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CELLULAR DETERMINANTS OF LOW-SHEAR BLOOD VISCOSITY

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ABSTRACT Low-shear viscometry is one of the methods commonly used to estimate the degree of red blood cell (RBC) aggregation in various bloods and RBC suspensions. However, it has been previously shown that alterations in RBC morphology and mechanical behavior can affect the low-shear apparent viscosity of RBC suspensions; RBC aggregation is also sensitive to these cellular factors. This study used heat treatment (48 °C, 5 min), glutaraldehyde (0.005-0.02%) and hydrogen peroxide (1 mM) to modify cell geometry and deformability. Red blood cell aggregation was assessed via a Myrenne Aggregometer ("M" and "M1" indexes), RBC suspension viscosity was measured using a Contraves LS-30 viscometer, and RBC shape response to fluid shear stress (i.e., deformability) was determined by ektacytometry (LORCA system). Our results indicate that low-shear apparent viscosity and related indexes may not always reflect changes of RBC aggregation if cellular properties are altered: for situations where RBC aggregation has been only moderately affected, cellular mechanical factors may be the major determinant of low-shear viscosity. These findings thus imply that in situations which may be associated alterations of RBC geometry and/or deformability, low-shear viscometry should not be the sole measurement technique used to assess RBC aggregation. © 1997 Elsevier Science Ltd

Introduction

Human red blood cells (RBC) in normal blood form linear and branched aggregates if the fluid shear forces affecting the cells are below a critical level (Chien,1975). This tendency for aggregation is known to be one of the major determinants of the shear-thinning behavior of blood: Formation of RBC aggregates at low shear rates results in higher apparent viscosity, while with increasing shear disaggregation occurs and the apparent viscosity decreases (Lowe and Barbenel, 1988). Thus, the degree of RBC aggregation

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is widely accepted as a very important determinant of low-shear viscometric behavior

of blood (Lowe and Barbenel, 1988). Based upon this principle, low-shear viscometry has been recommended as an indirect index of RBC aggregation (Rampling, 1988; Shiga, et al., 1990; Bull, et al., 1988) and has been used in numerous clinical studies.

In general, RBC aggregation is determined by two types of biophysical and physicochemical factors: 1) Suspending phase properties; 2) RBC properties. Previous literature reports have clearly demonstrated that RBC aggregation is dependent on the presence of specific macromolecules (e.g., fibrinogen, polymers such as dextran) in the suspending phase; these molecules induce aggregation either by forming cross-bridges between adjacent RBC membranes (Chien and Sung, 1987) or by generating an osmotic gradient that pulls fluid from the gap between cells thus forcing individual RBC into close contact (Janzen and Brooks, 1989). The molecular structure and concentration of these macromolecules can markedly influence the rate, strength and extent of RBC aggregation (Rampling, 1988; Shiga, et al., 1990; Chien and Sung, 1987), and there are numerous reports describing the effects of variations of such parameters as fibrinogen concentration and polymer size.

Experiments using standard, defined aggregating media (e.g., constant polymer concentration in an isotonic buffer), but with human RBC from different donors or with RBC having different properties, have revealed that cellular factors are also important in determining red cell aggregation behavior (Shiga, et al., 1990; Meiselman, 1993). RBC surface properties, shape and deformability are among the cellular properties which can affect the aggregation process (Nash, et al., 1987; Sowemimo-Coker, et al., 1989; Rampling and Pearson, 1994; Bohler and Linderkamp, 1993; Reinhart and Singh, 1990; Singh and Reinhart, 1991). In addition to the effects of these cellular properties on RBC aggregation, there is experimental evidence that RBC shape and deformability changes can influence the viscometric behavior of blood or red cell suspensions, independent of their effects on aggregation (Meiselman, 1978; Meiselman, 1981; Pfafferott, et al., 1982). Therefore, this possible confounding effect of RBC geometric and mechanical alterations on blood rheology raises potential questions regarding the validity of using viscometric data in order to infer RBC aggregation. Such concerns would seem to be of particular relevance to medically-related studies in which changes of the suspending phase (i.e., plasma) composition may be associated with changes of RBC properties.

The present study was thus designed to explore the effects of RBC geometric and mechanical alterations on the low-shear viscometric behavior of RBC-plasma and RBC-buffer suspensions, and to correlate this behavior with independent measures of RBC aggregation. Experimental alterations of RBC deformability and morphology were achieved via chemical and thermal treatment of the cells, with aggregation assessed using a cone-plate shearing system and RBC rheologic behavior tested by ektacytometry. Our findings indicate that the low-shear viscometric behavior of RBC suspensions can be affected by cellular rheologic and morphologic properties in addition to RBC aggregation, and that low-shear apparent viscosity may not always be a valid index to red blood cell aggregation.

