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IMPORTANCE OF CHANGES OF ERYTHROCYTES DEFORMABILITY IN DEVELOPMENT OF HEMORHEOLOGICAL DISTURBANCES

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Abstract: It has been known that a number of pathological processes in a human organism is accompanied by pronounced hemorheological disturbances. It was established a relationship between biochemical changes of the blood and its rheological characteristics. The contribution of erythrocytes to rheological blood properties is very essential. Therefore the erythrocyte deformability is a very important factor in rheology of blood. The relationship between rheological blood properties, form and sizes of capillary system was found in experiment.

Key words: circulation system, viscosity of blood, erythrocyte deformability, experiment, glass tubes experiment

Blood viscosity and erythrocyte deformability

A number of pathological processes which are accompanied by pronounced disturbances demands complex assessment of circulatory system state and at the same time accumulation of new facts on importance of discrete facts in disturbances development [5, 7].

In the literature, there are considerable amounts of information on diversified pathological states where relationship between biochemical changes of the blood and its rheological characteristics was established. For example, patients with purulent inflammations have an increased blood viscosity [8]. Under acute purulent-septic diseases of lungs and pleura, the blood fluidity also decreases. Analogous phenomena are typical for atherosclerosis and its basic implications, including myocardial ischemia [5].

Up to now the point of view was made up that determination of blood viscosity is necessary for valuable study of rheological properties of blood. Furthermore it is necessary to determine the value of hematocrit, deformability of erythrocytes and red blood cell aggregations, viscosity of plasma [5]. In connection with the fact that erythrocytes occupy almost a half of blood volume and tissues supply by oxygen depends on them, the contribution of erythrocytes to rheological blood properties (namely the role of their deformability and aggregation) is very essential. Therefore it is very interesting to analyze the contribution of changes in erythrocyte deformability to hemorheological disturbances which determine in a multitude of ways the state of microcirculation and state of central hemodynamics.

The microcirculation is connected not only with the release of substrate which are necessary for oxidizing processes in cells but with assimilation of cell metabolism products as well. The resistance to the blood flow depends on the complicated architecture of microvessels network which in its turn is controlled by vasomotor innervation (vasoconstrictive and vasodilative effects) and by humoral mechanisms.

The erythrocyte deformation decreases the resistance to the flow but erythrocyte aggregation increases the resistance. The other cell components of blood (leukocytes and thrombocytes) influence also erythrocyte behavior in the microvessels network [4].

The theoretical analysis shows that a erythrocytes form (disk) capable of deformation ensures its migration to direction of axis in parabolic velocity field [22, 30]. Rigid (incapable of deformation) particles near wall migrate also to the central axis with simultaneous motion of particles from axis to wall [35]. Therefore the formation of a cell-free layer due to the reduction of amount of deformation capable erythrocytes is less credible. This fact must be considered in diagnostics [48] and in analysis of facts contributing to pathological process development.

The most important factors for diagnostics are physiological and pathological ones of cell-free layer action which were established in experiment *in vivo* in isolated channel of rabbit myoseptum [45, 48]. In this research the phenomena were verified which have been established earlier in experiment in narrow glass tubes. But in addition the relationship between the thickness of cell-free layer and erythrocyte sizes was established. For example, the rabbit cells (volume is 75 μm^3) form more thin free-cell layer than human being cells (volume is 90 μm^3) with the same hematocrit. In microvessels with diameter under 10 μm (which is commensurable with erythrocyte diameters), there is no notion „free-cell layer thickness". In this case the velocity of erythrocytes motion is determined totally by erythrocyte deformability [48]. It is very important for methodical aspects of diagnostics by rheological blood properties. Even in more sizable vessels it is not always apparent either the erythrocytes deformability decreased or stenosing factors changed cell-free thickness [20], although these factors are interrelated. There is another point of view. In [9] it is proposed that the phenomenon of viscosity increase can be completely caused by loss of erythrocyte deformability.

It is of particular value that last years it is getting clear: a model of deformable cell motion by glass capillary of round section with constant diameter is necessarily inadequate to blood flow since this model does not simulate structure of blood vessels. The behavior of real erythrocytes in real vessels is a case of system interaction between structural cell membranes and structural blood vessels network. It is not simulated by structure of straightwalled glass tubes. A number of authors consider that there is another effect in nondeformable rigid particles: they are halted by capillaries of organism [41]. The consequence of this effect in old cells or in erythrocytes of patients is its influence on blood filterability.

Factors determining erythrocyte deformability

It is well known that the blood viscosity decreases at the high shear velocities due to erythrocytes deformation. This fact is connected with reduction of their amount in hydrodynamic effective volume [12, 14, 38]. The erythrocyte deformation contributes to release of oxygen from them, it takes place in capillaries [26, 31, 39]. The sufficient membranes area, the low shear modulus of membrane „material" representing a very complicated biocomposite system and high internal fluidity allow normal disc to deform and have different forms without appreciable changes of surface area or cell volume [12,14].

