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NANOHYPERTHERMIA OF MALIGNANT TUMORS. II. *IN VIVO* TUMOR HEATING WITH MANGANESE PEROVSKITE NANOPARTICLES

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Objectives: **To evaluate the ability of manganese perovskite nanoparticles (lanthanum-strontium manganite) to heat the tumor tissue *in vivo* under action of external alternating magnetic field. *Materials and Methods:* The magnetic fluid on the basis of nanoparticles of perovskite manganite was tested in the heating experiments using of alternating magnetic field of frequency 300 kHz and amplitude 7.7 kA/m. Guerin carcinoma was transplanted into the muscle of rat. Magnetic fluid was injected intramuscularly or intratumorally. Temperature was measured by copper-constantan thermocouple. *Results:* Temperature of magnetic fluid was increased by 56 °C for 10 min of alternating magnetic field action. Administration of magnetic fluid into the muscle followed by alternating magnetic field resulted in the elevation of muscle temperature by 8 °C after 30 min post injection. Temperature of the tumor injected with magnetic fluid and treated by alternating magnetic field was increased by 13.6 °C on the 30 min of combined influence. *Conclusion: In vivo* study with rat tissue has demonstrated that magnetic fluid of manganite perovskite injected in the tumor increases the tumor temperature under an alternating magnetic field. Obtained results emphasize that magnetic fluid of manganite perovskite can be considered as effective inducer of tumor hyperthermia.**

Key Words: **manganese perovskite, magnetic fluid, alternating magnetic field, tumor, hyperthermia.**

In the previous publication it was shown the possibility of synthesized magnetic fluid based on lanthanum-strontium manganite nanoparticles to generate the heat in externally applied alternating magnetic field (AMF) *in vitro* as well as lack of side effects of magnetic fluid administered into both non-tumor and tumor-bearing mice and rats [1]. The proposition to use the nanoparticles of perovskite manganite in the tumor hyperthermia is based on the postulate that these nanoparticles are able to demonstrate the ferromagnetic effect in the range of x=0.25–0.5 with Curie point = 0–95 °C that allows to avoid the overheating of surrounding normal tissues [2–7]. Samples of proposed nanocomposites were received in some laboratories, and their ability to be heated in the AMF in the system *in vitro* was shown [8–14].

It has to be noted that mentioned publications have shown the ability of nanoparticles of perovskite manganite to be heated by AMF action in model experiment [4, 8, 10, 13–14] and only one study presented theoretical calculations of the possibility of effective autostabilization of the temperature in the vicinity of T °C= 42 °C for nanoparticles of perovskite manganite [7]. Unfortunately, studies which could demonstrate the possibility to heat the tumor tissue with nanoparticles of perovskite manganite combined with AMF have not to be found in the accessible literature.

Taking into account this circumstance the current study was aimed to evaluate the ability of nanoparticles of perovskite manganite, in particular in the form of magnetic fluid under action of external AMF, to heat the biological tissues, especially tumor in the system *in vivo*.

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Abbreviation used: AMF – alternating magnetic field.

MATERIALS AND METHODS

Magnetic fluid. Magnetic fluid based on the synthesized nanoparticles of perovskite manganite ($La_{1-x}Sr_xMnO_3$) and water solution of agarose, has been used in current study. Characteristics of magnetic fluid were presented earlier [1].

Alternating magnetic field. The rat leg was treated by external AMF after injection into muscle or intramuscularly transplanted tumor (Guerin carcinoma). A high frequency generator worked out by Institute of Electrodynamics was used to induce an alternating current of 7.7 kA at a frequency of 300 kHz [1]. The current passes through a custom-made five-turn water-cooled coil of 30 mm in internal diameter and 30 mm in height to generate a magnetic field inside the coil.

Temperature measurement. Tumor temperature during heating was measured by means of cooper-constantan thermocouples (0.1 mm diameter; IF-Kyiv). The thermocouples were calibrated against a certified precision mercury-in-glass thermometer (Committee of State Standards, Ukraine) before and after each experiment. Temperature was recorded always with the power switched off.

