DIELECTROPHORETIC STUDY OF RADIATION PROTECTION ABILITY OF NANDIAMONDS

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Nanoparticles and dielectric parameters of human red blood cells

Cancer is a disease involving very complex phenomena at the subcellular level and at the cellular membranes. Modern therapy of malignant tumors is based on knowledge of the laws of the nature of growth and spread of the tumor process, as well as changes that occur against the background of a growing tumor and the biological effect of ionizing radiation [1,2]. The tumor affects all the systems of the body including the circulatory system. In malignant neoplasm's, quantitative and qualitative changes in the composition of erythrocyte membranes are often noted [3,4]. The complex of disorders found in the erythrocytes is associated with structural rearrangements of membranes and should be accompanied by a change in the degree of hydration of the membrane components. The change in hydration, in turn, affects the structure and functional activity of the biological system as a whole [5-8].

Nanotechnology is a new approach to understanding and mastering the properties of the matter at the nanoscale. It offers possible solutions to many current biomedical problems.

The study of the physical and chemical properties of the ultradispersed diamonds showed that each crystal of the nanodiamonds, in contrast to diamonds of other nature, has abnormally high adsorption characteristics (from 1 to 10 μg-eq/m^2), and a very large specific surface - up to 450 m^2/g. Each crystal of the ultradispersed diamonds, having a large number of non-conjugated electrons (3-7) • 1019 spin/cm^3, is essentially a powerful, plural free radical, and an electrophoretic charge of minus 78.44 mJ/mol. In addition, the ultradispersed diamonds crystal, having a chemically passive core of classical cubic diamond, has a rather chemically active surface "fringe" of human-functional functional groups (hydroxy, carboxy, hydroxy carboxyl groups, etc.). At the same time, it was found that, like diamonds of other nature, ultradispersed diamonds are chemically inert to any chemical and biochemical reagents, non-carcinogenic, non-mutagenic and non-toxic. At the same time, in the study of the sorption capacity of the ion cesium ultradispersed diamonds obtained in unequal conditions and purified by various methods, it was established that their sorption capacity varies in the range from 0.01 to 0.50 mol/kg. The largest sorption capacity (0.4-0.5 mol/kg) has samples obtained by purifying the diamond batch of ozone. Ultradispersed diamonds is defined as a complex composite material consisting of separate fractions that differ in content and composition of surface groups, as well as the size of aggregates. Thus, based on the literature data, a summary can be made:

1. The established properties of the ultradispersed diamonds allow considering the advisability of conducting nanodiamonds studies on the evaluation of their anti-radiation

Materials and methods

The studies were carried out on erythrocytes of blood of 40 sexually mature rats Vistar, weighing 160-180 g with subcutaneously transferred 20% suspension of Guerin's carcinoma cells. Rats were kept in vivarium on a standard diet.

The experimental group with Guerin's carcinoma of 20 rats received 1.0 ml of a diluted suspension of ND once per day with food during 5 days prior to the radiation treatment. The suspension has C=0.01 % of dry weight of ND in saline. The control group of 20 rats has not received ND with food.

Therefore, four groups of 10 rats have been studied: with both ND and X-ray (I); with ND without X-ray (II); without ND with X-ray (III); with neither ND nor X-ray (IV). In that way, 20 rats from the groups I and III were treated by X-ray with a dose of 5.8 Gy. Blood samples were collected in 30 days after the irradiation.

The irradiation of rats was carried out under the following conditions: the device RUM-3M, the voltage on the tube of 190 kV, the current of 12 mA, filters 0,5 Cu + 1,0 Al, tubes - 40 cm, the dose rate of 0,52 Gy/min, doses of 5.8 Gy. Animals were slaughtered in compliance with the rules of euthanasia for the 25th day from the beginning of the experiment.

The study of changes in dielectric properties of permeability and hydration of erythrocytes was obtained by microwave-dielectrometry at a frequency of 9.2 GHz, based on the relative measurements of the real (ε') and imaginary (ε'') parts of the complex permittivity. The value of the dielectric constant ε 'was determined by changing the resonance frequency (Δf) of the resonator with the sample relative to the empty resonator, and the value of ε' 'is the magnitude of the damping of the power of the microwave field, due to the introduction into the resonator of the dielectric [16]. The temperature of the test sample was measured by thermocouple copper-constantan with an accuracy of ± 0.1° C. The relative error of measurement ε' for the samples is 0.05%, ε'' - 0.1%. The results of experiments were processed by traditional methods of parametric statistics.

