TEMPERATURE FIELDS INDUCED IN RAT LIVER *IN VITRO* BY PULSED LOW INTENSITY FOCUSED ULTRASOUND

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Beneficial biological effects in soft tissues can be induced by focused ultrasound of low intensity (LIFU). For example, increasing of cells immunity to stress can be accomplished through the enhanced heat shock proteins (Hsp) expression induced by the low intensity focused ultrasound. The possibility to control the Hsp expression enhancement in soft tissues in vivo can be the potential new therapeutic approach to neurodegenerative diseases that utilizes the known feature of cells to increase their immunity to stresses through the Hsp expression enhancement. The controlling of the Hsp expression enhancement by adjusting the level of exposure to ultrasound energy would allow evaluating of ultrasound-mediated treatment efficiency. Our objective was to develop the numerical model capable of predicting in space and time temperature fields induced in multilayer nonlinear attenuating media by a circular focused transducer generating pulsed acoustic waves and to compare the results calculated for two-layer configuration of media: water - fresh rat liver with the experimental data. The measurements of temperature variations versus time at 5 points on the acoustic beam axis within the tissue sample were performed using 0.2-mm diameter thermocouples. Temperature fields were induced by the transducer with 15-mm diameter, 25-mm focal length and 2-MHz centre frequency generating tone bursts with the intensity I_{SPTA} varied between 0.45 W/cm^2 and 1.7 W/cm^2 and duration varied between 20 and 500 cycles at the same 20-% duty cycle and 20-min exposure time. Quantitative analysis of the obtained results allowed to show that, for example, for the acoustic beam with intensity I_{SPTA} = 1.13 W/cm^2 exposure time to ultrasound should not be longer than 10 min to avoid cells necrosis following the 43-^{o}C temperature threshold exceeding.

INTRODUCTION

In all cells there exist immune mechanisms protecting them against cytotoxic factors. Under stress conditions (induced by influence of multifarious harmful environmental factors,

among others by the temperature rise) cells synthesize special proteins, named the heat shock proteins (Hsp), protecting the regular structure and functioning of other cellular proteins. Thus, the enhanced Hsp expression increases immunity of cells to stresses. Recent investigations, reviewed in [1], have shown that exposure of tissue cultures to ultrasound energy may lead to the heat shock proteins (Hsp) expression enhancement. The possibility to control the Hsp expression enhancement in soft tissues *in vivo* by their exposure to ultrasound waves of various features can be the potential new therapeutic approach to the degenerative (among others neurodegenerative) diseases which utilizes the known feature of cells to increase their immunity to stresses through the Hsp expression enhancement. The controlling of the Hsp expression enhancement can be done by adjusting the exposure level to ultrasound energy. The exposure level depends on the ultrasonic regime of probes used and is determined by the tone bursts centre frequency, intensity, duration, duty cycle and exposure time.

Monitoring of ultrasound efficiency in the Hsp expression enhancement can be done by imaging the structure of cells exposed to ultrasound using methods of luminous (immuno-cytochemistry) or electron microscopy for various ultrasonic regimes.

The main aim of the proposed therapeutic application of ultrasound is targeted tissue heating at low intensity level as long as the temperature reaches the threshold of 43 ^{o}C. Raising the temperature above the physiological norm of 37 ^{o}C by no more than 6 ^{o}C may have a number of beneficial physiological effects, such as, for example, the enhanced Hsp expression or increasing the blood supply to the affected area. Tissue heating at temperatures higher than 43 ^{o}C may lead to an inability of cells to divide or to their death. The magnitude of the temperature rise depends on the ultrasound beam features as well as on the acoustic properties of tissues exposed to ultrasound and blood perfusion rate.