Animal and tumor models. All studies were conducted with strain IEPOR bread rats (Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine) weighting 150–200 g and bearing Guerin carcinoma transplanted intramuscularly into the right flank. The principle and method of transplantation were conventional. Animal were kept in Makrolon cages bedded with dust-free wood granulate, and had free access to a standard diet and tap water. All experiments had been approved by the regional animal ethics committee.

All 35 female rats were taken into experiment 7–9 days after transplantation (tumor volume 1.0–1.5 cm^3), anaesthetized with "calipsovet plus" at a dose of 0.1 ml/100g b.w., i.p. before the onset of experiment. Each animal was placed into a special prepared net-hammock suspended to make free leg. The net-hammock was fixed on the tripod so that the femur of animal was localized in the water-cooled magnetic induction coil with diameter of 3.0 cm (Fig. 1). Suspension of magnetic fluid of manganite perovskite was administered into the muscle (5 rats) or tumor (30 rats) at a dose of 0.5–0.6 ml (50 mg manganite per ml). Tissue temperature was measured when generator was switched off to avoid the influence of alternating magnetic field.

Fig. 1. Photograph of rat in the special created net-hammock (the femur is located in the coil of magnetic field generator)

In experiments *in vitro* temperature was measured every 5 min under the switched off generator but in experiments *in vivo* such procedure was repeated not so often to avoid the living tissue damage (every 15–20 min, in average).

RESULTS AND DISCUSSION

It was shown earlier that synthesized nanoparticles of lanthanum-strontium manganite did not display the toxicity or provoked the side effects in experimental animals with transplanted tumors [1].

At the first step of current study it was examined the ability of magnetic fluid of manganite perovskite to be heated in model experiment. Magnetic fluid was taken at a volume of 1.5 ml into the plastic tube which was located then in the coil of the generator of AMF in such way the tube setting up in the center of the field configuration. During the experiment temperature of magnetic fluid was measured when the generator was switched off. Obtained results were presented in Fig. 2. It was registered the increase of magnetic fluid temperature up to 72–75 °C within 10 min of AMF action. Fig. 3 represents the plot of temperature *versus* time after the onset of AMF action, and clearly demonstrates the increase of magnetic fluid temperature in comparison with the initial one. It has to be noted that specimens of magnetic fluid synthesized in different periods of time and used in this study displayed almost the same changes of temperature under AMF in experiment *in vitro* (Fig. 4).

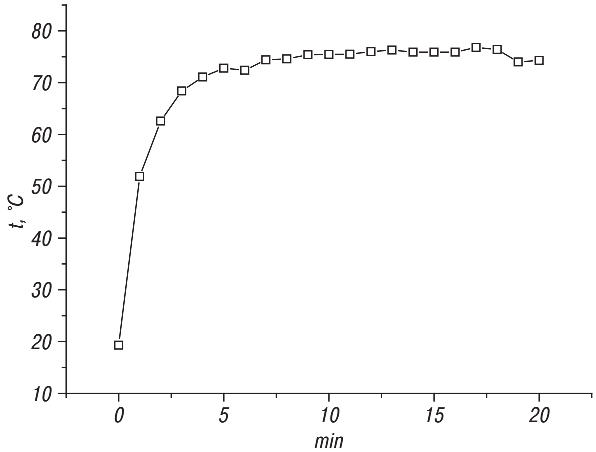

Fig. 2. Magnetic heating of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). Ordinate — temperature (T °C), abscisse — duration of AMF action (min)

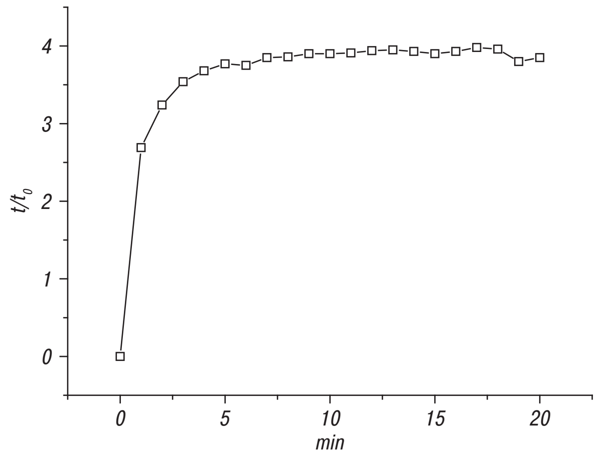

Fig. 3. Plot of temperature (t/t_0) *versus* time of the magnetic heating of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). Ordinate — temperature (t/t_0), abscisse — duration of AMF action (min)

