The Effect of Anticancer Nanotherapy on Lewis Lung Carcinoma

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ABSTRACT

The paper examines the effects of anticancer treatment by nanocomplex (NC) of iron oxide and doxorubicin (DOXO) nanoparticles on nonlinear dynamics of the growth of Lewis lung carcinoma. Mechanochemical synthesis (MS) and mehanomagnetochemical synthesis (MMS) of DOXO with ferric oxide nanoparticles in NC was processed in mechanomagnetoreactor. It showed that magnetic descriptions testifies in MS and MMS NC increased saturation magnetic moment with the typical curve of hysteresis for soft ferromagnetic. The effect of MS and MMS on DOXO was increased concentration of paramagnetic centers in NC. Animals with Lewis lung carcinoma were housed in 4 groups: group 1 – control (no treatment); 2 – MS NC-administration; 3 – MMS NC-administration; 4 – DOXO–administration. In the research of animals with Lewis lung carcinoma was shown, that MMS NC on the basis of nanoparticles from iron (II, III) oxide and DOXO had a greater antitumor effect than conventional DOXO.

***Keywords*:** nonlinear dynamics, nanocomplex, Lewis lung carcinoma, mehanomagnetochemical synthesis

1 INTRODUCTION

Cancer is often characterized as a chaotic, poorly regulated growth. Cancer can be viewed as a complex adaptive system. Complex adaptive systems can be described mathematically by nonlinear (dynamic chaos) theory including asymmetry, fractal structure and autocorrelation factor. Atypical shape of tumor cells and chaotic structures of blood flow is one from characteristic of cancer process. Atypical change of cell shape in conglomerates of tumor cells and structure of blood flow is accompanied by increase of dynamic chaos. Complex natural phenomena such as cancer are dynamical systems whose state changes by perturbation. The concept of dynamic chaos is hierarchical for host in contemporary ideas about role of chaos for potential application in oncology [1].

Transplanted animal tumors which can only be experimentally induced by transplanting living tumor cells significant influence on complex adaptive systems include developing of tumor formation for experimental animals. Anticancer nanotherapy offer some attractive possibilities in oncology [2].

The technology and mechanomagnetic reactor for mechanomagnetochemical dry synthesis of nanocomplex (NC) from nanoparticles of ferric oxide and antitumor anthracycline antibiotic doxorubicin (DOXO) has been developed recently. That is the effect of technology of mehanomagnetochemical synthesis (MMS), that drug can enhance the magnetic susceptibility and increase free radicals in NC. In future, clinical use of such changes in physical and chemical parameters will lead to better target drug delivery to the tumor and increase antitumor activity of drugs at a lower temperature gradient [3].

DOXO is antibiotic that has been shown to have a wide spectrum of clinical activity against a variety of solid tumors. The mechanisms of DOXO-induced cytotoxicity have been extensively studied and have been shown to include free radical formation and absorption of DOXO into the double helix of DNA resulting in topoisomerase II-mediated DNA damage. DOXO also causes depolarization of the membrane lipid bilayer in different cancer cell lines. Current forms of DOXO are highly toxic to the patient and can cause systematic complications, most notably cardiotoxicity. Systemic toxicity can seriously decrease the effectiveness of the drug since a lower dose must be administrated to avoid toxicity. Drug resistance is the single most important cause of cancer treatment failure and carries a massive burden to patients, healthcare providers, drug developers and society. It is estimated that multi drug resistance plays a major role in up to 50% of cancer cases [4]. Thus, problems related to the development multidrug resistance have led researchers to investigate alternative forms of nanocomplex of DOXO nanoparticles for cancer treatment.

This paper examines the effects of anticancer treatment by nanocomplex of nanoparticles of iron oxide and antitumor anthracycline antibiotic doxorubicin on nonlinear dynamics of the growth of Lewis lung carcinoma.

2 MATERIALS AND METHODS

2.1 Mechanomagnetochemical Effects

Mechanochemical synthesis (MS) and MMS of DOXO (Pfizer, Italy) with ferric oxide nanoparticles in NC were processed in original high-precision mechanomagnetoreactor (NCI, Ukraine) [3].

