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ANALYSIS OF THE MECHANISMS OF ACTION OF ULTRASOUND ON BIOLOGY CELLS

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АНАЛИЗ МЕХАНИЗОВ ДЕЙСТВИЯ УЛЬТРАЗВУКА НА БИОЛОГИЧЕСКИЕ КЛЕТКИ

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ABSTRACT

The analysis of our own and foreign researches about mechanism of ultrasound (US) action on biological objects is reflected in this article. This researches comprises last 50 years. It's shown that the biological effects are induced by US action, closely connected with the cavitation and depends of different parameters such frequency, US intensity, compound's concentration , temperature, pressing and etc. Lysis of cells is under the action of

ultrasonic waves is connected probably with the cavitation that is created acoustic microstreams and shearing voltage. It is shown that persistent action of US creates more sufficient changes than impulse one. In connection with the broad application of US in medical practice (physiotherapy, ophthalmology, surgery, internal diseases, microbiology)and food industry it is very important to study the ultrasonic waves action's mechanism on different objects.

АННОТАЦИЯ

В статье отражен анализ исследований механизма воздействия ультразвука (УЗ) на биологические объекты. Это исследование за последние 50 лет. Показано, что биологические эффекты вызваны действием УЗ, тесно связаны с кавитацией и зависят от различных параметров, таких как частота, интенсивность УЗ, концентрации соединения, температуры, давления и т. Д. Лизис клеток под действием ультразвуковых волн связан, вероятно с кавитацией, которая создается акустическими микропотоками и напряжением сдвига. Показано, что непрерывный УЗ создают более значимые изменения, чем импульсные. В связи с широким применением УЗ в медицинской практике (физиотерапия, офтальмология, хирургия, внутренние болезни, микробиология) и пищевой промышленности очень важно изучение механизмов действия ультразвуковых волн на различные объекты.

Keywords: US-action, cavitation, acoustic microstreams , hemolysis, lysis of cells

Ключевые слова: УЗ-действие, кавитация, акустические микропотоки, гемолиз, лизис клеток

Introduction

Ultrasonic waves of different power are used in the biological researches as physical factor with the selective action on biological objects. The results of experimental data by US action on biomacromolecules, bacteria, viruses, plant and animal tissues are reflected in monography and in papers of some authors – Miller[25-27],O'Brien[33],Nyborg[1,23,24]. A lot of experimental data shows that US has specific and selective action on structure of biomacromolecules, cells and cellular membranes. Ultrasonic energy is used efficiently in medicine and microbiological industry. Some different effects are noticed at the US action on cells and cellular suspension:

-heating of US medium (temperature effect);

-chemical damages that are induced by free radicals action in the cavitation process[5] and their transformation products;

-mechanic disruptions that are induced by impact waves and acoustic streamings (mechanical factor).

The persistent US action on biological structures gives more effect than impulse one. Let's see the influence of US field in details on cells in frames of above-mentioned factors. Heat-formation in cellular suspensions takes place in the results of transformation of acoustic energy to thermal as a result of US absorption. The degree of ultrasonic energy depends of medium viscosity, density and of periodic pressing of medium that creates adiabatic increasing of its temperature. Thermal effects may have the particular role in cell's life. Morphological pictures of cells changes are not similar by the action of heat and US. The border of thermal damaging of tissues less depends of their initial temperature, radiation regime and US frequency. Morphological changes aren't watched even after 8-hour irradiation if tissues temperature in the US field isn't more 42-43^{o}C[19].Effect of ultrasonic waves action on biological molecules and cells may be connect with the chemical reactions in the US field: 1) process in the gaseous medium; 2) on the border liquid-gas; 3) in the liquid phase in the result of interaction between active compounds of water sonolysis that are diffused in the collapse of cavitation bubbles[2,3,5].

Parameters that characterize the chemical reactions in the ultrasonic field depend from different factors: frequency and intensity of acoustic vibrations, temperature and pressure, nature and concentration of dissolved gases.

Both chemical and mechanical US actions take place in the cases when intensity of US vibrations is more than cavitation border's value. If low-size molecules (monomers, t-RNA, low-size proteins and peptides) are under the US influence the chemical action is over in its value. Mechanical forces play the main role if particles have a large molecular weight (DNA, DNP of viral particles)[12]. The dependence between effects and intensity of US [8] and time of its action is shown on the fig.1.