Materials and Methods

Preparation of blood samples

Venous blood samples were obtained from healthy adults, age range 28-54 years and of either gender, via withdrawal into sterile vacuum tubes containing (1.5 mg/ml blood). RBC were separated from the blood by centrifugation 1,400 g for 10 min and washed with 10 mM phosphate buffered saline (PBS; pH=7.4; osmolality 290 ± 3 mOsm/kg). The washed RBC were then resuspended in PBS at a hematocrit of approximately 5%. These low-hematocrit RBC suspensions were divided into four aliquots and exposed to: 1) 0.005% glutaraldehyde (GA; Sigma Chemical Company, St. Louis, MO, USA) at 25 °C for 30 min; 2) 1mM hydrogen peroxide (H_2O_2 , Sigma) at 25 °C for 30 min; 3) heat treatment at 48 °C for 5 minutes. The remaining aliquot was kept at 25 °C for 30 minutes and served as the control. In some experiments RBC were exposed to GA at concentrations ranging between 0.005% and 0.02% at 25 °C for 30 minutes. Following the treatment or control periods, the cells were washed three times in PBS and resuspended in either autologous plasma or PBS at a hematocrit of 40% and then used for viscosity and aggregation measurements. The washed RBC were also used for elongation index (EI) measurements by ektacytometry (see below). All analyses were completed within 5 hours after blood collection.

Viscosity measurements

The apparent viscosity of RBC suspensions was measured, at 25 °C, using a Couette viscometer (Contraves LS 30, Contraves AG, Zürich, Switzerland) at shear rates between 0.512 sec^{-1} to 94.5 sec^{-1} . For some experiments, the viscosity of RBC suspensions was also measured at higher shear rates ($300\text{-}1500 \text{ sec}^{-1}$) with a rotational cone-plate viscometer (Model 1/2 RVTDCP-200, Brookfield Engineering Labs, Stoughton, MA) operating at 25 °C.

Determination of RBC aggregation indexes

RBC aggregation was quantified using a photometric rheoscope (Myrenne Aggregometer; Myrenne GmbH, Roetgen, Germany) interfaced to a digital computer. This technique is based on the increase of light transmission through a red cell suspension which occurs when individual cells aggregate; gaps in the suspending medium between the aggregates allow more light to pass through the suspension. Two indexes of RBC aggregation were determined with the Myrenne system as described previously (Bauersachs, et al., 1989): 1) "M" which is measured by integrating light transmission for 10 sec, *at stasis*, following a brief period of high shear (600 sec^{-1}) to disrupt previously existing aggregates; 2) "M1" which is measured by integrating light transmission for 10 sec, *at a low shear rate of 3 sec^{-1}* , following the period of high shear. Note that M thus reflects aggregation in the absence of shear and M1 reflects aggregation when fluid movement tends to promote cell-cell interaction; both M and M1 increase with increasing RBC aggregation (Bauersachs, et al., 1989). All Myrenne studies were carried out at 40% hematocrit and at room temperature (22 ± 1 °C).

Measurement of RBC deformability via ektacytometry

RBC deformability (i.e., the ability of the entire cell to adopt a new shape in response to deforming forces) was determined, at fluid shear stresses between 0.5 and 50 Pascal (Pa), by laser diffraction analysis using an ektacytometer (LORCA, RR Mechatronics, Hoorn, The Netherlands). The system has been described elsewhere in detail (Hardeman, et al., 1994). Briefly, the sample is sheared in a concentric-cylinder Couette system made of glass, with a gap of 0.3 mm between the cylinders. The shape of the diffraction patterns resulting from RBC deformation are analyzed by a microcomputer, and elongation indexes (EI) are calculated; an increased EI indicates greater cell deformation and hence greater cell deformability. For these LORCA studies, the RBC were suspended in an isotonic, viscous

(24.8 mPa.s) PBS-dextran 70 (Sigma) solution at a cell count of 2×10^7 /ml; all measurements were carried out at 25 °C.

Miscellaneous techniques

The osmolality of solutions was determined by a freezing-point depression osmometer (Model 5004, Precision Systems, Inc., Natick, MA) and pH by a Orion Model 410A system (Orion Research, Boston, MA). The viscosity of the dextran solution used in ektacytometry was measured at 25 °C by the Brookfield cone-plate viscometer. Red blood cell morphology was determined by phase-contrast light microscopy of dilute wet mounts (i.e., 1-2 percent cells in their suspending medium).

Statistics

Results are expressed as mean \pm standard error (SEM). Statistical comparisons between groups were done by paired t-tests and p values smaller than 0.05 were accepted as being statistically significant.

