THE STUDY OF COMBINED EFFECT OF MICROWAVE ENERGY AND GOLD NANOPARTICLES ON TUMOR IN EXPERIMENT

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The aim of the investigation is to study the characteristics of the effect of microwave energy on tumors in the presence of gold nanoparticles.

Materials and Methods. The research was carried out on experimental tumor model cervical cancer. In the experiment there were used gold nanoparticles in the form of rods, 60x30 nm in size. Nanoparticles were injected to animals (mice) intravenously, 150 μg/kg. There was compared the efficiency of the following pulse sequences: 50 J (three times) and 150 J (once). The criteria of efficiency were the percent of tumor growth inhibition, and the increase of lifespan, as well as structural changes of tumor tissue revealed by optical microscopy.

Results. The highest anti-tumor activity was demonstrated by three times sequence of microwave energy of 50 J, just as with, so without gold nanoparticles. The effect of microwave freguency of low capacity could contribute to both significant changes of tumor structure, and tumor growth inhibition and the increase of lifespan of the animals. Gold nanoparticles in microwave therapy were shown to have no marked contribution to anti-tumor effect.

Key words: local microwave exposure; tumor model; gold nanoparticles; optical microscopy.

Local hyperthermia is recognized one of the most efficient modifying methods in combined oncotherapy, since it results in tumor sensibilization to chemotherapeutic agents and ionizing radiation. However, recently, intense interest of oncologists relates to local hyperthermia as an independent treatment modality [1]. There has been found that in the tumor heated to 43–45°C tumor cells die [2, 3], and according to some reports — apoptosis is triggered [4].

Currently, local hyperthermia develops in two directions: the selection of optimal sequences and sources of exposure for every tumor, and the use of nanoagents to improve treatment success [5]. In a wide variety of nanoagents known today, gold nanoparticles (GNP) are something special. They are biocompatible, have low toxicity, able to accumulate in a tumor and effectively convert energy of incident radiation into heat energy [6]. One more advantage of GNP is the possibility of *in vivo* control of their accumulation in a tumor using optical techniques [7–9]. Due to their ability to generate surface plasmon resonance, GNP improve the efficiency of laser hyperthermia [10–12], since their application enables to reduce by 10–25 times the dose of supply radiation required to initiate irreversible injuries [10].

Previously, the authors have found [13, 14] the enhancement of anti-tumor effect of laser hyperthermia of an experimental tumor due to GNP application. The use of these nanoparticles has been shown to make the exposure more targeted, and result in rapid and uniform heating deep

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in tumor tissue. Having succeeded in obtaining splendid results on laser hyperthermia with GNP, our science team has started to develop a new prospective modality for the treatment of experimental tumors — electromagnetic effect in microwave frequency. In this report we asked whether it is possible to improve significantly the efficiency of the technique by means of GNP application.

There are rare foreign reports on GNP successful application in electromagnetic hyperthermia [15, 16], but none has been reported in Russia.

The aim of the investigation was to study the characteristics of the effect of microwave energy on experimental tumors in the presence of gold nanoparticles.

Materials and Methods. The research was carried out on female CBA mice weighting 18–20 g. The effect of microwave exposure was studied on the model of transplantable solid cervical cancer tumor in mice. The tumor was modeled by subcutaneous injection of homogenized suspension of tumor tissue from a tumor-bearing animal.

When carrying out the experiments, ethical principles were kept inviolate according to European Convention for the protection of vertebrata used for experimental and other scientific purposes (the Convention was passed in Strasburg, 18.03.1986, and adopted in Strasburg, 15.06.2006).

The experiments were carried out on day 14 after tumor transplantation, when tumor node was formed completely and well palpated, on the day of the experiment the tumor size being 0.25±0.08 cm^3.

There was used the solution of gold nanorods. The size of nanoparticles was 60x30 nm, concentration in solution — 30 μg/ml, polyethyleneglycol stabilization — 6000 Da. GNP were injected intravenously in the dose of 150 μg per 1 kg of animal weight.

For local microwave exposure there was used the unit for controlled microwave thermal destruction — КСТД-1 developed in the Institute of Applied Physics of Russian Academy of Sciences (Nizhny Novgorod, Russia). The device has the following technical characteristics: wave length — 12.5 cm, oscillation frequency — 2.45 GHz, power output — from 0.5 to 450 W, impact depth — up to 5 mm, exposure time — from 100 to 400 ms. The exposure was achieved by contact method using an applicator, 10 mm in diameter. Tumor temperature was controlled on surface using infrared thermal recorder (IRTIS-2000 ME, Russia).

