Treatment of Myeloproliferative Disorders With Hydroxyurea: Effects on Red Blood Cell Geometry and Deformability

By K. Gunnar Engström and Eva Löfvenberg

Hydroxyurea (HU) is used in suppressing the bone marrow and producing fetal-like red blood cells (RBCs). These RBCs are large in size and may theoretically disturb the microcirculation. In five patients with myeloproliferative disorders (MPD), the RBC geometry and deformability were analyzed before and after 6 to 8 months of HU treatment. In untreated MPD, the RBC geometry and filterability was normal. After HU, the RBC membrane area increased 24% and the cell volume increased 29% (P < .005). This change resulted in a 12% increase in the minimum cylindrical diameter (MCD). From a static bending model of initial deformation, the RBC diametrical cross-section had a significantly increased section modulus. However, this increase in profile stiffness was

addressed this problem by analyzing the geometry and filterability of RBCs on previously untreated patients with MPD before and after 6 to 8 months of HU treatment. The RBC geometry was analyzed using cell curvature profile generation with the possibility of calculating and estimating the theoretical consequences of the HU-induced RBC change. These theoretical interpretations were also tested experimentally by RBC filterability measurements using both 5-µm and 3-µm Nucleopore membranes. The 5-µm pores measure the effects on initial bending deformation, whereas the 3-µm pores are sensitive to maximum deformation close to the critical minimum cylindrical diameter (MCD) limit.

MATERIALS AND METHODS

Patients and HU treatment. Five patients with newly diagnosed MPD were selected. Four of the patients had ET, whereas one was subgrouped to have MF with thrombocytosis. All five patients were about to begin receiving chemotherapy treatment because of thrombocytosis, symptoms, and/or thrombotic/hemorrhagic complications. The inclusion in the study had no influence on the clinical routine and treatment. The patients were analyzed immediately before HU and were then observed 6 to 8 months later during ongoing HU treatment. HU was administered daily, using an oral dose of 0.5 to 1 g/d. Normal controls were healthy age-matched hospital personal, who were studied in parallel. These controls were not the same individuals before HU and at the follow-up, thus producing 5 + 5 control observations.

Blood sampling and media. Blood samples were from venipuncture using standard heparinized 10-mL test tubes. The blood was centrifuged at 1,500g for 10 minutes with buffy coat removal. This was repeated three times to wash the RBCs, each time by topping up the suspension to 10 mL. Using this standard routine, most of the plasma, leukocytes, and platelets were removed. The final resuspension hematocrit for filtration analyses was set to 5%. The resuspending medium consisted of a Krebs-Ringer solution (135 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH₂PO₄, 1.2 mmol/L MgSO₄) buffered with 20 mmol/L HEPES and supplemented with 2.56 mmol/L CaCl₂, 5 mmol/L D-glucose, and 0.2 g/L human serum albumin (Fraction V Albumin; Sigma Chemical Co St Louis, MO). The pH was set to 7.40 and the final medium had an osmolality of 299 ± 1 mOsm. Medium osmolality was measured by freezing-point depression (Osmette Osmometer; Precision Systems Inc, Natick, MA). The medium was also prefilled through a 0.45-µm Millipore filter (Millipore Corp, Molsheim, France).

All experimentation was conducted on two parallel blood samples, one from the MPD patient and one from the matched control. These
blood samples were coded all through the study, after which time the patient identity was disclosed.

**RBC geometry.** About 5 µL of the whole blood was diluted in 1 mL of RBC suspension medium and incubated for 30 minutes before analysis. A chamber for RBC geometry measurement was made from coverslips on a microscope slide to make a rectangular compartment (18 × 10 × 0.17 mm). A drop of suspended RBCs was introduced into the slit entrance of the chamber, and the openings were sealed with immersion oil. RBCs were allowed to sediment with the chamber held upside down. After 2 minutes, the chamber was turned right side up and mounted in the microscope. A large proportion of the previously settled RBCs were thus hanging vertically from the upper glass surface. On these vertical RBCs, the diametrical cross-sections were focused on using bright field illumination (Carl Zeiss ×100/1.25 oil objective lens; Carl Zeiss, Oberkochen, Germany). A digitizing table with light-spot cursor (MOP-Videoplan; Kontron Bildanalyse GmbH, Munich, Germany) was optically superimposed on the microscopic image. The RBC diameter, maximum thoroidal thickness, and central thickness were measured. A thin diffraction band surrounded the RBC cross-sections due to differences in refractive indices between the RBC and the suspending medium to which the uniform shell rule was applied during measurements. A thin diffraction band surrounded the RBC cross-sections due to differences in refractive indices between the RBC and the suspending medium to which the uniform shell rule was applied during measurements.18 The method used here is consistent with that described in previous publications, to which readers are referred for further details.1,2,11,12 RBCs (50 cells per sample) were focused on one by one and measured at their diametrical cross-section, a procedure that required about 15 minutes of microscopy per blood sample.

