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A Targeted Drug Delivery System of Gd^{3+} for Neutron Capture Therapy against Cancer is Metalorganic Magnetic Nanoparticles

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Abstract

A method for producing nanoparticles based Cholesteric Liquid Crystal Dispersion (CLCD) of complexed [DNA-Gd] with a high content of gadolinium ions (up to 400 mg/ml). Preparation can be stored in the laboratory for 200 days without changes in the physico-chemical properties. It is shown that the formation of nanoparticles of gadolinium ions bind to DNA molecules by interaction with both phosphate groups, and with the nitrogen bases, breaking the regular the secondary structure of DNA. It was found that the formation of nanoparticles significantly reduced solubility of double-stranded DNA and appears uncompensated positive charge on the surface of the complex, impeding its aggregation. The technique of nanoparticles based on a set CLCD [DNA-Gd], including the processing of the original particles CLCD double-stranded DNA with aqueous $GdCl_3$. The magnetic properties of the nanoparticles, allowing maintain an active neutron diffusion exciting nuclide gadolinium directly on the hearth of a malignant tumor. Immobilization of nanoparticles performed by adsorption on macrophages defines active targeted delivery of gadolinium ions in lesions. The application of the local concentration of nanoparticles gadolinium is ~400 mg/ml, as a carrier for the neutron-exciting therapy of malignant neoplasms.

Keywords: Drug delivery system; Neutron capture therapy; Magnetic nanoparticles

Introduction

The most important task of molecular Biophysics and Pharmacy is to find new effective medicines, the combined effect of drugs, containing in its composition fragments, different types of therapeutic and biological activity. Currently, special importance attaches to study the molecular mechanisms of targeted delivery of drugs, will ensure the successful treatment with using existing tools in clinical therapy. One of the most used treatments for cancer is radiation therapy that causes degeneration of the tumor tissue and the inhibition of growth malignant cells. However, the use of ionizing radiation can lead to negative side effects on healthy tissues and of human organs in the form of local and remote consequences. There is reason to believe that the application of Neutron Capture Therapy (NCT) will solve many problems and improve clinical radiotherapy the survival rate of cancer patients.

Materials and Methods

We used calf thymus DNA («Sigma», USA), and polyethylene glycol (PEG) («Sigma», USA) molecular weight of 4000 Da and methacrylate macromonomer polyethylene oxide (M=4000 Da), chloride salts of rare earth elements - gadolinium, lanthanum, neodymium, praseodymium, samarium, terbium, ytterbium (99.99%).

Experiments on the toxicity nanoparticles CLCD [DNA-Gd] were carried out on white mice Vistar weighing 18-20 g, kept in a vivarium. The study of DNA and its fragments was carried out in a vertical chamber electrophoresis VE-2M (Russia) at 0-4°C and a voltage of 100 V for 60 minutes. Visualization of DNA was performed by UV exposure of the gel with the tracks of DNA at a wavelength of 312 nm, using the dye SYBR Green I. CLCD DNA performed according to the method [1] and was monitored by determining the optical density of DNA solution at 350-600 nm. Scanning was performed on samples of an atomic force microscope «P47-SPM-MDT» (Zelenograd) in tapping mode using standard silicon nitride cantilever (stiffness 5.5 N/ resonant frequency of 150 kHz, the radius of curvature of the needle 10 nm). X-ray analysis CLCD [DNA-Gd] conducted by small-angle diffractometer with a linear position-sensitive detector "Amur-K" (Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia) [2]. Extremum of Curves we count by Bragg's formula:

$$D=(\lambda/2)\sin(2\theta/2)$$

where λ – wavelength of X-rays (1,54 nm), 2θ – angle of density

Measurement of the magnetic moment of the sample magnetization performed on a SQUID magnetometer. Permanent magnetic field created a niobium-titanium tube, located in the helium vane. The magnetic field is measured by a Hall sensor with an accuracy of 2% and temperature - using Cu-CuFe-thermocouple with a statistical error 0.1 K. When atomic emission analysis CLCD [DNA-Gd] samples were ashed at a temperature of ~400°C, and dissolved in 5 M HCl. Gadolinium concentration in the resulting solution was determined by plasma atomic emission spectrometer JY-38P. Circular dichroism spectra were recorded with portable dichrometer SKD-2 (quartz cell with an optical length path of 1 cm) [3].

