

Levels of sCD40, sCD40L, TNF α and TNF-RI in the Culprit Coronary Artery During Myocardial Infarction

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Abstract

Background. Inflammatory processes are involved in the pathogenesis of coronary artery disease and acute myocardial infarction (AMI). Yet there is little known about concentrations of pro-inflammatory mediators in the cardiac milieu of patients suffering from AMI. The aim of this study was to evaluate blood samples directly obtained from the culprit coronary artery during acute myocardial infarction.

Materials and Methods. Serum samples were obtained from the culprit coronary artery of acute myocardial infarction patients (n=39) utilizing a X-sizer thrombectomy system. Sera from patients with stable angina (SA, n=34) and unstable angina (UA, n=37) served as controls. Levels of sCD40, sCD40L, TNF α and sTNF-RI were determined by enzyme-linked immunosorbent assays.

Results. Levels of sCD40L and sCD40 were increased in AMI group when compared to patients suffering from SA and UA. Levels of both inflammatory markers were highest in the culprit coronary artery. Increased concentrations of TNF α and sTNF-RI evidenced a further inflammatory response at the site of infarction.

Conclusions. We conclude that mediators of inflammation are heightened in the coronary blood flow during myocardial infarction. Our observations extend the current knowledge of the inflammatory cascade during myocardial infarction.

Key words: acute myocardial infarction; cytokines; culprit coronary artery; inflammation

Introduction

Inflammatory processes are thought to play a major role in the pathogenesis of atherosclerosis and myocardial infarction. During formation of atherosclerotic lesions leukocytes infiltrating the plaque release cytokines that promote local endothelial dysfunction and smooth muscle cell proliferation [8]. Increased systemic markers of inflammation in the clinical state of angina pectoris have been addressed in several studies and have been suggested as predictive markers for a later cardiovascular event [11, 19].

Inflammation following acute myocardial infarction (AMI) augments myocardial damage and thereby extends post-infarct myocardial dysfunction. Based on these findings, various anti-inflammatory strategies have been proposed in order to down-size myocardial loss (reviewed in [7]). However, as the inflammatory reaction is also prerequisite for healing and scar formation, suppression of the inflammatory cascade increases the risk of aneurysma formation and ventricular rupture. This could account at least partly for the failure of anti-inflammatory therapy during AMI in clinical practise [5, 20]. As a consequence a better understanding of the inflammatory processes at the site of myocardial infarction would be useful in order to develop further immunomodulatory strategies.

Early attempts made to elucidate the role of pro-inflammatory cytokines at the ischemic site during myocardial infarction included analysis of blood samples obtained from the coronary sinus. However, a major obstacle of coronary sinus sampling is that blood samples are often contaminated by atrial blood [12]. Recently, new technical tools in cardiac catheterization have been introduced, allowing direct blood suction from the culprit coronary artery [14, 16, 23]. Most of the work published has concentrated on the role of sCD40L and IL-6 at the site of infarction. Concentrations of other inflammatory proteins in the coronary blood flow are still unknown.

The aim of this study was to evaluate levels of sCD40, sCD40L, TNF α and sTNF-R1 in the culprit coronary artery and to compare them to systemic concentrations. Blood samples drawn from patients suffering from stable and unstable angina pectoris served as controls.

Materials and Methods

The study protocol was approved by the ethics committee of the University of Vienna (EC-No: 303/2005) and experiments were performed in accordance with the ethical standards laid down in the Declaration of Helsinki 1964. All patients gave written informed consent.

AMI patients and blood sample collection

Thirty-nine consecutive patients committed to the Medical University of Vienna undergoing emergency coronary angiography utilizing X-sizer thrombectomy (EndiCOR Medical Inc., San Clemente, CA, USA) were included in this study. Patients had to meet the following inclusion criteria: 1) chest pain at the time of coronary angiography, 2) new ST-segment elevations ≥ 2 mm in two or more chest leads, or new ST-segment elevations ≥ 1 mm in more than one horizontal plane lead observed within 20min of coronary angiography. Patients with a prior thrombolytic therapy, severe vessel tortuosity, calcification or difficult vascular access were excluded. A vessel diameter ≥ 3 mm, a large intraluminal contrast defect suggestive of thrombus, a TIMI flow of 0-1 after passage of the angiographic guide wire were mandatory for the decision of using the X-sizer thrombectomy. Patients received 250mg acetylsalicylic acid and were heparinized at an activated clotting time ≥ 300 s. Whole blood samples were either retrieved from the femoral artery or directly from the occluded coronary artery by the thrombectomy catheter system into serum tubes (Greiner, Kremsmünster, Austria) as described before [1]. Tubes were centrifuged at 1300g, (4°C, 10min) and serum samples frozen immediately at -80°C.

