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Effect of Reduced Red Cell "Deformability" on Flow Velocity in Capillaries of Rat Mesentery*

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Abstract. A graded reduction of "deformability" of red blood cells (RBC's) of rats was obtained by treatment with the SH-oxidizing agent, diamide. Rigidified RBC's were injected into rats by isovolemic exchange against 60% of the native RBC's and RBC flow velocities in capillaries of rat mesentery measured.

At normal mean arterial pressure RBC flow velocity decreases by 29% in rats receiving cells rigidified with 0.5 mmol·l^{-1} diamide. Surprisingly a further rigidification of erythrocytes by 1.5 mmol·l^{-1} diamide results in a decrease of flow by only 15%. During hypotension RBC flow velocities dropped precipitously to 8±15% for the 0.5 nmol·l^{-1} and to 2±6% for the 1.5 mmol·l^{-1} diamide group compared to velocities during normotension. By microscopy we observed a stop of flow in many vessels.

This result outlines the importance of a normal red cell "deformability" for the maintenance of sufficient perfusion of the microcirculation, in particular at low blood pressure gradients.

Key words: Red cell deformability – Capillaries – Rat mesentery – Hypotension – Flow resistance.

Introduction

The capillary is the site of exchange of nutrients and waste products. The flow rate of blood depends on driving pressure and capillary resistance. The latter is a function of vessel geometry (length, diameter) and of the apparent viscosity of the perfused blood. Apparent blood viscosity in turn depends on plasma viscosity, the concentration of red blood cells (RBC's), the tendency of RBC's to form aggregates, and RBC deformability. Mammalian red cell deformability depends on the viscoelastic properties of the cell membrane, the viscosity of the cytoplasm, and the surface area/volume ratio [1–11].

Cell "deformability" is a prerequisite for the passage of the cells in capillaries with a diameter smaller than the RBC diameter. Furthermore, in vessels, in which high shear stresses prevail, the major axis of the RBC becomes aligned with flow, the RBC elongates, and the membrane rotates around the cell content which leads to a lower blood viscosity [11]. For rigidified cells this effect will be less pronounced resulting in a higher viscous energy dissipitation compared to normal cells [1]. Although reduced RBC "deformability" is known to occur in various forms of anemia [9] and acidosis [2], little relevant data exist concerning the influence of a graded reduction of red cell "deformability" on blood flow in an intact microcirculatory network. Previously we showed that a variation of hematocrit (Hct) had only minor effects on RBC flow velocity in capillaries [3]. We now evaluate the effect of a rigidification of RBC's on their flow velocities in the capillaries of rat mesentery by isovolumic exchange of up to 65% of normal RBC's for modified cells.

Methods

The methods of preparation of rigidified RBC's by the SH-oxidizing agent diamide is described in an accompanying paper [7]. Briefly, RBC's were incubated for 15 min with 0.5 mmol·l^{-1} or 1.5 mmol·l^{-1} diamide, after pretreatment with 10 mmol·l^{-1} iodoacatate in order to block intracellular GSH [7]. The modified RBC's were resuspended in autologous plasma (Hct 40%) which had been cleared from thrombocytes by filtration (Millipore < 0.1 μm). The experiments were performed on anesthetized (Halothane) rats, weighing 210 ± 30 g (n = 23, ± S.D.).

The experimental protocol was as follows: after a control period of 30 min, 60–70% of the native red cells (blood volume was

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Table 1. Hemodynamics of the macro- and microcirculation of rats which received blood cells treated with iodoacetate (reference group). Results are given as mean values with corresponding standard deviations. The number of measured values are given in brackets

