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Decreased soluble cell adhesion molecules after tirofiban infusion in patients with unstable angina pectoris

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Abstract

Aim: The inflammatory response, initiated by neutrophil and monocyte adhesion to endothelial cells, is important in the pathogenesis of acute coronary syndromes. Platelets play an important role in inflammatory process by interacting with monocytes and neutrophils. In this study, we investigated the effect of tirofiban on the levels of cell adhesion molecules (soluble intercellular adhesion molecule-1, sICAM-1, and vascular cell adhesion molecule-1, sVCAM-1) in patients with unstable angina pectoris (AP).

Methods: Thirty-five patients with unstable AP (Group I), ten patients with stable AP (Group II) and ten subjects who had angiographycally normal coronary arteries (Group III) were included the study. Group I was divided into two subgroups for the specific treatment regimens: Group IA (n = 15) received tirofiban and Group IB (n = 20) did not. Blood samples for investigating the cell adhesion molecules were drawn at zero time (baseline; 0 h) in all patients and at 72 h in Group I.

Results: The baseline levels of sICAM-1 and sVCAM-1 were higher in Group I than in Groups II and III. They were higher in Group IA than in Group IB. However, the sICAM-1 and sVCAM-1 levels decreased significantly in Group IA after tirofiban infusion. In contrast, these levels remained unchanged or were increased above the baseline value in Group IB at 72 h.

Conclusion: The levels of cell adhesion molecules in patients with unstable AP decreased significantly after tirofiban infusion. Inhibition of platelet function by specific glycoprotein IIb/IIIa antagonists may decrease platelet-mediated inflammation and the ischemic end-point.

Background

The inflammatory response, initiated by neutrophil and monocyte adhesion to endothelial cells, is important in the pathogenesis of acute coronary syndromes, especially unstable angina pectoris (AP) [1-3]. Soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell

adhesion molecule-1 (sVCAM-1) are expressed on endothelial cell membranes and are responsible for the attachment of neutrophils, monocytes and lymphocytes [4-6]. sICAM-1 and sVCAM-1 are cell surface proteins inducible by tumor necrosis factor-α, interferon-γ and interleukin-1β [7,8].

Tirofiban blocks glycoprotein (GP) IIb/IIIa receptors and thus prevents fibrinogen binding to platelets. The main effect of GP IIb/IIIa inhibitors is their strong anti-platelet effect, which has been clinically proven in large-scale randomized trials [9-13]. Platelets play an important role in inflammatory process by interacting with monocytes and neutrophils [14]. In this study, we tested the effect of tirofiban on the levels of cell adhesion molecules in patients with unstable AP.

Methods

Patient population

Thirty-five patients with unstable AP (Group I), 10 with stable AP (Group II) and 10 subjects who had angiographically normal coronary arteries (Group III) were included in this study. Unstable AP was defined as a change in the pattern of chest pain, or a new onset of symptoms typical of myocardial ischemia, with or without ST-T wave changes on the electrocardiogram and without positive cardiac enzymes. Diagnosis of stable AP was based on significant coronary artery disease documented by coronary angiography, without recent change in the character, frequency or severity of anginal pain. Group III comprised 10 subjects who underwent coronary angiography due to suspicious or symptomatic positive exercise test findings but had normal coronary arteries.

All Group I patients were treated with aspirin, clopidogrel, a statin, parenteral nitrate, and sufficient unfractionated heparin infusion to achieve an activated clotting time of 300–350 s through 72 h. Group I was divided into two subgroups for the specific treatment regimens: Group IA (n = 15) received tirofiban and Group IB (n = 20) received no tirofiban. Tirofiban was administered as a 0.4 μg/kg per min bolus over 30 min followed by continuous infusion of 0.1 μg/kg per min for 72 h through an infusion pump.

Exclusion criteria

Patients with acute or chronic inflammatory disease, autoimmune disease, cancer, congestive heart failure, renal failure, congenital hemorrhagic disease, thrombocytopenia, thrombocytosis, significant ST segment elevation on ECG, significant increase in serum cardiac enzymes, current use of anticoagulant drugs or use of steroid or anti-inflammatory drugs within the last three months were excluded from the study.