Results

Treatment of RBC with 0.005% GA or 1 mM H_2O_2 resulted in similar, significant increases in the apparent viscosities of red cell-plasma suspensions, especially at lower rates of shear (Figure 1); at the lowest shear rate (0.512 sec^{-1}) the viscosities were 30-35% above control. The smaller increases of low-shear viscosity for RBC subjected to 5 minutes of heat treatment at 48 °C were not significantly different from control. A similar pattern of viscosity changes was observed for RBC suspensions prepared in the non-aggregating PBS medium: GA and H_2O_2 treated RBC exhibited

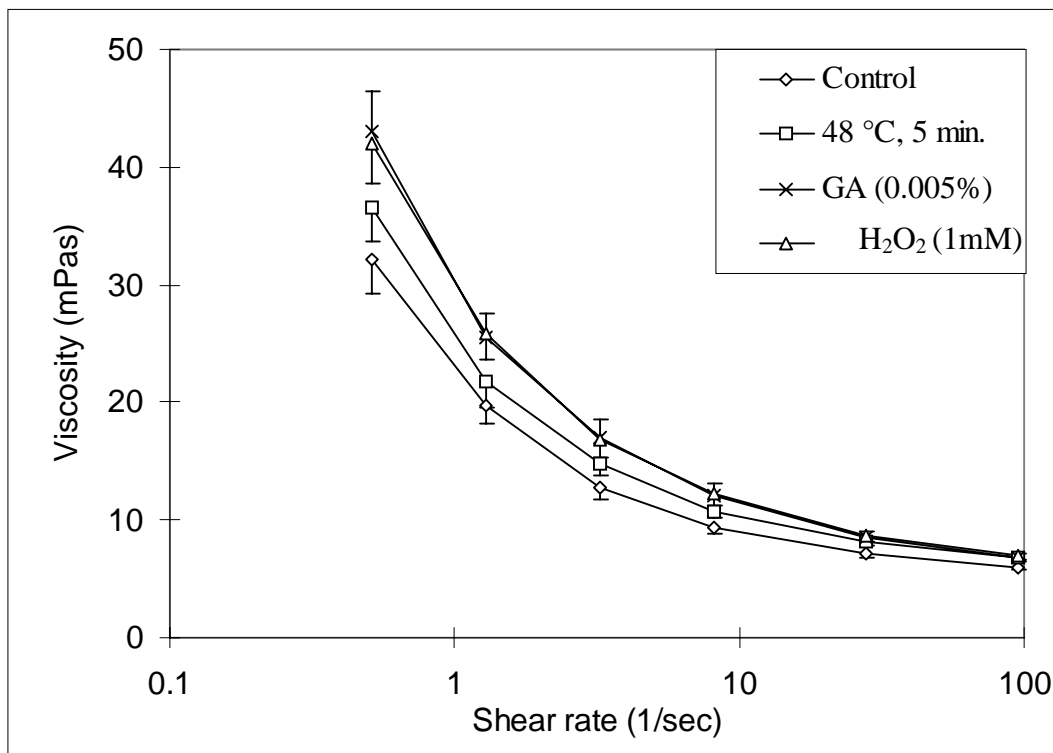


Fig. 1. Viscosity-shear rate data of 40% hematocrit RBC suspensions in plasma for control cells and cells treated with 0.005% glutaraldehyde (GA), 1 mM hydrogen peroxide or heat treated at 48 °C. Each point represents mean \pm SEM of five experiments.

increased low-shear viscosity versus control cells (Table 1). At higher shear rates (i.e., $\geq 27.7 \text{ sec}^{-1}$), all cell suspensions had apparent viscosities significantly greater than control regardless of the suspending media (i.e., plasma or PBS); increases above control were approximately 20-22% for GA or H_2O_2 treated cells and 7-10% for cells treated at 48°C .

The Myrenne "M" RBC aggregation index was found to be significantly lower for H_2O_2 and heat-treated RBC, whereas 0.005% GA-treatment did not affect this RBC aggregation index; none of the treatments significantly influenced the "M1" aggregation index (Figure 2). RBC deformability, as determined via ektacytometer EI values, was decreased versus control for the three treatment protocols at shear stresses $> 0.5 \text{ Pa}$ (Figure 3); the decreases for RBC incubated with GA or H_2O_2 were greater than those for heat-treated cells. Red cell morphology was normal (i.e., smooth, biconcave discs) for control, heat-treated and GA-treated RBC, whereas 50-60% of RBC were echinocytes in the H_2O_2 treated samples.