The absence of dependence between bactericidal US action on microorganisms suspension and nature of saturated gas gives conclusion that chemical products aren't significant for cells disruption. Degree of cell disruption isn't connect with the number of free radicals in the medium under US. Also the absence of dependence between cells disruption and generating free radicals shows that disruptive effects of US on cells defines probably by mechanical factors but not chemical ones [3,8].

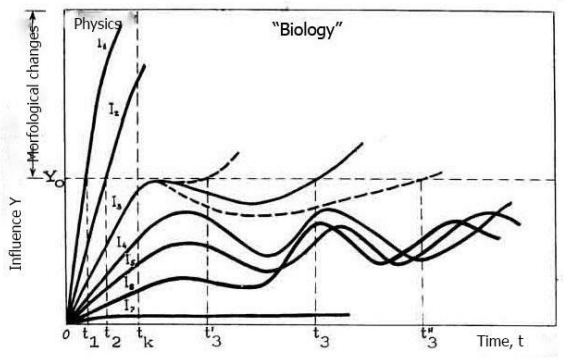

Fig.1. The dependence between biological effects and intensity of US (I_1 - >5 Vt/sm^2; I_2 – 5-1 Vt/sm^2; 1,0-0,4 Vt/sm^2)Some authors[8,9]supposed that prevailing mechanism of cells disruption may be "puncture" of cellular envelope as result of cavitation bubble's collapse near the cell. The emission of sonic wave is attended by loss of energy that is compensated of acoustic streams. Research of mechanism of US action on bacterial cells by high-frequency filming shows that cells disruption takes place as a result of vortical movement of medium under US action (low-frequency generator, 85 KHertz) [18,20] as simple second search of sound creating fluctuation of cellular membrane.

Acoustic streams may appear near the gaseous bubbles in the US medium that act on the membranes. In the data of Roy [4] amplitude of stabilized oscillating bubble is 30 mk. Microstreams induced by vibratory gaseous bubbles leads the death of microorganisms already in a few seconds of ultrasonic waves action with the amplitude pressure of 0, 1 atm. Some authors[40] supposed that cellular suspension under US action create small microstreams near the cell membranes and they may change cell's structure (fig.2).

There are different changes in the researched object in the result of mechanical action of US cavitation. These changes are in dependence of US-parameters, irradiation conditions and cell's state. Application of US based on US capability to create instantaneous disruption of bacteria, animal, plant cell and within cellular structures[20].

It's shown that only ultrasonic waves of definite intensity have disruptive action on cells. The border of cells disruption is defined by their nature, concentration and ultrasound conditions. Acoustic streams in suspension are appearing on pre-cavitation regime (0,05Vt/sm) and are capable only to "wash" macromolecules from surface of cell membranes. US intensity increases till values more than border of cavitation and forms the pulsing of gaseous bubbles in the medium, creating microstreams with the speed gradients 10^4 c $^{-1}$ [7].

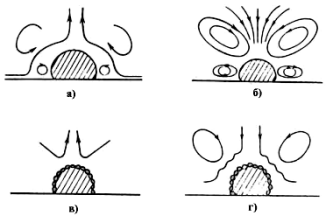

Fig. 2. The diagram of appearance of acousting streams

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Mechanical disruptions may be conditioned by shearing voltage in appearance of acoustic streams with the high speed gradients 10^4-10^5c^{-1} that will reach of 3-5x10^3 dyne/sm^2 [14,28].

The presence of gaseous microbubbles of resonance sizes in the medium increases the efficiency of US action in considerable extent.

There are gaseous bubbles in the regime of US action (intensity J= 1 Vt/sm^2 and frequency f=1 M Hertz) on cells, within cells or near the plasmatic membranes. These bubbles may leads to vibration in the cellular membranes in microstream. Thrombocytes begin to aggregate under the US action with the frequency of 1M Hertz and intensity 32 MVt/sm^2 if the shearing voltage will reach 50 dyne/sm^2 [23,24].

Leucocytes disruption in the field of stabile pulsating gaseous bubbles which frequency (about 20 KHertz) begins with the vibration amplitude increasing (its value is till 3). Process of haemoglobin extraction from erythrocytes at the 15-20min of US treatment conditioned of the creation of a large hydrodynamic forces near the vibrated bubble [8,28].The mechanism of disruption usually has also mechanical nature at the high-frequency US action (about1 MHertz) on cell suspension.The border of US intensity that cases cells death is depended both of US frequency and type of cells [3,8].

