

Endothelial Adhesion Molecules ICAM-1, VCAM-1 and E-Selectin in Patients with Acute Coronary Syndrome

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Introduction and objectives. The acute inflammatory response is an important phenomenon in the pathogenesis of myocardial damage during acute coronary syndrome. Endothelial dysfunction has been found in unstable angina and acute myocardial infarction, although the results are controversial. The purpose of this study was to determine the levels of the soluble endothelial adhesion molecules ICAM-1, VCAM-1 and E-selectin, in patients with unstable angina and acute myocardial infarction, compare the results in both groups, and analyze their relation with the degree of myocardial injury.

Method. Serum concentrations of ICAM-1, VCAM-1, and E-selectin were measured in 37 control subjects and 43 patients (32 with acute myocardial infarction and 11 with unstable angina). Measurements were made at the time of admission and ten days later using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, UK).

Results. There was a significant increase in E-selectin ($p < 0.05$) in patients with unstable angina at admission and ten days later. In contrast, patients with acute myocardial infarction showed no significant differences in E-selectin compared with the control group at admission or ten days later. A significant increase in VCAM-1 levels was demonstrated in both groups of patients and ICAM-1 levels in acute myocardial infarction, but the concentrations of VCAM-1 and ICAM-1 in both groups of patients at admission and ten days later did not differ significantly. There was no relation between soluble endothelial adhesion molecule levels and the severity of myocardial damage estimated by cardiac enzymes or electrocardiographic changes.

Conclusion. This study indicates that serum levels of E-selectin, measured at time of admission and ten days later, could be a marker for unstable angina and might be useful in the differential diagnosis with myocardial infarction.

Key words: *Unstable angina. Myocardial infarction. Ischemia. Endothelium.*

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Moléculas de adhesión endoteliales ICAM-1, VCAM-1 y E-selectina en pacientes con síndrome coronario agudo

Introducción y objetivos. La respuesta inflamatoria aguda es un importante fenómeno en la patogenia del daño miocárdico durante el síndrome coronario agudo, y se ha demostrado la existencia de disfunción endotelial con resultados controvertidos. El propósito de este estudio fue determinar los valores de las moléculas de adhesión endoteliales solubles ICAM-1, VCAM-1 y E-selectina en pacientes con angina inestable e infarto, comparar los resultados en ambos grupos y analizar su relación con el grado de daño miocárdico.

Método. Las concentraciones séricas de estas moléculas se estudiaron en 37 controles sanos y 43 pacientes, 32 con infarto de miocardio y 11 con angina inestable, en el momento del diagnóstico y 10 días más tarde, utilizando enzimoanálisis (ELISA) mediante estuches comerciales (R&D Systems, Reino Unido).

Resultados. Se observó un incremento significativo de la E-selectina ($p < 0,05$) en los pacientes con angina inestable en el momento del diagnóstico y 10 días más tarde. Sin embargo, en los pacientes con infarto de miocardio no se observaron diferencias significativas de las concentraciones de E-selectina en el momento de la admisión y 10 días después, al compararlos con el grupo control. Se demostró un incremento significativo de los valores de VCAM-1 en ambos grupos de pacientes y de los de ICAM-1 en el infarto de miocardio en relación con el grupo control, pero las concentraciones de VCAM-1 e ICAM-1 entre ambos grupos de pacientes en el momento de la admisión y 10 días más tarde no reveló diferencias significativas. No hubo correlación entre los valores de las moléculas de adhesión endoteliales solubles y la severidad del daño miocárdico, estimado por enzimas cardíacas y cambios electrocardiográficos.

Conclusión. Este estudio indica que los valores séricos de la E-selectina, medidos en el momento de la admisión y 10 días más tarde, podrían ser un marcador para la angina inestable y en el diagnóstico diferencial con el infarto agudo de miocardio.

