Effects of Low-Intensity Ultrahigh Frequency Electromagnetic Radiation on Inflammatory Processes

K. V. Lushnikov, Yu. V. Shumilina, V. S. Yakushina*, A. B. Gapeev, V. B. Sadovnikov, and N. K. Chemeris**

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Low-intensity ultrahigh frequency electromagnetic radiation (42 GHz, 100 μW/cm^2) reduces the severity of inflammation and inhibits production of active oxygen forms by inflammatory exudate neutrophils only in mice with inflammatory process. These data suggest that some therapeutic effects of electromagnetic radiation can be explained by its antiinflammatory effect which is realized via modulation of functional activity of neutrophils in the focus of inflammation.

Key Words: *ultrahigh frequency electromagnetic radiation; inflammatory reaction; neutrophils; active oxygen forms*

Studies of biological effects of ultrahigh frequency electromagnetic radiation (UHF EMR) started in the 1960s after introduction of this frequency band in radiotechnology. Pronounced therapeutic effects of UHF EMR were revealed [3,13]. Modern UHF therapy effectively uses low-intensity UHF EMR (causing no heating of the exposed object) for prevention and treatment of a wide spectrum of diseases, *i. e.* the effect of low-intensity UHF EMR is not etiotropic [3,13].

Despite wide clinical use of UHF radiation, the mechanisms underlying the therapeutic effects of UHF EMR remain unclear in many aspects. Based on our findings and published reports, we hypothesized that the pleiotropic effects of UHF EMR can be due to modulation of the regulatory systems, specifically of the immune system [7]. We previously demonstrated that UHF EMR had no effect on the parameters humoral immunity [6], but reduced the intensity of cell-mediated immunity in delayed-type hypersensitivity reaction [2], appreciably reduced the number of active phagocytes (phagocytosis percent) in the peripheral blood of intact animals [1,5], and reduced the intensity of inflammatory reaction (IR) [8].

Since IR underlies the pathogenesis of many diseases of different origin, we consider that the therapeutic effects of UHF EMR are explained by suppression of IR. We think that inflammatory processes are arrested under the effects of UHF EMR due to modulation of the neutrophil functional activity, because these cells play a leading role in the development of acute inflammation.

Here we studied the antiinflammatory effects of UHF EMR depending on the body functional status during the exposure and evaluated the effect of UHF EMR on functional activity of neutrophils of inflammatory exudate.

MATERIALS AND METHODS

Experiments were carried out on male outbred stock of NMRI mouse strain (25-30 g) from the collection of Laboratory of Biological Trials, Affiliated Department of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bio-Organic Chemistry, Russian Academy of Sciences. The mice were kept under 12:12 day:night regimen on standard laboratory diets with free access to water.

High-frequency generator G4-141 served as the source of UHF EMR. The stability of the generator frequency was ±0.03%, parasite deviation of the out-

Institute of Biophysics of Cell, Russian Academy of Sciences, Pushchino; *Department of Human and Animal Morphology and Physiology, L. N. Tolstoi State Pedagogical University, Tula; **Affiliated Department of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bio-Organic Chemistry, Russian Academy of Sciences, Pushchino. ***Address for correspondence:*** kolush@mail.ru. Lushnikov K. V.

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put signal frequency in the continuous generation mode was no more than 2 MHz. We used UHF EMR parameters whose efficiency was demonstrated previously (output signal 42 GHz, radiation intensity about 100 μW/cm^2) [11,12]. The animals were exposed in a distant zone (at a distance of 300 mm from the radiating piece) of the pyramidal funnel antenna with 32×32 mm^2 aperture in the presence of geomagnetic field with approximately 45 μT induction. The animals were not fixed during irradiation. Control animals received simulated exposure, for which they were placed into exposure zone with the high frequency generator switched on, but without output power. Single exposure to UHF EMR and simulated exposure were carried out for 20 min before and after IR induction.

