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Mitochondrial respiration inhibition after exposure to UWB pulses as a possible mechanism of antitumor action

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Abstract. The respiration of isolated mice liver mitochondria after exposure to nanosecond UWB pulses (0.15 – 36 kV/cm, 0.6 – 1.0 GHz centre frequency, 3 – 20 ns pulse duration) has been investigated. The respiratory control (RC, the ratio of oxygen consumption) was estimated. The possibility of mitochondrial membrane electroporation was detected as the decrease in the electrical resistance, according to the β-dispersion of the electric current. The monotonous decrease of RC after 1000 UWB pulses from 0.15 kV/cm was observed, the ohmic resistance of mitochondria suspension was reduced. The obtained data indicate the inhibitory effect of UWB pulses on a state of irradiated mitochondria and its membrane.

1. Introduction

It is known now that a pulsed ultra-wideband (UWB) radiation efficiently influences the biological objects. The UWB pulses of a microsecond duration have been used already for a tumor therapy in a combination with a chemotherapy, for an efficient targeted delivery of the chemotherapy, according to the mechanism of tumor cell membrane electroporation [1]. Even one nanosecond pulse of an electric field (20 kV/cm or more) can cause abnormalities in the development of irradiated fish eggs [2]. The nanosecond and ultrashort pulses of the UWB radiation (tens of kV/cm) can damage the membranes of intracellular organelles and temporary (reversible) violate the integrity of the plasma membrane, as well essentially increase the level of free intracellular calcium. All of them affect the level of cellular metabolism [3]. It is also known that microsecond electric pulses may reduce an ATP level in the irradiated cells [4]. The reducing amount of ATP, first, must be associated with a decrease in the production of ATP (by uncoupling of the oxidation and phosphorylation, and disruption of the electron transport chain (ETC) in the inner mitochondrial membrane). The UWB pulses are characterized by short pulse durations (tens of nanoseconds or shorter) and high pulse powers, and they should provide even more efficient action on the biological structures, such as mitochondria, and exercise their influence in a similar way. Mitochondria are the major cell organelles, in which the free chemical energy of food substrates is transferred and stored into the energy ATP molecules and further is expended for metabolic needs of a cell. From this point of view, it would be reasonable to study the effect of nanosecond UWB pulses on the coupling of oxidation and phosphorylation of isolated mitochondria (via assessing the intensity of mitochondrial respiration), as well as to identify a dependence of the effects on the field parameters and the number of affecting pulses.

2. Materials and methods

The mitochondria of an outbred white mice liver, which was isolated by the differential centrifugation [5], were used for the experiments. The suspensions of mitochondria were once exposed to 16 – 1000 nanosecond UWB pulses with a frequency of 0.6 – 1.0 GHz, a duration of 4 – 25 nanosecond, an amplitude of 0.1 – 36 kV/cm, and a pulse repetition rate of 13 pules per second (pps). Generation of high-frequency pulses was carried out by exciting the gyromagnetic precession in a nonlinear transmission line with a saturated ferrite. The RF pulse from a nonlinear transmission line was converted from a coaxial TEM mode into a TE_{01} mode of a rectangular waveguide by a specially designed converter [6-7]. The Eppendorf test tube with a suspension was placed in the center of the waveguide, where the amplitude of the electric field had a maximum value. The RF pulse was absorbed in the alcohol load after interacting with the sample. The mitochondria respiration was measured by an oxygen meter AKPM-02 (Russia). The respiratory control (the ratio of oxygen consumption) was calculated. The possibility of mitochondrial membrane electroporation was detected as a decrease in the electrical resistance, according to the β-dispersion of the electric current (measured by the LCR meter, Belarus). The irradiated and the sham mitochondrial suspensions were used. The sham suspensions were subjected to the same manipulations, as irradiated one, but without activating the radiation source. Six replicates were performed for each exposure experiment and sham. Statistical comparisons were made using the nonparametric Mann-Whitney test in Statistica 6.0 software.