2.2 Energy-Dispersive Spectroscopy

The elemental composition in NC was examined by scanning electron microscope JEOL JSM-6490LV (Jeol, Japan) with Oxford 250 energy dispersive spectrometer system.

2.3 Magnetic Studies

Magnetic descriptions were studied by the method of magnetometery on the vibration magnetometer "Vibrating Magnetometer 7404 VSM" ("Lake Shore Cryotronics", Inc., USA) in the magnetic fields with intensity up to 13 κOe. A sensitivity of magnetometer was 10^{-7} emu, that allows to perform measurements of magnetic moment on samples weighing units milligrams. Mass of the probed standards was determined by the electronic microbalance AB135-S/FACT with autoindemnification (firm "Mettler Toledo", Switzerland). The sensitivity of microbalance was 10^{-5} g.

2.4 Electron Spin Resonance (ESR) Spectroscopy

Electron paramagnetic resonance spectrums were registered on the modernized spectrometer RE1307 for the temperatures of liquid nitrogen (77 K) in a cylinder resonator with the mode H_{011}, on frequency 9.15 GHz. To measure the concentration of paramagnetic center in the sample and their identification by determining *g*-factors the studies were carried out on rebuilt EPR spectrometer RE-1307. Power microwave radiation was 40 mW. Magnetic field modulation frequency 100 kHz was carried out. Investigated samples placed in a quartz Dewar with an inner diameter of 4.5 mm.

2.5 Experimental Animals

In the study 40 C57BL/6 male mice weighing 19 ± 1 g bred in the vivarium of National Cancer Institute (Kyiv, Ukraine) were used.

2.6 Tumor Transplantation

The transplantation Lewis lung carcinoma were performed according to the established procedure [5]. Animals were housed in four groups: group 1 – control (no treatment); 2 – MS NC-administration; 3 – MMS NC – administration; 4 – DOXO–administration. Each group contained ten animals. Experimental animals were treated by conventional DOXO (Pharmacia & Upjohn) and NC in the dose 2.5 mg/kg. The treatment was performed four times by DOXO from 4 to 10 days after tumor transplantation every other two days. All animal procedures were carried out according to the rules of the regional ethic committee.

2.7 The Analysis of Nonlinear Kinetics of Tumor Volume and Statistics

Nonlinear kinetics of tumor volume was evaluated by growth factor φ according to autocatalytic equation

$$\frac{dx}{dt} = \varphi(x + x_0)(1 - x)\,, \tag{1}$$

where $x = \frac{\Phi - \Phi_0}{\Phi_\infty - \Phi_0}$ is relative tumor growth by time *t*; $x_0 = \frac{\Phi_0}{\Phi_\infty - \Phi_0}$ is relative tumor volume at the moment of time $t = 0$; Φ_0 and Φ_∞ is initial and limiting tumor volume accordingly; Φ is tumor volume at the moment of time *t* [6].

The solution of equation (1) is

$$\Phi = \Phi_0 + \Phi_0 \cdot \frac{e^{\varphi \frac{\Phi_\infty}{\Phi_\infty - \Phi_0} t} - 1}{1 + \frac{\Phi_0}{\Phi_\infty - \Phi_0} \cdot e^{\varphi \frac{\Phi_\infty}{\Phi_\infty - \Phi_0} t}}. \tag{2}$$

The effect of anticancer therapy on nonlinear dynamics of the growth of animal tumors was evaluated with the braking ratio:

$$\kappa = \frac{\varphi_c}{\varphi_{EI}}\,, \tag{3}$$

where φ_c is growth factor for control group of animals, φ_{EI} is growth factor for group after EI.

The volume of metastases in animals' lung was examined according to [7].

Statistical processing of numerical results was carried out using Statistica 6.0 (© StatSoft, Inc. 1984–2001) computer program with parametric Student's *t*-test.

3 RESULTS AND DISCUSSION

3.1 Energy-Dispersive Spectroscopy

The weight composition of NC elements in examined samples is shown in Table 1.

Examined object	Elements			
	Fe	C	O	Cl
Conventional DOXO	–	65.55	33.63	0.83
MS NC	1.08	72.08	25.26	1.20
MMS NC	2.16	72.08	23.22	2.56

Table 1: Weight composition (%) Fe, C, O and Cl in NC.