The goals of this study are 1) to model numerically in space and time the temperature field induced in multilayer configuration of media, relevant to real conditions of the neuro-degenerative disease treatment in rat liver, by a focused transducer with a circular aperture, 2) to examine the dependence of temperature elevations on the ultrasonic regime of the transducer (tone burst intensity, duration, duty cycle and exposure time) and 3) to compare the measurement data with the numerical predictions obtained under experimental boundary conditions to verify validity and accuracy of the model proposed. The pulsed acoustic pressure fields in two-layer configuration of media: water – rat liver were calculated using our original 3D (2D space + time) numerical solver for sources with axial symmetry. For prediction of temperature fields the Pennes bio-heat transfer equation was solved numerically.

1. NUMERICAL METHODS

It has been assumed that the positive z axis direction overlaps with the direction of the acoustic wave propagation. The circular spherically focused transducer with a radius a, focal length F and centre frequency f_0 is located in the cylindrical coordinate system as shown in Fig. 1.

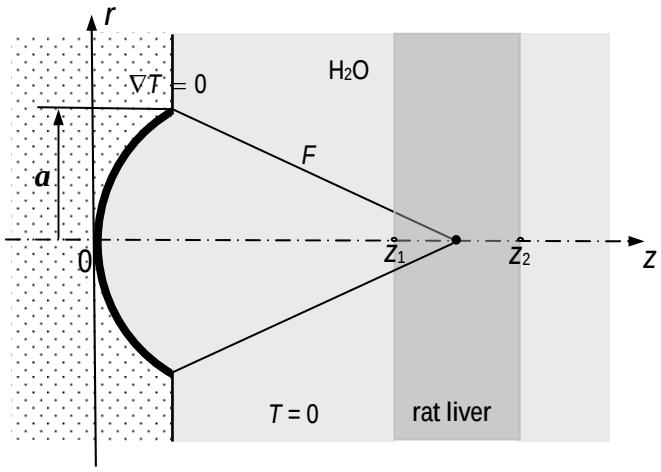

Fig.1. Geometric scheme for calculation the pressure and temperature fields induced in three-layer configuration of media: water - rat liver - water by circular focused transducer considered

The transducer is immersed in water and generates tone bursts with the intensity I_0, centre frequency f_0, pulse duration Δt and duty cycle d. The rat liver sample with thickness $l = z_2 - z_1$ is inserted in water perpendicularly to the acoustic beam axis. The water – sample interface is located at the specific axial distance z_1 from the face of the transducer. Then the focal plane occurs within the tissue sample. In previous publications [2, 3] have been shown that for any circular transducer with $ka >> 1$ and weak to moderate source pressure level the axial distance z_1 at which sudden growth of the second harmonics begins (for the tone burst generated from that transducer in water and propagating there) is specific for that transducer and constant independently on the source pressure amplitude. The weak to moderate source level means that in the nonlinear acoustic field produced by the source in water the ratio of the shock formation distance l_D to the Rayleigh distance R_0 is larger than about 0.3 [4].

The acoustic parameters of a rat liver are marked by the subscript s.

1.1. *Acoustic pressure field calculation*

In order to calculate pulsed acoustic pressure field generated from the circular focused source in multilayer nonlinear attenuating media with arbitrary attenuation frequency-dependence law the original 3D (2D space + time) numerical solver, recently developed in our lab, was used. The solver is a computer implementation of the numerical model based on the TAWE approach [5] to solve numerically the second order nonlinear differential wave equation for axisymmetric acoustic sources generating finite-amplitude tone bursts in a thermo-viscous fluid. The numerical model accounts for the effects of diffraction, absorption and nonlinear interactions of harmonics. The solver developed was a powerful research tool capable of fast predicting the 3D pulsed acoustic pressure fields produced in multilayer configuration of media by circular transducers driven by tone bursts of various frequency, intensity, duration, duty cycle and exposure time.

The model requires a number of input parameters that characterize boundary conditions of the source and media of propagation. The source parameters required for the model are: effective radius, focal length, pressure amplitude on the surface, apodisation function of radiating aperture, initial tone burst centre frequency, duration, envelope function and duty-cycle. The source parameters used for calculations were measured in water and are presented in Table 1. The media parameters required for the model are: density, sound velocity, attenuation coefficient and its frequency-dependence law, nonlinearity parameter. The

acoustic parameters for water and for rat liver being tested at physiological temperature were taken from the literature. The resulting data used in the numerical model are given in Table 2.

