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**SOME ASPECTS OF ULTRASONIC NANOMEDICINE**

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*In the paper the authors evolve their conceptions of the use of solid-phase inclusions in biological structures, as concentrators of acoustic energy for solving of some medical problems (destruction of tumorous tissue, drug delivery, increase of sound contrast). Special notice was attended to the possibility of synthesis of these inclusions directly in lesion focus. Hypothesis validity of solid-phase sonosensitization has been confirmed by experiments with germ cultures and animals.*

As far as we know, there is no settled definition for the term “ultrasonic nanomedicine”. We will use it as a conditional one for the definition of those jobs, which, in any event, refer to medical subjects and in which, as the basic acting principle, interaction of ultrasound and minor particles is used, regardless of their nature. The size of these particles ranges from  $10^{-9}$  to  $10^{-6}$  m. Their upper limit size approximately corresponds to modern definition of upper limit size of nanocluster. Appearance of this term is justified by a great number of articles in this area. This is the reason to regard it as a separate one.

To a first approximation, all the papers in the area of ultrasonic nanomedicine can be divided into three groups, where:

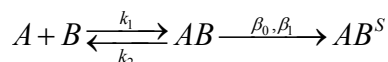
- ultrasound in combination with nanoparticles is the basic destructive factor of lesion focus;
- nanoparticles are the means of drug delivery to lesion focus, and ultrasound stimulates its elimination;
- nanoparticles are used for imaging of investigated area in ultrasonic field.

Conditional character of such division consists in the fact that physicochemical effects, serving as its basis, actually, are indivisible and have cross nature.

In present paper the authors [1-3] evolve their notion of the use of solid-phase inclusions in biological structures, as concentrators of acoustic energy. According to them, nanoclusters of certain nature, introduced in pathologic focus, stimulate in ultrasonic field excessive heat generation, reduce cavitation threshold and in the area of localization reduce mechanical strength of biological membrane. All these effects, in association with systemic drug therapy, result in local therapeutic action. At the same time, in certain conditions, solid-phase inclusions can serve as ultrasound scatterer, providing imaging of lesion focus. Drug delivery is also possible with the help of ultrasound and nanoclusters, but it requires additional device [4]. Thus, combinative application of ultrasound and solid-phase inclusions allows to realize practically all purposes of ultrasound nanomedicine, listed above.

At that, the basic task is control of solid-phase inclusions localization in proper place. There are two fundamentally different ways of their delivery to lesion focus – introduction to blood flow of ready nanoparticles and synthesis directly in the lesion focus. The last variant, in our opinion, is more preferred, as it differs in higher specificity of modifiers localization. Especially, it concerns ultrasonic therapy of malignant tumor, in which the specificity is provided for tumorous atypism, concerned with peculiarities of its growth, proliferation, metabolism and feed. As a result, physicochemical conditions in tumor differ from conditions in normal tissue, surrounding the tumor (pH, content of calcium ions, structure of bio membranes and others). This enables, by means of choosing of appropriate compound, to localize nanoclusters mainly in tumor, providing peculiar “biophysical focusing” of ultrasound.

We have worked out the mathematical model, describing dynamics of accumulation of modifier mass in porous matrix in conditions, relatively similar to phase formation *in vivo*, i.e. in situation of concentration constancy of one component in tumor (*A*) and the other component, decreasing in time according to exponential law concentration, introduced in blood flow (*B*). Equation, describing solid modifier formation is the following:

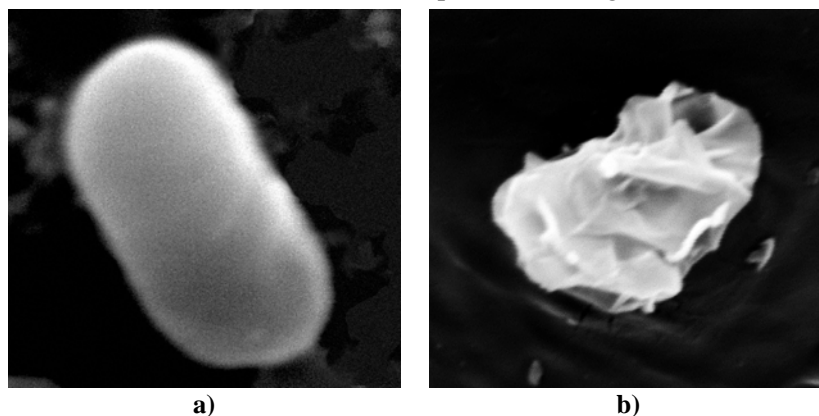


where index  $S$  concerns solid phase,  $k_1$ ,  $k_2$  – constants of the rate of direct and back homogeneous reaction,  $\beta_0$ ,  $\beta_1$  – constants of nucleation and growth of solid phase.

