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MAGNETICALLY SENSITIVE NANOCOMPOSITES AND MAGNETIC LIQUIDS BASED ON MAGNETITE, GEMCITABINE, AND ANTIBODY HER2

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The processes of adsorption of gemcitabine (GC) on the surface of nanosized singledomain magnetite (Fe_3O_4) have been investigated. Under the experiment conditions, the value of adsorption capacity A was ~ 37.2 mg/g, the extraction extent U = 33.13 %, the separation factor E = 82.58 mL/g. It has been determined that adsorption corresponds to the Freundlich model. The magnetic properties of nanocomposites (NC) Fe_3O_4@GC have been investigated. The average value of the thickness of the adsorbed layer of GC in the composition of Fe_3O_4@GC NC is 2.4±0.1 nm, as evaluated by the method of magnetic granulometry. Magnetic liquids (ML) have been synthesized based on magnetite and physiological solution (PS) stabilized with sodium oleate (Ol.Na) and polyethylene glycol (PEG), which contain GC and antibody (AB) HER2 (Fe_3O_4@GC/Ol.Na/PEG+PS). The properties of ML were investigated as well as cytotoxic/cytostatic activity with respect to HepG2 hepatocellular carcinoma of human liver.

Parameters of ML based on magnetite were found: Fe_3O_4 concentration – 14 mg/mL, Fe_3O_4 particle size – 4–22 nm, average size of Fe_3O_4 particles – 10.8 nm; the average size of Fe_3O_4 particles stabilized with sodium oleate – 16.8 nm, saturation magnetization M_∞ = 14.1±2.5 % Gs, hypsometric height – 25±10 % cm, viscosity η = 1.14±3 % mPa·s, density ρ_{ML} = 1.14 ± 1.0 % g/cm^3, the concentration of GC was 1.25 mg/mL, that of HER2 AB was 3.75 µg/mL. We have obtained the calculated and graphic data concerning the dependence of the surface area and the specific magnetization of saturation of Fe_3O_4@GC/Ol.Na/PEG on the thickness of GC layer, which can be useful for predicting of the nanoscale architecture of magnetically sensitive NC and ML in the manufacture of relative drugs. The synergistic nature of the influence of GC/Fe_3O_4/HER2 complex on HepG2 cells was revealed. It has been determined that IC50 for ML is 0.155 mg/mL, in the range of concentration of 0.025–0.1 mg/mL, ML is biocompatible with HepG2 cells. It has been shown that HER2 AB used alone in the investigated concentrations does not affect HepG2 cell viability/proliferation. In vitro, GC inhibits the proliferation of liver carcinoma cells, the IC50 value has been 0.002 mg/mL. The use of ML in complex with GC can increase the cytotoxic activity of the composite by 8–10 %. The ML+GC+HER2 AB complexes caused the synergistic effect and an increase in the cytotoxic activity, compared with GC used alone, up to 18–20 %, while GC contents reduced to 0.008 mg/mL.

The results of the studies indicate that the use of ML based on magnetite, gemcitabine, and antibody increases the effectiveness of the antitumor medicines with a significant reduction in their dose and, respectively, the toxico-allergic reactions of the body, and nanosized magnetite can be promising for the manufacture of magnetically sensitive adsorption materials for medical purposes, e.g. for detoxification of an organism after GC therapy.

Keywords: magnetite, magnetic liquids, gemcitabine, antibody HER2, hepatocellular carcinoma of human liver

INTRODUCTION

At the beginning of the 21st century, the works in the field of creation of medico-biological and biotechnological nanomachines and "nanoclinics" have got a priority [1–4]. In these works [5, 6], the ideas have been developed for creation of multilevel magnetically sensitive nanocomposites (NC) with a hierarchical nanostructure, which [7, 8] have a complex of functions characteristic for biomedical nanorobot: recognition of micro-biological objects in biological environments; targeted delivery of medicines to specific cells and organs, and deposit; complex therapy with chemo-, immune, neutron capture radiation, photodynamic, hyperthermic methods and real-time diagnostics; adsorption of cellular

decomposition remnants, ions of heavy metals, viral particles, and their removal from the body through an external magnetic field. It should be noted that for today, the concept of magnetically sensitive NC with multilevel hierarchical nanoscale architecture and functions of nanorobots was experimentally substantiated and confirmed through chemical construction of core-shell type nanostructures with complex multilevel hierarchical structure, their combined research and comprehensive check of the capability to perform these functions effectively [9–19].

For *in vitro* and *in vivo* systems, the benefits of using of magnetic liquid (ML) compared to the traditional use of cisplatin (CP) have been proven [20]. It has been found that the biggest cytotoxic activity of the nanocomposite, indicated in the MCF-7/CP resistance line, is related to the most active accumulation of ferrimagnetic nanoparticles in cells due to the high level of transferrin receptors and the disturbance of the antioxidant protection system of resistant cells. It has been shown that a magnetic liquid is capable to cause more pronounced cytomorphological changes and genotoxic effects in the cells of the resistance line compared to cells of the sensitive line. Thus, it has been found that ML on the basis of magnetite and cisplatin is characterized by the capability to redox regulation of cells with phenotype of medicine resistance, which testifies the prospect of its use for pathogenetically grounded targeted therapy of malignant tumors.

