In patients with coronary artery disease endothelial function is associated with plasma levels of C-reactive protein and is improved by optimal medical therapy

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Coronary artery disease; Inflammation.

Background. **Endothelial function is impaired in patients with coronary artery disease (CAD); in these patients plasma levels of C-reactive protein (CRP) and impaired endothelial function are related to future cardiac events. The aim of the present study was to evaluate the effects of medical therapy on endothelial function and CRP in patients with CAD.**

Methods. **Seventy-three patients (52 men, 21 women, mean age 66 ± 9 years) with CAD and 32 control subjects (25 men, 7 women, mean age 65 ± 11 years) were enrolled in the study. The endothelial function was evaluated by means of flow-mediated dilation (FMD) of the brachial artery following ischemia and CRP by means of a high-sensitivity assay. After baseline evaluation of CRP and FMD all patients received full medical therapy for 3 months and were then again tested for endothelial function and CRP.**

Results. **Compared to healthy controls, patients had significantly more impaired endothelial function (FMD 3.6 ± 3.2 vs 8 ± 2.4%, $p < 0.01$) and higher CRP plasma levels (1.6 ± 0.9 vs 0.9 ± 0.56 mg/dl, $p < 0.05$). At baseline a significant negative correlation was found between CRP plasma levels and FMD in patients with CAD ($r = -0.56$, $p < 0.05$) while no correlation was found in controls. Medical therapy resulted in a significant improvement in endothelial function (3.64 ± 3 vs 7.2 ± 3.5%, $p < 0.01$), and a decrease of CRP (-0.26 ± 0.19, $p < 0.01$); the changes in CRP and FMD were independent of the drug used. A positive correlation was found between the improvement in FMD and the degree of CRP reduction ($r = 0.57$, $p < 0.01$).**

Conclusions. **In patients with CAD plasma levels of CRP are associated with an impaired endothelial function suggesting a correlation between inflammation and the integrity of the endothelium. Full medical therapy reduces CRP with a parallel improvement in endothelial function.**

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Introduction

Although atherosclerosis is clearly multifactorial, it is now universally recognized that inflammation plays an important role in the development and evolution of atherosclerotic lesions[1-3]. A large body of evidence has shown a strong and consistent association between the clinical manifestations of atherothrombotic disease and systemic markers of inflammation, including white blood cell count and several hemostatic proteins that are also acute-phase reactants. C-reactive protein (CRP) is an acute-phase reactant, synthesized in the liver by hepatocytes in response to interleukin-6, and has been considered as being not only a nonspecific, sensitive marker of the acute inflammatory response but also an important risk factor for atherosclerosis and cardiovascular disease[4]. Several prospective epidemiological studies have shown that CRP may be linked to future cardiovascular events in healthy subjects, elderly patients and high-risk individuals[5] and that the concentration of CRP is directly correlated with the presence and severity of coronary, cerebral and peripheral arterial atherosclerosis.

The endothelium is a functional barrier between vessel wall and blood stream that plays a crucial role in the process of atherosclerosis, by its regulatory functions on the vasculature, such as control of vasomotor tone, local hemostasis, proliferative processes and immune response[6,7]. It is also known that the presence of coronary atherosclerotic lesions is associated with an

impaired endothelium-mediated regulation of the vascular tone[8] that may be evaluated by flow-mediated dilation (FMD) of the systemic arteries, a noninvasive parameter of endothelial function. Several experimental models have shown that endothelial dysfunction promotes thrombosis, vasospasm and vessel occlusion, and is implicated in the pathogenesis of acute myocardial infarction, stroke and other cardiovascular disorders. Consistent with the link between endothelial dysfunction and atherosclerosis, smoking, hypertension, diabetes and hyperlipidemia have all been associated with impaired endothelium-dependent dilation in studies in animals and humans[9]. Although the relationship between the inflammatory process and atherosclerosis has been extensively debated, there are few studies that have evaluated the presence of an association between CRP and endothelial function. Furthermore, if CRP is a marker of active atherosclerosis then it should be coupled with a dysfunctional endothelium and a reduced endothelial function. To this end, we assessed the relation between endothelial function, evaluated by means of FMD, and plasma levels of CRP in patients with coronary artery disease (CAD) and we have also evaluated the effects of medical therapy on both parameters.

