Investigation of Antiradiation and Anticancer Efficiency of Nanodiamonds on Rat Erythrocytes

Liliya Batyuk
Department of Medical and Biological Physics and Medical Information Science,
Kharkiv National Medical University,
Kharkiv, Ukraine
liliya-batyuk@ukr.net

Natalya Kizilova
Department of Applied Mathematics,
Kharkov National University,
Kharkiv, Ukraine
n.kizilova@gmail.com

Vladimir Berest
Department of Molecular and Medical Biophysics,
Kharkov National University,
Kharkiv, Ukraine
vpberest@hotmail.com

***Abstract*— Given the existing insufficiency in radioprotective agents and the expansion of contacts between people interacting with ionizing radiation during treatment or in technological processes, the study of the radiobiological aspects of the application of nanodiamonds is actual. The study is aimed at detection of the anti-radiation effectiveness of ultradispersed nanodiamonds roduced by detonation synthesis during a general radiation damage of rats with a dose of 5.8 Gy. The study has been performed on the red blood cells of rats who received 1.0 ml of a suspension of nanodiamonds one time every 5 days prior to the irradiation. The suspension was diluted to C=0.01 % by distilled water or by saline to a final concentration of 0.9% NaCl. Blood sampling had been performed 30 days after irradiation. Investigation of changes in the dielectric properties and hydration of the red blood cells has been carried out by the method of microwave-dielectrometry at a frequency of 9.2 GHz. The results indicate that irradiation of rats results in an increase in the decrement of the static permittivity of the blood erythrocyte suspension by 37% with respect to those in the control group (intact rats). The degree of hydration of erythrocyte membranes in animals receiving nanodiamonds during the irradiation was found increased by 8.4%, relative to control, and decreased by 29% relative to irradiation without nanodiamons uptake. Based on the data on the dielectric permittivity and hydration changes in the patients with different types of cancer, the conclusion on radioprotective and anticancer properties of NDs has been derived.**

Keywords—nanodiamonds; red blood cell membranes; dielectric permeability; hydration

I. INTRODUCTION

During the last decades a number of unique physical, chemical and biological properties of carbon nanoparticles has been discovered. The high strength, adhesive surface, magnetic moment, heat and electric conductivity of the nanoparticles are used in medicine and biology for the imaging, diagnostics and treatment purposes. One of them is ultradisperse nanodiamonds (NDs) of detonation synthesis, obtained by explosion in a confined space in the presence of organic substances containing carbon. 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 [1,2]. A study on the physical and chemical properties of ultradispersed NDs showed that each ND crystal, unlike the macro diamonds of different types, 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. Having a large number of unpaired electrons (3–7) • 10^{19} spin/cm^3, it is essentially a powerful multiple free radical whose electrophoretic charge is -78.44 mJ/mol. Having a chemically passive nucleus of classical cubic diamond, the crystal has a sufficiently chemically active surface “fringes” from human-safe functional groups (oxy, carboxy, and hydroxycarboxyl groups, etc.) and is chemically inert to any chemical and biochemical reagent. In water, ND particles behave like a poorly soluble weak organic acid. In dry form, NDs are a polydisperse light gray powder with a density of ~0.5 g/cm3, consisting of a conglomerate of one to hundreds of microns [3,4]. 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 [5]. In water, it is possible to obtain a relatively stable suspension of NDs, with an average size of aggregates of 300 nm, the dispersed composition of which does not change for a month or more [6-8]. The use of NDs in combination with chemo- and radiotherapy may be promising in the treatment of malignant tumors to prevent the mutagenicity of drugs. At the same time, an aqueous suspension of NDs can prevent the occurrence of mutations in normal cells [9].

The NDs are not carcinogenic, mutagenic or toxic [3,4,10]. It was found that adsorption of a low concentration of NDs does not affect the oxygenation state of red blood cells (RBC) of human and rat blood [11]. An in vivo animal model 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 NDs at different

conditions did not caused hemolysis, and the cell viability was not affected [12]. Importantly, the oxygenation/deoxygenation process was not found to be altered when NDs interacted with the RBC. The d=5 nm and d=100 nm cNDs with various concentrations (10–500 $\mu g/\mathrm{ml}$) did not changed the Activated Partial Thromboplastin Time (APTT test) [13]. Nevertheless some negative influence of the NDs on RBC has been reported [14]. The influence of the NDs on RBC deformability and aggregation in a concentration dependent manner has been reported in [12].