It has been experimentally shown that the use of ML containing NC on the basis of magnetite and CP, on the general and biochemical parameters of blood does not make more toxic effects on the body, compared with the official antitumor medicine cisplatin, in according doses.

In papers [16, 21–23] the results are presented concerning researches into NC made on the basis of nanosized Fe_3O_4 with modified (by hydroxyapatite, aminopropylsiloxane, polyacrylamide, *etc*.) surface, conjugated with CP and antibody (AB) CD95. *In vitro* NC were characterized by recognition of MCF-7 human breast cancer cells, determined activity of CP, CD95, and synergism of their combined activity with Fe_3O_4, which resulted *in vitro* to the death of cells in an amount that exceeded the effect of control samples of CP and CD95 in 1.4–2.7 times as dependent on the composition of the NC.

Taking all this into consideration, it is an actual task from the scientific and practical point of view to clarify the possibility of using of the above mentioned approaches to create new effective antitumor medicines based on ML, containing magnetically sensitive NC, other relevant chemotherapeutic medicines and antibodies [24–30]. Solving of this task can expand the functionality of NCs, improve their specificity, promote the creation of new multifunctional antitumor medicines for targeted delivery and local therapy, and provide new data concerning the interaction of oncological drugs with tumor cells.

Therefore, the purpose of this work is to synthesize new nanocomposites and magnetic liquids based on magnetite, gemcitabine and HER2 antibody, to investigate their properties and bioactivity in relation to HCC hepatic cells of HepG2 line.

EXPERIMENTAL PART AND DISCUSSION

In this work, gemcitabine – (2-deoxy-2′, 2) difluorocytidine monochloride) is used as a drug of the chemotherapeutic mechanism of action. It is a cytotoxic drug, an antimetabolite from the pyrimidine antagonists group. GC belongs to the List of the main medicines of the World Health Organization and the most effective and safe medicines needed in the healthcare system. It is characterized by considerable antitumor activity for the list of solid tumors (non-small cell lung cancer, pancreatic, bladder, breast and ovarian cancer), satisfactory endurance and the capability to combine successfully with other antitumor medicines. It is used for treatment of cholangiocarcinoma and other types of biliary cancer. The general side effects include bone marrow suppression, liver and kidney problems, nausea, fever, rash, shortness of breath and hair loss [31–34]. Therefore, for today, the possibility is actively studied to use GC in composition of magnetically sensitive NC and ML on their basis to create multi-functional antitumor medicines for targeted delivery and local therapy [35–38].

In this work as an AB, HER2 (Neu, ErbB-2, CD340) was chosen. It is a membrane protein, a tyrosine proteinkinase of the EGFR / ErbB epidermal growth factor receptor family encoded by the human gene ERBB2. Amplification of the HER2 gene plays an important role in the pathogenesis and progression of certain aggressive types of cancer [39–41]. HER2 is an

important biomarker and therapeutic target of the disease, associated with aggressiveness of the tumor and unfavorable prognosis. It is known that HER2 AB is considered to be one of the optimal for treatment of such diseases as gastrointestinal tract cancer, in particular in the presence of liver metastasis [42]. Therefore, for *in vitro* researches, we have chosen HER2 AB in combination with GC in composition of ML based on magnetite.

Hepatocellular carcinoma (HCC) is the most common primary malignant form of liver cancer, the result of malignant transformation of hepatocytes, a severe, high mortality disease. Every year in the world about 600 thousand cases of this disease are diagnosed. In order to test the antitumor activity of the synthesized ML *in vitro*, the cells of the HCC human liver of HepG2 line were selected in this work.

METHODS OF RESEARCH

Adsorption of GC on the surface of nanosized magnetite was carried out from aqueous solutions of different concentration. Gemcitabine TEVA (Pharmachemie BV, the Netherlands) was used for research.

Adsorption capacity of samples A (mg/g), extraction extent U (%), and separation factor E (mL/g) were determined by techniques described in [13, 14].

The amount of adsorbed substance on the surface of magnetite was determined by measuring the concentration of GC in contact solutions before and after adsorption using a spectrophotometer Spectrometer Lambda 35 UV/Vis Perkin Elmer Instruments.

Specific surface area (S_{sp}) of the samples was determined by nitrogen thermal desorption by means of a KELVIN 1042 device of "COSTECH Instruments" firm. The size of NP was appreciated by formula $D_{BET} = 6/(\rho S_{BET})$, where ρ is density of a particle of NC, S_{BET} is a value of specific surface area calculated by the polymolecular adsorption theory of Brunauer, Emmett and Teller (BET).

The hysteresis loops of the magnetic moment of the samples were measured using a laboratory vibration magnetometer of Foner type at the room temperature. The description of the installation and the method of measurement are set out in [43]. To prevent interactions, the demagnetized nanoparticles were distributed in the paraffin matrix with a volume concentration of ~ 0.05. For comparison, materials were used with known values of specific saturation magnetization (σ_s): a test sample of nickel and Fe_3O_4 NP (98 %) produced by Nanostructured & Amorphous Materials Inc., USA. The measurement tolerance of σ_s did not exceed 2.5 % with respect to the reference sample.

