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Ultrasonic Nanomedicine in the Therapy of Oncological Diseases

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In this paper the authors develop the concept of using solid-phase nanoscale inclusions in biological structures, as the concentrators of acoustic energy for ultrasound therapy of oncological diseases. Particular attention is paid to possibility of synthesis of these inclusions directly in the tumor tissue. The validity of the hypothesis of solid-phase sonosensitization has been confirmed in vitro on bacterial cultures. Superadditive effect of combined action of ultrasound and solid-phase sonosensitizer on bacterial cultures was shown. Experimental studies on animals showed that the ultrasound exposure of malignant tumors containing nanoparticles of gold and some complex compounds (e.g. theraphthal) results in a significant therapeutic effect, which is expressed in a considerable inhibition of tumor growth.

Keywords: Oncological diseases, Tumor, Ultrasound, Sonosensitization, Solid phase sonosensitizers.

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1. INTRODUCTION

Studies in which nanoparticles (micelles, liposomes, nanoemulsion, bubbles, etc.), introduced into the bloodstream, are the means of delivering drugs to the tumor, and ultrasound is a factor stimulating drug release, can be attributed to the ultrasonic nanomedicine.

The method of ultrasonic nanotherapy of malignant tumors, developed in this study, differs from those described in the literature by two statements: (i) nanoparticles and their aggregates are formed immediately in the tumor from a nontoxic and non-medicinal substance introduced into the bloodstream as a solution; (ii) the ultrasound exposure on a tumor containing nanoparticle aggregates causes effects leading to inhibition of its growth and, in some cases, to its total remission.

As a result of the metabolic atypia, physicochemical conditions in the tumor (decreased pH, an increased content of calcium ions in intercellular liquid, etc.) differ from conditions in normal tissues surrounding the tumor. These differences result in the possibility of solid phase formation mostly in the tumor. The solid phase segregates in the tumor after intravenous introduction of solutions of compounds whose calcium salts or acidic forms are insoluble under tumor conditions. Thus, the selectivity of the formation of nanoparticles and their aggregates mostly in the tumor can be achieved using the least specific, hence, most stable symptoms of its atypia.

The ultrasound-induced therapeutic effect on the biological systems modified by nanoparticle aggregates is achieved due additional acoustic energy release in regions where these aggregates are localized. This occurs due to the fact that aggregates locally change the ultrasound absorbance, increasing the heat release and intensity of cavitation processes [1].

The above considerations were put into the basis of the development of the method of ultrasonic tumor destruction in the presence of solid nanoparticles and their aggregates. We called the phenomena underlying this method and associated with the presence of the solid phase as the solid-phase sonosensitization, and nanoparticles and their aggregates as solid-phase sonosensitizers (SPSs). The effect of solid-phase sonosensitization was tested in experiments in vitro on bacterial cells and in vivo on mice.

2. EXPERIMENTAL

2.1 Solid-Phase Modifiers

Theraphthal™ – octasodium salt of cobalt octacarboxyphthalocyanine. Theraphthal is soluble in water compound. It was synthesized in Organic Intermediates and Dyes Institute (Moscow, Russia). In proper conditions it forms insoluble in water calcium salt of theraphthal.

2.2 In Vitro Experiments

2 mL of the suspension of bacterial cells and 2 mL of theraphthal solution (10^{-5} mol/L) or 2 mL of gold nanoparticles suspension (10^{-5} g/mL) were introduced into the thermostated vessel with an ultrasonic transparent bottom and were exposed to ultrasonic treatment (0.88 MHz, 1 W/cm^2) for 10 minutes at 38 °C. Then, 1 mL of the suspension was transferred to a sterile Petri dish. Bacterial cultures were grown on a PCA Standard Methods agar medium. The result of seeding was estimated after 24 hours by counting the number of colony-forming units.

2.3 In Vivo Experimens

Melanoma B16 was inoculated intramuscularly into BDF1 mice in the right paw according to the standard procedure [2, 3]. The initial tumor volume at 8th day after inoculation was $V_0 = 1.1 \pm 0.1$ cm^3. Sonosensitizers were injected intravenously 1 hour prior to ultrasonic

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treatment. Ultrasonic treatment of inoculated tumors was performed simultaneously with two frequencies (0.88 MHz 1 W/cm^2 and 2.64 MHz 2 W/cm^2) for 10 minutes at 40 °C. The dynamics of tumor growth was assessed by the change in its volume.

3. RESULTS

3.1 In Vitro Experiments

Fig. 1 shows scanning electron micrographs of bacteria after the action of ultrasound and theraphthal.