3. Results

The respiratory control by Chance [8] is the most informative indicator for the analysis of a mitochondria functioning. The RC is equal to the ratio of the mitochondrial oxygen absorption rate in the presence of the ADP to the oxygen absorption rate after the exhaustion of the ADP in suspension. The RC equal to 5 shows a good and efficient conjugation of the oxidation and the phosphorylation [5] for our isolated mitochondria (Figure 1, sham).
It was found that the nanosecond UWB pulses affect the rate of mitochondrial respiration. The effect was shown in a decrease of the oxygen consumption by mitochondria in the presence of ADP. As a result, the value of the RC was decreased. The effect depends on the electric field. The RC decreased monotonically with increasing a field strength to a value of 0.15 kV/cm or more (Figure 1). This indicates the inhibitory effect of the UWB pulses on mitochondria.

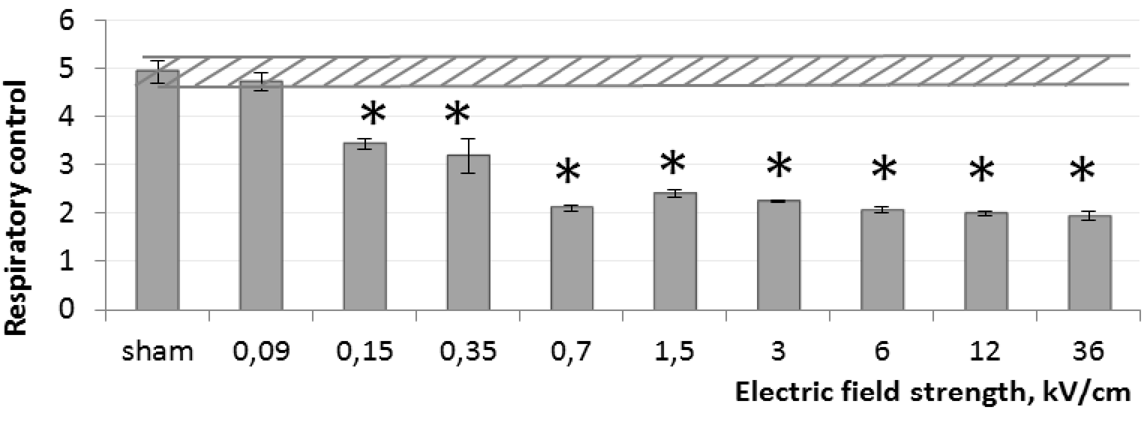

Figure 1. Changes of the RC value after exposure to 1000 nanosecond UWB pulses with field of 0.09–36 kV/cm per pulse. Shaded space is ± standard error of the mean of RC for sham mitochondria. * – differences of indicators are significant as compared with sham (p≤0.05).

The obtained effect of the respiration inhibition could be caused either by an electric field or by a total absorbed UWB radiation energy. Clarifying experiments were conducted in order to determine the contribution of the energy impact on the effect. In these experiments, the effect was measured after an exposure to the pulses of different intensity and different amounts, provided that the energy of RF pulses in the bunch (the RF power * pulse number = const) was the same. The RF energy was measured according to the waveforms from the E-sensor in the waveguide. It was found that the effect of the electromagnetic pulse with the same total energy from the UWB source provided the formation of an equal biological effect. In particular, there was a 30% reduction of the RC after an exposure to the UWB pulses with an incident total energy of 2.5 mJ per session (Figure 2A) and a 61% reduction after an exposure to the UWB pulses with a higher incident total energy of 10 mJ per session (Figure 2B). Thus, the increasing of energy leads to an increase in the biological effects.