Tab.1. The values of the source parameters used in the numerical model

Effective radius a (mm)	**Focal length** F (mm)	**Centre frequency** f_0 (MHz)	**Source pressure** P_0 (MPa)	**Focusing gain** G	**Pulse duration** (cycles)	**Duty cycle** (%)
7.5	25	2	0.1 ÷ 0.225	9.4	20 ÷ 500	20

Tab.2. The values of the media parameters at 37 ^{o}C temperature used in numerical model

Medium parameters		**Water**	**Rat liver**
Density	(kg/m^3)	993	1060
Sound velocity	(m/s)	1524	1615
Attenuation coefficient	(Np/(m· Hz^b))	$1.44 \cdot 10^{-14}$	$9 \cdot 10^{-6}$
Nonlinearity parameter B/A		5.6	7
Attenuation frequency-dependence index b		2	1
Specific heat	(J/(kg · ^{o}C))	4200	3600
Thermal conductivity	(W/(m · ^{o}C))	0.63	0.43

1.2. *Temperature field calculation*

In order to calculate temperature fields induced by ultrasound in biological tissues *in vivo* the Pennes bio-heat transfer equation [6] is used:

$$\rho_s C_s \frac{\partial T}{\partial t} = K_s \nabla^2 T - Q_b + Q_a + Q_m \ , \tag{1}$$

where ρ_s, C_s, K_s and T are the tissue density, specific heat, thermal conductivity and temperature, respectively, t depicts time, ∇^2 denotes the Laplacian. The first term on the right-hand side of Eq. 1 accounts for heat diffusion, the second term is responsible for losses due to blood perfusion, Q_a is the rate of heat sources production per unit volume due to ultrasound absorption and Q_m is the metabolism rate.

For tissues *in vitro* the accounting for the effects of both the metabolism and blood perfusion rates can be omitted. Then the equation (1) takes a form:

$$\rho_s C_s \frac{\partial T}{\partial t} = K_s \nabla^2 T + Q_a \ . \tag{2}$$

According to [7] Q_a is expressed as:

$$Q_a = \frac{P_0^2}{\rho c} \sum_{n=1}^{N} \alpha(f_r) \cdot n^b \left| C_n \right|^2 \ . \tag{3}$$

Here P_0 is the source pressure, $\alpha(f_r) = \alpha_1 \cdot f_r^{\,b}$, where α_1 is the sound absorption coefficient of the medium at 1 MHz, f_r is the pulse repetition frequency, b is the power index of the

frequency-dependence absorption law ($b = 2$ for water and $b = 1 \div 1.3$ for biological tissues), C_n is the amplitude of the n-th spectral component, $n = f / f_r$ is the number of the spectral component. Equation (2) has been solved numerically for the focused source with axial symmetry generating tone bursts in multilayer nonlinear attenuating media. To solve the problem the thermal insulation boundary conditions were assumed. First, the temperature gradient at the interface: source–water is assumed to be equal zero. Next, the acoustic energy is concentrated at the focus of the beam, therefore the temperature elevation around the focus area is large while at the peripheries, far from the acoustic beam axis, the temperature elevation is infinitesimal (see Fig. 1).

The original 3D (2D space + time) numerical solver, being the computer implementation of the numerical model, capable of predicting temperature fields induced in multilayer biological media during their exposure to pulsed focused ultrasound beams was developed. The solver requires a number of input parameters that relate to the media acoustic and thermal properties. The media thermal parameters required are: the heat capacity and thermal conductivity. The values of these parameters for the rat liver were taken from Holmes [8], the values of acoustic parameters were taken from [9] and are quoted in Table 2.