Macrophages isolated by intraperitoneal administration of 5 ml of Hanks' solution to bled line Vistar mice and then they were decapitated. The resulting liquid is centrifuged for 5 min at 1000 rpm/ min. The cells counted in Goryaev chamber and adjusted to 10^6 cells / ml. Viability of the alive cells was studied by staining their water

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trypan blue solution (0.5%) in the chamber Goryaeva 40 minutes, 24 hours and 72 hours. In the study of macrophages using fluorescence nanoscopy in a nutrient medium containing macrophages were added lipophilic dyes based aminostirilov - FM1-43 (Synaptogreen C3), FM4-64 (SynaptoRed C2), the concentration is 10-12 M. They provided staining and fluorescence of the cytoplasmic membrane of cells. Then cells were exposed to monochromatic light with a wavelength from 300 to 780 nm. Irradiation was consistent with a step of 1 nm. Recording the signal was recorded automatically by computer; the images are then successively superimposed on each other with software developed by DA Klimov (2005) of the Company "Sterionik" [4].

Methacrylate hydrogel obtained by radical polymerization hydrophilic monomers with crosslinking methylene-2-methacrylate. To do this in a cell with 2 ml polyethylene oxide was added 0.1 ml of methylene-2-methacrylate. The mixture was gently stirred and incubated overnight to equilibrate in the analyzed system. All experimental studies were carried out in 4-5 times replications. Statistical analysis of the results performed on a personal computer using Statistical data Origin-98 in a conventional manner using the Student t-test at the 95% significance level [5].

Results and Discussion

Visualization of nanoparticles [DNA-Gd] CLCD

These atomic force microscopy (AFM) shows (Figure 1) that the average particle size of ~$(4\text{-}5)\times10^2$ nm. This is almost the same as the CLCD particle size obtained from the original DNA. In processing CLDD DNA gadolinium chloride "fluid" nature of the molecular packing disappears, and they have hard-dimensional structure from that the particle size ranges from 100 to 700 nm. The largest number of particles of [DNA-Gd] has a diameter is about 500 nm.

X-ray spectra CLCD phases of the [DNA-Gd]

Registration curves of small angle X-ray scattering carried out at angles of 0.2 to 10 degrees. Figure 2 is a dependence of the Bragg peak 1 in the small-angle curves X-ray scattering phases CLCD complexes [DNA-Gd] from inverse distance S, which are X-rays in the crystal $(A^o)_1$. In analyzing the results of X-ray study found that for CLCD complex [DNA-Gd] is typical of small-angle X-ray reflections, which lies in the range of 31 to 53 A°. At concentration of gadolinium 3×10^{-5}-5×10^{-5} M characteristic Bragg peak, which determines the degree of order structure CLCD disappears (Figure 3, curve 2). Translational order in the neighboring DNA is broken down to its complete disappearance in concentration of $GdCl_3$ ~3×10^{-5} M. The amplitude abnormal band in the circular dichroism spectrum CLCD of [DNA-Gd] sharply increases. Most probably, this structure consists of alternating irregular fragments having the conformation of B-type right-helix and left-helicity Z-form DNA.

Determination of the concentration of gadolinium in CLCD [DNA-Gd] with magnetometric method

Magnetic moment Pm in sample was defined as $P_m=4.419\times10^{-9}$ N_{qm} where N_{qm} - indication of the quantum magnetometer. Specific magnetization of the sample was obtained by dividing Pm the mass of the sample. Magnetic susceptibility χ_{sp} expected the formula=χ_{sp} MJ_{sp}/B, where B - magnetic induction, J_{sp} – specific magnetization ($A \cdot m^2/kg$); M - magnetic constant ($4\pi\times10^{-7}$ H/m). Found that the number of DNA molecules in the same tablet 3.00×10^{15}. Accordingly, for each molecule of DNA has $N^*Gd/N_{DNA=}$2600 ions Gd^{3+}. The number of turns in the DNA helix is $(8\times10^6)/(6.6\times10^3)$~120, are on each turn of the spiral is located Gd^{3+}-ions 2600/120=21.7.

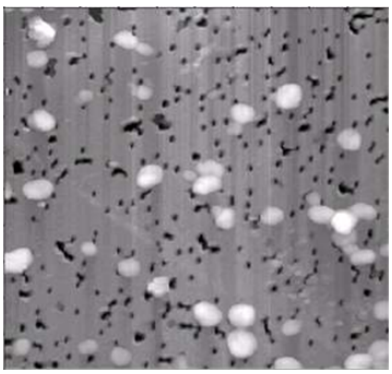

Figure 1: AFM-photo of CLCD nanoparticles formed from DNA treated with $GdCl_3$ and immobilized on surface of nuclear-membrane filter.

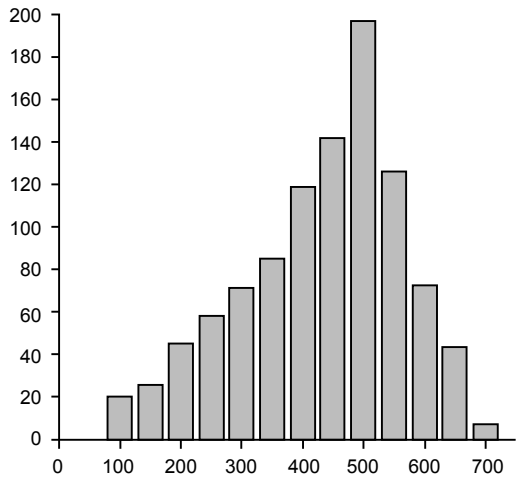

Figure 2: Size distribution of CLCD [DNA-Gd] nanoparticles.

