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THERAPEUTIC ULTRASOUND ACTION ON THE CELLULAR LEVEL

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Distinctive features of the therapeutic and surgical ultrasound action on biological structures are considered. Some possible mechanisms of therapeutic ultrasound on the separate cells and biological membranes are discussed.

Progress of the modern acoustics is conditioned largely by the development of its specialized and interdisciplinary directions. The biomedical acoustics is undeniably one of such important and perspective directions. In spite of its more than a semi-centennial history the biomedical acoustics still continues its development with a great dynamism and it gives new technologies for medicine. At the present time the acousticians themselves play the dominant role in this process. They introduce the physical conceptions for the medical applications but do not take into account in full measure some specific features (for example, structural and dynamic) of living organism as the object of an acoustic action. Such situation is observed in the many investigations concerning the potentials of the ultrasound (US) for medicinal purposes (leaving aside US as the diagnostic instrument). High intensity focused ultrasound ($I > 1000 \text{ W/cm}^2$) can be defined as strong (gross) action since it causes well-predicted high-temperature effects in biological tissues resulting to a total coagulation of tissue albumens or even tissue carbonization. Mentioned above the distinctness of final result and action mechanism based in general on the thermal effects allows to concentrate the main attention only on the physical effects such as temperature elevation analysis, contribution of cavitation and nonlinear effects taking place in some model continuum medium. In this case there is no exigency to take into account a cellular structure of tissues and organs. By this circumstance in many respects it can be explained a large quantity of investigations concerned with a given regime of ultrasound action on biological tissue study (so called **US surgery**¹).

It's well known however that a lot of medical indications require not surgical, but therapeutic treatment. With respect to US it means that its intensity must be not so high in comparison with US surgery to exclude an irreversible damage of tissue. In that case a required medical effect under low intensity ultrasound treatment ($I < 5 \text{ W/cm}^2$) (this is so called **therapeutic ultrasound**) can be achieved due a metabolism processes activation in each cell. It can cause a synergetic medical effect concerning whole organ or even organism. Therefore the theoretical analysis of US therapy must be connected with the complex analysis and the understanding of the processes on the cellular level. And the absence of the required attention on the side of the physicists to the US therapeutic problems is explained in part by this necessity. Moreover in recent time the tendency to the mixing and confluence of the "US surgery" and "US therapy" conceptions is marked among the physicists, who research the medical ultrasound problems. For example, the scientific societies have been created, the reviews have been written, the conferences have been organized under the title "Therapeutic ultrasound" where the US surgery problems were been dissolved. This process of mixing and confluence of two main medical conceptions is quite unnatural in the view of medicine.

Attempts of the scientists to carry out the target ("address") cell therapy (pharmacological, gene and/or biophysical therapy), where ultrasound plays in that process an important role of medicinal factor and governing mechanism, seems to be quite perspective. It should be noted that existing

¹) Given word-phrase used in medical acoustics represents a noninvasive US high-temperature process of tissue destruction. This is an equivalent of tissue excision in traditional surgery. Also the same phrase is used for the denote of another process, where low-frequency (some tens of kHz) US activate the surgery instruments in order to do tissue excision more effective.

interest to mentioned problem returns us to the necessity to analyze direct ultrasound action and/or secondary processes (such as cavitation, microjets) influence on single cells and there structure elements. Well-grounded traditions in that direction were laid by Russian scientist Elpiner [1, 2].

Under consideration of mechanisms and effects of US action on the separate cells and their structure elements (in the first place, on the biological membranes) it is necessary to be based on the following fact. Dosage and threshold characteristics, playing an important role in physical medicine and are traditionally referred to a given model biological tissue (see, for example, [3]), must be determined for the single cell. The main threshold effect for cell is a stopping its living activity caused by external action. In our case of US action the threshold characteristics of acting acoustical beam must be defined. The basic factors causing the possible cell death during ultrasound treatment are i) the thermal coagulation of the critical part of the cellular albumen and ii) acoustomechanical destruction (rupture) of cellular membrane.

