Erythrocyte Disaggregation Shear Stress, Sialic Acid, and Cell Aging in Humans

Alexandra L. Hadengue, Muriel Del-Pino, Alain Simon, Jaime Levenson

Abstract—Erythrocyte aggregation, which plays an important role in the physiological behavior of blood fluidity, was found to be enhanced in hypertension and hypercholesterolemia. While the role of macromolecule bridging force has been widely described, cellular factors related to membrane sialic acid content, which might contribute to the negative charge of cell surface causing the repulsion of erythrocytes, have been less studied. Cell age–dependent changes in membrane sialic acid content (in micromoles per gram of integral membrane protein) were investigated in 24 normotensive and 24 hypertensive matched subjects, each divided into 2 identical subgroups according to a cutoff of 6.2 mmol/L serum cholesterol. A progressive and significant (P<0.001) decrease in membrane sialic acid content associated with an increase (P<0.001) of disaggregation shear rate threshold (laser reflectometry in the presence of dextran) were observed with increased erythrocyte density (erythrocytes fractionated by density using ultracentrifugation) in both normotensive and hypertensive groups regardless of the cholesterol level. However, disaggregation shear rate threshold was significantly higher and sialic acid content was lower (P<0.001) in both hypertensive and normotensive subjects with hypercholesterolemia compared with either normotensive or hypertensive subjects with low cholesterol, respectively. A high membrane sialic acid content variance, beginning in the younger erythrocytes, was due mainly to triglyceride and LDL cholesterol levels (R²=0.49 for low, R²=0.43 for middle, and R²=0.54 for high densities, ie, young, mean, and senescent erythrocytes, respectively). We conclude that an early decrease in erythrocyte sialic acid content may influence the rheological properties of blood by increasing the adhesive energy of erythrocyte aggregates. (Hypertension. 1998;32:324-330.)

Key Words: erythrocyte aggregation ■ rheology ■ sialic acid ■ hypertension, essential ■ hypercholesterolemia

Erythrocyte aggregation is one of the main determinants influencing blood circulation at low shear rates by increasing blood viscosity and inducing “sludging” in the capillary.1 Aggregation of red blood cells is a reversible process that occurs when the bridging force due to the adhesion of macromolecules onto adjacent cell surfaces exceeds the disaggregation forces caused by electrostatic repulsion, membrane strain, and mechanical shearing.1,2,3

An increase in erythrocyte aggregation was found to be associated with cardiovascular risk factors such as hypertension,4 hyperlipoproteinemia,5,6 and smoking7 and in clinical situations such as myocardial ischemia,8 thromboembolic states,9 and retinal venous occlusion.10 In general, most studies have focused on the ability of plasma proteins and various polymers to induce erythrocyte aggregation. We showed that in hypertension and hypercholesterolemia the increase in erythrocyte aggregation could be attributed to an increase in the concentration of plasma fibrinogen.4,6 In addition, in hypercholesterolemia, concentrations of LDL cholesterol, apolipoprotein B, and lipoprotein AI:AI correlated positively with aggregation parameters.6,11 Cellular factors and erythrocyte aging have been less well studied.

Membrane-bound sialic acid with carboxyl groups contributes to the majority of the negative surface charge of the erythrocyte.12,13 It has been shown that neuraminidase-treated erythrocytes show a decrease in surface-charge density and an increase in aggregation induced by dextran.2,14,15 There have been few studies on the role of cell age in erythrocyte aggregation. Greater aggregation for aged versus young erythrocytes has been reported in healthy subjects.16,17 Furthermore, in a recent study we observed a diminished erythrocyte sialic acid content modulated by triglycerides and fibrinogen in hypercholesterolemic subjects, which might intensify the effect of fibrinogen on aggregation and disaggregation of erythrocytes and therefore contribute to the development of atherothrombotic complications.18

To our knowledge, the relation between cell age–dependent changes in sialic acid content and erythrocyte aggregation has not been extensively studied in subjects with cardiovascular risk factors. The purpose of this investigation was to examine the impact of sialic acid content of erythrocytes related to in vivo aging on the aggregation process in essential hypertension with or without primary hypercholesterolemia. We therefore measured the membrane sialic acid

© 1998 American Heart Association, Inc.
content of density-fractionated erythrocytes as well as their aggregation induced by an exogenous neutral polymer, dextran.

