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Investigation of the Effect of Magnetite Nanoparticles (MCS-B) on Human Platelet Aggregation

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ABSTRACT

Background: Currently, one of the main problems that happens in the treatment by extracorporeal methods of hemosorption is systemic shifts in homeostasis. Among these complications the most significant are blood cells traumatization and hemorrhage. Direct physical contact of blood with sorbent surface causes activation of a multistage reaction of thrombosis. Now, new methods of hemosorption using non-traumatic sorbents are being actively developed. Wide introduction of nanotechnological preparations (magnetite nanoparticles) in clinical medicine allows improving methods of hemocorrection, creating a new class magnetically sorbent.

The aim of this study is to know the activity of magnet-controlled sorbent inhibiting the aggregation of platelets in an in vitro model.

Materials and Methods: 0.9% NaCl, magnetite nanoparticles of magnet-controlled sorbent (MCS-B brand). Object of research: platelets in relatively healthy volunteers. Quantitative determination aggregation of platelets activity by using aggregometer A-1 was carried out by the Bornov's method in the modification of Zachary and Kinah.

Results and discussions: The results of the study showed that the use of NaCl saline solution shifts the colloidal suspension equilibrium of platelets towards a significant ($P<0.001$) increase in the rate and index of their aggregation. For the first time, the effect of 0.9% NaCl on function of platelets makes us reconsider the concept of safety of infusion solutions in patients with initial signs of platelet disorders hemostasis. On the contrary, the use of MCS-B nanoparticles significantly revealed ($P<0.05$) an increase in the stability of colloidal suspension of platelets. This is an important pathogenetic factor which affects the occurrence of correction of hemostasis in conditions of blood clotting disorders.

1. BACKGROUND

Currently, one of the main problems that happen in the treatment by extracorporeal methods of hemosorption is systemic shifts in homeostasis. Among these complications, most significant are blood cells trauma and bleeding. By contact with sorbent platelets are activated, aggregate and secrete activators to recruit other platelets. The interaction of platelets with the surface of sorbents depends not only of the roughness, porosity, charge, chemical sorbent activity, but also from initial platelet

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functions, their aggregation and adhesion ability. In addition, the level of platelets is reduced as a result of hemosorption. Despite the fact that these indicators are restored few days after the session of hemosorption and even exceed the initial levels, there is a real risk of hemorrhagic complications [1-5].

At the moment, methods of correction of hemostasis parameters remain one of the most relevant in clinical medicine. Modern medicine has in its arsenal pharmacological drugs that can actively inhibit the platelet functions, but excessive oppression can lead to the loss of their protective action. With current antiplatelet therapies invariably causing bleeding as an undesired adverse effect, novel therapies can be more beneficial if directed against specific platelet responses, populations, interactions, or priming conditions [6].

Now, new methods of hemosorption using non-traumatic sorbents are being actively developed. For example, until recently, a promising direction for the development of a new method of extracorporeal hemocorrection, in order to reduce the degree of damage to blood cells, was the attempt to use hemosorbents coated with albumin and other substances. However, the use of sorbents coated with albumin has not found wide practical application in connection with the spread of viral hepatitis, HIV infection, and increased allergization of patients [7].

Magnetic fields have one of the greatest effects on the circulatory system [8-11]. Therefore the next effective way to prevent the destruction of platelets is to conduct hemosorption in an alternating magnetic field. It turned out that during the first 10 minutes; more than 2 times less platelets are destroyed than under normal conditions. In the next 20 minutes, the degree of platelet destruction increases, but remains significantly lower than without the use of a magnetic field [7].

The therapeutic effect of magnetic fields on animals and humans was proved by V. I. Drozdov in 1879. Positive therapeutic effect of magnetotherapy was observed in burn and mechanical trauma, osteochondrosis, dental, ophthalmological and infectious diseases, hypertension, as well as other pathologies [8-13]. There is evidence that the change in the geomagnetic field of the Earth leads to a deterioration of health in cardiovascular diseases, up to an increase in mortality in them [14-16]. More detailed studies have shown that variable and pulsed fields can induce local currents in electrically excitable tissues whose levels exceed natural ones. This effect is the basis for additional biological changes. The magnetomechanical effect can be manifested by the appearance in diamagnetic and a paramagnetic molecule of torque, which orients them in such a way that a configuration is formed reduces their free energy in the field [12].

New directions in development science-nanotechnology in the field of Medicine allows to improve hemocorrection methods, to create a new class of biocompatible magnet-controlled sorbents where combining sorption and magnetic effects in one substance [13].

