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ANTITUMOR VECTOR SYSTEMS BASED ON BIOACTIVE LECTIN OF *BACILLUS SUBTILIS* IMB B-7724

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Combination of properties of lectins and magnetically sensitive iron-containing nanocomposites (NC) for use in oncology is actual and promising from scientific and applied point of view. The aim of the research is to synthesize and to study new iron-containing NC and magnetic fluids containing bioactive bacterial lectin, promising for use as prototypes of new effective antitumor vector systems for targeted drug delivery and combined local therapy of cancer with minimized side effects on the body and improved compatibility with other remedies.

To create vector systems, nanodisperse magnetite was synthesized by the Elmore reaction. The synthesis of aluminum-containing coating on the surface of Fe_3O_4 was carried out by double chemical modification with aluminum isopropylate. The obtained Fe_3O_4/Al_2O_3 NC was impregnated with sucrose solutions. Carbonization of the carbohydrate shell of NC was carried out in argon (500 °C). As a result, $Fe_3O_4/Al_2O_3/C$ NC was obtained.

The magnetic properties of nanostructures were measured using a laboratory vibration magnetometer of Foner type at room temperature. Adsorption immobilization of lectin was performed in 0.9 % NaCl solution in a dynamic mode at room temperature. Bacterial cytotoxic lectin of B. subtilis IMB B-7724 was used in the experiments. The amount of adsorbed substance (A) on the surface of nanocomposites was determined by measuring the concentration of lectin in the contact solutions before and after adsorption using a calibration graph. Measuring of the optical density and absorption spectra of lectin was performed on a spectrometer Lambda 35 UV/vis Perkin Elmer Instruments at λ = 280 nm.

Standard techniques and equipment were used for biological research.

The processes of adsorption immobilization of cytotoxic bacterial lectin of B. subtilis IMB B-7724 from physiologic saline on the surface of magnetite and carbon-containing $Fe_3O_4/Al_2O_3/C$ NC were studied at room temperature. It has been found that the adsorption capacity of lectin on the surface of magnetite is 25.3 mg/g, and $Fe_3O_4/Al_2O_3/C$ NC – 36.3 mg/g (at initial concentrations of lectin 0.06–0.4 mg/mL). The extraction extent of lectin R (%) was 12–38 % for magnetite and 46–67 % for $Fe_3O_4/Al_2O_3/C$ NC. The dependence of the adsorption capacity on time was studied.

A magnetic fluid (MF) based on single-domain Fe_3O_4, containing lectin was synthesized and investigated. Immobilization of lectin on MF particles was carried out in a dynamic mode at room temperature for 3 hours. The concentration of lectin in the composition of MF was 0.2 mg/mL. MF with immobilized lectin was further modified with PEG-2000. The synthesis of Fe_3O_4/ol.Na/lectin/PEG (ol.Na – sodium oleate) vector system was carried out in a dynamic mode for 3 hours. Modification of the surface of nanoparticles with polyethylene glycol was performed in order to increase the stability of the magnetic fluid, reducing the aggregation of particles.

To determine the effect of experimental samples on the viability of MCF-7 cells in vitro, the following samples were prepared: Fe_3O_4/ol.Na/PEG (MF), C_{Fe3O4} = 3 mg/mL; cytotoxic lectin of B. subtilis IMB B-7724 (CL), C_{CL} = 0.2 mg/mL; nanobiocomposite (NBC).

Nanobiocomposite based on MF and bacterial lectin was found to have a synergistic cytotoxic effect on MCF-7 human breast cancer cells, causing up to 40 % cell death. The IC_{50} values for the nanobiocomposite and lectin in relation to MCF-7 cells were 100 and 125 μg/mL, respectively.

The results of research show that the combination of properties of lectins and magnetically sensitive iron-containing NC for use in oncology is a promising direction in creating new effective antitumor vector systems for targeted drug delivery and combined local therapy of cancer. The use of natural components in vector systems is a way to minimize the side effects on the body and improve compatibility with other antitumor remedies.

***Keywords**: antitumor vector systems, targeted delivery, magnetosensitive nanocomposites, bioactive bacterial lectin, Bacillus subtilis IMB B-7724*

INTRODUCTION

As confirmed by many well-known oncologists in the world, biotherapy will become one of the foundations of cancer treatment in the XXI century. According to modern ideas, biotherapy of cancer is carried out using methods that activate the body's antitumor defenses by the immune system, as well as affect the factors and mechanisms that control the processes of angiogenesis and apoptosis. Biotherapy means are a wide range of different factors, including antitumor vaccines, sera, modern biotechnology products using monoclonal antibodies, cytokinins, cellular factors, regulators of genome activity and other molecular processes of cell life [1].