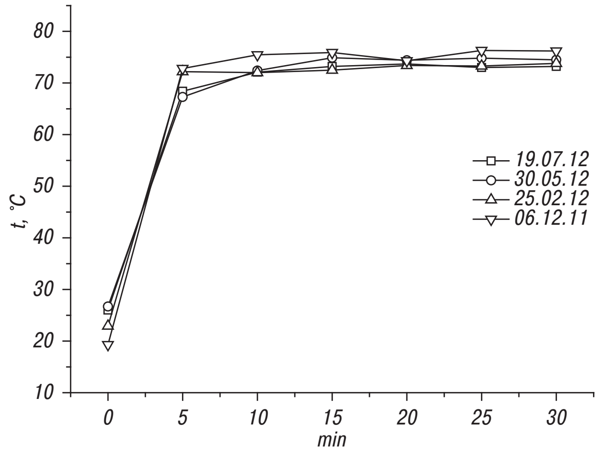

Fig. 4 Magnetic heating of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). Specimens of magnetic fluid synthesized in the different period of study were examined. Ordinate — temperature (T °C), abscisse — duration of AMF action (min)

At the second step it was provided the experiments *in vivo*. The heating of magnetic fluid was evaluated after it was injected into the muscle of intact animal leg or into the tumor in the femur as well as into the muscle of opposite leg of tumor-bearing rats. It was determined that the temperature of femur muscle of intact rat (magnetic fluid was administered into the muscle) was increased by 8 °C within 20–30 min of AMF action (Fig. 5). The temperature of tumor (administration into the tumor, Guerin carcinoma) was increased by 8.3 °C within 10 min, by 10.8 °C within 20 min, by 13.6 °C within 30 min of AMF (Fig. 6, 7). These results have distinctly shown that magnetic fluid on the basis of lanthanum-strontium manganite is able to increase the tumor temperature, i.e., "to heat" the tumor under influence of external AMF and can be considered as an effective inductor of tumor local hyperthermia.

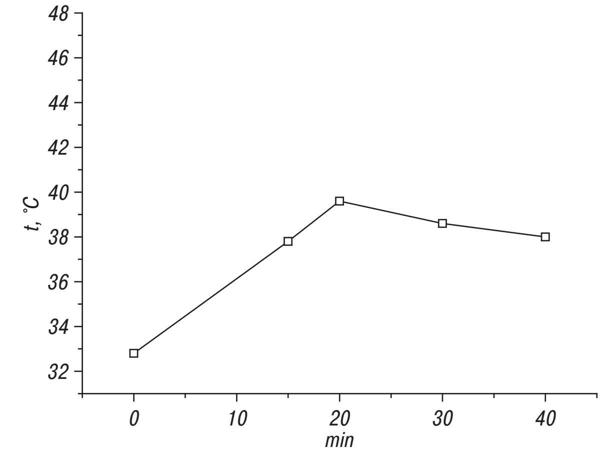

Fig. 5. Magnetic heating of rat muscle after intramuscular administration of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). Ordinate — temperature (T °C), abscisse — duration of AMF action (min)

Obtained results are the first demonstration of the possibility to heat the tumor up to therapeutically relevant temperature, i.e., 43–44 ^{0} with nanoparticles of manganite perovskite in the system *in vivo*. The temperature in muscle tissue surrounding the tumor node was not increased over 37–39 °C indicating the minimal possibility of the normal tissue overheating (Fig. 8). This observation confirms the idea about the self-controlled heating by exploitation of manganite substances [4, 12].