To study the effect of experimental samples on the viability of hepatocellular carcinoma cells of HepG2 line *in vitro* we used DMEM High glucose (Biowest, France, catalog No. L0102-500), fetal calf serum (FCS) (Biowest, France, catalog No. S181B-500), Versene solution (BioTestLaboratoria, Ukraine), a phosphate-salt buffer (Sigma, USA, Cat No. D1408), a physiological solution (Lekchem, Ukraine, series 71033007), ethyl alcohol (Ukrspirt, Ukraine), a crystalline violet dye (Sigma, USA, Cat No. C6158), plastic dishes for cell culture (TPP, Italy), 96-well cell culture plates (SPL, Korea).

The instruments were used: CO_2 incubator (Heal Force, China), an inverted microscope Axiovert 25 (Carl Zeiss, Germany), a Goryaev camera (Farmmedtech, Ukraine), a mini-shaker PSU-2T (BioSan, Latvia), a multi-band spectrophotometer (Labsystems Multiskan PLUS, Finland), automatic pipettes up to 20 μl (Eppendorf AG, Germany), 200 μl (Thermo Fisher Scientific Oy, Finland) and 1000 μl (Eppendorf AG, Germany).

The cells under research were cultured in a complete nutrient medium DMEM with 10 % FTS and 40 μg/mL of gentamicin in plastic dishes in a humidified atmosphere at 5 % CO_2 and 37 °C. The medium changes and transitions of the cells were carried out according to the standard method [44]. For the experiments we used cells that were in the exponential phase of growth. After 24–48 h after the last transition, the cells were planted for cultivation at a concentration of $1–1.5×10^4$ cells /well of 96-well cell plate. After 24 h, various amounts of experimental substances were added to the respective wells according to the study scheme and their cytotoxic activity was determined.

The vitality of cells in the experiment was evaluated by means of colorimetric method by colouring cells with crystalline violet. Adhesive cells are separated from the substrate after death. This property is used in order to determine the number of living cells after their treatment with the investigational agents. One of the ways to determine attached (living) cells is to dye them
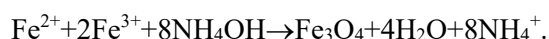
with crystalline violet dye which binds to proteins and DNA. The results were evaluated using a multi-spectrophotometer (wave length is 540 nm). The percentage of viable cells was calculated according to the formula:

$$I_R = (A540 \text{ (experiment)}/A540 \text{ (control)}) \times 100 \text{ \%}.$$

The cytotoxic and antiproliferative activity was determined using IC50 indicator.

SYNTHESIS AND PROPERTIES OF MAGNETITE

The synthesis of nanodisperse magnetite was carried out according to the method [7], by coprecipitation of iron salts according to the reaction:

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

The synthesized ensembles of Fe_3O_4 NP were characterized by dimensions of 3–23 nm. The average size of the NP (d_0) depended on the synthesis conditions and was 6–13 nm, the size distribution could be technologically managed. The specific surface area (S_{sp}) of synthesized magnetite, dependent on the average particle size, was $S_{sp} = 90–180$ m^2/g. In this work we used specimens for which $S_{sp} = 110\pm1$ % m^2/g. The value of mean diameter of the NP Fe_3O_4, calculated from the results of studies of X-ray diffractograms using the Scherrer formula, D_{XRD} was 10.5 nm. Functional OH groups with concentration of 2.2 mmol/g were detected in the study of infrared spectra of magnetite surface [7, 14].

The synthesized magnetite is characterized by satisfactory magnetic characteristics: the coercivity $H_c = 55.0\pm2.5$ % Oe, the specific magnetization of saturation $\sigma_s = 56.2\pm2.5$ % Gs ·cm^3/g, relative residual magnetization $M_r/M_s = 0.2\pm2.5$ %. Such characteristics are important for medical and biological applications [7, 44], for example, while transporting the medicine through blood vessels of small diameter, in which the embolization and aggregation of particles are extremely undesirable.

In [13, 14] it has been established that the synthesized magnetite with the given properties is characterized by superparamagnetism of nanoparticles and is in a completely single-domain state. It is known that super-paramagnetism is a form of magnetism, which is manifested in ferro- and ferrimagnetic particles.

If such particles are of sufficiently small size, they are converted into a singledomain state, that is, they become homogeneously magnetized through the entire volume at any values and directions of the field *H*. To the peculiarities of the singledomain state of these particles, one can also refer the existence of domains not only in alloys and compounds in the solid state, but also in liquid media (suspensions and colloids).

Adsorption of gemcitabine on a surface of nanosized Fe_3O_4. Adsorption of GC on the surface of magnetite Fe_3O_4 was carried out in a medium of saline solution in the range of concentrations $C_0 = 0.02–0.67$ mg/mL ($m = 0.03$ g, $V = 5$ mL, pH = 3.0) during 2 h in static mode at room temperature. The pH of GC solution medium in 0.9 % NaCl was given by 0.1 N HCl.

From the results of researches into the dependence of adsorption activity on pH, the maximal values of *A* (mg/g), *U* (%) and *E* (mL/g) for Fe_3O_4 are observed in the acid medium at pH = 3. According to the results of spectroscopic researches, at the value of pH = 3, up to 70 % of GC is in protonated form, which corresponds to the medicinal form of gemcitabine hydrochloride.

The isotherm of adsorption of GC on the surface of Fe_3O_4, and the results of its mathematical processing are given in Fig. 1.

