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Review Article

Reducing of Erythrocytes Destruction By Means of Medicine Nanotechnology (Magnet-Controlled Sorbent Brand of MCS-B)

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Abstract

The main purpose of this work is inhibition of erythrocytes hemolysison the whole blood by means of magnetite nanoparticles (magnet-controlled sorbent MCS-B). Conventional erythrocytes of person's venous blood were objects of the research. The time of appearing the signs of erythrocytes hemolysis was recorded with the help of visual method. In result of investigation it was established that magnetite nanoparticles not only reliably reduce hemolysis but also prolong time preservation of the blood, change activity adenosinetriphosphatases of erythrocytes and influence on transmembrane exchange, functioning of ion channels.

Keywords: Adenosinetriphosphatases; Haemolysis; Eryptosis; Nanoparticles (MCS-B); Transmembrane exchange

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Introduction

Metabolic restoration, prolongation of normal function of cells both inside and outside the organism is the main purpose of medical and biological trend in the 21st century. With rapid progress in nanotechnology, many nano-size materials have been extensively used in biomedical and pharmaceutical industry and industrial production. Nevertheless, the rapid growth of nanotechnology has raised biological safety concerns because of the unique dimensional and physicochemical properties of the nano-size materials [1]. Although the mechanism of toxicity of nanomaterials is complicated and markedly different from that of traditional biomaterials, current evaluations of nanotoxicology are still confined to testing the compatibility of materials using traditional methodologies. A standard research protocol to evaluate the nanotoxicity is lacking, severely limiting the development and applications of nanoparticles [2].

Fe_3O_4 Magnetic Nanoparticles (Fe_3O_4-MNPs) is the only nanomaterial that has been approved for clinical applications because of their relative safety, unique magnetic responsiveness, and their simple and controllable preparation [3,4]. Although studies concerning the potential risks of Fe_3O_4-MNP have been reported the biocompatibility evaluation relies mainly on in vitro cytotoxicity such as hemolysis testing, cell viability, oxidative damage, inflammatory reactions and genotoxicity, or on pharmacokinetics, and in vivo bio-distribution [5,6].

Erythrocytes are the main components in the circulation system and are also one of the first components that Fe_3O_4-MNPs contact when the nanoparticles are administered through intravenous injection. Fe_3O_4-MNPs are generally regarded as hemocompatible based on very low hemolytic activity [7]. Hemolysis testing is a well-accepted classical assay for acute toxicity screening in evaluating hemocompatibility and can reflect the breakage to the erythrocyte membrane. Accumulated evidence in recent years suggests that some nanoparticles have an adverse effect on erythrocytes pre-hemolysis after interacting with RBC in vitro and in vivo. Owing to the induction of specific structural changes in the lipid bilayer, these nanoparticles caused erythrocyte shape transformation decreased the deformability and oxygen-delivering ability modified the heme conformation and changed hemorheological properties [8-11]. Although it has been reported that MgNPs-Fe_3O_4 (100 mg/ml) can cause cellular membrane damage in cultured lung epithelial cells the impact of Fe_3O_4-MNPs at safe dose on cellular membrane of erythrocytes and circulatory properties beside hemolysis remains unknown [12].

The toxic effects determined based on the hemolysis, membrane injury, lipid peroxidation and antioxidant enzyme production were fairly size and dose dependent. For example, in particular, the smallest sized silver nanoparticles size of 15 nm (AgNPs15) displayed a greater ability to induce hemolysis and membrane damage than AgNPs50 and AgNPs100. Such cytotoxicity induced by AgNPs should be attributed to the direct interaction of the nanoparticle with the red blood cells (RBCs), resulting in the production of oxidative stress, membrane injury and subsequently hemolysis. Overall, the results suggest that particle size is a critical factor influencing the interaction between AgNPs and the RBCs [13].

In Ukraine, the first medical nanotechnology drug was synthesized and patented in 1998. These are such drugs as Intracorporeal Nanobiocorrector (ICNB), Magnet-Controlled Sorbent (MCS-B) and Micromage-B [14-16]. Basis of the drugs is magnetite of nanoparticles (Fe_3O_4) with the size ranging from 6 till 12 nm. Presence of adsorption layer provides high sorption activity for the magnetite of Nanoparticles (NPs). The total sorption surface of the magnetite NPs ranges from 800 to 1200 m^2/g, and intensity of the magnetic field that induced by each magnetite NPs is 300-400 kA/m.

The main purpose of study was reduced hemolysis of erythrocytes on the whole blood by means of nanoparticles of Magnet-Controlled Sorbent (MCS-B).

