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THRESHOLDS OF BIOLOGICAL ACTION OF ULTRASOUND

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The character of the regulative mechanisms in a cell depends on the degree to which the intracellular medium has changed and, hence, on the degree of change in the cell membrane permeability which is dependent on the intensity of ultrasound action. Therefore, for biological action, the threshold intensity is the intensity below which there appear no changes in the cell membrane permeability. Judging by well-known data, this threshold is $\leqslant 0.1$ kWm^{-2} (1 MHz). In a certain interval of higher ultrasound intensitities no visible changes are observed in the structure and function of cells, which is due to the development of the regulative processes. The upper limit of this interval represents another "registered" threshold of biological action of ultrasound (~ 1 kWm^{-2}). In a definite interval of ultrasound intensities exceeding 1 kWm^{-2} the observed biological effects are reversible. The upper limit of this interval (10 kWm^{-2}) can be taken as still another threshold.

The values of thresholds of biological action of ultrasound are of practical interest for ultrasound diagnostics, therapy and surgery. However, experimental determination of thresholds involves certain difficulties in each particular case, and extrapolation of values, characterizing the biological effect on threshold intensity of ultrasound, does not give the same results. There are a lot of cases [20] when bioeffects are produced at ultrasound intensities much less than the generally accepted curve obtained by means of extrapolation [18].

It seems possible to use another approach to the determination of thresholds of ultrasound bioeffects which is based upon a probable model of the mechanism of biological action of ultrasound.

A chain of successive reactions of cells to ultrasound action can be considered as such a model. The first reactions of this chain are due to physicochemical factors — mechanical, heat and chemical — which form the biological action of ultrasound. The effectiveness of separate factors which constitute the ultrasound action depends on the ultrasonic parameters and experimental conditions in different ways. Yet, each of these factors is capable of influencing the cell microenvironment and changing the substance transport through its membrane.

Thus, mechanical factors in the ultrasound field — variable displacements, gradients of vibrating velocity, radiation pressure, microstreamings — can change the cytoplasma viscosity [11], disturb the concentration gradients in the immediate vicinity of cell membrane [5], cause barodiffusion processes [15]. In all cases, the final result will be the change of conditions of transport of polar and non-polar molecules as well as ions through cell membrane.

Mechanical action upon cell membranes is greatly increased under the conditions of stable cavitation, which is observed at diagnostic ultrasound intensities [25] in certain cases.

The possibility of ultrasound action on the structure of the membrane itself was not taken into account in the above consideration. Nevertheless, already at rather small ultrasound intensities biomacromolecule desorption from the cell surface is observed [3, 17]. As a result, the conditions for monitoring membrane charges change, which also influences their permeability [2].

Intensive microstreamings are capable of breaking the integrity of cell membrane through the holes in which cell content flows out. This effect can be considered as a limit case of the change of conditions of substance transport through the cytoplasma membrane at ultrasound irradiation.

Also, an increase in temperature at the expense of absorption of ultrasound energy changes the conditions of substance transport in biological media. Each degree of temperature increase (in the field of 35-45°C) leads to a 2-3 per cent decrease in the viscosity of water and water solutions and a 3-5 per cent one in the viscosity of lipids. The coefficients of diffusion and self-diffusion increase correspondingly. The transfer conditions can be changed as well at the expense of thermodiffusion due to temperature gradients appearing when biological media [16] are irradiated by ultrasound.

According to calculations the Debye potential (otherwise called vibropotential) occurring in cell suspention and in tissues under the action of therapeutic ultrasound reaches a value comparable with the cell membrane potential [1]. The pulses of intensive ultrasound used in diagnostics can be responsible for tissue vibropotentials reaching hundreds of mV. Vibropotentials can cause depolarization of cell membranes and, therefore, an increase in permeability, at least with respect to ions.

The probability of the appearance of transient cavitation in biological tissues becomes possible if the intensity of ultrasound irradiation exceeds 3 kWm^{-2} (*SA*) [4]. In this case along with energetic microstreamings, thermal gradients and the Debye potentials, the permeability of cell membranes may be influenced by hydrogene peroxide [23] and, perhaps, by the ultraviolet component of ultrasound luminescence as well [19]. However, the effects due to hydrogene peroxide and ultrasound luminescence can most probably be neglected in comparison with those due to the influence of intensive microstreaming accompanying cavitation.

It follows from the above that the change in the cell membrane permeability is a universal reaction to ultrasound action no matter which of the ultrasound factors influencing the cells prevails in a particular case.

The change of transport of various substances through the cell membrane is, in turn, responsible for the disturbance of the composition of the intracellular medium and the cell microenvironment. The concentration of substances within the cell and near the membrane changes and along with it there are changes in the ratio of their concentrations. The disturbance of these compositions cannot but have an effect on the rates of biochemical reactions with the participation of enzymes being quite sensitive to the content of particular ions in the medium.