**Methods**

**Selection of Subjects**

This study was conducted in 48 subjects: 24 hypertensive patients with mild to moderate essential hypertension and 24 normotensive subjects. Cholesterol measurement enabled us to classify both populations into 2 subgroups: LC (cholesterol <240 mg/dL; 6.2 mmol/L) and HC (cholesterol ≥240 mg/dL; 6.2 mmol/L). Systemic blood pressure was measured with a sphygmomanometer 3 times for each subject after at least 10 minutes of rest in the supine position; hypertension was diagnosed if systolic blood pressure was ≥160 mm Hg (Korotkoff phase V) and/or diastolic blood pressure was ≥95 mm Hg (Korotkoff phase V). None of the normotensive patients were taking any medication. Fifty percent of the hypertensive patients had never taken any medication. The remaining 50% of hypertensive patients had been withdrawn from treatment at least 4 weeks before the study. None had cardiac, neurological, or renal complications or peripheral vascular disease. Primary hypercholesterolemia was confirmed by exclusion of diseases or factors causing secondary hypercholesterolemia.

**Sample Preparation**

Venous blood was collected after an overnight fast onto EDTA-anticoagulant and used within 4 hours. PBS was prepared as follows: Na2HPO4·7H2O 8 mmol/L, KH2PO4 1.5 mmol/L, KCl 2.7 mmol/L, NaCl 137 mmol/L, phosphate buffer (5P8; 4.1 and 4.9), which are insoluble in nonionic detergents and proteins 4.1 and 4.9), which are insoluble in nonionic detergents and proteins 4.1 and 4.9), which are insoluble in nonionic detergents. Blood was centrifuged at 2000g for 10 minutes for 1 hour at 37°C. After the plasma and buffy coat were removed, erythrocytes were density (ie, age)–fractionated by the method of density gradient centrifugation in long tubes with a small diameter. The top 10% of the packed cell column (relatively rich in younger cells), the intermediate fraction (80%), and the bottom fraction (10%, relatively older cells) were separately harvested and washed once in PBS. Erythrocytes separated by this method had characteristics of aged older cells) were separately washed once in PBS.

**Aggregation Measurements**

Dextran-induced aggregation was used to evaluate the influence of erythrocyte properties on cell age–dependent aggregation. Washed erythrocytes were resuspended in an artificial medium composed of Fluka). The aggregation parameters were measured with a laser technique (erythrouggregometer SEFAM) that has been previously described and validated. This technique measures the intensity of laser backscattered light with a blood suspension situated in a narrow gap between 2 coaxial cylinders. The inner cylinder is fixed, and the outer cylinder is transparent and rotatable. The outer cylinder can be adjusted to provide shear rates from 7 to 600 s⁻¹. The intensity of backscattered light by blood suspension is recorded as a function of time and shear rate. The variation of intensity of backscattered light as a function of time and shear rate allows the determination of the aggregation time, which represents the time of formation of aggregates and the DSRT (τy), which is related to the shear rate needed to break up the aggregates. After 10 minutes of incubation at 37°C, aggregation parameters were measured twice. To avoid the effect of cell concentration on erythrocyte aggregation, the samples were adjusted to a hematocrit level of 0.40±0.01 by addition of PBS, after duplicate determination by use of an Hermle centrifuge (Roucaire) at 12 000g for 3 minutes.

