Effect of Exogenous Frequency Exposure on Human Cells

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Effects of exogenous bioresonance oscillations on biological and morphological characteristics of continuous human lung and liver cells were studied. Proliferative activities and viability of cells decreased after exposure to frequencies of 8 and 78.5 Hz. This was paralleled by cytopathic disorders in lung and liver cells. The frequency of 72 Hz exhibited a less intense effect on both cell types. Cell contamination with mycoplasmas was provisionally suppressed after cell exposure according to F44 and F45 programs and to 97.5 and 69.5 Hz frequencies.

Key Words: *bioresonance exposure; human cell strains; mycoplasmas*

The development of science and technology, threat of disasters, exposure to a variety of environmental factors, such as ionizing radiation and electromagnetic fields, inevitably affect the population health. Study of the effects of physical factors of different intensity and protection from damage inflicted by them deserve special attention. However, virtually no studies in this direction were performed on human and animal cell cultures.

Living organisms represent a self-organized open system based on wave fluctuation processes. Each structural unit of the organism is characterized by an intrinsic frequency spectrum, certain frequency rhythm, modification of which leads to appearance of a pathological process. Study of bioresonance exposure indicates unambiguous effects of various frequencies. In some cases the fluctuations are synchronized, which serves as a stabilizing factor, in others this can lead to destruction of body cells.

Unambiguous effects of bioresonance exposure on the formation of various nonspecific adaptation reactions of the organism prompted us to carry out experiments on various cell strains.

Studies of the effects of electromagnetic field radiation emitted by various sources and of protective devices on human and animal continuous cells have been carried out at Laboratory of Tissue Cultures of D. I. Ivanovsky Institute of Virology since 1997 [6].

Studies of Russian devices showed that AMM applicator stimulated the proliferation of some cells (chicken embryo fibroblasts, HeLa, Hep-2), while ROTAN devices inhibited the growth of Vero and HeLa cells, involving deep damage to cellular DNA. Exposure of human fetal lung (HFL) fibroblasts and HeLa calls to low-intensity superhigh frequency (SHF) millimeter waves at frequencies of 30-300 Hz and λ=5.6 mm also stimulated proliferation of these cells [2].

"Vita" protective device stimulated the growth of HFL cell culture and protected the cells from radiation emitted by the computer. The efficiency of these protective devices (AMM, ROTAN-700, VITA) normalizing the immune and interferon status of mice and protecting from negative effects of electromagnetic fields was demonstrated.

We previously studied the effects of exogenous bioresonance radiation on tumor cells [5] and cell contamination with mycoplasma [4,7].

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Here we studied the direct effects of exogenous bioresonance oscillations on different cell strains from the collection of D. I. Ivanovsky Institute of Virology.

MATERIALS AND METHODS

The following cells were used in the study: HFL, Chang liver cells, and two human hepatocarcinoma cell strains (Lunet and CH5).

Morphological changes in HFL and Chang liver cells were studied after staining with azur and eosin after Romanowskii after 72-h growth. Specimens for electron microscopy were prepared routinely (fixation, dehydration, impregnation, and embedding in epoxy resins). Ultrathin sections were examined under a 100CX microscope.

Mycoplasmas were detected in the cells by the olivomycin method developed at Tissue Culture Laboratory [3].

The cells were exposed using Miniekspert-DT device (IMEDIS) for 3 days at the intensity of 100 μT, 3 min daily [9]. The studies were based on the human vibration model [1,2]. Frequencies used in experiment were close to frequencies characteristics of individual organs and systems, determined previously. The selected frequencies corresponded to the tables developed by Yu. V. Gotovskii [1,2] for liver and lung cells. The cells were exposed at 8, 72, and 78.5 Hz (effect on cell metabolism).

The results were evaluated by proliferative activity of cells by estimating the proliferation index (PI), cell viability, and morphological changes in cell cultures.

RESULTS

Proliferative activity and viability of 4 cell strains (HFL, Chang liver, Lunet, CH5) were evaluated after exogenous exposure to frequencies of 8, 72, 78.5 Hz; the results are summed up in Table 1.

Proliferative activity and viability of cells decreased in all variants of experiments with exogenous resonance exposure of the studied cells, the reduction being most pronounced after exposure at 78.5 Hz, which modified cell metabolism [1,2]. Exposure at a frequency of 8 Hz slightly affected liver cells and exhibited a more pronounced effect towards lung cells. Exposure at a frequency of 72 Hz had a less intense effect on human lung and liver cells.