In some cases, the change of medium composition within the cell can lead to an acceleration in enzymatic reactions, since in physiological conditions most enzymes function without realizing their catalytic possibilities to the full extent. It is at the expense of this kind of reserve that the regulation of rates of enzymatic reactions in the cell is carried out [12]. This means that the regulation of enzymatic reactions is likely to take place in cells at low ultrasonic intensity when the disturbance of membrane permeability is slight and when changes in the cell do not exceed the possibilities of its regulating systems.

As the ultrasound intensity increases the effect of suppression of enzymatic reactions in the cell becomes more likely, since as a result of depolarization of the cytoplasmatic membrane the concentration of potassium ions in the intracellular medium decreases as the concentration of sodium ions increases [27]. A lot of intracellular enzymes are activated by potassium ions. Their activation by sodium ions is observed to be much less [14].

As a result of suppresion of catalytic processes in the cell, after a while there appears to be a deficiency of some metabolites and the reparative systems of the cell speed up the synthesis of new enzymes. A large number of investigations have confirmed the fact of acceleration of protein synthesis in cells and tissues as well as increase of RNA content in these tissues necessary for new synthesis, if biological objects are irradiated by low intensity ultrasound [7, 8, 26, 28].

Summing up the results of the above reasoning one can build up the following chain — a hypothetical mechanism of ultrasound action on the cell: Physico-chemical ultrasound effects → disturbance of microenvironment of cell membranes → change of cell membrane transport → disturbance of composition of intracellular medium → change of rates of enzymatic reactions in the cell → appearance and development of reparative reactions in the cell.... A lot of well known facts can be accounted for by the mechanism suggested: for example, ultrasound causes a spontaneous contraction of muscles [9] and activates lymphocytes increasing cell membrane permeability with respect to calcium ions; it speeds up wound healing; it is responsible for an increase in the rate of synthesis of some proteins and RNA [8, 10, 26, 28]. These, as well as many other experimental facts, indicate that the suggested model — the mechanism of the biological action of ultrasound — reflects the actual state of affairs. The above model allows the thresholds of biological action of ultrasound to be determined.

The character of the regulative and reparative processes in the cell depends upon the extent to which the intercellular medium has changed and, hence, upon the extent of changes in the cell membrane permeability which is, in turn, dependent on the duration and intensity of the action influencing membrane permeability. The reparative processes will not come into being and develop provided the changes in membrane permeability are too small. And so one of the possible definitions of the threshold of biological action of ultrasound follows from this.

For the biological action of ultrasound the threshold ultrasonic intensity is that below which there appear to be no changes in the permeability of cell membranes and therefore no regulative and reparative processes aimed at the elimination of consequences caused by these changes start in the cells. According to many researchers [6, 22, 29, etc.), this threshold does not exceed 0.1 kWm^{-2}.

In an interval of higher ultrasonic intensities, disturbances arising in cytoplasmatic membranes do not, as a rule, result in visible changes in the structure and function of the cell, which is due to the development of regulative processes compensating for the consequences of the change of membrane permeability directly during ultrasound irradiation. The upper intensity boundary of this interval can be accepted as another "registering" threshold of the biological action of ultrasound.

By the registered threshold of biological action of ultrasound we shall denote the value of its intensity above which one can observe morphological, electrophysical, physiological and other changes in biological systems both in the process of irradiation and after it.

The registered threshold corresponds to that found by NYBORG [18] and is in the range of 1 kWm^{-2}.

The observed biological effects are reversible in the particular interval of ultrasound intensities exceeding 1 kWm^{-2}. The upper boundary of this interval ($\gtrsim 10$ kWm^{-2}) can be taken to be another threshold. Exceeding this threshold results in pronounced destructive changes and with this background reparative processes in cells are not revealed. The ultrasound intensity of 10 kWm^{-2} is considered to be maximum in modern physiotherapy. Exceeding this intensity value leads as a rule to the suppression of protein and RNA synthesis, then the suppression of exchange processes in a cell and certain biological functions of organism [13, 28].

If the changes in biological object under the action of ultrasound not exceeding the possibilities of the regulative systems of the cell are considered to be such a result, then the threshold of biological action of ultrasound must be quite small (< 0.1 kWm^{-2}). If the result of biological action of ultrasound is a registered change observed after ultrasound treatment as well (which coincides with the concept of "biologically significant effect" introduced by NYBORG [18]), then the threshold is approximately equal to 1 kWm^{-2}, although its value depends on the duration of irradiation. If we suppose that the results of ultrasound action are destructive changes in biological systems and these changes are due to transient cavitation or temperature increase to a level catastrophic for biological objects, then the threshold is $\geqslant 10$ kWm^{-2}.

All these three thresholds are relative and vary depending upon biological peculiarities and object state, duration of the ultrasound action and irradiation conditions, upon registered parameter and the sensitivity of the method used for registering this parameter.

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