**Total Sialic Acid and Integral Membrane Proteins**

To determine the total content of sialic acid (NANA) in erythrocytes, ghosts were prepared by hypotonic hemolysis with a buffer (5P8; composition Na2HPO4, 7H2O 5 mmol/L, adjusted to pH 8) and centrifugation at 12 000g for 20 minutes to obtain a white pellet of ghosts. Then ghosts were washed and stored as a suspension in PBS. Erythrocyte sialic acid content was measured by a colorimetric assay for an enzymatic determination (Boehringer) as follows: bound sialic acid was hydrolyzed from sialoglycoconjugates by neuraminidase. In the presence of NANA aldolase, NANA is cleaved into N-acetylmannosamine and pyruvate. The pyruvate formed was oxidized by pyruvate oxidase to H2O2, and the amount of formed H2O2 equivalent to the free NANA was converted by peroxidase to a red dye (absorbance, 550 nm). Erythrocyte sialic acid concentrations were determined from a calibration curve using N-acetyleneuraminic acid (2.4 mmol/L; from a kit, ref 784192, Boehringer) as a standard. Results are expressed in micromoles per gram of integral lipid bilayer spanning protein. The quantity of integral membrane protein was determined by the method of Bradford (Bio-Rad), which assays only soluble proteins obtained after a detergent treatment with Triton X-100 (Merck). The only integral proteins determined were the anion channel protein and a group of glycoporphins; peripheral or submembrane proteins (spectrin, actin, and proteins 4.1 and 4.9), which are insoluble in nonionic detergents such as Triton X-100, were not measured. Concentrations of proteins were measured from a calibration curve using an animal serum as the standard (Biotrol 33 plus, Biotrol). Results were expressed in micrograms per microliter suspension.

**Lipids and Apolipoproteins**

Levels of serum total cholesterol (TC), HDL cholesterol (HDL-C) after precipitation of LDL cholesterol and VLDL, and triglycerides (TG) were measured using phosphotungstic acid/magnesium chloride reagent. LDL cholesterol (LDL-C) was calculated according to Friedewald’s formula (which is accurate for triglyceride levels <4.5 mmol/L) as follows: LDL-C=TC−HDL-C−(TG/2.2).

**Ancillary Study: Removal of Sialic Acid From Erythrocytes**

Washed erythrocytes from 5 healthy subjects, kept at a constant adjusted hematocrit level (0.4), were incubated several times at 37°C with 10 mL/m/L neuraminidase (from Clostridium perfringens; activity, 0.5 IU/mg protein using N-acetyleneuraminyl-lactose; Sigma) in PBS during gentle shaking. After incubation, the suspension was cooled and the erythrocytes were washed with cold PBS twice. In each sample, aggregation measurements were performed with dextran 70 (40 g/L), and membrane sialic acid content was measured as described above.
Sialic Acid and Erythrocyte Rheology in Humans

**Statistical Analysis**

Variables are expressed as mean±SD. Comparisons among the 4 groups were performed with ANOVA. Comparisons between erythrocytes before and after treatment with neuraminidase were performed with paired Student’s t test. The linear correlations between parameters were performed with the least-squares method. The statistical analysis was carried out using an Apple Macintosh computer with Statview II (Abacus Concepts Inc) software. Statistical significance was considered to be *P*≤0.05.

**Results**

Table 1 shows the clinical characteristics of normotensive and hypertensive subjects, with each group separated according to cholesterol level into LC and HC subgroups. Age, body mass index, gender ratio, and percentage of current smokers were similar in the normotensive and hypertensive groups. By definition, hypertensives had higher blood pressure than normotensive subjects, and patients with high cholesterol had higher serum total cholesterol, LDL cholesterol, and triglyceride levels than subjects with low cholesterol.

**Normotensive Subjects**

In both LC and HC subgroups, there was a marked effect of cell density on the DSRT and aggregation time. Table 2 shows the progressive and significant (*P*<0.001) increase of DSRT with enhancing erythrocyte density. Compared with LC, erythrocytes of the HC subgroup were characterized at each density by an increased DSRT (*P*<0.001). The aggregation time decreased progressively with erythrocyte density (*P*<0.05) but was similar at each density in both LC and HC subgroups (Table 2).

The erythrocyte sialic acid content decreased progressively with increasing erythrocyte density in both LC and HC subgroups (Table 3). Furthermore, when compared with LC, erythrocytes of the HC subgroup were characterized at each density by a reduced sialic acid content (*P*<0.001). We observed a significant decrease (*P*<0.001) in integral membrane protein content when the erythrocyte density increased in both LC and HC subgroups (Table 3). However, no significant difference was shown in integral membrane protein content between LC and HC subgroups, whatever the density of erythrocytes. An enhanced total serum sialic acid (*P*<0.001) was observed in the HC compared with the LC subgroup (2093.9±76.2 versus 1980.7±69.0 μmol/L).