In recent years, researchers have growing interest in immunotherapy as one of the main methods of biotherapy for patients with cancer. In 2018, the Nobel Prize in medicine was awarded to James Allison (USA) and Tasuku Hondze (Japan) for a revolutionary technique of immunotherapy of cancer using T-cells [2].

It is known that the use of monoclonal antibodies in oncological practice is a method of passive immunotherapy [3, 4]. Clinical data suggest that passive immunotherapy is certainly effective and comparable to chemotherapy, but its toxicity is much lower. The combined use of these methods is still widely used, as it significantly expands the possibilities of treatment with modern drugs and helps to increase their effectiveness.

In our works [5, 6–8], the results have been shown of researches into magnetosensitive nanocomposites (NC) made on the basis of nanoscale one-domain magnetite (Fe_3O_4) with a modified surface (by hydroxyapatite, aminopropylsiloxane, polyacrylamide, *etc*.), conjugated with a chemotherapeutic drug – cisplatin (CP), and antibody (AB) CD95. *In vitro* NC were characterized by the recognition of MCF-7 human breast cancer cells, showed the activity of CP, CD95, and the synergism of their joint action with Fe_3O_4, which led *in vitro* to cell death in the quantity that exceeded the action of control samples CP and CD95 in 1.4–2.7 times, as dependent on the composition of NC.

In [9, 10] we synthesized magnetic fluid (MF) based on magnetite, gemcitabine (GC) and HER2 AB, studied their properties and bioactivity of the created colloidal systems against hepatocellular carcinoma (HCC) cells HepG2 of human liver. HER2 (Neu, ErbB-2, CD340) is a membrane protein, tyrosine protein kinase of the EGFR/ErbB epidermal growth factor receptor family, which is encoded by the human ERBB2 gene. Amplification of the HER2 gene plays an important role in the pathogenesis and progression of certain aggressive types of cancer [11–13]. HER2 is an important biomarker and therapeutic target of the disease, it is associated with tumor aggressiveness and unfavorable prognosis. It has been found that using MF based on physiologic saline and single-domain Fe_3O_4 in the complex with GC and HER2 AB, the antitumor activity of the composite can be increased *in vitro* by 8–10 %. MF+GC+HER2 complexes caused the synergistic effect and the increase in cytotoxic activity, compared to GC in monotherapy, up to 18–20 %, with decrease in GC contents to 0.008 mg/mL.

The revealed synergistic cytotoxic/cytostatic effect was explained by high biological activity of the complex with integrated ligand MF-GC-HER2 due to recognition of receptors of HepG2 tumor cells and pharmacological correction of endogenous iron metabolism provided by the use of HER2 and GC in the composition of iron-containing MF. Thus, it has been shown that using magnetic fluid based on magnetite, gemcitabine and HER2 antibody, the effectiveness of cytotoxic/cytostatic action of antitumor drugs increases with a significant reduction in their dose and, accordingly, toxico-allergic reactions of the body.

The said data led to the formulation of scientifically-practical issues related to this work, as an interdisciplinary direction on the border of nanotechnology, surface chemistry and physics, biology and medicine, based on the use of natural components in the composition of iron-containing bioactive NC and magnetic fluids [14–16] in creating effective vector systems for antitumor therapy with minimized side effects on the human body and improved compatibility with other drugs. Such natural components, which have unique properties, significant and not yet realized potential opportunities for practical use, include, in particular, lectins [17–21].

Lectins are known to be a group of substances of protein nature (proteins and glycoproteins) of non-immune origin which have

a capability to reversely and selectively bind carbohydrates and carbohydrate determinants of biopolymers without altering their covalent structure. They are substances of primary synthesis and are present in all kingdoms, types and classes of living organisms [17–20]. Lectins are capable to highly specific bind carbohydrate moieties on cellular surface and participate in cell recognition. For example, some pathogens use lectins to attach to the cells of an affected organism.

Many lectins were found to have cytotoxic properties, due to which they are considered promising for use in antitumor therapy. Their antitumor activity is carried out by various mechanisms, including apoptosis, autophagy and inhibition of tumor growth [18]. However, at present, lectins are more common used in medical research and *in vitro* experiments than in clinical practice. However, due to their involvement in cell recognition, more and more researchers are turning to the investigation of possibility to use lectins for the treatment of cancer. In this aspect, in our opinion, the combination of properties of lectins and magnetically sensitive iron-containing NC [14–16] is also interesting and promising from a scientific and applied point of view for use in oncology [22–28].

Therefore, the purpose of research in this work was the synthesis and study of new iron-containing NC and magnetic fluids containing bioactive bacterial lectin, promising for use as prototypes of new effective antitumor vector systems for targeted drug delivery and complex local therapy of cancer with minimized side effects on the body and improved compatibility with other drugs.

