Assessment of Protein Fractions of RBCs in Stroke under Influence of Nanodiamonds in vitro

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***Abstract*— Nanodiamonds have unique properties and demonstrate exceptional performance in various fields of investigation and biotechnology, especially in medicine as drug delivery system. Quantitative changes in the red blood cell (RBC) membrane proteins in acute ischemic stroke patients have been studied. The increased amount of ankyrin 2.4, 2.5 fractions, protein of band 3, protein of band 4.1, 4.2 and 4.9 fractions, protein of band 6, tropomyosin (protein of band 7) and protein of band 8 in patients with ischemic stroke have been detected. Increase in the content of spectrin, fractions 2.2, 2.3 (ankyrin), protein of band 2.5, 4.1, glucose transporter and protein of band 8, and decrease in the protein of band 4.2, 4.9 and an increase in the protein of band 4.2, 4.9 and p5 protein of band 5 (actin) from the samples with the nanodiamonds added have been observed. It is concluded that nanodiamonds plays an important role in regulation of the quantitative misbalances of the erythrocyte membrane proteins in the patients with acute ischemic stroke.**

Keywords— nanodiamonds; red blood cell membranes; stroke

I. INTRODUCTION

As it is known, a stroke is the second leading cause of death among the cardiovascular diseases [1], and a leading cause of mortality worldwide. The cases of stroke are predicted will have risen to 23 million, and 7.8 million related deaths in 2030 [2]. In our time, one of the determinants of the use of any medication in the prevention and treatment of the diseases are their toxicity. In this respect, the nanoparticles are given special attention as the promising medications [3-5], in use as agents for antitumor therapy [6], gene therapy [7], anti-inflammatory therapy [8], antiviral therapy [9] and antibiotic therapy [10,11] as well. Their unique chemical stability makes these materials suitable for the attachment of biomolecules, such as genes, proteins, and pharmacologically active substances. A plenty of nanoparticle agents have being applicated in stroke medicine and they have different features depending upon their application [12-19]. Nanoparticles differ in a) the sizes, ranging from tens of nanometers (gold and iron-oxide nanoparticles) to few hundreds of nanometers (polymeric and lipid-based nanoparticles) or as large as micron-sized particles; b) have the different shapes, from spherical nanoparticles to hemispherical, cylindrical; and c) have the surface properties, in which various electrostatic charges can be change to suit the required application [12]. Nanoparticles which have been utilized for diagnosis of stroke are perfluorocarbon particles, iron oxide particles, gold particles, polymeric particles, quantum dots, nanospheres, etc [20]. In medicine, one of the most widely used is ultradisperse nanodiamonds (NDs) of detonation synthesis [21-24]. Carbon particles formed in the form of NDs have an internal crystalline core and an external chemically active shell that determines the surface properties of the particles, obtained by explosion in a confined space in the presence of organic substances containing carbon [25,26]. A study on the physical and chemical properties of ultradispersed NDs showed that each ND crystal has anomalously high adsorption characteristics (from 1 to 10 μg-eq/m^2), and a very large specific surface, up to 450 m^2/g, and having a large number of unpaired electrons (3–7) • 1019 spin/cm^3, it is essentially a powerful multiple free radical whose electrophoretic charge is -78.44 mJ/mol. There are works devoted to the study of the therapeutic effect of nanoparticles, which can be attributed to toxicological assessment in vitro [27,28]. After the introduction, the transit of these nanoparticles through the bloodstream constantly occurs regardless of the route of administration [29-34]. As a rule, NDs in their original state (d=4-6 nm) do not exist for a long time, but are combined into primary clusters, 30-40 nm in size, which do not collapse, and larger aggregates of the order of 100 nm are formed [35]. In water, it is possible to obtain a relatively stable suspension of NDs, with an average size of aggregates of 300 nm, the

