Changes in blood rheology in patients with stable angina pectoris as a result of coronary artery disease


ABSTRACT We investigated several rheologic variables in 17 patients (11 men, six women, mean age = 52.1 ± 9.8 years) with chronic stable angina. None took any medication except for sublingual nitroglycerin for 2 weeks before the study, and all had angiographically proven coronary artery disease with no history of myocardial infarction. Rheologic measurements included hematocrit, whole blood and plasma viscosity (750 and 1500 sec⁻¹), degree of red cell aggregation via the zeta sedimentation ratio, and the extent and rate of red cell aggregation after stasis (Myrenne aggregometer). Compared with normal control donors, salient observations in the patients as a group included: (1) a small (6%) but significant increase in hematocrit, (2) a significant elevation in plasma viscosity (9%), (3) significant increases in whole blood viscosity at both shear rates (14% and 16%), (4) significant increases in the degree (12%), the extent (41%), and the rate (28% faster time constant) of red cell aggregation, (5) an elevated α₁ level (15% increase) and a significantly increased fibrinogen concentration (25% increase), both of which correlated with the enhanced red cell aggregation. Rheologic abnormalities were evident when patients with disease in either one vessel or two to three vessels were compared with controls, but differences between these subgroups of patients were not significant. We conclude that patients with angina have rheologic abnormalities that are compatible with disturbed blood flow and an enhanced tendency for coronary arterial thrombosis. Circulation 76, No. 1, 15–20, 1987.

THE RELATIONSHIPS between myocardial function, coronary artery disease, and blood rheology continue to be of interest to both basic science and clinical investigators. Compared with healthy control subjects, previous reports have indicated that patients with angina pectoris and ischemic heart disease (i.e., coronary artery disease) have increased whole blood viscosity,¹ ² plasma viscosity that is unaltered,³ ⁴ or, in the presence of unstable angina, elevated,⁵ elevated plasma fibrinogen levels,¹ and either increased⁶ or decreased⁷ red blood cell flexibility. Lowe et al.⁸ have reported that patients with two- or three-vessel disease have significantly higher blood viscosity and plasma fibrinogen levels than patients with no or one-vessel disease, whereas the data of Low et al.⁶ demonstrate differences in blood viscosity but not fibrinogen level in these two patient groups. Although fibrinogen is known to be a major determinant of red cell aggregation⁷ and thus of the rheologic behavior of human blood,⁸ ⁹ only limited data exist with respect to red cell aggregation in coronary artery disease; while patients with significant stenosis of at least one coronary artery have been shown to exhibit increased red cell aggregation,¹⁰ correlations with either fibrinogen levels or the extent of coronary artery disease have not been presented. The present study was thus designed to examine blood rheology in patients with stable angina pectoris as a result of coronary artery disease, with particular emphasis on the extent and dynamics of red cell aggregation.

Materials and methods

Patients. The patients selected for this study were among those participating in an ongoing long-term study of chronic
stable angina by the Division of Cardiology, USC School of Medicine. Their selection was based on all of the following entry criteria: (1) Angina defined as a dull pressure-like pain or discomfort in the chest reproducibly brought on by exertion or emotional upset. (2) A chronic stable angina pattern with less than a 50% variability in the weekly angina frequency for at least the most recent 2 months. (3) Significant coronary artery stenosis (i.e., 70% or greater), confirmed by coronary angiography, of at least one coronary artery (left anterior descending, left circumflex, or right coronary artery). (4) The absence of insulin-dependent diabetes, bronchospastic lung disease, acute myocardial infarction within the preceding 3 months, or other diseases with symptoms that could be confused with angina, such as esophageal disease. (5) The ability to perform an exercise treadmill test to confirm the presence of ischemia. A total of 141 patients were referred for possible inclusion in the study because of a history of symptoms suggestive of chronic stable angina. Only 28 were selected as possible candidates for this specific study: 43 refused either to participate or to undergo treadmill testing or coronary angiography, 21 had insulin-dependent diabetes or other medical or physical problems that precluded accurate evaluation of their symptoms, 18 were excluded because they were no longer having angina, 14 had normal coronary arteries at the time of angiography, 10 had such severe coronary disease that coronary angioplasty or bypass surgery was performed, three had a recent myocardial infarction, two were unable to perform on an exercise treadmill, and two died between the time of initial interview and the beginning of diagnostic testing. Because of the temporal demands of the rheologic assays (i.e., measurements within 4 to 6 hr after venipuncture; see below), 17 of the 28 patients meeting entry criteria were selected based solely upon whether the rheologic tests could be done at the time the patient entered the study.