Glutaraldehyde treatment had a dose-dependent effect on the apparent viscosity of 40% hematocrit RBC-plasma suspensions which was a function of shear rate (Figure 4): 1) At shear rates greater than 100 sec^{-1} the viscosity increased progressively with increased concentrations of GA; 2) At lower shear rates (i.e., below about 10 sec^{-1}) suspension viscosity increased with GA concentrations of 0.005% and 0.01%; 3) At the lower shear rates the viscosity for cells treated with 0.015% GA was less than for 0.01%, and treatment with 0.02% GA further decreased low-shear suspension viscosity such that it approached a level lower than control. This bi-phasic response was not seen for the aggregation indexes, where both M and M1 decreased progressively for GA concentrations between 0.01%-0.02%; the slight increases at 0.005% GA were not significant (Figure 5). The biconcave-discoid shape of the RBC was not altered in the samples treated with 0.005-0.02% GA and there was no evidence of echinocyte formation at any GA concentration.

Discussion

Low-shear viscometry is a technique commonly used to assess RBC aggregation (Chien, 1975; Lowe and Barbenel, 1988; Rampling, 1988), and

Table 1

Viscosity of 40% hematocrit RBC suspensions in PBS for control cells and cells treated with 0.005% glutaraldehyde, 1 mM hydrogen peroxide or heat treated at 48°C .

Shear rate (sec^{-1})	Control	HT	GA	H_2O_2
0.512	5.45±0.33	5.22±0.13	6.49±0.38*	6.92±0.33 [†]
1.285	5.19±0.29	5.16±0.21	6.44±0.32 [†]	6.62±0.39*
3.23	5.06±0.21	5.15±0.16	6.37±0.29 [†]	6.38±0.29 [†]
8.11	4.66±0.19	4.94±0.12*	5.86±0.24 [†]	5.70±0.23 [†]
27.7	4.14±0.14	4.45±0.08*	5.30±0.18 [†]	5.13±0.21 [†]
94.5	3.79±0.11	4.06±0.06*	4.50±0.11 [†]	4.58±0.16 [†]

Values are mPa.s and are mean \pm SEM of five experiments. Difference from control; *: $p < 0.05$, [†]: $p < 0.01$.

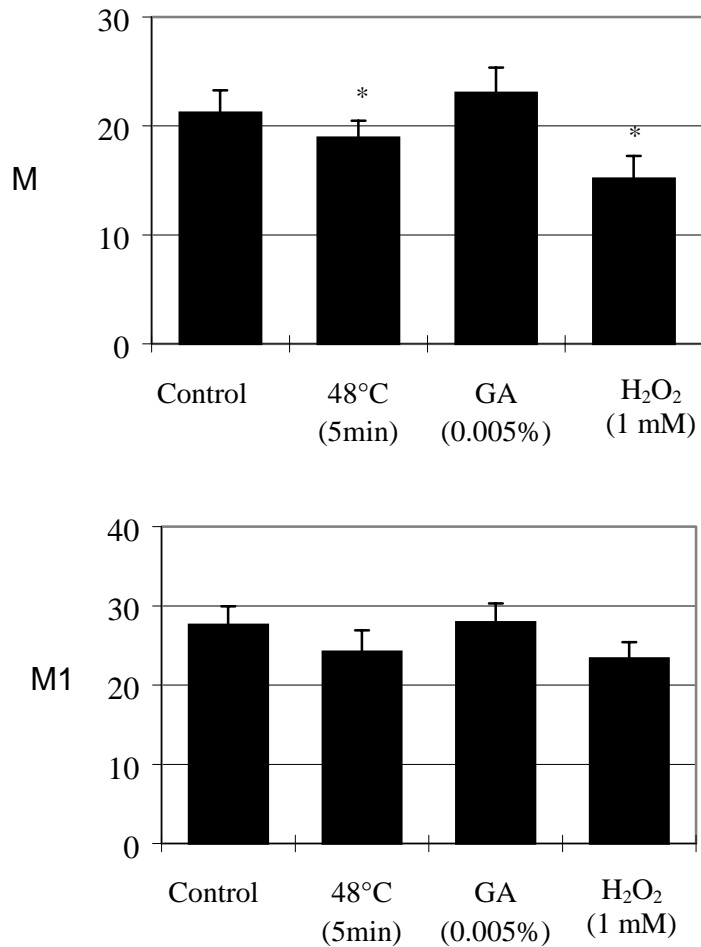


Fig. 2. M and M1 aggregation indexes for RBC-plasma suspensions at 40% hematocrit for control cells and for cells treated with 0.005% glutaraldehyde, 1 mM hydrogen peroxide) or heat treated at 48 °C. Mean \pm SEM of five experiments. Difference from control; *: $p < 0.05$.

