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THE INFLUENCE OF LOW-INTENSITY ULTRASOUND ON THE PROPERTIES OF CELL CULTURES

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In the present paper bovine kidney cell cultures were used as an experimental model for the study of the biophysical mechanism of ultrasonic action.

In the first series of experiments functional and morphological changes in the cells immediately after sonication were evaluated. A decrease in viability and degenerative morphological changes in the cells were found.

In the second series the sonicated cells were seeded in Roux bottles and grown in the optimal conditions. The growth properties of the cells were evaluated at different time intervals after sonication. Significant stimulation of the cell growth was demonstrated after the action of ultrasound intensity of 1.0 kWm^{-2}. However, after the action of ultrasound intensities above 3.0 kWm^{-2} the inhibition of the cell growth was found.

1. Introduction

Increased use of ultrasound devices in medical diagnostics, especially in obstetrics, has accentuated the question of its possible risk for the patient. In the 1976 WHO report [5] the value of 1 kWm^{-2} of ultrasound SATA intensity was recommended as the threshold of biological effectiveness "in vivo". However, changes in some morphological and functional properties of sensitive biological systems, for example isolated cells and cell cultures at ultrasound intensities lower than the recommended threshold of biological effectiveness, were found.

The effects observed in mammalian cells after ultrasound exposure include modification of macromolecular synthetic pathways, alteration of cell membrane properties, intracellular ultrastructural changes and alteration of the growth properties.

Ultrasonically induced functional alterations in the plasma membrane showed increased permeability, decreased active and non-mediated transport and changes in the electrophoretic mobility of cells. A mechanical stress mechanism of ultrasound action was suggested as the cause of an increase in the permeability of human erythrocyte membranes to potassium ions which were described by LOTA and DARLING [10]. BUNDY *et al.* [2] demonstrated a decrease in the transport of leucine in avian erythrocytes. In our laboratory [1], [7] there were described alterations of the electrophoretic mobility of erythrocytes treated with diagnostic ultrasound and changes in the aggregation ability of erythrocytes in polyethylene glycol solutions. SIEGEL *et al.* [11] reported that dispersed cultured human cells seeded in plastic Petri dishes showed significantly reduced cellular attachment after diagnostic ultrasound exposure.

Numerous reports have appeared describing ultrastructural damage to cells exposed to ultrasound. Electron microscopic examination of rat liver cells and fibroblasts irradiated with pulsed ultrasound revealed more free ribosomes, increased damage to mitochondria, endoplasmatic reticulum and lysosomal membranes and more cytoplasmic vacuolation [4], [6]. It has been suggested that cells are particularly susceptible to damage by ultrasound during mitosis, because major changes in the cell membrane and internal structure occur during this phase of the cell cycle [3].

2. Materials and methods

In the present study the Madine-Darby bovine kidney and primary calf kidney cell cultures were used as an experimental model for a complex study of the biophysical mechanism of ultrasonic action. The cell cultures were grown in the Minimum Essential Medium supplemented with 5-10 % calf serum in Roux bottles. Suspensions of approximately 10^6 cells in millilitre were prepared using trypsin.

The source of ultrasound was a laboratory CW generator operating at a frequency of 0.8 MHz. The sonication of cells over the ultrasound intensity range from 0.5 kWm^{-2} to 10.0 kWm^{-2} in the horizontal field in a 37°C water bath was carried out for 10 or 20 minutes. The incident intensity levels were controlled by means of callibrated hydrophone.

The morphological changes in sonicated and control cells were evaluated using both the optical and the electron microscope. The viability of cells was tested using trypan blue vital dye.

3. Results

Our preliminary experiments were directed to morphological changes in cells caused by sonication of cell monolayers at very low intensity levels of ultrasound (i.e. 0.5 kWm^{-2} and 1.0 kWm^{-2}). In micrographs of sonicated cells, disap-

pearing of protoplasmatic bridges, rounding off and desquamation of cells were proved.

