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Magnetic Nanotherapy by Magnetosensitive Nanocomplexes with Different Magnetic Properties of the Walker 256 Carcinosarcoma

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The magnetic nanocomplex (MNC) comprised of Fe_3O_4 nanoparticles (NP) and doxorubicin (DOXO) with a saturation magnetic moment $m_s = 10.5$ emu/g during magnetic nanotherapy initiated greater antitumor effect than MNC with $m_s = 8.55$ emu/g. After treatment of Walker 256 tumor the concentration of iron ions in the blood serum of animals with tumor increased and did not depend on the magnetic properties of MNC.

Keywords: Nanoparticles, Carcinosarcoma Walker 256, Iron Ions, Blood Serum, Magnetic Nanotherapy.

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1. INTRODUCTION

Magnetic nanoparticles (NP) hyperthermia appears well-suited as an effective tumor therapy: (1) the concentration of NP in the tumor is both sufficiently high and significantly higher than in surrounding, normal tissue, (2) the use of NP, which can combine both therapeutic and diagnostic capabilities in one dose, has the potential to lead toward personalized oncology and better outcomes for patients, (3) the particles possess a high enough specific absorption rate. However, there are a number of limitations of magnetic NP hyperthermia such as: (1) therapy by NP in the temperature range 43–70 °C can be accompanied by the formation of drug resistance due to the induction of heat shock proteins, (2) the temperature above 45 °C may shut down tumor tissue perfusion, and (3) targeted therapy with magnetic NP is often not suitable for disseminated and abdominal tumors [1].

To overcome the above problems, we have developed a new technology of magnetic nanotherapeutics based on multiple modes of actions, such as mild hyperthermia less 40 °C [2].

In this paper we studied an influence of magnetosensitive nanocomplexes (MNC) with different magnetic properties on the iron distribution in serum of animals with Walker 256 carcinosarcoma under magnetic nanotherapy.

2. MATERIAL AND METHODS

As a parts or drug components the NP of iron oxide Fe_3O_4 in diameter < 50 nm (Sigma-Aldrich) and doxorubicin (DOXO) (Pfizer, Italy) have been used. There were two types of synthesized samples MNC with different magnetic properties when exposed to 5 mT constant magnetic field (CMF) (MNC1) and 8 mT CMF (MNC2). Also, the effects of 5 mT and 8 mT CMF on the samples of iron oxide (labeled accordingly Fe_3O_4-1, Fe_3O_4-2) and DOXO (labeled accordingly DOXO1 and DOXO2) were examined during magneto-mechano-chemical activation.

The study of antitumor activity of DOXO, MNC, CMF and electromagnetic irradiation (EI) was conducted on 60 rats females weighing (175 ± 14) g from vivarium National Cancer Institute. Transplantation of tumor cells Walker 256 carcinosarcoma was performed by administration to the rat right thigh 20% cell suspension in a volume of 0.4 ml in the medium 199. DOXO were injected into animals at a dose of 1.5 mg/kg, MNC: DOXO at a dose of 1.5 mg/kg, Fe_3O_4 at a dose of 3 mg/kg. Drug administration was carried out in the tail vein of animals in a volume of 0.3 ml 0.9% NaCl solution. Treatment started 3 days after tumor transplantation and was performed five times every other day. Local EI of tumor animals was performed by experimental prototype device "Magnetotherm" (Radmir, Ukraine) with magnetic-dipole applicator that included needle localizer and neodymium permanent magnet with a maximum CMF magnetic induction of 0.4 T at a distance of 8 mm from the end of the dipoles and EMF frequency of 40 MHz with output power of 75 W. Temperature control was carried by fiber-optic thermometer TM-4 (Radmir, Ukraine). Intratumoral temperature did not exceed 38 °C.

The magnetic properties were studied by magnetometery using a "Vibrating Magnetometer 7404 VSM" (Lake Shore Cryotronics, Inc., USA) with magnetic fields up to 13 kOe. The magnetometer's sensitivity is 10^{-7} emu, and that allowed measurements of magnetic moment of samples weighing milligrams to be performed. The mass was determined by an electronic microbalance AB135-S/FACT with auto-identification (Mettler Toledo, Switzerland) which has a sensitivity of 10^{-5} g.