MATERIALS AND METHODS OF RESEARCH

Samples of magnetite and magnetite/carbon NC were used for research. In the composition of NC, carbon was used due to the known unique adsorption properties and biocompatibility.

Synthesis of nanosized magnetite and its main characteristics. Nanodisperse magnetite was synthesized by the Elmore reaction:

$$Fe^{2+}+2Fe^{3+}+8NH_4OH \rightarrow Fe_3O_4+4H_2O+8NH_4^+.$$

The ensemble of Fe_3O_4 nanoparticles (NP) was characterized by sizes of 3–23 nm, with their average size (d_0) ~ 11 nm, as determined by the Scherrer formula, and the specific surface area (S_{sp}) was 105 m^2/g. Magnetite NP were in a completely single-domain state, coercive force H_c = 81.0 Oe, saturation magnetization σ_s = 55.6 Gs·cm^3/g. In the study of IR spectra of magnetite surface, functional groups OH were revealed, the concentration of which was 2.2 mmol/g. The synthesis and properties of magnetite have been described in more detail in [16].

Synthesis of $Fe_3O_4/Al_2O_3/C$ NC. To protect magnetite from oxidation under the influence of the temperature (500 °C) required to obtain a carbon shell, the surface of magnetite was modified with a protective layer of alumina.

The synthesis of aluminum-containing coating on the surface of Fe_3O_4 was carried out by two-fold chemical modification with aluminum isopropylate. To remove isopropyl radicals from the surface of the synthesized NC, the modified samples were calcined in argon stream at the temperature of 500 °C for 2 hours. As a result of the polycondensation reaction, the surface of magnetite acquires an amphoteric character due to Al–OH groups [22].

The obtained Fe_3O_4/Al_2O_3 NC was impregnated using a rotary evaporator with sucrose solutions at the rate of 0.15–0.45 g of carbohydrate per 1 g of Fe_3O_4/Al_2O_3 nanocomposite. Carbonation of the carbohydrate shell of NC was carried out in argon medium (500 °C) with the heating rate of 10 deg/min.

Magnetic properties. Hysteresis loops of the magnetic moment of the samples were measured using a laboratory vibrating magnetometer of the Foner type at room temperature. The description of an installation and the measurement technique have been described in [19, 20]. Demagnetized NP were distributed in paraffin matrix with a volume concentration ~ 0.05 to prevent interaction. For comparison, we used materials with a known value of the specific saturation magnetization (σ_s): a tested sample of nickel and magnetite NP (98 %) manufactured by Nanostructured & Amorphous Materials Inc., USA. In relation to the reference sample, the measurement error σ_s did not exceed 2.5 %.

Adsorption immobilization of lectin. Adsorption of lectin was performed in physiologic saline in a dynamic mode at room temperature (g = 30 mg, V = 5 mL, pH = 7.0) in the concentration range C = 0.06–0.4 mg/mL. The amount of adsorbed substance (A) on the surface of nanocomposites was determined by

measuring the concentration of lectin in the contact solutions before and after adsorption using a calibration graph. Measuring of optical density (D) and recording of lectin absorption spectra were performed on a spectrometer Lambda 35 UV/vis Perkin Elmer Instruments at $\lambda = 280$ nm and a cuvette thickness $l = 1$ cm.

The adsorption capacity of samples A (mg/g) has been calculated by the formula: $A = (C_0 – C_{eq}) \cdot V/m$, where C_0 and C_{eq} are the concentration of the initial solution and the equilibrium concentration (mg/L), V is the volume of the solution (L), m is the portion of the adsorbent (g). The extraction extent R (%) was determined by the formula: $R = (1 – C_{eq}/C_0) \cdot 100$ %.

Vector system based on magnetic fluid and lectin. The synthesis of magnetic fluid (MF) composed of Fe_3O_4/ol.Na was carried out according to the technique [17]. The obtained MF was characterized by optimal values of the sizes of stabilized nanoparticles, saturation magnetization, sedimentation stability, dynamic viscosity and density [28, 29]. Immobilization of lectin on the particles of MF was carried out in a dynamic mode at room temperature for 3 hours. In the composition of Fe_3O_4/ol.Na MF, the concentration of lectin was 0.2 mg/mL.

The obtained magnetic fluid with adsorbed lectin was further modified with PEG-2000. In the colloidal solution of Fe_3O_4/ol.Na/lectin, the portion of PEG was added at the rate of 15 % (mass) of the polymer by weight of the portion of magnetite. The synthesis of Fe_3O_4/ol.Na/lectin/PEG vector system was carried out in a dynamic mode for 3 hours.

Modification of the surface of nanoparticles with polyethylene glycol was performed in order to increase the stability of the magnetic fluid, reduce particle aggregation [28, 29]. In addition, when using a magnetic fluid in a biological environment, the presence of polymer chains effectively prevents the adsorption of biomolecules onto the particles of MF, in particular, their agglutination by components that are part of the blood.

