RELATIONSHIP BETWEEN RED CELL DEFORMABILITY AND SHEAR STRESS IN CONDITIONS OF LOW SHEAR RATE IN VASCULAR DEMENTIA

Piotr Kowal and Renata Modestowicz

Rheological Laboratory, Department of Neurology, Karol Marcinkowski University of Medical Sciences, Poznan, Poland

SUMMARY – The aim of the study was to evaluate the effect of shear stress connected with low shear rate on red blood cell deformability in chronic brain ischemia. The study was carried out in a group of 18 patients with vascular dementia and control group of ten healthy subjects. Shear stress was calculated as a function of blood viscosity and basic shear stress for the applied range of shear rate. Red cell deformability (red cell transit time, RCTT) was investigated by filtration method, and the level of fibrinogen was assessed by use of a Chromotimer device. Then the relationship of particular shear stress with RCTT and fibrinogen was analyzed. In comparison to the control group, patients with vascular dementia showed higher levels of RCTT ($p<0.05$) and fibrinogen ($p<0.01$). Statistically significant correlations between RCTT and shear stress at a shear rate of 3.23 s^{-1} ($p<0.02$) and 0.695 s^{-1} ($p<0.05$) were found in the group of patients with vascular dementia. Results of the study suggested suggested that in the group of patients with vascular dementia, shear stress in the range of low shear rate considerably affected the RBC membrane, thus contributing to impairment of its elasticity.

Key words: *Dementia – physiology; Cerebrovascular disorders – blood; Blood viscosity – physiology; Erythrocyte deformability – physiology*

Introduction

The range of low shear rate (below 20 s^{-1}) is adequate for the blood flow in the microcirculation. Shear stress is the result of difference in the flow of adjacent fluid layers and it reaches the highest value close to the vessel wall. Blood as a polymer solution in which cellular elements (red blood cells, platelets, leukocytes) are suspended, belongs to other than newtonian fluids whose viscosity depends on shear rate. The level of hematocrit in the cerebral microcirculation makes about 70% of its value in large vessels[1], and the level of red blood cell (RBC) elasticity affects the transport of oxygen and glucose to the network of neurons. A rising shear stress influences the degree of RBD deformability[2], most probably in the course of cellular membrane permeability for cations[3]. In vascular dementia, a fall in the regional cerebral blood flow is observed, which is conditioned by hemorheologic disorder at the level of small vessels and capillaries, including impairment of RBC deformability[4]. According to Kaniewski *et al.*[5], ischemia does not lead to worsening of human RBC deformability in the conditions of high and low shear rate.

In the present study, an attempt was made to answer the question whether the level of RBC elasticity is influenced by shear stress connected with low shear rate in a model of chronic cerebral ischemia, i.e. vascular dementia.

Subjects and Methods

The study included a group of 18 patients with vascular dementia (ten female and eight male, mean age 67.5 years) and a control group of ten healthy subjects without any clinical signs of internal or neurologic disease (five

Correspondence to: *Piotr Kowal, M.D.,* Rheological Laboratory, Department of Neurology, Karol Marcinkowski University of Medical Sciences, Przybyszewskiego 49, 60-355 Poznan, Poland

Received February 4, 2002, accepted May 31, 2002

Table 1. Values of hemorheologic parameters in normal subjects (control group) and vascular dementia patients (VD group)

Parameter	Control group (n=10)	p	VD group (n=18)
Fibrinogen (mg/dl)	150.7 ±35.85	<0.01	238.8 ±91.22
RCTT	12.62± 2.66	<0.05	13.48± 2.93
Shear stress A (Pa)	7.89± 0.51x10^{-3}	<0.05	8.46± 0.73x10^{-3}
Shear stress B (Pa)	4.63± 0.34x10^{-3}	<0.05	4.98± 0.48x10^{-3}
Shear stress C (Pa)	1.61± 0.20x10^{-3}	<0.01	1.92± 0.32x10^{-3}
Shear stress D (Pa)	0.52± 0.08x10^{-3}	<0.02	0.66± 0.17x10^{-3}
Shear stress E (Pa)	0.18± 0.06x10^{-3}	<0.05	0.33± 0.15x10^{-3}
Mean shear stress (Pa)	6.12± 0.42x10^{-3}	<0.05	6.58± 0.59x10^{-3}