	Control period	Exchange period	Hypo-tension period	Reinfusion period
Mean art. pressure (kPa)	13.3 ± 0.8 (5)	12.7 ± 0.7 (5)	4.7 ± 0.41 (5)	13.1 ± 0.8 (5)
Red cell velocity ($mm \cdot s^{-1}$)	1.13 ± 0.51 (15)	1.25 ± 0.61 (15)	0.22 ± 0.32 (15)	0.94 ± 0.41 (15)
Relative velocity (%)	100	116 ± 35	18 ± 13	96 ± 56
Bleeding volume (%)			32 ± 4 (5)	
Hematocrit (%)	45 ± 2 (5)	42 ± 4 (5)	35 ± 3 (5)	41 ± 2 (5)

Table 2. Hemodynamics of the macro- and microcirculation of rats which received cells treated with 0.5 $mmol \cdot l^{-1}$ diamide (mean values and standard deviation)

	Control period	Exchange period	Hypo-tension period	Reinfusion period
Mean art. pressure (kPa)	12.8 ± 1.5 (8)	12.4 ± 1.3 (8)	4.7 ± 0.4 (8)	10.7 ± 2.3 (7)
Red cell velocity ($mm \cdot s^{-1}$)	0.91 ± 0.40 (25)	0.61 ± 0.32 (25)	0.04 ± 0.08 (25)	0.23 ± 0.34 (25)
Relative Velocity (%)	100	71.1 ± 19.1	8.3 ± 15.1	37 ± 45
Bleeding volume (%)			30 ± 8 (8)	
Hematocrit (%)	45 ± 4 (7)	42 ± 3 (6)	34 ± 3 (7)	40 ± 3 (3)

assumed to be 6% of the body weight) were isovolemically exchanged against rigidified cells. A hypotension period of 30 min was obtained by bleeding the animals to a pressure of 4.7 kPa. The pressure level was kept constant by taking small amounts of blood. After 30 min of hypotension the shed blood was reinfused. The reinfusion period was of varying time length (up to 1 h), depending on the recovery of red cell flow. During all experimental periods RBC velocities in capillaries of the mesentery were measured by means of intravital microscopy, closed-circuit TV-system, and crosscorrelation [13].

Results

a) Reference Group (Iodoacetate Treated Cells, Table 1)

During exchange of native erythrocytes against cells treated with iodoacetate, which does not alter RBC deformability [5], red cell velocity increased slightly but not significantly and dropped by about 80% during hypotension, from 1.13 ± 0.51 $mm \cdot s^{-1}$ to 0.22 ± 0.32 $mm \cdot s^{-1}$. During the reinfusion period the control velocity was almost reattained.

b) 0.5 $mmol \cdot l^{-1}$ Group (Table 2)

During exchange of native erythrocytes against cells regidified by 0.5 $mmol \cdot l^{-1}$ diamide [7], red cell velocity decreases from 0.91 ± 0.46 $mm \cdot s^{-1}$ to 0.61 ± 0.32 $mm \cdot s^{-1}$ and further to 0.04 ± 0.08 $mm \cdot s^{-1}$ during the hypotension period.

The standard deviations of flow velocities during the hypotension period are larger than the mean values, which reflects an asymmetric distribution of flow velocities. It is an indication of their spatial scattering. Flow stop was observed in many vessels, during the whole hypotension period, and slow but not zero flow in adjacent vessels. In most experiments however flow stop was observed in a whole microcirculatory unit, i.e. feeding arteriole, capillaries and draining venule at the same time. As an example the velocity distribution for the 1.5 $mmol \cdot l^{-1}$ diamide group during hypotension is plotted in Fig. 1.