**RBC profile calculations.** The above measurements were loaded into equation 1 expressing the RBC profile curvature, originally derived from Evans and Fung11 and later remodelled11,12:

\[
y = 0.5(1 - x^2)(C_0 + C_2x^2[1 - 0.561x^3])
\]

With the aid of equation 1, the RBC membrane area and volume were computed by solving the volume integral and using numerical computer integration for membrane area calculation.11 The cell area-to-volume ratio was calculated together with the surface area index, which describes the relative excess of membrane area beyond that required to enclose the cellular volume.2,14 MCD was then derived from the area (A) and volume (V) by a third order equation11:

\[
0 = -\pi(MCD)^{2/3} + A(MCD)/4 - V
\]

**RBC folding resistance.** It is possible to mathematically calculate the mechanical resistance to initial bending deformation of the RBC corpuscle in terms of a section modulus value. The section modulus only considers the RBC shape and size and not the membrane mechanical properties such as, eg, elastic shear, dilation, or surface bending moduli or their interrelations. The section modulus is calculated for the RBC corpuscle, consisting of a 0.01-µm-thick membrane shell model11 with linear elastic behavior.15 The unit becomes µm^3.

The static bending model. The corpuscle section modulus value is evaluated using a static bending model in which the RBC is stressed due to a pressure difference between the two sides of the cell (here set to a fictitious pressure of 1 kPa). The pressure generates a bending moment and hence a membrane tension, both having a maximum at the diametrical cross-section of the RBC;12 the higher the tension, the more deformable the RBC. The tension is mathematically given by the quotient of bending moment-to-section modulus, with the unit nano-Newton/µm^2 (nN/µm^2). This method of theoretical analysis is useful to evaluate the influence of RBC shape and size only on corpuscle deformability.11,12

**RBC filterability.** The capillary function of RBC was studied by a filterability model on resuspended cells. This was studied by means of a specially developed filtration system.1,2,16 This system uses a digital balance (Metler PM4880; Metler Toledo AG, Greifensee, Switzerland) in connection with a computer (IBM PS/2 Model 50; IBM UK Ltd, Greenock, Scotland) for high precision monitoring of the filtration flow. The accumulated weight is transmitted by a frequency of 7.5 Hz, with on-line calculation of flow rate versus time, and is not limited in volume. This sampling rate gives a sufficient resolution in time in relation to the obtained flow rate and the resolution of the balance, corresponding to 1 µL/0.13 seconds. All analyses were made at room temperature (22°C to 24°C).

A computer was programmed for communication with the balance for data sampling, experimental timing, calculation of statistics, and storage of filtration data. The computer program was here designed to find the peak in initial flow at filtration onset, thus avoiding artifacts associated with the acceleration of fluids.16 The filtration is driven by a constant hydrostatic pressure of 600 Pa. The filtration chamber and filter are vertically located to reduce cell sedimentation artifacts. Filterability was measured as a function of time and with respect to the initial filtration rate and clogging rate and, thus, with the possibility of detecting different measures of cellular rheology. Nucleopore polycarbonate membranes were used having pore diameters of 5 and 3 µm and a capillary length of about 11 µm (lots no. 54F8A36 and 62E0B32, respectively; Nucleopore Corp, Pleasanton, CA). In the configuration of the filtration system used, filters were cut from larger sheets (8 × 10 inches to 15-mm wide strips) and connected so that a roll of filter was made. These rolls (5 and 3 µm) were mounted on the filtrometer and allowed rapid change between each filtration. A blank filtration was used to calibrate each filtration. However, the variability in blank filtrations was negligible, because all filters were from the same batch; furthermore, within each experiment, all filters were from the same sheet. The nominal pore density was 4.0 × 10^6 pores/cm^2 and 2.0 × 10^6 pores/cm^2 for the 5-µm and 3-µm filters, respectively. The active filter area was 70.9 mm^2 (open area of the filter support), which produced a total number of filterable pores of 2.84 × 10^6 and 1.42 × 10^6, respectively.