Palabras clave: *Angina inestable. Infarto de miocardio. Isquemia. Endotelio.*

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INTRODUCTION

In 1985, Bevilacqua et al.¹ demonstrated that cytokines stimulate the endothelium, making it more adhesive for leukocytes. This observation revolutionized our understanding of the pathogenesis of inflammation² and later studies corroborated the endothelium as the center of events leading to the development of inflammatory lesions.^{2,3}

The adhesion of circulating leukocytes to the vascular endothelium is a fundamental step in leukocyte extravasation during inflammation. The adhesion molecule E-selectin mediates this process. The firm union and transendothelial migration depend on the interaction between intercellular adhesion molecule-1 (ICAM-1), vascular cytoadhesion molecule-1 (VCAM-1), and the integrins lymphocyte function antigen-1 (LFA-1, CD11a/ CD18) and very late activation antigen-4 (VLA-4, CD49/CD29) and the leukocytes.^{4,5} Endothelial adhesion molecules can separate from the cell surface and enter the blood circulation and the concentration of soluble molecules can reflect their expression on the endothelial surface.⁶ The acute inflammatory response is an important component in the pathogenesis of myocardial injury during acute coronary syndrome⁷⁻¹⁰ and endothelial dysfunction is related especially with leukocyte recruitment during the formation of the atherosclerotic lesion.¹¹⁻¹³ In clinical practice, the usefulness of the detection in serum of different markers of inflammation has been demonstrated, such as C reactive protein, amyloid A, troponin T, and cytokines, like interleukins (IL) 1 and 6, in association with the pathogenesis of acute coronary syndrome and its differential diagnosis. This is why some of them have been used as diagnostic and prognostic markers.^{8,14} In this syndrome, the value of soluble endothelial adhesion molecules as serum markers characteristic of endothelial dysfunction and inflammation has also been demonstrated, with controversial results.¹⁵⁻¹⁷ In view of the above arguments, the intention of this study was to determine the concentrations of the endothelial adhesion molecules ICAM-1, VCAM-1, and E-selectin in patients with unstable angina (UA) and acute myocardial infarction (AMI) at the time of diagnosis (admission) and 10 days later. The results obtained in both diseases have been compared and their relation with myocardial damage has been analyzed. Likewise, the participation of these serum markers and the possible immunological mechanisms associated in the inflammatory response of acute coronary syndrome have been determined, as well as their probable role in the diagnosis and prognosis of this disease in its different clinical forms.

PATIENTS AND METHOD

Patients and study groups

The study included 43 patients diagnosed and treated at the Institute of Cardiology and Cardiovascular Surgery of Havana, 31 men and 12 women (mean age, 66.4 years), 32 with typical AMI, 11 with UA, and 37 healthy subjects. The patients with AMI were diagnosed by the presence of a history of chest pain due to prolonged ischemia (more than 30 min), characteristic changes in the electrocardiogram (characteristic abnormalities of the T and ST segments, and presence of a pathological wave Q [width 0.04 s]) and increased concentration of the enzymes: creatine kinase (total CK>130 U), CK MB isoenzyme (CK-MB) (6% of total activity), and glutamic oxalacetic transaminase (GOT>29 U). The diagnosis of UA was based on a history of prolonged chest pain with changes in the T wave or depression of the ST segment and elevation of CK, CK-MB and GOT.

Exclusion criteria

The patients excluded from the study were patients with a previous history of arterial hypertension, diabetes mellitus, precordial pain or other signs of cardiac ischemia, electrocardiographic disturbances, a diagnosis of ischemic heart disease, patients with associated diseases that could cause disturbances in the immune system, patients that had been treated with anti-inflammatory or immunomodulating medications within 3 months of the onset of the study, and those who at the time of diagnosis presented signs and symptoms of infection or another possible associated acute disease.

Supposedly healthy individuals (blood donors), with no history of recent infection, or treatment with immunomodulators or other agents that have an effect on the immune system, with characteristics similar to those of the patients in the study sample were selected at the blood donation clinic of the Institute of Hematology and Immunology.

Sample characteristics

All the patients included in the study were in an age range and had a sex distribution similar to that of the control group, and the common risk factors of smoking and hyperlipidemia. The medical treatment of both clinical forms of acute coronary syndrome was based on the administration of beta-blockers, calcium channel blockers, nitrites, and aspirin. No invasive techniques like angioplasty and stent implantation were used, and fibrinolytic or antithrombotic agents were not administered to patients with AMI.