its use follows the classical description of the factors which affect the low and high shear rate rheological behavior of RBC suspensions (Chien, 1975). It is generally accepted that at constant hematocrit and temperature, low-shear blood viscosity is primarily determined by RBC aggregation, while high-shear viscosity is dependent on RBC deformability (Lowe and Barbenel, 1988). Thus it is tacitly assumed that the impact of RBC aggregation on the low-shear viscosity of RBC suspensions is evident when comparing the low-shear viscosities of a given RBC population in aggregating (e.g., plasma) and in non-aggregating (e.g., isotonic buffer) media (Chien, 1975; Shiga, et al., 1990; Meiselman and Baker, 1977). This assumption is based upon results of such comparisons for suspensions of normal RBC at low shear indicating large differences in apparent viscosity (aggregating \gg non-aggre-

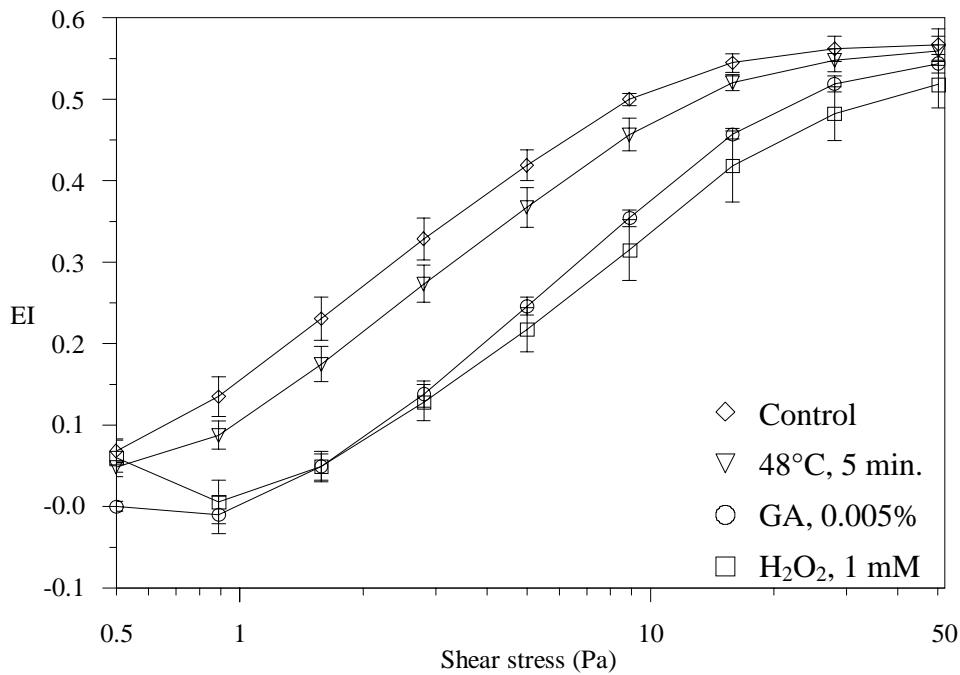


Fig. 3. Ektacytometer RBC elongation indexes (EI) for control cells and for cells treated with 0.005% glutaraldehyde, 1 mM hydrogen peroxide or heat treated at 48 °C. Each point represents mean \pm SEM of five experiments.

gating) which are enhanced by agents causing greater RBC aggregation (Chien, 1975; Lowe and Barbenel, 1988; Chien and Sung, 1987).

Based on the above considerations, interpretation of Figure 1 would yield the following conclusions: RBC treated with 1 mM H₂O₂ or 0.005% GA and then suspended in autologous plasma exhibit greater aggregation versus plasma suspensions of control or heat-treated RBC. However, it is obvious from the measurements of RBC aggregation in the Myrenne system (Fig. 2) that this interpretation does not correctly reflect the aggregation behavior of these cells: RBC treated with H₂O₂ had significantly decreased aggregation versus control, while 0.005% GA did not affect the Myrenne aggregation index. Further, the low-shear viscosity and aggregation results obtained with increasing levels of GA are not concordant (Figs. 4 and 5): 1) At 0.512 sec⁻¹, GA concentrations of 0.005, 0.01 and 0.015% increased apparent viscosity, whereas 0.01 and 0.015% GA significantly reduced the Myrenne indexes; 2) At 0.015% GA the Myrenne indexes decreased by about 80% while viscosity at 0.512 sec⁻¹ increased by approximately 20%; 3) At 0.02% GA the Myrenne indexes decreased by 95% while viscosity fell by only 15%.

