Increase in Erythrocyte Disaggregation Shear Stress in Hypertension

Seyed Mahmoud Razavian, Muriel Del Pino, Alain Simon, and Jaime Levenson

The aggregation and disaggregation behaviors of red blood cells (RBC) play an important role in the pathophysiological behavior of the blood circulation.1 The aggregation of RBC is a reversible phenomenon that occurs with macromolecules bridging the membranes of adjacent cells, and it is influenced by 1) the shearing force of blood, 2) the properties of erythrocytes (concentration, deformability, surface charge, and shape), and 3) the bridging force of high molecular weight plasma proteins.2-4 This phenomenon represents an equilibrium between adhesive forces (macromolecules bridging force), repulsive forces (electrical charges on RBC surface),5 and mechanical forces (shear stress).5,6 When the adhesive force is increased in pathological conditions, the shear stress required to break up RBC aggregates would become elevated. In hypertension, increases of red blood cell aggregation were largely extrapolated from blood viscosity measurements at low shear rates.7-11 In contrast, few studies have used direct quantitative methods to evaluate red blood cell aggregation in hypertension.12 Relatively little data are available on the shear stress needed to break up the aggregated RBC in normal and pathological conditions. In the present study, we used a new laser technique that allowed quantification of the aggregation kinetics and of the disaggregation shear rate of the aggregates.13,14 The simultaneous measurement of blood viscosity allowed us to determine the disaggregation shear stress as a representative index of the mechanical forces acting on RBC to disaggregate the rouleaux. Thus, the purposes of the present study are to determine in normotensive and hypertensive subjects the aggregation kinetics and the disaggregation shear rate of aggregates and to assess the shear stress needed to break up the aggregated RBC.

Methods

Twenty-one patients with mild-to-moderate hypertension, who were 44±2 years old (mean±SEM) and had a body mass index (weight/height$^2$) of 24.8±0.6 kg/m$^2$, and 17 normotensive control subjects, who were 40±2 years old and had a body mass index of 24.3±0.6 kg/m$^2$, participated in this institutionally approved study after giving informed consent. Systemic blood pressure was measured with a sphygmomanometer three times in each subject with the subjects in a reclining position, and hypertension was diagnosed when diastolic blood pressure was between 95 and 114 mm Hg (Korotkoff Phase V). All the subjects were either not taking any medication or had discontinued treatment at least 4 weeks before the study. None had cardiac, neurological, or renal complications or peripheral vascular disease; essential hypertension was documented by standard laboratory tests and timed intravenous pyelogram.

A new laser technique (erythroaggregameter, SEFAM, Nancy, France), which had been previously validated,13,14 was used to study the aggregation kinetic and disaggregation shear rate of blood samples. The blood was suspended in a narrow gap between two coaxial
cylinders; the inner cylinder was fixed; the outer cylinder was transparent and was rotated by a motor. Shear rates from 7 to 605 sec\(^{-1}\) can be applied to the outer cylinder. A ray of light provided by a laser diode irradiated the suspension. The backscattered light (BSL) by the blood suspension was recorded by a photodiode detector as a function of time and of shear rate. For determination of aggregation kinetics, the blood sample was sheared for 10 seconds at a high shear rate so that erythrocytes were disaggregated and oriented with the flow. Then, the shear rate was abruptly stopped, erythrocytes were no longer oriented, and a rapid increase in the BSL was recorded. The peak value characterizes the maximum steady-state value of BSL (BSLM). Thereafter a progressive decrease in the intensity of the BSL occurred that was related to the formation of the rouleaux. A mean aggregation index (AI) (related to aggregation kinetic) was calculated from the relative area above the light intensity curve during the first 10 seconds after abruptly stopping the shear rate (Figure 1). Thirty-two shear rates ranging from 7 to 605 sec\(^{-1}\) were sequentially applied to the sample, i.e., from the lowest shear rate (n = 1; shear rate, 7 sec\(^{-1}\)) to the highest shear rate (n = 32; shear rate, 605 sec\(^{-1}\)). Between successive shear rates, a lag time of about 5 seconds allowed the recording of BSLM. The decrease in shear rate induced a reduction in BSLM level. The variation of BSLM as a function of shear rate was then calculated as:

\[
\Delta \text{BSLM} = 1 - \frac{\text{BSLM}(\gamma_1)}{\text{BSLM}(\gamma_2)}
\]

where \(\gamma\) is shear rate. When the shear rate increased, \(\Delta \text{BSLM}\) decreased and approached zero (Figure 1). The disaggregation shear rate threshold was obtained by extrapolating the line of \(\Delta \text{BSLM}\) versus shear rate plot to \(\Delta \text{BSLM} = 0\).