Microwave frequency was exposed 5 h after GNP injection. It corresponded to maximum period of nanoparticle accumulation that we specified before [13] based *in vivo* researches using optic coherence tomography (OCT). There was compared the effect of two pulse sequences of microwave exposure: 150 J (single exposure) and 50 J (three times every other day).

The animals were divided into the following groups:

1) experimental (n=10) — combined effect of microwave energy and GNP;

2) test group (n=10) — with microwave exposure;

3) control (n=5) — with no exposure.

After microwave exposure we monitor tumor growth dynamics. The effectiveness of the technique was assessed by the criterion of tumor growth inhibition (TGI, %), increase of lifespan (IL, %) [17]. Minimum criteria of the method activity were considered TGI ⩾50%, IL⩾25%.

For morphological study tumor samples were taken 24 h after the exposure. The material was fixed in 10% neutral formalin, dehydrated in high-proof alcohol, and paraffin-embedded. Histological sections, 5 μm in depth, were received on microtome Leica SM2000R (Germany), died by hematoxylin and eosin, and examined using microscope Leica DM 1000 (Germany).

Results. The research had several aspects under consideration.

I. It is known that when electromagnetic field (EMF) of microwave frequency is used, the absorbed power per a tissue volume unit grows. Thus, in equal input of power, biotissue heating in microwave frequency range is several orders more effective, and therefore, enables to achieve high heating rate in the area under exposure (several seconds instead of several dozens of minutes). In the present study for the first time there was used electromagnetic effect with the frequency of gigaherz (GHz), it is three sequences as higher than used in available techniques of electromagnetic therapy nowadays.

II. The use of electrode bipolar system enables to make the supplied microwave energy be more targeted to pathological area and by that minimize the negative effect on surrounding healthy tissues. Currently, more frequently monopolar electrodes are used, when high frequency current goes through a patient's body uncontrolled.

III. The use of GNP. Based on mathematical calculations of dielecric permeability of medium containing GNP we supposed that nanorods GNP in EMF can act as dipoles, i.e. enhance EMF intensity (minimum threefold), and therefore, specific capacity released around GNP. Thus, in adequate GNP concentration it is possible to achieve high therapeutic effect in far less power input of radiation.

Preliminary studies. On a healthy mice skin area there were adjusted the sequences of microwave exposure (from 50 to 250 J) till visible changes (burn) appeared. Initial skin temperature was 37.0–37.4°C (See Table).

Microwave energy higher than 200 J was found to cause skin burns, and could not be used in further researches, therefore, the sequences of 200 J initiating hyperthermia effect were chosen and approved on cervical cancer tumor, and for control — 50 J, since it left the tissue unheated.

The effect of the selected sequences on cervical cancer tumor was assessed by light microscopy on day 1 and 5 after microwave exposure. Morphological analysis

Skin temperature and visible effect after microwave exposure

Skin manifestations	Mode, J				
	50 (t_1=20, t_2=5)	100 (t_1=20, t_2=10)	150 (t_1=20, t_2=15)	200 (t_1=20, t_2=20)	250 (t_1=20, t_2=25)
Tumor surface temperature, °C	37.6	40.0	42.0	45.0	46.3
Visible effect	None	None	None	None	Burn

Note: t_1 — number of pulses in a burst, t_2 — number of bursts

of cervical cancer tumor structure showed microwave exposure by energy of 200 J, both with and without GNP to have an undesirable effect — tumor tissue coagulation. After the exposure of 50 J, there were destructive changes on day 1, though on day 5 the tumor tissue consisted mainly of actively dividing cells. Therefore, 50 J microwave therapy is sparing, though one procedure is not enough to destroy tumor cells.

Thus, for further researches there was chosen three-time microwave exposure of 50 J with one-day interval, and a single microwave exposure of 150 J resulting in moderate hyperthermia.

The study of anti-tumor energy efficiency of 150 J (single exposure) and 50 J (multiple exposure). Numeric processing of infrared thermograms made immediately after microwave exposure showed microwave energy of 50 J to leave biological tissue unheated (36.65°C), while energy of 150 J resulted in insignificant heating (41.19°C). The use of GNP had no effect on total tumor heating. However, it may happen that there will be local microheating around every particle due to the increase of EMF intensity resulting in severe damage of tumor cells.

The analysis of tumor growth dynamics in different groups of animals after microwave exposure within 14 days after the exposure (Fig. 1) showed that combined impact of microwave energy, both 150 J (single) and 50 J (three times), with GNP had an anti-tumor effect in the form of tumor growth inhibition compared to a control group. The effect of microwave energy of 150 J without GNP also showed the tendency for tumor growth rate reduction. Statistically significant difference from the control group was observed in all groups except that with the sequence of 150 J without GNP. However, there was found no statistical difference in tumor growth dynamics in combined effect of microwave energy and GNP compared to simple microwave exposure.