Measurement of soluble adhesion molecules

TABLE 1. Concentrations of soluble endothelial adhesion molecules in unstable angina and acute myocardial infarction at the time of diagnosis and 10 days later

Adhesion molecules	Control subjects	Unstable angina		Acute myocardial infarction	
		Admission	10 days later	Admission	10 days later
VCAM-1	15.3±6.1	34.6±19.8 ^{a,d}	24.4±13.2 ^{a,d}	24.3±14.1 ^b	25.3±14.4 ^b
E-selectin	3.2±1.4	5.9±2.4 ^{a,e}	5.0±2.4 ^{a,e}	3.8±1.6 ^e	3.2±1.1 ^e
ICAM-1	18.5±6.9	26.6±21.1	24.9±10.6	27.7±12.4 ^c	29.4±8.8 ^c

Values are expressed as the mean±SD (ng/mL).

^aP<.05, ^bP<.01, ^cP<.001, when both groups of patients were compared with controls. ^dP<.05, when patients with angina were compared at the time of admission and 10 days later. ^eP<.05, when both groups of patients were compared at the time of admission and 10 days later.

and enzymes

Blood samples were obtained by puncture of the cubital vein at the time of admission, before beginning treatment, and 10 days later, in glass tubes without anticoagulant. The longitudinal study was made at 10 days because plasma cytokines have a short half-life, and the expression of soluble endothelial adhesion molecules depends on the degree of endothelial activation by the action of these molecules, and because the evolution of UA is generally fast and satisfactory. This time period made it possible to evaluate the concentrations of soluble endothelial adhesion molecules at the time of diagnosis and after 10 days if coronary blood flow was recovered.

The rapid and satisfactory evolution of patients with UA that required measurements of these molecules at 10 days of evolution, and the necessary exclusion criteria for this study, meant that only 11 patients with a diagnosis of UA were included in the study.

This investigation was approved by the ethics committees of the two participating institutions, in compliance with the principles of the Declaration of Helsinki.¹⁸

The serum was separated by centrifugation and stored at -80°C. No lipemic, hemolyzed, cloudy, or previously defrosted samples were used. Serum concentrations of ICAM-1, VCAM-1, and E-selectin were measured using commercial enzyme immunoassay kits (ELISA, R&D Systems, United Kingdom), which were used as instructed by the manufacturer. The test was repeated in triplicate for each sample. The 6 standards and control sera were measured in duplicate. The sensitivity was 2 ng/mL (10 ng/mL) at a dilution of 1/50; 0.35 ng/mL (7 ng/mL) and 0.1 ng/mL (2 ng/mL) at a dilution of 1/20 for VCAM-1, ICAM-1, and E-selectin, respectively. The intra-assay precision had coefficients of variation of 4.6%, 4.9%, and 4.7% for ICAM-1, VCAM-1, and E-selectin, respectively. The interassay precision had coefficients of variation of 7.4%, 8.9%, and 7.4% for ICAM-1, VCAM-1, and E-selectin, respectively.

Serum concentrations of CK, CK-MB and GOT were measured using commercial kits (Laboratory

Biomedical S.A. of C.V. LABISA, Tecnodiagnostics), in accordance with manufacturers' instructions.

Statistical analysis

All values were expressed as mean±standard deviation (X±SD). The differences between the two groups of patients and between each group and controls were analyzed statistically using the Student t test for unpaired data. The longitudinal study (concentrations at the time of admission and 10 days later) of both groups of patients was analyzed using the Student t test for paired data.

Comparisons of the concentrations of endothelial adhesion molecules in patients with normal and elevated concentrations of cardiac enzymes, and in relation to electrocardiographic changes in patients with AMI, without and with Q wave disturbances, were also made using the Student t test for unpaired data. The correlations between the concentrations of CK-MB, GOT, and the adhesion molecules were analyzed by linear regression analysis using the Pearson and Spearman method of correlation coefficients for patients with AMI and patients with UA, respectively.

RESULTS

In AMI, a significant increment in ICAM-1 and VCAM-1 was observed at the time of admission and 10 days later compared with normal controls. Nevertheless, no significant differences were observed in the concentration of E-selectin compared to healthy subjects. On the other hand, patients with UA later showed a significant increment in the soluble concentrations of E-selectin and VCAM-1 at the time of admission and 10 days later. High ICAM-1 concentrations were also observed, but the values showed no significant differences in relation to control subjects (Table 1; Figures 1-3).