In the first series of experiments the morphological and functional changes in Madine-Darby bovine kidney cell suspensions immediately after sonication were evaluated. One part of the sonicated cells was fixed using glutaraldehyde and prepared for electron microscopic examination. The other part of sonicated cells was incubated with trypan blue vital dye. After three minutes the viability of cells was tested. Electron microscopic examination of cells revealed ultrastructural damage to cells exposed to ultrasound of low intensity level. There were observed enlargement and damage to mitochondria, enlargement of endoplasmatic reticulum and vacuolation. Incubation of cells with trypan blue vital dye demonstrated changes in viability of cells after ultrasound exposure. In control conditions 13.9 per cent of cells were stained. Using ultrasound intensity of 1.0 kWm^{-2} 25.4 per cent of cells were stained and finally using ultrasound intensity of 5.0 kWm^{-2} 51.0 per cent of cells were stained.

In the second series of experiments the sonicated primary calf cells were seeded in Roux bottles and grown in the optimal conditions. The growth and morphology of the cells were evaluated microscopically at different time intervals after sonication, up to three weeks. The action of ultrasound of intensity above 3.0 kWm^{-2} caused the death of all sonicated cells. However, there was a difference in the behaviour of cells sonicated at different intensities of ultrasound. Cells exposed to ultrasound intensity of 3.0 kWm^{-2} or 5.0 kWm^{-2} were able to attach to the glass surface of Roux bottle, but could not divide themselves. Cells exposed to ultrasound intensity of 10.0 kWm^{-2} were not able to attach to the glass surface and died immediately. On the other hand, the action of ultrasound intensity of 1.0 kWm^{-2} stimulated significantly the growth of sonicated cells and formation of the monolayer. The control and sonicated cell cultures were observed over 8 passages after sonication and the difference in growth mentioned above was expressed over the whole period of observation.

4. Discussion

LIEBESKIND *et al.* [9] described morphological changes in the surface characteristics and post-sonication ultrastructural changes in cell cultures after pulsed diagnostic ultrasound exposure. The cells were examined up to 37 days after a single exposure and authors demonstrated abundant microvilli and cell pro-ections, indicating a hereditary change in the cell membrane after at least 50 generations (cell cycle time 16 hours). Shape changes in sonicated erythrocytes were described by HRAZDIRA [8]. The postsonication ultrastructural changes [9] included an increasing number and clustering of perichromatin granules, invagination of cytoplasm into the nuclear domain, separation of nuclear membrane leaflets and aggregation microtubules around the nucleus.

The authors showed that up to one week after sonication the cells continued to divide normally, but there were dramatic differences in mobility and surface behaviour. The authors concluded that low level pulsed ultrasound could alter both the cellular ultrastructure and metabolism. They suggested that persistance of disturbances in cell mobility many generations after sonication was especially important, and it can be speculated that if embryonic cells were to be subtly damaged by ultrasound, it might affect cell migration during ontogenesis.

In our laboratory, in accordance with the results mentioned above the alterations of growth properties of sonicated cells were observed. However, in contrast with results of LIEBESKIND *et al.* [9] significant stimulation of cell growth was demonstrated after the action of ultrasound intensity of 1.0 kWm^{-2} during 8 passages after sonication. Microstreaming and shear stress were suggested as the main components of ultrasonic action on cell culture growth stimulation.

5. Conclusions

In the first series of our experiments the Madine-Darby bovine kidney cell cultures were used. The decrease in viability and the degenerative morphological changes in cells immediately after the action of low intensity ultrasound were proved.

In the second series of experiments the primary calf cell cultures were sonicated using ultrasound intensity range from 1.0 kWm^{-2} to 10.0 kWm^{-2}. The growth properties of sonicated cells were evaluated at different time intervals after ultrasonic action. Significant stimulation of the cell growth was demonstrated after action of ultrasound intensity of 1.0 kWm^{-2} during 8 passages. However, after the action of ultrasound intensities above 3.0 kWm^{-2}, inhibition of the cell growth was found. In the future these findings would be completed by quantitative analysis of cell growth parameters with special respect to the behaviour of cells influenced by ultrasound over the intensity range from 1.0 kWm^{-2} to 3.0 kWm^{-2}. The results described have shown that further studies along this line are needed.

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