**TABLE 1. Characteristics of Study Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive Subjects</th>
<th></th>
<th>Hypertensive Subjects</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC (n=12)</td>
<td>HC (n=12)</td>
<td>LC (n=12)</td>
<td>HC (n=12)</td>
<td></td>
</tr>
<tr>
<td>M/F ratio</td>
<td>7/5</td>
<td>9/3</td>
<td>7/5</td>
<td>10/2</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>44±10</td>
<td>45±8</td>
<td>47±7</td>
<td>51±9</td>
<td>NS</td>
</tr>
<tr>
<td>(28–59)</td>
<td>(29–57)</td>
<td></td>
<td>(35–56)</td>
<td>(34–65)</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24±3</td>
<td>24±4</td>
<td>26±3</td>
<td>26±3</td>
<td>NS</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>128±8</td>
<td>129±9</td>
<td>159±15</td>
<td>164±14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80±10</td>
<td>81±10</td>
<td>104±8</td>
<td>102±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>33</td>
<td>42</td>
<td>42</td>
<td>33</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid levels, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.90±0.54</td>
<td>6.96±0.72</td>
<td>5.04±0.80</td>
<td>6.84±0.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.93±0.47</td>
<td>1.36±0.76</td>
<td>0.81±0.86</td>
<td>1.45±0.69</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.31±0.27</td>
<td>1.13±0.22</td>
<td>1.42±0.43</td>
<td>1.40±0.34</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.16±0.50</td>
<td>5.26±0.64</td>
<td>3.17±0.73</td>
<td>4.78±0.60</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SD.

**TABLE 2. DSRT and Aggregation Time for Density-Separated Erythrocytes Suspended in Dextran 70 in Normotensive and Hypertensive Subjects**

<table>
<thead>
<tr>
<th>Erythrocyte Density</th>
<th>DSRT, s⁻¹</th>
<th>Aggregation Time, s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Middle</td>
</tr>
<tr>
<td>Normotensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>58.1±4.5</td>
<td>63.4±5.5†</td>
</tr>
<tr>
<td>HC</td>
<td>68.8±7.3†</td>
<td>75.6±5.9†</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>61.3±4.2</td>
<td>67.4±4.2†</td>
</tr>
<tr>
<td>HC</td>
<td>67.3±4.4†</td>
<td>74.3±6.3†</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P*<0.05; †*P*<0.001 compared with low erythrocyte density; ‡*P*<0.001 compared with subjects with low cholesterol.
We observed a significant increase in DSRT among both the normotensive and the hypertensive subjects, subjects belonged to the LC or HC subgroups. However, hypertensive subjects showed no significant difference whether in density.

The comparison of DSRT, aggregation time, and erythrocyte density decreased with erythrocyte density. In addition, erythrocytes of the HC subgroup had a higher DSRT than those of the LC subjects. The aggregation time decreased with erythrocyte density. The Figure shows the behavior of DSRT as a function of increased time of incubation with neuraminidase (6.1% at 5 min, 14.9% at 7 min, 17.35% at 10 min, 26.3% at 15 min, and 33.5% at 20 min). A significant linear increase of DSRT was observed with membrane sialic acid content reduction. The membrane sialic acid content decreases progressively as a function of increased density.

### Hypertensive Subjects
Results observed in hypertensive patients were similar to those obtained in normotensive subjects. Table 2 shows that in both LC and HC subgroups, DSRT increased with erythrocyte density. In addition, erythrocytes of the HC subgroup had a higher DSRT than those of the LC subjects. The aggregation time decreased with erythrocyte density (P<0.05) but was similar when the LC subgroup was compared with the HC subgroup.

The erythrocyte sialic acid content decreased with erythrocyte density regardless of the cholesterol level (Table 3). Moreover, membrane sialic acid content was lower in the HC than in the LC subgroup at each erythrocyte density. The concentration of integral membrane proteins decreased progressively (P<0.001), but regardless of the density, erythrocytes had a similar integral membrane protein content in both LC and HC subgroups. There was no difference between LC and HC groups for total serum sialic acid (2045.1±81.9 versus 2105.7±64.3 μmol/L).