The longitudinal study of patients with AMI disclosed no significant differences. In patients with UA, a significant decrease in VCAM-1 concentration was appreciated 10 days after admission, but no significant

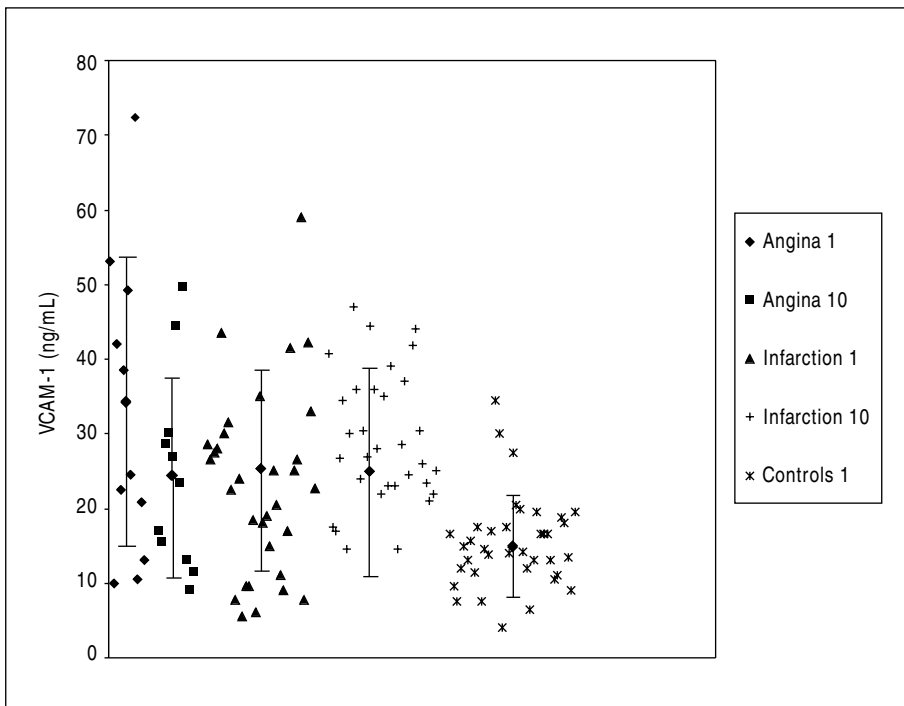


Fig. 1. Concentrations of VCAM-1 at time of admission and at 10 days.

modifications were found in the concentrations of E-selectin and ICAM-1 (Table 1).

Comparison of the two groups of patients revealed that the concentrations of VCAM-1 and ICAM-1, at the time of admission and 10 days later, did not differ significantly. However, there was a significant increase in the concentration of the E-selectin molecule in patients with UA at the time of admission

and 10 days later with respect to patients with AMI (Table 1).

No relation was appreciated between the concentrations of endothelial adhesion molecules, changes in cardiac enzymes (CK-MB and GOT), and electrocardiographic abnormalities (Tables 2-4).

