Leukocyte Rheology in Recent Stroke

E. Ernst, A. Matrai, and F. Paulsen

Eighteen patients with recent ischemic stroke were compared with an equal number of matched controls. Standardized suspensions of red cells as well as of red and white cells were filtered in a new filtration apparatus capable of discriminating between cell deformability and filter occlusion. Results show that red cell deformability, although slightly lower than in controls, is not significantly altered in stroke patients. Filter occlusion, however, was significantly higher in patients when red and white cell suspensions were filtered, but not when red cell suspensions were used, suggesting that white cell filterability is impaired after stroke, which could be due to decreased deformability and/or increased adhesiveness of leukocytes. Slowed white cell passage may also occur in the living microcirculation and may present an obstacle to nutritive flow in exchange vessels, possibly contributing to local ischemia and tissue necrosis after stroke. (Stroke 1987;18:59-62)

Subjects and Methods

Eighteen patients (11 women, 7 men) with a history of recent ischemic stroke were randomly selected from our stroke rehabilitation units. Average age was 61 ± 4 years. The acute event dated back 3–4 months and had in all cases been confirmed by computerized tomography (CT) scan during the acute phase. Concomitant diseases or cardiovascular risk factors were hypertension (n = 7), hyperlipoproteinemia (n = 2), smoking (n = 5), and diabetes (n = 3). One patient had suffered a myocardial infarction 4 years earlier and 1 had a history of claudication. Controls consisted of 18 patients matched for age (60 ± 5 years), sex (10 women, 8 men), and concomitant diseases or cardiovascular risk factors: hypertension (n = 8), hyperlipoproteinemia (n = 3), smoking (n = 4), and diabetes (n = 4). There were 2 individuals with a history of myocardial infarction and 2 suffering from claudication. Average body mass index was 26 in patients and 25 in controls. No individuals taking hemorheologically active drugs were admitted into the study.

Ten ml of venous blood was drawn from the supine patient after an overnight fast with minimal occlusion or suction and anticoagulated with 1.5 mg EDTA/ml. All measurements were terminated within 1 hour of drawing blood.

Preparation of Cell Suspensions for Filtration

Two ml of blood were used for erythrocyte and leukocyte counts (TOA cell counter) and subsequently for the recombination of blood cell suspensions with controlled numbers of cells in buffer. Eight ml of blood were centrifuged for 15 minutes at 3500g; theuffy coat was removed and discarded. Two ml of packed red cells were aspirated from the middle of the centrifuged red cell column and counted in the cell counter. This technique discards more than 98% of the leukocytes and 97% of the platelets, thus enabling platelet counts to be low in the following experiments. White cell count in this red cell mass was invariably less than 20/μl (Buerker chamber).

Standardized red cell suspensions (RCS's) containing 10^6/μl red cells, <0.03 x 10^3/μl leukocytes, and <4 x 10^3/μl platelets were prepared from each patient’s sample by adding 4500 x 10^6 red cells from the packed red cells to 4 ml phosphate-buffered, calcium-free saline solution. Standardized red and white cell suspensions (RWCS's) containing 10^6/μl red cells, 400/μl leukocytes and <20 x 10^3/μl platelets were composed by adding whole blood (from the uncentrifuged 2 ml) and packed red cells (from the centrifuged sample) to the buffer. RCS's and RWCS's contained roughly the same amount of plasma and EDTA (which was sufficient for complete anticoagulation) and thus differed only in terms of cell counts. The required volumes were calculated individually for each patient according to the cell counts obtained from whole blood and packed red cells. The amount of plasma trapped in the red cell mass was assumed to be 10% of the volume in each case. Three RCS's and 3 RWCS's were prepared from each patient's blood sample. Coefficients of variation for cell counts based on 3 measurements of identically reconstituted RCS's or RWCS's samples were 2% for red cell, 3% for leukocyte, and 3% for platelet count.
Filtration
The filtration apparatus has recently been described and its accuracy verified.\textsuperscript{7,8} In the "St. George's Filtrometer" (Carri Med, Dorking, England) cell suspensions are filtered at constant low pressure through a vertical filter (Nuclepore, batch 54P4B10, nominal pore diameter 5 \(\mu m\), diameter of filter 13 mm, effective filtration area 0.78 cm\(^2\)). In our prototype filtration rates are measured by optical detection of the retreating meniscus of the fluid in front of the filter at 39 sites in a horizontal glass tube.\textsuperscript{9} The filtered volume between each detector is 23 \(\mu L\). Thus the filtration process at the very beginning of filtering is quantified. The pressure head chosen to give satisfactory flow is 600 Pa. Variation of hydrodynamic conductivity is \(\pm 7\%\) in the batch of filters used.