Methods

Study population. The study population included 73 patients (52 men, 21 women, mean age 66 ± 9 years) with proven CAD and 32 healthy controls (25 men, 7 women, mean age 65 ± 11 years) who were hospitalized because of poorly controlled anginal symptoms. Patients were included if they had evidence of CAD defined as previous (> 3 months) myocardial infarction, angiographically proven coronary atherosclerosis (> 70% stenosis of one of the major coronary arteries) and if they had chronic stable angina with evidence of inducible myocardial ischemia (positive exercise testing or dobutamine stress echocardiography or evidence of reversible hypoperfusion at myocardial stress scintigraphy).

Patients with recent acute cardiac events (< 3 months), uncontrolled hypokaliemia, severe arrhythmia (Lown class > 3) and those scheduled to undergo coronary angiography or a revascularization procedure during the study period were excluded. Controls were chosen from hospital nonmedical staff and volunteers. Subjects underwent a full medical examination in order to clinically exclude the presence of cardiovascular disease.

Study design. Patients and subjects underwent evaluation of endothelial function by means of FMD and the measurement of CRP plasma levels at baseline and after 3 months. After baseline evaluation medical therapy was maximized in all patients.

In all subjects the endothelial function was evaluated by means of FMD of the brachial artery following reactive hyperemia and by a high-sensitivity assay of CRP plasma levels.

The study protocol was approved by the local Ethics Committee and all subjects gave their informed consent prior to participation.

Patients and controls were asked to refrain from smoking and drinking alcohol or caffeine-containing beverages and, also, to abstain from severe physical exercise during the 24 hours preceding the study. Patients and subjects were weighed and measured to calculate the body mass index and spent at least 20 min acclimatization period lying in a quiet room at a controlled temperature of 20 ± 1°C and a relative humidity of 65 ± 10%.

Blood samples for CRP were obtained 20 min prior to the investigation of the brachial artery endothelial function.

Brachial artery endothelial function. The endothelial function was assessed by measuring changes from baseline in the caliber of the brachial artery during reactive hyperemia, a procedure that increases blood flow and sheer stress through the vessel. Brachial artery FMD was measured with high-resolution ultrasound. In order to avoid interobserver variability, the same investigator studied each patient. Studies of the brachial artery reactivity were conducted according to a previously reported protocol[10]. In brief, all patients were studied in a quiet, temperature-controlled room (22 to 23°C). Participants were asked to avoid drinking beverages containing caffeine and to refrain from smoking for 6 hours preceding the study. After 15 min of rest in a supine position, the right brachial artery was imaged using an Acuson Sequoia C256 high-resolution ultrasound machine (Acuson Corporation, Mountain View, CA, USA) equipped with a 7.5 to 12.5 MHz linear-array transducer. The artery was scanned over a longitudinal section 3 to 5 cm above the elbow, the site where the clearest image can be obtained. The focus zone was set to the depth of the anterior vessel wall. Depth and gain settings were optimized to identify the lumen-vessel wall interface. The diameter of the right brachial artery was continuously measured 4 times: at rest, during reactive hyperemia, after a 10 min recovery period, and 5 min following sublingual nitroglycerin. A pneumatic tourniquet was placed around the forearm distal to the target artery and inflated to a pressure of 50 mmHg above the patients' systolic blood pressure for 5 min. Reactive hyperemia was induced by sudden cuff deflation. The brachial artery was continuously imaged for 30 s prior to and for 180 s after cuff release. Ten minutes following cuff deflation, a third scan was recorded to confirm that the basal conditions had been reestablished. To assess endothelium-independent vasodilation, sublingual nitroglycerin (0.4 mg) was administered, and a fourth scan was then recorded for 5 min. The ultrasound images were recorded directly onto the hard disk of the ultrasound machine, and then

transferred to a floppy disk. Image analysis was performed using a validated program. The diameter of the brachial artery was measured from the anterior to the posterior interface. For each condition, the mean arterial diameter was calculated from 4 cardiac cycles synchronized with the R-wave peak on the electrocardiogram. All measurements were made at end diastole. The diameter change was expressed as the percent change compared with the baseline diameter. In our group the intraobserver variability in diameter measurements is 0.38 ± 0.26% (range 0.1-1.2%), the coefficient of variation is 1.26%, and the coefficient of repeatability is 0.5%, as previously reported[10].