To fulfill the aim the following tasks are to be solved:

- Determine the dependence between time of appearing hemolysis and amount of processing of blood with MCS-B
- Investigate activity of transport adenosinetriphosphatase of erythrocytes: Na, K - ATPHase and Ca, Mg – ATPHase
- Investigate level of cytosolic calcium in erythrocytes
- Find optimum amount processing of blood by NPs of Magnet-Controlled Sorbent (MCS-B)

Material and Methods of Research

Material: NPs of MCS-B. The basis of MCS-B is Fe_3O_4. Physical and chemical properties of MCS-B (Figures 1 & 2; Tables 1 & 2):

- 0.1% colloidal solution of magnetite nanoparticles
- Size of magnetite of nanoparticles is 6-12 nm (the data were quantified from TEM micrographs)
- Total area of surface magnetite of nanoparticles Ss = 800-1200 m^2/g
- Magnetized of saturation is = 2.15 кА/m
- ζ - potential = - 19 mV

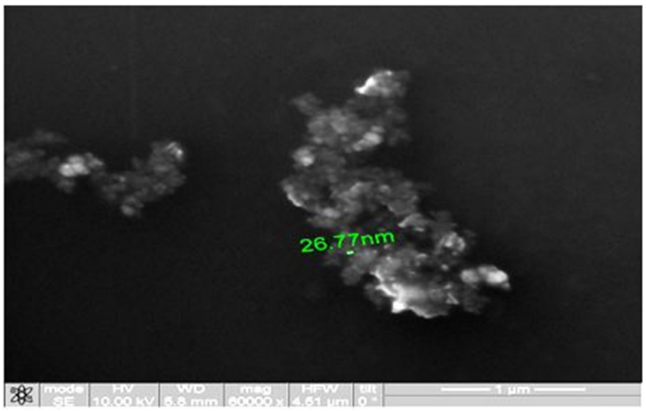

Figure 1: Study of Magnetite nanoparticles (MCS-B) with use microscope ion-electronic raster-type Quanta 200 3 D.

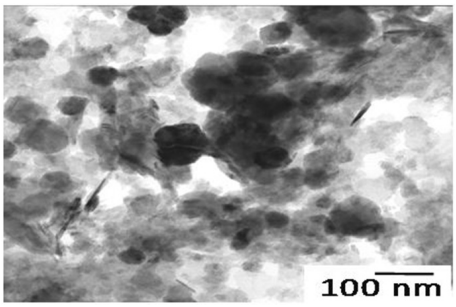

Figure 2: Study of Magnetite nanoparticles (MCS-B) with use translucent electronicmicroscope JEM-2100.

Phase	Formula	Space group	№ Card Database ICDD
Magnetite low	$Fe_{2.886}O_4$	227 : Fd-3m, choice-2	10861339 (ICDD)
Magnetite low, syn	Fe_3O_4	166 : R-3m, hexagonal	10716766 (ICDD)
Johannsenite	Ca Mn +2 Si_2O_6	15 : C12/c1, unique-b,cell-1	380413 (ICDD)

Table 1: X-ray analysis of ICNB in X-ray diffractometer Rigaku Ultima IV (CuKα, Kβ filter-Ni), one-coordinate DTeX semiconductor detector.

Phases (method of corundum numbers)	Content, %
Magnetite low	71
Magnetite low, syn(hexagonal)	29

Table 2: The phases of MCS-B (RIR - method; error 8±3%).

Object of research: conventional erythrocytes venous on the whole blood of the person. All researches *in vitro* were performed. The condition of erythrocytes venous of blood in 20 healthy volunteers was studied. The age of persons varied from 24 to 40 years. The researches included 2 stages: stage I was condition of erythrocytes on the 1st day of observation; stage II was condition of erythrocytes on the 21th day of observation. The biochemical investigations were performed only on stage I. The estimate of visually state of erythrocytes (hemolysis) was performed on stages I and II.

Research methods: 12 ml venous of blood was taken from patient. For preventing coagulation of blood citrate sodium was introduced. Then in each tube 3 ml of blood was introduced. The first tube was control. In second tube of test was added MCS-B in quantity of 1.5 ml with its following separation in during 30-40 sec by means of a constant magnetic field with the intensity 200 kA/m. In third tube of test the blood was twice processed by means of MCS-B. In fourth tube of test the blood was thrice processed by means of MCS-B. On stage I the activity of transport adenosinetriphosphatase (Na, K - ATPHase and Ca, Mg - ATPHase) and level of cytosolic calcium in erythrocytes were studied by the standard procedure of biochemical analysis [17]. The blood after performance of the biochemical investigation was stored in the refrigerating chamber at temperature +1°C. 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. An easy way to comply with the journal paper formatting requirements is to use this document as a template and simply type your text into it.

Results and Discussion

A visual condition of erythrocytes on stage I (1st day) is present on figure 3.

Studies have shown that visible signs of hemolysis in the control and tubes of test on stage I was not observed.