Working protocol

All patients gave informed consent. Blood samples for sICAM-1 and sVCAM-1 were drawn at baseline (0 h) in all patients and at 72 h in Group I. Medical stabilization was recommended for >24 h before any coronary intervention. All patients underwent coronary angiography to determine the severity and extent of their coronary artery disease and to plan their medical treatments before discharge.

Determination of sICAM and sVCAM levels

Blood samples for assays were obtained from antecubital vein and drawn into vacutainer clotted tubes. Sera were obtained by centrifugation of the tubes at 3000 rpm for 10 min. Samples were storaged at -70°C. sICAM-1 and sVCAM-1 concentrations were determined using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (Bendor Met Systems Test Kits, Vienna, Austria). Intra-assay coefficients of variation were 3.2%, 1.4% and 1.9% at sVCAM-1 concentrations of 460.0 ng/ml, 689.2 ng/ml and 1550.5 ng/ml respectively, and 4.1%, 2.5% and 7.1% at sICAM-1 concentrations of 169.4 ng/ml, 378.2 ng/ml and 939.7 ng/ml respectively. The overall intra-assay coefficients of variation were 3.1% for sVCAM-1 and %4.1 for sICAM-1.

Statistical analysis

All analyses were univariate. Groups were compared using chi square and Fisher's Exact test for nominal variables; the Kruskall Wallis nonparametric ANOVA for categorical variables; and the Mann Whitney U test for continuous variables. Post-hoc group comparisons were done by the Scheffe test. Values of $p < 0.05$ were considered significant. SPSS 10.0 Statistical software was used in the statistical analysis.

Results

Patient characteristics

Mean age was similar in all groups. Male sex was dominant in Group I. Female sex was dominant in Groups II and III. Clinical characteristics of the study groups are shown in Table 1. There were no significant differences between Groups IA and IB in the following variables: age, gender, diabetes mellitus, current smoker, hypertension, body mass index, leukocyte count, erythrocyte sedimentation rate, serum total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol. Statistical comparisons of possible confounders between the two acute coronary syndrome groups are shown in Table 2.

Cell adhesion molecules

Baseline levels of sICAM-1 and sVCAM-1 were higher in Groups IA and IB than in Groups II and III. Baseline levels of these molecules were similar in Group II and III (Figure 1). Table 3 shows the statistical comparison of cell

Table 1: Characteristics of the study groups

	Group IA (n = 15)	Group IB (n = 20)	Group II (n = 10)	Group III (n = 10)
Mean age (yr)	57.7 ± 10.2	57.8 ± 8.7	53.3 ± 7.4	56.8 ± 11.8
Male sex (%)	86	90	40	40
DM (%)	13	25	0	0
Current smoker (%)	80	45	60	70
HT (%)	60	35	70	30
BMI	33.7 ± 6.8	28.9 ± 4.5	26.1 ± 2.5	26.3 ± 3.1
WBC (/mm^3)	9044 ± 2097	8685 ± 2541	6200 ± 964	6530 ± 1240
ESR (mm/hr)	28.3 ± 16.2	22.4 ± 14	6.7 ± 1.9	8.5 ± 1.8
TC (mg/dl)	209.9 ± 69.1	181.2 ± 31.6	199.2 ± 46.5	200 ± 38.2
TG (mg/dl)	202 ± 120	156.8 ± 86.4	101.9 ± 32	113.6 ± 41.7
HDL-C (mg/dl)	40.5 ± 13	41.4 ± 9.9	32.8 ± 4.2	41.1 ± 9.8
LDL-C (mg/dl)	119 ± 47	109 ± 26.6	102.7 ± 24	107 ± 37.5
Extent of CAD				
1 vessel (%)	0	20	70	0
2 vessel (%)	54	20	30	0
3 vessel (%)	46	60	0	0

Group IA: Patients with unstable angina pectoris and tirofiban infusion, **Group IB:** Patients with unstable angina pectoris but no tirofiban infusion, **Group II:** Patients with stable angina pectoris, **Group III:** patients with normal coronary angiography, **DM:** Diabetes mellitus, **HT:** Hypertension, **BMI:** Body mass index, **WBC:** White blood cell, **ESR:** Erythrocyte sedimentation rate, **TC:** Total cholesterol, **TG:** Triglyceride, **HDL-C:** High density lipoprotein cholesterol, **LDL-C:** Low density lipoprotein cholesterol, **CAD:** Coronary artery disease.