To assess the device reproducibility, coefficients of variation for replicate tests were evaluated by performing 10 successive runs on the same blood aliquot of a single donor. We obtained an average coefficient of variation of 1.5% for AI and 3.3% for disaggregation shear rate threshold (Table 1). The variability within subjects was evaluated in 10 normotensive and 10 hypertensive subjects. For each subject five successive runs on the same blood sample adjusted to 40% hematocrit were performed. We obtained average coefficients of variation of 1.6±0.3% and 2.1±0.4% for AI and 2.9±0.5% and 4.0±0.4% for disaggregation shear rate threshold for blood samples from normotensive and hypertensive subjects, respectively (Table 1). To assess the long-term variability within subjects, five normotensive subjects were investigated twice. For each subject two successive runs on the same blood sample adjusted to 40% hematocrit were performed at 0 and 90 days after. We obtained an average coefficient of variation of 3.3±1.5% for AI and 2.5±1.1% for disaggregation shear rate threshold (Table 1).

Blood viscosity was determined in all subjects with a Couette viscometer with coaxial cylinders (Low Shear 30, Contraves AG, Zurich, Switzerland) that allowed measurements at several discrete shear rates over a wide range varying from 0.033 sec\(^{-1}\) to 240 sec\(^{-1}\). The measurements were taken at shear rates of 240, 96, 52, 11, 2.4, 0.52, and 0.11 sec\(^{-1}\) and were expressed as milliPascal·second (mPa·sec). Plasma viscosity was determined at 240 sec\(^{-1}\).

The shear stress needed to break up the aggregated RBC (disaggregation shear stress) was calculated for different blood samples from the determinations of disaggregation shear rate threshold and the blood viscosity at the shear rate equal to disaggregation shear rate threshold:

\[
\tau = \eta \cdot \gamma
\]

where \(\tau\) is disaggregation shear stress, \(\gamma\) is disaggregation shear rate threshold, and \(\eta\) is blood viscosity. Blood samples were obtained from the antecubital vein by clean venipuncture with disposable plastic syringes be-

**Table 1. Reproducibility of Red Blood Cell Aggregation and Disaggregation Parameters**

<table>
<thead>
<tr>
<th>Rheological parameters</th>
<th>Intra-assay variability (n=10)</th>
<th>Intrasubject variability (n=10)</th>
<th>Long-term intrasubject variability (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cell aggregation index</td>
<td>1.5%</td>
<td>1.6±0.3%</td>
<td>2.1±0.4%</td>
</tr>
<tr>
<td>Red blood cell disaggregation shear rate threshold (sec(^{-1}))</td>
<td>3.3%</td>
<td>2.9±0.5%</td>
<td>4.0±0.4%</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
between 8 and 9 AM after an overnight fast. Blood was treated with ethylenediaminetetraacetate, potassium salt (EDTA) anticoagulant and was used for the determination of AI, disaggregation shear rate threshold, blood viscosity, plasma viscosity, and microhematocrit. Plasma fibrinogen level was determined with a fibrometer (BioMérieux, Charbonnières-les-Bains, France) by a thrombin clotting method. Plasma protein concentrations were determined with a Gilford spectrophotometer (Ciba-Corning, le Vesinet, France). Microhematocrit was determined in duplicate by use of a Hermle centrifuge (Roucaire, Vélizy Villacoublay, France) (12,000g for 3 minutes). All rheological tests were performed at 37°C ±0.5°C.

Results

Compared with normotensive controls, hypertensive patients had higher systolic and diastolic blood pressures (164±3 versus 117±2 mm Hg, p<0.001, and 101±1 versus 74±1 mm Hg, p<0.001, respectively). As shown in Table 2, hypertension was associated with higher values of fibrinogen (23%, p<0.001), total protein concentration (10%, p<0.001), and plasma viscosity (6%, p<0.001). In comparison with normotensive subjects, the hypertensive patients (Table 2) showed higher values for AI (23%, p<0.001), disaggregation shear rate threshold (11%, p<0.02), blood viscosity at disaggregation shear rate threshold (13%, p<0.001) and erythrocyte disaggregation shear stress (26%, p<0.001) for the whole blood samples. When the hematocrit of the blood was adjusted to 40% (Figure 2), the hypertensive subjects again showed higher values for AI (26%, p<0.001), disaggregation shear rate threshold (20%, p<0.01), blood viscosity at disaggregation shear rate threshold (8%, p<0.05), and disaggregation shear stress (18%, p<0.001).