### Normotensive and Hypertensive Subjects
The comparison of DSRT, aggregation time, and erythrocyte sialic acid content values between normotensive and hypertensive subjects showed no significant difference whether subjects belonged to the LC or HC subgroups. However, among both the normotensive and the hypertensive subjects, we observed a significant increase in DSRT (P<0.001) and a significant decrease in erythrocyte sialic acid content (P<0.001) in the HC subgroups compared with the LC subgroups. Thus, both DSRT and erythrocyte sialic acid content were significantly different in hypercholesterolemic subjects only. Total serum sialic acid was increased (P<0.05) in hypertensive patients, whatever the cholesterol level, compared with the normotensive LC subgroup. There was no difference in integral membrane protein content between normotensive and hypertensive subjects.

Table 4 shows that in the overall population, sialic acid content at each erythrocyte density correlated negatively with levels of total and LDL cholesterol, triglycerides, and DSRT. In addition, total serum sialic acid was inversely related with membrane sialic acid content only for erythrocytes with low and middle densities. Because total and LDL cholesterol levels are highly related (r=0.95; P<0.0001), only LDL cholesterol was included in the multiple regression analysis (Table 5), together with triglycerides, age, and blood pressure, to determine the main risk factors influencing erythrocyte sialic acid content. This analysis showed that 49%, 43%, and 54% of the variance of sialic acid content of erythrocytes of low, middle, and high density, respectively, could be explained by LDL cholesterol and triglyceride levels.

### Ancillary Study: Removal of Sialic Acid From Erythrocytes
The Figure shows the behavior of DSRT as a function of membrane sialic acid content reduction. The membrane sialic acid content decreases progressively as a function of increased time of incubation with neuraminidase (6.1% at 5 min, 14.9% at 7 min, 17.35% at 10 min, 26.3% at 15 min, and 33.5% at 20 min). A significant linear increase of DSRT was observed with membrane sialic acid content reduction. The DSRT increase was observed as early as the 6.1% decrease in membrane sialic acid content (P<0.05).

### Discussion
In the present study, we investigated dextran-induced aggregation behavior of erythrocytes and its relation with membrane sialic acid content by erythrocyte density.

### TABLE 3. Membrane Sialic Acid and Integral Protein Content for Density-Separated Erythrocytes Suspended in Dextran 70 in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Membrane Sialic Acid, μmol/g Integral Membrane Protein</th>
<th>Integral Membrane Protein, μg/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Middle</td>
</tr>
<tr>
<td>Normotensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>232.7±11.4</td>
<td>202.4±13.6*</td>
</tr>
<tr>
<td>HC</td>
<td>214.8±9.6†</td>
<td>179.6±16.8†</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC</td>
<td>229.5±7.5</td>
<td>198.6±14.5†</td>
</tr>
<tr>
<td>HC</td>
<td>208.8±6.6†</td>
<td>175.7±8.4†</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.001 compared with low erythrocyte density; †P<0.001 compared with subjects with low cholesterol.

### TABLE 4. Correlation Coefficients Between Erythrocyte Sialic Acid Content by Erythrocyte Density and Clinical, Blood, and Rheological Variables in Overall Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>−0.16</td>
<td>−0.26</td>
<td>−0.08</td>
</tr>
<tr>
<td>Body mass index</td>
<td>−0.10</td>
<td>−0.13</td>
<td>−0.13</td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>−0.09</td>
<td>−0.08</td>
<td>−0.07</td>
</tr>
<tr>
<td>Diastolic pressure</td>
<td>−0.02</td>
<td>−0.03</td>
<td>−0.05</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.65*</td>
<td>−0.64*</td>
<td>−0.67*</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.62*</td>
<td>−0.60*</td>
<td>−0.64*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.47*</td>
<td>−0.43†</td>
<td>−0.48*</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.13</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Total serum sialic acid</td>
<td>−0.38†</td>
<td>−0.49*</td>
<td>−0.23</td>
</tr>
<tr>
<td>DSRT</td>
<td>−0.63*</td>
<td>−0.61*</td>
<td>−0.60*</td>
</tr>
</tbody>
</table>

*P<0.001; †P<0.01.
brane sialic acid content in essential hypertension with or without primary hypercholesterolemia. The use of dextran and not fibrinogen, which is a physiological bridging molecule, enabled us to study the specific influence of erythrocyte parameters independently of plasma environment. In addition, this study also examined the influence of the aging process of erythrocytes on both aggregation and membrane sialic acid content.