The erythrocyte filterability indices were calculated by means of a regression analysis of the flow rate, starting at the initial flow peak and during a 20-second filtration period. The filterability was expressed as RBC incremental volume (RBCiv) and clogging rate (RBCcr). The filtration rate decreased with time due to pore clogging of rigid erythrocytes and some contaminating leukocytes. The initial filtration rate (FRBC) was extrapolated from the regression line to time 0, thus reflecting the filtration rate before pore clogging. The RBC incremental volume (RBCiv) was calculated based on the initial filtration rate of blood (FRBC) and that of cell-free medium (Fm) and with correction for the RBC particle concentration (RBCc):

\[
RBC_{iv} = (Fm/F_{RBC} - 1)/RBC_c
\]

This equation expresses the relative decrease in filtration rate due to RBCs as compared with that of medium alone, Fm. The concentration of RBCs in the suspension, RBC, is also considered. More precisely, the RBCiv (expressed as nanoliters per RBC) is the volume of medium that is hindered from passing a narrow pore due to the presence of an RBC within it.

The decrease in filtration rate is due to clogging particles (RBCcr). The clogging rate can be evaluated by relating the slope, k (filtration rate as a function of filtration time) to the initial filtration rate, FRBC:

\[
RBC_{cr} = N_p k/F_{RBC}
\]

Np is the average number of initially open filter pores (see above). The unit for clogging particles will be RBC per second. The calculations in the present report are, in principle, in accordance with those described by Matrai et al,17 except that the filtration rate is expressed here as a function of time instead of as a function of volume.
Statistics. Data are presented as the mean values ± SEM. For statistical differences, both paired and unpaired t-tests were used for matched analyses with respect to differences induced by HU and for analysis versus control individuals, respectively. With unpaired testing, correction was made for unequal variance and different number of observations between groups. The different numbers of observation are indicated in the tables (n). Five levels of significance were tested: $P < .05^1$, $P < .02^2$, $P < .01^3$, $P < .005^4$, and $P < .001^5$.

RESULTS

Clinical response. The patients were diagnosed according to required criteria for MPD. The Hb concentration varied with the MPD spectrum. One patient had supranormal Hb and an MPD profile towards PV, but with normal RBC mass and classified as ET; the Hb was reduced after HU. In another patient, the Hb was subnormal (MF) and increased during HU (Table 1). In all included patients, the platelet counts were increased and became successfully reduced after HU ($P < .05$).

The mean corpuscular Hb concentrations (MCHC) were at normal levels in all patients and remained unaffected by HU (Table 1). The mean corpuscular volume (MCV) was normal but increased markedly during HU treatment. In the remaining three patients, an isolated ET was observed in which the Hb remained largely unaffected by HU (Table 1). In all included patients, the platelet counts were increased and became successfully reduced after HU ($P < .05$).

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Table 1. Patient Data

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age (yr)</th>
<th>Diagnosis</th>
<th>Treatment (mo)</th>
<th>Platelets (10⁹/L)</th>
<th>Hb (g/L)</th>
<th>MCHC (g/L)</th>
<th>MCV (µm²)</th>
<th>Symptoms at Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/70</td>
<td>ET</td>
<td>0</td>
<td>675</td>
<td>164</td>
<td>321</td>
<td>85</td>
<td>Headache, dizziness</td>
</tr>
<tr>
<td>2</td>
<td>M/62</td>
<td>ET</td>
<td>0</td>
<td>1,485</td>
<td>143</td>
<td>324</td>
<td>90</td>
<td>Visual disturbances</td>
</tr>
<tr>
<td>3</td>
<td>M/40</td>
<td>ET</td>
<td>0</td>
<td>1,770</td>
<td>146</td>
<td>348</td>
<td>91</td>
<td>Pulmonary hemorrhage</td>
</tr>
<tr>
<td>4</td>
<td>F/66</td>
<td>ET</td>
<td>0</td>
<td>736</td>
<td>142</td>
<td>326</td>
<td>101</td>
<td>Fatigue</td>
</tr>
<tr>
<td>5</td>
<td>M/70</td>
<td>MF</td>
<td>0</td>
<td>663</td>
<td>100</td>
<td>340</td>
<td>89</td>
<td>Fatigue, vertigo</td>
</tr>
<tr>
<td>6</td>
<td>M/62</td>
<td>ET</td>
<td>0</td>
<td>398</td>
<td>133</td>
<td>327</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M/40</td>
<td>ET</td>
<td>0</td>
<td>357</td>
<td>131</td>
<td>335</td>
<td>115</td>
<td></td>
</tr>
</tbody>
</table>