Morphological studies showed that control HFL culture at the level of passage 15 consisted of fibroblast-like cells with oval and elongated nuclei. The nucleoli were small, 2-4 per nucleus. The cytoplasm was finely reticular. The monolayer consisted of elongated cells, lying close to each other (Fig. 1, *a*). After passage 30, cell morphology changed: they became polygonal, with more round nuclei, honeycomb-like cytoplasm, the monolayer being incomplete.

Exposure of HFL cells (passage 15) to a frequency of 8 Hz was toxic for the cells, resulting in cell and nuclei destruction. Destruction of the cell layer, cell lysis, total, subtotal, and focal destruction of the culture with damage to cells and nuclei, such as modification of shape and size (shrinkage), cytoplasm vacuolation, chromatin margination, nuclear pyknosis and degeneration (Fig. 1, *b*), were observed. Loss of contact inhibition led to chaotic disposition of cells with formation of multilayer sites and eventually to cell death. A similar picture was observed after exposure to 78.5 Hz (Fig. 1, *c*).

No changes in cell morphology of this kind were observed after exposure of cells to a frequency of 72 Hz. Despite the heterogeneity of cell shape and size, and particularly of the nuclei, this variant was similar to the control by the predominant form (fibroblast-like) of cells and nuclei.

Experiments according to a similar protocol were carried on Chang liver cells (continuous human liver culture).

Control cultures primarily consisted of epitheliocyte-like and polygonal cells. Accumulations of nuclei of irregular shape and giant nuclei were seen. The cytoplasm was vacuolated; the nucleoli were large, 1-3 per nucleus (Fig. 1, *d*).

Exposure at a frequency of 8 Hz at the levels of passages 1 and 2 (3 sessions) caused no destruction of cells and nuclei so intense as in HFL cells. The monolayer was rarefied in comparison with the control. Spindle cells appeared; separate giant cells and nuclei and cytoplasm vacuolation were observed (Fig. 1, *e*).

Exposure at a frequency of 72 Hz did not impair the monolayer; the cells and nuclei changed little in comparison with the control, similarly as the HFL cells.

The monolayer did not form after exposure of cells at a frequency of 78.5 Hz. Separate cells of different shape and size were seen, mainly spindle and polygonal ones, and an appreciable number of giant cells and nuclei, sometimes fragmented. The cytoplasm was intensely vacuolated. Some cells underwent generation (Fig. 1, *f*). It seems that these cytopathic changes resulted from disorders in cell metabolism.

Electron microscopic studies confirmed the findings of morphological analysis.

Ultrastructural studies of HFL cells exposed to 8 Hz showed pathological changes in the morpho-