In accordance with TGI coefficients, the combined effect of microwave energy of 50 J (three times) and GNP has the most anti-tumor effect: maximum TGI — 68% on day 14. Simple microwave exposure of energy of 50 J (three times) also demonstrated anti-tumor activity: TGI was 62% on day 14. In other sequences TGI value did not exceed 50%.

The animals of both groups with three-time exposure of energy of 50 J had the maximum lifespan: IL in the group of animals with combined effect of microwave energy of 50 J with GNP was 58%, in the group of 50 J without GNP — 41.3%, while in other groups its value did not exceed 15%.

Morphological study of tumor structural changes after microwave exposure of different sequences. The analysis of tumor histological preparations 24 h after the treatment showed single microwave exposure of energy of 150 J to cause serious injuries of tumor tissue structure, such as necrosis, rarefaction of tumor area, chromatin condensation in tumor cell nuclei in these areas, full-blooded vessels. In combined exposure of microwave energy of 150 J and GNP on the areas with preserved dense tumor structure there were seen small necrosis foci, tumor cells with dystrophic changes in the form of vacuolar degeneration, cytoplasm vacuolization, nuclear clarification. Rarefaction areas were larger than those with no GNP used, and had hemorrhages.

However, threefold microwave exposure by energy of 50 J appeared to be more effective, since destructive changes were more expressed. In the bottom of the node there were found necrotic patches as well as the areas with tumor tissue rarefaction, segregation of cells from each other. Tumor cells decreased in size, they were roundish, with hyperchromatic nuclei and oxyphilic cytoplasm.

The use of GNP enhanced the effect of microwave frequency of 50 J exposed three times. In tumor cells of dense tumor structure there were observed destructive changes in the form of cytoplasm vacuolization, vacuolar degeneration, sometimes with cell membrane broken, swollen nuclei. These changes were more expressed, and involved a larger tumor volume.

For quantitative assessment of tumor morphological changes, for the purposes of the study, there were distinguished three areas: dense tissue area, rarefaction area, and necrosis area (Fig. 2). There were examined 60 areas, 100x100 μm in size.

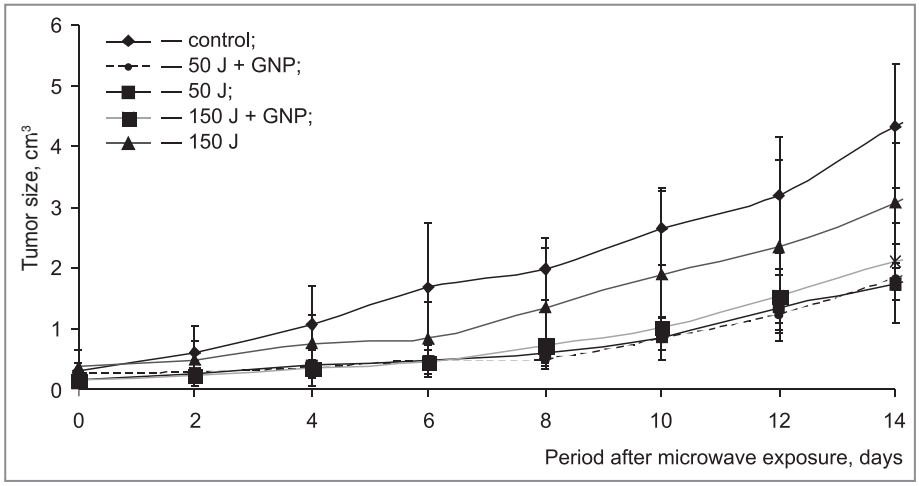

Fig. 1. Anti-tumor effect of microwave exposure by energy of 150 J (single) and 50 J (three times) with and without GNP on cervical cancer tumor. Mean values ± standard deviation are indicated

Quantitative analysis of tumor histological preparations revealed the changes in the proportion of rarefaction fields (the reduction of cell number per unit area), dense tissue, and necroses (Fig. 3). The tumor of control group consisted mainly of dense living tissue. The effect of microwave energy in all sequences resulted in significant increase of rarefaction area, and expanded necrosis area. There was observed a significant reduction of dense living tumor tissue in all groups compared to the control, but the lowest volume was found in the group of 50 J (three times) with GNP (about 25%). Thus, histological study