Magnetosensitive element iron in NC was on 50% more after MS, than after MMS. Similar effects have been observed with chlorine.

3.2 Magnetic Studies

Table 2 and Figure 1 shows that magnetic descriptions testifies in MS and MMS NC increased saturation magnetic moment m_s with the typical curve of hysteresis for soft ferromagnetic.

It should be noted the influence of mechanochemical activation and electromagnetic irradiation on saturation magnetic moment of examined samples. Conventional DOXO without influence was the diamagnetic.

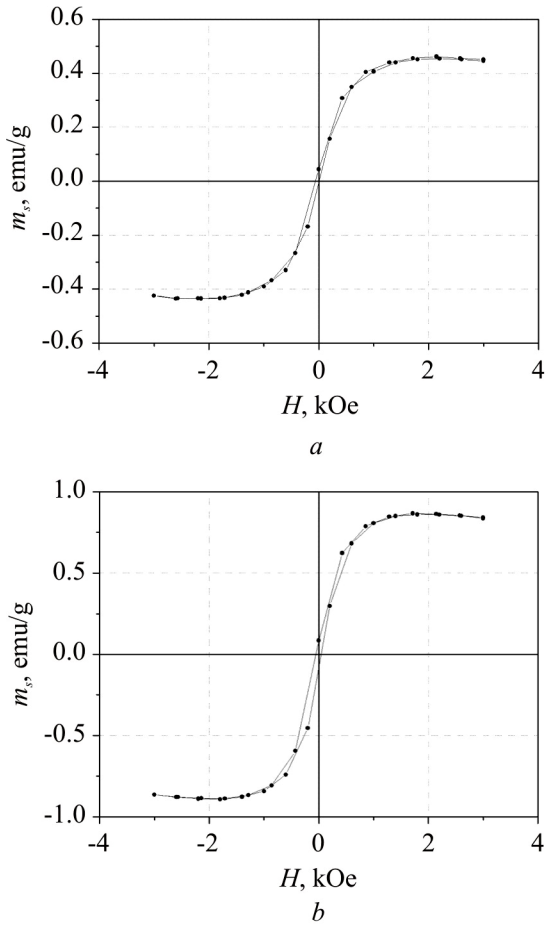

Figure 1: Hysteresis loops at 300 K: H – magnetic field, a – MS NC, b – MMS NC.

№	Examined object	Saturation magnetic moment m_s, emu/g	Saturation magnetic field H_{ms} (H for m_s), Oe	Coercive force H_c, Oe
1	Conventional DR	Diamagnetic $m_s = -0.200$ emu/g	–	–
2	MS NC	0.44890	2142.9	34.126
3	MMS NC	0.88039	1714.3	47.737

Table 2: Magnetic properties of the samples.

3.3 Electron Spin Resonance Spectroscopy

EPR spectrums of examined nanocomplexes are shown on Figure 2.

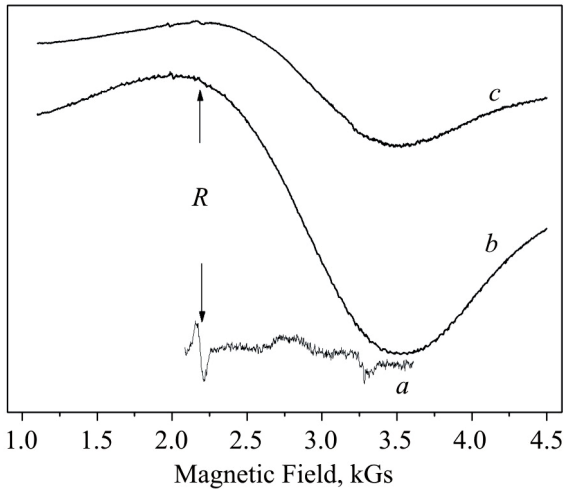

Figure 2: EPR spectra: a – conventional DOXO, b – MS NC, c – MMS NC.

The factor of spectroscopic splitting g and paramagnetic centers concentration is shown in Table 3.