There are shown that prevalent role in the modifying US action on cells have mechanical forces that are appearing in bubbles collapse-impact waves and hydrodynamic streams and microstreams.

When the frequency and intensity of US vibrations are varying and also the duration of US treatment it may be possible to control of these processes. US doesn't only disrupt the cells but it may stimulate and increase of cells vitality that were in poor physiological state

before, inhibition of cells division, alienation of cells death, increasing of cell membranes permeability, conformational changes in DNA molecule and others.

It's mentioned that continuous US creates more sufficient changes in cells structure that impulse one[10,22]. In the researches of US biological action usually the main parameters of it are intensity and total time of action [1-3,8].

There are changes in diffusion processes, intracellular viscosity, membrane conductance in the intensity 0,1-1,0 Vt/sm^2 and also there is the process of sonoluminiscence and biologically active substances and radicals are creating. Efficiency of US influence on cells and cellular structures also depends of microorganism's type, medium content, cells concentration, their morphological features, sizes, forms and functional state[10,16].

Sensitivity of different cells to US action varies very much. Amoeba's cells exist in the intensive radiation: there are 50% of vitality individuals in suspension (18) after 10 min. of US treatment (200 Vt/sm^2 (1MHertz) and also cells in suspension begin to disrupt at the 0,7 Vt/sm^2 (0,75 MHertz, 1min.)[31].

Braginskaya[3]has shown that there isn't correlation between increasing of dead cells number and decreasing of vitality cells number because some cells under US treatment are deformed(they aren't viable but cann't characterized as dead).

Some appreciable changes of physiological state are before the disruption of cells structure and if mechanical resistance of cells changes in wide limits (0,1-1,0 Vt/sm^2 1-10^4 s. at the 0,5-2 MHertz) in dependence of cells type, the border (threshold) of physiological changes also (0,1-0,5 Vt/sm^2 10-100 s. at the 0,5-2 МГц) isn't change. First of all cells resistance to US defines by the structure of their cellular envelope that is most under the influence of factors in US field. It's shown that US (0,88MHertz, 0,6 Vt/sm^2) in certain conditions sufficiently (on 60-100%) increases conductivity of bilayer lipid membranes and also their permeability for anion of borate tetraphenyl [7].

It's also discovered that focused US increases involving of channel-former antibiotic nystatin into matrix of phospholipid membranes (on 10%) that may be conditioned by changes in matrix mechanical properties and damages in the neighboring diffusion layers [18].

US effects not only on the processes of cell's vitality but also on structure and function of some cellular organelles. There are some damages in lysosomes membranes under the US action (2,5 Vt/sm^2, 1 MHertz, 1 min.) and lysis of cells in the rats liver [30]. We were watching the parallel effect in the action of low-frequency US (20KHertz) [10]. US in certain conditions (0,75 MHertz, 2 Vt/sm^2) may disrupt nucleus in cells but doesn't disrupt integral cytoplasmatic membranes [32,33]. These disruptions aren't conditioned of cavitation and microstreams and probably are explained by creation of resonance waves on the surface of nuclear membranes.

The created changes are repaired during 100 hours if US action isn't lethal for the cell and only mitochondrions need more time for repairing of their structure and function [30].

US action on cell isn't limited of influence on surface structures. There are power microstreams in the US field that are mixing the cell's content and changing the place of cellular organelles [10]. The source of these microstreams may be vibration of cytoplasmatic membrane or pulsating gaseous bubble if the distance between source and cell isn't more than 5x10^{-2}sm [24,26].

We discovered that during the long time there is development of processes post-morphological and post-functional interruptions in the cell after US radiation. For example electrophoretic mobility of erythrocytes decreases after US treatment (0,02-1Vt/sm^2; 0,8 MHertz и 0,4 MHertz, 3-180sec.) and repaires in 3-5 min after US switching.

Thrombocytes after US treatment (1 MHertz, 0,2-0,6 Vt/sm^2, 5 min.) on the electron microphoto aren't differ from control ones. They are functioning as intact thrombocytes and form thrombuses but in control and US irradiated cells there are functional and morphological differences after 30 min. of incubation at the 22^{0}C [26]. The time of recalcification of thrombocytes less changes during the process of US treatment(1 MHertz, 0,065-2 Vt/sm^2, 5 min) and decrease in 4-6 hours after irradiation [27].