Samples of MF for investigation in vitro. Samples of the synthesized magnetic fluids containing lectin were studied at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine (IEPOR NASU). Bacterial cytotoxic lectin of *B. subtilis* IMB B-7724 was used in the experiments.

In order to determine the effect of experimental samples on the viability of MCF-7 cells *in vitro*, the following samples were prepared:

1. Fe_3O_4/ol.Na/PEG (MF) $C_{Fe3O4} = 3$ mg/mL,
2. Cytotoxic lectin of *Bacillus subtilis* IMB B-7724 (CL) $C_{CL} = 0.2$ mg/mL,
3. Nanobiocomposite (NBC).

Equipment. The following equipment was used:

- a centrifuge OPN-2 (TNK “Dastan”, Kyrgyz Republic);
- CO_2 incubator (Heal Force, China);
- Goryaev chamber (Voles, Ukraine);
- an automatic pipette with a volume up to 200 μL (Ratiolab, Germany);
- an automatic pipette up to 1000 μL (Ratiolab, Germany);
- an inverted microscope with PrimaVert camera (Carl Zeiss, Germany);
- laboratory electronic scales (Kern and Sohn, Germany);
- a multiwell spectrophotometer (Labsystems Multiskan PLUS, Finland);
- a mini-shaker PSU-2T (Biosan, Latvia).

Materials and reagents.

Name of material or reagent	Manufacturer	Cat. No/Lot
Trypan blue	HyClon, USA	cat. No SV30084.01
Nutrient medium DMEM	Biowest, France	L0102-500
Fetal calf serum (FCS)	Biowest, France	No S181B-500
Versene solution	Vetline agroscience, Ukraine	Series 1
Gentamicin	Sigma, USA	G1397
Plate for cell culture, 96 wells	SPL, Korea	30096
Crystal violet	Sigma, USA	cat. No C6158
Ethyl alcohol	Ukrspirt, Ukraine	050219
Sodium chloride, physiological saline	Yuri-Pharm, Ukraine	C. AA8175/1-1

Cell cultivation. MCF-7 cells were cultured in complete DMEM medium with 10 % fetal calf serum (FCS) and 40 μg/mL of gentamicin in plastic ware for cell culture in humidified atmosphere at 5 % CO_2 and 37 °C. Changing of medium and reseeding of cells were performed according to the standard technique [26]. Cells were reseeded after reaching 80–90 % of the monolayer with the help of Versene solution. In the experiments, we used the cells being in the exponential growth phase.

Samples for studies were diluted with complete nutrient medium DMEM with 10 % FCS and 40 μg/mL of gentamicin to obtain concentrations:

– CL of *B. subtilis* IMB B-7724 – 200 μg/mL;

– MF – 300 μg/mL;

– NBC – 300 and 200 μg/mL, respectively.

Determination of cell viability by colorimetric method. In 24 hours after reseeding, MCF-7 cells were removed from the substrate with the aid of Versene solution, and the number of cells in suspension was assessed using trypan blue in Goryaev chamber. The number of cells has been determined by the formula: $X = (a/80)\times10^6$, where X is the number of cells in 1 mL; a – the number of cells counted diagonally in 5 large squares of the chamber.

To assess the effect of the experimental drugs on the viability and proliferation of human breast cancer (BC) cells, the cells were seeded in wells of a 96-well plate in DMEM nutrient medium with 10 % FCS at the concentration of 1×10^4/well. Cells were cultured in a humidified atmosphere at 5 % CO_2 and 37 °C for 24 hours.

A day later, in the appropriate wells of the plates, different concentrations of the experimental preparations were added in 3 repetitions:

– for lectin of *B. subtilis* IMB B-7724 – 200, 100, 50, 25, 12.5, 6.2, 3.1 μg/mL;

– for MF – 300, 150, 75, 37.5, 18.8, 9.4, 4.7 μg/mL;

– for nanobiocomposite – 300+200, 150+100, 75+50, 37.5+25, 18.8+12.5, 9.4+6.2, 4.7+3.1 μg/mL.

Physiologic saline was used as a negative control. Cells were cultured in a humidified atmosphere at 5 % CO_2 and 37 ºC for another 72 hours.

Calculation of results. After incubation, the results (number of living cells) were calculated visually (direct microscopy method) and by colorimetric method, staining living cells with crystal violet [30]. The nutrient medium was removed from the plates, and 50 μL of a solution of crystal violet (5 mg of dye in 1 mL of 70 % methyl alcohol) was added into each well. In 10 min, the dye was washed three times with running water. Further, the plate was dried at room temperature, and the dye was eluted with 96 % ethyl alcohol (100 μL/well) for 10 min using a mini-shaker PSU-2T at 300 rpm. The results were recorded using a multiwell spectrophotometer with a vertical beam path at the excitation wavelength of 540 nm. The number of living cells (X) in each well of the plate was expressed as a percentage, calculated by the formula:

$$X = \frac{A_1 \cdot 100\%}{A_0},$$

where A_0 is the average value of the optical density in the wells of the negative control, A_1 is the average value of the optical density in the well of the experimental group.

