Electromagnetic Oscillations as a Factor Modulating Blood Neutrophil Function

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Effects of various physical factors of electromagnetic nature on the synthetic and phagocytic activities of neutrophils were studied *in vitro* on the blood of patients with breast cancer. We found that alternating magnetic field, low-intensity laser, photodiod radiation, and their combinations induced mobilization of blood neutrophil function.

Key Words: *alternating magnetic field; low-intensive laser exposure; photodiod radiation; blood neutrophils*

Attempts to use physical factors of electromagnetic nature, such as alternating magnetic field (AMF), low-intensity laser (LIL), and photodiod radiation (PDR) for stimulation of nonspecific defense functions in different pathologies were undertaken [5,8,9]. The effects of these factors are realized at the level of regulation of both integral antistress reaction [3] and cellular and subcellular bioadaptive mechanisms. This determines principal possibility of correcting the immunological status of leukocytes [1,2,9]. However, the mechanisms underlying the effects of AMF, LIL, and PDR on functional potential of peripheral blood neutrophils, a key cell element of nonspecific resistance, remain unknown. We studied parameters of fluorescence of peripheral blood neutrophils, content of cationic proteins, and the state of oxygen-dependent mechanisms of bactericidal activity (NBT test) after *in vitro* exposure of the blood from cancer patients to AMF, LIL, PDR and their combinations before and after specific antitumor treatment.

MATERIALS AND METHODS

The study was carried out on whole blood samples from 10 donors and 35 patients with breast cancer (BC T3-4MoNx). Blood from the same patient was collected before and after antitumor therapy. Each blood sample was divided into equal portions (0.5 ml) and placed into special cuvettes; after obtaining cell monolayer the cells were exposed to the studied physical factors and their combinations.

The effect of AMF (10 mT induction, 1.5-3-6 Hz frequency, total exposure 15 min) was studied using Gradient-2 physiotherapeutic device (source of AMF) with a microprocessor. LIL and PDR had the same wavelength (λ=637 nm) in the red spectrum, the same power flow density at the lightguide end (7.5 mW/cm^2), and the same duration of exposure (up to 5 min). PDR possessed no coherence and polarization, while LIL was characterized by high polarization and coherence of optic radiation. The therapeutic dose was selected by modulating power flow density and duration of exposure, because it is known that the biological reactions during exposure to LIL and PDR depend not on the absolute dose of irradiation, but on its components. Ariadna-2 He-Ne laser was used for LIL exposure and Spectr-LTs physiotherapeutic device for PDR. Both devices were equipped with microprocessors.

Fluorescent spectral studies in blood smears were carried as described previously [4]. Acridine orange (MB) served as the fluorescent stain. The fluorescence spectra were recorded using a microspectrofluorimeter attached to a LUMAM-I3 microscope. Fluorescence

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was stimulated at 436 nm (DRSh-250-2 mercuric arch lamp) and measured at 530 and 640 nm without amendments for the device sensitivity. In preparations stained with acridine orange the main contribution to radiation at λ=530 nm was made by complexes of acridine orange monomer with double-stranded nucleic acids and at λ=640 nm by the complex with single-stranded ones. Hence, the ratio of fluoresce intensities in red (λ=640 nm) and green (λ=530 nm) bands is proportional to the ratio of single- to double-stranded nucleic acids and was expressed as coefficient a characterizing synthetic activity of the cell [4]. This parameter can be used for quantitative description (in arb. units) of fluorescence spectra of the entire cell population.

Cytochemical study of cationic proteins was carried out using the lysosomal cationic test [6]. To this end, the mean cytochemical coefficient (CCC) was determined in fast green-Azur A stained blood preparations [6]. At least 100 cells were counted in each preparation. The phagocytic activity of neutrophils (zymosan granules) and intensity of respiratory burst in spontaneous and stimulated NBT test were evaluated [7]. The studies were carried out at 24°C. The results were processed using Student's *t* test.