Colorimetric analysis with the Ferrozine (Ortho-Clinical Diagnostics, UK) has been used for the measurement of serum iron concentration by Vitros 250 (USA) chemistry analyzer.

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3. RESULTS AND DISCUSSION

The effect of MNC with different magnetic properties on the nonlinear kinetics of Walker 256 carcinosarcoma growth from the 3rd to the 15th day after tumor transplantation is shown at Table 1. According to the obtained data, a statistically significant difference was observed for anticancer effects of MNC1 as compared to MNC2 both without the influence CMF and EMF, and after combined action of CMF and EMF. The MNC antitumor effect was evaluated on the base of the inhibition coefficient of tumor growth. The MNC2 antitumor effect was 16.5 and 19% higher in the first and second case, respectively, as compared to MNC1.

Table 1 – An influence of MNC with different magnetic properties on the growth kinetics of Walker 256 carcinosarcoma after irradiation by CMF and EMF

N	Animal Group	Parameter	
		Growth Factor φ, day^{-1}	**Braking Ratio κ, Relative Units**
1	Control (without treatment)	0.43±0.01	1.00
2	Conventional DOXO	0.32±0.01*	1.35
3	MNC 1	0.40±0.01$^{*\#}$	1.09
4	MNC 2	0.33±0.02$^{*\circ}$	1.31
5	MNC 1 + CMF + EI	0.34±0.01$^{*\circ}$	1.27
6	MNC 2 + CMF + EI	0.28±0.02$^{*\circ\S}$	1.56

*, $^{\#}$, $^{\circ}$, § Statistically significant difference from 1, 2, 3, and 5 animal group, respectively, $p < 0.05$

When compared antitumor effect of MNC with conventional DOXO it should be noted that only in the 6th group of animals after joint action of MNC2 with CMF and EMF the braking ratio of tumor growth was increased by 15.5%. In further observation of changing in tumor size till 22 days after transplantation the trend for the breaking ratio remaining unchanged in spite of the fact that some animals with large tumor size in 1–4 groups were died.

An analysis of hysteresis loops (Fig. 1, Table 2) indicates that the samples of MNC, Fe_3O_4, DOXO1 and DOXO2 were soft ferromagnets. Conventional DOXO has diamagnetic properties. Greater saturation magnetic moment and the magnetic hysteresis loop area had MNC and DOXO samples synthesised or activated in nanoreactor with CMF induction of 8 mT. The coercive force in this case decreased. For Fe_3O_4-2 unlike MNC2 an increase in coercivity and reduction of magnetic hysteresis loop were reported as compared with Fe_3O_4-1.

Iron ions are acceptor of electromagnetic field. Magnetic nanotherapy can influence the reaction yields of iron ions between the tumor and the blood. To understand the distribution of the iron in the tumor and blood serum after magnetic nanotherapy we have analyzed the concentration of iron and iron-containing proteins in the serum blood of animals. In Table 3 an influence of MNC with different magnetic properties on iron concentration in blood serum of animals with Walker 256 carcinosarcoma after irradiation by CMF and EMF on the 15th day after tumor transplantation is shown. The content of iron ions in the blood serum increased in 2.2 times on the average in all studied groups, regardless of received treatment, as compared to the control group and animals without tumors.

A comparison obtained experimental results and published in the paper [3] confirms the previously known tendency to increase of the iron content in blood serum under the influence of chemotherapy of malignant tumors. According to the above cited authors this is due to the effect of hemolysis, assuming that 60% of iron in the body is found in hemoglobin.