The group of 17 patients finally enrolled in this investigation consisted of 11 males and 6 females, with a mean age of 52.1 years (SD = 9.8 years; range = 34 to 72). Seven of the patients had stenosis of one vessel, eight had stenosis of two vessels, and two had stenosis of three vessels. Each patient was taken off all medication, except for sublingual nitroglycerin taken as needed for episodes of chest pain, for at least 14 days before blood sampling and therefore was not receiving diuretics (which cause hemocoencentration) or agents that lower the fibrinogen concentration and thus blood viscosity. Seventeen university employees in apparent good health served as controls (11 men, six women; mean age = 53.6 ± 9.8 years, range 37 to 72).

Methods. Venous blood samples were taken from resting subjects into EDTA (1.5 mg/ml) for the rheologic tests and into no anticoagulant for the determination of serum proteins; all rheologic measurements were carried out within 4 to 6 hr after venipuncture. Whole blood hematocrit was determined by the microhematocrit method (13,000 g for 4 min; IEC MB centrifuge) and whole blood and plasma viscosity were measured via cone-plate viscometry at 25°C (750 and 1500 sec⁻¹ for whole blood, 1500 sec⁻¹ for plasma; Brookfield model ½ RVT-200 micro cone-plate viscometer). Fibrinogen was determined by a turbidometric method (DuPont ACA system) and serum proteins by gel electrophoresis.

Red cell aggregation was evaluated at room temperature (21 ± 1°C) with the use of cells that had been washed twice in autologous plasma and then resuspended in this plasma at a hematocrit of 40.0 ± 0.2%. Two separate techniques for red cell aggregation were used and are described below.

Zeta sedimentation ratio. The degree of red cell aggregation after a defined 3 min period of low gravity (7 to 8 g) dispersion and compaction was determined by the zeta sedimentation ratio method of Bull and Brailsford. Well-mixed cell suspensions are placed into 75 mm long by 2 mm inner diameter glass tubes, placed in the Zetafuge (Coulter Electronics, Hialeah, FL), and rotated in a nearly vertical position for four 45 sec periods; the tubes are rotated 180 degrees parallel to their long axis at the end of each 45 sec period. The “hematocrit” of the suspension, read after the 3 min period, is termed the “zetacrit.” The true hematocrit of the suspension in the tube is then determined via the conventional microhematocrit centrifuge method, and the zeta sedimentation ratio is calculated as the ratio of the true hematocrit to the zetacrit. Note that the value of the zeta sedimentation ratio increases with increasing red cell aggregation.

