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Thixotropic effect of some biochemical factors in ischaemic stroke

Piotr Kowal, Anna Marcinkowska-Gapińska

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

ABSTRACT

Introduction. Yield shear stress (YSS) well characterizes the thixotropic status of blood, that exemplifies a reversible loss of blood fluidity due to a low shear rate. At the stable haematocrit ratio YSS depends mainly on the fibrinogen level.
Aim. Since the role of other biochemical factors in the YSS phenomenon in cerebral ischaemia has not been well known, we have undertaken this problem in a group of stroke patients.
Material and Methods. The study was carried out in 36 patients with acute ischaemic stroke and in 12 controls. YSS was estimated by means of microviscometric method. In all subjects the concentration of the following biochemical factors were assayed: albumin, IgG, IgA, IgM, apolipoprotein A, and B, cholesterol, triglycerides, LDL, HDL and fibrinogen. Then the thixotroipic effect of all biochemical factors and their correlations to fibrinogen were estimated by means of mathematical formulas.
Results. We found a positive correlation in relation to the following thixotropic effects: for all subjects and separately for patients' group: Alb(YSS) ($p < 0.001$), ApoA(YSS) ($p < 0.001$), ApoB(YSS) ($p < 0.05$), chol(YSS) ($p < 0.01$), HDL(YSS) ($p < 0.05$); for patients group without additional diseases: Alb(YSS) ($p < 0.05$), ApoA(YSS) ($p < 0.005$), chol(YSS) ($p < 0.05$), HDL(YSS) ($p < 0.02$), LDL(YSS) ($p < 0.05$). There were not any significant correlations in controls.
Conclusions. Results of the study indicated that in the interaction between the red cells and fibrinogen some additional factors appearing or activating during ischaemic process may play a role.

Keywords: hemorheology, blood viscosity, plasma viscosity, blood proteins.

Introduction

The blood flow in blood vessels is a very complex matter. Under physiological conditions, the blood flow is determined both by the vascular factor and by the physical and physicochemical properties of blood [1–3]. In terms of rheological properties blood is a non-newtonian fluid, it is a suspension of cellular components of the plasma [1–4].

The main factors affecting the flow of blood are hematocrit, whole blood viscosity, plasma viscosity, aggregation and deformation of erythrocytes [2, 5]. In the low shear rate range aggregation of red blood cells is the major determinant of blood flow. The size of the aggregation of red blood cells is a function of flow conditions, the properties of the cell membrane, the presence of large molecules (fibrinogen, α 2-globulin, IgM), and metabolic factors, such as osmolarity, and pH of blood. The consequence of the creation and disintegration of aggregates of erythrocytes is the thixotropic character of blood [2, 6, 7]. Thixotropy is a phenomenon occuring under isothermal flow of a liquid remaining for a long time at rest, resulting in reversible decrease of shear stress with time at a constant shear rate. An example of this phenomenon is the sol gel

transition. The consequence of these properties is a specific hysteresis flow curve, as well as the dependence of rheological parameters on the "history" of fluid [8]. Yield shear stress (YSS) well characterizes the thixotropic status of blood, that exemplifies a reversible loss of blood fluidity due to a low shear rate [9].

Material and Methods

Research was conducted in a group of 36 patients with acute ischemic stroke and 12 individuals showing no clinical signs of neurological or internal medicine disease. In the control group there were 8 women and 4 men (mean age 65 years). The group of patients included 15 men and 21 women (mean age 68 years). Fourteen of them had hypertension, 9 were diabetes and 2 had angina pectoris. From the interview it was established that 1 patient had a heart attack, 1 had an episode of transient cerebral ischemia while 7 patients were subject to more than one risk factor for stroke.

From all subjects venous blood was collected into tubes with EDTA (1 mg/ml). In the case of patient blood collection took place within the first 48 hours from the onset of clinical signs of cerebral ischemia. The blood was centrifuged at 3000 rpm, and so isolated red blood cells were washed twice with 0.9 % saline. For the haemorheological study 30% suspensions of erythrocytes in their own plasma were prepared.

All measurements were made at 37°C. For each shear rate the value of shear stress was calculated. The values of yield shear stress (YSS) were determined from the dependence of the natural logarithms of shear tension and shear using numerical methods. The YSS value was estimated as the point of intersection of the extrapolated straight line with the axis of ordinates. The intensity of interaction of red blood cells and fibrinogen was determined by converting the calculated value of YSS to 100 mg of this protein. Blood samples collected from all the subjects were also used to obtain the serum, from which the contents of the following protein and lipid fractions were determined: albumin, IgG, IgA, IgM, apolipoprotein A_1 and B, cholesterol, triglycerides, LDL and HDL.

The value of YSS obtained for each sample was converted to 100 mg of a particular biochemical agent, thereby expressing the intensity of its effect on the interaction of red blood cells to fibrinogen (Thixotropic effect). Then we analyzed the relationship between that parameter and the level of fibrinogen.

