA activity (IU/ml)	0.74 (0.26–2.14)	1.35 (0.35–2.25)	0.34 (0.18–0.79)	1.34 (0.53–3.58)	0.015
t-PA antigen (ng/ml)	11.9 (9.1–18.3)	12.8 (8.1–19.7)	12.4 (10.1–15.8)	10.4 (8.8–16.5)	0.564
PAI-1 activity (IU/ml)	3.5 (0.1–13.3)	0.1 (0.1–5.6)	8.3 (1.4–19.1)	8.6 (0.1–16.6)	0.054
PAI-1 antigen (ng/ml)	67.7 (33.8–156.1)	41.5 (23.3–66.3)	143.4 (103.5–214.3)	66.5 (40.4–146.2)	0.003
t-PA/PAI-1 complexes (ng/ml)	50.4 (30.1–76.4)	36.4 (28.3–100.1)	55.3 (28.2–84.8)	52.4 (40.9–64.5)	0.679
Ratio of t-PA/PAI-1 activity	0.27 (0.025–7.59)	3.65 (0.29–22.02)	0.054 (0.007–0.49)	0.16 (0.029–2.62)	0.051
x-fold change					
t-PA activity	1.9 (1.4–3.6)	2.7 (1.9–7.6)	1.7 (1.2–3.0)	1.7 (1.2–2.3)	0.028
t-PA antigen	1.9 (1.5–3.0)	2.5 (1.5–3.3)	1.8 (0.8–2.9)	1.7 (1.1–2.3)	0.167
PAI-1 activity	0.62 (0.04–0.86)	0.048 (0.019–0.72)	0.69 (0.31–0.87)	0.83 (0.59–0.90)	0.028
PAI-1 antigen	0.83 (0.64–0.93)	0.84 (0.60–0.92)	0.88 (0.69–0.96)	0.72 (0.61–0.87)	0.331
t-PA/PAI-1 complexes	1.7 (1.3–2.6)	2.0 (1.4–2.8)	1.6 (1.1–3.1)	1.6 (1.2–2.2)	0.676

Values are median (interquartile range). Statistical differences were determined by the Kruskal-Wallis test.
CRP = C-reactive protein; PAI-1 = plasminogen activator inhibitor type-1; t-PA = tissue-type plasminogen activator; VO = venous occlusion.

CRP plasma levels (CRP ≥3 mg/l) showed no significant differences in age, blood pressure, and the presence of cardiovascular risk factors (Table 1). Body mass index was significantly higher in patients with high CRP plasma levels ($p < 0.05$). There was no difference of infarction size as determined by peak creatine kinase elevation. Plasma levels of sICAM were significantly higher in patients with elevated CRP levels, whereas plasma levels of TNF-alpha showed no statistical difference between the three groups. Patients with elevated CRP plasma levels showed an activation of coagulation as determined by elevated F1+2 plasma levels ($p = 0.025$).

The t-PA activity at baseline was significantly higher in patients with high CRP levels ($p = 0.005$), suggesting a chronic activation of the fibrinolytic system associated with subclinical inflammation of the vessels. Patients with low CRP levels showed significantly lower PAI-1 antigen levels than patients with elevated CRP levels ($p < 0.05$). During a standardized VO of the forearm, t-PA activity, t-PA antigen, and t-PA/PAI-1 complexes increased between 1.7- and 1.9-fold ($p < 0.0001$). In contrast, PAI-1 activity and PAI-1 antigen showed a significant decrease ($p < 0.0001$) (Table 2).

The CRP plasma levels showed a significant inverse correlation to the VO-induced relative increase of t-PA activity ($r = -0.28$, $p < 0.05$) (Fig. 1), whereas TNF-alpha ($r = -0.21$, $p = 0.16$) and sICAM ($r = -0.18$, $p = 0.21$) were not significantly associated with the response of t-PA activity during VO.

Table 2 illustrates that the VO-mediated increase in t-PA activity was 2.7-fold (1.9 to 7.6) in patients with CRP levels <0.1 mg/l and significantly higher compared with patients with medium CRP levels (1.7-fold increase [1.2 to 3.0]) and high CRP levels (1.7-fold increase [1.2 to 2.3]) ($p < 0.05$). Moreover, patients with signs of ongoing inflammation (medium and high CRP plasma levels) exhibited a blunted reduction of PAI-1 activity ($p < 0.05$). Accordingly, the ratio of t-PA/PAI-1 activity after VO tended to be higher in patients with low CRP-plasma levels ($p = 0.051$). The changes of antigen levels were comparable between the three groups of patients.

To investigate whether high CRP levels are an independent determinant of VO-induced response of t-PA activity, we performed a multivariate analysis that included CRP groups, plasma lipids, cardiovascular risk factors, and treatment with statins and beta-blockers as independent variables. To rule out that the blunted increase of t-PA activity

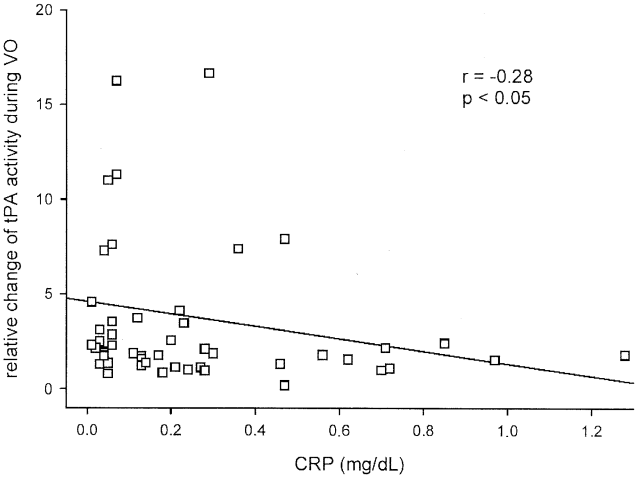

Figure 1. Correlation of relative increase of tissue-type plasminogen activator (t-PA) activity with plasma levels of C-reactive protein (CRP) during a standardized venous occlusion (VO) test of the forearm.

