BIOPHYSICS AND BIOCHEMISTRY

Some Biological Effects of Microwave Irradiation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 5, pp. 516-518, May, 1999
Original article submitted June 2, 1998

Morphological analysis and morphometry using immunoperoxidase methods showed that microwave (MW) radiation with a white noise type spectrum and radiation power density close to that emitted by the object stimulated proliferation of human fibroblasts *in vitro*. This effect is realized via stimulation of DNA synthesis. MV irradiation with the same parameters increased adhesive capacity of human blood neutrophils.

Key Words: *proliferation; fibroblasts; adhesion; microwave radiation*

Millimeter waves (λ=1-10 mm) corresponding to the microwave band and only recently introduced in clinical practice are now widely used for the treatment of various pathological states [1,3]. Biophysical and biochemical effects induced in cells by MW radiation [4] are extensively studied, but little is known on its effects at the tissue, cellular, and subcellular levels.

In the present study we investigated the effects of MV (5×10^{-18} W/cm^{2}Hz mean power, noise-like spectrum) on *in vitro* proliferative activity of human fibroblasts and functional activity of receptors in human neutrophils.

MATERIALS AND METHODS

A total of 6 strains of human skin fibroblasts grown out of skin samples from healthy donors were used [6]. Fibroblasts of passages 3-4 were exposed to MV 24 h after subculturing. The cells were grown on Costar plastic dishes in Eagle's medium (Institute of Poliomyelitis and Viral Encephalites, Russian Academy of Medical Sciences, Moscow) supplemented with 2% glutamine, antibiotics and 10% fetal bovine serum (Biolot, St. Petersburg) at 37°C and 5% CO_2 to a density of 12×10^4/cm^2. The cultures were exposed to MV with 5×10^{-18} W/cm^{2}Hz mean power and noise-like spectrum within 53-78 GHz frequency range generated by an Amfit-0.2-10-0.1 apparatus (Research Physical and Engineering Institute, Nizhnii Novgorod). Experiments were carried out in dry atmosphere at 18-20°C. The cells were exposed to MV for 10, 20, and 30 min; not exposed cells served as the control.

Morphological examination was carried out before and 24 h after irradiation. For evaluation of proliferative activity, the number of cells per square unit (visual field of inverted microscope at ×250) was determined and thymidine analog 5-bromo-2-deoxyuridine (BrdU) was added to the medium for 3 h. The number of dividing cells was determined using the immunoperoxidase method [2] and the labeling index was calculated.

For evaluation of adhesion of polymorphonuclear leukocytes (PNL), a pure neutrophil fraction was isolated from donor blood (n=11) by centrifugation in a Ficoll-Verografin density gradient. The following granular dextran sorbents were used as the neutrophil adhesion substrate: Sephadex G-25 (Pharmacia) opsonized with C3b complement component and Cytodex Cyt-3 (Pharmacia) coated with type I collagen. Sephadex was opsonized by 30-min incubation (37°C) with pooled sera from 10 donors. Noncovalently bound

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0007-4888/99/0005-0466$22.00

components were removed with 2 M NaCl (100°C for 10 min) [7].

The sorbents were suspended in Hanks' solution without phenol red to a concentration of 2×10^4/ml and 0.2 ml sorbent suspension was added to 0.2 ml neutrophil suspension (2×10^6 cells) preliminary exposed to MV for 20 min. Intact neutrophils served as the control.

Neutrophil adhesion capacity was assessed by the percentage of positive particles (3 and more bound neutrophils) after 2-fold washout [5].

The data were processed using standard tests for small samples.

RESULTS

Immediately before irradiation the cells consisted of spindle-shaped or sometimes stellate cells with long processes. Cell nuclei had clear-cut contour and 1-2 small nucleoli. The cells had compact homogenous cytoplasm, no signs of vacuolization were seen. About 5.33% cells were BrdU-positive.

Twenty-four hours after irradiation, cells exposed to 10-min irradiation did not differ from the control, while 20- and 30-min irradiation produced marked changes in cell morphology: it increased the number of dividing cells, the size of cell nucleus and nucleoli, and the number of nucleoli (to 3-4). Cell density in cultures and the percentage of BrdU-positive cells considerably increased. Thus, the effect of MV depends on the exposure. The experiments carried out with fibroblast cultures from 6 different samples yielded similar results (Tables 1 and 2).

The adhesive properties of PNL were assessed after 20-min exposure to MV radiation of different power (1 and 10 μW). MW radiation of a 1 μW power had no effect on cell adhesion, while MV radiation of a 10 μW power increased adhesion of PNL. It should be noted that MV of 1 and 10 μW power had practically no effect on C3b-dependent adhesion (68.3 and 72.8, respectively, *vs.* 69% in the control), while adhesion to Cytodex changed more considerably (52.1 and 70.8%, respectively, vs. 51.9% in the control).

Thus, MV increased adhesive capacity of PNL in experimental systems with specific sorbents (C3b-Sephadex and Cyt-3 Cytodex), which suggests enhanced expression of cell receptors to C3b complement component and type I collagen. The fact that MV radiation had practically no effect on neutrophil adhesion to C3b-Sephadex can be explained by maximum saturation of the membrane with these receptors.

TABLE 1. Effect of MV on Index of BrdU-Labeled Cells, %

Exposure, min	Human fibroblasts strains					
	1	2	3	4	5	6
Control	6	3	5.1	7	3	6
10	4.7	4	5.6	6.4	4.1	5.4
20	7	6	8.3	7.2	6	6.6
30	9	10	12	16.7	10.1	10.9

TABLE 2. Effect of MV Irradiation on Fibroblasts Density in Culture (Number of Cells$\times10^4$/cm^2)

Exposure, min	Human fibroblasts strains					
	1	2	3	4	5	6
Control	20.4	16.4	12.6	21.2	26.8	14.1
10	21.6	14	11.6	18.7	27	13.6
20	26.7	24.2	14	21.6	28.4	17
30	33.6	26.2	26.2	32	36.8	23.2

Thus, MV radiation with white noise-like spectrum and radiation power density close to that emitted by the object considerably stimulated proliferation of human skin fibroblasts and modulated adhesive capacity of human blood neutrophils *in vitro*. Irradiation with 1 μW MV power had no effect on PNL adhesive capacity since this power is below cell sensitivity threshold. The effect of MV on cell proliferation strictly depends on the exposure. The cell systems used in this study play an essential role in inflammatory reaction and tissue regeneration. The changes induced in these systems by MV with a power close to that emitted by cells is of particular importance and will be the subject of further investigations.

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