Thus, for our experiments using RBC with modified rheological or morphological properties suspended in the same aggregating medium, low-shear apparent viscosity did *not* correlate well with an independent measure of aggregation. However, it is of interest to note that RBC deformability, as estimated by EI at 5 Pa (Fig.3), was inversely correlated with low-shear viscosity at 0.512 sec⁻¹ (Fig.1) [$r=-0.45$, $p<0.05$, data from 5 experiments each using control, 0.005% GA, 1 mM hydrogen peroxide and 48 °C heat treated cells]. Therefore, the impairment of RBC deformability (Fig. 3) and the increase of low-shear viscosity (Fig. 1) were both more pronounced for cells treated with GA or H₂O₂ compared to heat-treated cells.

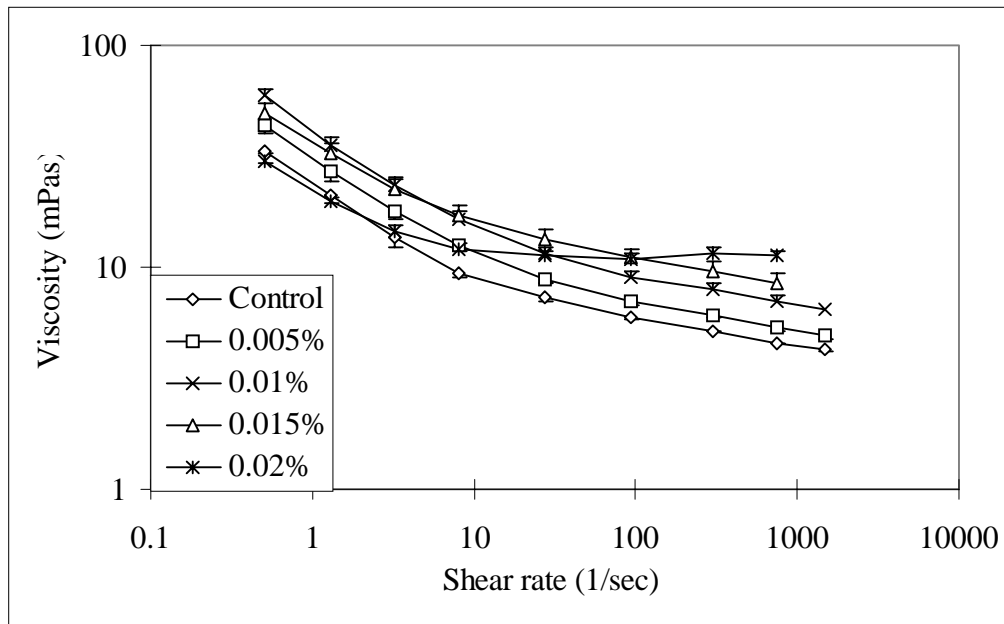


Fig. 4. Viscosity of RBC-plasma suspensions for control cells and for cells treated with various concentrations of glutaraldehyde. Each point represents mean \pm SEM of five experiments

Our viscometric findings are consistent with previously reported results for RBC suspensions with altered mechanical and/or geometrical properties. Meiselman and co-workers (Meiselman, 1978; Meiselman, 1981; Meiselman and Baker, 1977) reported that graded, isovolumic alterations of cell shape (i.e., discocyte to echinocyte) induced by various methods resulted in increasing levels of low-shear viscosity, while at higher shear rates the viscosity of these suspensions approximated control values. These graded discocyte to echinocyte shape changes were associated with stress-dependent alterations of RBC rheologic behavior, such that at low stress levels increased echinocytosis resulted in decreased cellular deformation, whereas at high stress cell deformation did not differ from control (Meiselman, 1981; Pfafferott, et al., 1982). The effect of shape-transformation was shown to be independent of RBC aggregation since the same pattern of increased low-shear viscosity with echinocyte formation was observed in both aggregating and non-aggregating media (Meiselman, 1978).

One possible explanation for the abovementioned shape-associated effect is to assume that at lower shear rates, cell-cell interactions might be enhanced by shape transformation and hence increased cell rigidity, thereby increasing the energy required for relative RBC-RBC movement during flow (Meiselman, 1978). It is known that a progressive echinocyte to ellipsoid-like shape change occurs with increasing shear rate and hence increasing shear stress (Meiselman, 1981; Hardeman et al., 1994; Schmid-Schonbein, 1975), and thus this smoothing, deformation and alignment of the cells would tend to decrease the perturbations of fluid streamlines, thereby decreasing apparent viscosity. This shear-dependent deformation and alignment concept also seems relevant to the viscometric behavior GA-treated RBC (Figure 4): increasing GA levels result in RBC whose resting shape is discocytic but whose ability to deform and align is progressively impaired. Thus, for such cells with a diminished ability to deform, there would be a smaller decrease of apparent viscosity at high shear (Chien, 1975; Shiga, et al., 1990).

