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SHORT COMMUNICATION

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BIOMAGNETISM OF DRUG-SENSITIVE AND DRUG-RESISTANT MALIGNANT TUMORS AFTER INJECTION OF FERROMAGNETIC NANOCOMPOSITE

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Magnetic signals emitted by living organisms, regardless of a biological species, are important biophysical indicators. The study of these indicators is very relevant and promising for the visualization of the tumor process and the development of technologies using artificial intelligence when it comes to malignant neoplasms, particularly resistant to chemotherapy. ***Aim:*** **To measure magnetic signals from transplantable rat tumors and their counterparts resistant to cytostatics for evaluating the features of the accumulation of iron-containing nanocomposite Ferroplat.** ***Materials and Methods:*** **Doxorubicin (Dox)-sensitive and Dox-resistant Walker-256 carcinosarcoma and cisplatin-sensitive and cisplatin-resistant Guerin's carcinoma transplanted in female Wistar rats were studied. The magnetism of tumors, liver and heart was determined using Superconductive Quantum Interference Device (SQUID) — magnetometry in a non-contact (13 mm over the tumor) way using specially designed computer programs. In a group of the experimental animals, a ferromagnetic nanocomposite (Ferroplat) was administered as a single intravenous injection and biomagnetism was assessed in 1 h.** ***Results:*** **The magnetic signals coming from Dox-resistant Walker-256 carcinosarcoma in the exponential growth phase were significantly higher in comparison with sensitive tumor. Intravenous administration of Ferroplat increased biomagnetism by at least an order of magnitude, especially in resistant tumors. At the same time, the magnetic signals of the liver and heart were within the magnetic noise.** ***Conclusion:*** **The use of SQUID-magnetometry with ferromagnetic nanoparticles as a contrast agent is a promising approach for visualization of malignant neoplasms with varying sensitivity to chemotherapy.**

Key Words: **tumor, magnetism, SQUID-magnetometry, drug resistance, ferromagnetic nanoparticles.**

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Magnetic signals coming from any part of a living organism, regardless of a biological species, are important biophysical indicators. The magnetism of the biological objects (organism, organ, cell) characterizes their biochemical and physiological state in normal and pathological conditions. The assessment of biomagnetism could be relevant in oncology both for diagnostic purposes and correct targeted hyperthermia of malignant neoplasms [1–7]. The combination of biomagnetism measurements and nanotechnologies (nanoparticles) with the use of Superconductive Quantum Interference Device (SQUID-systems) could be advantageous for improving the efficiency of diagnosis and therapy.

The targeted therapy with the use of ferromagnetic could be promising since these nanoparticles are able to facilitate intercellular and intracellular transport of antitumor drugs, the controlled release of the substances from the dosage form, and the delivery of the drugs to their targets [8–10]. In addition, there are all prerequisites for the use of nanoparticles in cancer immunotherapy [11–16]. Moreover, nanoparticle-based drug delivery may be beneficial for overcoming drug resistance in cancer therapy [17, 18]. In our previous studies [19], we demonstrated that the magnetic signals from the transplantable tumor are significantly higher compared to control animals. In addition, intravenous administration of the ferromagnetic nanocomposite resulted in a significant increase in the magnetic signal in tumors. At the same time, we have shown that ferromagnetic nanocomposite inhibited the growth of cisplatin-resistant (Cpt-R) Guerin's carcinoma by 40% as compared to 2% inhibition by the officinal form of cytostatic. The findings of our pharmacokinetic studies proved that ferromagnetic nanocomposite accumulates more intensively in a resistant tumor and causes the most significant manifestations of therapeutic pathomorphosis compared to a sensitive tumor [20].

In this study, we aimed to evaluate the features of the accumulation of iron-containing nanocomposite (Ferroplat) by tumor cells of Walker-256 carcinosarcoma and Guerin's carcinoma as well as their substrains resistant to cytostatics. With this aim in view, measuring equipment based on SQUID-magnetometry and special computer programs developed at the V.M. Glushkov Institute of Cybernetics, the NAS of Ukraine were used.