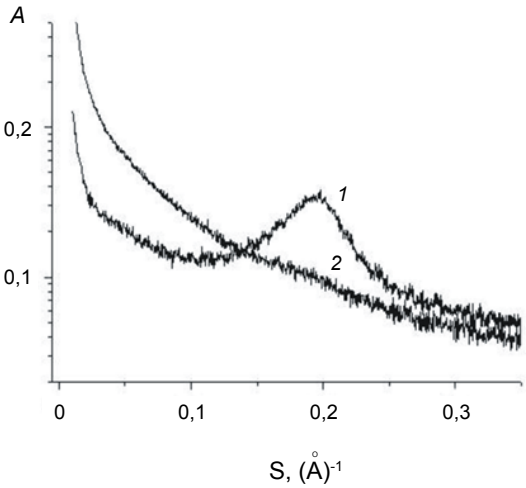

Figure 3: Curves of small angle X-ray scattering of CLCD DNA without Gd^{3+} (curve 1) in presence $GdCl_3$ (curve 2) at concentration of $GdCl_3$ 3×10^{-5}-5×10^{-5} M.

Analyst of gadolinium concentraion in CLCD [DNA-Gd] by neutron activation analysis

The mass of gadolinium in samples calculated using the formula: $mGd=m_H A_{Gd} T_H \eta / T_{Gd} A_H$, where m_{Gd}, A_{Gd}, T_{Gd} – mass activity and the measurement of samples CLCD complex [DNA-Gd]; m_H, A_H, T_H - weight, activity, and the measurement of the normalization of samples; η-correction for the decay of ^{159}Gd in the samples for the measurement and exposure. The latter can be calculated by the law of radioactive decay: $\eta=exp(0,693\Delta T/T1/2)$; ΔT - time difference extracts studied for CLCD and contral of samples, $T_{1/2}$ - half-life of the radionuclide ^{159}Gd CLCD study and normalization of the samples. In all our experiments, ΔT is small, so the amendment η close to 1. In the control sample with an accuracy analysis 2×10^{-3} gadolinium was detected. Found that in saturating conditions on one nucleoside in the DNA of the [DNA-Gd] with 1.5 gadolinium atom, that is, the local concentration of gadolinium in the particles up to 400 mg /ml (~30% of the mass of the particle).

Research of the stability of the nanoparticles CLCD [DNA-Gd]

Found that in the process of storing the amplitude of anomalous optical band increased by about 5%, which may indicate arranging the molecules of the crystal and a decrease of the free energy system CLCD [DNA-Gd]. X-ray studies CLCD [DNA-Gd], conducted after 200 days of storage in the laboratory, and not revealed significant differences in the properties of the crystal. The presence of CLCD [DNA-Gd] rigid DNA molecules containing phosphate groups with which ions of rare-earth metals form soluble compounds (solubility constant~10^{-12}), makes getting stabilized, do not change over time of their material properties with an extended range of conditions it existence. These data suggest that the stability nanoparticles CLCD [DNA-Gd] does not change with storage conditions laboratory within 200 days.

Estimation of cytotoxicity of nanoparticles CLCD [DNA-Gd]

Adding solution $GdCl_3$ (100 microliter, the concentration of 6×10^{-5} M) to the culture media to macrophages white mice resulted in 40 min of incubation to death of 40% of cells. After 24 and 72 hours, all of the cells when exposed to the ion gadolinium died. Introduction CLCD DNA into the medium in which are macrophages (100 microliter, concentration of DNA-2×10^{-7} M) accompanied by a significant decrease in the number of surviving cells. The experimental results showed that the inclusion of gadolinium ions in CLCD DNA nanoparticles significantly reduces its toxicity on macrophages. Obviously, these lanthanides is firmly retained within the nanoparticles and not washed medium. Nanoparticles derived CLCD [DNA-Gd], remain stability in biological tissues and can be used in medicine.

Simulation of the magnetic nanoparticles CLCD [DNA-Gd] in the capillaries of the tumor

Given the specific magnetic properties of gadolinium ions, a study of the magnetic susceptibility and control over nanoparticle magnetic field inside the system simulating tube of capillaries was done. In a quartz cell the size of $0.5\times1\times3$ cm^3 was poured 0.5 ml of a mixture containing nanoparticles, and the process of radical polymerization using 2-methyl-methacrylate. Then carefully layered another 1 ml hydrogel without gadolinium. The hydrogel was placed in a SQUID-magnetometer and exposed to a magnetic field for 2 h at 25°C. Registration of circular dichroism spectra showed that the action magnetic field diffusion of nanoparticles within the polymer net.