Patients’ data before and after HU treatment. The MPD were ET, of which one was classified as MF. The age, sex, and months of HU treatment are given together with platelet count, Hb concentration, MCHC, and MCV. Note that these data are from Coulter counter measurements, by which MCV is indirectly generated from a pore conductivity.

Effects of HU on calculated RBC deformability. The patients with MPD had normal RBC geometry and, thus, no difference in calculated deformability indices (Table 2). However, the HU-induced change in RBC size produced highly significant alterations in these calculated RBC deformability indices. These changes were found in terms of both maximum deformation, represented by MCD, and initial bending rigidity, as judged from the static bending model. The MCD increased significantly by 11.7% ± 0.9%; furthermore, the percentage of RBC having an MCD greater than 3.5 µm increased by 263% ± 39% (Table 2). This dramatic percentage increase suggests a larger resistance to pass a narrow capillary and with more capillary blockage.

The bending section modulus that was calculated for each RBC diametrical cross section indicated a significantly more rigid RBC membrane shell profile; this increase was by 23.6% ± 1.9% (Table 2). However, although the corpuscle profile was associated with a larger calculated bending resistance, when the larger projected surface area was taken into consideration and thus the larger pressure load on the RBC to initiate deformation, the generated membrane tension was almost the same before and after HU treatment (Table 2).

Effects of HU on the measured RBC filterability. The RBC filterability in patients with untreated MPD was not significantly different from those of normal controls. This was seen using both 3-µm and 5-µm pore filters, although there was a small tendency for a decreased filterability in MPD patients using the 5-µm filtration model (interpreted as a small increase in both $RBC_{iv}$ and $RBC_{cr}$, Table 3). After HU treatment, a significant reduction in filterability was recorded with 3-µm filters as the RBCiv increased by 63% (P < .02). There was also a 23% ± 13% increase in the the corresponding RBCcr, but without becoming significant. No significant changes were seen using the 5-µm pore size (Table 3).

DISCUSSION

The oxygen delivery from the blood to the surrounding tissue is promoted by the narrow dimensions of the capillaries. The RBC are folded in the capillaries because these are narrower than the cell diameter, of the order of 4 to 5 µm. The RBC therefore relies on its ability to deform (ie, cellular deformabil-
Impaired RBC deformability is thought to contribute to the viscosity increase, but more obviously in the microcirculation size may impair the blood flow in the macrocirculation by being larger in size \(2,14\) and are similar to the cells seen with HU treatment.\(^8\) In MPD, the administration of HU is aimed at altering the concentration of circulating RBCs and platelets by suppressing the abnormal myelopoiesis in favor of the fetal gene expression.\(^6\) This is done to reduce the clinical symptoms of MPD, such as thrombotic and hemorrhagic complications.\(^23\) However, the beneficial effects of HU are paralleled by a possible disadvantage of having large-size fetal RBCs. Intuition tells us that these large cells would more easily be trapped in the microcirculation. Our aim was to analyze this phenomenon by parallel experimental and theoretical observations on MPD patients before and after HU treatment and to compare the data to normal controls.

In each patient, the cross-sectional profiles of 50 random RBCs were carefully measured by computerized image processing. From triplicate size measurements, the RBC curvature equation was computer generated, and various RBC deformability indices were calculated. These indices were calculated according to (1) a static bending model\(^12\) to simulate the initial corpuscle bending deformation at capillary entrance and (2) MCD calculation to find the minimum capillary diameter the RBC can pass without lysis.\(^11,14\) The theoretical data were compared with those of experimental filtration measurements using (1) 5-µm pore membranes to simulate the initial deformation (cf, RBC static bending model) and (2) 3-µm pore membranes to analyze the final stage of deformation (cf, MCD).