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dispersed composition of which does not change for a month or more [36-38]. NDs in combination with chemo- and radiotherapy may be promising in the treatment of malignant tumors [39]. NDs are widespread used in medicine for diagnostic and therapeutic purposes with the possibility of influencing free radical reactions in cells caused by irradiation [37,19,40]. Their used to prevent the mutagenicity of drugs (an aqueous suspension of NDs can prevent the occurrence of mutations in normal cells) [41]. Since most therapeutic applications of nanoparticles are based on intravenous/oral administration, experiments on their interaction with human blood components are extremely important. A classical model for studying the properties of the pathogenesis of the disease at the cellular level is the cell membrane, in particular, the erythrocyte membrane, the main structural component of which is proteins and lipids. Erythrocytes are considered to be excellent carriers of nanoparticles, because they have unique features, including biocompatibility and long life in the bloodstream [25,26]. As carriers of drugs, erythrocytes can load drugs inside the cell; can carry drugs on the surface or inside the membrane, through the formation of transient membrane pores, using membrane-permeating peptides to import therapeutic proteins. In this context, it is extremely necessary to know to what extent the damage to red blood cells after exposure to nanoparticles contributes to general toxicity. The NDs are not carcinogenic, mutagenic or toxic [35,36,20]. It was showed that adsorption of a low concentration of NDs does not affect the oxygenation state of red blood cells (RBC) of human and rat blood [40]. Investigation in vivo shows NDs injected in blood attach to the RBC membrane and circulate with blood for more than 30 min; and ND do not stimulate an immune response. The oxygenation/deoxygenation process was not found to be altered when NDs interacted with the RBC. The d=5 nm and d=100 nm NDs with various concentrations (10–500) did not changed the Activated Partial Thromboplastin Time (APTT test) [31]. Some negative influence of the NDs on RBC, the influence of the NDs on RBC deformability and aggregation in a concentration dependent manner has been reported [30,32]. Due to impaired cerebral circulation in the peripheral blood, many functional and morphologically altered erythrocytes appear. The process of hemolysis of these cells is caused by disorders of the cytoskeleton of erythrocytes. Physicochemical properties of nanoparticles [18] determine their high adsorption properties with respect to various biological compounds and active used in medicine of stroke treatment [20]. NDs can possible creates a nanocomplexs with antitumor properties for drug delivery at the anticancer therapy in vivo and in vitro [39,40] and can interaction with the blood [30,32]. The ND-based complexes non-toxic and don't cause inflammation were found to be. The ND-based complexes are cheap to produce in large quantities [41]. The NDs and their complexes are widely recommended for medical applications while the mechanisms of their effect on biological objects have not yet been adequately studied. One of the conditions for the practical application of the NDs is the preliminary study of the effects of nanoparticles data to the various components of human blood. This article assesses the impact of NDs on the red blood cells through the study of quantitative changes in the composition of protein fractions membranes of red blood cells in patients with ischemic stroke.

II. Materials and Methods

The venous blood erythrocytes of patients and healthy donors were used as the object of the study. The study involved 36 patients aged 38-48 who underwent ischemic stroke. The control group consisted of 20 practically healthy donors of the same age. The effect of NDs in the concentrations of 0.01-0.00275% on the suspension of erythrocytes in a physiological solution containing $65.25 \cdot 10^6$ cells per 1 ml of the reaction mixture was analyzed. The NDs samples were kept in a physiologic solution with washed erythrocytes of blood of donors and patients for 60 minutes at room temperature. Before research, the mixture of erythrocytes with NDs was centrifuged at 7000 rpm for 10 minutes. In order to obtain erythrocyte mass, as well as to determine the number of fragmented erythrocytes, we used the method of blood fractionation in a gradient of ficolla-verographin density (p=0.077) [42]. Resistance of erythrocyte membranes to acidified solutions was checked with a tris-HCl hemolysing solution (0.004 N). For all further studies, red blood cells at the bottom of the test tube were used. Membranes of erythrocytes were obtained by the method of J. Dodge [43]. Isolation and separation of the membrane proteins of erythrocytes by one-dimensional disc-electrophoresis in a 10% polyacrylamide gel containing 1% sodium dodecyl sulfate was carried out according to the well-known Laemmli method [44] which was later improved by Fairbanks et al. [45]. The resulting gels were scanned on a densitometer. Calculations of the percentage content of individual protein fractions (before and after its introduction of nanodiamond suspension) were performed based on the analysis of densitograms. Statistical processing was carried out using the t-criteria Students.