The filtration technique has been described in detail elsewhere.\textsuperscript{9,10} Initial filtration rate is measured by extrapolating the filtration rate (in steps of 23 \(\mu L\)) of cell suspensions to zero time.\textsuperscript{9} Red cell deformability is expressed as incremental red cell volume (IrcV): 
\[ \text{IrcV} = \frac{(F_0 - F_m)}{F_0} \times \frac{1}{RCC} \]
where \(F_0\) and \(F_m\) are the initial filtration rates of the cell suspension and the suspending medium,\textsuperscript{9} and RCC is the red cell count in the suspension. IrcV is determined by the relative viscosity of red cells in the filter pores and is thus a measure of red cell deformability. It reflects the flow of suspending medium, hindered by the transit of an average red cell through an average pore.\textsuperscript{11} This method minimizes the effects of variation in red cell volume and the possible error in measuring low hematocrits. Filter occlusion is measured by the decrease in filter conductance during filtration of the first 0.25 ml.\textsuperscript{9} The concentration of particles initially occluding the pores of the filter is expressed in liters per milliliter as 
\[ \text{CP} = N_p \times 4 \times \left( \frac{F_0 - F_{0.25}}{F_0} \right) \]
where \(N_p\) is the initial number of free pores (3 \(\times 10^9\)); \(F_0\) is the filtration rate after 0.25 ml of the suspension has been filtered. \(F_0 - F_{0.25}\)/\(F_0\) is hence the relative decrease in filtration rate due to filter occlusion when 0.25 ml of suspension has been filtered.\textsuperscript{9}

Three RCS's and 3 RWCS's of every sample were filtered in duplicate within 10 minutes after suspending the cells in buffer (12 filtrations from each blood sample; 6 RCS's and 6 RWCS's). The mean of the 6 tests was taken for statistical analysis.

Statistics
Differences between patients and controls were evaluated by the nonparametric test of Wilcoxon and Wilcox.\textsuperscript{12} The null hypothesis was rejected when \(p\) was smaller than 0.01.

Results
Table 1 shows that there are no significant differences between patients and controls in any of the parameters recorded when filtering RCS's. However, there is a nonsignificant trend for IrcV to be higher after stroke. Blood cell concentrations are adequately controlled both in RCS's and RWCS's. Table 2 indicates that CP is significantly higher in stroke patients compared with controls when filtering RWCS. Again, absolute values for IrcV are higher in patients; the difference fails, however, to reach the level of significance.

Discussion
An impressive number of studies have shown red cell deformability to be abnormal after stroke and have deduced pathophysiological importance from this finding by considering filtration test analogous to microcirculation \textit{in vivo}.\textsuperscript{3-13,30} The methods then used to quantify red cell deformability are now known to be sensitive to white cell contamination.\textsuperscript{43} Pollock and colleagues showed that the difference in blood filtration is diminished to statistical nonsignificance when employing a filtration system that minimizes leukocyte artifacts.\textsuperscript{32} Therefore, higher leukocyte counts in the filtered suspension and/or fewer filterable leukocytes may have been responsible for previous findings.