Angina pectoris patients and blood sample collection

Blood samples from angina pectoris (AP) patients were obtained from patients attending the polyclinic of the Dept. for Cardiology, Medical University of Vienna. Stable angina (SA, n=34) was defined by typical exertional chest pain relievable by rest, glyceryl trinitrate administration or both. Patients featured a positive response to exercise ECG stress testing and catheterization revealed a $\geq 50\%$ diameter stenosis in ≥ 1 coronary arteries. Patients with unstable angina (UA, n=37) were defined according to the criteria of Braunwald [4]. All patients with UA class IIIB had diagnostic ST segment changes, T wave inversion or both. No patient included in the

study had evidence of an ongoing systemic or cardiac inflammatory process as defined by clinical history.

Blood samples were drawn by punctation of the vein, centrifuged and serum samples were kept frozen at -80°C until further tests were performed.

Detection of cytokines

Levels of sCD40, sCD40L, TNF α and sTNF-RI were determined utilizing commercially available enzyme-linked immunosorbent assays (ELISA, BenderMedSystems, Vienna, Austria). The assays were performed following the manufacturer's instructions. In detail plates were either supplied pre-coated or coated, sealed and incubated over night at 4°C. Plates were washed and blocked with PBS containing 0.05% Tween20 and 0.5% BSA for two hours. Standards, samples and a biotin conjugated detection antibody were added to each well. A streptavidin-horseradish peroxidase/3,3',5,5'-Tetramethylbenzidine-system was used for the enzymatic colour reaction. Plates were read at 450nm on a Wallac Multilabel counter 1420 (PerkinElmer, Waltham, MA, USA). Cytokine concentrations were calculated by comparing optical density (OD) values of samples with OD of known concentrations of the standards.

Statistical analysis

Statistical analysis were performed using SPSS software (SPSSInc., Chicago, IL, USA). Data are given as mean \pm standard of the mean. Normal distribution was verified using the Kolmogorov-Smirnov test. Paired two-sided T-tests for dependent and unpaired T-tests for independent variables were used calculating significances. Bonferroni-Holm correction was used to adjust p-values for multiple testings. P-values <0.05 were considered statistically significant.

Results

Characteristics of study patients

Table 1 presents clinical characteristics of patients. The mean age of the patients in the AMI group was 59.3 years. Nine patients suffered from diabetes, 23 from hypertension and 25 had either been former smokers or were still smoking. Average cholesterol level was 206.3mg/dL and triglycerides were 204.6mg/dL. The mean C-reactive protein levels were 3.6mg/dL (reference value 0-1mg/dL). This is in concordance to previously published data indicating a systemic inflammatory response during AMI [24] and correlates with significantly higher leukocyte counts in the AMI group when compare to SA and UA patients. In both AP groups, a similar proportion of patients had a history of myocardial infarction or previous coronary intervention. Established risk factors for coronary artery disease, total cholesterol concentrations and angiographic findings were comparable in both AP groups.

Levels of sCD40 and sCD40L

We were able to show that levels of the cleaved, soluble form of CD40 were heightened during AMI when compared to both AP groups (SA: 56.2±6.8pg/mL; UA: 49.5±4.8pg/mL; AMI femoral artery: 60.7±2.6pg/mL; AMI coronary artery: 62.5±4.8pg/mL; Figure 1a, 2a).

Our data are in line with previously published reports on the levels of sCD40L [21-23]. Serum concentrations increased from 724.3±223.8pg/mL in the SA to 1078.2±592.0pg/mL in the UA group (Figure 1b). In the AMI group levels of sCD40L were higher in the coronary blood flow (2149.2±160.6) when compared to systemic arterial blood concentrations (2005.4±127.5, Figure 2b). However, these findings did not reach statistical significance.

Levels of TNF α and sTNF-RI

Levels of TNF α and sTNF-RI are shown in Figure 1c, d and 2c, d. Concentrations of TNF α did not differ between AP and AMI patients. However, sTNF-RI significantly increased during AMI compared to the AP control groups.