Using human BC cell models *in vitro*, IC_{50} for test preparations was determined with the help of regression analysis.

Calculations of the mean value of the experimental indices (M) and the arithmetic mean error (m) were performed using the software package Excel 2016. To assess the significance levels of the difference in the mean values between the groups, Student's t-test was used. The calculations were performed using the software package STATISTICA 6.0. The difference was noted at a level of $p < 0.05$.

RESULTS AND DISCUSSION

The measured hysteresis loops of magnetite particles and composites are shown in Fig. 1.

Table 1 shows the magnetic characteristics of magnetite, Fe_3O_4/Al_2O_3 and Fe_3O_4/Al_2O_3/C NC.

Studies have shown that the magnetic characteristics of single-domain magnetite are not become worse under the chosen conditions of formation of Al_2O_3 and carbon shells (500 °C for 2 h in argon atmosphere) during the synthesis of Fe_3O_4/Al_2O_3 and Fe_3O_4/Al_2O_3/C NC.

Research into the processes of adsorption of bioactive bacterial lectin of B. subtilis IMB B-7724 onto the surfaces of magnetite and Fe_3O_4/Al_2O_3/C nanocomposite. The study of the adsorption of lectin onto the surface of Fe_3O_4

and $Fe_3O_4/Al_2O_3/C$ NC dependent on time was carried out in static mode at pH = 7, the volume of the solution was 5 mL, the weight of the adsorbent was 0.03 g, the lectin concentration was 0.116, 0.221 and 0.375 mg/mL (Fig. 2, *a, b*). The high rate of sorption in the first 30 min is characteristic for the rapid movement of sorbate molecules to a sorbent surface and may indicate the rate of chemical binding to the active centers of the sorbent. The equilibrium between the solution and the sorbent for concentrations of 0.116 and 0.221 mg/mL is reached in 90 min.

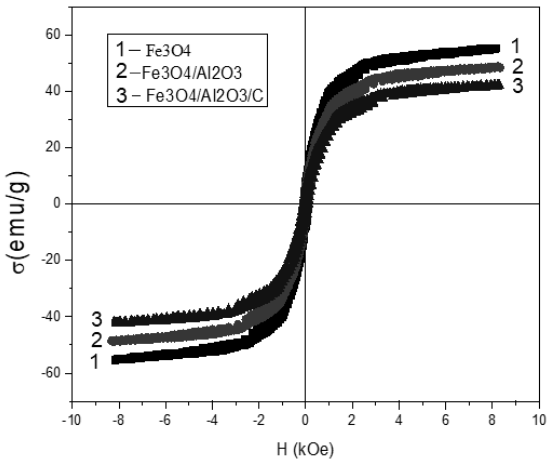


Fig. 1. Loops of magnetic hysteresis of Fe_3O_4 and NC Fe_3O_4/Al_2O_3, $Fe_3O_4/Al_2O_3/C$, carbonized with sucrose

Table 1. Magnetic characteristics of magnetite, Fe_3O_4/Al_2O_3 and $Fe_3O_4/Al_2O_3/C$

Sample	$\sigma_{(H=8\ kOe)}$, emu/g	σ_r, emu/g	σ_s, emu/g	σ_r/σ_s	$\sigma_s^{NC}/\sigma_s^{Fe3O4}$	H_c, Oe
Fe_3O_4	55.6*)	7.37*)	57.9	0.12	1	81
Fe_3O_4/Al_2O_3	48.7*)	6.31*)	51.0	0.12	0.88	82
$Fe_3O_4/Al_2O_3/C$, carbonized with sucrose	42.4*)	5.36*)	44.3	0.12	0.76	94

Note: *) error ± 2.5 %, H_c, Oe – coercive force, σ_s, emu/g – specific saturation magnetization (its value is obtained by extrapolation of the experimental curve $\sigma(H^{-1})$ to the *y*-axis), $\sigma_{H=8\ kOe}$, emu/g is the specific magnetization in the field of 8 kOe, σ_r, emu/g is the residual specific magnetization, σ_r/σ_s is the relative residual magnetization, $\sigma_s^{NC}/\sigma_s^{Fe3O4}$ is the mass fraction of magnetite in NC

