Blood Filtrability in Cerebrovascular Disorders, With Special Reference to Erythrocyte Deformability and ATP Content

SHIGERU SAKUTA, M.D.

SUMMARY Erythrocyte deformability was determined in 48 patients with cerebrovascular disease and 116 healthy controls as an index of blood filtrability in microcirculation. Hemo-rheological and blood chemical changes were also studied to assess their effect on filtrability. Erythrocyte deformability, determined by Reid's method, was expressed as the deformability index (DI). DI and ATP levels decreased with advancing age in controls and patients; these levels were lower in patients. In the controls, DI was inversely proportional to hematocrit, hemoglobin, fibrinogen, and lactate; in patients, it was directly proportional to albumin. There were no significant differences in DI and ATP levels in patients with cerebral infarction and cerebral hemorrhage. The values for DI and ATP levels were the same in arterial and venous blood.

SEVERAL STUDIES dealing with occlusive vascular diseases of the heart and extremities, have shown that hemo-rheological considerations are very important in approaching microcirculatory disturbances. Blood viscosity is a complex of factors, including plasma viscosity, erythrocyte deformability and volume, blood coagulability, and aggregation of erythrocytes and platelets.

Reid et al. have developed a simple, clinically applicable method for measuring erythrocyte deformability. We used their method to investigate hemo-rheological changes in patients with cerebrovascular disorders by measuring erythrocyte deformability and several factors responsible for changes in blood viscosity.

Subjects and Methods

The present study evaluated 48 patients with cerebrovascular disease ranging in age from 43–88 years. In all patients at least 6 months had elapsed since their stroke. None of the patients was receiving drugs which may have affected erythrocyte deformability or other viscosity factors. There were 116 healthy controls: 32 were less than 39, 84 were more than 40 years of age. All subjects resided in 2 rural communities in Hirosaki, where annual screening for circulatory diseases has been carried out for many years under the auspices of Hirosaki University.

Fasting blood samples were drawn from the antecubital vein with 3.8% sodium citrate solution as the anticoagulant. To evaluate differences in hemo-rheological and chemical properties between venous and arterial blood, additional samples were obtained from the femoral artery of 14 controls soon after venipuncture. Statistical analysis was by the paired t-test.

Blood filtrability was measured by the filtration technique of Reid et al. The apparatus consisted of a simple hydrostatic system which produces a pressure gradient of 20 cm of water across a membrane filter (Nuclepore Corp.) contained in a membrane holder (Nuclepore, Pop-Top, 13 mm). The holder had an intake and outlet connection accommodating a plungerless graduated 1 ml syringe containing 1 ml of blood. Whole blood was filtered through the membrane via 5 μm diameter cylindrical channels under a pressure of 20 cm of water. Measurements were made within 30 minutes of venipuncture at room temperature. Each sample was filtered 3 times and the mean value was used for further statistical analysis. This method was reproducible; in preliminary examinations, the coefficient of variation was 4.6%. Results were expressed as the volume of blood filtered per minute and designated as the deformability index (DI).

Adenosine triphosphate (ATP), lactate and pyruvate levels in whole blood were determined by the method of Bücher, Gutmann and Wahlefeld and Czok and Lamprecht, respectively. Hematocrit and hemoglobin were measured by conventional autoanalytical methods. Fibrinogen, α1-antitrypsin, α1-macroglobulin and antithrombin III were determined by single radial immunodiffusion methods, serum total protein and albumin by the biuret method, lactate dehydrogenase (LDH) activity by the method of Wróblewski and La Due. Serum total cholesterol was assayed by the modified method of Zak-Henly, triglyceride by the acetylaceetone method, lipid peroxide by Satoh's method. Serum VLDL, LDL, and HDL-cholesterol were measured by the heparin-Ca++ precipitation method. Platelet adhesiveness was determined by the modified method of Salzman. Statistical evaluation was by the Student's t-test for unpaired samples.

Results

1. DI and ATP Levels in Arterial and Venous Blood

DI, determined for 14 arterial and venous blood control samples, was the same at 0.80 ± 0.08 ml/min. ATP levels in arterial and venous control samples were 28.9 ± 3.4 mg/dl and 29.1 ± 3.1 mg/dl, respec-
tively. Analysis by the paired t-test revealed that neither DI nor the ATP levels were indicative of arterio-venous gap.

2. DI in Controls and Patients

In controls and patients, DI decreased with advancing age (table 1). In controls 20–29 years of age, DI was significantly higher, and in all subjects over 70 years it was significantly lower, than in all other age groups. In patients, DI was lower than in controls of the same age range; in 50–69-year-old patients, this difference was statistically significant.

