Platelet Activation in Different Clinical Forms of the Coronary Artery Disease (Roll of P-Selectin and others Platelet Markers in the Stable and Unstable Angina)

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Objective: Markers of platelet activation are elevated in coronary artery disease. We sought to identify the presence and the potential associations of different markers of platelet activation.

Methods: We studied patients with unstable angina (n=28), patients with stable angina (n=36) and patients without coronary artery disease (n=30); sex and age matched. Blood levels of the adhesion molecule P-selectin, Thromboxane B2 and Serotonin were measured by enzyme immunoassays.

Results: When we compared the groups the results were: sP-selectin, thromboxane B2 and serotonin levels were significantly higher in patients with unstable angina than in patients with stable angina.

Conclusion: These markers of platelet activation were able to identify unstable forms of coronary artery disease.

Key words: Platelet activation, coronary artery disease.

The coronary artery disease, represented by the atherosclerosis, is the most prevalent disease of our time and its trombotic complications are responsible for an exceedingly high number of deaths and disabilities. Over the past few years, experimental investigation, clinical and pathologic observations have led to a better understanding of how a thrombus forms and also of its incidence in coronary artery disease[1].

Platelets have been recognized as being in the initiation and propagation of coronary thrombosis[2-9]. At the site of the ruptured plaque, platelets form an initial monolayer and a variety of activators (collagen, adenosine diphosphate [ADP], epinephrine, serotonin) promote platelet activation.

Platelet aggregation and thrombus formation secondary to plaque disruption also have been implicated as major pathogenic mechanisms underlying the acute coronary syndrome of unstable angina. The pathophysiology of unstable angina is different from that of effort-induced angina due to increased myocardial oxigen demand[14]. The hypothesis of disease process in unstable angina has been strongly supported by results of previous studies[10-16]. The disease process of unstable angina appears to involve a transient thrombus formation mediated by a complex cascade of cellular interactions between the vascular endothelium and platelets at the atherosclerotic coronary artery[15].

Cell adhesion molecules play a key role in cellular interactions in diverse disease processes, including coronary thrombosis, atherosclerosis, restenosis after coronary angioplasty, and reperfusion injury[15,16]. P-selectin is a member of the selectin family and is an integral membrane glycoprotein found in both α-granules of platelets and Weibel-Palade bodies of endothelial cells. These molecules contain na N-terminal lectin-like domain, an epidermal growth factor-like domain, a variable number of consensus repeats of a sequence found in complement regulatory proteins, a transmembrane domain, and a short cytoplasmic tail[16,17]. After cellular activation by agonists such as thrombin, P-selectin is rapidly redistributed to the cell surface and binds a sialylated carbohydrate structure expressed on neutrophils and monocytes through a calcium dependent lectin-like mechanism. Thus, P-selectin mediates platelet-leukocyte and endothelium cell-leukocyte adhesive interactions[17-19].

In the present study, we examined the plasma levels of soluble P-selectin, serotonin and thromboxane B2 in patients with unstable and stable angina to further understand the pathophysiology of these distinct syndromes.

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Characteristic	Stable angina n=36	Unstable angina n=28	Control n=30
Age (mean ± S.D)	56,9 ± 9,1	57,8 ± 12,3	56,2 ± 8,7
Men/women (n)	30/6	25/3	23/7
Dislipidemic (n)	18	14	13
Smoking	10	14	8
Hypertension	14	11	11
Sedentarism	24	25	22
Obesity	18	18	15
Family history of CAD	10	10	8
Previuos MI	22	8	0
Previous history of revascularization and/or coronary angioplasty	18	4	0

CAD - *coronary artery disease;* MI - *myocardial infaction.*

Table 1 - Clinical profile of the study groups

Methods

Study patients - The study population consisted of a group of patients with unstable angina (n=28), a group of patients with stable angina (n=36) with indication for coronary revascularization and a control group without CAD (n=30). Detailed clinical information, including history of angina pectoris, coronary artery disease risk factors, medication, and prior interventions were prospectively colected. All patients had granted their written informed consent to participate. Patients with ongoing infections, diabetes mellitus, malignancy, chronic liver disease, renal insufficiency, connective tissue disease, or being treated with anti-inflammatory or anticoagulant drugs (including clopidogrel, aspirin or glycoprotein IIbIIIa receptor inhibitor), were excluded from the study. Patients received conventional drug therapy for unstable angina or for stable angina. All patients were waiting a surgical myocardial revascularization without aspirin to avoid the interaction with the platelet activation. When anticoagulation was indicated with heparin in patients with unstable angina, were selected only patients in use of the low-molecular-weight heparin, that does not affect the platelet function. Their clinical profile is shown in Table I.