Table 2: Statistical comparisons of possible confounders between the two acute coronary syndrome groups

	Group IA (n = 15)	Group IB (n = 20)	Significance
Mean age (yr)	57.7 ± 10.2	57.8 ± 8.7	0,84[c]
Male sex (%)	86	90	0,60[a]
DM (%)	13	25	0.43[a]
Current smoker (%)	80	45	0.79[a]
HT (%)	60	35	0.18[b]
BMI	33.7 ± 6.8	28.9 ± 4.5	0.07[c]
WBC (/mm^3)	9044 ± 2097	8685 ± 2541	0.49[c]
ESR (mm/hr)	28.3 ± 16.2	22.4 ± 14	0.11[c]
TC (mg/dl)	209.9 ± 69.1	181.2 ± 31.6	0.27[c]
TG (mg/dl)	202 ± 120	156.8 ± 86.4	0.37[c]
HDL-C (mg/dl)	40.5 ± 13	41.4 ± 9.9	0.27[c]
LDL-C (mg/dl)	119 ± 47	109 ± 26.6	0.73[c]

a = Fisher's exact test **b** = Chi square test **c** = Mann Whitney U test **Group IA:** Patients with unstable angina pectoris and tirofiban infusion, **Group IB:** Patients with unstable angina pectoris but no tirofiban infusion, **DM:** Diabetes mellitus, **HT:** Hypertension, **BMI:** Body mass index, **WBC:** White blood cell, **ESR:** Erythrocyte sedimentation rate, **TC:** Total cholesterol, **TG:** Triglyceride, **HDL-C:** High density lipoprotein cholesterol, **LDL-C:** Low density lipoprotein cholesterol.

Table 3: Statistical comparison of cell adhesion molecule levels among all groups

Cell adhesion molecule	Group IA (n = 15)	Group IB (n = 20)	Group II (n = 10)	Group III (n = 10)	Post hoc*
sICAM-1 Baseline ng/ml	633.37 ± 136.8	450.0 ± 138.9	196.4 ± 100.2	93.6 ± 43.6	1 > 2 > (3 = 4)
sVCAM-1 Baseline ng/ml	390.3 ± 96.9	283.9 ± 75.5	162.0 ± 52.5	105.0 ± 33.5	1 > 2 > (3 = 4)

* Scheffe test **Group IA:** Patients with unstable angina pectoris and tirofiban infusion, **Group IB:** Patients with unstable angina pectoris but no tirofiban infusion, **Group II:** Patients with stable angina pectoris, **Group III:** patients with normal coronary angiography,**sICAM-1:** Soluble intercellular adhesion molecule-1, **sVCAM-1:** Soluble vascular cell adhesion molecule-1.

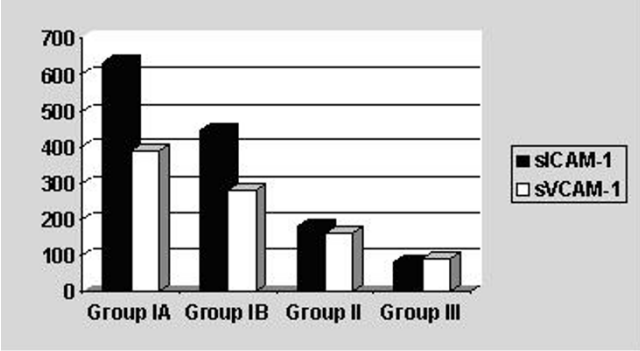

Figure 1
Levels of the cell adhesion molecules in all groups. Group IA: Patients with unstable angina pectoris and tirofiban infusion, Group IB: Patients with unstable angina pectoris but no tirofiban infusion, Group II: Patients with stable angina pectoris, Group III: patients with normal coronary angiography, sICAM-1: Soluble intercellular adhesion molecule-1, sVCAM-1: Soluble vascular cell adhesion molecule-1.