Energy costs and hence energy characteristics of US field needed to achieve a total thermal coagulation of cellular albumens can be evaluate by using of Arrenius equation. It defines a response rate in terms of its activation energy:

$$\Omega = \frac{k_B}{h} \cdot e^{\frac{\Delta S}{R}} \int_0^t T \cdot e^{-\frac{\Delta H}{RT}} dt, \quad (1)$$

where R is absolute gas constant, k_B is Boltzmann constant, h is Planck's constant, ΔS is reaction entropy, ΔH is reaction enthalpy, T is absolute temperature, temporal temperature elevation $\delta T(t)$ in case of short time interval t and for small volume element without taking into account a heat diffusion can be expressed as follows $\delta T = q \cdot t / \rho C$ where q is energy density of volumetric heat sources, ρ and C are density and thermal capacity of biological structure, Ω is some dimensionless characteristic of reaction result, for example $\Omega = \ln \left[\frac{C(t=0)}{C(t)} \right]$, where $C(t=0)$, $C(t)$ are the molecules numbers (concentrations) of initial (immutable) kind at initial time $t = 0$ and end point t of reaction.

Using averaged values of the parameters for thermal albumen denaturation:

$$\Delta S \sim 60 \left(\frac{kcal}{mole K} \right), \quad \Delta H \sim 45 \left(\frac{kcal}{mole} \right), \quad (2)$$

and determining formally the cells thermal death by equality $\Omega = 1$ (what it is equivalent to transfer of 63 % of all albumen number from native into denatured state) by applying some modifications [3] to equation (1) we will find the corresponding thermal energy density $q \approx 0.7 J/cm^3$ per unit of time. Taking into account the effectiveness of the acoustical energy into the thermal energy due the ultrasound absorption described by the simple equality $q = 2\alpha \cdot I$, where α is a coefficient of ultrasound absorption in biological tissue and I is wave intensity, it can be found the threshold value of ultrasound intensity needed to achieve a thermal destruction of cells. For example, at the frequency 1 MHz where averaged coefficient of ultrasound in tissue is $\alpha = 0.05 cm^{-1}$ we get the threshold wave intensity $I_t \sim 7 W/cm^2$. It should be noted that in US surgery used more intensive ultrasound ($I > 10^3 W/cm^2$). This circumstance is accounted by existing of the heat diffusion, bloodflow perfusion in whole mass of tissue and higher power inputs needed to achieve boiling up and carbonization of all cells "killed" by heat in comparison with theoretical predictions.

Mechanical mechanism of cells destruction during acoustical treatment can be discussed only for strong impulse (shock-wave) regime when in spite of need big peak level there are no thermal effects and the influence of the cavitation is excluded. Evaluations and experiments show that the single pulses to be typical for shock-wave lithotripsy (SWL) (pulse duration 0.01 – 0.05 ms and peak pressure 10 – 20 MPa) destroy grossly the cell membranes and under real simultaneous acoustical treatment of a great number of cells the part of surviving cells tends to be zero.

Thus the use of medical US with the parameters described above gives the egregious uncontrolled cellular strictures (the cellular membrane chiefly) damage under the main factors such as thermal and acoustomechanical and the total cell death as consequence. The US action with the lower level of considered parameters ($\sim 0.5 - 5 \text{ W/cm}^2$ in the case of the continuous US and the peak pressure is equal to $0.1 - 1 \text{ MPa}$ under using of pulse regime) can govern the cells metabolism without their destruction for the medical purposes. It is precisely these levels of parameters (intensity or peak pressure), the required medical purposes and application conditions (without cell damage) define the term “**therapeutic ultrasound**” and hence therapeutic regime of ultrasound treatment.

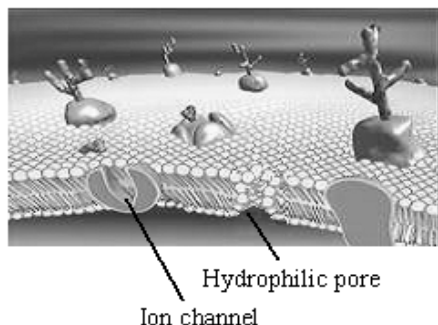


Fig. 1. Cellular membrane structure

Therapeutic action of the US on cells can be realized mainly by means of the influence on the barrier and exchange function of the cellular membranes that are the lipid bilayer with built usually represents albumen structures built other (albuminous mainly) structural elements. (Fig. 1). During normal conditions the exchange processes through the cellular membrane is realized by the endogenous transport systems: transmembrane diffusion and special ion channels (Fig. 1). Ultrasound can change transport properties of the membrane by three ways. The first way is the alteration of the distance between lipids and their mutual orientation caused by tensile deformation and

tensile strain. The second way is membrane capacity and potential changes (for example by means of the bilayer thickness variation). These parameters define in great part the transport properties of the membrane. And the last way is an alteration of inner and external relatively the membrane. This circumstance acts on diffusion too.

