DOI 10.26724/2079-8334-2021-3-77-194-198
UDC 616.15-008.7+616-008.9]:616-005.1:615.326:549.731.1-022.532

E.M Vazhnichaya, R.V. Lutsenko, O.V. Semaka, T.O. Deviatkina, N.M. Deviatkina, Yu.A. Kurapov[1], S.Ye. Litvin[1]
Poltava State Medical University, Poltava, [1]Electric Welding Institute after E.O. Paton, Kyiv

BLOOD GASES AND ELECTROLYTES UNDER USE OF MAGNETITE NANOPARTICLES IN BLOOD LOSS

e-mail: vazhnichaya@ukr.net

Blood gases, acid-base balance, and electrolytes were studied under the conditions of correction of acute blood loss in abino rats with magnetite nanoparticles (5–8 nm) obtained by electron-beam technology and incorporated into sodium chloride crystals. It was shown that blood loss decreases total hemoglobin and the volumetric concentration of oxygen, diminishes sodium concentration and increases potassium concentration in the blood. Dissolved and injected intraperitoneally after the blood loss, magnetite nanoparticles (1.35–6.75 mg iron/kg) reduce the partial pressure of carbon dioxide, increase the partial pressure of oxygen, saturation of hemoglobin with oxygen and volumetric oxygen concentration, increase hydrogen index and sodium content, and reduce potassium concentration in the blood. These positive changes develop against the background of an increase in total hemoglobin. They surpass some effects of the traditional iron preparation and can be the basis for further research aimed at the use of magnetite nanoparticles in acute posthemorrhagic syndrome.

Key words: blood gases, electrolytes, acid-base balance, blood loss, magnetite nanoparticles.

О.М. Важнича, Р.В. Луценко, О.В. Семака, Т.О. Дев'яткіна, Н.М. Дев'яткіна, Ю.А. Курапов, С.Є. Литвин

ГАЗИ КРОВІ ТА ЕЛЕКТРОЛІТИ ПРИ ВИКОРИСТАННІ НАНОЧАСТИНОК МАГНЕТИТУ ПРИ КРОВОВТРАТІ

Гази крові, кислотно-лужний баланс та електроліти досліджували за умов корекції гострої крововтрати у білих щурів наночастинками магнетиту (5–8 нм), отриманими електронно-променевою технологією та включеними в кристали хлориду натрію. Показано, що крововтрата викликає падіння загального гемоглобіну, зменшує об'ємну концентрацію кисню, знижує концентрацію натрію та підвищує концентрації калію в крові. Розчинені та введені внутрішньоочеревинно після крововтрати наночастинки магнетиту (1,35–6,75 мг заліза/кг) знижують парціальний тиск вуглекислого газу, збільшують парціальний тиск кисню, насичення гемоглобіну киснем та об'ємну концентрацію кисню, а також збільшують водневий показник і вміст натрію та зменшують концентрацію калію в крові. Ці позитивні зміни розвиваються на тлі зростання загального гемоглобіну. Вони перевершують окремі ефекти традиційного препарату заліза і можуть бути основою для подальших досліджень, спрямованих на застосування наночастинок магнетиту при гострому постгеморагічному синдромі.

Ключові слова: гази крові, електроліти, кислотно-лужний баланс, крововтрата, наночастинки магнетиту.

The work is a fragment of the research project "Pharmacological study of biologically active substances and drugs for the development and optimization of indications for their use in medical practice" (state registration No. 0120U103921).

Iron oxide nanoparticles (NPs), including magnetite (Fe_3O_4) NPs, belong to the family of inorganic nanomaterials which were a focus of research during the last decades [11]. Magnetite NPs were approved for clinical applications because of their relative safety, unique magnetic have are responsible for a simple and controllable preparation [4]. These NPs are explored as magnetic resonance imaging contrast agents, tumor-targeting photothermal therapy, and drug carriers, diagnostic and therapeutic tools in the regenerative medicine [4, 11]. Therapeutic applications of magnetite NPs also encompass iron supplementation in the iron-deficient anemia, especially in patients with chronic renal disease and hemodialysis [10].