Shear stress at shear rate: A=20.4 s^{-1}; B=11.2 s^{-1}; C=3.23 s^{-1}; D=0.695 s^{-1}; E=0.204 s^{-1}; RCTT=red cell transit time

female and five male, mean age 65.4 years). In the patient group, the diagnosis of vascular dementia was made on the basis of NINDS-AIREN criteria[6].

In all study subjects, blood viscosity was determined at a shear rate of 20.4, 11.2, 3.23, 0.695 and 0.204 s^{-1}, using a Low Shear 30 (Contraves, Zürich) microviscosimeter at a temperature of 37 °C and stable hematocrit of 30%. For each shear rate, the value of shear stress was calculated as a function of blood viscosity and basic shear stress for the shear rate range used. The principle of calculation was taken over from the operating manual for this particular microviscosimeter:

τ=0.000652 x z Pa/1% x b (dynamic conditions), where 0.652 Pa=value of basic shear stress for the shear rate range used; z=value of viscosimeter readings; b=10^{-3}.

RBC deformability was investigated by a St. George's Filtrometer (Carri Med, Darking, England). A 10% erythrocyte suspension in PBS at pH 7.4-7.6 was filtered under a constant subpressure using 5-mm filters (Nucleopore Co., Pleasantown, USA). The finding was expressed as a mean of two measurements of the red cell transit time (RCTT).

The level of fibrinogen was determined by use of a Chromotimer device (Behring). Then, correlations of RCTT and fibrinogen values were analyzed according to particular shear stress levels and mean shear stress, i.e. according to the overall shear stress distribution in the group of vascular dementia patients and in the control group of healthy subjects. As the distribution of the given feature was approximately normal, Pearson's correlation coefficient (r) was used. Between-group differences were assessed by Student's t-test.

Results

Comparison of the results obtained in the group of patients with vascular dementia and control group of normal subjects yielded statistically significant differences between the two groups, i.e. higher values of fibrinogen ($p<0.01$), RCTT ($p<0.05$), mean shear stress ($p<0.05$), and shear stress at shear rates of 20.4, 3.23, 0.695 and 0.204 s^{-1} ($p<0.05$, $p<0.01$, $p<0.02$ and 0.05, respectively), in the former (Table 1).

In the control group, there was no statistical correlation between RCTT and shear stress. The same applied to the mean shear stress as a manifestation of the dynamics of distribution within the analyzed range of shear rate. In the group of patients with vascular dementia, a statistically significant positive correlation was recorded for shear stress values at shear rates of 3.23 s^{-1} ($p<0.02$) and 0.695 s^{-1} ($p<0.05$). A statistically significant positive correlation was found between RCTT and mean shear stress for the analyzed distribution of shear stress ($p<0.05$) (Table 2).

Concerning the correlation between the level of fibrinogen and shear stress, significant correlations were observed for shear rates of 20.4, 11.2, 3.23 and 0.204 s^{-1} ($p<0.01$, $p<0.02$, $p<0.001$ and $p<0.001$, respectively) in the control group, and for all shear rates ($p<0.001$) in the group of patients with vascular dementia (Table 3).

Discussion

The red cell deformability appears to be an independent factor affecting blood viscosity. This term defines the RBC capability to change spatial structure under the in-

Table 2. Correlation coefficients between RCTT and shear stress at particular shear rates in normal subjects (control group) and vascular dementia patients (VD group)

Parameter	Control group (n=10)	VD group (n=18)
Shear stress A	0.085	0.455
Shear stress B	– 0.055	0.556**
Shear stress C	0.191	0.465
Shear stress D	0.417	0.497*
Shear stress E	0.253	0.290
Mean shear stress	0.067	0.489*