US-treatment of the cells in suspension or culture depends of US parameters and irradiation conditions and may be the reason both the stimulation and depression of vitality processes. US of biochemical processes in the cells increases biological activity and resistance to environment. The marrow cells were irradiated by US (0,8MHertz, 0,3-0,7 Vt/sm^2, 20 min) and injected to control animals; they give the beginning of a large number of colonies on the surface and in the spleen's parenchyma. These colonies grow faster and colonies differentiation increases in this case[27].

US treatment (0,9 и 2,6 MHertz, 10 min, 0,5-1,2 Vt/sm^2) less decreases the number of vitality cells in marrow suspension but after some days of their storage at the 4^{0}C in US-irradiated probes there are more vitality cells than in control ones.

The time of disruption of a half of marrow cells in suspension after US treatment increases twice – from 5 to 9 days that decrease losses in cells storage that may be use in transplantation [3].

The increasing of US intensity till values more than 1,0-1,5 Vt/sm^2 as a rule inhibits the cells biological functions. Continuous US (1MHertz, 0,8-2,6 Vt/cm^2, 60 min) inhibits the speed of growth of amniotic human cells in culture and the threshold of inhibitory action is between 0,8 and 1,7 Vt/sm^2 [24,34].

Usually animal cells in culture exist good US irradiation except cavitation or heating. Survivor cells as a rule are capable to normal growth and development but colonies created by these cells sometimes reach the sizes of ones that were formed from unirradiated US cells[16].

US action also decreases the speed of cells division. Animal cells are more sensitive to US and the speed of cells division decreases at the very low US intensity. So five-minute of US-irradiation with the intensity of 60 MVt/sm^2 and with the frequency of 1

MHertz inhibits mitotic index in tissues of rats liver [21].The US irradiation (0,1Vt/sm^2, 2MHertz, 5 min.) of *Erlich adenocarcinoma* cells inhibits tumor's growth that was forming at the reinjection of these cells[3]

US may increase the processes that are going more slowly in certain conditions [8].US is used for intensification of some processes that are connected` with the fermentation[3] and make possible to solve some technological problems.

Conclusion

We use this information and may consider that the main parameter of the process is only duration of US if you choose stationary regime at the disintegration of cells by US action (concentration of cells, US intensity, volume of suspension, distance of immersion of the oscillator head to medium). The inductions time is very important factor in the process of US action; during of this period the speed of destruction is very small and replace the fast one of transformations in the suspension after US treatment. Processes in the US medium in induction period may be essentially for the next transformations in cells in US field.

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РОЛЬ ОПИОИДНОЙ СИСТЕМЫ В МЕХАНИЗМАХ АНТИНОЦИЦЕПТИВНОГО ДЕЙСТВИЯ ОСЛАБЛЕННОГО ЭЛЕКТРОМАГНИТНОГО ПОЛЯ

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THE ROLE OF THE OPIOID SYSTEM IN THE MECHANISMS OF AN ANTINOCYCEPTIVE ACTION OF A DILUTED ELECTROMAGNETIC FIELD

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АННОТАЦИЯ

Исследована роль опиоидной системы в механизмах изменения ноцицепции моллюсков *Helix albescens* в условиях ослабленного электромагнитного поля. Показано, что эффективность антиноцицепции опиоидной системы на разных этапах действия электромагнитного фактора неодинакова.

ABSTRACT

The role of the opioid system in the mechanisms of change in the nociception of Helix albescens mollusks under conditions of a weakened electromagnetic field has been investigated. It is shown that the effectiveness of antinociception of the opioid system at different stages of the action of the electromagnetic factor is not the same.

Ключевые слова: электромагнитное экранирование, ноцицепция, опиоидная система, налоксон, моллюски *Helix albescens.*

Keywords*:* electromagnetic shielding, nociception, opioid system, naloxone, Helix albescens mollusks.

ВВЕДЕНИЕ

Изучение механизмов действия электромагнитных факторов привлекает внимание многих исследователей. Ранее нами были показаны фазные изменения ноцицептивной чувствительности моллюсков *Helix albescens* при действии электромагнитных факторов различной интенсивности [1]. Однако механизмы изменения ноцицепции под влиянием факторов электромагнитной природы не изучены.

В 1993 г. A.H. Frey впервые высказал предположение о том, что в реакцию организма на действие электромагнитных факторов вовлечена опиоидная система, которая является древней сигнальной системой [2]. В дальнейшем были получены многочисленные экспериментальные