The surface of magnetite NPs is typically modified with a biocompatible coating with a purpose to stabilize the NPs in biological media, to prevent them from possible aggregation and oxidation, to improve their biocompatibility, and/or to attach functional molecules such as targeting ligands or drugs [4, 11]. Chemical structure, molecular weight and surface density of coatings are critical parameters that influence the dispersion stability, cytotoxicity and blood circulation time of such NPs [11].

As a rule, constructed magnetite NPs must stay in the blood during few hours and be eliminated gradually, but in some cases, rapid inclusion of nano-iron into the metabolic processes is necessary, for example in restoration of hematologic parameters after the acute blood loss. Previous investigations showed the effectiveness of ultrasmall magnetite NPs in the inorganic sodium chloride (NaCl) matrix for correction of red blood cells count, hemoglobin concentration, and hematocrit in the laboratory animals with this pathology [1] that make mentioned biocompatible particles prospective for the control of acute posthemorrhagic syndrome in humans. Information about the use of magnetite or other iron oxide NPs for

the urgent treatment of acute hemorrhage and acute posthemorrhagic syndrome is not enough, especially when we attach the state of oxygen-transporting function of the blood considered as blood gases, hydrogen index (pH), and electrolytes that needs following investigation.

The purpose of the work was to study the influence of magnetite NPs incorporated in the NaCl crystals on blood gases, acid-base balance, and electrolytes under the conditions of acute blood loss in the laboratory animals.

Materials and methods. The synthesis of NPs was carried out by condensation of mixed molecular fluxes of iron (Fe) and NaCl in a vacuum electron-beam installation [1]. The microstructure and content of elements in the condensate were examined using scanning electron microscopes VEGA 3 (Tescan, Czech Republic) and CamScan (Cambridge, UK) with an X-ray prefix INCA-200 Energy (Oxford Inca Energy 200 EDS, UK).) [7]. Studies of morphology and phase composition of particles were performed by transmission electron microscopy using a HITACHI H-800 microscope (Hitachi, Japan) [6]. The hydrodynamic diameter and hydrodynamic size distribution of NPs in colloidal solutions containing 1 or 5 mg of NPs condensate in 1 ml of distilled water were determined by dynamic light scattering using a laser correlation spectrometer ZetaSizer-3 (Malvern Instruments Co. Ltd., UK). [9].

In vivo experiments were performed on 45 male Wistar rats weighing 183–221 g. The experiments did not raise objections from the Commission on Bioethics of the Poltava State Medical University. Two series of experiments were performed. The grouping of experimental animals in both these series was as follows: 1 – intact animals (intact control); 2 – acute blood loss with a solvent administration (control pathology); 3 – acute blood loss with the administration of a reference iron preparation (referent group); 4 – acute blood loss with magnetite NPs administration (experimental group).

Acute blood loss was modeled by cardiac puncture and removal of 25 % of circulating blood [12]. The procedure of blood extraction was performed under the ether anesthesia in a surgical stage. 3 hours after the blood loss, rats were anesthetized and undergone thoracotomia. Samples of blood were obtained from the left ventricle of the heart. Euthanasia was performed under the ether anesthesia (3–4 ml/kg body weight) by taking blood from the heart until it stops.

For pharmacological correction, NPs powder was dispersed in water for injections and administered to albino rats intraperitoneally at the dose of 6.75 mg Fe/kg body weight as colloidal solution with a concentration of 5 mg magnetite NPs condensate per 1 ml immediately after the blood loss (series 1) or similar at the dose of 1.35 mg Fe/kg as colloidal solution with a concentration of 1 mg NPs condensate per 1 ml (series 2). A reference preparation was iron hydroxide (III) polyisomaltosate known as Ferrum Lek (Lek, Slovenia), which was administered at the dose of 3.25 mg Fe/kg (series 1) or 1.25 mg Fe/kg (series 2).

Partial pressure of carbon dioxide (pCO_2) and oxygen (pO_2), total hemoglobin (tHb), hemoglobin saturation with oxygen ($\%sO_2$), volumetric concentration of oxygen (ctO_2), pH, concentration of bicarbonate (HCO_3^-), sodium (Na^+), potassium (K^+), and calcium (Ca^{2+}) were determined in blood samples using a blood gas and electrolytes analyzer OPTI CCA-TS (OPTI Medical Systems Inc., USA) [8]. The body temperature of rats was measured with a digital thermometer Medisana FTF (Medisana, Germany). It is an initial parameter for the blood gases analysis and in this study ranged within 37.5–37.8° C, the normal values for this species of animals [5].