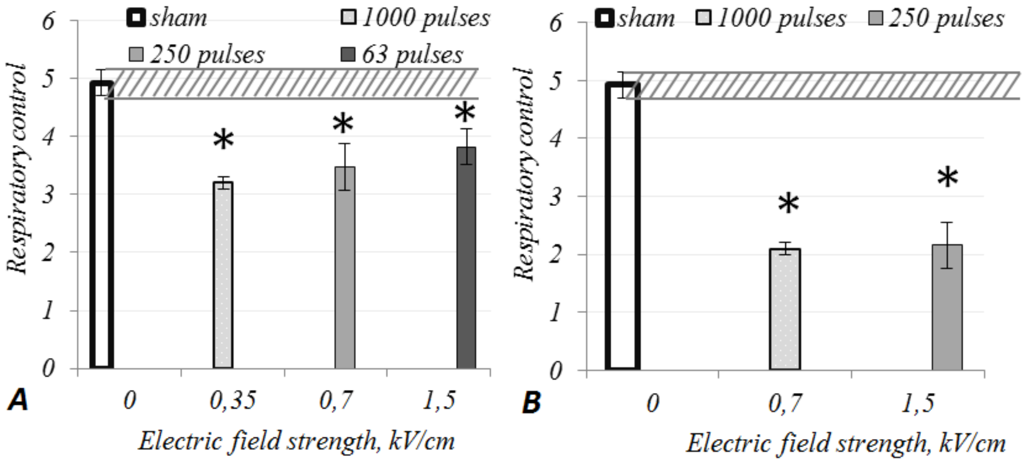

Figure 2. RC changes after exposure to 1000 nanosecond UWB pulses with incident total energy of 2.5 mJ (***A***) and 10 mJ (***B***). (Field intensity from 0.35 to 1.5 kV/cm per pulse, the number of pulses from 1000 to 63, center frequency 0.6 GHz, pulse duration 25 ns). Symbols are similar to Figure 1

The drop of the RC indicates a decreased conjugation of an oxidation and phosphorylation, as well as a broken integrity of the mitochondrial membrane. Under such conditions, it should be a disturbed ATP production by the irradiated mitochondria. A confirmation of this assumption validity could be the data of Frandsen S.K. [4], in which the decrease in the ATP level after an exposure to microsecond pulses was described. The authors of the work linked this effect with an electroporation of the cell membranes and the intracellular structures. Our additional experiments were conducted to check the possibility of an electroporation after the UWB pulses. In those experiments, the resistance of a mitochondrial suspension to the alternating current (on AC frequencies 10^4-10^6 Hz, β-dispersion) was measured. The resistance to a current of such frequencies characterized the physicochemical state of the biological membranes. It has been found that the UWB reduces a resistance of mitochondria, measured at frequencies of β-dispersion. In particular, an exposure to 1000 pulses of the UWB with a field of 3 kV/cm reduced an ohmic resistance by 23%. The observed decrease in the mitochondrial membrane resistance may be due to the formation of additional structures of a nonspecific conductivity (electropores) induced by the exposure to the UWB radiation.

4. Conclusions

The obtained data indicate that the UWB pulses of a nanosecond duration reduce the level of the mitochondrial respiration and break the oxidative phosphorylation coupling. The observed changes could be due to a break of a membrane structure in some irradiated mitochondria.

The reduced mitochondrial suspensions resistance after the exposure to UWB pulses is a result of the electroporation of a lipid bilayer of an inner mitochondrial membrane. This should lead to a decrease in a proton potential on the inner mitochondrial membrane, and consequently to the oxidation and the phosphorylation uncoupling, and therefore, reduce the RC. As a result, it can affect either changes in a cellular metabolism or a deficiency of the ATP, or the generation of reactive oxygen species (as a result of a disruption of the respiratory chain), or the oxidative modification of the lipid and protein components of mitochondrial membranes [9, 10]. Moreover, the RC reduction may be due to the loss of mitochondrial cytochrome *c* ratio or a shift of the ATP synthetase /ATPase activity relations (caused by a decrease of the proton potential) [10, 11]. All these processes can explain the observed decrease in the oxygen consumption of mitochondria, reducing the RC and the ohmic resistance of mitochondrial suspensions.

Nevertheless, the effects depended on the electric field and the number of pulses, as well as on the total energy per pulse. This circumstance is consistent with the opinion of T. Schunc [12] that it is the amount of energy, which determines the efficiency of the electroporation. The temperature of irradiated samples increases due to the energy growth, and then the increase in the effect, upon more energy absorption, can be explained by the addition in a mobility of fatty acid residues of the mitochondrial membrane lipids, which will provide the increase of the electroporation probability. Changes of intracellular processes are possible in cases when the UWB radiation affects the functioning of mitochondria. This makes it possible to control the metabolism of cells, including tumor cells, by the UWB radiation with optimal parameters. The observed dependence of the effect on the number of pulses is fundamentally important because it will minimize the effect on any cell (including the cells around the tumor) while retaining the required result. In addition, a research in this area could contribute to the development of the perspective methods of a directed transport of biologically active substances into cells using the electroporation induced by the UWB exposure.

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