Influence of the information drug "Cholesterol plaque" on saponin hemolysis of rabbit erythrocytes in vitro

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SUMMARY

The effect of the information preparation "Cholesterol plaque" was investigated at three values of the coefficient of amplification of the apparatus "IMEDIS-BRT-A", associated with dilutions D3, D12 and D60, on the state of cholesterol in erythrocyte membranes in vitro. The state of erythrocyte membranes was assessed by the method of chemical erythrograms according to I.A. Terskov and I.I. Gitelzon using saponin as a hemolytic. The results obtained allow us to consider the effect of the drug "Cholesterol plaque" on the erythrocyte membrane as contributing to the stabilization of the state of cholesterol and strengthening of its bonds with the stroma. It is shown that the action of the drug "Cholesterol plaque" is characterized by an increase in the viscoelastic properties of erythrocyte membranes.

Key words: saponin hemolysis of blood erythrocytes, cholesterol,"IMEDIS-BRT-A".

RESUME

We studied the influence of the information preparation "Cholesterol plaque" with three amplification coefficients of the apparatus "Imedis-BRT-A" associated with potencies D3, D12, D60 on the condition of cholesterol in the membrane of erythrocytes in vitro. The state of the membrane was evaluated by chemical erythrogramms according the the method of IA Terskov and II Gitelzon using saponin as a haemolytic. The results showed that the influence of the preparation "cholesterol plaque" on the erythrocyte membrane facilitated the stabilization of cholesterol and strengthened its binding with the stroma. Results showed that increase of viscoelastic qualities the erythrocyte membrane is typical for this preparation.

Atherosclerosis, as a particular manifestation of the accumulation of cholesterol in the body (cholesterolosis), is the main cause of the onset and development of cardiovascular pathology, which ranks first in mortality among other diseases [1]. An increase in cholesterol content is accompanied by a change in tissue metabolism and disruption of their functions, which is associated with its accumulation in the plasma membranes of cells. For example, only in the vascular wall, this process affects its mechanical properties, which leads to impaired blood flow and the development of subsequent pathologies. Long before the clinical manifestations of atherosclerosis in the intima and subendothelial layer of the arteries, a complex pathogenetic mechanism of the formation of atherosclerotic plaques is triggered, which in themselves cause partial or complete blockage of blood vessels,

heart attacks and strokes. Stopping or slowing down atherogenesis precisely at the preclinical stage is considered an extremely promising way to reduce mortality and the incidence of cardiovascular diseases.

The action of existing pharmacological drugs (class of statins), which help to reduce blood cholesterol, is aimed at reducing cholesterol synthesis in the liver, as well as affecting the smooth muscle layer of the vascular wall [2]. However, an exhaustive explanation of such an impressive effectiveness of statins has not yet been found - the expected effect from a simple decrease in plasma cholesterol levels was much smaller. In addition, the reduction in the risk of acute coronary complications under the influence of statins was noted earlier than a significant change in plasma lipid levels.

It has long been established that there is a dynamic balance between the content of cholesterol in blood plasma and in plasma membranes, in particular, in the membrane of erythrocytes. Thus, by studying the effect of an informational preparation on the state of cholesterol in erythrocyte membranes, one can indirectly evaluate its effect, which manifests itself at the organismal level.

In this regard, the purpose of these studies was to study the effect of the information drug "Cholesterol plaque" on the state of cholesterol in the membranes of erythrocytes under conditions in vitro.

Materials and methods

As a carrier, a physiological (0.9%) NaCl solution in 10 ml ampoules was used, on which the information preparation “Cholesterol plaque” was created using the “IMEDIS-BRT-A” apparatus manufactured by the “IMEDIS” Center in the “transfer” mode by transfer. which was an experimental test. The cholesterol plaque information drug was used at 3 amplification factors associated with dilutions D3, D12 and D60.

Similar ampoules with saline, but without transferring the informational properties of the drug, served as control samples. Then, two 2 ml samples were prepared from each ampoule (experimental and control).