The tested MPD patients had normal RBC geometry and a filterability resistance comparable to that of the matched controls. In the literature there are no absolute answers as to how the circulating blood cells are affected in MPD; this may depend on the wide spectrum of MPD diseases with dysplastic erythropoiesis and, in many cases, possible coexistence of iron deficiency\(^24\) and thus altered RBC geometry. The present patients showed normal RBC geometry, which makes the interpretation of the HU more strict. Note, our microscopy data suggest slightly oversized RBCs (in MPD patients and normal controls), with an average cell volume of 105.7 ± 3.3 \(\mu\text{m}^3\) and membrane area of 141.4 ± 3.0 \(\mu\text{m}^2\) in the control group. These recordings are about 10% larger than that recorded using micropipette aspiration of RBC (93.2 ± 1.5 \(\mu\text{m}^3\) and 133.3 ± 1.6 \(\mu\text{m}^2\), respectively).\(^25\) Similar reference values on RBC geometry are available based on microscopy interference holography,\(^13\) although larger size reference values, similar to those recorded here, also exist.\(^26,27\) Thus, the exact geometry of the average RBC is not known, and the variations in reference value depend on the used techniques. This is due in part to the optical limits with light microscopy. With the present data, the RBC geometry is derived from diametrical cross-sections. This technique requires a consistent tracing routine of a diffraction band that outlines the cell and is the result of the light resolution and the difference in refractive indices between the RBC and the surrounding medium. The RBC diffraction phenomenon was first analyzed by Ponder\(^28\) and later reevaluated by Korpman et al.\(^10\) With the Korpman rule,\(^10\) the cell membrane is thought to be found in the middle of the diffraction band, which is the guideline today. However, there is probably no exact tracing

### Table 2. Geometry and Related Parameters of RBC Before and After HU Treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MPD</th>
<th>MPD + HU</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (µm)</td>
<td>8.12 ± 0.12</td>
<td>8.92 ± 0.07</td>
<td>8.10 ± 0.09</td>
</tr>
<tr>
<td>Thoroid (µm)</td>
<td>2.58 ± 0.03</td>
<td>2.97 ± 0.06</td>
<td>2.60 ± 0.04</td>
</tr>
<tr>
<td>Central (µm)</td>
<td>1.10 ± 0.08</td>
<td>1.12 ± 0.10</td>
<td>1.11 ± 0.04</td>
</tr>
<tr>
<td>Area (µm²)</td>
<td>141.3 ± 3.5</td>
<td>174.7 ± 3.6</td>
<td>141.4 ± 3.0</td>
</tr>
<tr>
<td>Volume (µm³)</td>
<td>104.9 ± 2.6</td>
<td>145.3 ± 4.4</td>
<td>105.7 ± 3.3</td>
</tr>
<tr>
<td>Area/Vol (—)</td>
<td>1.368 ± 0.015</td>
<td>1.224 ± 0.015</td>
<td>1.359 ± 0.016</td>
</tr>
<tr>
<td>Surface area index (—)</td>
<td>1.320 ± 0.015</td>
<td>1.314 ± 0.008</td>
<td>1.314 ± 0.006</td>
</tr>
<tr>
<td>MCD (µm)</td>
<td>3.20 ± 0.04</td>
<td>3.57 ± 0.05</td>
<td>3.22 ± 0.04</td>
</tr>
<tr>
<td>MCD &gt; 3.5 µm (%)</td>
<td>16.8 ± 2.9</td>
<td>58.8 ± 6.9</td>
<td>17.8 ± 3.7</td>
</tr>
<tr>
<td>CMSM (µm³)</td>
<td>0.125 ± 0.003</td>
<td>0.154 ± 0.004</td>
<td>0.126 ± 0.03</td>
</tr>
<tr>
<td>CMBT (nN/µm²)</td>
<td>501 ± 26</td>
<td>532 ± 9</td>
<td>490 ± 11</td>
</tr>
</tbody>
</table>