C-reactive protein plasma levels. Blood samples were obtained at baseline and after 3 months of full medical therapy from patients with CAD and from the control group. Venous blood samples were taken after at least 10 hours of fasting, in a supine position after 20 min of rest with a Vacutainer system (Becton Dickinson, Meylan, France). Baseline blood samples were collected in tubes containing EDTA or trisodium citrate (1:9 vol/vol). The blood samples were immediately placed on ice and centrifuged within 1 hour of collection. The plasma was divided into aliquots and stored at -80°C until laboratory analysis. All the serum samples were assessed in duplicate. The obtained plasma samples were thawed and assayed for CRP by means of a high-sensitivity assay with a coefficient of variation < 5% (hs-CRP, Dade Behering, Marburg, Germany).

Statistical analysis. Values are given as mean ± SD or as percentages where appropriate. The differences in mean values between groups were assessed using the Wilcoxon test for paired data. All calculated p values are two-tailed and considered as significant when < 0.05.

Analysis of covariance for repeated measurements using baseline values as constant covariates was used to test the statistical difference between measurements within groups. The correlation between variables was calculated with a correlation coefficient. A multivariate analysis was performed in order to determine which variables were associated with an improvement in endothelial function. In the analysis plasma levels of cholesterol, LDL cholesterol, degree of CAD, CRP levels, the development of acute ischemic syndromes, cigarette smoking, hypertension, diabetes, and the new use of any cardioactive or antiplatelet or lipid-lowering drug were entered as independent variables. A second analysis was performed using the different pharmacological classes of drugs as independent variables.

Results

The clinical features of the patients and controls are shown in table I. Patients had a higher incidence of hyperlipidemia and a lower incidence of cigarette smoking than controls. The use of cardiovascular drugs for the control of symptoms or myocardial ischemia and the control of risk factors is shown in table II.

Compared to controls, patients had a significantly more impaired endothelial function (FMD 3.6 ± 3.2 vs 8 ± 2.4%, $p < 0.01$) and higher CRP plasma levels (1.6 ± 0.9 vs 0.9 ± 0.56 mg/dl, $p < 0.05$) (Table III). A significant negative correlation was found between baseline plasma levels of CRP and FMD in patients with CAD at baseline ($r = -0.56$, $p < 0.05$) while no correlation between FMD and CRP levels was found in controls (Fig. 1). The correlation did not change when CRP levels were plotted after log transformation in order to account for the non-normal distribution. During follow-up 1 patient was admitted for occurrence of acute ischemic episodes and 3 underwent a percutaneous revascularization procedure. After 3 months no significant change in FMD or CRP levels was detected in controls (Table III). In patients optimal medical therapy resulted in a significant improvement in endothe-

Table I. Clinical characteristics of the study patients.

	Patients (n = 73)	Controls (n = 32)
Age (years)	66 ± 9	65 ± 11
Sex (M/F)	52/21	25/7
Body mass index (kg/m^2)	27 ± 5	28 ± 4
Risk factors for CAD (%)		
Cigarette smoking	12	25
Hyperlipidemia	82	37.5
Hypertension	62	25
Diabetes	38	–
Family history of CAD	24	37.5
CAD (n =)		
Previous MI	15	–
Documented CAD	46	–
Inducible ischemia	54	–

CAD = coronary artery disease; MI = myocardial infarction.

Table II. Use of cardiovascular drugs at baseline and after optimization of medical therapy.

	Baseline	After optimization of therapy
Beta-blockers	23	41
Nitrates	48	39
Calcium channel blockers	35	37
Trimetazidine	6	14
ACE-inhibitors	21	34
Diuretics	21	23
AT_1-blockers	8	11
Aspirin/antiplatelet agents	34	61
Anticoagulants	45	62
Statins	–	12

Table III. Brachial artery diameters.

