Thrombosis Research 124 (2009) 683–688

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

The role of platelets CD40 ligand (CD154) in acute coronary syndromes

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ARTICLE INFO

Article history:
Received 17 March 2009
Received in revised form 25 June 2009
Accepted 30 June 2009
Available online 4 August 2009

Keywords:
Acute Coronary Syndrome
CD154
Flow Cytometry
Platelets

ABSTRACT

Background: Despite of the proof of the biological function of CD154 on platelets, there has been little information about its role either in patients with stable angina or in those with acute coronary syndrome (ACS).
Objective: This study aimed to investigate the expression of CD154 on platelets and its role in ACS.
Methods: The study included 50 patients with ACS (24 patients with acute myocardial infarction (AMI) and 26 patients with unstable angina (UA)), 20 patients with stable angina (SA) and 18 healthy volunteers. CD154 and CD62 expression on platelets were analyzed by flow cytometry. Their relations to the clinical and laboratory data were assessed in the studied group.
Results: Patients with AMI and UA had higher levels of platelets CD154 and CD62 as compared to those with SA and among patients with AMI, UA and SA versus healthy volunteers. Platelets CD154 showed significant positive correlations with the studied pro-inflammatory markers (Ox-LDL, CRP and fibrinogen), segmental wall motion score and the studied risk factors. There were significant negative correlations between platelet CD154 and serum nitric oxide among patients.
Conclusions: CD154 may be used as a marker of thrombo-embolic events. Nitric oxide may have an anti-atherogenic effect. There is an association between platelet activation and severity of coronary artery disease among patients with ACS.

Introduction

Acute coronary syndrome (ACS) is a term used to describe a constellation of symptoms resulting from acute myocardial ischemia. Acute myocardial ischemia is usually but not always caused by atherosclerotic plaque rupture or erosion with superimposed intracoronary thrombosis [1]. Inflammation is known to be a major driving force underlying the initiation of coronary plaques, their unstable progression, and eventual disruption, and it contributes significantly to thrombotic complications that occur in ACS. Therefore, atherosclerosis is defined as a chronic inflammatory response to vascular injury that caused by a variety of agents that activate or injure endothelium and promotes lipoprotein infiltration, retention and modification combined with inflammatory cell entry, retention and activation [2].

In addition, endothelial cells (ECs), smooth muscle cells (SMCs), leukocytes and platelets express CD40 receptor and their ligand (CD40L and referred to CD154). Thus, CD40-CD40L interaction plays an important role in initiation and progression of atherosclerosis [3]. CD40 receptor is constitutively expressed on B-cells, monocytes, macrophages, ECs, and SMCs [4]. CD 40 L (CD154) is a transmembrane protein. It was originally identified on stimulated $CD4^+$ T-cells, and later on stimulated atheroma associated cells including ECs, SMCs and macrophages and on activated platelets [5].

Despite of the proof of the biological function of CD154 on platelets, there has been little information about its role either in patients with stable angina or in those with ACS.

The purposes of this work are to investigate whether patients with ACS show significant differences in the expression of platelet CD154 in comparison to patients with stable angina and healthy volunteers relating the results to those of platelet P-selectin (CD62-P) as a well established marker of platelet activation. Secondly, to study

Abbreviations: ACS, Acute coronary syndrome; ECs, endothelial cells; SMCs, smooth muscle cells; CD40L, CD40 ligand; CAD, coronary artery disease; AMI, acute myocardial infarction; UA, unstable angina; SA, stable angina; BMI, body mass index; WHR, waist hip ratio; SWMSI, segmental wall motion score index; C-RP, C-reactive protein; Ox-LDL, oxidized low density lipoprotein level; NO, serum nitric oxide; GMFI, geometric mean fluorescence intensity.

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doi:10.1016/j.thromres.2009.06.028

the relations of platelet CD154 to the clinical, cardiographic and laboratory data in ACS. Finally, to study the relation between platelet CD154 with different potential risk factors for coronary artery disease (CAD).

Patients and methods

The study was conducted on 50 consecutive patients with ACS. Relying on a serial electrocardiogram (ECG) evaluation and serum cardiac enzymes measurement (creatine phosphokinase (CK), CK-MB iso-enzyme and troponin T), patients were categorized according to criteria of Antaman and Braunwald [6] as follows:

- Patients with ST elevation myocardial infarction (STEMI): They were 18 patients with: 1-Typical anginal pain of great severity, long duration and not relieved by sublingual nitroglycerine. 2-Serial ECGs showed ST segment elevation ⩾0.2 mV in adjacent chest leads and ⩾0.1 mV in adjacent limb leads with or without pathological Q waves. 3-Serum CK levels were twice the upper limit of normal and CK-MB and troponin T were elevated.
- Patients with non ST elevation myocardial infarction (NSTEMI): They were six patients characterized by: 1-Typical aforementioned anginal pain. 2-Serial ECGs showed ST segment depression 0.05 mV with T wave inversion and infrequently pathological Q waves. 3-Serum CK levels were twice the upper limit of normal and CK-MB and/or troponin T were elevated.

