BIOPHYSICS AND BIOCHEMISTRY

Modulation of Rat Leukocyte Respiratory Burst by Ultrahigh Frequency Electromagnetic Radiation

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Electromagnetic radiation of ultrahigh frequency modulates luminol-dependent chemiluminescence of rat blood leukocytes activated by latex beads. This effect is frequency-dependent, it either stimulates (at 41 GHz) or inhibits (at 69 GHz) the chemiluminescence response by a factor of 2.00 and 0.63, respectively.

Key Words: *polymorphonuclear leukocytes; chemiluminescence; millimeter waves; reactive oxygen species*

Animal and human phagocytes (neutrophils and macrophages) produce reactive oxygen species (superoxide anion and peroxide), which have strong cytotoxic effect during antimicrobial and antineoplastic defense [9]. In various pathological processes (pneumonia, asthma, atherosclerosis, cardiac diseases, and malignant neoplasms), phagocytic activity (respiratory burst) varies in a wide range [3]. Suppression of phagocytic activity impairs defensive functions, while the extra activity of these cells can damage healthy cells or even the phagocytes themselves in the host organism [1,7]. Therefore, new immunomodulators can be useful for pharmacological correction of the activity of phagocytic cells.

Recently, noninvasive medical treatment with electromagnetic radiation (EMR) has been applied [2]. It was shown that the indices of blood cell activity change during treatment with EMR of ultrahigh frequencies (UHF) in the millimeter wavelength range [8].

We explored the possibility of modulating the respiratory burst of rat leukocytes by UHF EMR. Detailed examination of EMR effects in the wide frequency range is a laborious task, therefore, we chose only two frequencies (41 and 69 GHz) that provided strong modulating effect on calcium-dependent chlorine current in the algal plasmatic membrane [5].

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MATERIALS AND METHODS

Polymorphonuclear leukocytes (PMNL) were isolated from peripheral blood of Wistar rats weighing 220-230 g by differential centrifugation after lysis of erythrocytes with ammonium chloride [10]. Chemiluminescence (CL) of rat PMNL was estimated in the incubation medium (0.5 ml) containing 0.9% NaCl, 10 mM Tris-HCl, 6.6 mM glucose, 0.3 μg/ml peroxidase, and 5×10^{-5} M luminol (pH 7.9) at 37°C. The CL response was induced by addition of latex beads 1 μ in diameter to the suspension of PMNL (10^7/ml).

Irradiation of neutrophils by UHF EMR (frequency range 37-78 GHz) was performed with G4-141 and G4-142 electric generators. Radiation was transmitted from generators via a polyethylene waveguide with an elliptic cross-section 4×6 mm and

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length 120 mm. The output power was 20-30 mW according to measurements with M5-49 and M5-50 thermistor transducers connected to the measuring bridge. Irradiation was performed at 20-22°C with constant shaking. This procedure averaged heterogeneity of cell irradiation over the entire volume and prevented cell attachment to the surface of vials, which could be an additional factor affecting cellular activity. The suspension temperature during irradiation was controlled with an MTZ-18 microthermistor transducer (diameter 0.5 mm). The control cells were kept in the same volume of incubation medium under constant shaking.

Cell content varied in different series of experiments, so the control values also changed. The results were statistically analyzed using Student's *t* test.

RESULTS

After addition of latex beads to the cells, CL intensity increased considerably following a latent phase of 1-2 min and reached the maximum after 4-5 min (Fig. 1). Then CL intensity dropped, decreasing by about 2 times after 10 min. EMR at 41 GHz increased the amplitude of CL response and decreased duration of the latent phase. It should be noted that the maximum rate of CL development and the moment of maximum CL was not changed at this time. Evidently, it means that irradiation at 41 GHz affected the system of induction of CL response, but not the direct generators of CL response, i.e., peroxidase and myeloperoxidase. By contrast, 69 GHz irradiation decreased the amplitude of CL response and the rate of CL development, but did not markedly affect the duration of the latent phase. This effect of radiation at 69 GHz indicates that the target of UHF EMR at this frequency differs from the target for 41 GHz EMR. Figure 1 shows that EMR at 69 GHz changes qualitatively the dynamics of CL response: it looses the maximum and gradually transits to the steady state phase. Irradiation at 41 GHz significantly stimulates the maximum CL response (by about 2 times), while irradiation at 69 GHz suppresses the maximum CL response to the level of 0.63 compared with the control value (Table 1).