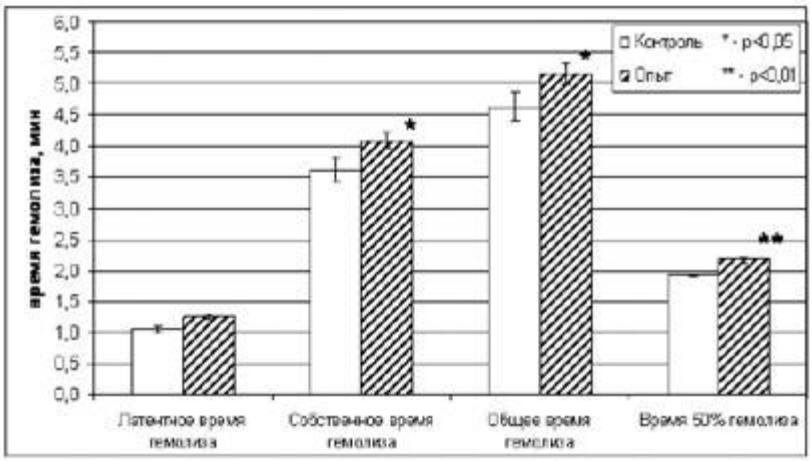
The test object of the study was the erythrocytes of the blood of rabbits taken without the use of coagulants by puncture of the marginal ear vein. Blood in the amount of 20 μl was suspended in 2 ml of control and experimental samples; the resulting suspension was incubated for 30 min. at a temperature of 18 ° C. The state of erythrocyte membranes was assessed by the method of chemical erythrograms according to I.A. Terskov and I.I. Gitelzon [3] using a computerized measuring complex as part of a PC and a PC564i attachment (Velleman, Belgium) operating in the recorder mode. A 0.0008% saponin solution (Merck, Germany) prepared in 0.9% NaCl solution was used as a hemolytic. Photometric registration of hemolysis kinetics was carried out in a thermostated cuvette at a temperature of 24 ° C using a differential photometer. The obtained kinetic dependences were used to determine the total, latent and proper hemolysis time, as well as the time of 50% hemolysis. More than 264 measurements were made in total. Credibility

differences in the compared parameters were calculated using the Student's t-test using the statistical functions of the Microsoft Excel program. Differences were considered significant at p <0.05.

Results and discussion

The studies were carried out in 3 experimental series: in the first series, the effect of the information drug "Cholesterol plaque" was studied at the values of the gain of the device "IMEDIS-BRT-A" associated with dilutions D3, in the second - D12, and in the third - D60.

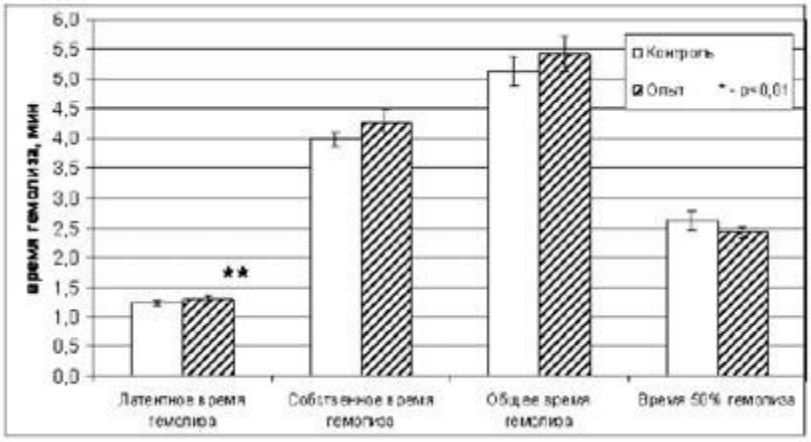
The results of the first experimental series are shown in Fig. 1. From the given data it follows that the intrinsic, total (p <0.05) and time of 50% hemolysis (p <0.01) change statistically reliably. A different picture is observed in the dynamics of the parameters of saponin hemolysis upon dilution of the information preparation D12, shown in Fig. 2, where only the latent time of hemolysis (p <0.01) is a reliable variable indicator, while the other parameters are not statistically significant, although their absolute values retain the same tendency towards an increase in hemolysis times as in D3, with the exception of 50% hemolysis. The effect of the information drug "Cholesterol plaque" in the D60 dilution is characterized by a similar dynamics of changes in indicators that at D3, but differs in a higher reliability (p <0,



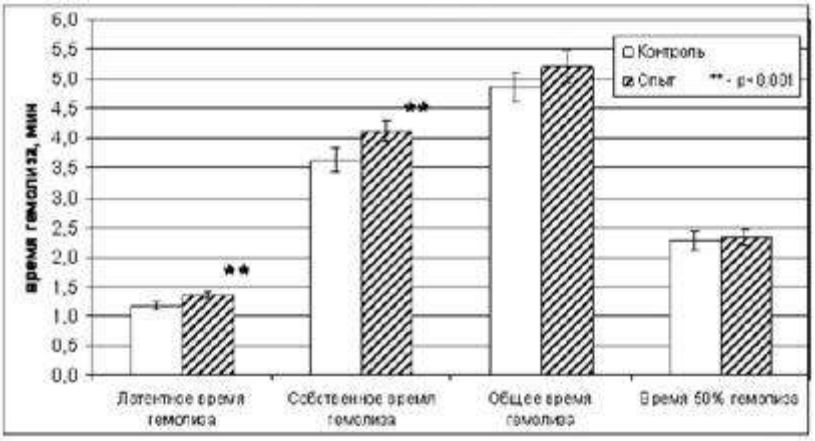
Rice. 1. The main parameters of saponin hemolysis of intact blood erythrocytes rabbits (control) and after incubation for 30 min. (experience) in the presence information preparation "Cholesterol plaque", obtained with the amplification factor of the device "IMEDIS-BRT-A", associated with dilution D3.