The use of magnetite nanoparticles for hemosorbent completely excludes the possibility of mechanical traumatization of blood elements. Each particle of magnetite is a subdomain of an elementary magnet that induces a constant magnetic field. The study of the General toxic effect of magnetite was carried out in an acute experiment on laboratory animals. Magnetite (Fe_3O_4) was nontoxic in acute experiments [14-16]. Wide introduction of nanotechnological preparations (magnetite nanoparticles) in clinical medicine allows to improve extracorporeal hemocorrection methods and to outline fundamentally new approaches to solving problems of correction of artificial hemostasis [17].

There are few reports regarding the interaction between nanoparticles and platelets. Unfortunately, most of them show evidence of the procoagulant effects of employed nanomaterials, along with their potential health risks if the exposed person is going through pathological processes such as cardiovascular disease or metabolic syndrome. Nanoparticle-platelet interaction and endothelial injury may result in the activation of the coagulation cascade, the formation of blood clots, and the partial or total occlusion of blood vessels by thrombi. These effects will depend not only on the size, charge, hydrophobicity, or type of cover but also on the intrinsic characteristics of the nanoparticles [18].

In Ukraine, the first biocompatible magnetite nanoparticles for medical use were manufactured and patented by Andrey N. Belousov in 1998. These are intracorporeal nanobiocorrector (ICNB brand), biocompatible nanoparticles of magnet-controlled sorbent (MCS-B brand), and biologically active nanodevice (Micromage-B brand) [19-22].

Activity of the enzyme link of the antioxidant system in red blood cells, modulation metabolic processes in leukocytes after processed by magnetite nanoparticles have been discovered in the blood of a human. [23-25]. The biocompatible magnetite nanoparticles has nonspecific and modulated effect on metabolic processes and has been demonstrated in previously the complex investigations. Nonspecific activation of the metabolic processes, increase potential of organelle cells and adaptive mechanisms, acceleration of reparative processes on levels of the macromolecules and membranes have been proved after injection of the biocompatible magnetite nanoparticles on the study of ultrastructure of the reticuloendothelial system (lungs, liver, kidneys). [26-28]. Selective adsorption protein on surface of the membrane of cells (by according to the principle of magnetophoresis) by means MCS-B is prevent the oxidation modification of proteins by way of stabilizing the active groups, normalizating a state of membrane receptors, increasing activity of enzymes' membrane-bound [29-31]. For MCS-B both sorption and indirect effects are inherent, which are caused by the action of a constant magnetic field created by nanoparticles [32, 33]. However, when platelets come into contact with any material, they can adhere to its surface and begin a cascade of signals that eventually leads to fibrin cross-linking and clot formation. It can lead to the appearance of potentially

deadly thrombi that can cause strokes or cerebrovascular accidents [34]. If a nanomaterial has the capacity to induce platelet aggregation and alter the normal process of coagulation, it could lead to bleeding or thrombosis. Hence, it is important to know the thrombogenic properties of nanoparticles especially when considering its potential nanobiomedical applications.

The above was the basis for choosing the topic of this study, devoted to the study of the influence of MCS-B on aggregation of platelets. The use of MCS-B may be approach to problems solving in the correction of hemostatic systems and suppression aggregation of platelets in the optimal regime.

The *aim of this study* is to know the activity of magnet-controlled sorbent (MCS-B) inhibiting the aggregation of platelets in an in vitro model.

2. MATERIALS AND METHODS

a) The basis of MCS-B is magnetite nanoparticles (Fe3O4). The size of nanoparticles is from 6 to 12 nm; the total sorption surface magnetite of nanoparticles is from 800 to 1200 m2/g; magnetization of saturation Is = 2.15 kA/m; volume concentration q = 0.00448; viscosity η = 1.0112 cSt; ζ - potential = - 19 mV; saline NaCl. Magnetite nanoparticles have been produced in Laboratory of Applied Nanotechnology of Belousov. Magnetite nanoparticles were synthesized by co-precipitation method. The main physics and chemical properties of MCS-B:
   - Concentration of the colloidal solution of magnetite nanoparticles in physiology solution of NaCl is 4.5%.
   - Size of magnetite nanoparticles is 6-12 nm;
   - Total area of surface magnetite of nanoparticles Ss = 800-1200 m2/g;
   - Magnetization of saturation Is = 2.15 κA/m;
   - ζ - potential = - 19 mV.

b) 0.9% NaCl solution.

2.1. OBJECT OF RESEARCH

Object of research: platelets in relatively healthy volunteers. Normal initial state of the thromboelastography (TEG) was selection criteria. The number of volunteers was 14 people. Under aseptic conditions, venous blood in the amount of 16 ml was taken from the ulnar vein. The collected volume of blood in equal parts (4 ml) was distributed to 4 heparinized test tubes. Conditionally 4 test tubes were divided into four experiments:

- Experiment 1: control tubes with venous blood.
- Experiment 2: tubes with venous blood + 2 ml saline NaCl.
- Experiment 3: tubes with venous blood + 2 ml 4.5% colloidal solution of MCS-B.
- Experiment 4: tubes with venous blood + 2 ml 4.5% colloidal solution of MCS-B with the subsequent removal of it from the blood by means Belousov's method [19, 20].