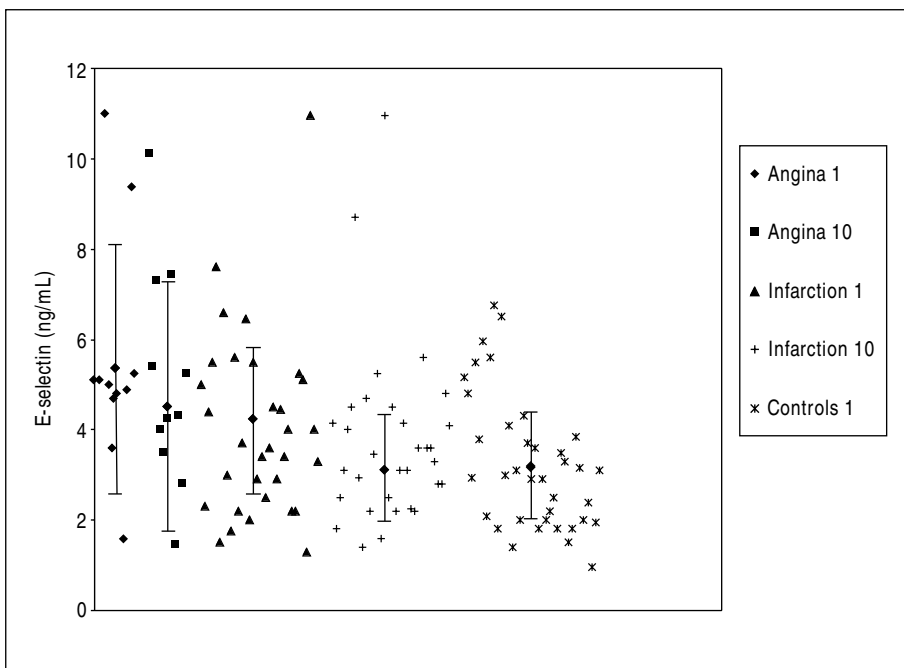


Fig. 2. Concentrations of E-selectin at time of admission and 10 days.

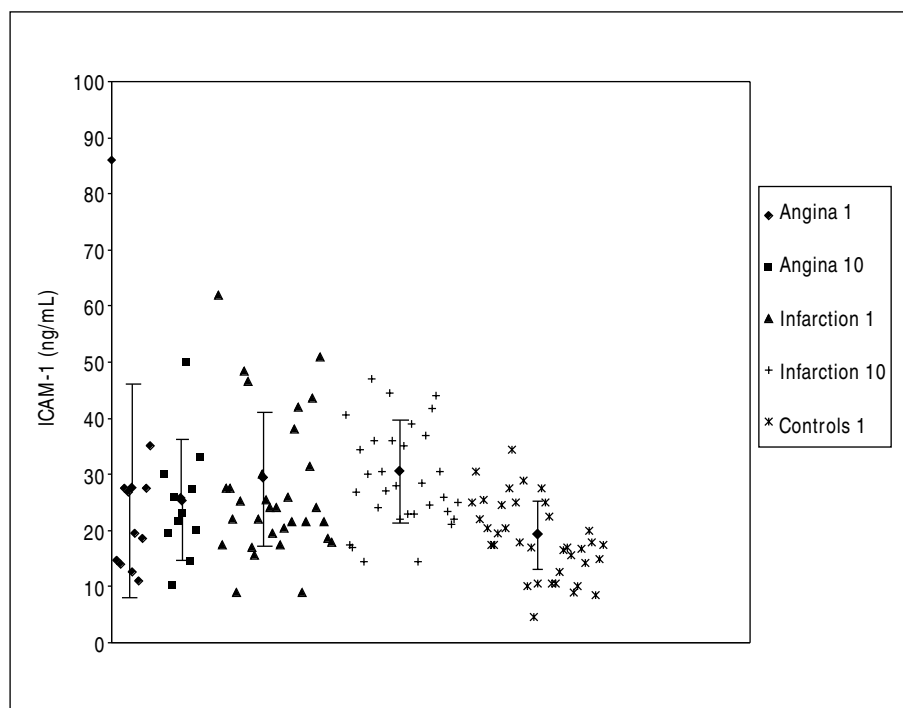


Fig. 3. Concentrations of ICAM-1 at time of admission and 10 days.

TABLE 2. Comparison of the concentrations of endothelial adhesion molecules and electrocardiographic status of the Q wave in acute myocardial infarction

Adhesion molecules	Acute myocardial infarction			
	With Q wave disturbances (n=22)		Without Q wave disturbances (n=10)	
	Diagnosis	10 days	Diagnosis	10 days
VCAM-1	22.5±13.3	24.7±15.2	30.8±16.2	27.7±10.9
E-selectin	3.8±1.6	3.2±1.1	3.5±1.5	3.3±1.3
ICAM-1	27.0±12.5	29.6±9.2	30.2±12.5	28.5±8.1

Values are expressed as the mean±SD (ng/mL).

DISCUSSION

Our results indicated high values of soluble ICAM-1 and VCAM-1 in patients with AMI at the time of ad-

mission and 10 days later. A characteristic pattern of release of these adhesion molecules was observed. Initially, the concentrations of these molecules rose in the acute phase of myocardial ischemic damage, when coronary blood flow is reduced. Nevertheless, the E-selectin pattern during longitudinal follow-up differed from the previously described pattern for the ICAM-1 and VCAM-1 molecules, since it did not reveal significant differences compared with normal values at the time of admission and 10 days later, despite the persistence of raised ICAM-1 and VCAM-1 values. In the patients with UA, high VCAM-1, ICAM-1, and E-selectin values were obtained at the time of admission and 10 days later, but the ICAM-1 values obtained did not demonstrate significant differences with respect to normal controls and patients with AMI.