3. ATP Levels in Controls and Patients

The ATP levels also tended to decrease with advancing age in controls and patients (table 2). In 20–29-year-old controls, ATP was significantly higher than in the other age groups; in controls over 70 years of age, it was significantly lower than in the other age groups, excepting 40–49 and 60–69-year-old controls. In patients, there were no significant age-related differences in the ATP levels; however, these values were lower than in control subjects, irrespective of the age groups. Like DI, the ATP levels in 50–69-year-old patients were significantly different from controls of the same age range.

4. DI and ATP Levels in Cerebral Hemorrhage and Infarction

The 48 patients with cerebrovascular disease were classified into those with cerebral hemorrhage (10) and those with cerebral infarction (38). Their DI and ATP levels were compared. As shown in table 3, DI and ATP values were lower in patients with cerebral infarction, but the difference was not statistically significant.

5. Relationship Among DI and Several Laboratory Values

In the controls and patients, DI was directly proportional to ATP levels (fig.). Coefficients of the correlation between DI and various laboratory values are presented in table 4. In controls, DI was inversely proportional to hematocrit, hemoglobin, lactate, and fibrinogen contents; in patients it was directly proportional to albumin and A/G ratio.

Furthermore, in the controls, albumin was directly proportional to the hematocrit and hemoglobin concentration (N = 61, r = + 0.386, p < 0.01 and N = 61, r = + 0.427, p < 0.01, respectively).

Discussion

Hemo-rheological observations on separated blood components dispersed in buffer solution or plasma have been reported. Red cells and plasma proteins influence blood flow. Clinical studies of hemo-rheological changes should include observations on the effect of different components of blood cells and plasma. The method of Reid et al. is clinically applicable because it is a simple procedure using an easily prepared apparatus. In a previous paper, we

**Table 3** DI and ATP Levels in Cerebral Hemorrhage and Cerebral Infarction

<table>
<thead>
<tr>
<th>Case</th>
<th>DI (ml/min)</th>
<th>ATP (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M ± SD</td>
<td>M ± SD</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>10</td>
<td>0.77 ± 0.21</td>
</tr>
<tr>
<td>Infarction</td>
<td>38</td>
<td>0.67 ± 0.14</td>
</tr>
</tbody>
</table>

**Table 1** DI (ml/min) in Controls and Patients

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases</th>
<th>M ± SD</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>9</td>
<td>1.16</td>
<td>0.21</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>0.98</td>
<td>0.12</td>
</tr>
<tr>
<td>30-39</td>
<td>23</td>
<td>0.98</td>
<td>0.19</td>
</tr>
<tr>
<td>40-49</td>
<td>34</td>
<td>0.97</td>
<td>0.19</td>
</tr>
<tr>
<td>50-69</td>
<td>24</td>
<td>0.85</td>
<td>0.15</td>
</tr>
<tr>
<td>60-69</td>
<td>17</td>
<td>0.65</td>
<td>0.20</td>
</tr>
<tr>
<td>70-</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** ATP levels (mg/dl) in Controls and Patients

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases</th>
<th>M ± SD</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>9</td>
<td>32.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>27.8</td>
<td>3.6</td>
</tr>
<tr>
<td>30-39</td>
<td>23</td>
<td>26.0</td>
<td>4.8</td>
</tr>
<tr>
<td>40-49</td>
<td>34</td>
<td>27.2</td>
<td>5.4</td>
</tr>
<tr>
<td>50-69</td>
<td>24</td>
<td>26.2</td>
<td>4.6</td>
</tr>
<tr>
<td>60-69</td>
<td>17</td>
<td>21.9</td>
<td>7.6</td>
</tr>
<tr>
<td>70-</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Cases</th>
<th>M ± SD</th>
<th>M ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>9</td>
<td>22.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>20.6</td>
<td>3.1</td>
</tr>
<tr>
<td>30-39</td>
<td>23</td>
<td>20.3</td>
<td>3.1</td>
</tr>
<tr>
<td>40-49</td>
<td>34</td>
<td>19.0</td>
<td>3.3</td>
</tr>
<tr>
<td>50-69</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70-</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.01: statistically significant differences from control value. | *p < 0.01: statistically significant differences from control value. | *p < 0.01: statistically significant differences from control value. | *p < 0.01: statistically significant differences from control value. | *p < 0.01: statistically significant differences from control value. |
confirmed the high reproducibility of the results. The negative pressure of 20 cm of water used in the present study is about 15 mm Hg — which is close to the pressure of the capillary system in the brain. The DI value determined in vitro may reflect an aspect of the microcirculatory blood flow in the brain. In order to pass through capillaries with a smaller diameter than that of the cells, mammalian erythrocytes must deform. Erythrocyte deformation may begin in capillaries in a proximal portion near the arterioles. Although DI should be determined in arterial blood, in many subjects there are sampling difficulties. Our results on DI and ATP values disclosed an absence of arterio-venous difference so that venous blood values may be applicable in clinical practice.