III. Results and Discussions

As is known all red blood cells are a heterogeneous population. Under physiological conditions, the erythrocyte is stable, which is ensured by the rate of formation and destruction of the cell. In conditions of pathology of various genesis, the erythrocyte system is characterized by molecular and biochemical changes that have features of both compensatory adaptive and disadaptive character. Morphofunctional changes in erythrocytes caused by various causes contribute to the disturbance of the regulatory function of the maintenance of homeostasis, and the increase in the heterogeneity of the erythrocyte pool. The increase in the specific gravity of the transformed forms of erythrocytes reflects a violation of the membrane structure and cell metabolism and is an unfavorable diagnostic and prognostic feature. With a detailed examination of the morphological changes in the structure of red blood cell membranes and the induction of these mechanisms, the question naturally arises of the possibility of use the erythrocyte as a model for studying

the electrical properties of biological disperse systems taking into account the proteins distribution in the cell membrane, which of depends of mechanisms of various diseases. The results of the study of the assessment of membrane proteins in patients with ischemic stroke are presented in the table. Sixteen of basic proteins fractions during electrophoresis of membrane proteins of erythrocytes were obtained. Comparative analysis of the protein spectrum of erythrocyte membranes of patients and healthy people revealed significant changes in the content of spectrin, ankyrin, and bands of proteins 2, 3, 4.1, 4.2, 6, 7 and 8.

TABLE I. THE CHANGE IN THE CONTENT (%) OF DIFFERENT FRACTIONS OF THE MEMBRANE PROTEINS OF ERYTHROCYTES OF PATIENTS WITH ISCHEMIC STROKE ($X \pm Sx$)

Membrane protein of erythrocyte	**Control group (n = 20)**	**Ischemic stroke (n = 36)**	**Ischemic stroke + ND (n = 36)**
Band 1 (spectrin α chain)	11,8 ± 0,7	15,7 ± 0,8*	14,3 ± 1,0
Band 2 (spectrin β chain)	11,4 ± 0,7	14,1 ± 1,2*	9,0 ± 0,5**
Band 2.1 (ankyrin)	3,4 ± 0,4	5,3 ± 0,4*	4,5 ± 1,0*
Band 2.2	4,1 ± 0,7	6,2 ± 0,9*	8,0 ± 0,7*
Band 2.3	3,8 ± 0,7	5,1 ± 0,5*	6,3 ± 1,0*
Band 2.4	4,4 ± 0,8	2,6 ± 0,1**	4,0 ± 0,6*
Band 2.5	3,8 ± 0,6	2,4 ± 0,2*	4,1 ± 0,3
Band 3	21,0 ± 0,6	16,5 ± 1,0*	19,0 ±1,0
Band 4.1	4,9 ± 0,3	2,7 ± 0,4**	5,4 ± 0,7
Band 4.2 (paladin)	6,3 ± 0,8	4,0 ± 0,4	5,8 + 0,9*
Band 4.5 (GLUT1) (glucose transporter)	8,0 ± 0,4	10,2 ± 0,6**	8,7 ± 1,3*
Band 4.9	3,3 ± 0,5	1,0 ± 0,4	2,9 ± 0,7*
Band 5 (actin)	7,3 ± 0,7	5,5 ± 0,2	6,3 ± 0,7
Band 6 (GAPDH) (glyceraldehyde-3-phoshate dehydrogenase)	5,8 ± 0,9	2,6 + 0,3*	3,7 ± 0,6**
Band 7 (stomatin)	4,9 ± 1,0	3,9 ± 0,6	4,6 ± 0,7
Band 8 (glutathion-S-transferase)	3,8 ± 0,8	2,2 ± 0,7*	5,0 ± 0,6*