To determine the influence of hematocrit on RBC aggregation parameters, the erythrocyte concentrations of blood samples were varied by adding or removing calculated volumes of autologous plasma. AI did not change significantly by varying hematocrit from 30% to 40% to 50% (14.9±1.7, 15.8±1.3, and 16.3±0.8, respectively, for normotensive subjects and 19.1±1, 20.3±0.8, and 18.9±1.1, respectively, for hypertensive subjects). In contrast, as shown in Figure 3, by increasing hematocrit from 30% to 50%, blood viscosity at disaggregation shear rate threshold increases while disaggregation shear rate threshold decreases. Furthermore, a hematocrit-independent disaggregation shear stress was obtained. At any level of hematocrit, blood viscosity at disaggregation shear rate threshold, disaggregation shear rate threshold, and disaggregation shear stress were significantly higher in hypertensive patients compared with normotensive subjects (p<0.05, 0.01, and 0.001, respectively).

Table 3 shows correlations in the overall population between the aggregation parameters and hematocrit, plasma proteins, fibrinogen, AI, and AI(40%) were both correlated positively with plasma proteins (p<0.05) and fibrinogen (p<0.001) but not with hematocrit. Disaggregation shear rate threshold was negatively correlated to hematocrit (p<0.001). Disaggregation shear rate threshold correlated positively with plasma proteins (p<0.01) and fibrinogen (p<0.001) only when hematocrit of blood was adjusted to 40%. Finally, disaggregation shear stress was positively correlated to plasma proteins (p<0.05) and fibrinogen (p<0.05). A strong correlation was observed in the whole population between blood viscosity at disaggregation shear rate threshold and AI (r=0.52, p<0.001) (Figure 3). Such a correlation with AI was not significant for blood viscosity at 240 sec⁻¹.

Discussion

Two main parameters can be quantified with the laser erythroaggregameter used in the present study: red blood cell aggregation related to the aggregation parameters and erythrocyte concentrations of blood samples were varied by adding or removing calculated volumes of autologous plasma. AI did not change significantly by varying hematocrit from 30% to 40% to 50% (14.9±1.7, 15.8±1.3, and 16.3±0.8, respectively, for normotensive subjects and 19.1±1, 20.3±0.8, and 18.9±1.1, respectively, for hypertensive subjects). In contrast, as shown in Figure 3, by increasing hematocrit from 30% to 50%, blood viscosity at disaggregation shear rate threshold increases while disaggregation shear rate threshold decreases. Furthermore, a hematocrit-independent disaggregation shear stress was obtained. At any level of hematocrit, blood viscosity at disaggregation shear rate threshold, disaggregation shear rate threshold, and disaggregation shear stress were significantly higher in hypertensive patients compared with normotensive subjects (p<0.05, 0.01, and 0.001, respectively).

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Discussion

Two main parameters can be quantified with the laser erythroaggregameter used in the present study: red blood cell aggregation related to the kinetics of RBC aggregates and disaggregation shear rate that provides the shear threshold required to dissociate the aggregates. The former parameter determines the reversible tendency of RBC to form aggregates, and the latter estimates the shear resistance of RBC aggregates. There are many similarities between this new technique and the infrared reflectometric method for measuring RBC aggregation under shear flow. As previously described, the aggregation parameters measured by this new technique are sensitive to experimental conditions. The reliability of the method was confirmed.
by the low coefficients of variation found in the intra-assay and within-subjects (short- and long-term) results of the present study. Furthermore, the low values of coefficients of variation were similar between normotensive and hypertensive subjects.

Several rheological abnormalities have been reported in hypertension; the elevation of whole blood viscosity is one that is widely recognized. The hyperviscosity magnitude observed in hypertensive patients was found to be inversely related to shear rate. Because at low shear rates RBC aggregation is one of the major determinants of increased blood viscosity, low shear blood viscosity has been used as an indirect evaluation of rouleaux formation. By focusing directly on RBC aggregation measurements, we differentiated two major abnormalities in hypertension. The enhanced RBC aggregability further confirmed that it led to the increased blood viscosity in low shear conditions. The strong association between the blood viscosity measured at disaggregation shear rate threshold and the AI further corroborates that red blood cell aggregation is a major determinant of blood viscosity at low shear rate. Such an association disappears at higher shear rate as indicated by the nonsignificant relation between AI and blood viscosity at 240 sec⁻¹. The absence of correlation between aggregability and high shear blood viscosity is well established. At high shear rate, the blood viscosity values, at a given hematocrit, reflect red blood cell deformability. The second major abnormality observed with this direct technique was the elevation of disaggregation shear rate threshold, which indicates that the adhesive force between the RBC in the rouleaux is stronger in hypertensive than normotensive subjects. The forces that disaggregate the rouleaux are important because red blood cell aggregation may occur physiologically in vivo. However, the simple determination of disaggregation shear rate does not indicate the forces needed to break up the aggregates. Disaggregation stress stress, the product of disaggregation shear rate and the blood viscosity measured at this shear rate, represents the hydrodynamic force acting to break up the aggregates. To our knowledge this is the first time that abnormalities of disaggregation shear stress have been quantified in hypertensive patients.