Discussion

The role of inflammation in the pathogenesis of myocardial infarction has been targeted in many studies. However, most of the knowledge regarding local effects/mechanisms of pro-inflammatory cytokines in the myocardium was gained by necropsy studies or animal models. Insight in the inflammatory process *in vivo* is limited to sparsely clinical studies analyzing blood samples obtained from the coronary sinus.

One major obstacle of the measurement of inflammatory markers from the peripheral circulation is that they may not reflect intra-coronary levels. Moreover, systemic processes can occult measured concentrations and thus may lead to wrong implications [12]. In order to overcome these problems we utilized a X-sizer thrombectomy system to directly obtain blood samples from the culprit coronary artery in our patient series.

Our data corroborate previous reports on the levels of sCD40L in the coronary blood flow during AMI. In addition to these findings, we analyzed concentrations of sCD40, TNF α and sTNF-RI at the site of infarction.

The sCD40/sCD40L system has been shown to be a relevant factor in the pathogenesis of myocardial infarction. sCD40L – an important protein in immunologic function - is released by activated thrombocytes at the site of infarction and thereby bridges the coagulatory and inflammatory system [9]. It has been shown that increased systemic levels of sCD40L during myocardial infarction are useful diagnostic and prognostic markers [22]. Furthermore, the cleaved, soluble form of the sCD40L receptor (sCD40) is able to bind and neutralize sCD40L in the blood circulation. Thereby, increased concentration of sCD40 in our patients during myocardial infarction could be interpreted as a natural regulatory mechanism to prevent an overshoot of sCD40L effects.

Besides sCD40/sCD40L, TNF α seems to be an important cytokine during AMI. It is mainly secreted by resident, intramyocardial macrophages and the myocardium itself [13]. Increased TNF α levels have been detected after ischemia and ischemia/reperfusion injuries on a mRNA and protein level by analysis of peripheral blood samples [3, 10, 15]. Deleterious effects of TNF α on the myocardium are mainly mediated through TNF-RI, whereas ligation of TNF-RII seems to be cardioprotective [18]. The cleaved sTNF-RI is involved in the functional neutralization as well the clearance of TNF α from the circulation [6]. Analysis of peripheral blood samples from

our patient collective corroborate previous published data on TNF α during acute myocardial infarction and coronary artery disease [3, 17]. Interestingly, TNF α levels were not higher in the coronary blood flow when compared to systemic concentrations as one would have expected on basis of molecular [10] and histopathologic examinations [2]. An explanation for this discrepancy could be heightened levels of circulating sTNF-RI, that effectively demonetize TNF α .

To the best of our knowledge this is the first published evidence of intra-coronary levels of sCD40, TNF α and sTNF-RI during myocardial infarction. Furthermore, we were able to confirm published data on levels of sCD40L in the culprit coronary artery. In recent history new therapeutic strategies of myocardial infarction based on an alteration of the inflammatory response have failed. An incomplete knowledge of the inflammatory processes as well as fundamental differences between animal models and disease process in human is made responsible for the disappointing results of these clinical trials [7]. We therefore conclude that a better understanding of the inflammatory cascade is pivotal for developing new anti-inflammatory interventions. Further studies are needed to extend our data on the inflammatory processes taking place in the cardiac milieu during acute myocardial infarction.

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Figure Legend

Figure 1

Levels of sCD40, sCD40L, sTNF α and TNF-RI are shown in Figures 1. Compared to the AP control groups a significant increase of concentrations of sCD40 (SA: 56.2 \pm 6.8pg/mL; UA: 49.5 \pm 4.8pg/mL, AMI: 60.7 \pm 2.6pg/mL), sCD40L (SA: 724.3 \pm 223.8pg/mL; UA: 1078.2 \pm 592.0pg/mL; AMI: 2005.4 \pm 127.5pg/mL) and sTNF-RI (SA: 0.43 \pm 0.08ng/mL; UA: 0.43 \pm 0.07ng/mL; AMI: 1.46 \pm 0.32ng/mL) was observed. Levels of TNF α did not differ between study groups.

Figure 2

Figure 2 depicts comparisons between systemic cytokine levels and their concentrations in the culprit coronary artery. None of the parameters measured in the coronary bloodstream evidenced a significant increase/decrease when compared to systemic serum samples.