For quantitative description of equilibrium processes in the investigated range of concentrations the Langmuir, Freundlich, and BET [45] models were used. Linearized equations of these models are used to determine the values of the constants (for the Langmuir model $R^2 = 0.849$, the Freundlich – 0.996, BET – 0.948), included in the isotherm equation.

In our experiments, there has been a deviation in the adsorption character from the Langmuir scheme, which may be caused by the presence of several types of binding centers, characterized by different values of equilibrium constants (energy heterogeneity) and/or the effects of a compatible adsorption. Therefore, we have used the Freundlich model (Fig. 1 *a*, Table 1), that describs the isotherm of adsorption on a heterogeneous surface: $A = K_F C_p 1/n$, in the linearized form $lnA = lnK_F + 1/n\ lnC$.

The experimental values of the adsorption capacity *A* were ~ 37.2 mg/g, the extraction extent *U* = 33.13 %, the separation coefficient *E* = 82.58 mL/g at $C_0 = 0.67$ mg/mL ($m = 0.03$ g, $V = 5$ mL, pH = 3.0).

The results of the studies indicate that nanosized magnetite may be promising for the producing of magnetically sensitive adsorption materials for medical purposes, for example, for detoxification of an organism after GC treatment.
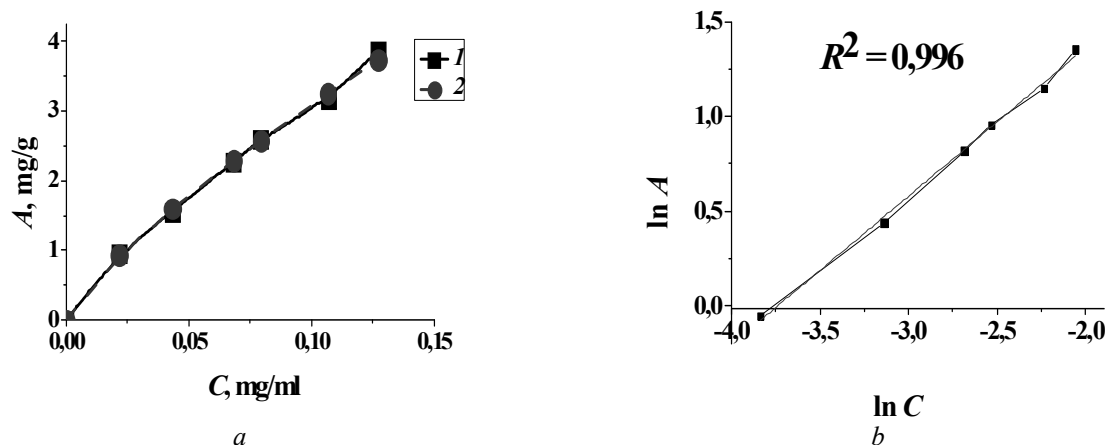

Fig. 1. *a* – Isotherm of adsorption of GC on surface of Fe_3O_4 (*1*) and that calculated from the Freundlich equation (*2*); *b* – the linearized form of the Freundlich isotherm

Table 1. The data of calculations of the adsorption constants of GC on Fe_3O_4 surface according to Freundlich model

Surface	C, mg/mL		A_{exp}, mg/g	Freundlich model			
	C_o	C_p		A_{calc}, mg/g	K_F	n	R^2
Fe_3O_4	0.15	0.12	3.87	3.72	18.7	1.28	0.996

Magnetic properties of NC Fe_3O_4@GC. The magnetic properties of Fe_3O_4@GC nanocomposites have been investigated. The measured hysteresis loops of magnetite NP, and NC with immobilized GC, are shown in Fig. 2 (σ is specific magnetization, H is magnetic field strength). Table 2 shows the magnetic properties of magnetite and NC with adsorbed gemcitabine derived from experimental hysteresis loops.

In the table: H_c, Oe – coercive force; σ_s, emu/g – specific magnetization of saturation of NC; σ_r, emu/g – residual specific magnetization of NC; σ_r/σ_s – relative residual magnetization; α_{Fe3O4}^{calc} – calculated mass concentration of Fe_3O_4 in NC, %.
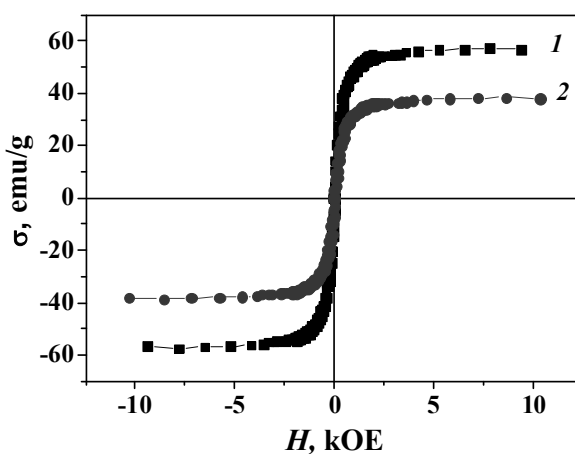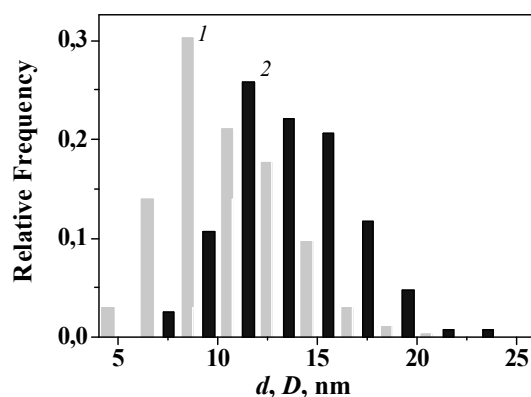