2. MATERIALS AND METHODS

2.1. *Experimental Facility*

The block-scheme of the experimental facility used for measuring the temperature fields induced in soft tissues by focused acoustic tone bursts is shown in Fig. 2.

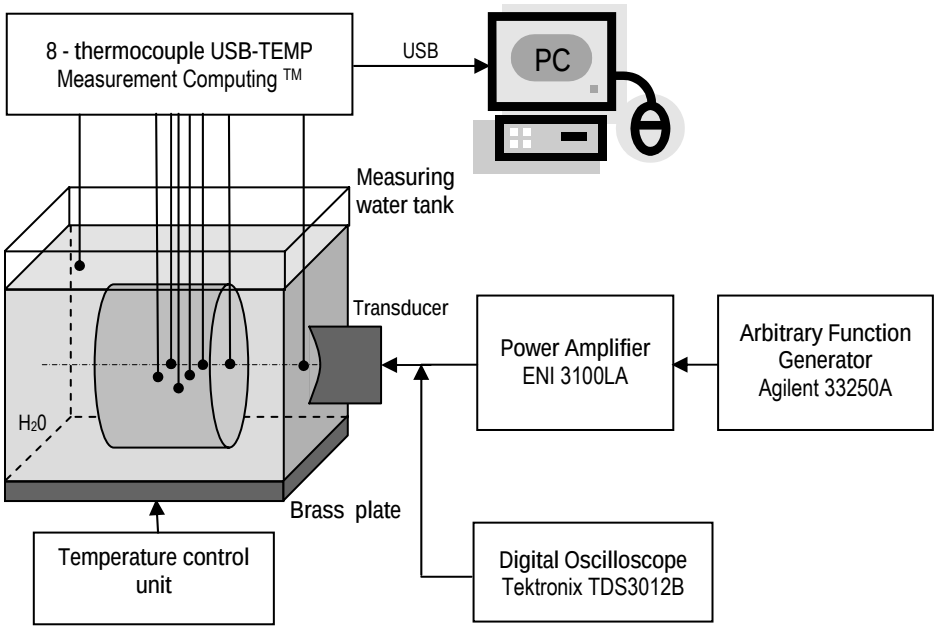

Fig.2. The experimental facility employed to make temperature field measurements

The acoustic pressure tone bursts were generated at the surface of the PZT (Ferroperm Pz26, Denmark) circular focused transducer with a resonance frequency $f_0 = 2$ MHz, radius $a = 7.5$ mm and focal length $F = 25$ mm. The transducer was air-backed, had not a quarter wavelength matching layer and was driven at its resonance frequency by tone bursts with

varied number of cycles (20, 50, 100, 500) at the same duty cycle d = 20 %. The transmission electronics were based on an arbitrary function generator (Agilent 33250A, Colorado Springs, USA) that defined the generated tone bursts frequency and duty cycle. Then the signal was amplified with a power amplifier (55 dB gain) ENI 3100LA (ENI, Rochester, NY, USA). The waveform of the tone burst was displayed with a Tektronix TDS3012B digital storage oscilloscope (Tetkronix, Beaverton, USA). The output of the amplifier excited a piezoelectric transducer to produce the pulsed ultrasound field. The voltage applied to the transducer was varied from 31.6 V_{pp} to 61 V_{pp} to produce the average acoustic power on the transducer face verying between 0.8 W and 3 W. The acoustic power was measured with a power balance (Ohmic Instruments UPM-DT-1E, Easton, USA). The transducer was mounted in a water tank with controlled temperature. The source pressures were determined using two methods. First, with a calibrated bilaminar PVDF membrane hydrophone (Sonora Medical Systems Inc. SN S5-153, preamplifier P-159, Longmont, CO, USA) with a 0.513-mm diameter sensitive element and next, with a power balance. The measurements with the hydrophone were carried out in water at a 5-mm distance from the transducer face at 37 ^{o}C temperature and gave the average pressure amplitudes at the transducer face varied from 0.116 MPa to 0.226 MPa. The agreement between two methods was within 10 %.