The amount and concentration of MCS-B colloidal solution was used in accordance with the previously developed Belousov's method [19, 20].

Quantitative determination aggregation of platelets activity by using aggregometer A-1 was carried out by the Bornov's method in the modification of Zachary and Kinah [35-38].

Statistically processing the obtained results was carried out by parametrical method of variation statistics by Student criterion. Processing the obtained data was carried out by means of Excel.

3. RESULTS AND DISCUSSIONS

To identify the main mechanisms of action of magnetite nanoparticles (MCS-B) on platelet aggregation parameters, different variants of blood processing by MCS-B were investigated. Taking into account the fact that a physiological solution was used as a carrier of MCS-B fluid, the effect influence of physiological solution of NaCl on platelet aggregation parameters were studied for the purity of the experiment. Platelet aggregation indicators depending on different variants of blood treatment with solutions are presented in table 1.

The data of table 1 show that the use of 0.9% NaCl in comparison with the control, causes a significant ($P<0.001$) increase in speed by 0.025 optical density/min and platelet aggregation index by 9.1 %. At the same time, there was no significant decrease in platelet disaggregation index. In the third experiment, where MCS-B was added to venous blood compared with the control significantly ($P<0.001$) marked a sharp decrease by 0.0108 optical density/min and platelet aggregation index by 12.4%. An increase was also observed in platelet disaggregation index in a significant manner ($P < 0.001$). In the fourth experiment, where MCS-B was added to venous blood with the subsequent removal, there was a significant ($P<0.001$) increase in rate by 0.017 optical density/min and platelet aggregation index by 7%, compared with control group. Significant increase in platelet disaggregation was not observed. However, compared with the 2nd experiment, where 0.9% NaCl was added to the blood, an increase of platelet disaggregation index by 5% ($p<0.01$) was reliably revealed. For clarity, the dynamics of platelet aggregation in different versions of the experiments is presented graphically in Fig. 1.

Comparative analysis of the results of the 3rd and 4th experiments showed that the main reason of the differences effects that were obtained is associated with different methods of applications of MCS-B. In the third experiment, the MCS-B nanoparticles were not removed from the blood. Since MCS-B nanoparticles have sorption activity, a certain amount of them is sorbed on the surface of protein structures of platelet membranes. The constant

magnetic field induced by MCS-B nanoparticles (300-400 kA/m) changes not only the bioelectric charge of platelet membranes [39], but also affects intracellular biochemical

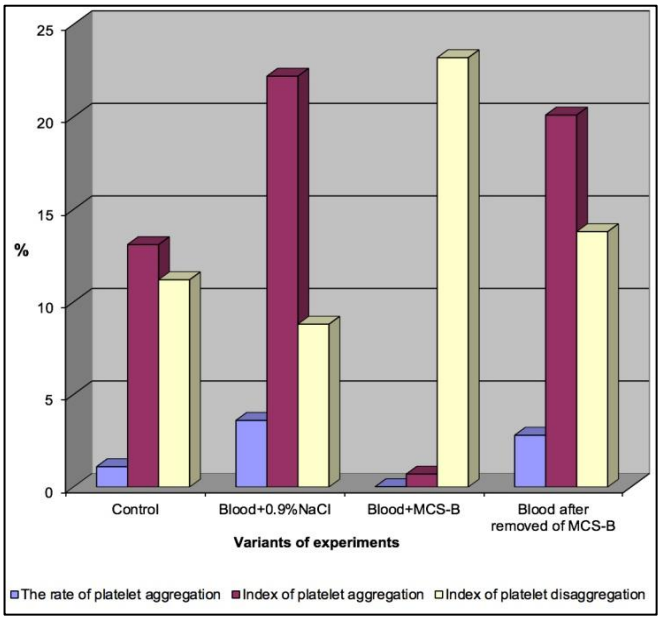

Figure 1: *Dynamic indicators of platelet aggregation in different variants of the experiment (n=14). MCS-B: magnet-controlled sorbent.*

processes, and the activity of enzyme systems. Ultimately this caused activation of disaggregation, decreased of rate and platelet aggregation index in the third experiment. The data of I. M. Movshovich and M. A. Shilo confirm the above. The constant magnetic field slows down erythrocyte sedimentation rate (ESR) and improves microcirculation. This is associated with disaggregating effect of magnetic field, decrease in viscosity and hemostatic potential of blood with improvement of its rheological properties [40].