	Patients		Controls	
	Baseline	Follow-up	Baseline	Follow-up
CRP (mg/dl)	1.6 ± 0.9*	1.34 ± 0.7**	0.9 ± 0.56	0.9 ± 0.84
FMD (%)	3.6 ± 3.2*	7.2 ± 3.5**	8 ± 2.4	8.4 ± 3.2

CRP = C-reactive protein; FMD = flow-mediated dilation. * $p < 0.01$ compared to controls; ** $p < 0.01$ compared to baseline.

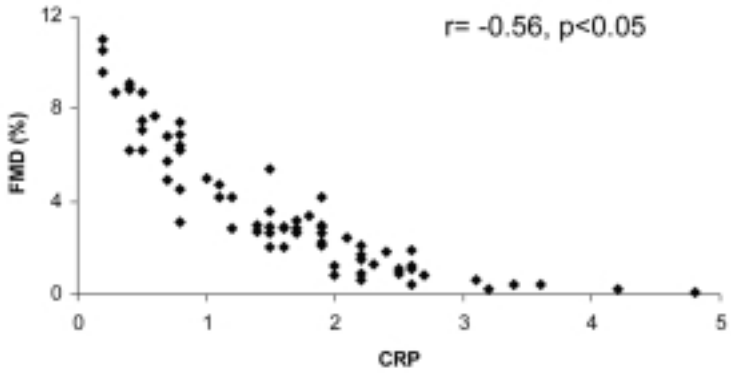

Figure 1. *Correlation between baseline endothelial function (flow-mediated dilation-FMD) and C-reactive protein (CRP). A significant negative correlation was found.*

lial function (3.64 ± 3 vs 7.2 ± 3.5%, $p < 0.01$) and in decreased CRP levels (-0.26 ± 0.19, $p < 0.01$). The changes in CRP and FMD were independent of the drug used (Table II). Multivariate analysis revealed that optimization of medical therapy and the decrease in CRP levels were the only independent predictors of an improvement in endothelial function during follow-up. However, when taken individually, none of the classes of drugs were found to be significantly associated with an improvement in endothelial function. A positive correlation was found between the improvement in FMD and the degree of CRP reduction ($r = 0.57$, $p < 0.01$) (Fig. 2).

Discussion

The present study shows that increased CRP plasma levels are associated with an impaired endothelial function in patients with CAD and that medical therapy, independently of the drug used, resulted in a significant improvement in endothelial function and in a decrease of CRP plasma levels. This is suggestive of a correlation between inflammation and the integrity of the endothelium. Our data are in agreement with those of a previous study by Fichtlscherer et al.[11] in which the impairment of normal endothelial function was related to elevated CRP plasma levels suggesting a link between systemic in-

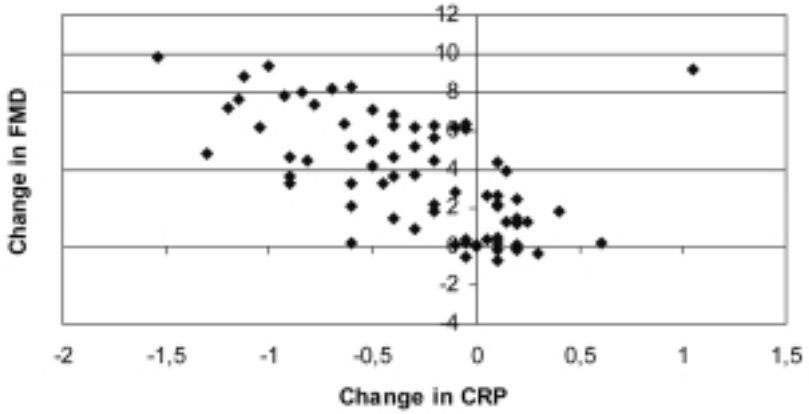

Figure 2. *Correlation between improvement in endothelial function (flow-mediated dilation-FMD) and C-reactive protein (CRP) after optimization of medical therapy.*