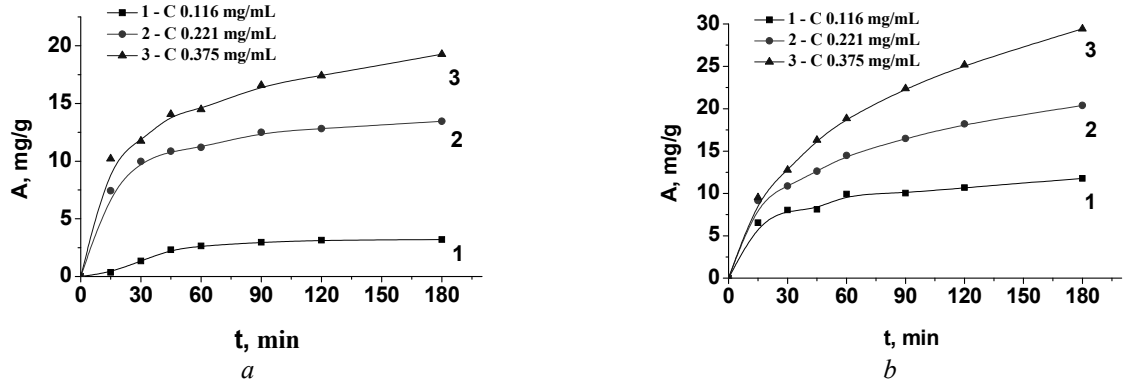
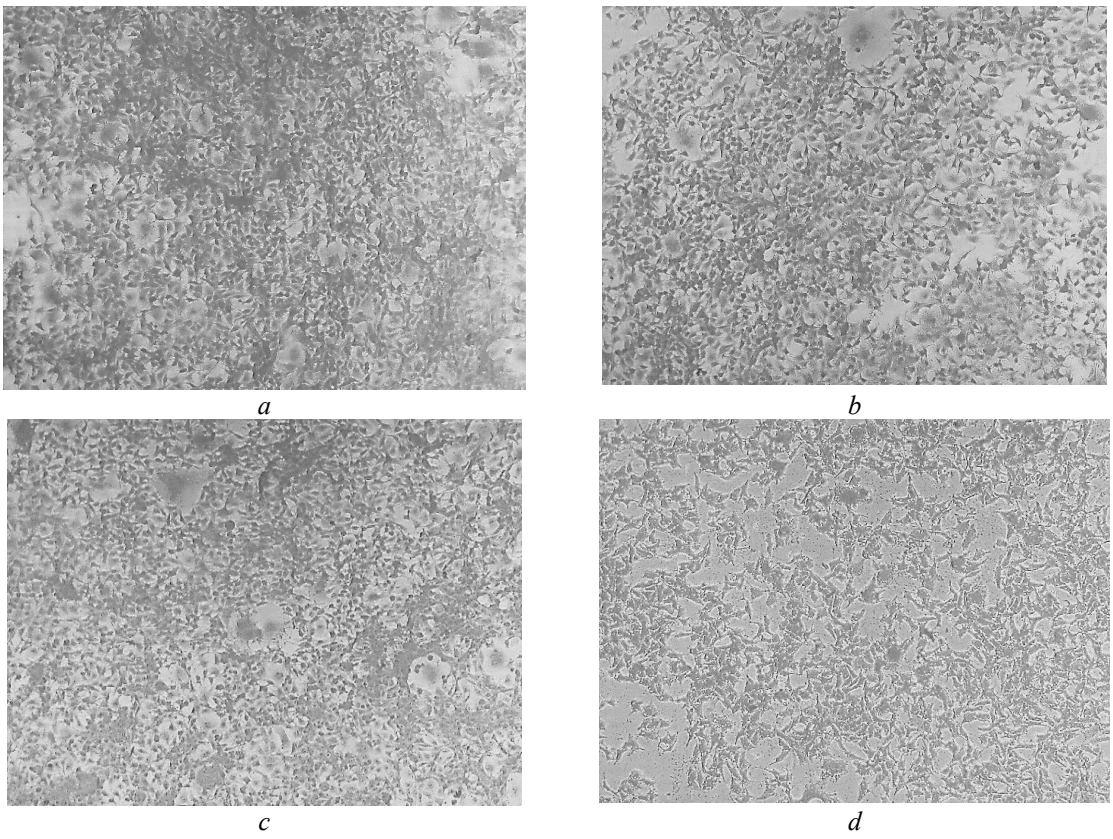


Fig. 2. Time dependence of lectin adsorption on the surface of Fe_3O_4 (*a*) and on the surface of $Fe_3O_4/Al_2O_3/C$ NC (*b*)

The extraction extent of lectin *R* (%) was 12–38 % for magnetite and 46–67 % for $Fe_3O_4/Al_2O_3/C$ NC. Adsorption saturation of the surface was observed.

Study of the effect of nanobiocomposite on the viability of MCF-7 human BC cells. Visualization of the effect of the studied preparations on the number of living human BC cells is shown in Fig. 3.