We also studied the dependence of the CL response amplitude on the irradiation dose. Figure 2 shows that activating effect increases with the exposure period during the first 5 min and becomes saturated after 10 min. Exposure of control cells for 10 min under identical conditions, but without EMR, did not change the CL response. All the previously described experiments were performed with preliminary irradiation of the PMNL that had not been activated with latex beads.

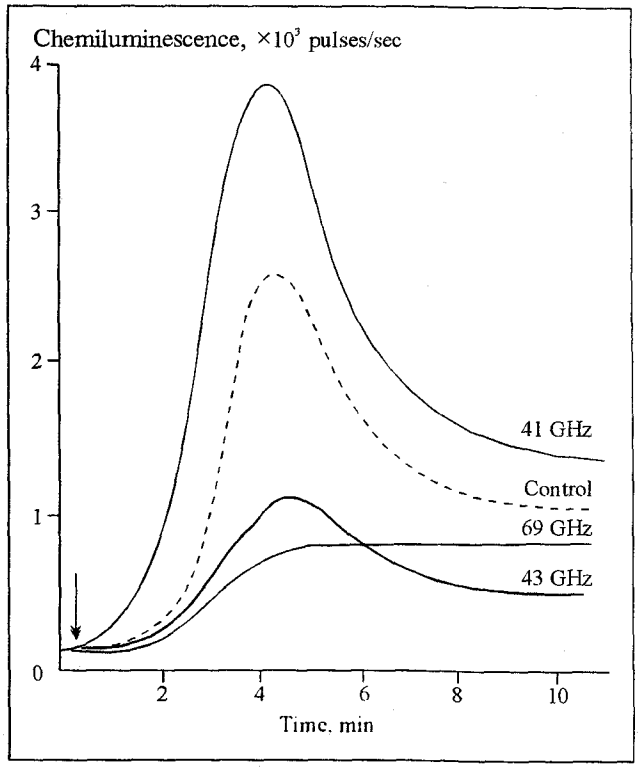

Fig. 1. Typical plots of chemiluminescence of the polymorphonuclear leukocytes induced by latex beads with preliminary 3-min UHF EMR at 41, 43, and 69 GHz with constant stirring. Here and in Fig. 3: the addition of latex beads is indicated with an arrow.

To study the nature of UHF EMR influence on activated cells, experiments were performed with two identical samples of PMNL which were irradiated during the quasistationary activation phase 10 min after addition of latex beads (Fig. 3). Five periodic radiation stimuli (stimulation duration 1.5 min) enhanced the CL response to UHF EMR at 41 GHz, while at 69 GHz it decreased the CL response. These findings agree with the effect of UHF EMR on resting neutrophils.

The opposite effects of different frequencies on CL response of neutrophils testifies that the mode of action of EMR cannot be explained by thermal effects. It was shown [5] that incubation of human leukocytes at 37, 40, and 43°C inhibits generation of

TABLE 1. Maximum CL of Rat Polymorphonuclear Leukocytes after Irradiation with UHF EMR at 41 and 69 GHz ($X \pm m$)

Frequency of UHF EMR	CL, ×10³ pulses/sec		Degree of influence of UHF EMR/control
	control	UHF EMR	
41 (n=10)	2.70±0.83	5.38±1.04	1.99*
69 (n=5)	1.82±0.17	1.15±0.18	0.63*

Note. *p<0.05 compared with control.