Patients with newly diagnosed MPD were analyzed before and after treatment with HU \((n = 5)\). Healthy controls were studied in parallel \((n = 5 ± 5)\). A drop of whole blood was suspended at low hematocrit in balanced KRH medium and introduced into a chamber under the microscope. RBCs hanging vertically from the upper chamber cover slip were focused on and, from interactive measurements of the RBC diameter, thoroidal thickness, and center thickness, the cell geometry and deformability parameters were calculated. This geometry was based on a computer iterative search for the RBC curvature polynomial equation (see equation 1). The membrane area, cell volume, area-to-volume ratio, and surface area index are given. The MCD was computerized representing the minimum pore diameter the RBC can pass through without lysis. The RBC corpuscle membrane section modulus (CMSM) and the corpuscle membrane bending tension (CMBT) were also generated from a static RBC bending model. Five levels of significances were tested: \(P < .05\), \(P < .02\), \(P < .01\), \(P < .005\), and \(P < .001\). No significant differences were found between patients with MPD before treatment and controls.

### Table 3. RBC Pore Filterability Before and After HU Treatment

<table>
<thead>
<tr>
<th>RBC (µm)</th>
<th>MPD</th>
<th>MPD + HU</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-µm (nL)</td>
<td>5.49 ± 1.37</td>
<td>16.10 ± 2.98</td>
<td>5.11 ± 0.57</td>
</tr>
<tr>
<td>3-µm ——</td>
<td>31.9 ± 4.3</td>
<td>37.6 ± 2.7</td>
<td>34.1 ± 2.8</td>
</tr>
<tr>
<td>5-µm (nL)</td>
<td>0.857 ± 0.124</td>
<td>1.019 ± 0.148</td>
<td>0.693 ± 0.052</td>
</tr>
<tr>
<td>5-µm ——</td>
<td>2.51 ± 0.41</td>
<td>1.61 ± 0.44</td>
<td>1.58 ± 0.24</td>
</tr>
</tbody>
</table>

Patients with newly diagnosed MPD were analyzed before and after treatment with HU \((n = 5)\). Healthy controls were studied in parallel \((n = 5 ± 5)\). Whole blood was centrifuged and washed three times with removal of plasma and white blood cells. The RBCs were resuspended in balanced KRH medium to 5% hematocrit. The RBC filterability was tested through both 3-µm and 5-µm Nucleopore membranes at 600 Pa constant pressure. From the measurements of initial filtration rate and clogging slope as a function of filtration time, the RBC incremental volume \((\text{RBC}_i)\) and the RBC clogging rate \((\text{RBC}_c)\) were calculated (see the Materials and Methods). Five levels of significances were tested: \(P < .05\), \(P < .02\), \(P < .01\), \(P < .005\), and \(P < .001\). No significant differences were found between patients with MPD before treatment and controls.
rule for the membrane edge, a fact that may account for the apparent oversizing of the normal RBC in this study. As an example of optical effects, by assuming an error on the linear scale corresponding to the light-microscopy resolution of approximately 0.2 µm, an RBC diameter of 8 µm has a potential optical error of ±2.5%. The second and third power errors, membrane area and cell volume, are 5.1% and 7.7%, respectively. This example simplifies the true calculations, because the cell diameter is only one of three measured parameters from which the RBC curvature is synthesized. The optical limits may thus introduce apparent discrepancies in baseline cell size. However, this does not affect the interpretations made in this study because of the systematic nature of the membrane-tracing routine. Note also that electronic orifice sizing of RBC (ie, Coulter counter) is affected by a shape factor correction and does not represent exact estimates of the RBC geometry, especially not shape-changed RBCs. In MPD with discoid RBCs, one would assume that the shape-factor error would be rather consistent to yield a good estimate of the true MCV. In this study, the Coulter counter indicated a 30.9% ± 5.3% increase in MCV due to HU, which did not differ significantly from the microscopy data (39.0% ± 6.3%). Microscopy measurements on vertical RBCs and diametrical cross-section profiles are far more time-consuming than using Coulter counting; however, the microscopy technique has been made more available due to modern computer technology and the use of a modified RBC curvature equation. Furthermore, with the curvature equation, not only the RBC volume is derived but also membrane area and various deformability indices based on the RBC cross-sectional profile (see below).