TABLE 3. Comparison between concentrations of endothelial adhesion molecules and the results of cardiac enzymes in unstable angina

Adhesion molecules	Unstable angina							
	Glutamic oxalacetic transaminase				CK-MB			
	Raised (n=2)		Normal (n=9)		Raised (n=2)		Normal (n=9)	
	Diagnosis	10 days	Diagnosis	10 days	Diagnosis	10 days	Diagnosis	10 days
VCAM-1	36.7±13.4	33.8±9.3	34.6±19.8	24.43±13.2	36.7±13.4	33.8±9.3	34.6±19.8	24.4±13.2
E-selectin	4.4±0.7	3.9±0.3	5.9±2.4	5.0±2.4	4.4±0.7	3.9±0.3	5.9±2.4	5.0±2.4
ICAM-1	22.2±8.4	23.5±2.2	26.6±3.4	24.9±10.6	22.2±8.4	23.5±2.2	26.63±21.1	24.9±10.6

Values are expressed as the mean±SD (ng/mL).

TABLE 4. Comparison between the concentrations of soluble endothelial adhesion molecules and the results of cardiac enzymes in acute myocardial infarction

Adhesion molecules	Acute myocardial infarction							
	Glutamic oxalacetic transaminase				CK-MB			
	Raised (n=20)		Normal (n=12)		Raised (n=24)		Normal (n=8)	
	Diagnosis	10 days	Diagnosis	10 days	Diagnosis	10 days	Diagnosis	10 days
VCAM-1	24.0±14.3	25.6±4.7	21.3±13.6	23.7±13.2	23.4±14.4	25.0±14.7	23.7±13.5	24.9±12.8
E-selectin	3.9±1.6	3.2±1.1	3.6±1.4	3.2±1.2	3.8±1.6	3.1±1.1	3.4±1.1	3.2±1.2
ICAM-1	27.5±12.3	30.0±9.1	26.8±9.2	30.6±8.7	27.4±12.1	29.8±9.0	27.1±10.6	29.0±8.0

Values are expressed as the mean±SD (ng/mL).

ICAM-1

Previously, the presence of high ICAM-1 values has been communicated in AMI^{15,16,19} and UA.^{17,20-22} In a recent study, it was reported that patients with AMI present elevated ICAM-1 and E-selectin concentrations in peripheral blood, but E-selectin values reached their maximum level 4 to 6 h after admission, returning to baseline values within 24 h.¹⁵ Studies *in vitro* have also demonstrated differences in the kinetics of the endothelial expression of E-selectin and ICAM-1.²³ Nevertheless, other studies have not found an increment in E-selectin in AMI.^{16,17}

Activation of the microcirculation is the main component of the inflammatory response. The vascular endothelium develops and expresses molecules that initiate the local immigration of leukocytes.^{24,25} The ICAM-1 molecule can be released (specifically and non-specifically) by damaged or inflamed tissue as a result of non-specific proteolysis.²⁶ This observation could explain high ICAM-1 values in patients with acute coronary syndrome and their even higher concentrations in AMI.

VCAM-1

VCAM-1 is expressed by the arterial endothelium in early atherosclerotic lesions in an experimental model of atherosclerosis in the rabbit (Watanabe rabbit), and could be responsible for attracting the mononuclear cells that contribute to the development of the atherosclerotic lesion.¹² Other authors have demonstrated that VCAM-1 is widely expressed on the endothelial cells of occluded arteries during accelerated atherosclerosis.²⁷ Coronary atherosclerosis is the most frequent cause of heart disease due to ischemia and plaque rupture with thrombosis is the main cause of acute coronary syndromes, such as UA, AMI, and sudden death.²⁸ These observations could explain the high concentration of VCAM-1 in acute coronary syndromes and the existence of a significant decrease in serum concentrations of this molecule 10 days later

in UA, when coronary blood flow has improved.

