

The influence of magnetite nanoparticles (MCS-B) on the hemolysis of erythrocytes

A.N. Belousov

Laboratory of Applied Nanotechnology,
pr. Lenina, 31-v, fl. 32, Kharkov, 61072, Ukraine

E-mail: anb@vlink.kharkov.ua

Web site: www.nanolab.com.ua

ABSTRACT

In Ukraine the first medical nanotechnology drugs were synthesized and patented in 1998. These are such drugs as intracorporeal biocorrector (ICBB), magnet-controlled sorbent (MCS-B) and Micromage-B.

Basis of the drugs is magnetite nanoparticles (Fe_3O_4) with the size ranging from 6 till 12 nm.

Presence of adsorption layer provides high sorption activity for the magnetite nanoparticles. The total sorption surface of the magnetite nanoparticles ranges from 800 to 1200 m^2/g , and intensity of the magnetic field induced by each magnetite nanoparticle is 300-400 kA/m.

The main purpose of the work is to reduce hemolysis of erythrocytes by means of nanoparticles of magnet-controlled sorbent (MCS-B).

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

- to determine the dependence between time of appearing hemolysis and amount of processing of blood with MCS-B;

- to investigate activity of transport adenosinetriphosphatase of erythrocytes: Na, K - ATPase and Ca, Mg - ATPase;

- to find optimum amount processing of blood with nanoparticles of magnet-controlled sorbent (MCS-B).

This work for the first time describes (in comparison with control) the indices characterizing dependence of time of appearing hemolysis on frequency rate of processing the blood by nanoparticles of MCS-B.

It was established, that extracorporally processing the blood by nanoparticles of MCS-B reliably reduces activity of Ca, Mg - ATPase of erythrocytes.

The researches has proved that now nanoparticles of MCS-B are able not only to considerably reduce hemolysis, and thereby prolong storage time of the blood, influence activity of adenosinetriphosphatases of erythrocytes, regulate transmembrane exchange, but also to extracorporally influence cellular apoptosis.

Thus:

1. The optimum frequency rate (1-2 times) of processing the blood by nanoparticles of MCS-B inhibiting hemolysis was found.

2. It was established, that activity of Ca, Mg - ATPase of erythrocytes decreases with increasing frequency rate of processing the blood by nanoparticles of MCS-B.

3. Activity of Na, K - ATPase of erythrocytes in extracorporally processing the blood by nanoparticles of MCS-B does not change ($p > 0.05$).

4. The minimum indicator of activity of Ca, Mg - ATPase is 6.01 ± 1.2 protein mmol/mg in mines which time of appearing hemolysis practically does not differ from the control was found.

Keywords: magnetite nanoparticles, MCS-B, erythrocytes, hemolysis, apoptosis.

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. Having solved this task the mankind will closely approach the mystery of longevity, treatment of previously incurable diseases, make a significant advance in the sphere of microbiology, transplantology, growing and storage of cells.

Will the nearest future allow to purposefully managing metabolism of cells, treating earlier incurable diseases etc? What should tools and methods be to meet this purpose? All these questions can be answer by the modern trend of science, i.e. nanotechnology.

Nanotechnology is a real breakthrough in both the science of the 21st century and life in general. Protection of environment, ozonosphere, manufacture of any fabric, any sort of fuel, physiological immortality of the organism are only a short list of what this branch of science will bring in our life [1].

American National Institute of Health (NIH) has included nanomedicine in the top five of priority branches of development of medicine in the 21st century. Scientists from the US National Institute of Cancer consider that nanotechnology will help to treat cancer at its earliest stages and to avoid side-effects [2].

In Ukraine the first medical nanotechnology drugs were synthesized and patented in 1998 [3]. These are such drugs as intracorporeal biocorrector (ICBB), magnet-controlled sorbent (MCS-B) and Micromage-B [4,5,6].

Basis of the drugs is magnetite nanoparticles (Fe_3O_4) with the size ranging from 6 till 12 nm.

Presence of adsorption layer provides high sorption activity for the magnetite nanoparticles. The total sorption surface of the magnetite nanoparticles ranges from 800 to 1200 m^2/g , and intensity of the magnetic field induced by each magnetite nanoparticle is 300-400 kA/m.

The main purpose of the work is to reduce hemolysis of erythrocytes by means of nanoparticles of magnet-controlled sorbent (MCS-B).

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

- to determine the dependence between time of appearing hemolysis and amount of processing of blood with MCS-B;
- to investigate activity of transport adenosinetriphosphatase of erythrocytes: Na, K - ATPHese and Ca, Mg – ATPHese;
- to find optimum amount processing of blood with nanoparticles of magnet-controlled sorbent (MCS-B).

MATERIALS AND METHODS

Material: colloid solution of magnet-controlled sorbent (MCS-B). The basis of MCS-B is magnetite nanoparticles (Fe_3O_4). The size of particles is from 6 to 12 nm; the total sorption surface of magnetite nanoparticles is from 800 to 1200 m^2/g ; magnetisation of saturation $I_s = 2.15 \text{ kA/m}$; volume concentration $q = 0.00448$; viscosity $\eta = 1.0112 \text{ cSt}$; ζ – potential = - 19 mV.

Object of research: erythrocytes of venous blood of the person.

All researches were performed in vitro. The condition of erythrocytes of venous blood in 20 healthy volunteers was studied. The age of persons varied from 24 to 40 years. The researches included 3 stages: stage I - initial condition of erythrocytes; II – condition after processing by nanoparticles of MCS-B; III - condition of erythrocytes on the 21st day of observation.