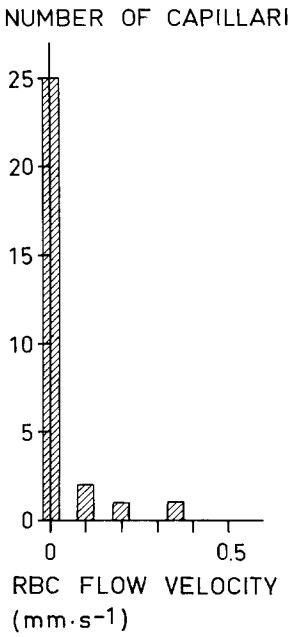

Fig. 1. Velocity distribution of the 1.5 $mmol \cdot l^{-1}$ diamide group during *hypotension period.* It clearly demonstrates an asymmetric velocity distribution: 25 capillaries have a zero velocity, 4 vessels have a velocity greater than zero

The velocities during the reinfusion period amounted to 0.23 ± 0.34 mm · s^{-1} showing again an asymmetric velocity distribution due to flow stop in several vessels during the whole reinfusion period. Only in those vessels in which no stagnation of flow during the hypotension period was observed control velocities were approximately regained.

c) 1.5 mmol·l^{-1} Group (Table 3)

Surprisingly a further rigidification of erythrocytes by a treatment with 1.5 mmol·l^{-1} diamide [7] leads to a smaller decrease of RBC flow velocities during the exchange period. During the hypotension and reinfusion period the flow behavior of cells treated with 1.5 mmol · l^{-1} diamide was comparable to that of cells treated with 0.5 mmol · l^{-1} diamide.

Table 3. Hemodynamics of macro- and microcirculation of rats which received red blood cells treated with 1.5 mmol · l^{-1} diamide (mean values and standard deviation)

	Control period	Exchange period	Hypo-tension period	Reinfusion period
Mean art. pressure (kPa)	12.0±2.3 (9)	11.7±1.5 (9)	4.5 ±0.7 (9)	10.5±1.5 (7)
Red cell velocity (mm · s^{-1})	0.97±0.54 (33)	0.83±0.47 (33)	0.03±0.08 (28)	0.12±0.32 (24)
Relative Velocity (%)	100	86 ±24	2.1 ±6.1	9.1 ±21.0
Bleeding volume (%)			36 ±11 (8)	
Hematocrit (%)	44 ±3 (9)	40 ±4 (9)	34 ±3 (8)	40 ±2 (5)

Discussion

In order to describe the capillary resistance to flow of cells accurately, we would need detailled additional information on pressure gradients along the capillary as well as measurements of geometrical parameters such as length and diameter. Up till now however, there are no data available on pressure gradients in capillaries of the rat mesentery. Moreover, considering the anatomical irregularities such as tapering of capillaries in rat mesentery, it seemed useless to give a definite diameter. Thus, we chose RBC-flow velocity as an indicator of flow behavior of blood in the microcirculation after a change of flow properties (rigidified cells) and of flow conditions (changed mean arterial pressure). A prerequisite for this experimental series was the knowledge of the fate of the modified RBC's in the circulation. In an accompanying paper [7] we show that RBC's treated with iodoacetate and diamide are, after injection into rats, retained in the circulation to almost 100% for the duration of our present experiments (2.5 h). This can also be concluded indirectly from the hematocrit values which remained constant during the exchange and reinfusion period.

Although a treatment of RBC's with iodoacetate impairs cell metabolism it does not influence "deformability" (elongation index) and flow velocity at normal driving pressure (Table 1). RBC flow velocities are in the range we obtained for untreated cells (control period). At low perfusion pressure the velocities of the iodoacetate group are about 30% lower than those of untreated cells. This may reflect a slight decrease in shear elongation, not measurable in the rheoscope, but becoming effective in vivo at low driving pressures. Finally we failed to observe the slight reactive hyperemia after reinfusion of shed blood usually found in experiments with untreated cells [3]. In these experiments, hyperemia amounted to 20%. Thus iodoacetate treatment of RBC's seems to produce discrete effects on flow velocity. However, these effects of iodoacetate treated RBC's on flow velocity are exceeded by far by those obtained with diamide treated cells. Compared to velocities before exchange (control period), both groups of diamide treated cells show, at normal pressure, a decrease of red cell velocity by 30% for 0.5 mmol · l^{-1} and by 14% for 1.5 mmol · l^{-1} diamide respectively. During hypotension velocities further diminished, flow frequently came to a complete stop, on average it was smaller than 10% of control flow. Moreover, velocities recovered only very incompletely during reinfusion period (Tables 2 and 3) presumably due to secondary events occurring during the hypotension period, e.g. hypoxia, thrombus formation, local intravascular coagulation despite of heparinisation of the animals, or of swelling induced by local acidosis.