Myrenne aggregometer. Both the extent and rate of red cell aggregation at zero shear rate were measured with an instrument (MA-1 Aggregometer, Myrenne GmbH, Roeigen, F.R.G.) that uses the light transmission method of Schmid-Schoenbein et al. This technique is based on the increase of light transmission through a red cell suspension that occurs when individual cells aggregate into rouleaux or rouleaux-rouleaux complexes; gaps in suspending medium between the cell aggregates produce the increased light transmission. The suspension under study is placed between the transparent cone and plate of the instrument and then sheared at 500 sec⁻¹ to disperse all cell aggregates. The drive motor is then instantly stopped and the light transmission abruptly drops due to a transient state of random cellular orientation. After this rapid drop, the transmission increases with time at a rate proportional to the rate of cell aggregation (i.e., at a rate proportional to the formation of cell-free gaps between aggregates). Two measures of red cell aggregation were obtained with this device: (1) Aggregation index. The instrument integrates the light transmission for 10 sec after the above-mentioned abrupt drop in transmission, then displays the numeric result of this integration, i.e., the aggregation index. Note that, like the zeta sedimentation ratio, the value of the aggregation index increases with increasing red cell aggregation. (2) Aggregation time constant. Via external computer-controlled analog-to-digital conversion of the light transmission signal, the resulting time-light transmission data after the abrupt drop can be fitted to a semilogarithmic model by least squares linear regression. The inverse of the resulting slope during the early phase of aggregation provides the aggregation time constant (in seconds), which decreases with increasing rates of cell aggregation.

Differences in mean values were analyzed by the two-tailed Student’s t test and correlations between variables by single and multiple Pearson correlation coefficients.

Results

Hematologic and rheologic results for the patients and the control subjects are listed in table 1. In the initial comparison of the angina group with the controls, the following observations were made: (1) The patients had a small but significant increase in hematocrit (6% increase, p < .02). (2) The patients had a significantly higher level of fibrinogen (25% increase, p < .02) and a 15% increase in α2, which failed to achieve significance (0.05 < p < .07). (3) Values for total protein, albumin, α1, β, and γ were not significantly different in patients and control subjects (5% or less increase, p > .20). (4) The plasma viscosity in the patients was significantly elevated (9% increase, p < .005). (5) At both shear rates, the patients had significantly higher levels of whole blood viscosity (at 750
sec⁻¹ a 14% increase, p < .001; at 1500 sec⁻¹ a 16% increase, p < .001). (6) The degree of red cell aggregation, as judged by the zeta sedimentation ratio, was significantly elevated in the patients (12% increase, p < .001). (7) As measured via the Myrenne aggregometer, the patients had a greater extent of red cell aggregation (41% increase of the aggregation index, p < .001) and a faster rate for this aggregation process (28% decrease of the aggregation time constant, p < .001).

The zeta sedimentation ratio and the aggregation index data for the pooled patients demonstrated significant correlations with the plasma levels of α₂ and fibrinogen (figures 1 and 2): for α₂, r = .71 (p < .01) for the zeta sedimentation ratio and r = .54 (p < .05) for the aggregation index; for fibrinogen, r = .53 (p < .05) for the zeta sedimentation ratio and r = .73 (p < .01) for the aggregation index. Multiple correlation coefficients, obtained by simultaneous linear regression of either the zeta sedimentation ratio or the aggregation index against α₂ and fibrinogen were also highly significant (r = .79, p < .001, for both the zeta sedimentation ratio and the aggregation index), indicating that more than 62% of the variation of either of these two aggregation variables can be explained by the combined influence of α₂ and fibrinogen.¹⁶ The zeta sedimentation ratio and the aggregation index were found to exhibit a significant cross-correlation (r = .71, p < .001), but no meaningful relationships existed between the aggregation time constant and either the zeta sedimentation ratio or the aggregation index nor between the plasma concentrations of α₂ and fibrinogen (r = .36, p > .2).

Separation of the patient population into subgroups of those with one-vessel disease and those with two- or three-vessel disease was carried out to examine the effect of the extent of coronary artery disease (table 1). The patients with two- and three-vessel disease were pooled owing to the small number of patients with stenosis of three vessels. With the exception of whole blood hematocrit in the one-vessel disease group and fibrinogen concentration in the two- or three-vessel disease group, all of the above-mentioned hematologic and rheologic abnormalities were evident and significant when either of the subgroups were compared with the control subjects. There was also a significant increase in total protein in the one-vessel disease group. Note that the percentage changes from control for these hematologic and rheologic variables were comparable for the two subgroups of patients. No significant dif-