MATERIALS AND METHODS

The studies were carried out on 56 female Wistar rats weighing 120–150 g bred in the vivarium of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine. The experimental procedures were carried out in accordance with the "Regulations on the Use of Animals in Biomedical Experiments" and the provisions of Directive 2010/63/EU of the European Parliament and the Council of the European Union "On the Protection of Animals Used for Scientific Purposes" of September 22, 2010. The study was approved by the institutional bioethics committee.

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Abbreviation used: Cpt – cisplatin; Cpt-R – cisplatin-resistant; Cpt-S – cisplatin-sensitive; Dox – doxorubicin; Dox-R – doxorubicin-resistant; Dox-S – Dox-sensitive; FP – Ferroplat; SQUID – superconductive quantum interference device.

The tumor strains of Walker-256 carcinosarcoma and Guerin's carcinoma were obtained from the National Bank of cell lines from human and animal tissue (R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine). The doxorubicin-resistant (Dox-R) substrain of Walker-256 carcinosarcoma and Cpt-R substrain of Guerin's carcinoma were developed in our previous study [21]. Doxorubicin (Dox) (EBEWE, Austria) inhibited growth of Dox-R Walker-256 carcinosarcoma by 7–10% (5 i.p. injections daily at a dose of 1.5 mg/kg, starting from the 4th day after tumor transplantation) as compared to 72–75% inhibition of the initial Dox-sensitive (Dox-S) strain. Cisplatin (Cpt) (EBEWE, Austria) inhibited growth of Cpt-R Guerin's carcinoma by 0–3% (5 i.p. injections once every two days at a dose of 1.2 mg/kg, starting from the 8th day after tumor transplantation) as compared to 93–97% inhibition of the initial Cpt-sensitive (Cpt-S) strain.

The rats were transplanted with Dox-S and Dox-R Walker-256 carcinosarcoma or Cpt-S and Cpt-R Guerin's carcinoma subcutaneously ($2.5 \cdot 10^6$ cells per animal).

The ferromagnetic nanocomposite Ferroplat (Fe_3O_4+cisplatin) (FP) developed in O.O. Chuiko Institute of Surface Chemistry (National Academy of Sciences of Ukraine) by the team of Prof. P.P. Gorbik was used in the study. FP was injected once into the tail vein at a dose of 2 mg of Cpt/kg of body weight.

In the exponential phase of tumor growth (for Walker-256 carcinosarcoma — 5 days, for Guerin's carcinoma — 10 days after tumor transplantation), non-contact SQUID-magnetometry was performed over the tumor (13 mm). In a FP group, biomagnetism was measured in 1 h following single intravenous FP injection in the exponential phase of tumor growth.

The properties of FP and the method of non-contact SQUID-magnetometry were described in detail in our previous article [19].

Statistical processing of data was performed using the software Statistica (v. 7.0) and Student's *t*-test when the data complied with the conditions of normality and equal variance. The difference was considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In the exponential growth phase, in Dox-R Walker-256 carcinosarcoma the maximum value of $\mathbf{E}_m$ (energy characteristic; $\mathbf{E}_m = \Sigma \mathbf{ß}_z^2$; value, proportional to magnetic field energy in limits of measurement area) was significantly higher (by 32%) than in Dox-S Walker-256 carcinosarcoma (Table 1). Upon FP injection, the maximum value of $\mathbf{E}_m$ in both Dox-S and Dox-R Walker-256 tumors increased by approximately 12-fold. However, $\mathbf{E}_m$ in the Dox-R tumor was 31% higher than in the Dox-S. Similar results were obtained when comparing the integral values of $\mathbf{E}_m$ that were by 25% higher in Dox-R Walker-256 tumors (Table 2). After the FP injection, the integral values of $\mathbf{E}_m$ in the Dox-S tumor increased by 13 times, and in the Dox-R by 14 times (Table 2).