The experimentally induced reduction of "deformability" of RBC's potentially influences hemodynamics in a) arterioles, b) capillaries or c) venules.

a) Arterioles

In arterioles and capillaries high shear stresses prevail under normotension [10]. Native RBC's exposed to high shear stresses become elongated and aligned to the flow field, the membrane rotating in a "tanktread" like motion around the cell interior [11]. This passive adaptation greatly reduces viscous energy dissipation, sometimes to such an extent that the presence of red cells does not significantly raise the plasma viscosity

[11]. This holds for well deformable cells. However, a fixation of RBC's with glutaraldehyde increases blood viscosity measured in viscometric flow at shear rates greater than 100 s^{-1} [1] to 6-fold that of native biconcave RBC's. For human RBC's treated with diamide an increase of apparent viscosity of up to 30% was found [4]. From these observations it is expected that in arterioles, in which high shear stresses prevail, infusion of diamide treated cells increases the apparent viscosity of blood. This results in an increase of precapillary resistance and may be responsible for the observed reduction of flow.

b) Capillaries

Rigidification of RBC's is expected to increase the resistance to flow due to a thinning of the lubrication layer between cell and vessel wall which leads to higher stresses at the capillary wall in the neighborhood of passing cells and to an increased contribution of the cell to the overall pressure drop along the capillary [12]. At low driving pressure this effect will be even more pronounced [12]. It might come to a "seize up" [6], leading to a sharp rise in resistance due to the development of frictional forces between cell and vessel wall.

Although there is no experimental evidence for "seize up" in glas capillaries even at very low velocities (5 $\mu m \cdot s^{-1}$) [8, 9], sickle cells, poikilocytes and hereditary spherocytes [9] have the tendency to occlude capillaries at bifurcations, capillary bendings, or capillary narrowing on their infusion into vessels of mouse cremaster muscle. At low driving pressure we now observed a stagnation of flow in many vessels upon infusion of RBC's rigidified with diamide.

c) Venules

For human RBC's it has been shown [4] that with increasing diamide concentration there is a concomitant decrease in aggregate formation. Aggregate formation is only possible at low shear stresses which prevail in venules. Difference in aggregate formation could explain the slightly lower RBC-flow velocity of the 0.5 mmol $\cdot$ l^{-1} diamide group compared to the 1.5 mmol $\cdot$ l^{-1} diamide group during normotension, since on the basis of the deformability properties of RBC's we expected a treatment with 1.5 mmol $\cdot$ l^{-1} diamid hydrodynamically to be more effective than a treatment with 0.5 mmol $\cdot$ l^{-1}. The unexpected opposite result may be ascribed to a residual capacity of aggregate formation of the 0.5 mmol $\cdot$ l^{-1} and the complete loss of this capacity of the 1.5 mmol $\cdot$ l^{-1} diamide group. The residual formation of aggregates might increase the resistance to flow in the venules, thus contributing to an already impeded capillary perfusion due to a reduced deformability of the diamide cells.

Concluding Remark

A reduced RBC "deformability" leads to a much more pronounced disturbancy of red cell flow than a change in hematocrit [3]. The present and former [3] findings demonstrate that not only flow conditions (driving force) but also flow properties of blood as such (hematocrit, RBC-deformation) determine whether or not red cells disturb blood flow.

By now it is not possible to localize the site of the microvascular bed where the reduced deformability of RBC's becomes effective and to quantify the influence of nervous control and autoregulation of vessel diameter on RBC-flow.

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