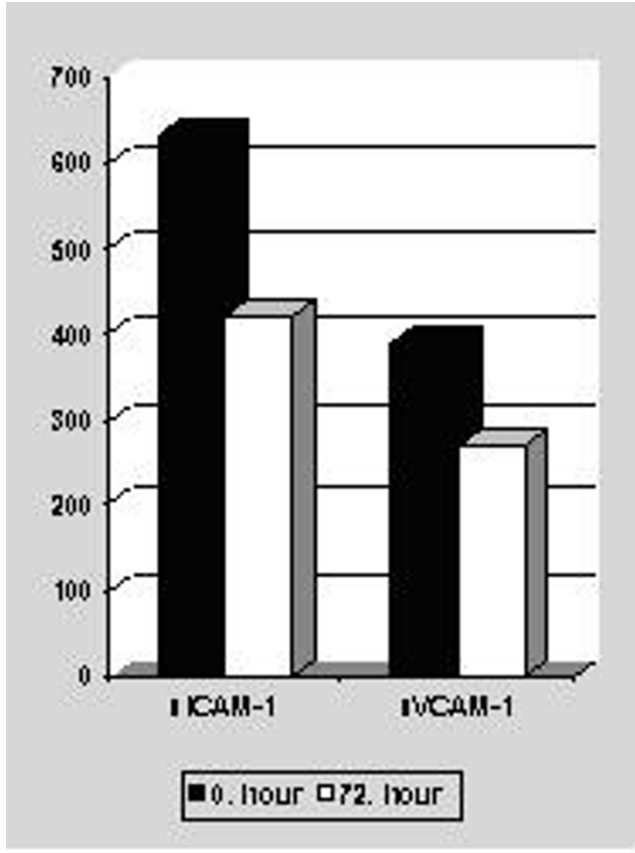

Figure 2
Levels of the cell adhesion molecules in patients with unstable angina pectoris who received tirofiban infusion. sICAM-1: Soluble intercellular adhesion molecule-1, sVCAM-1: Soluble vascular cell adhesion molecule-1.

adhesion molecules levels among the all groups. Although the baseline levels were higher in Group IA than in Group IB, sICAM-1 and VCAM-1 decreased significantly in Group IA after tirofiban infusion (Figure 2). In contrast, sICAM-1 and sVCAM-1 levels remained unchanged or increased above baseline in Group IB at 72 h (Figure 3). Table 4 shows the statistical comparison of cell adhesion molecule levels between the two acute coronary syndrome groups.

Coronary angiography

Multivessel coronary artery disease was found in 100% of Group IA and 80% of Group IB. Based on the coronary angiographic results, 26.7% of Group IA patients were treated by medical therapy, 53.3% PTCA and 20% CABG. In Group IB, 25% of the patients were treated by medical therapy, 50% PTCA and 25% CABG. These differences between the two subgroups were not statistically significant. The coronary angiographic results are shown in Table 1.

Discussion

This study suggests that tirofiban infusion suppresses the elevation of soluble cell adhesion molecule levels in patients with unstable AP. Such patients were selected because it was expected that inflammatory activities would be triggered in their vascular systems.

The importance of inflammation in acute coronary syndromes is well established. It is known that GP IIb/IIIa antagonists inhibit platelet activation by blocking the fibrinogen binding receptor [15]. However, can the clinical improvements demonstrated in multi-central studies be attributed solely to inhibition of platelet activation?