NDs are widespread and used in medicine for diagnostic and therapeutic purposes with the possibility of influencing free radical reactions in cells caused by irradiation [5, 9, 15]. On the basis of detonation nanodiamond and doxorubicin antitumor drug a nanocomplex with magnetic properties of a soft ferromagnetic has been produced by mechano-magnetochemical synthesis [16]. The combined action of the synthesized nanocomplex and local radio-frequency hyperthermia has been shown to initiate the tendency for an increase of the growth inhibition of Walker-256 carcinosarcoma as compared to the monoeffects of the nanocomplex and officinal doxorubicin [16]. The NDs and ND-doxorubicin complexes have been found promising materials for drug delivery at the anticancer therapy in vivo and in vitro [17]. Due to their complex fingered surface structure they can discharge slowly their drag load into the cancer tissues. ND surface modification and coating can optimize the ND interaction with the blood [11]. The epirubicin adsorbed NDs can kill even chemoresistant hepatic cancer stem cells [18]. The problem is the convenient anticancer drugs tend to become ineffective because cancer cells quickly pump them out before they have had time to do their work, and this sort of drug resistance accounts for 90% of treatment failure in malignant cancer [19]. Recently novel ND-based anticancer drug targeted delivery systems based on the Epidermal Growth Factor (EGF) and the antitumor drug cisplatin (EFG-ND-CP complex) has been elaborated [20]. The ND-based complexes were found to be non-toxic and don't cause inflammation. They are also cheap to produce in large quantities [19].

Thus, on the basis of literature data, it can be concluded that 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.

II. Materials and Methods

In the process of searching for variants of ND suspensions with sufficient stability and higher dispersion, the anti-radiation efficiency of NDs had been tested in experiments on rats in the direction of revealing the possible preventive effect of NDs in acute radiation sickness (adaptation scheme). The studies have been performed on RBC of mature rats weighing 180-200 g, which had been divided into 4 groups (5 animals per group). The first group was a control group. According to the adaptation scheme, the suspension of NDs diluted with distilled water was administered to rats of the groups 2 and 3 orally with a dose q=1.0 ml daily once for 5 days. The suspension was diluted to C=0.01 % by distilled water or by saline to a final concentration of 0.9% NaCl. The former suspension was administered to group 3 of the rats one day before the X-ray irradiation. The rats of the 3-rd and 4-th groups had been irradiated using the RUM-3M laboratory equipment at standard technical conditions: U = 190 kV, I = 12 mA, dose rate 0.52 Gy/min, dose 5.8 Gy, filters 0.5 mm Cu + 1.0 mm Al. Blood sampling was performed on the 30th day after irradiation. Investigation of the changes in the dielectric properties of permeability and hydration of RBCs was carried out by the method of microwave-dielectrometry at a frequency of f=9.2 GHz, according to the relative measurements of the real (ε') and imaginary (ε") parts of the complex dielectric permittivity [20, 21].

Since the dielectric relaxation of most of the water in the solution suspension is described by the Debye equation with a central dispersion frequency of 20 GHz (20-25 ° C), the frequency of the dielectric relaxation f_d of water molecules in the systems under study was calculated by the formula

$$f_d = f\,(\varepsilon' — \varepsilon_\infty)/\varepsilon'', \quad (1)$$

where f_d and f are the frequency of the dielectric relaxation and the frequency of the microwave field, respectively, ε_∞ = 5.5 (the dielectric constant of the investigated objects in the infrared range).

The use of the microwave-dielectrometry method to study the degree of hydration of substances dissolved in water is based on the difference in the complex permittivity of free and bound water: the molecules of bound water and solute in the centimeter wavelength range are less mobile than free water molecules. This leads to a decrease in the permittivity (ε' and ε") of the solution compared to the permittivity values (ε' and ε") of free water. Evaluation of the degree of hydration was calculated by the formula:

$$\Delta\varepsilon_S = \varepsilon_S{}^W — \varepsilon_S{}^S, \quad (2)$$

where ΔεS is the decrement of the static permittivity of the solution with respect to the solvent (water), εSW and εSS are the statistical dielectric permittivities of the solvent and solution, respectively [22]. The obtained data have been compared with the control data by the Fisher method. The corresponding temperature dependencies are presented in Fig.1 a,b.

III. Results and Discussions

It was found that in the group 2 after introduction of NDs during 30 days no significant deviations from the biological control parameters have been detected. All rats were healthy, there was no mortality, and they gained weight. This confirms the existing opinion about the non-toxicity of NDs when they enter into the body with usual food. In the group 4 (pure irradiation at a dose of 5.8 Gy), a typical radiation sickness of rats has been developed (LD70/30). Animals, starting from 3 days, were ruffled, and this sign in the majority, was kept till 30 days. The death of irradiated rats started from 8 days and reached a maximum on 12-15 days (50%).