Results

In **Table 1** we showed the distribution of the linear correlation coefficients between the level of fibrinogen and the strength of the thixotropic effect for selected biochemical factors of blood. Statistically significant positive correlations were demonstrated for the following factors when considering the total sample and separately groups of patients: Alb(YSS) ($p < 0.001$), ApoA(YSS) ($p < 0.001$), ApoB(YSS) ($p < 0.05$), chol(YSS) ($p < 0.01$), HDL(YSS) ($p < 0.05$); for patients without additional diseases: Alb(YSS) ($p < 0.05$),

Table 1. A summary of linear correlation coefficients between the fibrinogen and the level of the thixotropic effect for selected biochemical factors of blood in patients with acute cerebral ischemia and in the control group

Dependence	I n = 12	II n = 36	III n = 18	IV n = 48
Fib/IgG(YSS)	0.0005	0.2928	0.0230	0.2452
Fib/IgA(YSS)	0.1377	0.2801	0.0350	0.1554
Fib/IgM(YSS)	-0.4063	0.1280	-0.0640	0.1165
Fib/Alb(YSS)	-0.1069	0.6659*****	0.5830*	0.5804*****
Fib/ApoA(YSS)	0.1223	0.6659*****	0.7110****	0.5883*****
Fib/ApoB(YSS)	0.0935	0.4103*	0.4200	0.3005*
Fib/Chol(YSS)	0.1187	0.4535***	0.7170****	0.3987***
Fib/Tgc(YSS)	0.0851	0.0655	0.1820	0.0448
Fib/HDL(YSS)	0.2355	0.4096*	0.6530**	0.3984*
Fib/LDL(YSS)	0.0442	0.3217	0.5360*	0.2478

I – control group, II – ischaemic stroke, III – stroke patients group without additional diseases, IV – whole group; the significance level of differences: * $p < 0.05$; ** $p < 0.02$; ***$p < 0.01$; **** $p < 0.005$; ***** $p < 0.001$;

ApoA(YSS) ($p < 0.005$), chol(YSS) ($p < 0.05$), HDL(YSS) ($p < 0.02$), LDL(YSS) ($p < 0.05$). When considering only the control group no statistical significance for any of the examined relationships was found.

Discussion

Former findings show the role of synergism of some protein components of plasma with the interaction between red blood cells and fibrinogen in patients with ischemic stroke [10].

The results of studies of the effect of fibrinogen on the level of the thixotropic effect of some lipid fractions seem to confirm the supporting role of lipids in the aggregation of erythrocytes. In the applied method of mathematical analysis thixotropic effect level denotes the YSS value relative to 100 mg of a biochemical factor. It turns out that the increase of fibrinogen level significantly increases the thixotropic effect exerted by apolipoprotein A_1. As in the control group a negative correlation of the lipid fraction with the low shear rate value of blood viscosity was found, it suggests some duality of apolipoprotein A_1 function. The impact of fibrinogen on the thixotropic effect of apolipoprotein B, cholesterol and HDL from a statistical point of view is much weaker. Among the protein fraction, only albumins show an equally strong positive correlation of the thixotropic effect with fibrinogen level as in the case of apolipoprotein A_1. These phenomena also occur when analyzing the group of patients with no other additional diseases but they are not observed in the control group. This suggests the involvement of some additional factors in the process of red blood cell interactions with fibrinogen. These factors either appear upon occurrence of acute ischemic stroke or have been present before and only get activated by this event.

Some light was shed on this issue by the reports on the impact of the immunological phenomena on blood clotting disorders [11] and also on the presence of antiphospholipid antibodies and antycardiolipin in the part of the population of patients with cerebral ischemia (especially in patients over the age of 50) [12–15].

According to Donner *et al.* [16], in the presence of immunoglobulins albumins effect on the aggregation of red blood cells seems to be a complex phenomenon, depending on the ratio of albumins to globulins. Albumin molecules do not induce rulonization of erythrocytes, however they have inhibitory or stimulatory effect on the aggregation of red blood cells stimulated by other proteins [16, 17].

In the process of erythrocyte aggregation fibrinogen plays an important role [9, 18, 19]. Fibrinogen has particularly strong affinity to the cell membrane of red blood cells. Aggregation of red blood cells in a solution of pure fibrinogen starts from the moment when the concentration of the protein is in the range of 2 g/l, however the presence of other proteins may reduce this threshold. According to Janzen *et al.* [20], aggregation of red blood cells is also possible in the absence of these macromolecules by means of so called depletion mechanism, but such phenomenon is more related to an experimental model. On the other hand, Skalak and Cheng [21] noted that even when red blood cells are not aggregated flow dysfunction can occur in the microcirculation as a result of hydrodynamic interactions between cells.

Conclusions

Mathematical model analysis of the contribution of various biochemical factors in the phenomenon of thixotropy shows that the process of interaction of red blood cells with fibrinogen takes place with the help of additional unknown factors which in the acute phase of cerebral ischemia either appear or get activated.

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Conflict of interest statement
The authors declare no conflict of interest.

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Correspondence address:
Anna Marcinkowska-Gapińska
Rheological Laboratory, Department of Neurology
Poznan University of Medical Sciences, Poland
49 Przybyszewskiego Street, 60-355 Poznań, Poland
email: margap@ump.edu.pl