Fig. 2. Hysteresis loops of: *1* – magnetite, *2* – composite Fe_3O_4@GC. $T = 300$ K

Table 2. Magnetic characteristics of magnetite and NC with adsorbed GC

Sample	H_c, Oe	σ_s, emu/g	σ_r, emu/g	σ_r/σ_s	$\alpha_{Fe3O4}{}^{calc}$, %
Fe$_3$O$_4$	41	57.7	10.4	0.18	100
Fe$_3$O$_4$@GC	44	39.0	6.07	0.15	68

Fig. 3. Histograms of relative frequencies of the diameters of NP Fe$_3$O$_4$ and NC Fe$_3$O$_4$@GC, (*d*, *D*, respectively)

The histograms of the relative frequencies of the diameters of NP Fe$_3$O$_4$ *d* and NC Fe$_3$O$_4$@GC *D* (*1*, *2*, respectively) were constructed (Fig. 3) using the method of magnetic granulometry and assuming that the thickness of GC layers depends hardly on the diameter of NP Fe$_3$O$_4$.

The obtained results are typical for core-shell type structures. The average value of the thickness of the adsorbed layer of GC in the composition of NC Fe$_3$O$_4$@GC is 2.4±0.1 nm, as evaluated by the method of magnetic granulometry.

Synthesis and properties of magnetic liquids. Magnetic liquids (ML) based on magnetite and physiological solution (PS) stabilized with sodium oleate (Ol.Na) and polyethylene glycol (PEG) containing GC (Fe$_3$O$_4$@GC/Ol.Na/PEG + PS) were synthesized.

PS was used as the dispersion medium of samples of magnetic liquids for research. It should be noted, that the use of distilled water as dispersion medium, does not change the magnetic properties of colloidal systems substantially.

Nanosized magnetite was used as a dispersed phase, in the singledomain state, or related NC Fe$_3$O$_4$@GC.

To prevent aggregation, Fe$_3$O$_4$ nanoparticles and NC were stabilized with sodium oleate (C$_8$H$_{17}$CH=CH(CH$_2$)$_7$CO–O–Na), (dynamic mode, 1 h) and polyethylene glycol (PEG-2000).

The mass of sodium oleate $m_{Ol.Na}$ used to stabilize the surface of the NP and NC in the composition of the ML was calculated taking into account the concentration of hydroxyl groups on the surface of magnetite. The calculation was made using the formula: $m_{Ol.Na} = B \cdot M \cdot m$, where *B* is the concentration of hydroxyl groups (2.2 mmol/g) on the surface of starting nanosized magnetite, *M* – molecular weight of sodium oleate (304 g/mol), *m* is the mass of Fe$_3$O$_4$ or NC.

It is known that PEG interferes with adsorption interactions of liquid components with protein molecules [45], which is important for medical applications of magnetic liquids. Additional modification with PEG-2000 was carried out in a dynamic mode using a shaker, the amount of polymer was 10–15 % of the weight of the bulk of NP Fe$_3$O$_4$, or NC [13, 14].

Fig. 4 shows the hysteresis loops of aqueous ML Fe$_3$O$_4$@Ol.Na/PEG+PS (*1*) and Fe$_3$O$_4$@GC/Ol.Na/PEG+PS (*2*). The thickness of the layer of GC is ~ 2.3 nm. The thickness of the layer of the stabilizer ~ 3 nm, and the volumes of water in the ML are the same.

As known, taking into account the distribution of nanocomposite particles by volumes *p*(*V*), the magnetization curve of ML (Fig. 5) can be represented as follows [46]:

$$M(H) = \varphi_\rho \int_0^\infty p(v)v^{-1}\left[M_s v_c L(x) + \chi v_s H\right]dv \quad (1)$$

where φ_ρ is the volume fraction of the magnetic component in ML, determined by its density,

$$p(v) = \frac{1}{v\sigma_{\ln v}\sqrt{2\pi}} \cdot \exp\left[-\ln^2(v/v_m)/\left(2\sigma_{\ln v}^2\right)\right],$$

v – volume of a NC particle, M_s – magnetization of the saturation of the core, v_c – volume of the core, $L(x) = cthx - x^{-1}$ – Langevin's function, $x = \left(\frac{M_s v_c H}{k_B T}\right)$, χ – magnetic susceptibility of the shell, v_s – shell volume. The main contribution to the magnetization of ML is introduced by the superparamagnetism of the cores and the paramagnetism of the shells of the core@shell particles. Compared to superparamagnetism of cores in the fields $H = 0$–10 kOe, in most cases the paramagnetism of the shells (the second term in (1)) can be neglected.

The modification of ML Fe$_3$O$_4$@GC/Ol.Na/PEG+PS with AB HER2 (receiving ML Fe$_3$O$_4$@GC/Ol.Na/PEG/AB HER2+PS) was performed dynamically with use of a shaker.