The hyperviscosity state resulting from increased erythrocyte aggregation is a function of the level of plasmatic macromolecules. Indeed RBC aggregation and disaggregation may be influenced by the level of plasma proteins such as fibrinogen or globulins and by the hematocrit value. Our study showed that in hypertension, the increase in RBC aggregation is associated with higher fibrinogen and total protein concentrations. The role of hematocrit on the kinetics of RBC aggregation as well as on disaggregation parameters was also investigated in the present study. The disaggregation shear rate decreased with increasing hematocrit and therefore with increasing blood viscosity. The disaggregation shear stress, however, was independent of hematocrit in both normotensive and hypertensive subjects. We observed that the influence of hematocrit on the AI remained small as previously reported by Chabanel and Samama. Some reports have shown that the aggregation kinetic is dependent on the hematocrit being in the 30–50% range. However, although Donner et al reported that a rise in hematocrit is associated with an increase in the kinetics of aggregation, Schmid-Schönbein et al and Agosti et al observed the reverse effect. In fact, RBC aggregation is a complex process that involves rouleaux and a network of rouleaux formation, each of which could vary with hematocrit.

### Table 3. Correlation Coefficient Between Biochemical and Aggregation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AI (unit)</th>
<th>AI (40%) (unit)</th>
<th>(\gamma) (sec⁻¹)</th>
<th>(\gamma) (40%) (sec⁻¹)</th>
<th>(\tau) (dyn/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ht (%)</td>
<td>0.12</td>
<td>0.10</td>
<td>-0.58*</td>
<td>0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>Prot (g/l)</td>
<td>0.35†</td>
<td>0.38†</td>
<td>0.21</td>
<td>0.43†</td>
<td>0.39†</td>
</tr>
<tr>
<td>Fib (g/l)</td>
<td>0.62*</td>
<td>0.61*</td>
<td>0.31</td>
<td>0.52*</td>
<td>0.37†</td>
</tr>
</tbody>
</table>

AI, red blood cell aggregation index; AI (40%), AI at hematocrit adjusted to 40%; \(\gamma\), red blood cell disaggregation shear rate threshold; \(\gamma\) (40%), \(\gamma\) at hematocrit adjusted to 40%; \(\tau\), red blood cell disaggregation shear stress; Ht, hematocrit; Prot, plasma proteins; Fib, fibrinogen. †p<0.05, ‡p<0.01, *p<0.001.
The fact that hypertensive patients had an increased shear resistance of their RBC aggregates may be a relevant finding, particularly concerning the risk of vascular complications that generally are associated with a low flow status. Indeed, RBC aggregate more rapidly at stasis, and the shear forces necessary to disperse the aggregates would be higher than in normal flow status. Such conditions may exist in ischemic necrosis or infarction, which are well-known complications in hypertension, and might contribute to the induction or aggravation of the course of the disease. Furthermore, abnormal aggregations were reported in such clinical situations as cardiogenic pulmonary edema, myocardial ischemia, thromboembolic states, and retinal venous occlusion. The latter is an excellent model of a low flow state at arteriovenous crossing sites. Arteriosclerotic changes compromise the venous return at these points to create a low flow area that could lead to venous occlusion; therefore, branch retinal occlusion is seen in hypertensive or arteriosclerotic individuals. It was recently reported that by using the same methodology as in the present work, the abnormal RBC aggregation and disaggregation shear conditions observed in retinal vein occlusion could predispose to the onset and development of this complication. The decreased wall shear rate and stress reported recently by our group in the large artery of hypertensive patients may also be relevant to RBC aggregation when vascular changes occur. In poststenotic regions with vessel compression and thrombus formation, shear rate can become very low and favor RBC aggregation, inducing a further increase in blood viscosity and retardation of blood circulation.

In conclusion, the elevation of RBC aggregation and the increased shear resistance of their RBC aggregates can play a major role in the pathophysiology and development of cardiovascular complications in hypertensive patients.

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