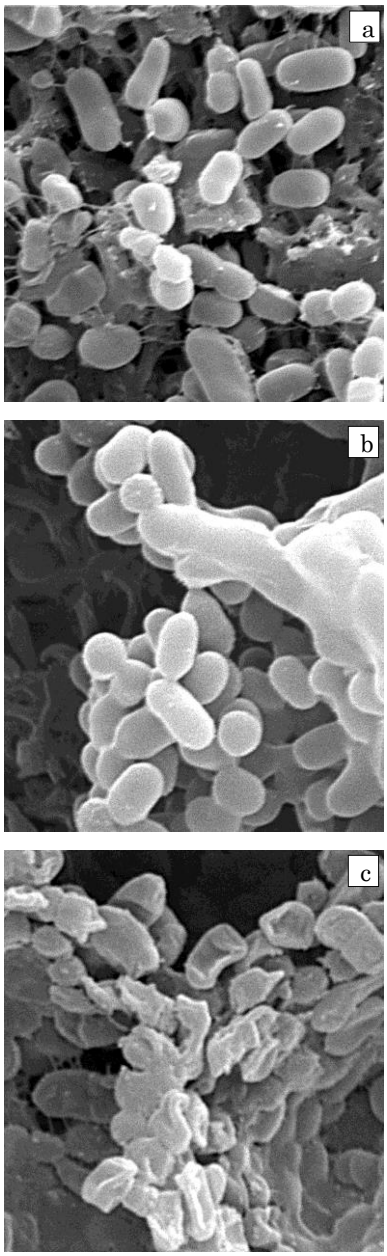

Fig. 1 – Electron micrographs of Enterococus spp. Cells: untreated cells (a), after ultrasonic treatment (b) and after combined action of ultrasound and theraphthal (c)

It is evident that as a result of ultrasonic treatment some of the bacteria exposed to destruction apparently accompanied by the leakage of cytoplasm (Fig. 1b). However, most of the cells keep normal form. Bacteria pretreated with theraphthal show a change in the shape and the destruction of the membranes that cover almost all the treated cells (Fig. 1c). Thus combined action of theraphthal and ultrasound leads to the destruction of cell membranes and organelles. In our opinion, theraphthal reacts with calcium ions contained in the cells and forms a solid phase of nanoparticles of calcium salts. This phase provides sonosensitizing action, stimulating the destruction of the cells under the action of ultrasound.

Data on destruction of bacterial cells are in accordance with data on their survival. Thus ultrasound exposure leads to about 10 % decrease of bacteria viability. Bacteria treated with theraphthal or gold nanoparticles show no change in viability. Combined action of ultrasound and sonosensitizer (theraphthal or gold nanoparticles) leads to significant decrease of viability (30-60 %). Hence this combined action results in a superadditive effect.

3.2 In Vivo Experimens

At the N.N. Blokhin Cancer Research Center, Russian Academy of Medical Science, the experimental possibility of applying the solid-phase sonosensitization effect to ultrasonic therapy of oncological diseases is preclinically studied over several years [4]. The experiments are performed on animals with various tumor types, different therapy schemes, and include estimation of the therapeutic efficiency, harmlessness, and the effect on metastatic disease. These studies showed a high therapeutic efficiency of the method, i.e., tumor regression by 75-80 % on average with an increase in the animal lifetime by two times, good exposure tolerance, and the absence of the effect on metastatic disease.

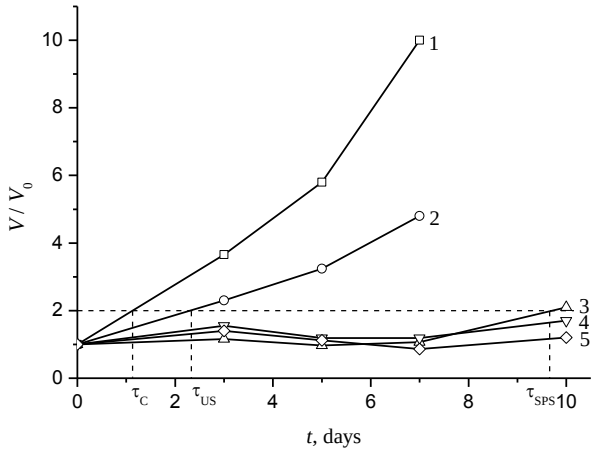

Fig. 2 – Growth dynamics of tumors containing SPS after ultrasound exposure for BDF1 mice and melanoma B16 tumor. Conditions: simultaneous exposure to ultrasound of two frequencies: 0.88 MHz, 1 W/cm^2 and 2.64 MHz, 2 W/cm^2; the exposure time is 10 minutes at 40 °C; V_0 is the tumor volume to the beginning of ultrasound exposure, t is the time of observation of tumor volume V growth after ultrasound exposure. (1) control; (2) ultrasound; (3) theraphthal, 30 mg/kg + ultrasound; (4) gold nanoparticles, 7 mg/kg + ultrasound; (5) ZnPc, 12 mg/kg + ultrasound