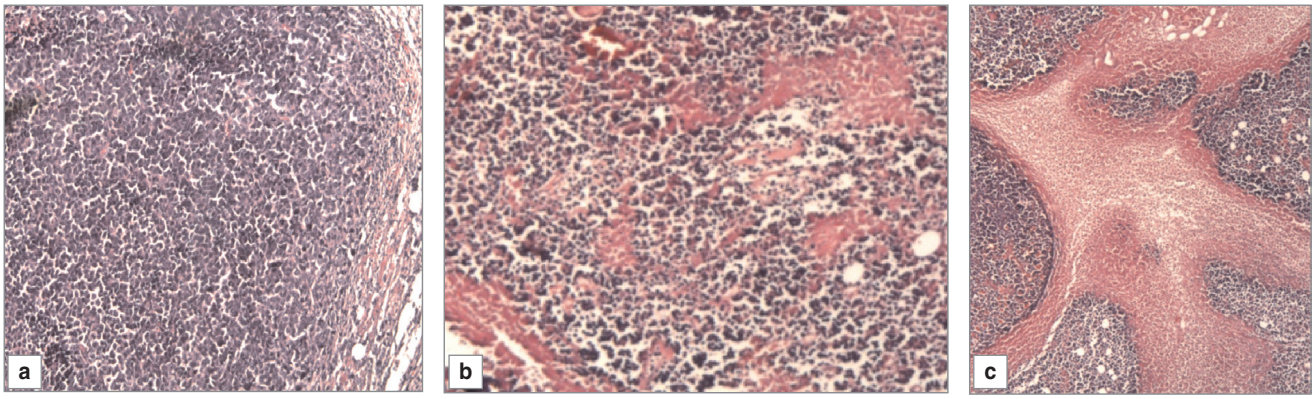

Fig. 2. Structural changes of cervical cancer tumor against the background of microwave exposure with GNP of different sequences: *a* — the area of dense living tissue, *b* — rarefaction area, *c* — necrosis area

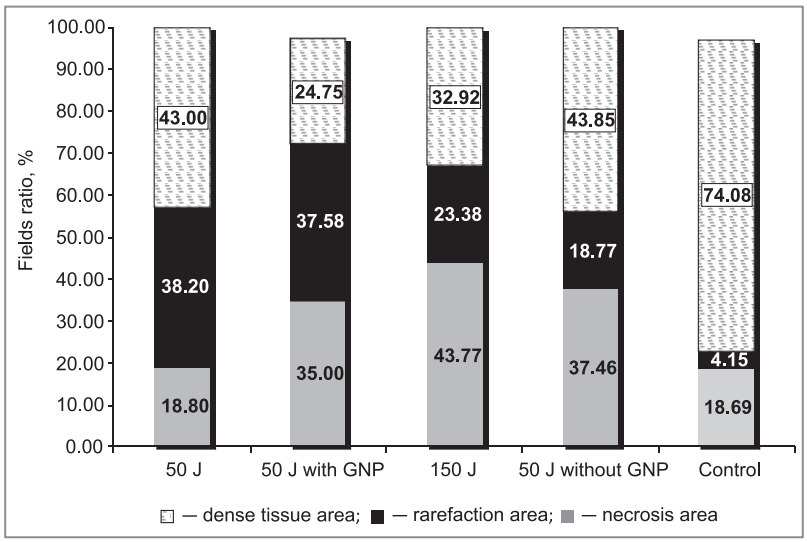

Fig. 3. Quantitative analysis of histological preparations of cervical cancer tumor after microwave exposure in different sequences

showed the effect of microwave exposure (in any of the sequences under study) on tumor tissue to be marked, though in the sequence of 150 J there prevailed necrotic areas, and in 50 J (three times) sequence — rarefaction areas. In comparison with simple microwave therapy, a combined effect of microwave energy and GNP results in the reduction of living tissue volume, and the increase of necrotic fields.

Conclusion. The study of the effect of microwave energy of 150 J (single exposure) and 50 J (three times exposure) with gold nanoparticles and GNP-free on experimental cervical cancer tumor model substantiated that all the approved sequences irrespective of the use of gold nanoparticles lead to marked destructive changes of tumor tissue. However, according to the values of tumor growth inhibition and the increase of lifespan, the sequence of 50 J (three times) both with gold nanoparticles and GNP-free has the highest anti-tumor activity, but no statistically significant differences between these groups were found.

In contrast to laser hyperthermia, where gold nanoparticles significantly improve the efficiency of this method due to plasmon resonance, in microwave therapy these particles have no marked effect on anti-tumor activity. We failed to confirm the hypothesis on the effect of gold nanoparticles due to the increase of electromagnetic field intensity (under given sequences and GNP concentrations used).

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