N. F. Lezhenina and V. N. Rodionov also note that the use of magnetic hemotherapy in combination with hemosorption allows reducing the dose of anticoagulants [41]. Reasoning about the mechanism of the effects obtained it should be said that the magnetic field can cause synchronization of initially weak oscillations of several groups of cells (trigger reaction), which is confirmed by scientific works of a number of authors [39, 42, 43].

Such in-phase oscillations can be as an information signal for various regulatory systems of the body, which leads to various macroscopic effects in the form of conformational restructuring of cellular structures. Self-oscillations, probably occurring in the blood cell membranes, when the vibrational part of the membrane begins to act as a pump, have a significant impact on the ionic and molecular transport of substances through the cell membrane. The magnetic field affects the processes of lipid peroxidation and redox processes, elements of cell structures, and protein-enzyme molecules which have a dipole moment [44]. Adding to the list of mechanisms of influence of magnetic fields on biological processes, it should be noted the process of structuring water with a change in the orientation of the nuclear spins of hydrogen in its molecule, which probably also affects the course of enzymatic processes [45, 46].

Thus, the effect of predominance of magnetic properties of MCS-B nanoparticles over sorption ones was observed in the third experiment. MCS-B nanoparticles increase the negative charge of the membrane, which leads to a decrease in their aggregation.

In contrast in the fourth experiment where MCS-B was extracted from venous blood using a constant magnetic field (20-25 mT) the sorption properties of the nanoparticles prevailed over the magnetic ones [17]. The mechanism of action of MCS-B was primarily associated with the effect of sorption of surface proteins of blood cell membranes [23]. The result of the above effect is a reliable decrease in the platelet aggregation rate ($P<0.05$) and an increase in the platelet disaggregation index ($P<0.01$) compared to the experiment in which 0.9% NaCl was used.

4. SUMMARY

The results of the study showed that the use of NaCl saline solution in conditions of severe disturbances of blood microcirculation and rheology in order to improve them is not only doubtful, but also unsafe. In this case, the colloidal suspension equilibrium of platelets is shifted towards a significant ($P<0.001$) increase in the rate and index of their aggregation. On the contrary, in result on the use of MCS-B nanoparticles significantly ($P<0.05$) revealed an increase in the stability of colloidal suspension of blood cells (platelets). In conditions of impaired microcirculation and rheology of blood this is an important factor for occurrence of sanogenetic effects. In the case of correction of the platelet link of the blood coagulation system, blood treatment by MCS-B nanoparticles is preferable to the introduction of 0.9% NaCl. These results are probably due to the restoration of the Zeta potential of platelets [20].

5. CONCLUSIONS

I. The use of magnet-controlled sorbent (MCS-B brand) allows inhibiting the aggregation of platelets in an in vitro model.

II. The presence of MCS-B nanoparticles in the blood, compared with the control, significantly ($P<0.001$) reduces by 0.0108 optical density/min index and platelet aggregation index by 12.4 %. At the same, time of the platelet disaggregation index has been increased significantly ($P<0.001$) by 12%.

Table 1: Influence of different variants of solutions for blood treatment on platelet aggregation (n = 14; M±m)

Indicator of platelets aggregations	1 (Control)	2 (blood + 0.9% NaCl)	3 (blood + MCS-B)	4 (blood after removed of MCS-B)
The rate of platelet aggregation (optical density/min)	0.011±0.002	0.036±0.003 $p_1<0.001$	0.0002±0.0001 $p_1<0.001$ $p_2<0.001$	0.028±0.002 $p_1<0.001$ $p_2<0.05$ $p_3<0.001$
Index of platelet aggregation (%)	13.1±1.2	22.2±1.1 $p_1<0.001$	0.7±0.1 $p_1<0.001$ $p_2<0.001$	20.1±1.3 $p_1<0.001$ $p_2>0.05$ $p_3<0.001$
Index of platelet disaggregation (%)	11.2±1.2	8.8±1.1 $p_1>0.05$	23.2±1.2 $p_1<0.001$ $p_2<0.001$	13.8±1.1 $p_1>0.05$ $p_2<0.01$ $p_3<0.001$

MCS-B - magnet-controlled sorbent; p_1-in comparison with the first experience (control); p_2-in comparison with the 2nd experience (blood + 0.9%NaCl); p_3-in comparison with the 3rd experience (blood + MCS-B)

III. Treatment of venous blood by MCS-B with its subsequent removed significantly (P<0.001) increases aggregation rate by 0.017 optical density/min and platelet aggregation index by 7% compared with the control. Compared with 0.9% NaCl the use of MCS-B nanoparticles, the platelet disaggregation index significantly increased (P<0.01) by 5%.

IV. The use of 0.9% NaCl in comparison with the control causes a significant (p<0.001) increased in aggregation speed by 0.025 optical density/min and platelet aggregation index by 9.1%. At the same time, there was no significant decrease in the platelet disaggregation index.

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