Thus, despite on sorption activity of MCS-B on surface proteins of erythrocyte membrane the hemolysis visually was not registered [18]. For determine reactions of transmembrane exchange on stage I were studied activity adenosinetriphosphatases (Na, K - ATPHase and Ca, Mg - ATPHase) of erythrocytes and level of cytosolic calcium. Results of the research activity adenosinetriphosphatases of erythrocytes are presenting in table 3.

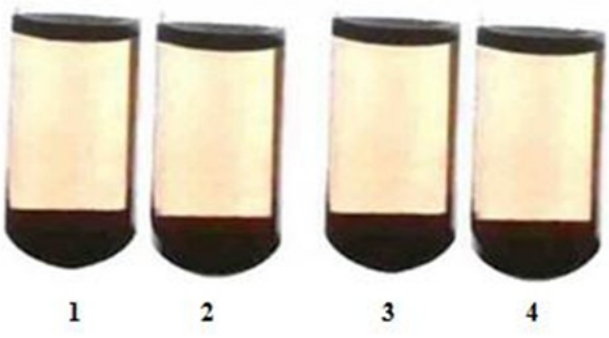

Figure 3: A visual picture condition of erythrocytes on 1st day.

Notes: 1 - control; 2 - after single processing by MCS-B; 3 - after double processing by MCS-B; 4 - after triple processing by MCS-B.

Adenosine-triphosphateses	Control	Frequency rate processing of MCS-B		
		Single	Double	Triple
Na, K - ATPHase, protein mmol/mg in mines	6.34±0.5	6.11±0.6*	5.89±0.7*	5.93±0.4*
Ca, Mg - ATPHase, protein mmol/mg in mines	23.64±0.6	21.17±0.7**	18.45±0.5***	17.63±0.3***

Table 3: Font results of research activity adenosinetriphosphatases before and after processing of erythrocytes by NPs of MCS-B (M±m; n=20).

Note: * - p>0.05; ** - p<0.01; *** - p<0.001

So, the dates of table 3 are demonstrating that single processing of blood by MCS-B reliably reduces (in comparison with the control) activity of Ca, Mg - ATPHase of erythrocytes - by 2.47±0.6 protein mmol/mg in mines (p<0.01), double - by 5.19±0.5 protein mmol/mg in mines (p<0.001), triple - by 6.01±0.5 protein mmol/mg in mines (p<0.001). On the contrary, the reliable changes activity of Na, K - ATPHase in any test tubes (in comparison with the control) were not detected (p>0.05).

It is known that if the enzyme level is reduced, this would lead to an increase in cytosolic calcium [19]. That is why cytosolic calcium was determined. Results of research the level of ion Ca^{2+} in erythrocytes before and after processing by NPs of MCS-B are presenting in table 4.

Ion	Control x10^{-8}	Frequency rate processing of MCS-B		
		Single x 10^{-7}	Double x 10^{-7}	Triple x 10^{-7}
Ca^{2+}, m/ml.cell	1±0.1	4.9 ±0.1*	5.6±0.2 *	6.5 ±0.1*

Table 4: Results research the level of ion Ca^{2+} in erythrocytes before and after processing by NPs of MCS-B (M±m; n=20).

Note: * - p<0.001 in comparative with control.

The dates of table 4 are demonstrating that level of cytosolic calcium with highly reliable (p<0.001) is increasing and depends from frequency rate processing by MCS-B.

It is known that increasing the concentration of cytosolic Ca^{2+} leads to a change in shape, decrease deformability, reduced life of erythrocytes and activation of eryptosis [20]. Also, increasing intracellular Ca^{2+} connected with increasing of erythrocytes aggregation and is the main reason of microcirculatory disorders [21]. If you follow the logic, in ours event the increase of cytosolic Ca^{2+} in erythrocytes must destroy them.

However, in this paper the results of studies reliably indicate the opposite. A visual condition of erythrocytes on stage II (21th day) is present on figure 4.

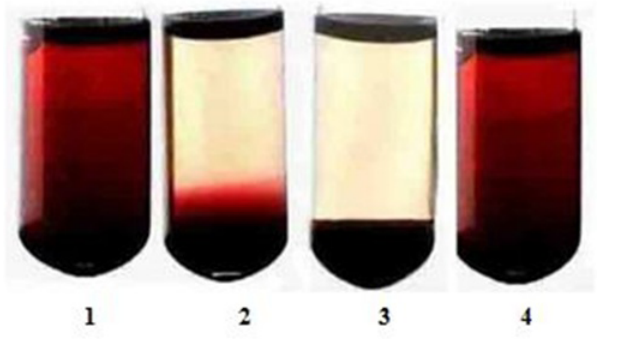

Figure 4: A visual picture condition of erythrocytes on 21th day.