(Relatively with respect to control: * - $p \leq 0.05$; ** - $p \leq 0.01$)

The obtained data show that more quantitative disintegration is observed in the content of membrane proteins of erythrocytes used ND in vitro. The higher levels of spectrin and fractions 2.2, 2.3 of ankyrin in the group with ND testify to the presence of a denser "packing" of the spectrin molecules in the structure of the membrane protein. Spectrin is one of the main high-molecular membrane proteins and, together with actin forms a protein network on the plasma surface of erythrocyte membranes, thereby maintaining the integral integrity of the membrane and ensuring high erythrocyte deformability. Spectrin with the membrane binds through the protein of the band 2.1. Ankyrin is a peripheral protein of the erythrocyte cytoskeleton and binds to spectrin in a ratio of 1: 1. Fractions 2.1, 2.2, 2.3, 2.4, 2.5 refer to different polypeptides of the membrane protein of ankyrin Perhaps, therefore, an increase in the amount of spectrin is accompanied by a compensatory increase in the number of different fractions (2.1, 2.2, 2.3). Reduction of protein 3, 4.9, 5 and 6 content can strongly influence the mechanisms of the transmembrane transport of cations. The protein of band 4.9 plays an important role in the fixation of spectrin with actin and protein of band 3. Disturbances in the interaction of membrane proteins, reduction in their normal content in oxidative degradation lead to profound structural and functional changes, as well as to the emergence of new conduction channels (change in cation transport activity) in the erythrocyte membrane. This, in turn, affects the processes of cellular metabolism and disturbs the normal life of the cell, resulting in a large number of damaged fragmented red blood cells appear in the blood. With a deficiency of protein 4.9, the interaction of spectrin-actin-protein of band 3 will be violated. The violation of interaction effect between proteins of the erythrocyte cytoskeleton, protein-lipid interactions in particular are lead to a decrease in fluidity and an increase in the rigidity of the membrane what is the reason the neurology diseases. With ND the interaction of the protein of band 3 is weakened.

IV. CONCLUSIONS

The carried out researches testify, that at neurological disease compensatory activity of composition of membranous proteins is observed. To correct the detected disorders of ischemic stroke it is necessary to study the possibility of using various ND for treatment and drug delivery.

REFERENCES

[1] E. Perevedentseva, Y.C. Lin, M. Jani, and C.L. Cheng, "Biomedical applications of nanodiamonds in imaging and therapy," Nanomedicine, vol. 8, pp. 2041–2060, 2013.

[2] V.N. Mochalin, O. Shenderova, D. Ho, and Y. Gogotsi, "The properties and applications of nanodiamonds," Nat. Nanotechnol., vol. 7, pp. 11–23, 2012.

[3] B.J. Panessa-Warren, J.B. Warren, S.S. Wong, and J.A. Misewich, "Bioligical cellular response to carbon nanoparticle toxity," J. Phys. Condens. Matter., vol.18, no. 33, pp. 2185–2201, 2006.

[4] M.I. Sriram, S.B.M. Kanth, K. Kalishwaralal, and S. Gurunathan, "Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model," Int. J. Nanomedicine, vol.5, pp. 753-762, 2010.

[5] P. Gopinath, S.K. Gogoi, A. Chattopadhyay, and S.S. Ghosh, "Implications of silver nanoparticle induced cell apoptosis for in vitro gene therapy," Nanotechnology, vol. 19, no. 7, pp.075104-075115, 2008.

[6] S. Shin, M. Ye and H. Kim, H. Kang, "The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells," Int. Immunopharmacol, vol. 7, pp. 1813-1818, 2007.

[7] H.H. Lara, N.V. Ayala-Nuñez, L. Ixtepan-Turrent, and C. Rodriguez-Padilla, "Mode of antiviral action of silver nanoparticles against HIV-1," J. nanobiotechnology, vol. 8, pp. 1-8, 2010.

[8] C. Lok et al., "Silver nanoparticles: partial oxidation and antibacterial activities," JBIC Journal of Biological Inorganic Chemistry, vol. 12, pp. 527-534, 2007.