E-selectin

Our results indicated a significant increment in the concentration of E-selectin at the time of admission and 10 days later in the group of patients with UA. E-selectin is particularly interesting because it is only found in the activated endothelium, in contrast with other adhesion molecules, which have a more widespread tissue distribution. The demonstration of soluble E-selectin in blood can thus be considered conclusive evidence of endothelial activation. This adhesion molecule is biologically active, mediating the adhesion of neutrophils to the endothelial surface, as well as the adhesion of monocytes, eosinophils, basophils, and natural-killer cells.^{3,29} E-selectin facilitates the early phase of polymorphonuclear adhesion to the endothelial cell, constituting an early serum marker of the inflammatory response and promoting cellular damage by ischemia.^{30,31} Brief episodes of ischemia during coronary angioplasty make possible the soluble stimulus capable of inducing integrin expression in neutrophils.^{32,33} It also has been observed in studies of patients with UA that the appearance of chest pain 48 h after coronary angiography is related with significantly higher neutrophil activation values, suggesting that the degree of activation is related with later episodes of angina at rest.³²

On the other hand, elevated interleukin-1 beta (IL-1 β) concentrations have been found in patients with ischemic heart disease, particularly in individuals with minimum coronary artery disease and angina.³⁴ The precise function of IL-1 β in coronary artery disease is still undetermined, but during the inflammatory response endothelial cells express E-selectin in response to IL-1 β .^{34,35} In light of this evidence, ischemic episodes could act as triggers of a sequential mechanism consisting of endothelial activation with the expression and release of E-selectin in blood, increased IL-1 β values that promote the expression of E-selectin by endothelial cells and neutrophil activation by soluble

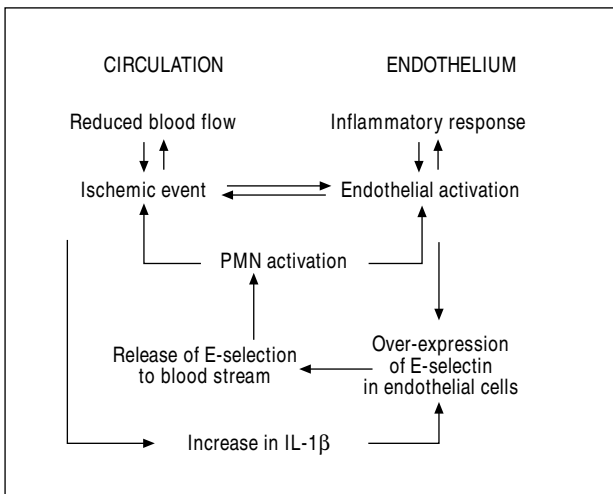


Fig. 4. Sequential mechanism showing how an endothelial activation reaction could prolong the elevation of soluble E-selectin values in blood.

E-selectin, induction of ischemic cellular damage and, consequently, more release of E-selectin by activated endothelial cells (Figure 4). These integrated reactions probably explain why E-selectin behaves differently from what is described by the some authors¹⁵ in AMI.

Findings found by other authors facilitate the understanding of our results if we consider that AMI is accompanied by a more intense inflammatory response than UA,^{14,16,17,36-38} which is associated to an inflammatory mechanism unrelated to the presence of necrosis or the extension of coronary lesions.⁸

Relation with the degree of myocardial damage

In our study, no relation could be demonstrated between increased concentrations of adhesion molecules and variations in cardiac enzymes and electrocardiographic changes. This suggests that the presence of high concentrations of adhesion molecules is not associated with the severity of myocardial damage, at least as measured by these parameters in acute coronary syndrome. This concurs with reports by various authors for other serum markers related with the diagnosis and prognosis of this acute coronary syndrome³⁸, and with evidence that specific elevations of E-selectin concentrations could indicate endothelial activation or damage as a particular component of a specific pathological process.²⁹

CONCLUSIONS

Our study suggests that high concentrations of the endothelial adhesion molecule E-selectin measured at the time of admission and days later could be a marker for UA and useful in the differential diagnosis with

AMI as a complement to the information acquired from conventional tests. However, other studies are needed to clarify the true value of increased E-selectin concentrations in the diagnosis of UA and its differential diagnosis with AMI, as well as their correlation with other serum markers of known value in the diagnosis of acute coronary syndrome, such as PCR and troponin T, which could be an instrument of value in the prognosis of patients with coronary diseases.

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