Aseptic IR was induced by injection of 25 μl nonopsonized zymosan in a concentration of 5 mg/ml Hanks' solution (Sigma) under the aponeurotic plate of the left hind paw. A similar volume of the solvent was injected into the contralateral paw. The severity of inflammation was evaluated by measuring the size of paw edema with a micrometer. The inflammation index (II, relative increase in the thickness of the left paw in comparison with the right one) was calculated [10]:

$$II=\frac{D_l-D_r}{D_r}\times 100\%,$$

where D_l is thickness of the left paw and D_r thickness of the right paw.

In order to evaluate functional activity of inflammatory exudate neutrophils, IR was induced by intraperitoneal injection of zymosan (5 mg/ml, 150 μl). The animals were sacrificed 5 h after zymosan injection and the peritoneal cavity was washed with 3 ml cold Hanks' solution. Cell suspension was washed from exudate proteins by centrifugation in Hanks' solution. The resultant suspension contained about 80% neutrophils. Cell viability in the Trypan blue test was about 90%. Functional activity of inflammatory exudate neutrophils was evaluated by in the luminol-dependent chemiluminescence test by the production of reactive oxygen species (ROS, "respiratory byrst") characterized by a potent cytotoxic effect. Phorbol ether (phorbol-myristate acetate, Sigma) in a final concentration of 1 μM served as the activating agent. The summary production of ROS was calculated in proportion to the area under the kinetic curve reflecting the chemiluminescence intensity and expressed in arbitrary units [11,12].

The data were statistically processed using Student's *t* test.

RESULTS

We previously showed that the percent of phagocytosis of peripheral blood neutrophils from intact animals decreased as early as 30 min after single exposure to UHF EMR and remained low during at least 24 h, but this decrease was not observed after irradiation of animals with inflammatory reaction [1,5,8]. Now we evaluated the effect of UHF EMR on the development of IR depending on the functional status of the organism by the moment of exposure. The animals were exposed to UHF EMR 1 h before (n=12) and 1 h after (n=16) IR induction in the hind paw pad, the size of paw edema was measured during 8 h, and II was calculated. II of irradiated animals was compared to that in controls (n=29). Three-to-six hours after IR induction, II decreased by 15-30% (p<0.05) in animals exposed to UHF EMR after IR induction, but did not change under the effect of exposure carried out before zymosan injection (Fig. 1). Hence, the antiinflammatory effect of UHF EMR was observed only in animals with developing IR.

In order to clear out the effect of UHF EMR on cells infiltrating the inflammatory focus during the first hours of IR we evaluated functional activity of neutrophils from the inflammatory focus in exposed animals. The effect of UHF EMR on ROS production by inflammatory exudate neutrophils was studied in animals exposed 1 h before (n=5) and 2 h after (n=5) induction of IR in the peritoneal cavity. Similarly as in the previous experimental series, ROS production by neutrophils of irradiated animals was compared to the corresponding parameters in sham-irradiated controls (n=10). It was shown that irradiation before IR induction did not modify ROS production by peritoneal neutrophils. By contrast, ROS production by neutrophils from animals irradiated after zymosan

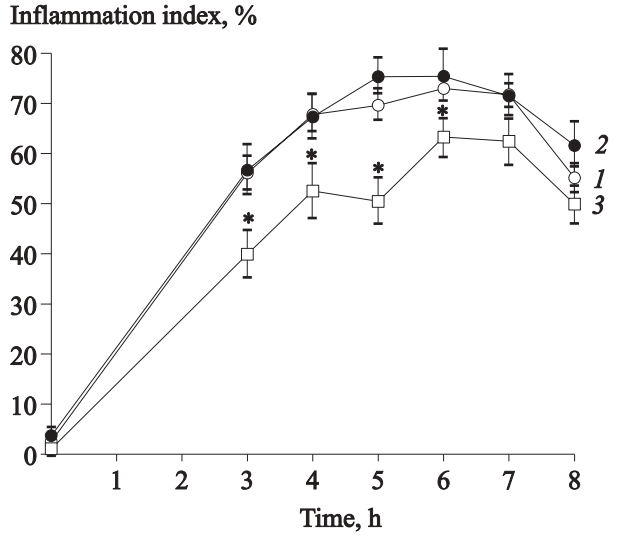

Fig. 1. Time course of inflammation index in control animals (*1*), animals exposed to ultrahigh frequency electromagnetic radiation (UHF EMR); 42 GHz, 100 μW/cm^2, 20 min) before (*2*) and after (*3*) zymosan induction of inflammatory reaction in the hind paw. *p<0.05 compared to the control.

