SECTION I. BIOTECHNOLOGY

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Mechano-associated bioeffects caused by high-intense focused ultrasound

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Abstract

High-intensity focused ultrasound therapy (HIFU) has become a serious alternative to surgical treatments. The main therapeutic effect of HIFU is mainly associated with thermal destruction of the affected tissue. However, the energy of the acoustic wave of the emitter becomes heat and forms mechanical shifts, the significance of which has not been studied well yet. In order to solve some problems, experimental studies were conducted that made it possible to identify some bioeffects of vortex microflows generated by mechanical shifts. Experimental results on biological objects showed that after HIFU exposure, the number of glycogen clumps in the liver sharply decreased, and the isolated scattered tumor cells were found on the periphery of the tumor tissue. Thus, acoustic waves refracting in biological tissues generate thermal and mechanical effects that can cause a decrease in glycogen clumps in hepatocytes and the separation of tumor cells from the mother tumor.

Keywords: high-intensity focused ultrasound, experiment, mechanical shifts, vortex microflows, thermal destruction, tumor cells, glycogen clumps.

In recent years, high-intensity focused ultrasound therapy (HIFU) has been widely introduced into clinical practice as a serious alternative to surgical methods of treatment in oncology. Nowadays it is actively used in the treatment of benign and malignant neoplasms of the liver, mammary glands, prostate gland, connective tissue, kidneys, brain, and other organs [1, 2]. The HIFU method has a number of significant advantages, namely: non-invasiveness, repeatability of the action to achieve a positive clinical effect, the absence of post-procedural changes in healthy tissues, which significantly reduces the operational-anesthetic risk [1, 3]. The energy of the acoustic wave generated by a focused ultrasonic emitter, propagating in heterogeneous biological tissues, partially transforms into heat, and partially is transmitted in the form of a mechanical impulse [4].

The therapeutic effect is based on thermal destruction, the role of a mechanical impulse is practically not taken into account. However, the velocity gradient in a heterogeneous biological environment can reach significant values. In particular, at a HIFU intensity of $8.2 \text{ kW} / \text{cm}^2$ and a frequency of 1.4 MHz, the velocity gradients reach $10^7 \dots 10^9 \text{ s}^{-1}$ and are more than enough to rupture cell membranes, disrupt intercellular and intracellular structures, and large molecules. Pulsating cavitation bubbles are another source of mechanical microflows. The maximum speed of the micro-flow near the pulsating bubble can be estimated based on the equations:

$$v = U^2 / \omega \alpha, \text{ where}$$

v – the maximum speed of the micro-flow;

U – radial speed of a bubble;

$\omega = 2\pi f$ – circular frequency;

α – average radius of a bubble.

Given the significance of the vortex flows, the aim of the study was to investigate mechanically associated HIFU-induced biological effects. Mature male Wistar rats ($m=286.0 \pm 3.8$ g) were subjected in the study ($n=19$). The experiments were conducted in accordance with Guidelines for the use of laboratory animals for scientific and educational purposes in Pavlov First Saint Petersburg State Medical University, compiled based on the international ethical guidelines for the care and use of laboratory animals (European Directive 2010/63/EU). The animals had ad libitum access to standard laboratory K-120 chow (“Inform-feed”, Russia) and filtered tap water. All subjects were housed under a 12 hrs/12 hrs light/dark cycle. The rats were housed in groups of four in a plastic cage in a ventilated room ($22-25$ °C, of 50–70% humidity). The quarantine duration was 14 days. All animals were divided into two groups. First experimental group ($n=10$) received transplantation of the cellular homogenate of Pliss lymphosarcoma (line from the N. N. Petrov Research Institute of Oncology). The transplantation of a solid Pliss lymphosarcoma sample was injected under aseptic conditions in the region of the right thigh (s.c.). A 10% cell suspension diluted in 0.9% sterile saline was administered in a volume of 0.2 ml. Measurements of Pliss lymphosarcoma size in animals were carried out with 2-3 days interval within 15 days from the implantation. After the tumor reached a certain size, the animals were anesthetized by intravenous administration of Zoletil 50 (VIRBAC, France) and Xila (Interchemie werken “De Adelaar BV”, Netherlands) in equal volumes at a dose of 0.5 ml / kg and placed on a TCAT-2 temperature-controlled table Temperature Controller (Physitemp, USA), with constant maintenance of rectal temperature in the range of $37.0-37.8$ °C. Subsequently, the animals were exposed to high intensity ultrasound using a H-148 S\N 010 (Sonic Concepts, Inc., USA) emitter under the following conditions: frequency 1.4 MHz, intensity 8.2 kW/cm^2 , exposure 300 ms. The tumor surface was previously coated with a viscous Aquagel gel.