flammation and ischemic coronary syndromes. CRP is an acute-phase protein, produced by hepatocytes in response to most forms of tissue injury, infection, and inflammation. Its synthesis is regulated by cytokines, including interleukin-6, interleukin-1, and tumor necrosis factor-α. The latter has been recently related to cardiovascular events[3,4,12]. Several epidemiological studies have shown that CRP may be considered a nonspecific but sensitive marker of the acute inflammatory response in atherosclerotic disease[11]. In particular, elevated CRP serum levels have been reported in patients with acute myocardial ischemia, myocardial infarction, unstable angina, and chronic stable angina[3,13-16]. In these patients high CRP plasma levels have been associated with an increased inflammatory activity, a rapid progression of atherosclerotic disease, and a poor prognosis[14-16]. In fact, inflammation plays an important role not only in the onset but also in the development and evolution of atherosclerotic lesions. Histopathological and immunocytochemical observations have suggested that active inflammatory processes may destabilize the fibrous cap tissue, thus triggering plaque rupture and enhancing the risk of coronary thrombosis[3]. Therefore, a large body of evidence suggests a link between inflammation and atherogenesis and an association between an acute systemic inflammatory response and a transiently increased risk of an acute cardiovascular event[17]. Recent data suggest that inflammation and infection may initiate and promote atherosclerosis or its complications by adverse effects on the vascular endothelium and that endothelial dysfunction may be the link between inflammation and the risk of an acute cardiovascular event[9,18-20]. It is known that the endothelium is a functional barrier between the vessel wall and the blood stream that plays a key role in the regulation of arterial tone and in maintaining the physiologic equilibrium between procoagulant and anticoagulant forces. Experimental studies have demonstrated that by the inhibition of platelet aggregation, monocyte adhesion, vascular smooth muscle cell proliferation and thrombosis a functionally intact endothelium exerts potent antiatherosclerotic and antithrombotic effects. Early clinical studies found an impaired endothelial function in angiographically diseased arteries and in normal vessels in patients with atherosclerosis elsewhere. These observations have led to the concept that endothelial dysfunction occurs very early in the disease process. Afterwards, endothelial dysfunction in both the coronary and brachial arteries has been found in many diseases/disorders associated with an increased cardiovascular risk, such as smoking, essential hypertension, diabetes and dyslipidemia, even in the absence of evidence of atherosclerotic lesions, suggesting that the endothelium is both a target and a mediator of atherosclerosis[8,18,20]. Recently Fichtlscherer et al.[11] demonstrated an inverse correlation between CRP levels and the forearm blood flow responses to acetylcholine in males with documented CAD. In the present study, besides the inverse correlation between CRP and endothelial function we have shown that decreasing CRP levels over time were associated with a normalization of the endothelium-mediated forearm blood flow responses after 3 months. For this reason we hypothesize that optimization of medical therapy in coronary patients not only reduces the levels of the markers of vascular inflammation but also improves endothelial function. It is important to note that in the present study the improvement in either inflammatory markers or endothelial function was not related to any specific class of drugs suggesting that it is the optimization of medical therapy rather than single classes of drugs that may induce the beneficial effects. Recent experimental studies have shown that the exposure of endothelial cells to proinflammatory cytokines induces procoagulant activity, upregulates chemotactic and adhesion molecules for monocytes and T lymphocytes, secretes colony-stimulating factors that induce differentiation of monocytes into macrophages, and impairs endothelium-dependent vascular relaxation. All of these alterations in endothelial function, termed "endothelial activation", modify the physiological protective regulatory balance, which is a critical factor in the onset and progression of atherosclerotic vascular disease. Therefore, the exposure of endothelial cells to proinflammatory cytokines leads to the expression of cell-surface adhesion molecules and impairs endothelium-dependent vascular relaxation, suggesting that the inflammatory response and endothelial dysfunction may provide a link between systemic inflammation and cardiovascular disease. Another mechanism by which CRP may interfere with endothelial function is through a decrease in nitric oxide production. As shown by Verma et al.[21], this is partly mediated through a post-transcriptional effect on the stability of the endothelial nitric oxide synthase mRNA.

In conclusion, the correlation between CRP levels and endothelial function supports the hypothesis that inflammation is associated with a clinically measurable alteration of the integrity and functionality of the endothelium. Medical therapy improves endothelial function probably by reducing the degree of vascular inflammation.

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