The small number of patients with NSTEMI (6 patients) that might yield no statistically significant results if they were considered as a separate group, had encouraged us to add them to patients with STEMI and all (24 patients) had evolved the group of acute myocardial infarction (AMI).

- Patients with unstable angina (UA): They were 26 patients who had the same characteristics of patients with NSTEMI except for the serial level of serum CK, CK-MB and troponin T over 24-72 hours period remained negative.
- Patients with stable angina (SA): They were diagnosed according to criteria of Schwartz and Goldberg [7]. They characterized by: 1-Anginal pain that occurred after a constant level of exertion for >60 days with no change in frequency, duration, severity, precipitating factors, or ease of relief. 2-Resting ECGs; in some patients they were normal, in others they showed T wave inversion or Q waves suggestive of a previous myocardial injury and in the remainders they showed ST segment depression with or without T wave inversion. 3-Diagnosis of SA in this group was confirmed with stress ECG test as described by Mark et al. [8] and/or myocardial perfusion scanning as described by Iskanadria and Verani [9].

Patients with non-cardiac diseases that may interfere with the findings such as infection, advanced liver disease, renal failure, diabetes mellitus, malignancy, collagen diseases, hyperthyroidism, inflammatory bowel disease and acute cerebrovascular stroke were excluded. Diagnosis of liver disease was done by means of physical, laboratory and radiological evaluation. Diagnosis of renal failure was defined when estimated glomerular filtration rate ≥50 ml/min [10] and diagnosis of diabetes mellitus was defined as fasting blood sugar ≥6.9 mmol/L, 2 hours postprandial blood sugar ≥11 mmol/L, random blood sugar ≥11 mmol/L or current intake of oral hypoglycemic medications or insulin [11].

Patients with cardiac diseases other than coronary artery disease, except for minor mitral regurgitation and patients with overt right or left ventricular failure, those who had subjected to coronary artery bypass graft or percutaneous transluminal coronary angioplasty and those who had treated by thrombolytic and antiplatelet agents within three months before the study were excluded as well.

All the study subjects were subjected to the following after informed written consent in accordance with Assiut University ethical committee guidelines.

- Thorough clinical history and examination that included estimation of systemic arterial blood pressure, body mass index (BMI) and waist hip ratio (WHR).
- Evaluation of standard 12 – leads electrocardiogram (ECP-2155 Fukuta Denshi, Japan)
- Evaluation of M- mode 2D- Doppler echocardiography (Agilent HP-Sonos 4500-USA):

Two-dimensional echocardiography was used to detect resting segmental wall motion (SWM) abnormalities. SWM score (SWMS) was calculated by summing the scores for each segment. SWMS index (SWMSI) was calculated by summing the scores for each segment and dividing them by the number of analyzed segments [12].

- Coronary artery angiography was performed in 30 patients (6 AMI, 8 UA and 16 SA) for diagnostic and therapeutic purposes using Philips Integris 3000 system. It could not be done to all patients because of lack of financial support, impaired left ventricular systolic function or patient's refusal.
- Laboratory investigations that included assessment of cardiac enzymes, serum blood sugar, kidney functions, complete lipogram, liver function tests, prothrombin time and concentration, complete blood count, erythrocyte sedimentation rate, serum antinuclear antibody, thyroid profile, serum C-reactive protein (C-RP), plasma oxidized low density lipoprotein level (Ox-LDL), Serum nitric oxide (NO), plasma fibrinogen level (Table 1). In patients with AMI and UA, peripheral venous blood sample was withdrawn immediately after hospital admission and before administration of thrombolytic or anticoagulant therapy.

Isolation of LDL

To prevent oxidation and proteolytic degradation of LDL; plasma was separated by low-speed centrifugation of blood sample with a single vertical spin in a vertical ultracentrifuge rotor using the modified method of Chung et al [13]. The isolated LDL was dialyzed for 48 hours against 0.15 mmol/NaCl containing EDTA. The protein content of the LDL was measured by the modified technique of Lowry et al [14]. LDL was sterilized by passage through a 22 um milli-pore filter, and used within two weeks of preparation.

Estimation of Ox-LDL

The following solutions were added in succession; 0.2 ml of isolated LDL solution, 0.2 ml of sodium dodecyl sulphate, 1.5 ml of 20% acetic acid adjusted to PH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. The volume was then completed to 4 ml with distilled water. The mixture was heated at 95 °C for 60 minutes in a water bath and then cooled with tap water. One ml of distilled water and 5 ml of a mixture n-butanopyridine (1: 5, volume for volume) were added to the mixture.

The mixture was shaken vigorously and centrifuged at 4000 rpm for 10 minutes. The organic layer (n-butanol phase) was taken and its absorbance at 532 nm was measured. 1,1,3,3 tetramethoxypropane was used as an external standard to prepare standard concentrations of malonaldehyde and the procedure was repeated to prepare a standard curve. From this curve the peroxide concentration was deduced from the corresponding absorbance using the regression equation for the standard curve and Ox-LDL level was calculated [15].