The present results imply that the phenomenon formerly observed might in part be due to altered white cell rheology after stroke. However, it does not completely rule out red cell rigidification immediately after stroke, since nonacute patients were studied. If EDTA and 5-\(\mu m\) filter pores are used, platelets are not candidates for blocking the pores of the filter.\textsuperscript{39} Thus altered thrombocyte function is unlikely to have a marked effect on the results. The mechanism leading to altered rheological behavior of white cells is as yet unclear.

Table 1. Filtration Rates and Cell Counts in Red Cell Suspensions in Stroke Patients and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stroke</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IrcV (fl)</td>
<td>1042 ± 56</td>
<td>994 ± 69</td>
</tr>
<tr>
<td>CP (10^3/(\mu L))</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>White cells (10^3/(\mu L))</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Red cells (10^3/(\mu L))</td>
<td>995 ± 27</td>
<td>1004 ± 22</td>
</tr>
<tr>
<td>Platelets (10^3/(\mu L))</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
</tr>
</tbody>
</table>

No significant differences.
Values are mean ± SD.
IrcV = incremental red cell volume as a measure of red cell deformability.
CP = clogging particles as a measure of white cell rheology.

Table 2. Filtration Rates and Cell Counts in Red and White Cell Suspensions in Stroke Patients and Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stroke</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IrcV (fl)</td>
<td>1064 ± 61</td>
<td>1017 ± 74</td>
</tr>
<tr>
<td>CP (10^3/(\mu L))</td>
<td>0.21 ± 0.03</td>
<td>0.11 ± 0.04*</td>
</tr>
<tr>
<td>White cells (10^3/(\mu L))</td>
<td>0.40 ± 0.01</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>Red cells (10^3/(\mu L))</td>
<td>1002 ± 24</td>
<td>1016 ± 20</td>
</tr>
<tr>
<td>Platelets (10^3/(\mu L))</td>
<td>7 ± 6</td>
<td>12 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SD.
IrcV = incremental red cell volume as a measure of red cell deformability.
CP = clogging particles as a measure of white cell rheology.
*p < 0.005.
Possibly crossover experiments with normal leucocytes suspended in pathological plasma and vice versa would provide further insight.

Leucocytes may represent a significant obstacle to the microcirculation, hindering nutritive flow and oxygen exchange. It can be shown in experimental myocardial infarction that size of the necrosis correlates with number of white cells in the perfusate. Similarly it can be demonstrated that peripheral resistance depends on the number of leucocytes, a phenomenon which is more pronounced in low flow states. Finally, an epidemiological study identified the total white cell count as a predictor for cerebral infarction during a two-year observation period. This is contrary to cerebral hemorrhage, where there is a weak inverse relationship.

Thus the present findings indicating decreased leucocyte filterability after stroke may concern an important pathophysiological event. The impaired rheological properties of white cells could increase the size of the infarction by obstructing collateral perfusion and amplifying the effects caused by increased numbers of leucocytes and the overall hemodynamic situation, which can induce low flow systemically and/or locally. In addition to mechanically blocking capillaries, white cells release oxygen radicals, hydrogen peroxides, and proteolytic enzymes and induce endothelial swelling, further increasing tissue damage. Such effects can be synergistic in their harm to tissue.

The observed phenomenon of reduced white cell filterability might be more pronounced when studying acute cases of stroke. Our study shows that reduced filterability is present well after the acute event, which holds true also for other hemorheological sequelae of ischemic stroke. In acute patients it would be worthwhile correlating rheological abnormality with prognosis to test the role of leucocytes in this condition. A causative influence of leucocyte counts on the prognosis of stroke was suggested in an epidemiological study. Another trial has shown that blood filtration tests may have some prognostic value in ischemic stroke. More work is required to define which leucocyte subpopulation is responsible for the phenomenon and by which mechanism white cells are rendered less filterable. In principle this could, of course, be due to decreased deformability and/or increased adhesiveness.

References

30. Ernst E, Matrai A, Schönhaber H, Paulsen HF, Magyarosy I:


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