RESULTS

Fluorescent spectral analysis of the blood provided neutrophil fluorescence spectra for all examined groups. In intact donors the fluorescence spectrum was presented by two fluorescence bands at λ=530 nm and λ=640 nm (green and red bands). In breast cancer patients before treatment (no exposure) the fluorescence spectrum was presented by only one band with λ_{max}=530 nm. After a course of antitumor treatment in the group without exposure the fluorescence spectrum was presented by two bands with peaks at λ=530 nm and λ=640 nm (green band predominated).

After exposure of the blood to AMF, LIL, and PDR both peaks at λ=530 nm and λ=640 nm were recorded in both donors and patients before and after treatment (green band slightly predominated, Fig. 1). Both peaks (λ=530 nm and λ=640 nm) were recorded after combined AMF+LIL and AMF+PDR exposure, but the red band now predominated. Each fluorescence spectrum was characterized by the quantitative parameter α. All physical factors and their combinations significantly increased parameter α in donors and breast cancer patients before and after treatment (Table 1). The mean values of parameter α in the groups exposed to combinations of physical factors (AMF+LIL and AMF+PDR) were higher than after exposure to a single factor and were significantly higher in patients after treatment than before it (Table 1). The mean

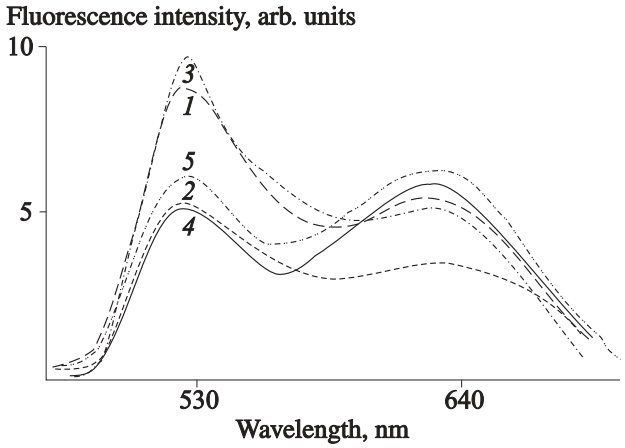

Fig. 1. Typical fluorescence spectra of peripheral blood neutrophils from patient K. with breast cancer after treatment and exposure to low-intensive laser (LIL; *1*), photodiod radiation (PDR; *2*), alternating magnetic field (AMF; *3*), AMF+LIL (*4*), and AMF+PDR (*5*).

values of parameter α were low, if the fluorescence spectrum contained only one peak at λ=530 nm in the green band. These data can indicate inhibition of synthetic processes and hence, suppression of functional activity of blood neutrophils. The appearance and certain predominance of the red band (λ=640 nm) in the fluorescence spectrum and increase in parameter α probably attest to activation of synthetic processes in neutrophils after exposure to physical factors and particularly to their combination. The maximum values of parameter α and the presence of similar peaks at λ=530 nm and λ=640 nm in the red and green bands was characteristic of the blood of breast cancer patients after treatment and exposure to AMF+LIL and AMF+PDR. It was previously shown that exposure to physical factors of electromagnetic nature acts as early trigger in the chain of complex interrelated reactions of cell stimulation. This transition is explained by increased production of RNA, chromatin decondensation, and changes in acridine orange binding, which correlates with transcription activity [5].

In donors the mean CCC values were virtually the same (Table 1). In breast cancer patients before treatment the differences were significant only in the groups exposed to combinations of physical factors (AMF+LIL and AMF+PDR). In breast cancer patients after treatment (without exposure) CCC values were the lowest ($p<0.05$), while after exposure to AMF, LIL, and PDR and their combinations CCC values were significantly higher and approached those in donors. The parameters of spontaneous NBT test in the blood of breast cancer patients did not change after exposure(in all variants), but parameters of stimulated NBT test significantly ($p<0.05$) decreased after exposure to AMF+LIL and AMF+PDR. The parameters of spontaneous NBT test increased after treatment in all groups

TABLE 1. Effects of AMP, LIL, and PDR on Functional Activity of Neutrophils (arb. units. $M \pm m$, n=100)