Fig. 4. Hysteresis loops of aqueous ML Fe$_3$O$_4$@Ol.Na/PEG+PS (thickness of the stabilizer layer ~ 3 nm) (*1*) and Fe$_3$O$_4$@GC/Ol.Na/PEG+PS (*2*)

The optimal parameters of the experimental ML on the basis of magnetite were evaluated: concentration of Fe$_3$O$_4$ – 14 mg/mL, size of Fe$_3$O$_4$ particles – 4–22 nm, average size of Fe$_3$O$_4$ particles – 10.8 nm; the average size of Fe$_3$O$_4$ particles stabilized with sodium oleate – 16.8 nm; saturation magnetization $M_\infty = 14.1\pm2.5\,\%$ Gs, hypsometric height – $25\pm10\,\%$ cm, viscosity $\eta = 1.14\pm3\,\%$ mPa·s, density $\rho_{ML} = 1.14\pm1.0\,\%$ g/cm^3, the concentration of GC was 1.25 mg/mL, HER2 AB – 3.75 µg/mL.

The concentration of GC and AB HER2 in such ML is determined by the therapeutic necessity. In the starting ML, the concentration of GC and AB HER2 has been 1.25 mg/mL and 3.75 µg/mL, respectively, which allows one providing the necessary dosages of the medicine in the experimental samples by diluting the initial ML. In addition, such a liquid is characterized by satisfactory rheological properties and sedimentation resistance.

Investigation of influence of experimental samples on viability of HepG2 hepatocellular carcinoma cells of human liver in vitro. For the research, the series of experimental samples were used:
1. ML: Fe$_3$O$_4$@Ol.Na/PEG+PS (control 1),
2. Gemcitabine (control 2),
3. AB HER2 (control 3),
4. ML + GC: Fe$_3$O$_4$@GC/Ol.Na/PEG+PS,
5. ML + AB: Fe$_3$O$_4$@Ol.Na/PEG+PS+HER2,
6. ML + GC + AB: Fe$_3$O$_4$@GC/Ol.Na/PEG+PS + HER2,
7. GC + HER2 (control 4),
8. PS (control 5).

The concentration of magnetite for all systems was $C_{Fe3O4} = 3$ mg/mL, the concentration of gemcitabine $C_{GC} = 0.25$ mg/mL, that of antibody $C_{AB} = 0.75$ µg/mL.

According to the results of research into the influence of experimental samples on the viability of HCC cells of human liver of HepG2

line, features were revealed of the influence of the composite system ML+GC+AB (Fe$_3$O$_4$@GC/Ol.Na/PEG+PS+HER2) and of each component separately (ML, GC, AB, PS, respectively) on HCC cells.

In particular:

1. IC50 for ML was 0.155 mg/mL (control 1). In samples with a ML concentration more than 0.19 mg/mL, the number of living cells is not determined, which is, likely, due to high optical density of samples.

2. The HER2 AB in monoapplication (control 3) in the studied concentrations does not affect the viability or proliferation of hepatocellular carcinoma of human liver cells of the HepG2 line, as its effect does not decrease the cell viability and does not differ practically from the influence of PS (control 5).

3. The cultivation of HepG2 cells at the same time in the presence of ML and AB HER2 at concentrations less than 0.05 mg/mL and 0.013 µg/mL, respectively, did not practically affect the viability of cells of liver carcinoma. However, the complex application of ML and HER2 at concentrations of 0.1 mg/mL and 0.025 µg/mL, respectively, reduced the number of viable cells of the said line to ~ 85.9 %.

4. IC50 for GC was 0.002 mg/mL (control 2). The influence of GC in monoapplication at the concentration of 0.008 mg/mL left ~ 78 % of cells in a viable state. The use of GC in this concentration in combination with ML (0.1 mg/mL) showed a synergistic effect and increased the effectiveness of cytostatic drug up to ~ 10 % (the number of alive cells was ~ 68 %).

5. The combined application of GC and AB HER2 (control 4) at concentrations of 0.008 mg/mL and 0.025 µg/mL, respectively, also showed a synergistic effect, that resulted in a decrease of number of viable cells to ~ 65 %.

6. The use of a composite system consisting of GC and HER2 at concentrations of 0.008 mg/mL and 0.025 µg/mL, respectively, and ML (0.1 mg/mL with respect to Fe$_3$O$_4$) results in a reduction of the number of viable cells of HepG2 liver carcinoma to ~ 55 %, that indicates a significant synergistic effect of the said components.

The revealed synergistic cytotoxic/cytostatic effect can be explained by the high biological activity of the complex Fe$_3$O$_4$-GC-HER2 with the integrated ligand due to recognition of HepG2 tumor cell receptors and pharmacological correction of endogenous iron exchange, which is ensured by the use of iron-containing ML, GC, and HER2 antibodies.

Indeed, in mechanisms of implementation of the apoptosis program due to formation of the medicinal effect of NC, violations of the exchange of endogenous iron in tumor cells play an essential role [47]. The said violations cause an increased need of iron for cells, which is satisfied by the accumulation of a significant number of Fe$_3$O$_4$ nanoparticles with ML. The high level of "free iron" in the form of accumulated Fe$_3$O$_4$ and acidic medium in the cells, causes accelerated formation of iron ions and active forms of oxygen (Fenton reaction), which in its turn, leads to oxidative stress of cells and apoptosis. Thereby an increase occurs also in the effectiveness of both GC and HER2 AB. As an example we can take the increase up to ~ 10 % in the action of GC at the concentration of 0.008 mg/mL in the composition of ML and the appearance of cytotoxic action of the ML+HER2 complex at a level of ~ 10 % at the concentration of HER2 of 0.025 µg/mL, compared to the absence of activity of AB in these doses in monoapplication.