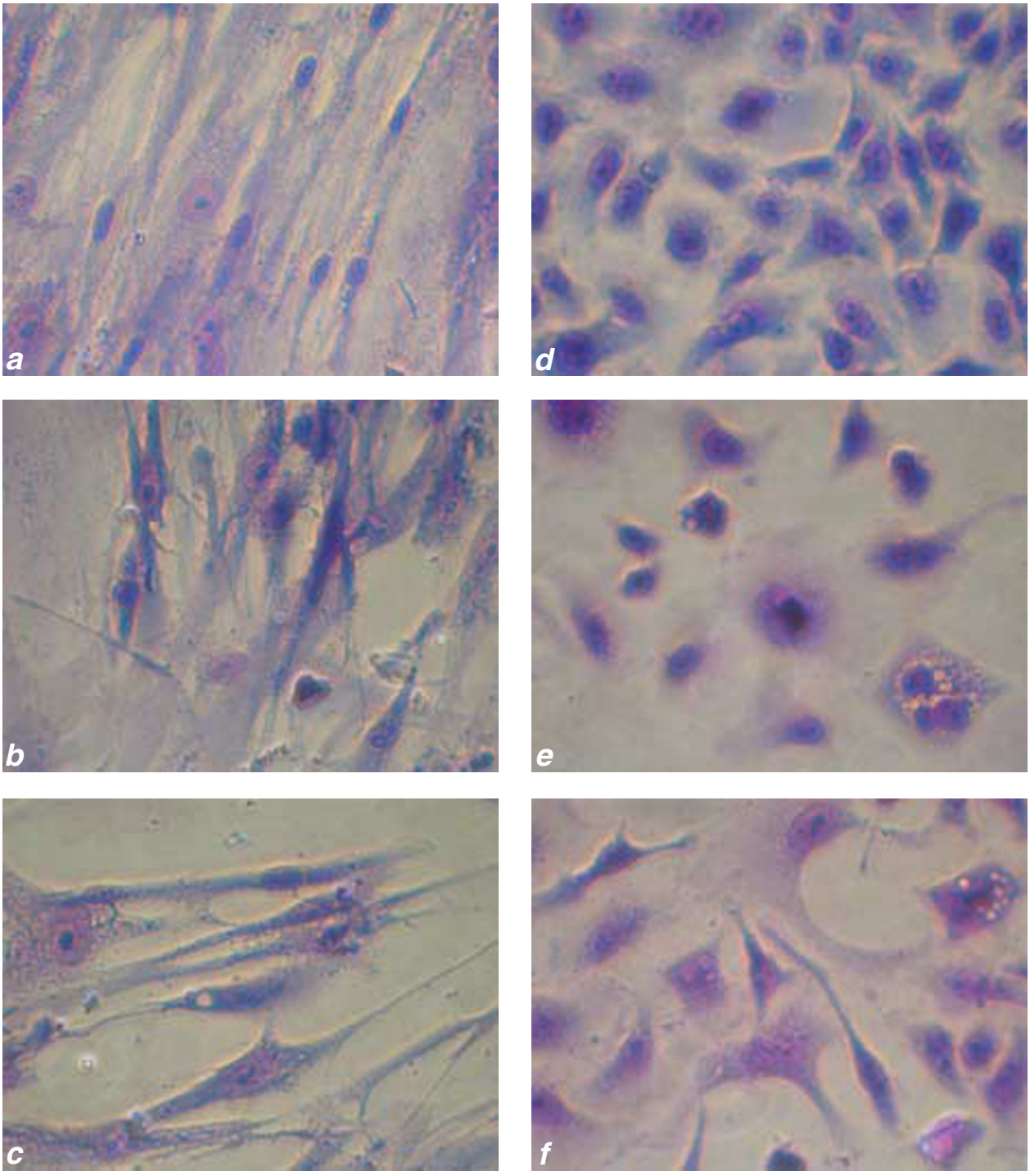

Fig. 1. Morphology of HFL (*a-c*) and Chang liver cells (*d-f*). *a*) control HFL culture, passage 15; *b*) HFL culture after bioresonance exposure at a frequency of 8 Hz; *c*) HFL cell culture after bioresonance exposure at a frequency of 78.5 Hz; *d*) control Chang liver cell culture; *e*) Chang liver cell culture after bioresonance exposure at a frequency of 8 Hz; *f*) Chang liver cell culture after bioresonance exposure at a frequency of 78.5 Hz. Hematoxylin and eosin staining (×400).

logy (structure of the contents) of the nuclei and cytoplasm in the majority of cells. Nuclear wrinkling and pyknosis, enlargement of the perinuclear space in the nuclear membrane were noted. Liquefaction and clarification of the hyaloplasm, numerous vacuoles, impaired structure of the endoplasmic reticulum, mitochondrial swelling, matrix condensation, and cryst destruction were seen in the cytoplasm. The structure of the cell plasma membrane was impaired in some cases (Fig. 2, *a*).

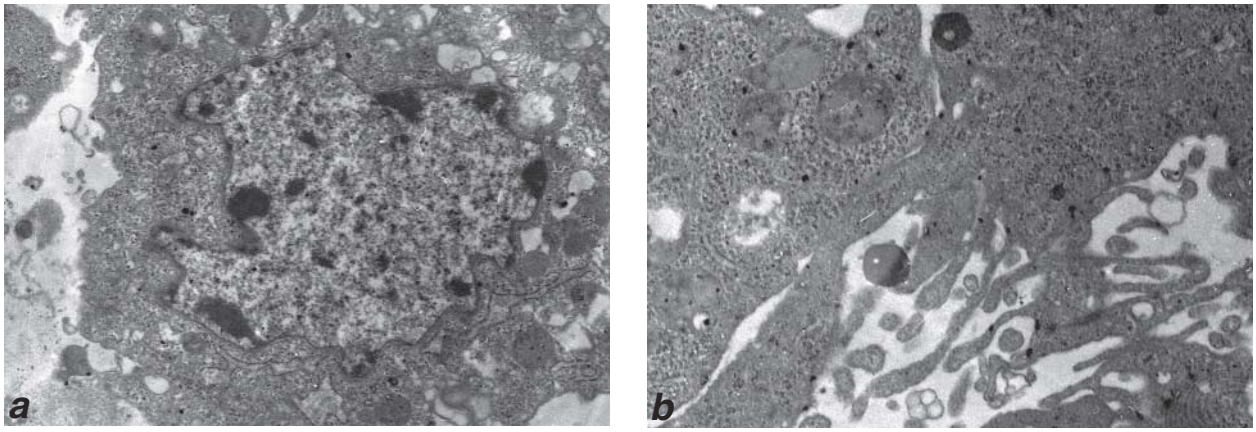

Fig. 2. Ultrastructure of HFL cell culture after bioresonance exposure at a frequency of 8 Hz (*a*; ×12,000) and 72 Hz (*b*; ×12,000). *a*) wrinkled pyknotic nucleus, numerous vacuoles and electron-dense incorporations in the cytoplasm; *b*) scanty intracytoplasmic vacuoles and ribosomal structures in the cytoplasm.