Considering the influence of the informational preparation "Cholesterol plaque" on the kinetics of saponin hemolysis of erythrocytes, it is advisable to proceed from the following concepts.

In the mechanism of chemical hemolysis, the cell membrane is considered a critical target, and changes in its physicochemical properties cause a violation of the barrier function and subsequent hemolysis. Hemolysis itself is a multistage process, which can be characterized by the rate, assessed, as a rule, by the release of hemoglobin molecules from the cell. The rate of hemolysis depends on the concentration of the hemolytic, its nature and the state of the erythrocyte membrane. Determined by the release of hemoglobin, the resistance of erythrocytes to the action of hemolytics is the result of three processes: the time required for the hemolytic to overcome the membrane impermeability barrier, the rate of disintegration of intracellular structures, and the time during which the mechanical strength of the membrane resists the osmotic pressure growing inside the cell.



Rice. 2. Basic parameters of saponin hemolysis of intact blood erythrocytes rabbits (control) and after incubation for 30 min. (experience) in the presence of the information drug "Cholesterol plaque" amplification factor apparatus "IMEDIS-BRT-A", associated with the dilution of D12.



Rice. 3. Basic parameters of saponin hemolysis of intact blood erythrocytes rabbits (control) and after incubation for 30 min. (experience) in the presence of the information drug "Cholesterol plaque" amplification factor

apparatus "IMEDIS-BRT-A", associated with the dilution of D60.

Saponin, as a lipophilic hemolytic, has a high affinity for membrane lipids and predominantly for cholesterol. Solubilization of intramembrane lipids by saponin causes the formation of large morphologically detectable pores in the membrane, which leads to disruption of osmotic equilibrium and hemolysis, accompanied by the release of hemoglobin from the cell [4]. A change in the state of membrane lipids, and, first of all, cholesterol, affects the kinetics of hemolysis of erythrocytes by saponin, which leads to a change in the temporal parameters of the hemolytic process and, first of all, latent and proper time.

Plaque formation occurs as a result of the accumulation of fatty acids, cholesterol, collagen and elastin fibers, macrophages and other substances in the arterial wall. Recent studies have shown that lipoproteins and pre-lipoproteins by themselves cannot form an atherosclerotic plaque. To do this, they must first undergo a process of peroxidation by free radicals. The result is highly toxic products. Once in the arterial wall, they are captured by macrophages, which, capturing a huge amount of low and very low density lipoproteins, die due to the inability to "assimilate" them, and all cholesterol is poured into the vascular wall, forming a soft cholesterol plaque. Such a plaque is not, strictly speaking, a stationary formation. In it there is a constant flow of lipoproteins inward and outward. At this stage (for example, during fasting, an increase in the - / - -lipoprotein index, cleansing the blood of large amounts of cholesterol, etc.), the soft plaque is still capable of shrinking and even completely absorbing. However, at a certain stage of its existence, calcium salts begin to accumulate in it, and plaque calcification occurs. A hard calcified plaque irritates the vascular wall, which begins to thicken, forming a fibrous membrane around the calcified plaque. By this, the vessel “separates” itself from the plaque. As a result, there is a sharp narrowing of the vessel lumen with impaired blood circulation and a decrease in its elasticity.) the soft plaque is still capable of shrinking and even completely absorbing. However, at a certain stage of its existence, calcium salts begin to accumulate in it, and plaque calcification occurs. A hard calcified plaque irritates the vascular wall, which begins to thicken, forming a fibrous membrane around the calcified plaque. By this, the vessel “separates” itself from the plaque. As a result, there is a sharp narrowing of the lumen of the vessel with impaired blood circulation and a decrease in its elasticity.) the soft plaque is still capable of shrinking and even completely absorbing. However, at a certain stage of its existence, calcium salts begin to accumulate in it, and plaque calcification occurs. A hard calcified plaque irritates the vascular wall, which begins to thicken, forming a fibrous membrane around the calcified plaque. By this, the vessel “separates” itself from the plaque. As a result, there is a sharp narrowing of the lumen of the vessel with impaired blood circulation and a decrease in its elasticity. By this, the vessel “separates” itself from the plaque. As a result, there is a sharp narrowing of the vessel lumen with impaired blood circulation and a decrease in its elasticity. By this, the vessel “separates” itself from the plaque. As a result, there is a sharp narrowing of the vessel lumen with impaired blood circulation and a decrease in its elasticity.