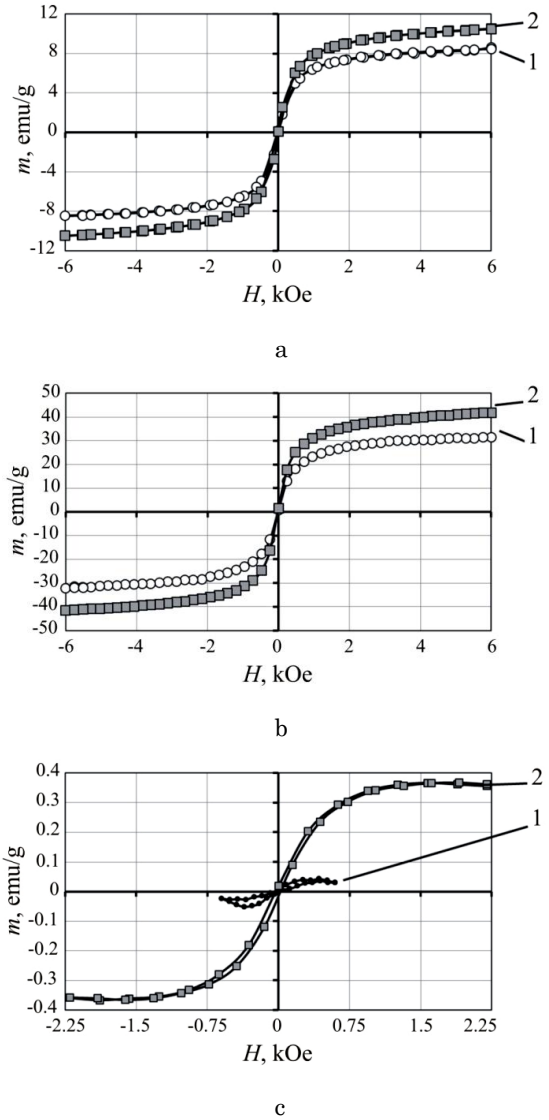

Fig. 1 – Hysteresis loops. MNC (a), Fe_3O_4 (b) and DOXO (c) at 38 °C. H – magnetic field; m – magnetic moment. B – CMF induction during magneto-mechano-chemical synthesis in nanoreactor: 1 – MNC1 (B = 5 mT), 2 – MNC2 (B = 8 mT)

Table 2 – Magnetic properties of the samples at 38 °C

Examined object		**MNC1**	**MNC2**	**Fe_3O_4-1**	**Fe_3O_4-2**	**DOXO1***	**DOXO2**
CMF induction during magneto-mechano-chemical activation or synthesis in nanoreactor, mT		5	8	5	8	5	8
Magnetic hysteresis loops	Saturation magnetic moment m_s, emu/g	8.55	10.5	31.8	41.6	0.05	0.37
	Coercive field H_c, Oe	13.1	4.4	15.2	18.62	30.1	24.8
	Area of hysteresis loop, erg/g	136.3	1135.4	5613.3	1657	17	36.7

*Conventional DOXO is diamagnetic, magnetic moment $m = -1.18$ emu/g at 3000 Oe

Table 3 – An influence of MNC with different magnetic properties on the concentration of iron in blood serum of animals with Walker 256 carcinosarcoma after irradiation by CMF and EMF

N	**Animal group**	**Iron, µmol/l**
1	Control (without treatment)	15.4±1.2
2	Conventional DOXO	38.4±2.6*
3	MNC 1	31.0±3.9*
4	MNC 2	38.2±3.4*
5	MNC 1 + CMF + EI	34.1±3.1*
6	MNC 2 + CMF + EI	35.3±4.6*
7	Animals without tumor	16.7±0.77#o+§£

*, #, o, +, §, £ Statistically significant difference from 1, 2, 3, 4, 5, and 6 animal group, respectively, $p < 0.05$

Thus, it is possible to state that the use of magnetic nanotherapy technology opens up the opportunities for further implementation in clinical practice the effect of the controlled local toxicity based on the selection of optimal magnetic properties of MNC.

4. CONCLUSION

Magnetic nanotherapy by the MNC comprised of Fe_3O_4 NP and DOXO with saturation magnetic moment m_s=10.5 emu/g and hysteresis loop area of 1135.4 erg/g has initiated greater growth inhibition factor of Walker-256 carcinosarcoma on 23% as compared to the MNC with lesser saturation magnetic moment m_s=8.55 emu/g and hysteresis loop area of 136.3 erg/g. The content of free iron complexes was minimal in Walker-256 carcinosarcoma after magnetic nanotherapy by MNC with a large hysteresis loop area. The concentration of iron ions in the blood serum increased in 2.2 times on the average in all studied groups, regardless of received treatment, as compared to the control group and animals without tumors. This opens up the opportunities for further development of the technology magnetic nanotherapy for oncology clinics.

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