The obtained material was statistically processed using the Statistica for Windows 8.0 software. All data of the in vivo experiments were expressed as mean (M) ± standard error of the mean (m). The probability of the difference between the groups was evaluated by one-way ANOVA variance analysis with the Fisher LSD as a posteriori test. A p-value < 0.05 was considered statistically significant.

Results of the study and their discussion. Transmission electron microscopy of fine chips of the original condensate revealed the presence of a nanosized substance with a predominant phase composition corresponding to Fe_3O_4, or magnetite. In the condensate formed at a substrate temperature of 45 °C, magnetite NPs had a polycrystalline structure and an average core size ranged between 5–8 nm and can be characterized as ultrasmall particles iron oxide. The elemental composition of condensate was: iron – 26.9 %; sodium – 22.5 %; chlorine – 34.4 %; oxygen – 16.2 %.

It was found that in the sample with 1 mg of condensate per 1 ml of colloidal solution 99.9 % of particles were with a hydrodynamic size of 13–120 nm (a maximum – 23 nm). In the colloidal solution prepared by dissolving of 5 mg of condensate in 1 ml of aqueous medium, 64 % of particles had hydrodynamic diameter 296–182 nm with a maximum of 213 nm.

In the series 1 of *in vivo* experiments, 3 hours after the blood loss pCO_2, pO_2 and $\%sO_2$ did not differ significantly from those in the intact rats (Table 1).

Table 1

The influence of a bigger dose of magnetite nanoparticles included in the NaCl crystals (6.75 mg Fe/kg) on blood gases, pH, and electrolytes under the conditions of acute blood loss (M±m)

Parameters	Groups of animals			
	Intact animals (n=7)	Blood loss + solvent (n=5)	Blood loss + Ferrum Lek (n=5)	Blood loss+ magnetite nanoparticles (n=5)
pCO_2, mmHg	37.2±2.8	38.9±1.5	34.0±0.9	33.1±1.0
pO_2, mmHg	63.1±4.8	66.0±3.5	67.8±2.7	73.8±5.4
$\%sO_2$, %	85.7±5.6	85.9±5.1	89.4±2.5	94.9±1.5
ctO_2, ml/dl	16.1±1.0	9.6±1.2*	12.3±1.1	13.7±0.5$^{\#}$
tHb, g/L	136.6±3.7	81.6±4.1*	95.8±2.1$^{*,\#}$	102.4±3.1$^{*,\#,@}$
pH, units	7.38±0.02	7.36±0.01	7.40±0.02	7.43±0.02$^{\#}$
Na^+, mmol/L	141.3±0.7	136.4±0.3*	140.0±0.6$^{\#}$	138.9±0.8$^{*,\#}$
K^+, mmol/L	3.5±0.2	3.7±0.1	3.6±0.1	4.1±0.1*
Ca^{2+}, mmol/L	0.85±0.03	0.91±0.05	0.92±0.03	0.89±0.03

Notes. * – p<0.05 as compared to intact animals; $^{\#}$ – p<0.05 as compared to Blood loss + solvent; $^{@}$ – p<0.05 as compared to Blood loss + Ferrum Lek; n – Animals amount in the group.

At the same time, the blood loss with a solvent administration was characterized by a decrease in ctO_2 (p<0.001). The described status took place against the background of a decrease in tHb by 55 g/L (p<0.001). Blood pH in this group remained at the level of intact animals. The change of electrolyte balance caused by the blood removal was manifested as a small, but probable decrease in the content of Na^+ (p<0,001). There were no changes in the concentrations of K^+, Ca^{2+}, and bicarbonate after the blood loss in the series 1.

The use of Ferrum Lek at the dose of 3.25 mg Fe/kg did not affect blood gases as compared to the control pathology. The reference preparation normalized the content of Na^+ (p<0,001) without affecting other electrolytes and blood pH as compared to the blood loss. At the same time, Ferrum Lek increased tHb by 14.2 g/L (p<0.001).