Immobilization of nanoparticles on the membranes of macrophages

Cells were incubated for 40 min with nanoparticles containing ions Eu^{3+}, and then washed twice with Hank's solution, followed by centrifugation, and cytoplasm staining was performed membrane dyer FM1-43 and FM4-64 (concentration-10^{-12} M). Process immobilization of nanoparticles on macrophages monitored by Fluorescent Nanoscope and obtain three-dimensional images of cells with nanoparticles immobilized on membranes. Since macrophages tend to concentrate in the area of the tumor, we can expect that immobilization of nanoparticles on their membranes will lead to the death ray focus tumor cells, i.e., locally, thereby reducing the possible dose Gadolinium and intensity of the neutron flux.

NCT simulation using nanoparticles immobilized on macrophages

The cell suspension was cultured in plastic vials of 1 ml culture medium RPMI-40 (1×10^6 cells/ml) at 5% CO_2 and 37°C. Samples 1-4 were exposed neutrons (Table 1). It may be noted that the thermal neutrons are not have radiation effects on metabolism of macrophages, because they do not have sufficient energy to radiation damage at the cellular level. For example, for a single thermal neutron irradiation in control samples after 40 minutes was 95% of viable cells and 72 hours after exposure -86%.

Development of the method of standardization and quality control CLCD of the [DNA-Gd] using optical methods

For quality control, drug concentration and purity of DNA needed absorption spectra (λ_{max}=258 nm), i.e., dependence optical density of the wavelength of the light falling on the object: D=εlC, where D - optical density, ε - molar extinction coefficient, l - optical path length, C - concentration (M). In the study DNA analysis of the absorption band is measured its maximum.

Conclusion

Currently, numerous studies are going on to examine the possibility of gadolinium as neutron exciting nuclide. One of the main reasons in limiting the use of gadolinium as a promising material for NCT is because of a toxic free gadolinium. The need to obtain drug compound that provides a high concentration of gadolinium in the tumor and a considerable time after the introduction of its localization in the lesion, and the safety of its use involves a number of approaches to solving this problem. This thesis proposes a method nanoparticles based CLCD complex [DNA-Gd], based on the processing of particles CLCD original double-stranded DNA with aqueous $GdCl_3$. Shows that gadolinium interacts with double-stranded DNA binding as to its phosphate groups, and with the bases. In this case, structural changes in the form of DNA, as evidenced by changes in the circular dichroism spectra of the initial low-molecular DNA nanoparticles produced by the complex of DNA and CLCD rare earth element gadolinium.

Number of sample	% of survived cell after exposition, time		
	40 minutes	24 h	72 h
1- control sample (exposed)	95 ± 5	84 ± 4	86 ± 4
2 - cell + CLCD DNA	95 ± 5	84 ± 4	72 ± 3
3 - cell + nanoparticales	0	0	0
4 - nanoparticles	-	-	-
5 – cell without exposition (control)	100 ± 5	98 ± 5	93 ± 5

Table 1: Surviving of macrophages after exposed neutrons.

The existence of independent particles supports the hypothesis when uncompensated positive charge on the particles CLCD [DNA-Gd]. This stabilization of the spatial structure of nanoparticles CLCD, in turn, prevents the aggregation and the formation of a homogeneous phase of the [DNA-Gd]. AFM image of single nanoparticles suggests that the processing of double-stranded DNA solubility $GdCl_3$ significantly reduced, and there is a tough space structure. Method AFM shows that this structure has a shape close to spherical, and found using an atomic force microscope diameter close to 500 nm. The nanoparticles are composed of about 10^3 DNA molecules, and one molecule in average of 1.5 gadolinium atom. The nanoparticles contain 80% water and 20% of nucleoside of the [DNA-Gd]. They maintained for 200 days significant concentration of gadolinium (~400 mg/ml) and can be immobilized on macrophages isolated from human tissue for the purpose of targeted delivery to the lesions. In studying the process of transport of nanoparticles in the pockets of defeat, we attempted to use the paramagnetic properties of nanoparticles and monitor targeted delivery of neutron exciting nucleotide with a strong magnetic field.

Measurements of the magnetic moment of nanoparticles containing gadolinium ion, show that this rare earth element strongly associated with molecules DNA. Adsorptive immobilization of nanoparticles on macrophage white mice leads to increased stability of the nanoparticles and is connected, first turn, forced deceleration in the aqueous phase and following directed interaction of nanoparticles with the centers tissue damage. Our experimental data, calculations and analysis literature data allow us to recommend the nanoparticles created on CLCD basis of the [DNA-Gd], as agent for the neutron-capture therapy.

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