After intravenous FP administration to animals with transplanted Guerin's carcinoma, the maximum values of $\mathbf{E}_m$ increased by 17 times in Cpt-S tumor and by 18 times in Cpt-R tumor ($p < 0.05$) (Table 3). The same result was obtained when analyzing the integral values of $\mathbf{E}_m$ (Table 4). FP injection led to an increase of this index in the Cpt-S Guerin's carcinoma by 20.4 times, and in the Cpt-R by 25.7 times.

We also measured the biomagnetism of the liver and heart of experimental animals. It turned out that the magnetic signals coming from these organs practically corresponded to the magnetic noise.

The results obtained evidence that the increase of magnetic signals in tumors could be related with the degree of neoplasms sensitivity to cytostatics with different mechanisms of action.

Due to their affinity for iron ions, resistant tumors accumulate a larger number of ferromagnetic

Table 1. Maximum values of energy characteristic $\mathbf{E}_m$. Values obtained in the exponential growth phase of Walker-256 carcinosarcoma (5 days after tumor transplantation)

Group of animals	Maximum values of $\mathbf{E}_m$, a.u.	
Magnetic noise (before and after measurements)	0.40 ± 0.13; n = 4	
Rats with Dox-S		**(FP)**
Walker-256 carcinosarcoma	1.19 ± 0.08; n = 7	14.06 ± 1.81**; n = 7
Rats with Dox-R		**(FP)**
Walker-256 carcinosarcoma	1.57 ± 0.09*; n = 7	18.42 ± 1.99**; n = 7

Notes: The mean ± standard deviation is indicated. *$p < 0.05$ compared with Dox-S Walker-256 carcinosarcoma; **$p < 0.05$ compared with animals that did not receive FP.

Table 2. Values of the integral of the energy characteristic $\mathbf{E}_m$. Values obtained in the exponential growth phase of Walker-256 carcinosarcoma (5 days after tumor transplantation)

Group of animals	The values of integral of the energy characteristic $\mathbf{E}_m$, a.u.	
Magnetic noise (before and after measurements)	5.99 ± 0.60; n = 4	
Rats with Dox-S		**(FP)**
Walker-256 carcinosarcoma	16.28 ± 1.47; n = 7	215.37 ± 21.15**; n = 7
Rats with Dox-R		**(FP)**
Walker-256 carcinosarcoma	20.30 ± 1.44*; n = 7	293.01 ± 22.17**; n = 7

Notes: The mean ± standard deviation is indicated. *$p < 0.05$ compared with Dox-S Walker-256 carcinosarcoma; **$p < 0.05$ compared with animals that did not receive FP.

Table 3. Maximum values of energy characteristic $\mathbf{E}_m$. Values obtained in the exponential growth phase of Guerin's carcinoma (10 days after tumor transplantation)

Group of animals	Maximum values of $\mathbf{E}_m$, a.u.	
Magnetic noise (before and after measurements)	0.41 ± 0.10; n = 4	
Rats with Cpt-S Guerin's carcinoma		**(FP)**
	1.60 ± 0.19; n = 7	27.09 ± 2.35*; n = 7
Rats with Cpt-R Guerin's carcinoma		**(FP)**
	1.95 ± 0.40; n = 7	35.06 ± 3.86*; n = 7

Notes: The mean ± standard deviation is indicated. *$p < 0.05$ compared with animals that did not receive FP.