Magnetite NPs at the dose of 6.75 mg Fe/kg increased ctO_2 by 1.4 times (p<0.001) and did not affect the partial pressure of oxygen and carbon dioxide or $\%sO_2$ as compared to a control pathology. They increased tHb by 20.8 g/L (p<0.005) Magnetite NPs contributed to an increase in the pH (p<0.05) as compared to the blood loss with a solvent administration. The use of magnetite NPs produced an increase in Na^+ level (p<0.05). Changes in the content of K^+ were minimal, but the concentration of this electrolyte became higher than the intact control (p<0.02). Investigated NPs did not influence the concentration of Ca^{2+} and bicarbonate compared to control pathology.

In the series 2, after the blood loss pCO_2 and pO_2 were at the level of intact control (Table 2).

Table 2

The influence of a small dose of magnetite nanoparticles included in the NaCl crystals (1.35 mg Fe/kg) on blood gases, pH, and electrolytes under the conditions of acute blood loss (M±m)

Parameters	Groups of animals			
	Intact animals (n=7)	Blood loss + solvent (n=5)	Blood loss + Ferrum Lek (n=6)	Blood loss + magnetite nanoparticles (n=5)
pCO_2, mmHg	42.0±2.4	47.9±2.6	44.0±0.9	37.2±1.9$^{\#,@}$
pO_2, mmHg	67.0±8.1	62.0±6.1	77.8±2.7$^{*,\#}$	97.4±7.6$^{*,\#,@}$
$\%sO_2$, %	89.7±3.7	81.8±5.8	82.4±2.5	95.2±2.7$^{\#,@}$
ctO_2, ml/dl	17.9±1.0	10.1±0.6*	15.3±0.5$^{*,\#}$	14.1±0.7$^{*,\#}$
tHb, g/L	135.2±2.9	95.5±4.0*	111.7±6.1$^{*,\#}$	106.0±2.8*
pH, units	7.41±0.01	7.37±0.03	7.42±0.01	7.46±0.01$^{*,\#}$
Na^+, mmol/L	139.0±0.3	135.4±1.0*	143.0±0.6$^{*,\#}$	137.4±0.8$^{\#,@}$
K^+, mmol/L	3.2±0.1	3.7±0.1*	3.5±0.1	3.1±0.1$^{\#}$
Ca^{2+}, mmol/L	1.0±0.01	0.97±0.02	1.11±0.03$^{*,\#}$	0.92±0.02$^{*,@}$
HCO_3^-, mmol/L	26.9±0.9	27.5±0.6	29.1±0.6*	26.6±0.8$^{@}$

Notes. * – p<0.05 as compared to intact animals; $^{\#}$ – p<0.05 as compared to Blood loss + solvent; $^{@}$ – p<0.05 as compared to Blood loss + Ferrum Lek; n – Animals amount in the group.

$\%sO_2$ in this group also did not differ from this parameter of the intact rats. ctO_2 decreased 1.8-fold (p<0.001) as compared to intact control. tHb in the animals from the control pathology group decreased by 39.7 g/L (p<0.001) as compared to that in the intact animals. Changes in electrolytes and acid-base balance 3 hours after the moment of blood loss were characterized by the lowering of Na^+ concentration and an enhance of the content of K^+ in the absence of other changes.

Ferrum Lek at the dose of 1.25 mg Fe/kg did not change pCO_2 and $\%sO_2$. The administration of the reference preparation increased pO_2 by 15.8 mmHg as compared to control pathology ($p<0.05$). It enhanced ctO_2 by 1.5 times ($p<0.001$) in a comparison to the blood loss with a solvent. Due to the action of Ferrum Lek, tHb increased by 16.2 g/L ($p<0.02$) as compared to control pathology. In the reference group, pH and HCO_3^- remained at the level of control group. The content of Na^+ and Ca^{2+} increased ($p<0.001$), and the content of K^+ did not differ from this parameter in the animals with a blood loss and solvent injection.