Parameter		Donors	Patients with breast cancer	
			before treatment	after treatment
No exposure	NBT_s	19.4±3.5	22.2±1.4	16.3±1.3^{**++}
	NBT_z	42.9±4.2	40.2±2.1	39.3±1.0^{+}
	CCC	2.27±0.91	1.75±0.10^{o}	0.61±0.20^{o**+}
	α	0.1200±0.0001^{+}	0.078±0.001^{o+}	0.0900±0.0001^{o**+}
LIL	NBT_s	—	23.2±1.3	23.8±1.5*
	NBT_z	—	38.0±1.4	39.3±2.1^{+}
	CCC	2.20±0.16	1.92±0.60	2.11±0.70^{*+}
	α	0.3300±0.0009^{*+}	0.42±0.11^{*+}	0.48±0.09^{*+}
PDR	NBT_s	—	25.0±1.4	28.2±1.0*
	NBT_z	—	46.6±3.3	49.3±3.3
	CCC	2.13±0.14	1.92±0.17^{o}	2.09±0.05^{*+}
	α	0.4200±0.0009^{*+}	0.47±0.13^{*+}	0.68±0.16^{*+}
AMF	NBT_s	—	23.2±1.3	24.5±1.4*
	NBT_z	—	40.2±1.2	46.1±2.2^{+}
	CCC	2.18±0.13	1.82±0.22	2.13±0.14^{*+}
	α	0.4700±0.008^{*+}	0.55±0.17*	0.54±0.18^{*+}
AMF+LIL	NBT_s	—	23.2±1.2	23.2±1.4*
	NBT_z	—	38.0±1.4	40.1±1.2
	CCC	2.72±0.11	2.17±0.10^{o*}	2.72±0.1*,**
	α	0.68±0.07*	0.770±0.009*	1.08±0.12^{o**}
AMF+PDR	NBT_s	—	23.5±1.1	29.9±2.8*
	NBT_z	—	33.0±1.3	57.8±1.3*,**
	CCC	2.68±0.17	2.78±0.10*	2.66±0.11*
	α	0.710±0.008*	0.88±0.11*	1.21±0.33^{o*,**}

Note. NBT_s: spontaneous NBT test; NBT_z: zymosan-stimulated NBT test. $p<0.05$ compared to: ^{o}donors, *patients without exposure. **patients exposed before treatment, $^{+}$exposure to AMF+PDR, $^{++}$exposure to AMF+LIL.

of patients, while the parameters of stimulated NBT test increased only after exposure to AMF and AMF+PDR (Table 1).

These results indicate that the studied physical factors produce a biostimulatory effect, which is most pronounced in combined exposure (AMF+LIL and AMF+PDR). It is known that the content of cationic proteins and NBT reduction to formazan in blood neutrophils reflect the intensity of "respiratory burst" and the status of oxygen-dependent mechanisms of bactericidal activity [6,7]. The mean CCC values and results of NBT test prove that the number of cells containing cationic protein granules and their saturation with these granules increased.

The sensitivity of neutrophils to the studied exposures increased in breast cancer patients after treatment. Presumably, these types of exposure stimulate the intensity of intracellular oxygen-dependent reactions and stimulate their traditional inductor zymosan. The fact of oppositely directed effects of AMF+PDR on the results of spontaneous and stimulated NBT test in patients before and after treatment is worthy of note. It seems that the initial decrease in stimulated NBT test values indicates certain exhaustion of neutrophilic oxygen-dependent potential, which is restored after exposure to these factors in the group of patients with breast cancer after treatment.

Hence, *in vitro* exposure of the blood to AMF, LIL, PDR, and their combinations leads to pronounced mobilization of metabolic processes in blood neutrophils — an important cellular component of natural nonspecific resistance; this opens new vistas for the correction of the immune status. Universal electromagnetic nature of different physical factors probably determines triggering of the common system of intimate mechanisms regulating blood neutrophil function as a model of wave-like interactions between the environment and the organism.

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