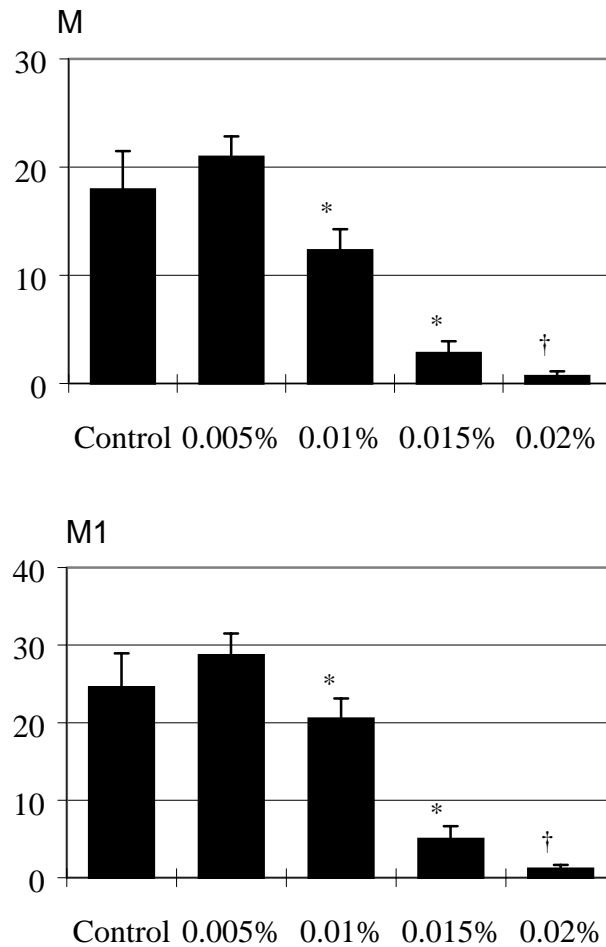


Fig. 5. M (upper panel) and M1 (lower panel) aggregation indexes of RBC-plasma suspensions for control cells and for cells treated with various concentrations of glutaraldehyde. Values are mean \pm SEM of five experiments. Difference from Control; *: $p < 0.05$; †: $p < 0.01$.

It is well-known that RBC aggregation is influenced by cellular properties as well as the composition of the suspending medium (Nash et al., 1987; Sowemimo-Coker, et al., 1989; Meiselman, 1993). Our results imply that if RBC aggregation is altered due to the changes in cellular properties (e.g., cell geometry, cellular deformability), then the absolute magnitude of low-shear apparent viscosity may not uniquely reflect RBC aggregation. However, there are literature suggestions for using viscometric data to calculate RBC aggregation indexes: 1) the difference between low and high-shear viscosity scaled by high-shear viscosity $(V_{LS} - V_{HS}) / (V_{HS})$ (Bull, et al., 1986; Bull et al., 1988); 2) the ratio of apparent viscosity in aggregating and non-aggregating media $(V_A) / (V_{NA})$ at shear rates below 1 sec^{-1} (Brooks, et al., 1974). As shown in Table 2, neither of these two indexes were significantly different from control for heated and H_2O_2 treated RBC, yet

Table 2.

Myrenne "M" aggregation parameter and RBC aggregation indexes calculated from RBC-plasma viscometric data for control cells and for cells treated with 0.005% glutaraldehyde (GA), 1 mM hydrogen peroxide or heat treated at 48 °C.

	M index	V_{LS}	V_{HS}	$(V_{LS} - V_{HS}) / V_{HS}$	V_A / V_{NA}
Control	21.21 ± 2.05	32.13 ± 2.96	5.97 ± 0.22	4.35 ± 0.37	3.97 ± 0.42
HT	18.91 ± 1.57*	36.54 ± 3.17	6.73 ± 0.27*	4.39 ± 0.30	4.68 ± 0.43
GA	23.02 ± 2.32	42.99 ± 2.91 [†]	6.87 ± 0.21*	5.22 ± 0.25*	4.43 ± 0.27
H ₂ O ₂	15.17 ± 2.05*	41.99 ± 3.42 [†]	6.95 ± 0.27 [†]	5.00 ± 0.28	4.04 ± 0.28

$V_{LS} = V_A$ = apparent viscosity in plasma at 0.512 sec⁻¹; V_{HS} = apparent viscosity in plasma at 94.5 sec⁻¹; V_{NA} = apparent viscosity in PBS at 0.512 sec⁻¹. Data are mean ± SEM of five experiments. Difference from control: *: p < 0.05, [†]: p < 0.01.