All rat liver samples being tested were obtained from the breeding ground of the Institute of Experimental Medicine PAS and stored in 1% saline solution. Experiments were done within 2 hours after slaughter. The rat liver was degassed and inserted in a cylindrical container with a 30-mm diameter and 20-mm height. The container had a transparent for sound, 20-μm thick polyethylene foil stretched over each end and was immersed in saline solution tank. The axis of both the sample container and transducer overlapped. The distance z_1 between the transducer centre and the water - rat liver sample interface was specific for the transducer considered and equal to 17 mm. In previous publications [2] it was shown that for circular sources with ka >>1 producing in water weak to moderate nonlinear fields the axial distance at which sudden growth of the natively generated second harmonics occurs is specific for this source and constant independently on the source pressure. In this connection, the distance z_1 was chosen as the distance at which sudden growth of the second harmonics occurs for the tone burst generated from the transducer considered in water (see Fig. 3). The thermocouples with a diameter of 200 μm were inserted in the tissue sample using thin, 0.6-mm diameter hypodermic needles fixed on the tank cover in order to ensure their parallelism and precise position along and across the acoustic beam axis. The uncertainty of the thermocouple tip position was ± 0.5 mm. Due to small diameter of thermocouples their influence on measurement results were negligible. The 8-thermocouple module was used to detect the temperature rises induced by ultrasound in rat liver at chosen points on and off the acoustic beam axis. The thermocouple tips were placed in the nodes of the 5-mm size grid to avoid the thermocouples interaction influence on measurement results.

All measurements were performed at 37 ^{o}C in the temperature-controlled 1-% saline solution tank and complied with the ethical regulations regarding animal research. The temperature variations detected by thermocouples were recorded *versus* time by the USB-TEMP unit (Measurement Computing, Norton, USA) with 1 second step and transferred to the PC memory *via* USB slot. For processing and visualizing the data obtained the software TracerDAQ was used. In order to meet the requirements of the LIFU applications, such as heat-responsive gene therapy or neurodegenerative diseases treatment through the Hsp expression enhancement, the temperature elevation induced by ultrasound beam in tissue *in vivo* at the focal area should not exceed 43 ^{o}C.

3. RESULTS AND DISCUSSION

The 3D (2D space + time) temperature elevations *versus* time in the fresh rat liver samples irradiated by the pulsed LIFU generated from the circular focused transducer with a 2 MHz centre frequency, chosen dimensions and varied ultrasonic regime parameters were measured. The ultrasonic regime parameters varied as follows: the source acoustic intensity I_{SPTA} was varied between 0.452 W/cm^2 and 1.7 W/cm^2 (acoustic power N_0 = 0.8 W ÷ 3W), tone burst duration Δt = 20 ÷ 500 cycles at the same 20-% duty cycle, exposure time t = 0.5 ÷ 20 min.

The 3D temperature fields induced in the fresh rat liver by the LIFU of varied level were predicted by calculation first, the acoustic pressure field using the TAWE approach [4] to the numerical solution of the second order nonlinear wave equation and next, the temperature field using the numerical solution of the Pennes bio-heat transfer equation (2) for tissues *in vitro*. The input parameters required for the numerical models related to the source conditions were measured, related to the media of propagation were determined experimentally or taken from the literature and are tabulated in Table 1 and Table 2.

In order to determine the distance z_1 the axial pressure variation of the fundamental and two higher harmonics for the tone burst generated in water from the transducer considered was calculated. The distance z_1 was determined as the axial distance at which sudden growth of the 2nd harmonics appears. Fig. 3 shows the axial pressure distribution of the 1st, 2nd and 3rd harmonics for the 8-cycle tone burst with various initial pressure propagating in water.