Table 3. Multivariate Analysis of Relative t-PA Response During VO, Including Risk Factors, Medical Treatment, and Baseline t-PA Activity

	Univariate p Value	Beta	p Value
CRP	0.016	−0.365	0.022
Age	< 0.001	0.719	< 0.001
Hypertension	0.207	−0.409	0.004
Gender	0.312	−0.027	0.841
Smoking	0.144	0.005	0.976
Body mass index	0.648	0.286	0.071
Total cholesterol	0.971	−0.301	0.725
LDL cholesterol	0.274	0.373	0.650
HDL cholesterol	0.291	−0.004	0.990
Triglycerides	0.196	0.222	0.487
Diabetes	0.237	−0.154	0.226
Family history of CAD	0.575	0.103	0.477
Statins	0.993	−0.002	0.986
Beta-blockers	0.646	0.071	0.576
Baseline t-PA activity	0.140	0.007	0.959
Adjusted R^2		0.407	0.002

Abbreviations as in Tables 1 and 2.

upon VO in the groups with medium and high CRP levels is merely due to the increase of baseline t-PA activity, we also included t-PA activity at baseline. Table 3 shows that the only statistically significant determinants for the increase of t-PA activity during VO were age, hypertension, and CRP levels.

DISCUSSION

We stratified patients with stable CAD, six months after a first STEMI, according to their CRP levels, into the generally accepted three clinical risk groups, namely patients with low CRP levels and low risk for future cardiovascular events (CRP <1 mg/l), patients with medium CRP levels and moderate risk (1 to 2.9 mg/l), and patients with high CRP plasma levels and high risk for future cardiovascular events (CRP ≥3 mg/l) (15). Patients with high CRP plasma levels show at baseline a markedly increased activation of coagulation and fibrinolysis as determined by a significant elevation of F1+2 and t-PA activity. In contrast, an acute prothrombotic stimulus is not able to further increase the endothelial release of t-PA activity in patients with high (≥3 mg/l) as well as medium (1 to 2.9 mg/l) CRP levels. The blunted increase of t-PA activity upon VO was not merely due to the increase of baseline t-PA activity as shown by performing a multivariate analysis that included t-PA activity at baseline. Patients with low CRP levels showed also a significantly higher consumption of free PAI-1 during VO compared with patients with CRP levels >1 mg/l. A multivariate analysis that included cardiovascular risk factors and medical treatment known to have anti- or profibrinolytic effects showed that CRP is an independent predictor of the t-PA response after a standardized VO. We suggest that a reduced t-PA release consecutively diminishes the capacity to counteract thrombus formation after rupture or erosion of coronary plaques and thus increases the risk for acute coronary events.

How chronic inflammation may be linked with a diminished fibrinolytic capacity is still under investigation. Most studies have focused on PAI-1 as the fibrinolytic parameter being responsible for a reduced fibrinolytic potential. The proinflammatory cytokines TNF-alpha and interleukin-1 induce an increase of PAI-1 secretion in vascular cells (6), and PAI-1 has been shown to be a part of the acute-phase response induced by proinflammatory cytokines in hepatocytes (10). We showed that patients with medium or high CRP plasma levels show significantly higher levels of PAI-1 antigen at baseline compared with patients with CRP levels <1 mg/l. The CRP plasma levels may reflect, in part, the presence and severity of atherosclerosis (16), and coronary plaque burden is inversely related with substance-P–induced t-PA release from the coronary artery (17). Accordingly, the blunted fibrinolytic response as observed in our study may be related to the extent of atherosclerosis that may be reflected by plasma levels of CRP.

Plasma levels of the proinflammatory cytokine TNF-alpha and the soluble adhesion molecule sICAM did not correlate with t-PA release upon VO. Although TNF-alpha may directly lead to a prothrombotic and hypofibrinolytic phenotype of vascular cells, and sICAM may be a marker for inflammatory activation of endothelial cells, in our study neither parameter was associated with the fibrinolytic capacity. One may speculate that the acute-phase reactant CRP might show a better correlation to the blunted fibrinolytic response upon VO because CRP is a very sensitive but, in contrast to TNF-alpha and sICAM, nonspecific marker for inflammatory processes (10).

C-reactive protein may also promote atherogenesis and thrombosis by perturbing normal endothelial cell function by inducing tissue factor expression, the production of proinflammatory cytokines, the expression of adhesion molecules, and the down-regulation of nitric oxide and prostacyclin production by endothelial cells (18,19). Taking together the results of a study showing that CRP induces the production of PAI-1 in endothelial cells (20) and our study, one could speculate that CRP may also promote atherothrombosis through the reduction of the fibrinolytic potential by affecting endothelial cells.

We have shown that the endothelial release of t-PA activity upon VO, reflecting the fibrinolytic capacity, is diminished in patients with medium and high CRP plasma levels independently from cardiac risk factors and medical treatment. This could explain, in part, the moderate and high risk for acute cardiovascular events in these patients, and one could speculate that this impaired endogenous fibrinolytic capacity might be an important contributor to the increased coronary event rate associated with elevated CRP levels. Thus, patients after a first myocardial infarction with medium (1 to 2.9 mg/l) and high (≥3 mg/l) CRP levels may benefit from intensive antithrombotic treatment and treatment with statins or modulators of the renin-

angiotensin-aldosterone system that—besides their effects on plasma lipids or hypertension—have also anti-inflammatory properties that may improve endogenous fibrinolytic capacity.

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