In both hypertensive and normotensive populations, our results show an increase of DSRT during in vivo aging of human red blood cells, suggesting marked direct effects of cellular factors on the aggregation of age-separated cells. This observation is in line with data in the literature reporting an increased dextran- or autologous plasma–induced aggregation of senescent erythrocytes. An original finding of our study was that the increased DSRT was linked to the cholesterol level, independently of blood pressure, regardless of the age-related changes of cells. The fact that the force needed to break the aggregates was related to hypercholesterolemia and not to hypertension is not in contradiction with a previously observed elevation of DSRT in the whole blood of hypertensive subjects, in which the enhanced level of plasma proteins such as fibrinogen or globulins may strongly influence the bridging aggregation force between red cell membranes.

The results of the present work also show that a hypercholesterolemia-associated increase in DSRT was observed even in the younger red blood cells, suggesting an intrinsic cellular abnormality. It is known that geometric as well as mechanical properties and internal viscosity are altered with aging of erythrocytes. Smaller cell volume, surface area, or deformability during aging have been reported, whereas hemoglobin content remained constant, so the cytoplasmic hemoglobin concentration increased as did cell density. The decrease in surface area might be explained by a reduction in the concentration of phospholipids or a loss of membrane fragments, including proteins. In the present study, the important difference in membrane sialic acid content between young and old erythrocytes (30%) contrasts with the small difference in membrane protein content (8%). This may be explained by the fact that only proteins related to the anion channel protein and glycoporphins, and not the total protein content, were determined. In addition, Greenwalt et al have reported that the area loss is most likely accompanied by some thickening or increase in density of the membrane underlying the protein network. Moreover, these authors have observed the presence of a new glycoprotein on the surface of older cells, which was probably generated by modification of preexisting proteins. These observations are in agreement with the small decreased integral membrane protein content observed during erythrocyte aging in both normotensive and hypertensive subjects. However, Seaman et al have stated that the loss of sialic acid can be explained on the basis of the loss of membrane area during in vivo aging.

A limitation of our study was the lack of erythrocyte mean corpuscular volume measurements in subpopulations of erythrocytes, which did not allow the surface area calculation during cell aging. Nevertheless, in young erythrocytes from hypercholesterolemic subjects, where the membrane surface area would not yet be reduced by in vivo aging, the membrane sialic acid content was significantly decreased and associated with an increased DSRT when compared with young erythrocytes from subjects with low cholesterol.

The reduced membrane sialic acid content of old versus young erythrocytes in both hypertensive and normotensive subjects could partially explain the increase of DSRT with erythrocyte in vivo aging. Indeed, a negative relation between sialic acid and DSRT was demonstrated in the present study with age-related changes. However, the relevance of this finding to erythrocyte aggregation is complicated by the results of earlier studies of cell electrophoresis in saline or buffer that reported no differences of density-surface charge between old and young erythrocytes despite a loss of membrane sialic acid content. However, an increased electrophoretic mobility with cell age has been observed in erythrocytes suspended in dextran or plasma. The differences detected in polymer solutions are most likely a consequence of cell- and polymer-specific interactions. The decreased membrane sialic acid content may be due to a possible alteration of glycoporphin A, a major sialoglycoprotein of human erythrocyte membrane that may contribute to the rouleaux formation. Removal of sialic acid residues of the saccharide chains of glycoporphin A during red cell aging results in exposure of a new antigen recognized by immunoglobulin G, leading to the normal process of elimination of aged cells by macrophage. It has been shown that the
sialosaccharide chains of glycoprotein A are also recognized by macrophage scavenger receptors, which selectively bind and take up chemically modified LDL such as oxidized LDL. The cell age–dependent loss of sialic acid may be also related to an alteration of membrane permeability to calcium. Indeed, sialic acid of membrane glycoprotein is the main calcium-binding component of the outer surface of the membrane. Furthermore, it has been reported that an accumulation of calcium inside the erythrocyte may lead to changes in cytoskeletal conformation and viscoelasticity of the membrane, so the cells would become less deformable.