Myrenne "M" index indicated significant decreases in aggregation. In contrast, the significantly higher value of $(V_{LS} - V_{HS}) / (V_{HS})$ for GA-treated RBC is not consistent with the unaltered "M" index. Table 3 presents the two calculated viscometric indexes for plasma suspensions of RBC treated with various concentrations of GA. Here again the indexes seem to be of limited value: 1) the $(V_{LS} - V_{HS}) / (V_{HS})$ parameter fails to reflect the decrease of "M" at 0.01% GA and exhibits significant decreases only when aggregation is essentially abolished (0.015 and 0.02% GA); 2) the ratio $(V_A) / (V_{NA})$ does reflect the decreases of aggregation at 0.01 and 0.015% but fails to detect this decrease at 0.02% GA (Figure 5).

In overview, our results indicate that low-shear viscometry and indexes calculated from viscometric data should be used with care when attempting to assess RBC aggregation. If RBC with normal rheologic and morphologic characteristics are employed, either the absolute magnitude of low-shear apparent viscosity or one or more of the calculated indexes seem appropriate for testing the effects of altered suspending medium properties (e.g., fibrinogen level, polymer properties). However, in situations where altered RBC shape or rheological behavior are expected (or can not be ruled out), then extreme caution is required: use of viscometric data *alone* may yield accurate yet non-relevant information regarding RBC aggregation. Further, it is important to note that the Myrenne Aggregometer system used herein may be subject to potential artifacts associated with altered RBC properties: 1) M and M1 values reflect the integrated difference between high-shear light transmission and that at stasis or at low shear (Bauersachs *et al.*, 1989). Thus, if RBC deformability is markedly decreased, high-shear light transmission through the RBC suspension being tested would be reduced (Bauersachs *et al.*, 1992), thereby possibly affecting the integrated difference; 2) Although the Myrenne Aggregometer uses IR rather than visible light, it is possible that alterations of RBC cellular and/or membrane mechanical properties could lead to altered membrane optical characteristics and thereby changes the aggregation indexes. Such artifacts seem relatively unlikely however, inasmuch as Myrenne indices correlate well with RBC sedimentation rates for normal and pathologic blood (Schmid-Schönbein *et al.*, 1990), provide data for density-separated RBC (Meiselman, 1993)

Table 3.
Myrenne "M" aggregation parameter and RBC aggregation indexes
calculated from RBC-plasma viscometric data for control cells and for
cells treated with increasing concentrations of glutaraldehyde.

	M index	V_{LS}	V_{HS}	$(V_{LS} - V_{HS}) / V_{HS}$	V_A / V_{NA}
Control	17.97 ± 3.44	29.58 ± 3.71	6.01 ± 0.19	4.49 ± 0.49	3.74 ± 0.27
0.005% GA	20.07 ± 2.06	44.01 ± 3.92*	7.00 ± 0.28*	5.24 ± 0.33	4.3 ± 0.32
0.01% GA	11.12 ± 2.31*	59.21 ± 8.12*	9.01 ± 0.58*	5.56 ± 0.36	5.98 ± 0.31 [†]
0.015% GA	2.81 ± 1.24*	49.49 ± 5.51*	11.06 ± 0.91 [†]	3.47 ± 0.36*	4.91 ± 0.24*
0.02% GA	0.93 ± 1.24 [†]	29.87 ± 2.61	10.93 ± 0.58 [†]	1.72 ± 0.16 [†]	3.33 ± 0.09

$V_{LS} = V_A$ = apparent viscosity in plasma at 0.512 sec⁻¹; V_{HS} = apparent viscosity in plasma at 94.5 sec⁻¹; V_{NA} = apparent viscosity in PBS at 0.512 sec⁻¹. Data are mean ± SEM of five experiments. Difference from control: *: p < 0.05, [†]: p < 0.01.

agree with direct microscopic observations of red cell aggregation (Rampling and Whittingstall, 1986a), and are "extremely sensitive" to changes of fibrinogen levels in the RBC suspending medium (Rampling and Whittingstall, 1986b).

Given the above concerns, and the lack of a "gold standard" for the calibration of methods to quantitate RBC aggregation behavior (Schmid-Schönbein et al., 1990), it therefore seems appropriate to employ one or more additional measures of RBC aggregation: 1) erythrocyte sedimentation rate (ESR) or Zeta Sedimentation Ratio (ZSR, Bull and Brailsford, 1974); 2) light transmission through (Bauersachs, et al., 1989) or light reflectance from (Hardeman, et al., 1994) RBC suspensions in known geometries and under defined shear conditions; 3) electrical admittance measurements (Pribush, et al., 1996); 4) direct observation via light microscopy of dilute RBC wet-mounts or calculation of an index based on quantitative microscopic analysis of individual RBC and RBC clusters per unit volume of suspension (Chien, 1975).

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