Shear stress at shear rate: A=20.4 s^{-1}; B=11.2 s^{-1}; C=3.23 s^{-1}; D=0.695 s^{-1}; E=0.204 s^{-1}; level of significance of Pearson's correlation coefficient (r): *p<0.05; **p<0.02; RCTT=red cell transit time

fluence of mechanical factors such as vascular bed morphology. It is a manifestation of the viscid-elastic quality of cell membrane, viscosity of intracellular fluid, and RBC morphology[7,8]. The increase in red cell rigidity (the process of aging, hemoglobinopathy, disturbance in acid-base balance, electrolyte balance or osmolarity) is associated with possible adverse effects on perfusion within microcirculation[9]. Impairment of red cell deformability is also one of the factors affecting RBC aggregation with regard to shear rate. The increase in RBC aggregation in turn leads to an increase in platelet aggregation, which then increases viscosity and decreases blood flow[1]. Platelet aggregation induced by shear stress occurs due to the contribution of adhesive proteins such as fibrinogen (the range of low shear rate) and von Willebrand's factor (the range of high shear rate)[10].

The results of the study provided evidence that in the presence of lesions of chronic cerebral ischemia, shear stress in the conditions of low shear rate affected the level of RBC elasticity. The analyzed range of shear rate stimulated enhanced RBC aggregation. In contrast to platelet aggregates, RBC aggregates were observed to depend just on shear rate and could be disaggregated at a high shear rate. The phenomenon was only recorded in patients with vascular dementia and not in the control group of normal subjects. The analysis of distribution of statistical significance of correlation coefficients between RCTT and shear stress for particular shear rates showed that there was evident but nonsignificant correlation at 0.204 s^{-1} only. It may suggest that in the conditions of most pronounced RBC aggregation, the effect of shear stress on RBC elasticity was not observable. Fibrinogen plays a key role in the process of RBC aggregation, as demonstrated in the studies of acute stage of cerebral ischemia[11], as well as in a selective plasmapheresis model[12]. The analysis of quantitative contribution of fibrinogen molecules in the formation of bonds between RBCs indicates that it is a dynamic process dependent on a variety of factors[13]. According to some authors[14], extracellular ligands stimulate interaction between integral membrane proteins influencing the level of RBC elasticity and their susceptibility to shear stress.

The present study confirmed strong correlation between the level of fibrinogen and shear stress (the range

Table 3. Correlation coefficients between fibrinogen and shear stress at particular shear rates in normal subjects (control group) and vascular dementia patients (VD group)

Parameter	Control group (n=10)	VD group (n=18)
Shear stress A	0.791*	0.750**
Shear stress B	0.719*	0.730**
Shear stress C	0.864**	0.822**
Shear stress D	0.367	0.760**
Shear stress E	0.860**	0.786**
Mean shear stress	0.799*	0.752**

Shear stress at shear rate: A=20.4 s^{-1}; B=11.2 s^{-1}; C=3.23 s^{-1}; D=0.695 s^{-1}; E=0.204 s^{-1}; level of significance of Pearson's correlation coefficient (r): *p<0.01; **p<0.001

of low shear rate) in both patient group and control group. The level of fibrinogen and values of particular shear stress were significantly higher in the patient group. Accordingly, it could be suggested that in the group of patients with vascular dementia, shear stress in the range of low shear rate considerably affected the RBC membrane, thus contributing to impairment of its elasticity.