When rats were administered with magnetite NPs at the dose of 1.35 mg Fe/kg, pCO_2 decreased by 10.5 mmHg ($p<0.005$) in comparison with control pathology. This parameter under the action of NPs was probably lower than that with administration of the reference preparation ($p<0.05$). The use of magnetite NPs for the treatment of blood loss was characterized by an increase in pO_2 of 35.4 mmHg ($p<0.001$) as compared to the blood loss with a solvent administration, and this level was probably higher than that under the influence of Ferrum Lek. In this group, $\%sO_2$ enhanced ($p<0.05$) as compared to control pathology, which was also higher than this parameter in the reference group ($p<0.05$). Treatment of the blood loss by magnetite NPs increased ctO_2 ($p<0.01$) against that in the animals with the blood loss, which was expressed to the same extent as under the influence of the reference preparation. Under the action of magnetite NPs, an increase in tHb was 10.5 g/L ($p<0.1$) in comparison with a blood loss and solvent The administration of magnetite NPs caused some changes in the block of acid-base and electrolyte balance: there was an increase in pH ($p<0.002$), increased Na^+ concentration ($p<0.05$), and decreased K^+ content ($p<0.01$) as compared to the control pathology. In this group, the content of sodium, calcium and bicarbonate were lower than in the reference group.

Concerning to the discussion of the obtained results, it should be noted that blood gases, pH, and electrolytes are rarely studied when using iron oxides NPs and normative data on their values in laboratory rats are limited. Comparison of mentioned parameters of the intact animals in both series of our experiments shows that they are similar to those under the phenobarbital anesthesia in albino rats and differ, first, by lower pO_2 from the parameters of non-anesthetized rats or rats anesthetized by ketamine [15]. This may be due to the peculiarities of the etheric anesthesia used in the presented investigation as well as the blood sampling from the left ventricle of the heart of animals, and not from the cannulated artery.

Regarding the concentration of transported oxygen (ctO_2) in arterial blood and Na^+ content, changes in the body of animals with control pathology were similar in both series of experiments and are naturally explained by the loss of hemoglobin, which almost entirely determines the content of oxygen transported by blood and ctO_2, and hydremia as a compensatory reaction in the acute blood loss when it comes to Na^+ concentration [2]. An increase in the K^+ concentration in the blood loss was only observed in the series 2 of experiments and, apparently, was due to the release of these ions from the cells, primarily erythrocytes, under the conditions of hypoxia caused by the blood removal [2].

Magnetite NPs, like the standard parenteral iron drug Ferrum Lek, have been shown to improve oxygen transport, electrolytes, and acid-base balance in the acute blood loss, apparently because of restoring hemoglobin content and its associated buffer system. It can also be assumed that the synthetic identity of magnetite NPs deposited in the NaCl crystals determined their biological identity [11], which provided not only rapid inclusion in erythropoiesis by the macrophage mechanism, but also by the direct interaction with erythrocytes in other ways, in particular by modifying their membranes [14], although some authors reject this possibility [13]. It can also be assumed that the compensation of the reduced Na^+ content is partially carried out due to Na^+ ions presented in the NPs condensate and in the injectable form of Ferrum Lek as auxiliary substances.

Matching the effects of two doses of magnetite NPs showed higher efficiency of the smaller of them relating both blood gases and electrolytes, although a degree of the recovery of tHb against its level in the intact animals, taken as a conditional norm, was almost the same. Better expression of the positive changes at a lower dose of injected nano-iron suggests dose-dependent effects of magnetite NPs, which is more commonly described in a relation to the toxicity of this nanomaterial [6]. The higher efficiency of the low dose of investigated NPs can be explained by their smaller hydrodynamic size, which is consistent with the literature data on increasing solubility and reducing NPs aggregation in the dilute solutions [3].

In general, the improvement in blood gases and pH and the elimination of electrolyte disturbances, which occurs along with the accelerated recovery of hematological parameters during the first 3 hours in the correction of acute blood loss by magnetite NPs deposited in the inorganic NaCl matrix, indicates that such NPs have prospects for the use in the acute blood loss, competing with whole blood and erythrocytes

transfusions in patients with massive bleeding and a high risk of symptomatic anemia that is a traditional first-line therapy for anemia during the serious illness or trauma.

Conclusion

So, in albino rats, early period of the acute loss of 25 % circulating blood with a solvent administration is accompanied by a decrease of ctO_2, lowering of Na^+ concentration, and an enhance of K^+ content in the blood alongside the significant drop of tHb level.