Thus, the combined effect of ML, GC, and HER2 on the HepG2 cells significantly exceeds their effects in monouse at the same concentrations, which results in synergistic effect.

So, *in vitro*, an example of the effect of a new magnetocarried colloidal system, containing magnetite, antitumor component GC and HER2 antibody, on HCC human liver cells of HepG2 line shows the possibility to achieve a cytotoxic effect at substantially lower concentrations of chemo- and immunotherapeutic medicines and to create conditions for reducing of toxico-allergic reactions of the organism as a whole. In addition, the revealed experimental data indicate that the investigated MLs can be promising for use in the method of targeted delivery and local therapy of oncological diseases.

CONCLUSION

The processes of adsorption of GC on the surface of nanosized singledomain magnetite (Fe$_3$O$_4$) have been studied. In the experiment, the value of the adsorption capacity *A* reached

~ 37.2 mg/g, the extraction extent U = 33.13 %, the separation factor E = 82.58 mL/g. The adsorption matching to the Freundlich model has been established.

The NC Fe_3O_4@GC were synthesized and their magnetic properties were investigated. The average value of the thickness of the adsorbed layer GC (2.4±0.1 nm) in the NC Fe_3O_4@GC was evaluated through the method of magnetic granulometry.

The ML were synthesized based on magnetite and PS, stabilized with Ol.Na and PEG, containing GC and HER2 AB (Fe_3O_4@GC/Ol.Na/PEG + PS). The properties of ML were studied as well as cytotoxic/cytostatic activity in relation to HCC of human liver of HepG2 line.

The obtained data can be useful for predicting of the nanoscale architecture of magnetically sensitive NC and ML in the production of medicines on their basis.

The synergistic nature of the effect of GC/Fe_3O_4/HER2 complex on HepG2 cells was revealed. It has been shown that HER2 AB alone does not affect HepG2 cell viability/ proliferation in investigated concentrations.

GC suppressed cell proliferation of liver carcinoma, the IC50 value was 0.002 mg/mL *in vitro*. The use of ML in combination with GC can increase the cytotoxic activity of the composite up to 8–10 %. The ML + GC + HER2 complexes caused a synergistic effect and increased the cytotoxic activity, compared with GC in monouse, to 18–20 %, while GC content reduced to 0.008 mg/mL.

The results of the studies indicate that the use of ML on the basis of magnetite, gemcitabine and antibody increases the effectiveness of the antitumor medicines with a significant reduction in their dose and, respectively, the toxico-allergic reactions of the body, and nanosized magnetite may be promising for the manufacture of magnetically sensitive adsorption materials for medical purposes, for example, detoxification of an organism after GC therapy.

Магніточутливі нанокомпозити та магнітні рідини на основі магнетиту, гемцитабіну і антитіла HER2

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Досліджено процеси адсорбції гемцитабіну (ГЦ) на поверхні нанорозмірного однодоменного магнетиту (Fe₃O₄). В умовах експерименту значення адсорбційної ємності А становило ~ 37.2 мг/г, ступінь вилучення U = 33.13 %, коефіцієнт розділення Е = 82.58 мл/г. Встановлено відповідність адсорбції моделі Фрейндліха. Досліджено магнітні властивості нанокомпозитів (НК) Fe₃O₄@ГЦ. Методом магнітної гранулометрії оцінено середнє значення товщини адсорбованого шару ГЦ у складі НК Fe₃O₄@ГЦ, яке становить 2.4±0.1 нм. Синтезовано магнітні рідини (МР) на основі магнетиту і фізіологічного розчину (ФР), стабілізовані олеатом натрію (Ol.Na) і поліетиленгліколем (PEG), що містять ГЦ та антитіло (АТ) HER2 (Fe₃O₄@ГЦ/Ol.Na/PEG+ФР). Досліджено властивості МР та цитотоксичну/цитостатичну активність щодо гепатоцелюлярної карциноми (ГЦК) печінки людини лінії HepG2. Встановлено параметри МР на основі магнетиту: концентрація Fe₃O₄ – 14 мг/мл, розмір частинок Fe₃O₄ – 4–22 нм, середній розмір частинок Fe₃O₄ 10.8 нм; середній розмір частинок Fe₃O₄, стабілізованих олеатом натрію – 16.8 нм; намагніченість насичення M∞ = 14.1±2.5 % Гс, гіпсометрична висота – 25±10 % см, в'язкість η = 1.14±3 % мПа·с, густина ρMP = 1.14 ±1.0 % г/см³, концентрація ГЦ становила 1.25 мг/мл, АТ HER2 – 3.75 мкг/мл. Отримані розрахункові та графічні дані щодо залежності питомої площі поверхні і питомої намагніченості