Research methods: 3 ml intake of venous blood of a patient was performed. For preventing coagulation of blood citrate sodium was introduced. The first test tube was control. Into the second test tube MCS-B was singularly introduced in quantity of 1.5 ml with its following separation by means of a constant magnetic field with the intensity of 200 kA/m. In the third test tube there was the blood processed twice by MCS-B. In the fourth tube there was the blood processed thrice.

The suspension of blood cells after performance of the biochemical investigation was stored in the refrigerating chamber at temperature +1°C. On the 14th day the signs of hemolysis were registered visually.

On stages I and II the activity of transport adenosinetriphosphatase of erythrocytes was studied: Na, K - ATPHese and Ca, Mg – ATPHese by the standard procedure of biochemical analysis [7].

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.

RESULTS AND DISCUSSION

As a result of the research it was established, that in the control and test tubes where the blood was processed by

nanoparticles of MCS-B, on the 1st day of observation visible signs of hemolysis it were not observed (Fig. 1).

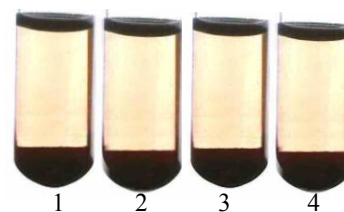


Figure 1: A visual picture of condition of the erythrocytes on the 1st day of observation.

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

However the signs of hemolysis on the 21st day were determined in the control and test tubes where the blood was processed thrice by nanoparticles of MCS-B.

On the contrary, in the test tubes where blood was processed by nanoparticles of MCS-B once or twice, hemolysis was practically not observed (Fig. 2).

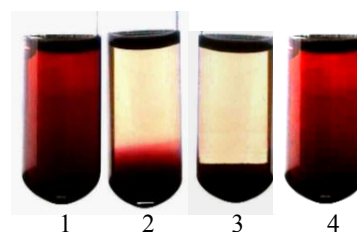


Figure 2: A visual picture of condition of the erythrocytes on the 21st day of observation.

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

Results of the research of activity of adenosinetriphosphatases of erythrocytes are presented in table 1.

Table 1. Results of research of activity of adenosinetriphosphatases before and after processing of erythrocytes by nanoparticles MCS-B ($M \pm m$; $n=20$).

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

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

So, the data of table 1 demonstrate that singular processing of blood by MCS-B reliably reduces (in comparison with the control) activity of Ca, Mg – ATPHese 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, reliable differences concerning changes of activity of Na, K - ATPase in any test tubes (in comparison with the control) were not detected (p > 0.05).

Thus, as a result of the research, the optimum frequency rate of extracorporeal processing of blood by nanoparticles of MCS-B essentially slowing down hemolysis was founded. The minimum value of activity of Ca, Mg - ATPase erythrocytes was 18.45±0.5 protein mmol/mg in mines. The subsequent depression of activity of Ca, Mg - ATPase leads to acceleration of hemolysis of erythrocytes.

CONCLUSION

This work for the first time describes (in comparison with control) the indices characterizing dependence of time of appearing hemolysis on frequency rate of processing the blood by nanoparticles of MCS-B.

It was established, that extracorporally processing the blood by nanoparticles of MCS-B reliably reduces activity of Ca, Mg - ATPase of erythrocytes.

The researches has proved that now nanoparticles of MCS-B are able not only to considerably reduce hemolysis, and thereby prolong storage time of the blood, influence activity of adenosinetriphosphates of erythrocytes, regulate transmembrane exchange [8], but also to extracorporally influence cellular apoptosis.

Thus:

1. The optimum frequency rate (1-2 times) of processing the blood by nanoparticles of MCS-B inhibiting hemolysis was found.

2. It was established, that activity of Ca, Mg - ATPase of erythrocytes decreases with increasing frequency rate of processing the blood by nanoparticles of MCS-B.

3. Activity of Na, K - ATPase of erythrocytes in extracorporally processing the blood by nanoparticles of MCS-B does not change (p > 0.05).

4. The minimum indicator of activity of Ca, Mg - ATPase is 6.01±1.2 protein mmol/mg in mines which time of appearing hemolysis practically does not differ from the control was found.

REFERENCES

- [1] Chasis J.A., Mohandas N. J. Cell Biol., 1986, v.103, 343 rivers
- [2] Phoenix C., Drexler E. "Safe exponential manufacturing". Nanotechnology 15 (8):869-872 (Aug 2004). <http://www.iop.org/EJ/abstract/>
- [3] <http://www.nanolab.com.ua>
- [4] Belousov A.N. The method of producing magnetic liquid for transport and retention of medicines in the organism: The State patent №14817A UA A61N2/00/(Ukraine). – Appl. 21.06.96. Publ. 18.02.97. Bull. №2. - 3 p.
- [5] Belousov A.N. The sorbent for extracorporeal detoxication of biological liquids: The State patent №24322A UA A61N2/00/(Ukraine). – Appl. 19.06.97; Publ. 17.07.98. Bull. №7. - 4 p.
- [6] Belousov A.N. "Micromage-B" is product of treatment-and-prophylactic: The State patent №30538A UA A 23L 1/304/(Ukraine). – Appl. 25.05.98. Publ. 15.11.00. Bull. № 6-11. - 3 p.
- [7] Severina S.E. Transport adenosinetriphosphates. In: Modern methods of exploration. Moscow State University Publishing house, 1977, 194 p.
- [8] Belousov A.N. Influence of magnetite - a preparation of nanotechnology on cellular metabolism // Bulletin of problems biology and medicine. Poltava, 2004. - №2. - P. 34-37.