Fig. 2 shows the tumor growth dynamics in several experimental series using octasodium salt of cobalt octacarboxyphthalocyanine (theraphthal), octasodium salt of zinc octacarboxyphthalocyanine (ZnPc), and gold nano-

particles as SPS. We can see in Fig. 2 that the time of tumor size doubling in the experiments using SPS (τ_{SPS}) increases ten times in comparison with the control group (τ_C) and five times in comparison with the case of only ultrasound exposure (τ_{US}). This means that the therapeutic efficiency of ultrasound exposure in the presence of SPS significantly increases. Similar results were also obtained for other tumor types (carcinoma Ca755, Ehrlich carcinoma, and Lewis carcinoma). In therapeutic efficiency, these results were comparable to the results of treatment using optimum chemotherapeutic schemes.

Tumor growth inhibition and its complete remission in certain cases probably result from destruction of tumor cell membranes and cellular organelles. Fig. 3 compares the electron micrographs of mitochondria of melanoma B16 tumor cells unexposed and exposed to ultrasound (0.88 MHz, 1 W/cm^2 + 2.64 MHz, 2 W/cm) in the presence of SPS nanoparticles (theraphthal). The experiment was performed on BDF1 mice. In the micrograph of tumor exposed to combined action of ultrasound and theraphthal (Fig. 3b), we can clearly see mitochondria with destructed membrane structures (cristae). Similar defects of mitochondria were almost absent when using the same ultrasound exposure parameters without theraphthal (Fig. 3a).

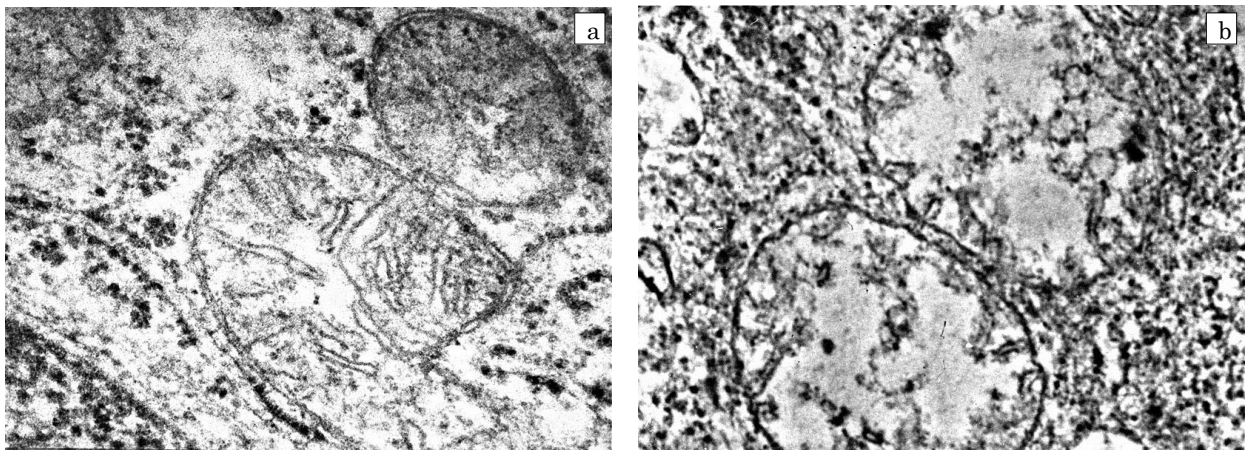

Fig. 3 – Electron micrographs of mitochondria of melanoma B16 tumor cells. Control (a) and ultrasound exposure in the presence of theraphthal nanoparticles (b)

4. CONCLUSION

Analysis of the results shows that nanoparticles of various substances are effective "amplifiers" of antitumor action of ultrasound. The results of these studies formed the basis of the method of ultrasonic therapy of cancer with the use of solid-phase nanoinclusions synthesized directly in the tumor. Clinical trials of this method are carried out at the N.N. Blokhin Cancer Research Center, Russian Academy of Medical Science.

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