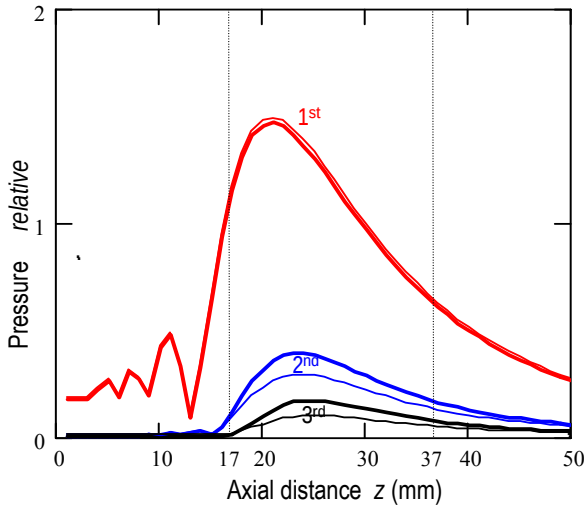

Fig.3. Axial pressure distribution of the 1st, 2nd and 3rd harmonics for the 8-cycle tone burst with the initial pressure amplitude P_0 = 0.412 MPa (thin lines) and P_0 = 0.583 MPa (thick lines) generated from the circular focused transducer considered and propagating in water

It is evident from Fig. 3 that sudden growth of the 2nd and appearance of the 3rd harmonics occurs at the axial distance z = 17 mm from the source independently on the source pressure. In this connection the path-length of the tone burst propagating through the water layer between the transducer centre and the input window of the tissue sample was determined to be z_1 = 17 mm (see Fig. 3). All calculations were done for the beams produced in the three-layer configuration of parallel media: 17-mm layer of water □ 20-mm layer of tissue □ 13-mm layer of water. The calculation and measurement results are presented in Figs. 4 - 6.

Figure 5 shows an example of the axial acoustic power variation (averaged over the repetition period) for the tone burst with the initial pressure amplitude p_0 = 0.184 MPa (I_{SPTA} = 1.13 W/cm^2) propagating through the three layer structure of media.

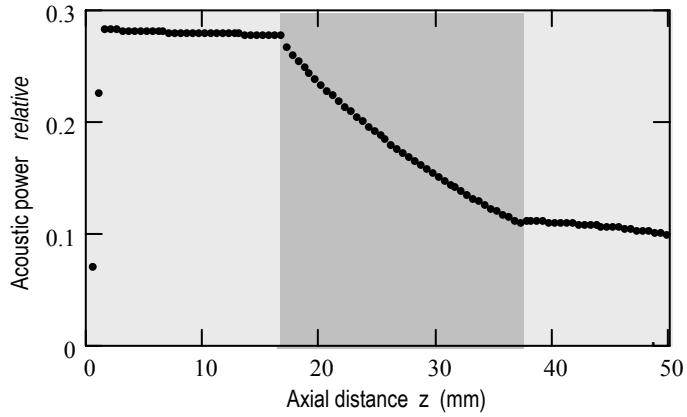

Fig.4. Axial variation of the acoustic power for the 20-cycle tone burst with initial intensity of 1.13 W/cm^2 and 20% duty cycle propagating through the three-layer configuration of media: 17-mm water layer - 20-mm rat liver layer - 13-mm water layer

The temperature field pattern in the (r, z) plane for the pulsed acoustic beam from Fig. 4 produced in three layer structure of media considered after the 20-min heating is shown in Fig. 5.