### Table 1

**Hematologic and rheologic data**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 17)</th>
<th>All patients (n = 17)</th>
<th>One-vessel disease (n = 7)</th>
<th>Two- or three-vessel disease (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hematologic measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>41.1 ± 2.3</td>
<td>43.5 ± 2.9º</td>
<td>43.0 ± 2.7</td>
<td>43.9 ± 3.1º</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>73 ± 4</td>
<td>76 ± 5</td>
<td>77 ± 4ª</td>
<td>75 ± 6</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>46 ± 3</td>
<td>47 ± 4</td>
<td>49 ± 4</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>α₁ (g/l)</td>
<td>2.3 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>α₂ (g/l)</td>
<td>6.2 ± 1.3</td>
<td>7.1 ± 1.6</td>
<td>6.7 ± 1.5</td>
<td>7.3 ± 1.6</td>
</tr>
<tr>
<td>β (g/l)</td>
<td>8.2 ± 1.6</td>
<td>8.2 ± 1.7</td>
<td>8.7 ± 1.4</td>
<td>7.8 ± 1.8</td>
</tr>
<tr>
<td>γ (g/l)</td>
<td>10.1 ± 2.4</td>
<td>10.6 ± 2.7</td>
<td>9.9 ± 1.6</td>
<td>11.1 ± 3.2</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.05 ± 0.51</td>
<td>3.80 ± 0.99ª</td>
<td>3.97 ± 1.02ª</td>
<td>3.66 ± 1.00</td>
</tr>
<tr>
<td><strong>Rheologic measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma viscosity (mPa·sec)</td>
<td>1.74 ± 0.06</td>
<td>1.89 ± 0.17º</td>
<td>1.94 ± 0.22ª</td>
<td>1.85 ± 0.12º</td>
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<tr>
<td>Blood viscosity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>750 sec⁻¹ (mPa·sec)</td>
<td>5.03 ± 0.32</td>
<td>5.72 ± 0.66³</td>
<td>5.80 ± 0.87ª</td>
<td>5.66 ± 0.53ª</td>
</tr>
<tr>
<td>1500 sec⁻¹ (mPa·sec)</td>
<td>4.59 ± 0.27</td>
<td>5.33 ± 0.58³</td>
<td>5.41 ± 0.76ª</td>
<td>5.28 ± 0.46³</td>
</tr>
<tr>
<td>Zeta sedimentation ratio</td>
<td>0.52 ± 0.03</td>
<td>0.58 ± 0.05³</td>
<td>0.57 ± 0.05c</td>
<td>0.58 ± 0.06c</td>
</tr>
<tr>
<td>Aggregation index</td>
<td>19.8 ± 3.7</td>
<td>28.0 ± 4.6³</td>
<td>29.1 ± 4.7²</td>
<td>27.3 ± 3.4³</td>
</tr>
<tr>
<td>Aggregation time constant (sec)</td>
<td>1.75 ± 0.26</td>
<td>1.26 ± 0.21³</td>
<td>1.24 ± 0.23³</td>
<td>1.27 ± 0.20³</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation for "n" donors. 1 mPa·sec = 1 cp.
Significance levels compared with control: *p < .05; *p < .02; *p < .01; *p < .025; *p < .005; *p < .001. No significant differences were found between the one- and two- or three-vessel disease groups.
The results of this study demonstrate that patients with angina pectoris due to coronary artery disease have elevated plasma and whole blood viscosity, and in addition enhanced red cell aggregation that can be related to their plasma levels of α2-protein and fibrinogen. Note, however, that the elevation of whole blood viscosity is most likely due to the combined effects of increased whole blood hematocrit and plasma viscosity. At the high shear rates used in the present study there is an approximately 4% increase in whole blood viscosity per unit increase of hematocrit. Our results are thus in general agreement with previous reports (see, for example, refs. 1, 2, and 10), but do differ in certain areas: (1) Lowe et al. indicate that patients with two- or three-vessel disease have a higher hematocrit compared with patients with angina and zero/one-vessel disease, whereas we found no differences in hematocrit between our patient subgroups. (2) In previous studies no differences in plasma viscosity were found between control subjects and patients with stable angina, or between control subjects and either those with zero/one- or two- to three-vessel disease, and both types of comparisons showed significant differences in the present study. (3) Elevated fibrinogen levels were previously only detected in patients with two- or three-vessel disease, whereas both our patients as a group and the one-vessel subgroup, but not the two- or three-vessel disease subgroup, showed increases.