The effect of different concentrations of cytotoxic lectin of *B. subtilis* IMB B-7724, MF, NBC preparations on the viability of human BC cells was studied *in vitro*. The results are given in Table 2.



a *b* *c* *d*

Fig. 3. Visualization of the effect of the studied preparations on the number of living human BC cells (cells are stained with crystal violet): *a* – control (MCF-7 cells without preparations); *b* – MCF-7 + MF 75 μg/mL; *c* – MCF-7 + lectin 50 μg/mL; *d* – MCF-7 + NBC 75 + 50 μg/mL

Table 2. Viability of MCF-7 human BC cells after their treatment with the test preparations

Concentration of lectin, μg/mL	Cell viability, %	Concentration of MF, μg/mL	Cell viability, %	Concentration of NBC, μg/mL	Cell viability, %
200	5.5±1.8	300	3.1±0.8	300+200	0.6±0.1
100	69.8±4.1	150	55.5±7.4	150+100	0.7±0.3
50	98.7±2.4	75	85.8±1.1	75+50	61.2±4.8
25	107.8±2.8	37.5	100.8±1.6	37.5+25	93.7±3.4
12.5	105.1±2.2	18.8	103.0±2.6	18.8+12.5	94.0±1.9
6.2	103.1±3.6	9.4	102.5±3.5	9.4+6.2	95.0±2.8
3.1	97.3±2.1	4.7	99.2±4.2	4.7+3.1	96.8±1.8

To assess the cytotoxic effect of the studied preparations on human BC cell cultures, we used the value of IC_{50} (cytotoxicity index) – the concentration of half-maximal inhibition, the concentration of drug required for 50 % inhibition of the test response (number of alive tumor cells) *in vitro*.

On the base of results presented in Table 2, using the method of regression analysis, the values of IC_{50} were calculated for the test preparations on human BC cell models (Table 3).

Table 3. The value of IC_{50} for the test preparations on the model of MCF-7 human BC cells

Cell line	Preparation	The value of IC_{50}, μg/mL
MCF-7	Cytotoxic lectin of *B. subtilis* IMB B-7724	125.5 ± 6.4*
	MF	222.8 ± 11.3#
	NBC	80.8 ±5.3# + 53.9 ± 4.0*

Note: *p, #p < 0.05 is statistically reliable deviation in comparing the values of the given groups

When realizing comparative analysis of the cytotoxic activity of NBC to the experimental compounds in mono-use on human BC cells, a synergistic effect of the nanobiocomposite and the complex of preparations on MCF-7 cells was revealed (Table 3). The treatment of tumor cells with NBC or the complex of preparations led to a statistically reliable decrease in the value of IC_{50} for lectin by 57 %, and for MF, the value of IC_{50} decreased by 64 %, compared to mono-use.

The said data show that the combination of properties of lectins and magnetically sensitive iron-containing NC for use in oncology is a promising direction for creation of new effective antitumor vector systems for targeted drug delivery and complex local therapy of cancer with minimized side effects on the body and improved compatibility with other remedies.

CONCLUSIONS

The processes of adsorption immobilization of cytotoxic bacterial lectin of *B. subtilis* IMB B-7724 from physiologic saline onto the surface of magnetite and carbon-containing $Fe_3O_4/Al_2O_3/C$ NC were studied at room temperature. It has been found that the adsorption capacity of lectin on the surface of magnetite is 25.3 mg/g, and $Fe_3O_4/Al_2O_3/C$ NC – 36.3 mg/g (at initial lectin concentrations of 0.06–0.4 mg/mL). The extraction extent of lectin R (%) was 12–38 % for magnetite and 46–67 % for $Fe_3O_4/Al_2O_3/C$ NC. The dependence of the adsorption capacity on time was studied.

In order to create new magnetically sensitive antitumor vector systems based on bioactive compounds, the nanobiocomposite (magnetic fluid based on physiological saline, magnetite and lectin of *B. subtilis* IMB B-7724) was synthesized. The effect of nanobiocomposite on the viability of MCF-7 human breast cancer cells was studied *in vitro*. The nanobiocomposite based on MF containing magnetite and bacterial lectin was found to have a synergistic cytotoxic effect on MCF-7 cells, causing up to 40 % cell death. The IC_{50} values for the nanobiocomposite and lectin in relation to MCF-7 cells were 100.1 and 125.5 μg/mL, respectively.

The results of research show that the combination of properties of lectins and magnetically sensitive iron-containing NC for use in oncology is a promising direction for creation of new effective antitumor vector systems for targeted drug delivery and complex local therapy of cancer. The use of natural components in vector systems is a way to minimize the side effects on the body and improve compatibility with other antitumor remedies.