Figure 1a

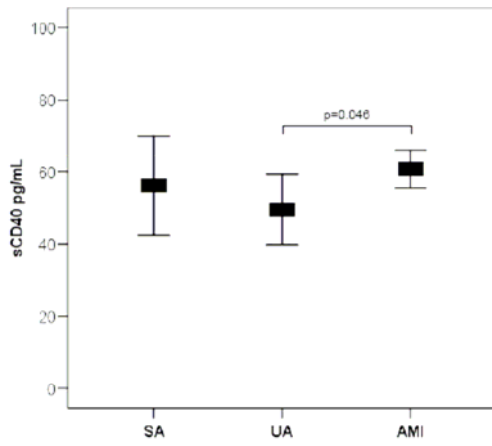


Figure 1b

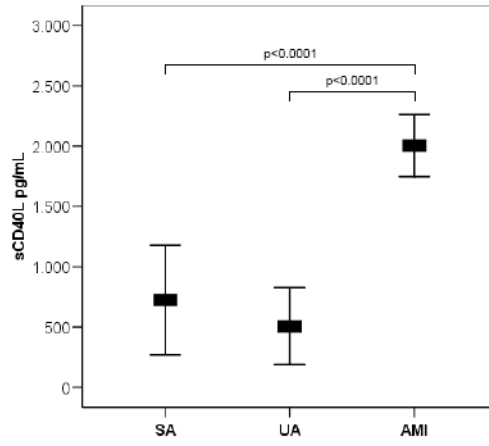


Figure 1c

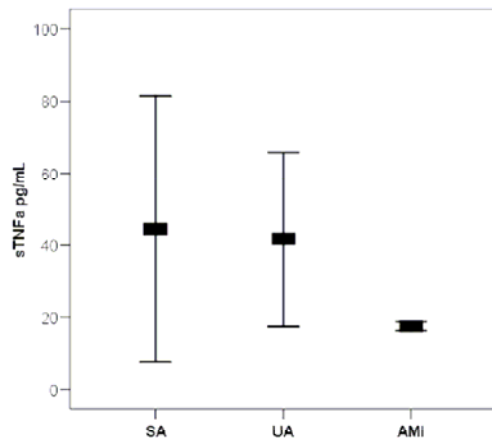


Figure 1d

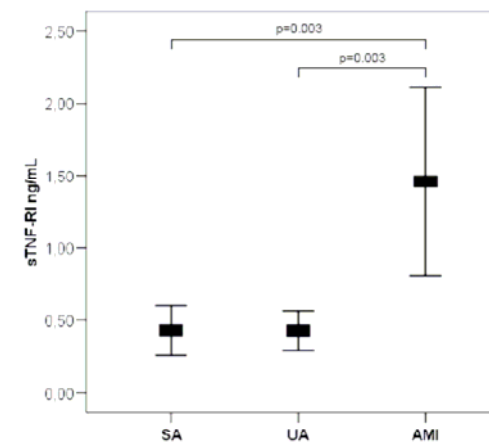


Figure 2a

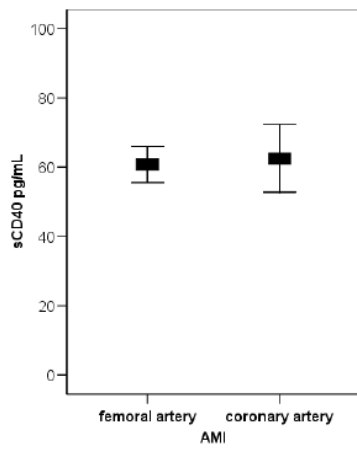


Figure 2b

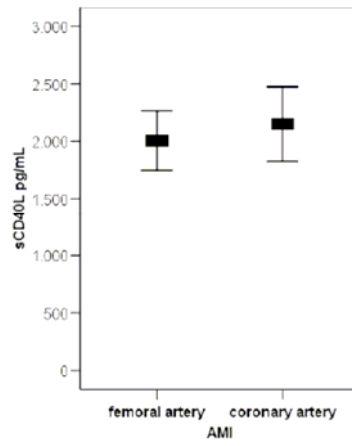


Figure 2c

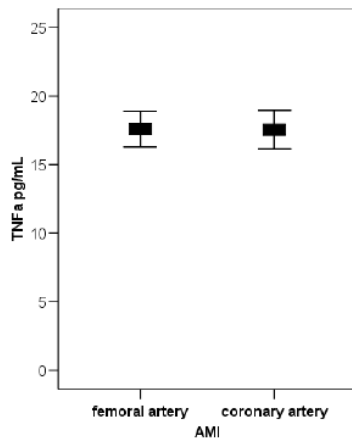


Figure 2d