The age-related decrease in DI and ATP levels suggests that the formation of ischemic lesions in aged subjects with marked arteriosclerotic changes, may be accelerated by diminished blood flow. Thus, the hemo-rheological approach to cerebrovascular disorders, in which focal brain ischemia is of importance, is reasonable.

Although the influence of erythrocyte deformability on hemo-rheological properties has been suggested, few reports have dealt with the changes in erythrocyte deformability in cerebrovascular disorders. Ott et al.
noted high blood viscosity in patients with cerebral infarction in the acute stage, but they made no reference to erythrocyte deformability.

DI is affected by the blood viscosity which, in turn, is related to erythrocyte deformability and the plasma.\textsuperscript{25, 26} The effects of hematocrit and hemoglobin content on DI are clearly demonstrated in the inversely proportional relationships between these 2 parameters and DI in control subjects (table 4).

ATP plays a major role in the energy metabolism of erythrocytes, and according to Weed et al.\textsuperscript{27} and Meiselman and Baker,\textsuperscript{27} ATP depletion was associated with a decrease in the deformability of erythrocytes. The close relationship in the present study between DI and ATP values is indicative of the considerable influence of the ATP level on DI.

The focal diminution of blood flow brought about by arteriosclerotic changes results in increased blood viscosity and a fall in the pH of stagnant blood. Plasma viscosity, affected in the controls by plasma proteins such as albumin, globulin and fibrinogen, probably further affects the changes in blood filtrability. In our controls, DI was inversely correlated with fibrinogen and in the patients, it was directly proportional to albumin and the A/G ratio (table 4). Wells et al.\textsuperscript{28} have suggested that albumin inhibits the effects of fibrinogen with respect to both viscosity and red cell aggregation.

The relationship between DI and plasma proteins may be explained as follows: In the controls, fibrinogen may exert a strong effect on DI while the effect of albumin may be weak. The directly proportional relationship between DI and albumin in patients may mean that a decrease in DI, associated with high fibrinogen levels, is inhibited by albumin. Some reports have indicated the significance of albumin in cerebrovascular disorders.\textsuperscript{29-31} The close relationship in control subjects between albumin and hematocrit and hemoglobin is evidence for an indirect association of albumin with the pathophysiological status brought about by abnormal hemo-rheological conditions. Kimura et al.,\textsuperscript{32} reported an epidemiological study, indicating that low dietary proteins were associated with a high incidence of cerebrovascular accidents in hypertensive individuals. The present data provides further support of the significance of albumin in these disorders.

La Celle\textsuperscript{33} observed ATP-depletion and a resultant decrease in erythrocyte deformability in blood stored in acid citrate dextrose solution. De Mendonca et al.\textsuperscript{34} reported a depletion of the ATP level in erythrocytes if the suspending medium had a low pH. In the present study, a decrease in erythrocyte deformability was accompanied by the acceleration of the anaerobic metabolism. Based on these considerations, it is probable that erythrocyte deformability may be impaired in ischemia when acidosis occurs with anaerobic metabolism and an accelerated production of lactate.

That arteriolar thrombus formation is initiated by the adhesion and aggregation of platelets is widely recognized. Danta\textsuperscript{35} reported a high level of platelet adhesiveness in patients with cerebral infarction.

Acheson et al.\textsuperscript{36} have questioned the role of platelets and the significance of platelet adhesiveness in cerebral infarction.

The interaction between plasma and cellular components in thrombus formation is of importance. In the present study, DI was not related to platelet adhesiveness, a finding which may indirectly support the view that plasma fibrinogen and albumin are more important in microcirculatory disturbances by impaired erythrocyte deformability than platelet adhesiveness. Chien,\textsuperscript{37} who reviewed the data of others,\textsuperscript{36-37} suggested that erythrocytes are drawn into the central core of the blood flow and that fluctuations in the erythrocyte paths lead to radial platelet dispersion. The lack of a relation between DI and platelet adhesiveness noted in the present study may be ascribable to differences in the flow patterns of erythrocytes and platelets.

Impaired deformability of erythrocytes is not specific for cerebrovascular disorders as this phenomenon has also been reported in peripheral vascular disorders.\textsuperscript{38, 39} A low DI value may be indicative of the existence of ischemia and/or lesions in some part of the body. ATP-depletion, which leads to impaired erythrocyte deformability,\textsuperscript{37} supports the possibility that there may be metabolic depletion. DI and ATP levels may be possible indicators of the efficacy of treatment and the extent of microcirculatory deterioration.

Acknowledgment

I am grateful to Professor Seitoku Mizuno, M.D., and Dr. Shigeru Takamatsu for their helpful advice. I am also indebted to Miss U. Petralia for editing the manuscript.

References

13. Sakurabayashi I, Kawai T: Clinical aspects of quantitative


Blood filtrability in cerebrovascular disorders, with special reference to erythrocyte deformability and ATP content.

S Sakuta

doi: 10.1161/01.STR.12.6.824

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/12/6/824