Examined object	g-factor	C, mg^{-1}
Conventional DOXO	1.97	$5 \cdot 10^{17}$
	2.003	$8 \cdot 10^{17}$
	2.005	$4 \cdot 10^{17}$
MS NC	2.3	$4 \cdot 10^{19}$
MMS NC	2.25	$4 \cdot 10^{19}$

Table 3: The changes in paramagnetic centers concentration (C, mg-1).

The effect of MS and MMS on DOXO was increased concentration of paramagnetic centers in NC. After MS and MMS g-factor of spectroscopic splitting increases up to 2.3 and 2.25 for NC.

During work of mechanomagnetoreactor as a result of mechanochemical effect and electromagnetic irradiation in the chamber with grinding pellets from metal initiated

mechanical tension into dried DOXO, that disintegrated drug and produced semiquinone anion free radicals and ions Fe^{2+} and Fe^{3+} effectively. It was suggested, therefore, that free radicals and ions formed in the above natural products are metal complexes of the corresponding radicals and ions induced by solid state electron and ions transfer mechanism from the active metal surface, part of which is further immobilized in biopolymeric fibers [8]. Free radicals and ions refer to particles with have very small size, from ~ 1 nm. Free radicals and ions produced self-assembling nanoparticles in solid states of drug and disintegrated in nanoparticles after solution.

3.4 The Growth Kinetics and Metastasis of Lewis Lung Carcinoma

Our study shows that the growth kinetics of Lewis lung carcinoma and dissemination of tumor are dynamically similar chaotic (nonlinear) processes (table 4). Groups: group 1 – control (no treatment); 2 – MS NC; 3 – MMS NC; 4 – Conventional DOXO.

Group	Parameters				
	Growth factor $\varphi_{12\text{-}17}$, day^{-1}	Braking ratio $\kappa_{12\text{-}17}$	Growth factor $\varphi_{12\text{-}21}$, day^{-1}	Braking ratio $\kappa_{12\text{-}21}$	Mean metastasis volume
1	0.317 ± 0.002	1	0.332 ± 0.008	1	4.7 ± 3.48
2	$0.276 \pm 0.004^{*}$	1.15	$0.284 \pm 0.003^{*}$	1.17	10.0 ± 5.9
3	$0.221 \pm 0.003^{*+}$	1.43	$0.242 \pm 0.005^{*+}$	1.37	0.23 ± 0.09
4	$0.253 \pm 0.004^{*+\bullet}$	1.25	$0.250 \pm 0.001^{*+}$	1.33	0.44 ± 0.15

* Statistically significant difference from control group, $p < 0.05$;
$^{+}$ Statistically significant difference from MS NC, $p < 0.05$;
$^{\bullet}$ Statistically significant difference from MMS NC, $p < 0.05$.

Table 4: The growth kinetics and dissemination of Lewis lung carcinoma ($M \pm m$).

Growth factor for tumor volumes on 12 and 17th day after tumor transplantation was maximal and braking ratio was minimal after treatment by MMS NC (3 groups animals). Since 17th day after transplantation tumor volumes for animals from 3 groups this tendency was similar. The most significant antimetastatic tendency has been registered after treatment by MS and MMS NC.

What is the interrelation between the physicochemical and antitumor molecular effects? We suppose that during MMCS iron atoms become by ions Fe^{2+}. Under the attraction of dipoles water molecules, thus formed in DOXO of NC structure of the hydroxyl free electrons $OH^{\bullet}$.

$$2Fe \rightarrow 2Fe^{2+} + 4e;\ Fe^{2+} \rightarrow Fe^{3+} + e;$$
$$2H_2O + O_2 + 4e \rightarrow 4OH^{\bullet} \quad (5)$$

MMS NC stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication in tumor cells. Schematically this reaction can be written down as:

$$NC + [TOP{+}DNA] \rightarrow DNA^{*}, \quad (6)$$

where $[TOP{+}DNA]$ is topoisomerase II complex, DNA^{*} is damaged DNA .

CONCLUSION

In the research of animals with Lewis lung carcinoma was shown, that mechano- magnetochemically synthesized nanocomplex on the basis of nanoparticles from iron (II, III) oxide and doxorubicin had a greater antitumor effect than conventional doxorubicin.

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