In such a manner, the erythrocyte deformation is determined by the following factors [12, 14, 20, 26, 38, 44, 46]:

1) rigidity of erythrocyte membrane;
2) cell form;
3) intracellular viscosity.

The viscoelasticity of erythrocyte membrane is controlled by mechanical properties and structure of the cytosceleton and lipid bilayer. The cytosceleton proteins play important role in maintenance of erythrocyte form and in provision of membrane rigidity, especially under interaction of spectrin, ankerin and a number of other cytosceleton proteins. The erythrocyte deformation ability breaks down on genetic level under oxidation deterioration of

protein cytosceleton and under disruption of interaction between cytosceleton proteins and cytoplasmic constituents.

The authors of the paper [2] analyze influence of infections and burn toxins on animal erythrocyte deformability and draw the conclusions that deterioration of high-molecular proteins in erythrocyte membrane decreases their elasticity.

It should be recorded that the changes of lipid constitution of erythrocyte stroma are also very significant. For example, the increase of cholesterol - phosphatide index is characteristic for blood of sick animals under both mentioned pathologies. The observed variations of indexes in experimental animals confirm this facts under burn.

The relationship between loss of filterability, content of spectrin proteins fraction and increase of portion of more „light" proteins fraction gives a signal of possible changes of erythrocytes form. This fact was recorded in [6] under burn toxemia of patients. The relationship between ability of erythrocyte membranes for deformation and state of spectrin proteins was recognized for many years [32], now this relationship has a new interpretation. There appears to be rigidity is connected with influence of spectrin proteins on possibility of conservation of erythrocyte membrane areas with negative curvature [1].

The membrane rigidity increases with increase of cholesterol content in lipid bilayer. It decreases erythrocyte ability for deformation and simultaneously denudes cell of negative curvature. The last fact was showed by morphodensitometry [14].

The concavo-concave disc form of erythrocytes having great surface area (140 μm^2) with cell volume 90 μm^3 ensures the cell deformability. The form changes due to genetic disruption of cytosceleton proteins, metabolic break down (during blood conservation) or interaction between some medicinal preparation and lipid bilayer can impede deformation. The quantitative characteristics of form loss were introduced by morphodensitometry [3].

The erythrocyte deformation depends on concentration of intracellular hemoglobin. Pathological changes of hemoglobin structure cause disruption of erythrocyte deformation (denatured hemoglobin, crystallized hemoglobin in crescent cells at crescent cell anemia, change of hemoglobin viscosity at the sting by malaria parasites).

It has been observed *in vivo* that erythrocytes can deform in a blood stream to different forms (crooked, folded, forms of torpedo, parachute, slipper and other forms) [11]. The modifications of such forms move often by series. The analogous types of erythrocyte deformation are responsible for drop in blood stream resistance at venous sections under microcirculation [27].

The erythrocytes processed by diamide, glutaraldehyde and other preparations are used as a model of „hardened" cells [16] which have an enhanced stream resistance [47, 48] caused by pressure overfall at the ends of glass tube named sometimes as capillary. The heat treatment of blood specimens contributes also to the increase of resistance [23]. The processing of cells by glutaraldehyde and formaldehyde is responsible for considerable resistance increase in the blood channel of experimental animals [25, 28, 37]. Therewith the blood returns totally to the norm due to perfusion of test animals by composition containing normal cells.

By this means the erythrocyte deformability by series in the stream can be modeled by glass tubes with diameter which is considerable superior to the erythrocytes sizes. The stream by series („Indian file") observed in narrow glass tubes is a function of vessel diameter and hematocrit [19]. As this takes place, the erythrocyte deformation can be symmetrical about some plane [19, 24]. The erythrocyte orientation becomes asymmetrical at low stream velocities (smaller than 0.2 mm/sec) [40].

The rigid erythrocytes processed by glutaraldehyde [21] and subjected to heat treatment [36] have higher stream resistance in experiments with the use of glass tubes. Thus

spherocytes (rigid cells) remain in parietal space as shown under treatment of erythrocytes by glutaraldehyde [25].

The normal erythrocytes (discocytes) can pass through tubes with diameter 2.7 - 2.8 ❍m [13] which is close to the sizes of organism capillaries. The change of erythrocyte form can be theoretically predicted by elastic models of cell membranes [20, 34, 40, 42]. At the same time, however, the erythrocytes deformation is quite different [11] in model capillaries because the shear effort can be dissimilar in magnitude. The erythrocytes in organism microvessels are dissimilar in age and new cells deform easier than old ones [31].