[9] S.W. Wijnhoven et al., "Nano-silver-a review of available data and knowledge gaps in human and environmental risk assessment," Nanotoxicology, vol. 3, pp. 109-138, 2009.

[10] V.P. Bogatyrjova et al., "Surface and electrochemical properties of dynamical synthesis nanodiamonds," Superhard Materials, vol.6, pp. 42–46, 1999.

[11] S.I. Chukhaeva, P.Ya. Detkov, A.P. Tkachenko, and A.D. Toropov, "Physico-chemical properties of fractions extracted from the ultradisperse diamonds," Superhard Materials, vol.4, pp. 29–31, 1998.

[12] K. Kawata, M. Osawa,and S. Okabe, "In vitro toxicity of silver nanoparticles at noncytotoxic doses to HepG2 human hepatoma cells," Environ. Sci. Technol., vol. 43, pp. 6046-6051, 2009.

[13] R.P. Asha, K.M.G. Low, M.P. Hande, and S. Valiyaveettil, "Cytotoxicity and genotoxicity of silver nanoparticles in human cells," ACS nano, vol. 3, pp. 279-290, 2008.
[14] Y.S. Kim et al., "Twenty-eight-day oral toxicity, genotoxicity, and gender-related tissue distribution of silver nanoparticles in Sprague-Dawley rats," Inhal Toxicol, vol. 20, pp. 575-583, 2008.
[15] J.H. Jung et al. "Twenty-eight-day inhalation toxicity study of silver nanoparticles in Sprague-Dawley rats," Inhal Toxicol, vol. 19, pp.857-871, 2007.
[16] J.H. Sung et al., "Subchronic inhalation toxicity of silver nanoparticles," Toxicological Sciences, vol. 108, pp.452-461, 2009.
[17] Y.S. Kim et al., "Subchronic oral toxicity of silver nanoparticles," Particle and fibre toxicology, vol. 7, no. 20, pp. 1-11, 2010.
[18] E. Park et al., "Repeated-dose toxicity and inflammatory responses in mice by oral administration of silver nanoparticles," Environ. Toxicol. Pharmacol., vol. 30, pp. 162-168, 2010.
[19] O. Bar-Ilan, R.M. Albrecht, V.E. Fako, and D.Y. Furgeson, "Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos," Small, vol. 5, pp. 1897-1910, 2009.
[20] K. Stuart and S. Sikha, "Nanotechnology for the Detection and Therapy of Stroke," Adv. Healthcare Mater, pp. 1-18, 2014.
[21] I.N. Kulakova, T.M. Gubarevich, V.Yu. Dolmatov, and A.P. Rudenko, "Chemical properties of ultradisperse detonation diamonds," Superhard Materials, vol.1, pp. 46–53, 2000.
[22] V. Yu. Dolmatov, "Ultradisperse detonation synthesis diamonds: properties and applications," Suvvesses in Chemistry, vol. 70, pp. 687–708, 2001.
[23] A.S. Barnard, S.P. Russo, and I.K. Snook, "Simulation and bonding of dopants in nanocrystalline diamond," J. Nanosci Nanotechnol., vol. 5, pp. 1395–1407, 2005.
[24] J.Y. Raty et al., "Quantum confinement and fullerenelike surface reconsructions in nanodiamonds," Phys. Rev. Lett., vol.90, pp. 370–401, 2003.
[25] C. H. Villa, A. C. Anselmo, S. Mitragotri, and V. Muzykantov, "Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems," Adv. Drug Deliver. Rev. vol. 106 (PtA), pp. 88-103, 2016.
[26] A. V. Gothoskar, "Resealed Erythrocytes: A. Review," Pharma. Tech., pp. 142-143, 2004.
[27] M. Hamidi, A. H. Zarrin, M. Foroozesh, N. Zarei, and S. Mohammadi-Samani, "Preparation and in vitro evaluation of carrier erythrocytes for RES-targeted delivery of interferon-alpha 2b," Int. J. Pharm., vol. 341, no. 1, pp. 125–133, 2007.