Platelets play an important role not only in the coagulation system but also in the inflammatory response [16-18]. Platelet activation can increase inflammation through several mechanisms. Platelets release a wide range of growth factors and inflammatory mediators from intracellular storage organelles. Products of activation may aid neutrophil accumulation and enhance inflammation. Activated leukocytes and platelets potentate each others' effects. The important mediators released by platelets are P-selectin (CD62p), CD40p, platelet activating factor, macrophage chemotactic factor-1, interleukin-1, thrombospondin and fibronectin. These mediators are rapidly expressed by activated platelets [19-24].

Table 4: Statistical comparison of cell adhesion molecule levels between the two acute coronary syndrome groups

Cell adhesion molecule	Group IA (n = 15)	Group IB (n = 20)	Significance
sICAM-1 Baseline ng/ml	633.3 ± 136.8	450.0 ± 138.8	0.001
sVCAM-1 Baseline ng/ml	390.3 ± 96.9	283.9 ± 75.5	0.003
sICAM-1 (0–72nd hour) difference ng/ml	209.7 ± 139.2	-25.0 ± 110.8	<0.0001
sVCAM-1 (0–72nd hour) difference ng/ml	121.7 ± 50.9	-23.4 ± 51.6	<0.0001

Group IA: Patients with unstable angina pectoris and tirofiban infusion, **Group IB:** Patients with unstable angina pectoris but no tirofiban infusion, **sICAM-1:** Soluble intercellular adhesion molecule-1, **sVCAM-1:** Soluble vascular cell adhesion molecule-1.

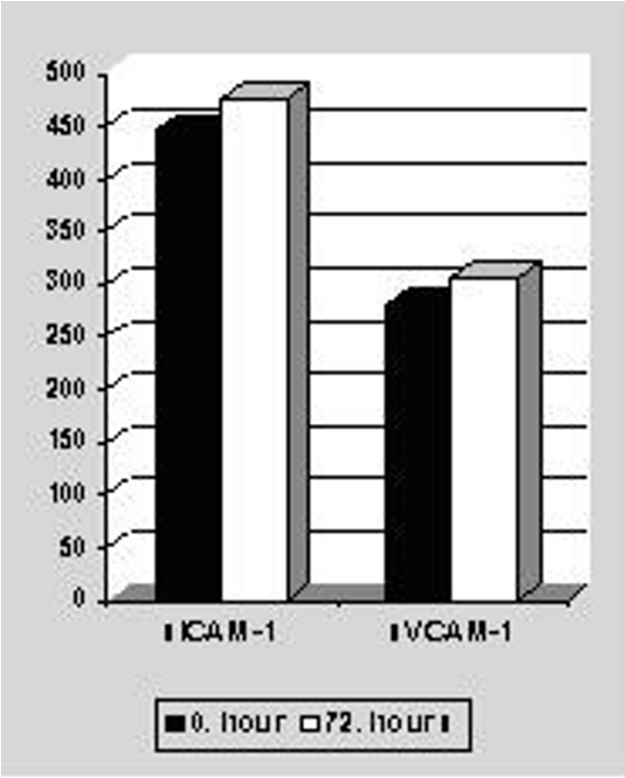

Figure 3
Levels of the cell adhesion molecules in patients with unstable angina pectoris but no tirofiban infusion. sICAM-1: Soluble intercellular adhesion molecule-1, sVCAM-1: Soluble vascular cell adhesion molecule-1.

Inhibition of platelet function by specific GP IIb/IIIa antagonists may decrease plateletmediated inflammation. Tirofiban may not only inhibit platelet activation but also suppress inflammatory processes [25,26]. This effect is not cross-reactivity; it can explain the antiinflammatory properties of tirofiban.

In a group (IA) of high risk patients with unstable AP, the high baseline levels of some cell adhesion molecules (sICAM-1, sVCAM-1), significantly decrease after adding tirofiban to the usual treatment of intravenous unfractionated heparin, aspirin and others. In patients with UAP but lower risk, the baseline values of sICAM-1 and sVCAM-1, although higher than those of the Groups II and III, were not affected by the treatment, that not includes tirofiban.

Inflammation, especially platelet-mediated, may be suppressed and the ischemic end-point will be reduced after GP IIb/IIIa antagonist therapy.

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