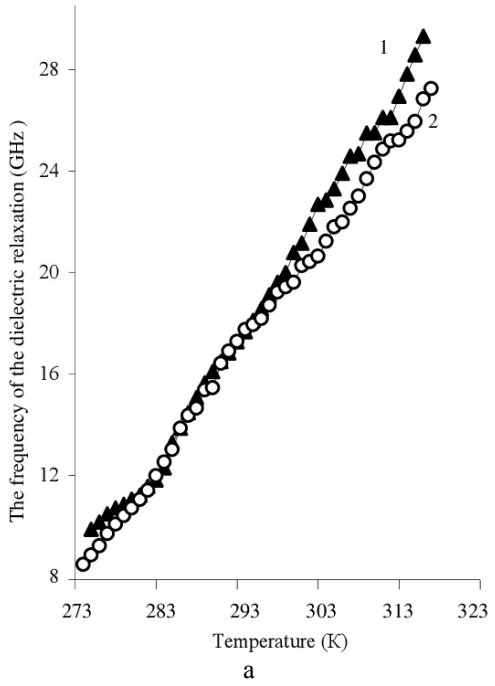

Fig. 1. Temperature dependencies of the frequency of dielectric relaxation for the group 2 (1.0 ml of a 0.01% suspension of nanodiamonds) (a), and the group 4 (irradiation 5.8 Gy) (b) of the animals groups compared to the control group 1.

As shown in Table. 1, irradiation of rats with the dose of 5.8 Gy leads to an increase in the decrement of the static permittivity (DSP=ΔεS) of the erythrocyte suspension by 37% with respect to the control group (intact animals). The results obtained can be explained by the appearance (or increase in the number) of defects in the lipid bilayer membrane due to the deformation of cells under the influence of X-ray irradiation. In the group 2, where the rats received an aqueous solution of NDs, the DSP of the erythrocyte suspension increases by 4%. In the group 3, under irradiation conditions, the DSP of the erythrocyte suspension increases by 8.4% relative to the control group. This phenomenon can be explained in terms of reducing the number of free water molecules with the growth of hydration of erythrocyte membranes in rats under conditions the NDs use, as a result of structural rearrangement of cell membranes. As shown by the available experiments [26], under conditions of local fractional X-ray irradiation without drug intervention, an increase in the DSP of the erythrocyte suspension relative to the control group is observed. Infringement due to irradiation in the erythrocyte membranes of protein-protein and protein-lipid interactions, activates lipid peroxidation in erythrocytes. Therefore, lysophospholipids and free fatty acids are formed that disorganize the structure of the lipid bilayer of the membrane, disrupting the interaction of polar heads of phospholipids with specific areas of the protein and rupturing native hydrophobic contacts, which leads to a structural rearrangement of the erythrocyte membrane.

The oral administration of the suspension of NDs to the rats in group 1 results in a decrease in the frequency of the dielectric relaxation fd of water molecules in the RBC suspension relative to the frequency of the dielectric relaxation of water molecules in the suspension of RBCs of rats in group 2 (Fig.1a). Under the conditions of X-ray irradiation of rats, a significant decrease in the frequency of the dielectric relaxation fd of water molecules in the erythrocyte suspension relative to the control parameters is observed (Fig. 1b), which can be associated with an increase in the amount of bound water in the membrane systems that is reflected in the hydration of cell membranes (Table 1).

Therefore, the electron-acceptor compounds NDs lead to a decrease in the dehydration of blood erythrocyte membranes in irradiated rats, which indicates the presence of adaptive properties in NDs.

TABLE I. DEPENDENCE OF THE DECREMENT OF THE STATIC PERMITTIVITY ($\Delta\varepsilon_S$) OF THE ERYTHROCYTE SUSPENSION UNDER CONDITIONS OF X-RAY IRRADIATION AND THE USE OF NANODIAMONDS (X ± SX)

N	Group	*n*	$\Delta\varepsilon_S$
1	Control	5	8,3 ± 0,3
2	1.0 ml of a 0.01% ND suspension	5	8,6 ± 0,2*
3	irradiation 5.8 Gy + 1.0 ml 0.01% ND suspension	5	9,0 ± 0,3*
4	irradiation 5.8 Gy without NDs	3	11,4 ± 0,4*

*Relatively with respect to control ($p \leq 0.05$)

IV. CONCLUSIONS

It was shown that administration of the ND suspension to the rats orally during 30 days did not show a significant deviation of the value of the DSP of the erythrocyte suspension from the value of the DSP of the erythrocyte suspension of the rat from the biological control, what indicates the non-toxicity of NDs when they are administered orally with food. On the model of radiation sickness, it was

shown that under the conditions of x-ray irradiation of rats at a dose of 5.8 Gy, an increase in the index of the degree of hydration of the membrane of the RBCs has been observed. The degree of hydration of the RBC membranes in the rats injected with the NDs under X-ray irradiation conditions increased by 8.4%, relative to control, and decreased by 29% relative to pure irradiation, which suggests that NDs may possess radioprotective and anticancer properties.

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