Another original finding of our study was the lack of difference in erythrocyte sialic acid content between normotensive and hypertensive subjects, whereas hypercholesterolemia was associated with decreased erythrocyte sialic acid content in both normotensive and hypertensive groups compared with subjects with low cholesterol. The observation of similar levels of membrane sialic acid content in both hypertensive and normotensive groups was in good agreement with the literature data reporting that total sialic acid content in erythrocyte membrane did not differ between normotensive patients and those with essential or renal hypertension. To determine whether the decrease in membrane sialic acid content in hypercholesterolemia could play a direct role in erythrocyte aggregation, we carried out an additional study using neuraminidase to reduce membrane sialic acid content by the amount of the difference seen between subjects with low and high cholesterol. We observed an increase of DSRT associated with the decrease of membrane sialic acid content, suggesting a specific effect of sialic acid. This result was in good agreement with previous studies and further suggested a specific effect on the increased DSRT in hypercholesterolemic subjects.

The finding that levels of triglyceride, LDL cholesterol, and membrane sialic acid are negatively related confirms results of our previous study. Moreover, in the present work, we observed that the high variance of membrane sialic acid content, beginning even in young erythrocytes, was mainly explained by the levels of triglycerides and LDL cholesterol. Triglycerides may be involved in the decrease of membrane sialic acid content by an inhibition of sialyltransferase activity, the enzyme responsible for the transfer of NANA residues on the glycoproteins. Another mechanism that may also participate in the decreased erythrocyte sialic acid content is the activation by triglycerides of sialidase linked to the membrane by a glycosylphosphatidylin anchor, able to remove sialic acid of intact erythrocytes. Regarding LDL, an interaction with membrane erythrocyte that is not altered by sialic acid removal has been observed. A biological function proposed for this binding may be an exchange of cholesterol between the cell membrane and lipoproteins. Nevertheless, it has not been demonstrated whether an exchange of sialic acid with LDL, which is a sialolipoprotein, exists. Contrasting with the decrease in membrane sialic acid content, an enhanced serum sialic acid was observed in hypertensive and/or hypercholesterolemic subjects. Furthermore, serum sialic acid correlated negatively with membrane sialic acid content of young and middle-age erythrocytes. The elevation of serum sialic acid could result from a release of sialic acid bound from membrane glycoprotein A, but the mechanism is unclear. An increased fibrinogen level, as observed in hypertension and hypercholesterolemia, may induce an enhanced serum sialic acid content. It was also observed that serum sialic acid level was affected by triglycerides and LDL cholesterol and was increased in atherosclerotic patients or after myocardial infarction. The inverse relationship between increased serum sialic acid and decreased membrane sialic acid and DSRT may be one of the mechanisms that links blood rheological modifications to cardiovascular risk factors, as suggested by long-term follow-up indicating that serum sialic acid is a predictor of cardiovascular mortality.

In conclusion, the aging process of erythrocytes was accompanied by a progressive increase of DSRT related to a decrease of membrane sialic acid. In addition, an increased DSRT and an altered red blood cell membrane sialic acid content were observed only in patients with hypercholesterolemia regardless of the age of cells, suggesting that in hypertension, enhanced erythrocyte aggregation is influenced mainly by increased macromolecule bridging force.

Acknowledgments

This work was supported by a grant from the Institut National de la Santé et de la Recherche Médicale (Contrat Recherche INSERM No. 4U010B). The authors express their gratitude to Dr Véronique Atger for constructive discussion of this article. We wish to thank Nelly Poulain for her excellent technical assistance and Isabelle d’Argentre for her secretarial assistance.

References

Sialic Acid and Erythrocyte Rheology in Humans


Erythrocyte Disaggregation Shear Stress, Sialic Acid, and Cell Aging in Humans
Alexandra L. Hadengue, Muriel Del-Pino, Alain Simon and Jaime Levenson

Hypertension. 1998;32:324-330
doi: 10.1161/01.HYP.32.2.324

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/32/2/324

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/