Experimental investigations show that erythrocytes in capillaries fold along their diameter or asymmetrically [19, 29, 40]. According to the theoretical analysis the erythrocytes tend to take the maximal asymmetric form. It allows to minimize pressure or maximize stream [33].

Consideration of the problem of erythrocyte deformability in real circulatory system

According to the observation of some authors erythrocytes in blood vessels are subjected to deformation to a smaller extent than erythrocytes in glass tubes with the same diameter and free-cell layer in blood vessels is more thin [47].

The sensitivity of microvessel network *in vivo* to the confinement of erythrocytes processed by glutaraldehyde depends on its topography [25, 37]. For example, lungs, spleen, liver and bone marrow of animals are sensitive to the decrease of erythrocyte defofmability. It increases the stream resistance in appropriate microvessels [37]. The same is observed under infusion of homozygous falciform erythrocytes to mouse [15] and rat [17, 18]. The resistance increase can be compensated by transfusion of normal cells to rabbit in the wake of transfusion of erythrocytes deformed by chemical treatment [48].

By this means we see relationship between microvessel (or its model) diameter size and discocyte ability to sufficiently light deformability. This fact was observed in experiments with glass tubes, in experiments *in vivo* and at the analysis of some clinical findings.

The systemic deterioration of blood circulation hemodynamics forces to pay attention to phenomena connected with loss of erythrocyte deformation properties. They influence on microcirculation both directly by increase of blood viscosity and mediately by changes of parietal and axial flow in capillaries. In the process the structure of this flow has many layers. It forces to consider a motion of cell population in glass tubes as a model of cell motion only for sufficiently large blood vessels. The modeling of erythrocyte behavior on blood channel capillaries can be correct only by filtration methods. Therewith it is noteworthy to consider a form of pores of filter capillary space.

The free-cell layer decreases to a greater extent than in glass tubes. It can be explained by different conditions of stream in vessels with structures. These vessels do not have a simple cylindrical form.

It is obvious other parameters of description for blood cell behavior (kinetics of their aggregation, stability and aggregates size) need in more detail analysis.

Conclusions

The influence of erythrocyte deformation properties on rheological behavior of blood was analyzed. The relationship between rheological blood properties, form and sizes of capillary system was found. The microcirculation network must be also considered as a complicated, spatially oriented medium.

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РОЛЬ ИЗМЕНЕНИЙ ДЕФОРМИРУЕМОСТИ ЭРИТРОЦИТОВ В РАЗВИТИИ ГЕМОРЕОЛОГИЧЕСКИХ НАРУШЕНИЙ

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Известно, что многие патологические состояния человека связаны с изменением реологических характеристик крови (реология - наука о деформациях и течении тел). У больных гнойными воспалениями, гнойно-септическими заболеваниями легких и плевры, при атеросклерозе и его осложнениях, в том числе при ишемической болезни сердца, текучесть крови понижается.

К настоящему времени сложилась точка зрения, что для глубокого изучения реологических свойств крови необходимо определение не только вязкости крови, но и ее составляющих: величины гематокрита, деформируемости эритроцитов и агрегаций эритроцитов, вязкости плазмы. В связи с тем, что эритроциты занимают почти половину объема крови и от них зависит снабжение тканей кислородом, вклад эритроцитов в реологические свойства крови является наиболее существенным.

Изменения в деформируемости эритроцитов во многих случаях определяют циркуляцию крови в капиллярах и в центральных отделах кровеносной системы. Изменение нормальной дисковой формы эритроцитов уменьшает сопротивление потоку крови, а образование агрегатов эритроцитов увеличивает его. Исследование влияния различных факторов на деформируемость эритроцитов , описанные в данной работе, проводились in vivo и при движении крови в стеклянных трубках, хотя имитация капилляров крови гораздо сложнее.

Эксперименты показывают, что деформируемость эритроцитов зависит от жесткости мембраны эритроцитов, формы клетки, внутриклеточной вязкости. В работе подробно анализируется влияние различных факторов, определяющих деформируемость эритроцитов (изменение белкового состава, концентрация внутриклеточного гемоглобина, обработка различными препаратами). Показана зависимость деформируемости эритроцитов от величины диаметра микрососуда (или его модели).

Отмечается, что движение клеток в стеклянных трубках может моделировать движение крови только в достаточно крупных кровеносных сосудах.

Исследования рассмотренных вопросов представляют большой интерес для понимания процессов центрального и капиллярного кровообращения и построения биомеханической модели данного процесса.

Ключевые слова: кровеносная система, нарушения вязкости крови, деформируемость эритроцитов, эксперимент, моделирование течения крови с помощью стеклянных трубок

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