Протипухлинні векторні системи на основі біоактивного лектину *Bacillus subtilis* IMB B-7724

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Актуальним і перспективним з наукової і прикладної точок зору є поєднання властивостей лектинів і магніточутливих залізовмісних нанокомпозитів (НК) для застосування в онкології. Метою досліджень роботи є синтез та дослідження нових залізовмісних НК та магнітних рідин, що містять біоактивний бактеріальний лектин, перспективних для використання як прототипу нових ефективних протипухлинних векторних систем для адресної доставки лікарських засобів та комплексної локальної терапії онкологічних захворювань з мінімізованими проявами побічного впливу на організм та покращеною сумісністю з іншими лікарськими засобами.

Для створення векторних систем нанодисперсний магнетит синтезували за реакцією Елмора. Синтез алюмінійвмісного покриття на поверхні Fe_3O_4 здійснювали двократним хімічним модифікуванням ізопропілатом алюмінію. Одержаний НК Fe_3O_4/Al_2O_3 було імпрегновано розчинами сахарози. Карбонізацію вуглеводної оболонки НК здійснювали в середовищі аргону (500 °C). В результаті отримували НК $Fe_3O_4/Al_2O_3/C$.

Магнітні властивості наноструктур вимірювали за допомогою лабораторного вібраційного магнітометра фонерівського типу за кімнатної температури. Адсорбційну іммобілізацію лектину проводили у 0.9 % розчині NaCl в динамічному режимі за кімнатної температури. В дослідах використовували бактеріальний цитотоксичний лектин Bacillus subtilis IMB B-7724. Кількість адсорбованої речовини (А) на поверхні нанокомпозитів визначали вимірюванням концентрації лектину в контактних розчинах до та після адсорбції з використанням калібрувального графіка. Вимірювання оптичної густини та спектрів поглинання лектину здійснювали на приладі Spectrometer Lambda 35 UV/vis Perkin Elmer Instruments при λ = 280 нм.

Для біологічних досліджень використовували стандартні методики і обладнання.

Вивчено процеси адсорбційної іммобілізації цитотоксичного бактеріального лектину B. subtilis IMB B-7724 на поверхні магнетиту та карбонвмісного НК $Fe_3O_4/Al_2O_3/C$ за кімнатної температури. Встановлено, що адсорбційна ємність лектину на поверхні магнетиту становить 25.3 мг/г, а НК $Fe_3O_4/Al_2O_3/C$ – 36.3 мг/г (за вихідних концентрацій лектину 0.06–0.4 мг/мл). Ступінь вилучення лектину R (%) складав 12–38 % для магнетиту і 46–67 % для НК $Fe_3O_4/Al_2O_3/C$. Вивчено залежність адсорбційної ємності від часу витримки у розчині лектину.

Синтезовано та досліджено магнітну рідину (МР) на основі однодоменного Fe_3O_4, що містить лектин. Іммобілізацію лектину на частинки МР здійснювали в динамічному режимі за кімнатної температури протягом 3 годин. Концентрація лектину в складі МР становила 0.2 мг/мл. МР з іммобілізованим лектином додатково модифікували ПЕГ-2000. Синтез векторної системи Fe_3O_4/ол.Na/лектин/ПЕГ (ол.Na – олеат натрію) здійснювали в динамічному режимі впродовж 3 годин. Модифікування поверхні наночастинок поліетиленгліколем проводили з метою підвищення стабільності магнітної рідини, зменшення агрегації частинок.

Для визначення впливу експериментальних зразків на життєздатність клітин лінії MCF-7 in vitro готували наступні зразки: Fe_3O_4/ол.Na/ПЕГ (МР), C_{Fe3O4} = 3 мг/мл; цитотоксичний лектин Bacillus subtilis IMB B-7724 (ЦЛ), $C_{цл}$ = 0.2 мг/мл; нанобіокомпозит (НБК).

Встановлено, що нанобіокомпозит на основі МР і бактеріального лектину виявляє синергічний цитотоксичний ефект на клітини лінії MCF-7, що спричиняє загибель до 40 % клітин. Значення IC50 для нанобіокомпозиту та лектину для клітин MCF-7 складали відповідно 100 та 126 мкг/мл.

Результати досліджень свідчать, що поєднання властивостей лектинів і магніточутливих залізовмісних НК для застосування в онкології є перспективним напрямком створення нових ефективних протипухлинних векторних систем для адресної доставки лікарських засобів та комплексної локальної терапії онкологічних захворювань. Застосування природних компонентів в складі векторних систем є

шляхом до мінімізації проявів побічного впливу на організм та покращення сумісності з іншими протипухлинними лікарськими засобами.

***Ключові слова**: протипухлинні векторні системи, цілеспрямована доставка, магніточутливі нанокомпозити, біоактивний бактеріальний лектин, Bacillus subtilis IMB B-7724*

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