насичення НК Fe₃O₄@ГЦ/Ol.Na/PEG від товщини шару ГЦ можуть бути корисними для прогнозування наноархітектури магніточутливих НК і МР при виготовленні лікарських засобів на їхній основі. Виявлено синергічний характер ефекту впливу комплексу ГЦ/Fe₃O₄/HER2 на клітини HepG2. Встановлено, що IC50 для МР становить 0.155 мг/мл, у діапазоні концентрацій 0.025–0.1 мг/мл МР є біосумісною з клітинами HepG2. Показано, що АТ HER2 у монозастосуванні в досліджених концентраціях не впливає на життєздатність/проліферацію клітин HepG2. ГЦ пригнічує проліферацію клітин карциноми печінки, значення IC50 становило 0.002 мг/мл in vitro. Використання МР в комплексі з ГЦ дозволяє підвищити цитотоксичну активність композиту на 8–10 %. Комплекси МР+ГЦ+АТ HER2 спричиняли синергічний ефект та підвищення цитотоксичної активності, порівняно з ГЦ у монозастосуванні, до 18–20 %, при цьому вміст ГЦ зменшувався до 0.008 мг/мл.

Результати досліджень свідчать, що використання МР на основі магнетиту, гемцитабіну та антитіла підвищує ефективність дії протипухлинних препаратів при істотному зменшенні їх дози та, відповідно, токсико-алергічних реакцій організму, а нанорозмірний магнетит може бути перспективним для виготовлення магніточутливих адсорбційних матеріалів медичного призначення, наприклад, для детоксикації організму після терапії ГЦ.

Ключові слова: магнетит, магнітна рідина, гемцитабін, антитіло HER2, гепатоцелюлярна карцинома печінки людини

Магниточувствительные нанокомпозиты и магнитные жидкости на основе магнетита, гемцитабина и антитела HER2

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Исследованы процессы адсорбции гемцитабина (ГЦ) на поверхности наноразмерного однодоменного магнетита (Fe₃O₄). В условиях эксперимента значение адсорбционной емкости A составило ~ 37.2 мг/г, степень извлечения U = 33.13 %, коэффициент разделения E = 82.58 мл/г. Установлено соответствие адсорбции модели Фрейндлиха. Исследованы магнитные свойства нанокомпозитов (НК) Fe₃O₄@ГЦ. Методом магнитной гранулометрии оценено среднее значение толщины адсорбированного слоя ГЦ в составе НК Fe₃O₄ @ ГЦ, которое составляет 2.4±0.1 нм. Синтезированы магнитные жидкости (МЖ) на основе магнетита и физиологического раствора (ФР), стабилизированные олеатом натрия (Ol.Na) и полиэтиленгликолем (PEG), содержащие ГЦ и антитело (АТ) HER2 (Fe₃O₄@ГЦ/Ol.Na/PEG+ФР). Исследованы свойства МЖ и цитотоксическое/цитостатическое действие в отношении гепатоцеллюлярной карциномы (ГЦК) печени человека линии HepG2. Установлены параметры МЖ на основе магнетита: концентрация Fe₃O₄ – 14 мг/мл, размер частиц Fe₃O₄ – 4–22 нм, средний размер частиц Fe₃O₄ – 10.8 нм; средний размер частиц Fe₃O₄, стабилизированных олеатом натрия – 16.8 нм; намагниченность насыщения $M_\infty = 14.1\pm2.5$ % Гс, гипсометрическая высота – 25±10 % см, вязкость $\eta = 1.14\pm3$ % мПа·с, плотность $\rho_{МЖ} = 1.14\pm1.0$ % г/см³, концентрация ГЦ составила 1.25 мг/мл, АТ HER2 – 3.75 мкг/мл. Полученные расчетные и графические данные о зависимости удельной площади поверхности и удельной намагниченности насыщения НК Fe₃O₄@ГЦ/Ol.Na/PEG от толщины слоя ГЦ могут быть полезными для прогнозирования наноархитектуры магниточувствительных НК и МЖ при изготовлении лекарственных средств на их основе. Выявлен синергический характер эффекта воздействия комплекса ГЦ/Fe₃O₄/HER2 на клетки HepG2. Установлено, что IC50 для МЖ составляет 0.155 мг/мл, в диапазоне концентраций 0.025–0.1 мг/мл МЖ характеризуется биосовместимостью с клетками HepG2. Показано, что АТ HER2 при моноиспользовании в исследованных концентрациях не влияет на жизнеспособность / пролиферацию клеток HepG2. ГЦ подавляет пролиферацию клеток карциномы печени, значение IC50

составляло 0.002 мг/мл in vitro. Использование МЖ в комплексе с ГЦ позволяет повысить цитотоксическую активность композита на 8–10 %. Комплексы МЖ + ГЦ + АТ HER2 вызывали синергический эффект и повышение цитотоксической активности, по сравнению с ГЦ в моноприменении, до 18–20 %, при этом содержание ГЦ уменьшалось до 0.008 мг/мл.

Результаты исследований свидетельствуют, что использование МЖ на основе магнетита, гемцитабина и антитела повышает эффективность действия противоопухолевых препаратов при существенном уменьшении их дозы и, соответственно, токсико-аллергических реакций организма, а наноразмерный магнетит может быть перспективным для изготовления магниточувствительных адсорбционных материалов медицинского назначения, например, для детоксикации организма после терапии ГЦ.

Ключевые слова*: магнетит, магнитная жидкость, гемцитабин, антитело HER2, гепатоцеллюлярная карцинома печени человека*

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