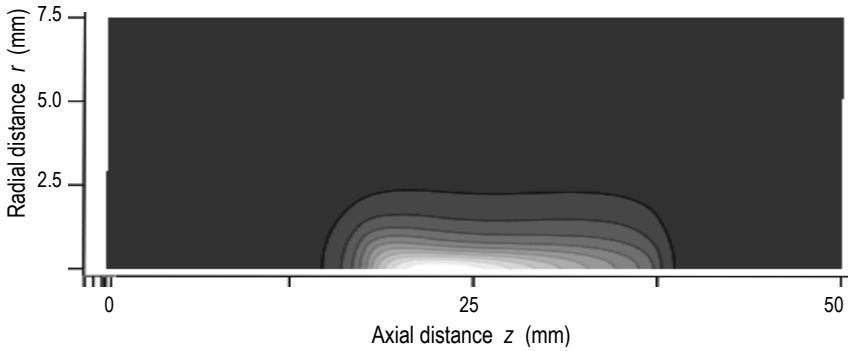

Fig.5. Grey-scale pattern of temperature field T (r, z) induced in three-layer configuration of media water - rat liver - water by the acoustic pressure field generated from the transducer considered after 20-min exposure time. The source pressure amplitude p_0 = 0.184 MPa

Fig. 6 illustrates the temperature elevations *versus* time measured and simulated numerically at the focus (z = 21 mm) and at 3 other points on the acoustic axis for the tone bursts with the initial intensity of I_{SPTA} = 1.13 W/cm^2. It is evident from this figure that at the first stage of heating the temperature accretion rate predicted theoretically overestimates (within 1 ^{o}C) those measured. The discrepancy decreases with exposure time extension. After 20-min heating the calculated and measured curves overlap. Good agreement between the theoretical results and measurement data for all cases considered has verified the validity and accuracy of our numerical model.

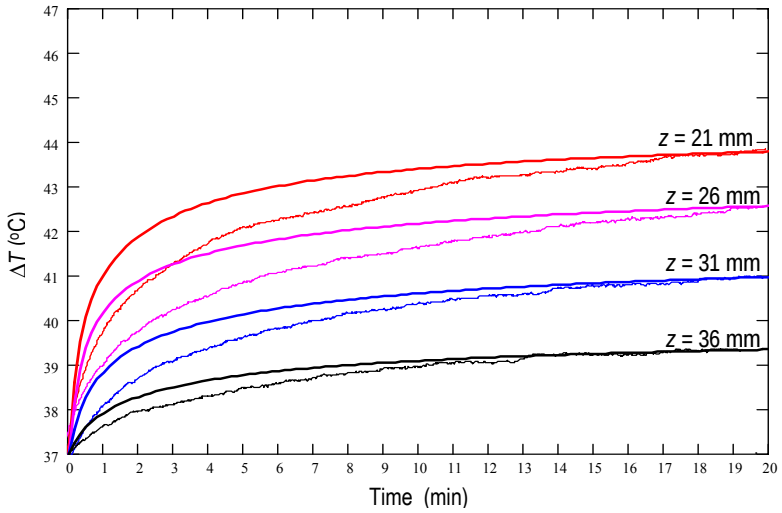

Fig.6. Temperature elevation profiles *versus* exposure time at the beam focus (z = 21 mm) and at 3 other points on the acoustic axis. Calculated (solid lines) and measured (points) results for the 20-cycle tone bursts with initial intensity I_{SPTA} = 1.13 W/cm^2 and 20 % duty cycle

4. CONCLUSIONS

The effect of the pulsed ultrasonic beam properties, determined by the initial tone burst pressure amplitude, duration, duty cycle and exposure time, on the temperature rise in the sample of rat liver immersed in water were investigated theoretically and experimentally. The acoustic parameters of the rat liver and water were taken from literature. The theoretical results were compared with those measured during 20-min exposure time and fitted to them by adjusting the values of the rat liver thermal conductivity, specific heat, absorption coefficient and its frequency-dependence power law. The quantitative analysis of the obtained results has shown that the numerical model is most of all sensitive to thermal conductivity and attenuation measurements accuracy. The experimental results enabled estimation of time and level of tissue exposure to pulsed ultrasound beams generated from a 2 MHz circular focused transducer with a 15-mm diameter and 25-mm focal length in order to avoid the temperature rise of tissue above 37 ^{o}C by more than 6 ^{o}C. As shown in Fig. 6 in order to avoid the rat liver overheating the exposure time for beam with intensity 1.13 W/cm^2 should not exceed 10 min.

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