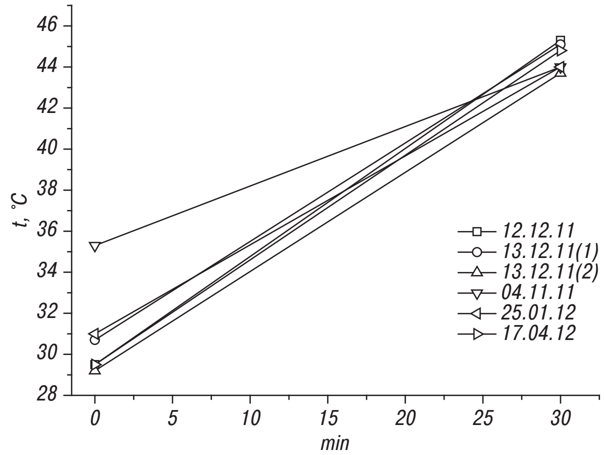

Fig. 6. Magnetic heating of Guerin carcinoma after intratumoral administration of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). It is given individual curves of rat tumor heating; experiment on 13.12.11: 1 and 2 — Guerin carcinoma was transplanted into both femurs of the same rat. Ordinate — temperature (T °C), abscisse — duration of AMF action (min)

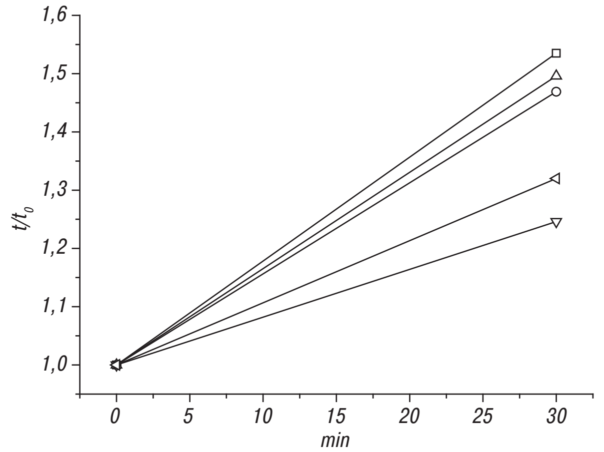

Fig. 7. Plot of temperature (t/t_0) versus time of the magnetic heating of Guerin carcinoma after intratumoral administration of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). It is given individual curves of rat tumor heating. Ordinate — temperature (t/t_0), abscisse — duration of AMF (min)

It should to be also noted that the analysis of obtained results has indicated that there is necessity to discuss the problems of coating, agglomeration of nanoparticles as well as their size that may influence the efficacy of heating, the distribution of nanoparticles in the tumor tissue as well as the manifestation of their magnetic properties in the specific tumor microenvironment.

The therapeutic relevance of nanohyperthermia method using manganite perovskite magnetic fluid and external AMF as single mean as well as in combination with chemotherapy is under evaluation now in experiment with transplanted tumors *in vivo*. Moreover, the advantage of nanohyperthermia in comparison with conventional radiofrequency hyperthermia has to be assessed obligatory, as we suppose.

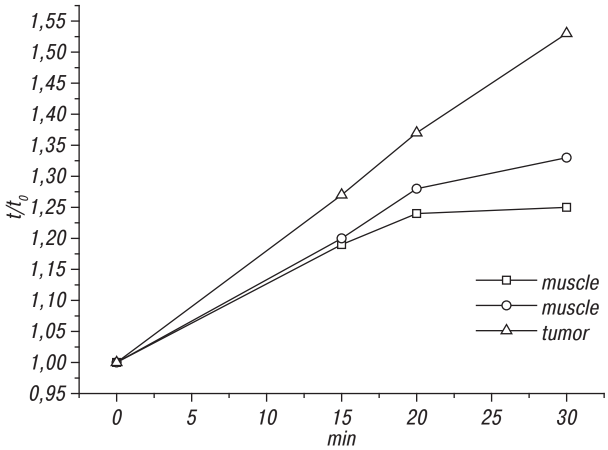

Fig. 8. Plot of temperature (t/t_0) versus time of the magnetic heating of muscle and Guerin carcinoma after intramuscular or intratumoral administration of manganite perovskite magnetic fluid under applied AMF (300 kHz, 7.7 kA/m). Ordinate — temperature (t/t_0), abscisse — duration of AMF action (min)

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