The results of calculations, in the presence of appropriate database, enable to determine time period, where ultrasonic influence on tumor in these conditions would be the most effective.

Experimental results on bacterial cells *E. coli* can serve as a confirmation of the assumptions about the important part of combinative action of ultrasound and nanoprecipitates in cancerous growth therapy.

The culture of bacterial cells was incubated in teraphthal solution (octosodium salt of cobalt octocaroxyphthalocyanine), which was sorbed on bacterial cells. In bacterial medium, containing calcium ions, teraphthal forms solid phase of calcium salt, localized on the surface of bacterial cells. Ultrasonic influence on bacteria, being in hard contact with calcium salt of teraphthal, results in decrease in number of viable cells (fig. 1). The higher teraphthal concentration is and the more powerful ultrasound is the less the number of survived cells. Appreciable and replicable effect of teraphthal influence on decrease of the number of survived cells in ultrasonic field is revealed in intensity value, which ranges from 0.6-2.0 watt/cm<sup>2</sup>, at teraphthal concentration of 10<sup>-4</sup>-10<sup>-5</sup> M. Qualitative picture of complete bacterial destruction in ultrasonic field is represented in figure 1.



**Fig. 1.** Electron microscope images of bacteria – *Escherichia coli*: a) native bacterium; b) bacterium after combinative influence of ultrasound and teraphthal.

It is supposed that amplified effect of ultrasonic influence on viability of bacterial cells results from the fact that solid inclusions of calcium salt of teraphthal on cells membrane promote the reduction of mechanical strength of membranes and the increase of cavitation effects due to local decrease of cavitation strength of medium.

Similar picture of bio membranes destruction can be observed directly in organelles of tumor cell. Electron micrographs of cells mitochondria of melanoma tumor B-16, influenced and not influenced by ultrasound (0.88 MHz, 1 watt/cm<sup>2</sup> + 2.64 MHz, 2 watt/cm<sup>2</sup>) in presence of nanoparticles of teraphthal are compared in figure 2. The experiment was carried out on mice of line BDF-1. In micrograph of tumor, influenced by ultrasound, mitochondria with destructed cristas are clearly visible.

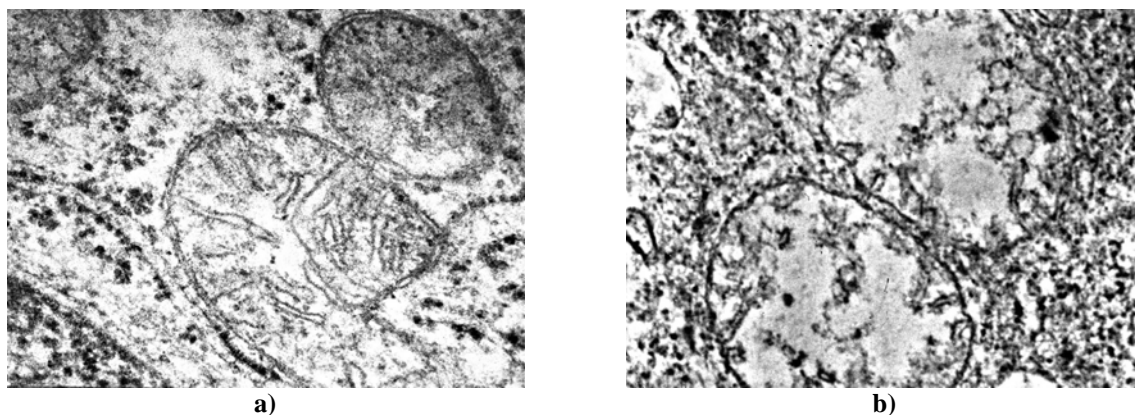


Fig. 2. Electron micrographs of cells mitochondria of melanoma tumor B-16: a) control, b) influenced by ultrasound in presence of nanoparticles.

The above-mentioned effects of combinative influence of ultrasound and nanoparticles had irreversible, destructive character, caused by cavitation processes. At the same time, additional heat effects of ultrasonic influence on polymer mediums, resulting from their modification by nanoclusters, enable to create materials for controlled drug delivery.