[28] Y. Zhu, J. Li, W. Li, et al., "The Biocompatibility of Nanodiamonds and Their Application in Drug Delivery Systems," Theranostics, vol.2, pp. 302–312, 2012.
[29] L.-W. Tsai et al., "Nanodiamonds for Medical Applications: Interaction with Blood in Vitro and in Vivo," Int J Mol Sci., vol.17, pp. 1111-1118, 2016.
[30] Y.-Ch. Lin, L.-W. Tsa and E. Perevedentseva, "The influence of nanodiamond on the oxygenation states and micro rheological properties of human red blood cells in vitro," J. Biomed. Opt., vol. 17, pp.101512, 2012.
[31] J. Mona, C.-J. Kuoa, E. Perevedentseva, A.V. Priezzhev, and C.-L. Cheng, "Adsorption of human blood plasma on nanodiamond and its influence on activated partial thromboplastin," Diamond & Related Materials, vol. 39, pp. 73–77, 2013.
[32] B.J. Panessa-Warren, J.B. Warren, S.S. Wong, and J.A. Misewich, "Biological cellular response to carbon nanoparticle toxity," J. Phys. Condens. Matter, vol. 18, pp. 2185–2201, 2006.
[33] V.L. Feigin et al., "Global burden of stroke and risk factors in 188 countries, during 1990–2013: A systematic analysis for the global burden of disease study 2013," Lancet Neurol., vol. 15, pp. 913–924, 2016,
[34] K. Strong, C. Mathers, R. Bonita, "Preventing stroke: Saving lives around the world," Lancet Neurol., vol. 6, pp. 182–187, 2007.
[35] B. Godin, J.H. Sakamoto, R.E. Serda, A. Grattoni, A. Bouamrani, M. Ferrari, "Emerging applications of nanomedicine for the diagnosis and treatment of cardiovascular diseases," Trends Pharmacol. Sci., vol. 31, no. 5, pp. 199-205, 2010.
[36] A.P. Puzyr et al., "Nanodiamonds with novel properties: A biological study," Diam. Relat. Mater., vol. 16, pp. 2124-2128, 2007.
[37] V.Yu. Dolmatov, "Biological activity of the ultradisperse detonation synthesis diamonds," Materials of the 6-th Intern. Conf., August 19–23, 2002, Tomsk, pp. 370–371, 2002.
[38] N.P. Dubiago et al., "Ultradisperse carbons materials as antioxidant and antitumor's preparations," Advances in current natural science, vol. 8, pp. 78-79, 2008.
[39] V.E. Orel et al., "Magnetic characteristics and anticancer activity of a nanocomplex consisting of detonation nanodiamond and doxorubicin," J. Superhard Materials, vol.34, pp. 179–185, 2012.
[40] Y. Li, Y. Tong, R. Cao, Z.Tian, B. Yang, and P.Yang, "In vivo enhancement of anticancer therapy using bare or chemotherapeutic drug-bearing nanodiamond particles," Int. J. Nanomedicine, vol. 9, pp. 1065–1082, 2014.
[41] M. Turner, "Diamonds deliver on cancer treatment," Nature News. 09 March 2011.
[42] Z.S. Barkagan and I.V. Tamarin, "Evaluation of the degree of erythrocyte damage in disseminated intravascular coagulation," Laboratory, vol. 3, pp. 34-39, 1998.
[43] J. Dodge, C. Mitchell, and D. Hanahan, "The preparation and chemical characteristics of haemoglobin free ghosts of erythrocytes," Arch. Biochem. Biophys., vol. 100, no. 1, pp. 119-130, 1963.
[44] U.K. Laemmli, "Cleavage of structural proteins during the assembly of the head," T4. Nature, vol. 227, no. 5259, pp. 680-685, 1970.
[45] G. Fairbanks, T.L. Steck, and D.F.H. Wallach, "Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane," Biochemistry, vol. 10, pp. 2606–2617, 1971.