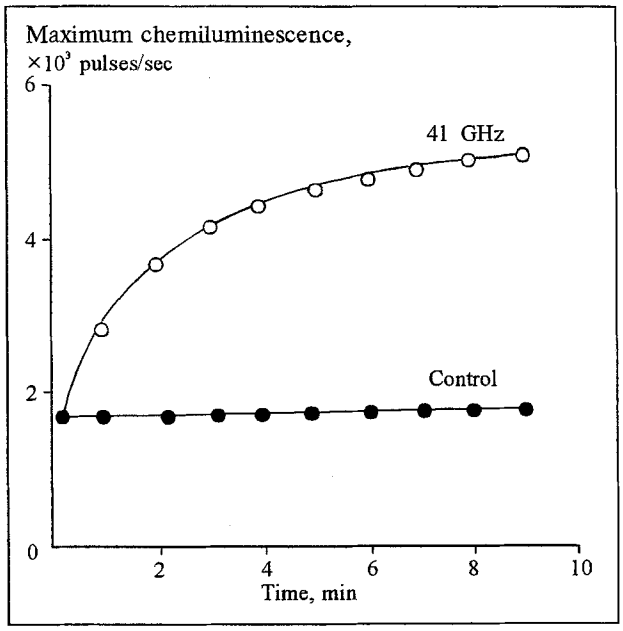

Fig. 2. Dependence of maximum chemiluminescence on duration of UHF EMR at 41 GHz.

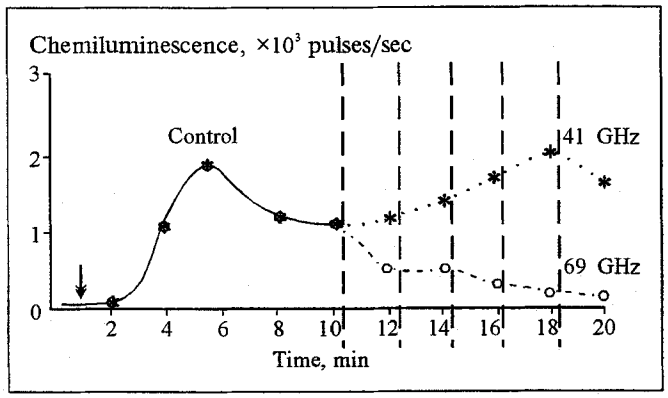

Fig. 3. Opposite effect of millimeter waves on the late quasistationary phase of chemiluminescence. Dashed lines show the intervals of irradiation. UHF EMR was performed for 1.5 min. CL was measured for 30 sec.

superoxide anion by 10-12% beginning from 40°C and exposure time 60 min. In our experiments heating of irradiated cell suspension did not exceed 1°C in respect to the control, indicating that inhibitory effect cannot be explained by heating. The literature data on EMR effect on phagocytes are limited. The effects of EMR, which regulate bacteriophagocytosis, were observed previously with different lasers by varying wavelength and dose of irradiation [6,12,13]. It was shown that variation of the dose of irradiation from a He-Ne laser increases the CL response of mouse phagocytes by about 2 times at the optimal dose of 220 J/m² [11]. Here the same effects are demonstrated for EMR of the range corresponding to the quanta of much lower energy, which could decrease the effects of EMR on other cellular systems. One cannot exclude the possibility that the effect of UHF EMR on phagocytes is realized in the whole organism and thus can be used in the beam therapy. It should be stressed that higher adsorbing efficiency of the skin in respect to UHF EMR underlies the high selectivity of its effect on the skin in comparison with laser irradiation.

Considering the skin as a primary target of different external influences [4], and as an organ participating in generation of both local and global responses that result from activity of the entire immune system, our findings show that UHF EMR is an efficient and selective immunomodulator of the integumentary tissues. Thus, rat PMNL are highly sensitive to UHF EMR which modulates the respiratory burst of phagocytes in a wide range (about 0.6-2.0 times in comparison with the control cells) by changing irradiation frequency. Depending on frequency, either activation or suppression of phagocytic activity can be attained. This effect is not due to heating of the cells.

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