Unstable angina group - Patients admited to the emergency department with typical angina at rest and ST segment depression on the eletrocardiogram had a diagnosis of unstable angina. From a group of 48 patients screened, 20 patients with elevated CK-MB enzyme and troponin I were excluded from the study. The remaining 25 men and 3 women had ≥ 75% luminal diameter stenosis of at least one coronary artery.

Stable angina group - The 30 men and 6 women included in the stable angina group had typical exertional angina, no angina at rest, a positive exercise test, and ≥ 75% luminal diameter stenosis of at least one coronary artery.

Control group - The 23 men and 7 women without coronary artery disease.

Blood sampling and measurent of the platelet markers - In patients with unstable angina, venous blood was collected immediately after their admission to the emergency department, before initiation of anticoagulant therapy. In patients with stable angina blood samples were collected before angiography. All blood samples were obtained from an antecubital vein. The anticoagulated samples was separated and stored at –80^{0}C for measurements of platelet markers. Concentrations of sP-selectin, Serotonin and Thromboxane B2 in stored plasma were measured with enzyme-linked immunosorbent assay kits (Bender MedSystems Diagnostics GmbH, Immunotech/Coulter and Amersham Pharmacia, respectively).

Statistical analysis - The plasma levels of sP-selectin, serotonin and thromboxane B2 were compared by one-way ANOVA with the computer program SYSTAT 9. A P-value <0,05 was considered statiscally significant.

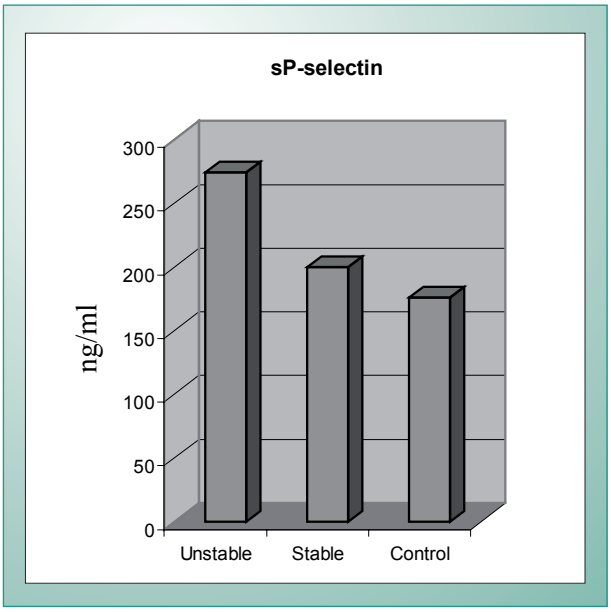

Fig. 1 *- sP-selectin levels.*

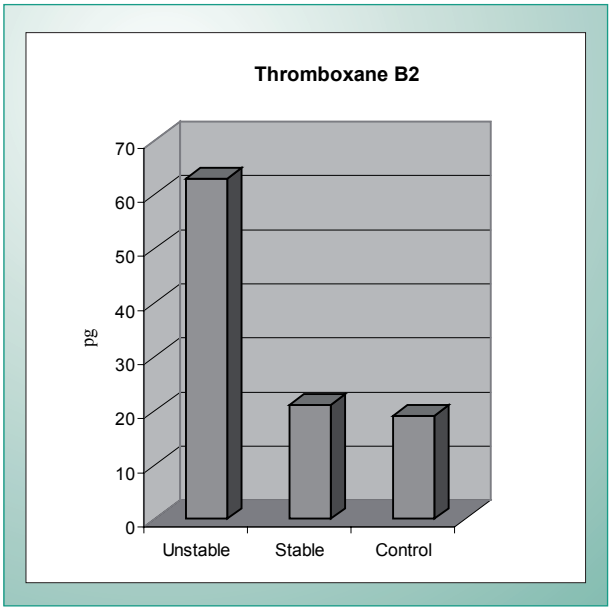

Fig. 2 *- Thromboxane B2 levels.*

Results

Plasma sP-selectin levels - The plasma soluble P-selectin levels in the unstable angina group were 274,2 ± 131,9 ng/mL, in the stable angina group were 199,7 ± 15,78 ng/mL and in the control group were 176,20 ± 43,90 and are presented in figure 1. The plasma sP-selectin levels were significantly higher in the unstable angina group than stable angina or control group (P <0,002).

Thromboxane B2 levels - The plasma thromboxane levels in the unstable angina group were 62,89 ± 14,58 pg/mL, in the stable angina group were 21,03 ± 6,44 pg/mL and in the control group were 19,09 ± 3,28 and are presented in figure 2. The plasma thromboxane levels were significantly higher in the unstable angina group than stable angina or control group (P <0,001).