Table 4. Values of the integral of the energy characteristic $\mathbf{E}_m$. Values obtained in the exponential growth phase of Guerin's carcinoma (10 days after tumor transplantation)

Group of animals	The values of integral of the energy characteristic $\mathbf{E}_m$, a.u.	
Magnetic noise (before and after measurements)	6.11 ± 2.60; n = 4	
Rats with Cpt-S Guerin's carcinoma		**(FP)**
	20.79 ± 5.04; n = 7	423.56 ± 36.58*; n = 7
Rats with Cpt-R Guerin's carcinoma		**(FP)**
	18.95 ± 5.54; n = 7	487.70 ± 57.15*; n = 7

Notes: The mean ± standard deviation is indicated. *$p < 0.05$ compared with animals that did not receive FP.

nanoparticles compared to sensitive tumors, which is due to the impaired endogenous iron metabolism and the antioxidant defense resulting in increased iron consumption to meet metabolic and proliferative needs of tumor cells [21]. Moreover, the oxidative damage imposed by nanocomposite in resistant cells also leads to an increase of lipid peroxidation, which causes structural and functional rearrangements of cells membranes and contributes to the reduction of their invasive properties [22, 23]. Therefore, the enhancement of the magnetic signal in the tissue of resistant neoplasms is probably explained by the ability of the nanocomposite for redox-regulation in cells with the phenotype of drug resistance.

It should be noted that magnetic nanoparticles such as magnetite (Fe_3O_4) have superparamagnetic properties [24–26]. Such particles are characterized by magnetic anisotropy. In addition, iron nanoparticles smaller than 100 nm (and the nanoparticle size in FP is approximately 32.7 nm) penetrate cells more easily and faster.

Thus, the data obtained using non-contact SQUID-magnetometry characterize the features of the *in vivo* FP accumulation in cells of drug-sensitive and drug-resistant malignant tumors. In our opinion, SQUID-susceptometer systems using ferromagnetic nanoparticles as a contrast agent can be used in the near future for effective diagnostics and antitumor chemotherapy. Moreover, such approach is prospective for the development of artificial intelligence models, for cancer diagnosis and assessment of therapy response.

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COMPETING INTERESTS

The authors declare that they have no competing interest.

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БІОМАГНЕТИЗМ ЧУТЛИВИХ ТА РЕЗИСТЕНТНИХ ДО ЦИТОСТАТИКІВ ЗЛОЯКІСНИХ ПУХЛИН ПІСЛЯ ВВЕДЕННЯ ФЕРОМАГНІТНОГО НАНОКОМПОЗИТУ

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***Мета*:** Магнітні сигнали, які надходять від живих організмів, незалежно від біологічного виду, є важливими біофізичними показниками. Дослідження цих показників є дуже актуальним і перспективним напрямком для візуалізації пухлинного процесу та створення технологій з використанням штучного інтелекту, коли мова йде про злоякісні новоутворення, у першу чергу резистентні до хіміотерапії. Тому в цій роботі ми оцінили магнітні сигнали, які надходять від експериментальних злоякісних пухлин різного гістогенезу та чутливості до цитостатиків. ***Матеріали та методи*:** Як моделі пухлинного росту на щурах-самицях лінії Wistar використовували чутливу та резистентну до доксорубіцину карциносаркому Walker-256, а також чутливу та резистентну до цисплатину карциному Герена. Магнетизм пухлин, печінки та серця визначали за допомогою SQUID-магнітометрії безконтактним методом, використовуючи спеціально розроблені комп'ютерні програми. Окрім того, деяким експериментальним тваринам вводили одноразово внутрішньовенно феромагнітний нанокомпозит (Ferroplat) та через 1 год визначали біомагнетизм. ***Результати*:** Магнітні сигнали, які надходять від резистентних пухлин в експоненційній фазі росту, були або достовірно вищими, або фіксувалася чітка тенденція до їх збільшення порівняно з чутливою пухлиною. Внутрішньовенне введення Ferroplat мінімум на порядок підвищувало біомагнетизм, особливо в резистентній пухлині. Водночас магнітний сигнал печінки та серця практично відповідав магнітному шуму. ***Висновки*:** Застосування SQUID-магнітометрії з використанням у якості контрастного агента феромагнітних наночастинок є перспективним підходом до візуалізації злоякісних новоутворень з різним ступенем чутливості до хіміотерапії.

Ключові слова: пухлина, магнетизм, SQUID-магнітометрія, лікарська резистентність, феромагнітні наночастинки.