After HU treatment, the thrombocytopenia was reduced and, in most cases, normalized. From a clinical perspective, the HU treatment was successful, with no signs of disturbed microcirculation. The RBC concentration was reduced in the patient having Hb in the upper range, whereas it was increased in the patient with a clinical profile towards MF. The cell size was dramatically increased, whereas the average MCHC remained at normal level. In this study, we analyzed the start and end points before and after 6 to 8 months of HU. Our previous research has indicated a close to linear increase in MCV during this initial period, two separate pools of RBC were seen: those with normal MCV and those with HU-affected geometry, respectively. This was followed by a complete replacement with HU-RBC. Previously in the literature, the terms responders versus nonresponders have been attributed the pattern of HbF increase in HU-treated MPD patients. Although we did not measure HbF or detailed temporal dynamics of RBC geometry change, it is tempting to look at the different end-point RBCs as possible responders or nonresponders. All patients showed a dramatic increase in MCV, whereas the MCHC varied. However, there was no correlation between the rate of MCV increase versus MCHC change in a way that could reflect different response patterns. Further research may highlight this issue.

Our Coulter counter data in HU-treated MPD patients seem comparable to results in sickle cell anemia. However, our 131% ± 5% increase in MCV appears somewhat larger than in many sickle cell reports. This may be attributed the hemolytic anemia in sickle patients with an increase in reticulocytes and thus an increase in MCV before HU treatment. It could also reflect errors associated with the shape-factor correction of sickle RBCs in Coulter counting. In our study on MPD, the average MCHC appeared unaffected by HU; this is again paralleled by observations in HU-treated dogs and in many sickle cell studies.

The RBC microscopy measurements, as with the Coulter data, also showed a dramatic increase in RBC size; the cell diameter and thickness were significantly increased, whereas the central thickness (dimple) remained unchanged. The RBC curvature calculation produced a significant increase in both RBC membrane area and cell volume. These changes resulted in a significant decrease in RBC area-to-volume ratio, whereas the surface area index remained unchanged. The calculation of MCD gave a significant increase of 12%.

When the RBC cross-sectional profile of HU-treated patients was analyzed using the static bending model, a significantly larger section modulus was generated, suggesting a greater required force to initiate deformation of the corpuscle. However, the larger RBC diameter after HU also resulted in a larger projected surface area and thus deforming pressure load. This increased pressure load balanced the increase in section modulus to generate similar membrane tension and deformation in the RBC before and after HU.

The filtration analyses of HU-RBC correlated well with the theoretical observations and geometry. With 3-µm pore membranes, a significantly increased filtration resistance was produced by HU. This increase drives the attention to the increase in MCD and the percentage of RBC having an MCD larger than 3.5 µm from 17% to 59% after HU (cells that would effectively be trapped within the filter). With 5-µm pores, there were no significant differences associated with HU treatment. This lack of difference using the 5-µm filtration correlated with the theoretical findings given by the static bending model. The RBC geometry change after HU produced a more stiff corpuscle shell profile; however, this profile was balanced by a larger projected cell area and hence deforming pressure load. The resistance to initiate RBC folding was remained larger without change after HU treatment. It is noteworthy that, with 5-µm pores, the RBC will required only minor folding to pass for which the static bending model appears relevant.

In conclusion, patients with MPD have RBCs of normal shape, geometry, and rheological function. With HU treatment, the bone marrow is suppressed to produce fetal-like large RBCs. The present detailed microscopic measurements of individual RBC cross-sectional profiles confirm this geometry change; not only is the cell volume increased, but also the membrane area. However, in addition to mere cell geometry, the calculations were extended to also analyze the mathematical deformability; an obvious impairment in micropore function (MCD increase) was seen, whereas the initial corpuscle bending deformability appeared unaffected. These calculations were confirmed by 3-µm and 5-µm filterability measurements, respectively. Because the capillaries in the circulation are larger in diameter than 3 µm, we suggest that the HU-induced change in RBC geometry is acceptable from a perspective of cellular microrheology.
REFERENCES


28. Ponder E: The measurement of the diameter of erythrocytes. V.-The relation of the diameter to the thickness. Quart J Exp Physiol 20:29, 1930


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