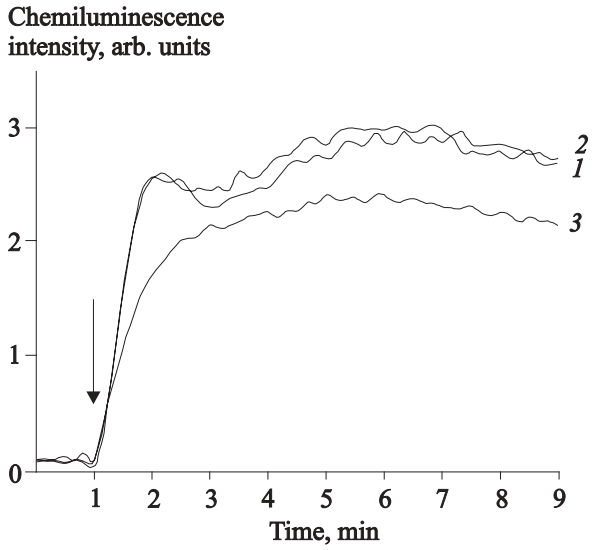

Fig. 2. Original records of chemiluminescence kinetics reflecting production of active oxygen forms by inflammatory exudate neutrophils in control animals (*1*) and animals exposed to UHF EMR before (*2*) and after (*3*) inflammation induction. Arrow: phorbolmyristate acetate injection.

injection decreased by 24±6% ($p<0.05$) compared to the control (Fig. 2).

Phorbol ether used for the induction of the respiratory burst is a structural analog of diacylglycerol and activates proteinkinase C without involving the receptor system of the cell. Active proteinkinase C phosphorylates a series of subunits in the NADPH-oxidase complex, activated NADPH-oxidase and produces ROS [4]. The kinetics of ROS production in cells from control animals is clearly biphasic (Fig. 2). Phase 1 corresponds to activation of NADPH oxidase via proteinkinase C and rapid mobilization of intracellular Ca^{2+}. Subsequent increase of ROS production is explained by the release of extracellular Ca^{2+} and further activation of enzyme systems responsible for ROS production [4]. In irradiated animal cells (UHF EMR exposure after induction of IR) phase 1 of the respiratory burst is suppressed and phase 2 reduced, which probably attests to changes at the level of Ca^{2+} signaling and interaction of proteinkinase C with NADPH oxidase complex.

Hence, UHF EMR inhibits ROS production by inflammatory exudate neutrophils, due to changes at the level of intracellular signaling systems of these cells. Presumably, these changes can modulate other neutrophil functions during inflammation: degranulation, production and secretion of inflammatory cytokines, *etc.*, which probably underlies suppression of IR under the effect of UHF EMR.

It is believed that UHF EMR energy is absorbed in the surface skin layers [3], *i. e.* the zone of direct effect of radiation includes only skin structures [7,9] with microcirculatory bed of the skin and blood cells. However, changes in the functional activity of neutrophils caused by whole body exposure to UHF EMR are due to mediated, but not direct effect, because *in vitro* irradiation of intact animal blood did not modify neutrophil phagocytic activity [1,8]. Presumably, the biological effect of UHF EMR is realized with participation of an intricate system of neuroendocrine regulation [7]. Manifestation of the antiinflammatory effect of UHF EMR only if the exposure is carried out after IR induction can be determined by the total systems reaction aimed at suppression of the pathological process. Hence, antiinflammatory effect of UHF EMR is observed only in animals with IR and is mediated by modulation of the functional activity of neutrophils in the inflammatory focus.

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