The reasons for the above-mentioned differences are
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presently unclear, although the more restrictive criterion for coronary artery disease used in the present study (i.e., 70% or greater stenosis) vs the 50% or greater benchmark used in the earlier report may provide a partial answer. Note that although cigarette smoking has been shown to increase fibrinogen, Lowe et al. found no relationship between smoking history and the elevation of fibrinogen in their patients. Thus, the exact mechanism responsible for the elevation of fibrinogen in patients with coronary artery disease remains uncertain, although increased biosynthesis and turnover of this protein have been implicated.

The fibrinogen–red cell aggregation results reported herein are consistent with those from other studies of the effects of this protein on cell-cell interaction. Schmid-Schoenbein et al. showed that increased amounts of exogenous fibrinogen added to red cell suspensions caused a marked decrease in the aggregation time constant, and the higher endogenous levels of fibrinogen that occur in normal pregnancy and nephrotic syndrome also increase the rate of aggregation. Our aggregation results also agree with investigations in which elevated zeta sedimentation ratio values were found to be associated with fibrinogen-induced cellular aggregation. Furthermore, they are supported by a large body of rheologic data that demonstrate a positive correlation between red cell aggregation and fibrinogen concentration. Likewise, the effects of α1 on red cell aggregation (figure 1) are also not unexpected, inasmuch as high molecular weight proteins (e.g., macroglobulins) have been shown to enhance aggregation, and the α2-protein has been linked to the elevation of low shear rate blood viscosity after acute myocardial infarction.

The enhanced red cell aggregation found in this study suggests that altered blood flow behavior should be associated with coronary artery disease. The rheologic properties of human blood have been studied by several investigators. Salient features of its rheologic behavior include: (1) Normal blood behaves as a non-Newtonian fluid, with viscosity increasing with decreasing shear rate. (2) Hematocrit influences both viscosity and the degree of non-Newtonian behavior, so that at and above the normal range, blood exhibits a nonlinear increase in viscosity with hematocrit and is markedly non-Newtonian. (3) At constant hematocrit, increased red cell aggregation causes increased blood viscosity, especially at shear rates below 100 sec⁻¹. (4) The extent of cellular aggregation is an inverse and reversible function of shear rate, such that red cell aggregates (i.e., rouleaux) seen at stasis or at low rates of shear are disrupted at high shear rates. (5) Fibrinogen and macroglobulins are the plasma proteins mainly responsible for red cell aggregation and the non-Newtonian flow properties of blood.

The above-mentioned increase in blood viscosity due to red cell aggregation, coupled with reports indicating that an elevated fibrinogen level is an important risk factor for cardiovascular death, myocardial infarction, and cardiovascular disease, are consistent with investigations suggesting that altered blood rheology and blood flow may be of importance in the development of vascular occlusion and thrombosis. Several experimental studies have shown that platelet deposition and residence time increase near a region of disturbed flow and that the extent of this abnormal platelet behavior is a function of the rheologic properties of blood. Furthermore, the convective transport of relevant solutes (i.e., oxygen, ADP, thrombin, other plasma components) is also influenced by the rheology of blood, such that increased red cell aggregation and thus increased blood viscosity reduce the rate of convective movement. Areas of low oxygen tension or concentrated procoagulants would be favored by increased blood viscosity, particularly in regions of low and/or disturbed flow (e.g., near regions of stenosis).

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