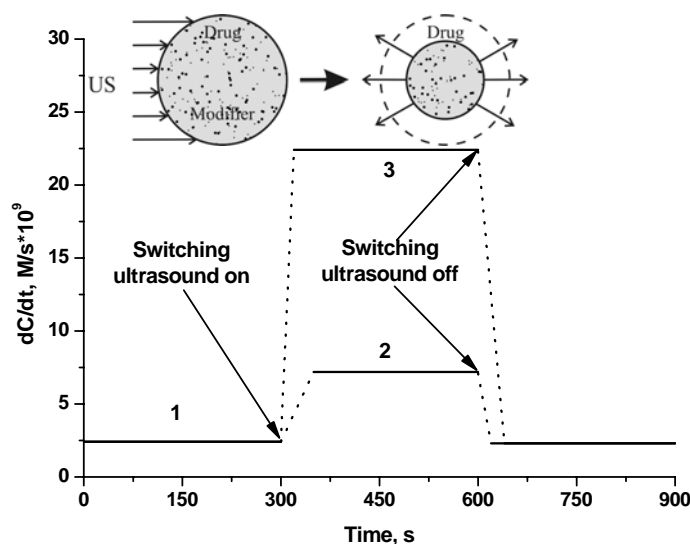
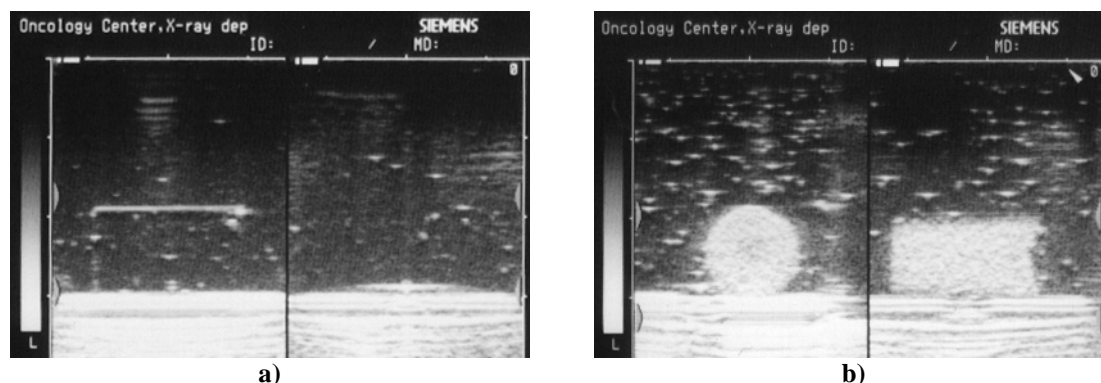


Fig. 3. The influence of ultrasound on dynamics of ferrocen release out of samples, not containing and containing modifier. Characteristics of ultrasound: frequency – 2.64 MHz, intensity – 1 watt/cm<sup>2</sup>.

The basis for such materials can be, for example, heat-sensitive polymeric hydrogel, which is able to undergo structural phase change at certain temperature. In ultrasonic field hydrogel is heated due to absorption of ultrasound, intensified by solid-phase inclusions. On reaching critical temperature abrupt change in its volume occurs, which, in its turn, can be used for the drug substance release out of gel, which was previously introduced in it. At that, hydrogel does not undergo irreversible destructions and after discontinuation of ultrasonic influence totally recovers its volume. Thus, the container can be reused and the duration of its functioning is determined by initial quantity of the substance, introduced in gel.

Figure 3 represents data on the speed of drug substance ferrocen release out of hydrogel (polydiethylacrylamide) containers – modified by solid-phase inclusions and unmodified. Area 1 in graph corresponds to background release of ferrocen outside ultrasonic field, 2 and 3 – ferrocen release out of gel under the influence of ultrasound. Represented data show that the speed of substance

release increases under the influence of ultrasound, at that, it is 3 times higher for the sample with modifier (HAP), than for the sample without modifier.



**Fig. 4.** Ultrasonic images a) agarose gel; b) agarose gel, modified by crystals of barium sulfate.

The increase of ultrasonic contrast of investigated object is the most vital issue of ultrasonic diagnostics. The use of nanoclusters to this effect, gradually concentrated in investigated area gives a fundamental opportunity to work out due regard to the problem solving. Their effectiveness is determined by the nature of modifiers and their way of interaction with polymeric matrix. Figure 4 shows an example of the increase of sound contrast of agarose gel by means of its modifying by crystals of barium sulfate. This model experiment confirms realism of such approach.

Thus realistically we can assert that the study of the effects of ultrasonic influence on biological structures, containing solid-phase modifiers of different nature, has great potential in the view of ultrasonic nanomedicine development.

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