The ultrastructure of HFL cells exposed to 72 Hz differed from that exposed to 8 Hz: a trend to normalization was observed. The monolayer consisted of cells contacting between each other, with less manifest disorders in the structure of intracellular organelles. Cell cytoplasm was characterized by compact contents with orderly ribosomal structures (polysomes). Rare cytoplasmic vacuoles, liposomes, rough endoplasmic reticulum were detected. Lamellar incorporations were revealed. The nuclei were round, with chromatin lumps and nucleoli. The morphology of plasma membrane was normal: numerous protrusions and pseudopodias were seen on the cell surface (Fig. 2, *b*).

Cell destruction was most pronounced after exposure to a frequency of 78.5 Hz.

Hence, the results confirm that resonance electromagnetic exposure in *in vitro* system leads to modification of metabolic processes and cell morphology.

Proliferative activity of cells decreased in all variants of bioresonance therapy (BRT), particularly after exposure to 78.5 Hz. Cell viability also somewhat decreased. Exposure at a frequency of 8 Hz caused more significant changes in lung cells compared to liver cells. The frequency of 72 Hz caused the least changes in lung and liver cells.

Morphological analysis showed the type of destructive changes in HFL and Chang liver cells under the effects of exposure at frequencies of 8 and 78.5 Hz. Exposure to 72 Hz caused minimum changes in both cell types.

It seems that cytopathic disorders in the cells observed after exposure to 8 and 78.5 Hz (changed shape of the cells destruction of the monolayer, cytoplasm vacuolation, pyknosis and fragmentation of the nuclei, chromatin destruction) result from metabolic disorders in cell cultures.

Our previous studies of the effects of exogenous bioresonance radiation on tumor cells showed

TABLE 1. Proliferation and Viability of Cell Cultures Exposed to Different Frequencies

Cell culture	Frequency, Hz	PI		Viability, %	
		control	experiment	control	experiment
HFL	8	1.9	1.3	96	84
	72	1.9	1.6	96	84
	78.5	1.9	1.3	96	79
Chang liver	8	3.3	2.5	92	74
	72	3.3	2.8	92	84
	78.5	3.3	1.5	92	81
Lunet	8	1.8	1.3	—	—
	78.5	1.8	1.2	—	—
CH5	8	2.0	1.6	—	—

Note. "—": not studied.

inhibition of growth activities of HeLa (human uterine cervical epidermal carcinoma), A 549 (human lung carcinoma), and L 41-M (bone marrow cells of a patient with leukemia) tumor cultures exposed in a Miniekspert-T device at frequencies of 100, 410, and 22.5 Hz [5]. Reduction (1.5-3 times) of proliferative activity in comparison with the control depended on the intensity of oscillations. Degenerative changes were irreversible, the cultures were not restored and were incapable of further passages.

We previously studied the effects of frequency resonance exposure on decontamination of cultures from mycoplasmas [4,7] on HeLa, RK-13 (rabbit kidney), and Vero E6 (monkey kidney) cells intensely contaminated with mycoplasma. Exposure of cells according to F 44 and F 45 modes at 100 μT for 3 min was optimal. Additional exposure in the F-mode at 97.5 and 69.5 Hz and 30 μT for 1 min had a favorable impact on cell regeneration. After exposure of non-contaminated cells, PI decreased to 3.3 with 90% viability *vs.* intact control (PI 4.0, viability 98%). Exposure of contaminated HeLa cells resulted in PI reduction to 1.3 *vs.* 1.9 in the control at 85% viability *vs.* 93% in the control. These cells were incapable of further passages. Irradiation of cell suspension in the same, but inverse mode (counterphase) resulted in a 2-fold reduction of the contaminated cells' PI at 99% viability. Experiments on HeLa cells showed temporary suppression of mycoplasma contamination over 4 passages and normalization of their morphological.

Two of the studied cells strains (Lunet and CH5) were contaminated by mycoplasma, however none of the BRT frequencies (8, 72, 78.5 Hz) used in experiments eliminated mycoplasma.

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