Magnetite NPs (5–8 nm) included in the NaCl crystals when were dissolved and administered intraperitoneally decreased pCO_2 increased pO_2, $\%sO_2$, ctO_2 as well as increased pH, enhanced Na^+ concentration, and diminished K^+ content in the animals with acute blood loss. These positive changes develop against the background of tHb growth. They exceed effects of a traditional iron preparation in some points and may be a basis for the further investigations directed towards the use of magnetite NPs for the urgent condition caused by acute hemorrhage. The study of the influence of magnetite NPs coated with high molecular weight substance and 3-hydroxypyridine derivative on blood gases, pH, and electrolytes in the experimental blood loss will be the next step of our research.

References

1. Vazhnycha OM, Mokliak YeV, Movchan BO, Kurapov YuA, vynakhidnyky ta patentovlasnyky. Sposib likuvannia hostroyi krovovtraty za dopomohoyu nanochastynok oksydu zaliza (II, III) Patent Ukainy No 103401. 2013 Zhov 10. [in Ukrainian]

2. Vasilev AG, Haytsev NV, Balashov AL, Balashov LD, Kravtsova AA, Trashkov AP., Pahomova MA. O patogeneze sindroma ostroy krovopoteri. Pediatr. 2019; 10(3): 93–100. [in Russian]

3. Baalousha M, Sikder M, Prasad A, Lead J. Merrifield R, Chandler GT. The concentration-dependent behavior of nanoparticles. Environ Chem. 2015 Oct 8; 13(1): 1–3.

4. Dadfar SM, Roemhild K, Drude NI, von Stillfried S, Knüche R, Kiessling F, Lammers T. Iron oxide nanoparticles: diagnostic, therapeutic and theranostic applications. Adv Drug Deliv Rev. 2019 Jan 1; 138: 302–25.

5. Dangarembizi R, Erlwanger KH, Mitchell D, Hetem RS, Madziva MT, Harden LM Measurement of body temperature in normothermic and febrile rats: Limitations of using rectal thermometry. Phys Behav. 2017 Oct 1; 179, 162–7.

6. Gokduman K, Bestepe F, Li L, Yarmush ML, Usta OB. Dose-, treatment- and time-dependent toxicity of superparamagnetic iron oxide nanoparticles on primary rat hepatocytes. Nanomedicine London. 2018 Jun; 13 (11): 1267–84.

7. Goldstein JI, Newbury DE, Michael JR, Ritchie NWM, Scott JHJ, Joy DC. Scanning electron microscopy and X-ray microanalysis. 4th ed. New York: Springer, 2018. 550 p.

8. Hasan A. Handbook of Blood Gas / Acid–Base Interpretation. London: Springer, 2009. 362 p.

9. Jeevanandam J, Danquah MK, editors. Research advances in dynamic light scattering. New York: Nova Science Publishers Inc, 2020. 330 p.

10. Macdougall IC, Strauss WE, Dahl NV, Bernard K, Li Z. Ferumoxytol for iron deficiency anemia in patients undergoing hemodialysis. The FACT randomized controlled trial. Clin Nephrol. 2019 Apr; 91(4): 237–45.

11. Mulens-Arias V, Rojas JM, Barber DF. The intrinsic biological identities of iron oxide nanoparticles and their coatings: unexplored territory for combinatorial therapies [Internet]. Nanomaterials. 2020 Apr 27 [cited 2020 Oct 8], 10, 837. Available from: https://doi.org/10.3390/nano10050837

12. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010 Jul–Dec; 1(2): 87–93.

3. Pham BTT, Jain N, Kuchel PW, Chapman BE, SA Bickley, Jones SK, Hawkett BS. The interaction of sterically stabilized magnetic nanoparticles with fresh human red blood cells. Int J Nanomedicine. 2015 Oct 23; 10: 6645–55.

14. Ran Q, Xiang Y, Liu Y, Xiang L, Li F, Deng X, Xiao Y, Chen L, Chen L, Li Z. Eryptosis indices as a novel predictive parameter for biocompatibility of Fe3O4 magnetic nanoparticles on erythrocytes [Internet]. Sci Rep. 2015 Nov 5 [cited 2020 Oct 5]; 5: 16209. Available from: https://www.nature.com/articles/srep16209

15. Subramanian RK, Sidharthan A, Maneksh D, Ramalingam L, Manickam AS, Kanthakumar P, Subramani S. Normative data for arterial blood gas and electrolytes in anesthetized rats. Indian J Pharmacol. 2013 Jan-Feb; 45 (1): 103–104.

Стаття надійшла 13.06.2020 р.