Increased Adhesiveness of White Blood Cells in Patients with Unstable Angina: Additional Evidence for an Involvement of the Immune-Inflammatory System

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Summary

Background: Data are being accumulated on the presence of inflammatory response in patients with acute coronary syndromes.

Hypothesis: The study was undertaken to confirm that the adhesive state of the peripheral blood leukocytes can provide information on an inflammatory process in patients with unstable angina pectoris.

Methods: The study included 21 patients with unstable angina, 32 patients with stable angina, and 13 patients with chest pain and no evidence of coronary artery disease.

Results: The percentage of aggregated leukocytes in patients with unstable angina ($5.8 \pm 3.2\%$) was significantly ($p < 0.02$) increased compared with that of patients with no evidence of active coronary disease ($3.2 \pm 2.6\%$).

Conclusion: Additional evidence for an existing smoldering inflammatory process in unstable angina pectoris can be provided by testing the percentage of aggregated leukocytes in peripheral blood.

Key words: leukocytes, leukocyte adhesion/aggregation, unstable angina pectoris

Introduction

Immune-inflammatory mechanisms might participate in the pathogenesis of unstable angina pectoris (UAP).¹ Inflam-

mation is known to be present in patients with UAP, and this is probably not a response to the ischemic process itself.² Several studies suggested that atherosclerosis is accompanied by a chronic inflammatory response and that the appearance of UAP might be a result of such a response.³ One of the markers of inflammation in patients with UAP is the increased expression of adhesive molecules on the surface of white blood cells.⁴ In this paper, we addressed the question of whether increased leukocyte adhesiveness can be visualized in the peripheral blood of patients with UAP. This diagnostic modality could permit identification of individuals who might potentially benefit from a more aggressive anti-inflammatory approach.

Methods

Study Subjects

The study population included 66 patients (50 men, 16 women) with a mean age of 65 years (range 28–82) who were admitted for coronary angiography as a part of an evaluation of chest pains. Thirteen patients had chest pain; there was no evidence of an acute coronary event, and the coronary arteries were found to be normal on the coronary angiogram. Thirty-two patients had stable angina pectoris and were admitted on an elective basis following a thallium scan that was positive for reversible ischemia in the majority. Twenty-one patients had rest pain with or without electrocardiographic ischemic change, but there was no evidence of myocardial infarction. These patients were given the conventional treatment of aspirin, nitrates, and heparin, and they were catheterized on a semi-elective basis following stabilization.

Protocol

In order to exclude the possibility that acute stress was related to the episode of chest pain and that ischemia affects the adhesive state of the white blood cells in the peripheral blood,⁵ we evaluated our patients at only about 4 days following the last episode of pain and ischemia. In addition, we excluded from the present study patients with any infection/inflamma-

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tion that alone would explain the appearance of increased leukocyte adhesiveness in the peripheral blood.⁶ For the same reason, we also excluded patients with severe unstable angina who had C-reactive protein (CRP) concentrations above 0.5 mg/l, the upper normal value in our laboratory.

The state of leukocyte adhesiveness/aggregation in the peripheral blood was evaluated by using a simple slide test.⁷ Briefly, blood was drawn into a syringe containing sodium citrate (one volume of 3.8% sodium citrate and three volumes of whole blood). Several large drops of blood were placed on a slide that was held for 2 to 3 s at a 45° angle so that the blood could slip down by gravity, leaving a fine film. The slides were dried in an incubator or at room temperature while in a completely horizontal position. To cause hemolysis, the dried slides were refrigerated at -18°C for 10 min. Fixation was done with absolute methanol and staining with hematoxylin. The percentage of aggregated leukocytes on the slides was determined by counting 300 white blood cells at random. For example, if 30 of 300 were in aggregates, whether one aggregate of 30 cells or two aggregates of 15, the percentage of aggregated leukocytes was 10%. Cells were considered aggregated when three or more nuclei were located less than one cell diameter apart (Fig. 1).

Two slides were prepared from each patient, and the final result was a simple mean of both. Variation between duplicates did not generally exceed 20%, although larger differences were seen from time to time because of the presence of nonhomogeneous distribution of the aggregates.⁸ This was also the variation noted when two independent observers examined slides from the same patient, and when the same sample was tested or retested, differences did not exceed 15%. The intra-assay coefficient of variation was calculated from 15 slides that were prepared from the same donors, and the interassay coefficient of variation was calculated when the same normal donor was examined on successive days for 15 consecutive days, the respective values being 0.2 and 0.3.

FIG. 1 Increased state of leukocyte adhesiveness/aggregation in the peripheral blood of a patient with unstable angina.

The composition of the aggregates was generally similar to the differential white blood cell count (WBCC), that is, it consisted mainly of neutrophils in cases of neutrophilia and of lymphocytes in cases of lymphocytosis. Mixed aggregates of polymorphonuclear and mononuclear leukocytes were seen frequently.⁹

The concentrations of CRP were determined by using laser nephelometry. The fibrinogen concentration was determined by the method of Clauss, and the WBCC and differential count were performed on a Technicon H1 autoanalyzer.