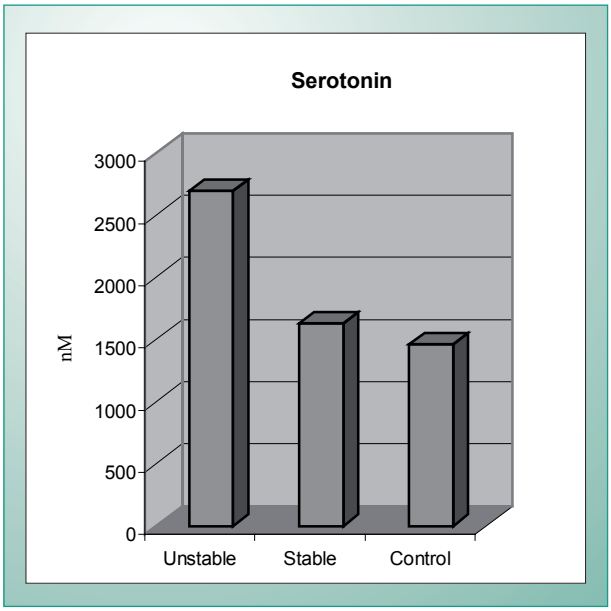

Fig. 3 *– Serotonin levels.*

Serotonin levels - The plasma serotonin levels in the unstable angina group were 2692,61 ± 358,42 nM, in the stable angina group were 1631,36 ± 315,2 nM and in the control group were 1462,20 ± 166,36 and are presented in figure 3. The plasma serotonin levels were significantly higher in the unstable angina group than stable angina or control group (P <0,001).

Discussion

In the present study, plasma levels of soluble P-selectin, thromboxane B2 and serotonin were higher in patients with unstable angina than in patients with stable angina. These observations suggest that activation of platelets takes place in the circulation during unstable angina.

Soluble P-selectin - Coronary plaque disruption resulting in thrombus formation and/or platelet aggregation is considered the most important mechanism responsible for the development of acute coronary syndromes, including unstable angina[19]. Recent studies[19-21] have show that the increase levels of sP-selectin in patients with unstable angina may be due to plaque rupture and thrombus formation, as well as to an interaction of platelets by activated leukocytes before plaque disruption, and sP-selectin level could be used as a marker of plaque destabilization in unstable angina[22]. Nevertheless, the level of sP-selectin in patients with multivessel disease could be higher than in those with single-vessel disease[23]. In conclusion, sP-selectin may indirectly reflect clinical condition of patients with coronary artery disease, with potential diagnostic and therapeutic implications[22-26].

Ault et al[21] reported that there is evidence of continued activation of platelets after an acute ischemic coronary event. Platelet associated P-selectin is a sensitive measure of platelet activation; this marker remained elevated for up to one month after clinical stabilization after unstable angina or myocardial infarction. Persistent platelet activation may be a consequence of sustained inflammatory stimuli[20,21]. The authors also found a weak correlation between platelet activation parameters and levels of serum C-reactive protein[21].

Thromboxane B2 - The levels of TXB2 were estimated by measurement of 11-dehydro-tromboxane B2, the most abundant enzymatic metabolite of TXA2 in plasma. This study demonstrated increase of thromboxane levels in patients with unstable angina when compared with stable angina. These findings are consistent with the concept that stable angina reflects restricted blood flow through a fixed stenosis and with the observation that tromboxane A2 inhibitors and antagonists do not alter exercise induced myocardial ischemia[27]. They also suggest that the increase in thromboxane B2 in unstable angina is likely to be an event of primary importance and is not merely secondary to myocardial ischemia. In addition, the incidence of myocardial infarction and death is decreased by 30 to 50 percent in patients with unstable angina treated with aspirin[21,27].

Serotonin - Clinical studies[28-30] have demonstraded that platelets are activated and aggregate at the sites of coronary

artery stenosis and endothelial injury. Activated platelets release serotonin in substantial quantities causing vasoconstriction and recurrent aggregation of platelets with cyclic flow reductions. Serotonin also acts as a growth factor stimulating mitogenesis and migration of arterial smooth muscle cells[31]. Recent studies propose that serotonin is useful as a novel marker for atherosclerotic vascular disease[32-34].

The present study shows that sP-selectin, thromboxane B2 and serotonin are increased in the unstable angina when compared with stable angina. Thus, these markers of platelet activation may help us to understand the pathophysiology of unstable coronary artery disease, and may indirectly reflect clinical condition of patients with coronary artery disease, with potential diagnostic and therapeutic implications.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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