Notes: 1 - control; 2 - after single processing by MCS-B; 3 - after double processing by MCS-B; 4 - after triple processing by MCS-B.

This picture is demonstrates that on stage II in tube of control the strongly pronounced sign of hemolysis was detected. Also, the strongly pronounced sign of hemolysis in tube of test after triple processing the blood by MCS-B was determined. In tube of test of blood that was single time processed by MCS-B the hemolysis was less pronounced. The sign of hemolysis in the tube of test after double processing the blood by MCS-B was not observed.

Thus, visual investigations have shown that maximum inhibition hemolysis of erythrocytes was determined after double processing by MCS-B. Opposite after triple processing of blood by MCS-B was not inhibited hemolysis of erythrocytes.

Represented the results of studies have shown that frequency rate processing of blood by MCS-B reliably influences on activity of hemolysis. However, appearance of hemolysis has not linear dependence on rise level of cytosolic calcium and frequency rate processing of blood by MCS-B. This does not contradict the mentioned before mechanisms, but only it proves multidirectional impact of nanoparticles MCS-B on the micro-cellular space, including the water sector, protein molecules and phospholipids. Maintaining membrane potential is necessary for normal functioning of ion channels that are very sensitive to any changes in them. Nanoparticles of MCS-B alter the bioelectric potential of erythrocyte membrane [22]. As a result, some ion channels open and ions including Ca^{2+} that is a regulator of many enzymes begin flow in cells according to concentration gradient. Therefore, increasing of intracellular concentration is a signal to start a series of processes such as synthesis of adenosinetriphosphate (universal cell "battery") that is necessary for starting metabolic reaction [23]. The following sequence of events is: the action of a constant magnetic field of magnetite nanoparticles (300-400 kA/m) → membrane potential of cells changes → opening of ion channels, including calcium (Ca^{2+} begins to flow into the cell on the concentration gradient) → intracellular Ca^{2+} increases → activation of the Ca - dependent enzymes. Schematic illustration of the mechanism of the effect MCS-B on erythrocytes is present on figure 5.

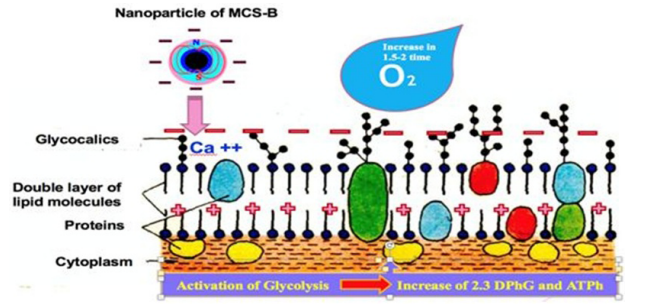

Figure 5: Schematic illustration of MCS-B impacts on erythrocytes.

In result the energy appears that needs for further intracellular metabolic processes such as the activation of glycolysis in erythrocytes. As a result, 1.5 to 2 times oxygen capacity is increased, bioelectric charge membranes of erythrocytes are modulated catabolic processes in leukocytes are inhibited [23, 25] and eryptosis process is declining [19,23-25].

Thus, as a result of the research, the optimum frequency rate of extracorporeal processing of blood by NPs of MCS-B for maximum of slowing down of hemolysis was determined.

It was established, that extracorporally processing the blood by NPs of MCS-B reliably reduces activity of Ca, Mg - ATPHase of erythrocytes and increases level of cytosolic calcium.

This paper has shown that mechanism of inhibition hemolysis by NPs of MCS-B is not connects with increasing level of cytosolic Ca^{2+} and activity of adenosinetriphosphates (Ca, Mg - ATPHase).

Running a few steps forward next investigations have shown that activity of hemolysis depends on condition polarization of the water molecules micro-cellular space of erythrocytes. This is confirming Gilbert N. Ling's theory about multi-layer organization polarization of water [26]. However, this scientific information will publish in next article.

Conclusion

- For inhibiting of hemolysis the optimum frequency rate (1-2 times) of processing the blood by NPs of MCS-B was determined
- It was established that extracorporally processing the blood by NPs of MCS-B reliably reduces activity of Ca, Mg - ATPHase of erythrocytes and increases level of cytosolic calcium
- After processing of blood by means NPs of MCS-B the activity of Na, K - ATPHase of erythrocytes does not change ($p>0.05$)
- Manifestation of hemolysis has not linear dependence on rise level of cytosolic calcium and frequency rate processing of blood by MCS-B
- Likely that the nanoparticles of MCS-B are changing the state polarization of water molecules of micro-cellular space of erythrocytes. It is influences on activity of hemolysis, activity of ATPHases, opening of ion channels that in whole explains the decline of eryptosis mechanism.

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