References

1. WOOD JH, KEE DB. Hemorheology of cerebral circulation in stroke. Stroke 1985;16:37-41.
2. MAZERON P, MÜLLER M, AZOUZI H. Deformation of erythrocytes under shear: a small-angle light scattering study. Biorheology 1997;34:99-110.
3. JOHNSON RM. Membrane stress increases cation permeability in red cells. Biophys J 1994;67:1876-81.
4. LECHNER H, WALZL M, WALZL B. Hämorheologie und H.E.L.P. bei Multiinfarct Demenz. Wien Klin Wochenschr 1992;104:290-3.
5. KANIEWSKI WS, HAKIM TS, FREEDMAN JC. Cellular deformability of normoxic and hypoxic mammalian red blood cells. Biorheology 1994;31:91-101.
6. WETTERLING T, KANITZ RD, BORGIS KJ. Comparison of different diagnostic criteria for vascular dementia (ADDTC, DSM-IV, ICD-10, NINDS-AIREN). Stroke 1996;27:30-6.
7. CHIEN S. Principles and techniques for assessing erythrocyte deformability. Blood Cells 1978;3:71-99.
8. DINTENFASS L. Blood as a near-'ideal' emulsion – a retrospective on the concept of the red cell as a fluid drop, its implication for the structure of the red cell membrane. Biorheology 1990;27:149-61.
9. SAKUTA S. Blood filtrability in cerebrovascular disorders, with special reference to erythrocyte deformability and ATP content. Stroke 1981;12:824-35.
10. RUGGERI ZM. Glycoprotein Ib and von Willebrand factor in the process of thrombus formation. Ann N Y Acad Sci 1994;714:200-10.
11. TANAHASHI N, GOTOH F, TOMITA M, SINOHARA T, TERAYAMA Y, MIHARA B. OHTA K, NARA M. Enhanced erythrocyte aggregability in occlusive cerebrovascular disease. Stroke 1989;20:1202-7.
12. KOWAL P, WALZL M, WALZL B, LECHNER H. The influence of the HELP system on yield shear stress in vascular disease. Clin Hemorheol 1993;13:701-6.
13. KOWAL P. Quantitative study of fibrinogen molecule contribution to the inter-red cell connections in selected clinical groups of stroke patients. Clin Hemorheol Microcirc 1998;18:37-41.
14. PAULITSCHKE M, NASH GB, ANSTEE DJ, TANNER MJ, GRATZER WB. Perturbation of red cell membrane rigidity by extracellular ligands. Blood 1995;86:342-8.

Sažetak

ODNOS IZMEĐU DEFORMABILNOSTI ERITROCITA I OŠTEĆENJA MEMBRANE ERITROCITA U UVJETIMA NISKOG STUPNJA NAPREZANJA KOD VASKULARNE DEMENCIJE

P. Kowal i R. Modestowicz

Cilj ovoga ispitivanja bio je procijeniti učinak mehaničkog oštećenja membrane eritrocita (*shear stress*) povezanog s niskim stupnjem naprezanja (*low shear rate*) na deformabilnost eritrocita kod kronične moždane ishemije. Ispitivanje je provedeno u skupini od 18 bolesnika s vaskularnom demencijom i kontrolnoj skupni od desetoro zdravih ispitanika. Oštećenje membrane eritrocita izračunato je kao funkcija viskoznosti krvi i osnovnog mehaničkog oštećenja uz primijenjeni raspon stupnja naprezanja. Deformabilnost eritrocita (vrijeme prolaska eritrocita (*red cell transit time*), RCTT) ispitivana je metodom fitriranja, dok je razina fibrinogena procijenjena primjenom uređaja Chromotimer. Tada je analizran odnos pojedinog oštećenja membrane eritrocita s RCTT i fibrinogenom. U usporedbi s kontrolnom skupinom bolesnici s vaskularnom demencijom imali su više razine RCTT ($p<0,05$) i fibrinogena ($p<0,01$). U skupini bolesnika s vaskularnom demencijom nađene su statistički značajne korelacije između RCTT i oštećenja membrane eritrocita kod stupnja naprezanja od 3,23 s^{-1} ($p<0,02$) and 0,695 s^{-1} ($p<0,05$). Rezultati ispitivanja ukazali su na to da je u skupini bolesnika s vaskularnom demencijom mehaničko oštećenje membrane eritrocita kod niskog stupnja naprezanja znatno utjecalo na membranu eritrocita i time doprinjelo smanjenju njezine elastičnosti.

Ključne riječi: *Demencija – fiziologija; Cerebrovaskularne bolesti – krv; Viskoznost krvi – fiziologija; Deformabilnost eritrocita – fiziologija*