The severity of coronary disease was scaled on a number from 0 to 4 according to the number of affected coronary arteries. Each main artery (i.e., left anterior descending, left circumflex, right coronary artery, or left main coronary artery) or its tributaries was given a number, and the severity of coronary disease was a simple sum of all the diseased arteries. An obstruction of a coronary artery >70% and of the left main coronary artery >50% was considered to be significant.

Statistical Analysis

The SPSS statistical package version 6.1 was used for statistical analysis. We used the ANOVA test for individual group significance and the Bonferroni and Scheffe procedures for intergroup analysis of significance. A *p* value of <0.05 was considered significant.

Results

The laboratory findings are given in Table I and illustrated in Figure 2. Patients with UAP showed an increased state of leukocyte adhesiveness compared with patients without an unstable coronary condition. The state of leukocyte adhesiveness/aggregation was the only laboratory parameter to reach a statistically significant difference between patients with UAP and those with stable angina (*p* = 0.018 for the Bonferroni procedure and *p* < 0.05 for the Scheffe procedure). No correlation was found between any of the laboratory variables and the severity of the coronary artery disease, as judged by

TABLE I Results (mean ± SD; range in brackets) of the laboratory values according to diagnostic groups

Diagnosis	LAAT (%)	Fibrinogen md/dl	WBCC (cmm × 10 ³)	Number of vessels involved
Normal coronaries	3.3 ± 1.5 (2.3–7.7)	395 ± 107 (198–578)	7.6 ± 1.3 (4.8–9.3)	0
Stable angina	3.8 ± 1.9 (2.2–10.8)	390 ± 76 (248–548)	7.5 ± 1.8 (4.0–11.4)	2.2 ± 1.0 (0–4)
Unstable angina	5.5 ± 3.4 (2.5–12.0)	402 ± 87 (250–596)	7.8 ± 1.1 (6.3–10.9)	2.3 ± 1.1 (1.0–4.0)

Abbreviations: LAAT = leukocyte adhesion/aggregation test, WBCC = white blood cell count, SD = standard deviation.

FIG. 2 The leukocyte adhesiveness/aggregation test (LAAT), white blood cell count (WBCC), and fibrinogen concentrations in patients with normal coronary arteries, stable angina, and in those with unstable angina pectoris. Statistically significant differences were seen in patients with unstable angina and the other two groups only from the state of leukocyte adhesiveness/aggregation in the peripheral blood. □ = LAAT, ■ = WBCC, ■ = fibrinogen.

the number of involved vessels. Heparin administered to 14 of our patients suffering from unstable angina did not affect the adhesive state of the white blood cells.

Discussion

The findings of this study demonstrated that the state of leukocyte adhesiveness/aggregation in the peripheral blood as revealed in our slide test is a valid and sensitive marker of inflammation.⁶ They showed that an increased state of leukocyte adhesiveness is present in patients with UAP, and this probably represents an increased inflammatory reaction in these patients. Previous studies have shown that the state of leukocyte adhesion/aggregation in the peripheral blood is increased in patients with myocardial infarction.^{10, 11} We excluded patients with myocardial infarction and those with severe unstable angina who had an increased concentration of CRP protein because it is our experience from previous studies that the state of leukocyte adhesiveness/aggregation in the peripheral blood is expected to increase during myocardial infarction.¹² It was the aim of the present study to explore whether our slide test would be sensitive enough to detect a minor inflammatory response that did not result in increased values of the commonly used acute phase response markers (WBCC as well as fibrinogen or CRP concentrations). Our test was, indeed, proven to be a sensitive marker of inflammation in patients with UAP.

Inflammation is an important feature of atheroma and is associated with activation and proliferation of macrophages, endothelial cells, smooth muscle, as well as with the generation of cytokines and growth factors, the activation and deposition of complement, and the presence of proinflammatory

mediators.¹³ Fibrinogen is one of the proteins that is increased during inflammation and in atherosclerotic patients.¹⁴ We had shown in an earlier study that fibrinogen might be involved in the increased adhesive state of white blood cells in the peripheral blood.¹⁵ Although we found no significant changes in fibrinogen concentrations among the various groups in the present study, this does not exclude eventual participation of this protein in the phenomenon of increased leukocyte adhesiveness/aggregation in the peripheral blood, since the ligand for fibrinogen on the surface of white blood cells, namely the CD11b/CD18 adhesive molecule,¹⁶ might be activated in patients with UAP.⁴ Such activated receptors might bind fibrinogen which will serve as a bridge between one leukocyte and another.¹⁷

The potential harmful role of sticky leukocytes in ischemic disease was already discussed by us and by others in the past.^{10, 18} One can improve the outcome of myocardial ischemia by reducing the adhesive properties of the white blood cells.¹⁹ Our findings might therefore present a new approach to identify individuals with an increased inflammatory response and UAP. This means of identification might be helpful in singling out those patients who might potentially benefit from a more aggressive anti-inflammatory treatment modality. A recent study²⁰ has indeed proposed that patients with atherosclerosis and an increased inflammatory response might benefit from anti-inflammatory treatment more than the individuals with a low-grade or absent inflammation.

Conclusions

Patients with unstable coronary artery syndrome may present with increased leukocyte adhesiveness in their peripheral blood. This identification may have both diagnostic and therapeutic implications.

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