Another group of drugged animals (9 rats), under the control of vital functions, were subjected to a middle laparotomy, and a rubber wound expander was introduced into the surgical wound and the ribs were moved aside, increasing the transverse size of the surgical field by subsequent removal into the surgical wound of the liver, and its most accessible surface was visually examined to eliminate the focus of the injury and to carry out subsequent manipulations. During preparation, the liver surface was constantly irrigated with a warm isotonic solution of 0.9% sodium chloride, which prevented rapid drying of the parenchymatous organ surface. The selected liver area was irradiated with HIFU in the following mode: frequency 1.4 MHz, intensity 8.2 kW/cm^2 in an averaged focal spot of 0.6 mm diameter, exposure 400 ms. The zone of thermal damage was at a depth of 1.0-1.5 mm from the liver surface. Previously, the surface of the parenchymatous organ was covered with a viscous Aquagel gel allowing acoustic vibrations to pass.

The efficiency of ultrasonic exposure was evaluated using a Seek Thermal Comhact PRO mobile thermal imaging camera for Android with micro-USB (Seek Thermal, USA).

Immediately after the completion of the experiment, a biopsy sample was taken in animals near the foci of thermal destruction and subjected to histological examination cells. Subsequently, biopsy specimens taken from the liver were fixed in alcohol-acetic acid-formalin solution. In liver samples, glycogen was detected by the PAS-reaction. During histochemical processing of the histological preparation, it was found that the samples observed a significant decrease in glycogen in the liver compared to the control. Sinusoidal capillaries of the liver were moderately dilated (Fig. 1).

Fig. 1. Histochemical preparation of rat liver near the zone of direct HIFU-induced exposure. PAS-reaction with hematoxylin tint. OK. x10, lens x10 (a); OK. x10, lens x40 (b)

The research of histological preparations from tumor tissue near the focus area by light microscopy showed that the tumor tissue is an accumulation of densely located small and large irregularly shaped cells with distinct boundaries. Their hyperchromic nuclei of various shapes occupy most of the cell, and the moderately basophilic cytoplasm looks like of a thin rim. In some areas, the tumor cells are highly scattered, and the spaces between them are filled with a structureless eosinophilic mass. Separate cavities of a round or oval shape, devoid of contents, are located in the tumor tissue. In the stroma of the tumor, there is vascular congestion, moderate edema and hemorrhage. Bundles of edematous striated muscle fibers, some of which are necrotic, are found in the tumor tissue. Inside the tumor tissue, connective tissue layers containing rarely located tumor cells, as well as blood formed elements, are visible in various thicknesses. Both on the surface of the tumor and outside it, a small number of randomly lying tumor cells are found in the connective tissue (Fig. 2).

Fig. 2. Inoculated Plissa lymphosarcoma immediately after ultrasound exposure. a - central zone, b - peripheral zone. Hematoxylin and eosin stain. x400

Discussion. A decrease in the number of glycogen granules in the liver cells, scattered tumor cells lying outside the tumor tissue indicate the significance and intensity of the ultrasonic microflows generated by high-intensity focused ultrasound.

HIFU propagation in heterogeneous living tissues forms an acoustic field in the form of a set of spatiotemporal distributions of the characteristics of mechanical disturbances with the formation of interference maxima in local zones due to the superposition of acoustic waves from external ultrasonic and internal sources. The response of biological structures to such effects should be determined, on the one hand, by the nature of the energy distribution, the degree of manifestation of wave and nonlinear effects, and, on the other, by the characteristics of the high-frequency focused acoustic field. The combined functioning of systems, organs, tissues, cells, subcellular and molecular structures generates its own acoustic field, should be taken into account. It can be assumed that the intrinsic fluctuations of biological structures are incapable of create conditions for the interference of oscillations of biostructures and an external high-frequency acoustic field. However, molecular vibrations are capable of initiating high-frequency acoustic radiation comparable in frequency characteristics with HIFU and extend to the phenomenon of superposition of acoustic waves, witch effect poorly understood. The validity of such a theoretical calculation is still worth confirming experimentally.

Most notably, the mechanical vibrations manifest themselves in the form of a mechanical impulse, generating acoustic flows that generate microflows. Vortex microflows can cause changes in the cytolemma, cell membranes, as well as initiate transmembrane “breaks”. The inertial (unstable) ultrasonic cavitation can be source of mechanical pulses, which can occur in the form of pressure pulses and shock waves.

Thus, focused ultrasonic vibrations, which generating an architectonic complex acoustic field in biological tissues, can generate thermal and mechanical effects that affect the formation of the thermal pattern, the spatiotemporal continuum of vortex microflows in the focal zone and perifocal regions. This phenomenon is confirmed by a reducing aggregation of glycogen clumps and a separation of cells from the main tumor.

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The usage of innovative techniques for plastic closure of wound defects of the toes and wrist's fingers

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Abstract

The work is devoted to assessing the effectiveness of the usage of nitrogen monoxide in the regime of NO-therapy in the treatment of patients who underwent autologous skin