The red blood cells (RBC) were washed out from blood plasma with saline and then diluted to the standard concentration 35%. The dielectric properties of the RBC have been studied by microwave-dielectrometry at the frequency f=9.2 GHz. This frequency corresponds to the gamma-dispersion range which is determined by mobility of water molecules. Molecules of free water have higher rotational mobility while the water molecules bound by the RBC membranes are bound and form hydration shell over the membrane. The RBC suspension has been placed in a capillary (d=0.4 mm) and located in the resonator of the dielectrometer at a fixed temperature. The real and imaginary parts of the dielectric permittivity of the suspension activity.

2. Additional studies are required to determine the toxicity of nanodiamonds.

3. Mechanisms of the effect of ND on biological objects are still insufficient.

To a certain extent, these issues are addressed in this paper. In this work the dielectric permittivity of erythrocytes membranes on experimental model of Guerin's carcinoma was studied.

Results

It was established that in groups 1 and 2, after the introduction of the nanodiamonds for 30 days, there were no deviations from the biological control indices (group 4). All the rats in these groups were healthy, there was no mortality, and they were gaining weight. This confirms the perceived non-toxicity of the nanodiamonds when it is introduced per os. In rats of group 3 (pure irradiation 5.8 Gy), a typical radiation sickness (LD70 / 30) developed. Animals, from 3 days were shaken and this sign in most individuals was kept up to 30 days. The death of irradiated rats began with 8 days and reached a maximum of 12-15 days (50%). All this indicates that the nanodiamonds present in the stomach and intestines at the time of x-radiation can perform a definite radioprotective effect, protecting first of all one of the most radiosensitive tissues - mucus of the intestinal tract, and thus weakening the weight of acute radiation sickness. The collected material will be supplemented and finally processed**.** The obtained results show that in the suspension of erythrocytes of rats-tumor carriers with graft-derived Geren carcinoma prior to the use of the nanodiamonds, an increase in the dielectric relaxation frequency f_d of water molecules in the inter membrane space is observed in comparison with the frequency of dielectric relaxation of water molecules in the surrounding solution. Based on published data suggests that this effect is due to a decrease in the number of bound water systems resulting from structural alterations of erythrocyte membranes under conditions of malignant neoplasms. In the conditions of local fractional x-irradiation of rat-tumor carriers, there is a probable decrease in the frequency of dielectric relaxation of f_d water molecules in the suspension of erythrocytes of animals relative to control parameters, which may be due to an increase in the number of bound water in the membrane systems and is reflected in the hydration of the membranes cells (Table 1). In animals with graft carcinoma Gerena, there is a probable increase in the rate of hydration of erythrocyte membranes ($\Delta\varepsilon_s$) by 20% relative to control.

Experiment	$\Delta\varepsilon_s$
Control (IV)	8,5 ± 0,3
Tumor	10,2 ± 0,4*
Tumor + inradiation in a dose of 5.8 Gy (III)	9,8 ± 0,3*
Tumor + irradiation in a dose of 5.8 Gy + ND (I)	9,1 ± 0,3
Tumor + ND (II)	9,7 ± 0,4*

Note. * - relative to control ($p \leq 0,05$).

have been measured. The first value corresponds to physical dielectric permittivity determined by the thickness of the hydration shell and dielectric permittivity of the membrane. The second value is the loss factor, a measure of the dissipative loss associated with the polarizable charges moving in the electric field.

Conclusions

1. In the model of experimental carcinogenesis, an increase in the frequency of the dielectric relaxation of water molecules in the suspension of rat erythrocytes in tumorous rats compared with intact animals is shown, which is accompanied by a change in the state of water and an increase in the amount of free water.
2. It was established that in the conditions of local fractional x-radiation at a dose of 5.8 Gy there is a moderate decrease in the degree of hydration of membranes of erythrocytes of rats with grafting Guerin's carcinoma.
3. It was shown that under the influence of local fractional x-radiation at a dose of 5.8 Gy and administration of ND one hour prior to irradiation, there is a general tendency to decrease the frequency of dielectric relaxation of water molecules, which is due to changes in the structure of protein-lipid complexes, and suggests the benefit of the idea of reduction dehydration of erythrocyte membranes of rat-tumor carriers.

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