Ion channels can be in active (open) or passive state (“close”). It should be noted that there are different kinds of ion channels activated by different ways. In particular there is a large group of so-called mechanosensitive ion channels activated by alteration of membrane tension caused by different ways (for example, deformation) in comparison with the same characteristic in unperturbed state. It shows an additional possibility to govern this process during ultrasound treatment. It is important to note that difficult albumen structure of each ion channel and size of its reach-through hole are fixed and acting external exposure including induced by US elevation of membrane tension increases the probability of the channel activation and as a consequence its carrying capacity.

Significant and controlled alteration of the membrane permeability can be achieved by forming of additional reach-through holes (membrane pores) considered as structure defects of the membrane. There is a possibility to form such pores during fluctuations of lipid molecules packing in lipid bilayer. However external factors of pore formation are more effective and various. Among these are the biological and chemical action of the albumen-toxins that dissolve lipid bilayer, significant elevation of membrane tension due osmotic shock, local bilayer destruction with further pore formation caused by external electric potential (electroporation) and by ultrasound treatment (sonoporation). In comparison with another factors causing pore formation the ultrasound is characterized by several mechanisms of influence upon the membranes such as the direct action of ultrasound wave on bilayer resulting to an additional membrane strain and as a consequence the pore

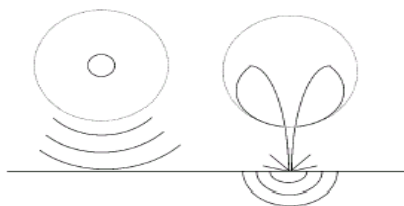


Fig.2 Collapsing bubble action on the membrane

formation possible, also the contribution of cavitation effects caused by translating acoustical wave (Fig. 2). During symmetric collapse of cavitation bubble a secondary shock wave with peak pressure $\sim 10 \text{ MPa}$ is generated. This situation is equivalent to the action of initial wave (Fig. 2(1)). When collapsing bubble is located in the vicinity of the membrane it changes the form and generates a microjet with characteristic velocity $\sim 100 \text{ m/s}$ that during the process of interaction with the membrane can cause pore formation (Fig. 2(2)).

Usually pore is considered as a round hole in stretched

membrane and characterized by radius r and bilayer thickness h (Fig. 3). It should be noted that such easy structure could have a limited radius and be stable or transform to unstable state during external exposure that causes rupture of the membrane. The cause of such defect behavior consists in the

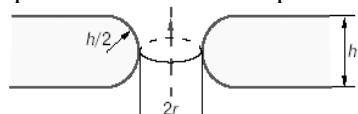


Fig. 3 Lipid pore structure

opposite action of two membrane surface tension components. Laplace pressures on interface water – lipids is defined by opposite signs of two boundary radiuses of curvature. The pressure concerned with pore radius r prevents pore from its unlimited growth and another pressure defined by curvature of the defect edge tends to extend the pore.

Increased attention to the problem of membrane pore formation and its dynamics is associated with the possibility of the targeted drug delivery into the cells. The using of ultrasound for these purposes make this process noninvasive and also the current utilization of the diagnostic ultrasound allows to control the procedure at most.

Under consideration of US action on the cells it is necessary to take into account physical-chemical effects appearing on the corresponding microscales. For example ultrasound destroys the thin surface water layer at the interface water – lipids that plays an important role in vital functions of the cells and has an another characteristics in comparison with the same parameters of water volume phase [4, 5]. It results not only to a fast alteration of transmembrane exchange processes but can induce a deferred (retarded) reaction of the cellular system.

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