The reduction in the risk of coronary heart disease, the number of complications, the risk of death, and the need for hospitalization and surgery, proven in large clinical trials, has led to a significant increase in the indications for active prescription of statins [2]. In most developed countries, statins are recommended to be prescribed to all persons with hypercholesterolemia with ineffective diet therapy, as well as in the early hospital period of unstable angina pectoris and myocardial infarction. In the United States, they adhere to the recommendations to prescribe statins in the acute period of myocardial infarction to all persons, and regardless of the initial level of cholesterol and low density lipoprotein plasma. However, European researchers are more conservative. They rightly indicate that

elevated plasma lipid levels.

Our results allow us to consider the effect of the drug "Cholesterol plaque" on the erythrocyte membrane as a combination of stabilizing the state of cholesterol and strengthening its bonds with the stroma. Regulating, depending on the magnification of the IMEDIS-BRT-A apparatus, the statin-like action of the drug allows, apparently, to more subtly influence the exchange of membrane cholesterol with blood plasma.

It should also be noted that the effect of statins on the mechanical (viscoelastic) properties of erythrocyte membranes, which was found in patients during their treatment with simvastine, was found in a number of studies [5, 6]. The mechanical properties of erythrocyte membranes are directly related to the rheology of blood and, first of all, to the rate of its flow in the vessels, since erythrocytes are quantitatively the predominant corpuscular elements [7]. The increase in the elasticity of erythrocyte membranes caused by the action of statins is similar to the action of the drug "Cholesterol plaque" obtained in our experiments. This is evidenced by a statistically significant increase in the intrinsic hemolysis time at various values of the coefficient of amplification of the device "IMEDIS-BRT-A" associated with the dilutions of the drug D3 ($p < 0.05$), D60 ($p < 0,001$) and a trend ($p > 0.1$) at D12 (Fig. 1; Fig. 2; Fig. 3). Since the main mechanism of hemolysis after a violation of the integrity of membranes as a result of pore formation occurs according to the osmotic type, an increase in the time of proper hemolysis time associated with the action of the drug is characteristic of an increase in the viscoelastic properties of erythrocyte membranes.

conclusions

Received experimental results demonstrated significant changes in some parameters of saponin hemolysis of erythrocytes under the action of the drug "Cholesterol plaque", as well as the dependence on the value of the gain of the device "IMEDIS-BRT-A", which is non-linear. As a result of the analysis, it becomes obvious that the nonlinear dependence of the beneficial effect of the information drug "Cholesterol plaque" on the state of cholesterol in the erythrocyte membrane is due to more subtle mechanisms. It also cannot be ruled out that a similar effect will be observed when it acts on the structure and function of the vascular endothelium, contributing to the maintenance of their optimal elasticity. It can also be assumed that the improvement of endothelial function and blood rheology against the background of the action of the information drug "Cholesterol plaque" will not depend on the level of plasma cholesterol.

Literature

1. Lopukhin Yu.M., Archakov A.I., Vladimirov Yu.A., Kogan E.M. Cholesterol (Cholesterol biomembranes. Theoretical and clinical aspects). - M .: Medicine, 1983 .-- 352 p.

2. Jula A., Marniemi J., Rönnemaa T., Virtanen A., Huupponen R. Effects of diet and Simvastatin on fatty acid composition in hypercholesterolemic men. A randomized controlled trial // Arterioscler. Thromb. Vasc. Biol. - 2005. –V.25, N.9. - P. 1952-1959.

3. Terskov I.A., Gitelzon I.I. Chemical (acid) erythrogram method // Biophysics. - 1957. - T.11, issue 2. - S. 259-266.

4. Baumann E., Stoya G., Volkner A., Richter W., Lemke C., Linss W. Hemolysis of human erythrocytes with saponin affects the membrane structure // Acta Histochem. - 2000. - V.102, N.1. - P. 21–35.

5. Coccia R., Spadaccio C., Foppoli C., Perluigi M., Covino E., Lusini M., Chello M. The effect of simvastatin on erythrocyte membrane fluidity during oxidative stress induced by cardiopulmonary bypass: a randomized controlled study // Clin. Ther. - 2007. - V.29, N.8. - P. 1706-1717.

6. Broncel M., Bała A., Koter-Michalak M., Duchnowicz P., Wojsznis W., Chojnowska-Jezierska J. Physicochemical modifications induced by statins therapy on human erythrocytes membranes // Wiad. Lek. - 2007. - V.60, N.7-8. - P. 321–328.

7. Ivens I., Skeylak R. Mechanics and thermodynamics of biological membranes. - M .: Mir, 1982 .-- 304 p.

Gotovsky, M.Yu. Influence of the information preparation "Cholesterol plaque" on saponin hemolysis of rabbit erythrocytes in vitro / M.Yu. Gotovsky, L.B. Kosareva // Traditional Medicine. - 2009. - No. 1 (16). - P.7-11.

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