Effect of Terahertz Electromagnetic Irradiation at Nitric Oxide Frequencies on Concentration of Nitrites in Blood Serum of Albino Rats under Conditions of Immobilization Stress

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The terahertz electromagnetic irradiation at NO emission and absorption spectrum frequencies elevated the concentration of nitrites in blood serum of albino rats subjected to acute immobilization stress.

Key Words: *terahertz electromagnetic irradiation; nitric oxide; nitrites*

Currently, NO generating system is considered as a special stress-limiting system activated when the organism is subjected to various stress factors or during adaptation to the repetitive action of environmental challenges [8,9].

The study of the effect of various stressors on NO synthesis resulted in a widely spread view that the decrease in serum NO concentration reflects disadaptation of the organism to stress, while enhanced NO deposition in the vascular endothelium attest to adaptation of the organism to the stress factors [5,8-10].

Modern medicine employs some NO donors such as organic nitrates to elevate NO concentration in the blood. However, pharmacological stimulation of NO synthesis can be accompanied by detrimental and sometimes hazardous side effects. Consequently, it is important to search for noninvasive physical stimulators of the synthesis of endogenous NO based on natural physical regulation. One of the promising ways is the use of low-intensity electromagnetic irradiation in the millimeter and submillimeter frequency range [3].

In recent years, a new avenue of informational medicine appeared, terahertz therapy [1,3]. The terahertz range of electromagnetic radiation is mostly interesting by the fact that it encompasses emission and absorption molecular spectra (EAMS) of various cell metabolites, NO included [13].

Endogenous NO is permanently synthesized in organs, tissues, and cells from L-arginine in the reaction catalyzed by NO-synthases, the family of P-450-like enzymes [11]. Oxidation transforms NO into nitrites and then nitrates [10,14]. NO concentration in the blood mostly depends on activity of endothelial NO-synthase, which is characterized by pronouncedly lower rate of catalysis compared to macrophagic NO-synthase (production of NO by macrophages is one of the mechanisms of non-specific organism's resistance), although the amount of endothelial isoform is much greater than that of macrophagic enzyme [4,12].

Apart from direct measurement of NO-synthase activity (by citrulline production), it can also be assessed by the concentration of stable metabolites, nitrites and nitrates. The concentration of nitrites is an objective criterion of activity of nitroxidergic system [5].

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In light of this, our aim was to examine the effect of electromagnetic irradiation in the terahertz frequency range (THF-waves) corresponding to NO EAMS on nitrite concentration in the plasma of the animals subjected to acute immobilization stress.

MATERIALS AND METHODS

Experiments were carried out on blood serum of randombred male albino rats weighing 180-220 g. The animals were maintained under vivarium conditions on standard ration. The experiments were performed in compliance with the ethic requirements of World Medical Association Declaration of Helsinki on Human Treatment of Experimental Animals. Acute stress was simulated by tight supine fixation of the rats for 3 h [6,7].

The rats were irradiated at NO EAMS frequencies of 150.176-150.664 GHz using a KVCh-NO generator developed in Extremely High Frequencies (KVCh) Medicotechnical Association in cooperation with Istok Company and Central Research Institute of Measuring Equipment [2]. The irradiator was placed at a distance of 1.5 cm over the skin and directed to the shaved skin locus (3 cm^2) situated above the sternum xiphoid process. The total radiation power and its density were 0.7 mW and 0.2 mW/cm^2, respectively. The irradiation dose was determined by power density on the skin and total exposure time. Stressed rats were subjected to a single radiation exposure for 30 min.

NO production was blocked with non-specific inhibitor of NO-synthase L-NAME (Sigma) in a dose of 4 mg/kg [4].

The rats were randomized into seven groups: group 1, intact controls (n=20); group 2, experimental rats exposed to THF-irradiation for 30 min (n=15); group 3, unexposed rats receiving L-NAME (n=15); group 4, experimental controls subjected to acute immobilization stress (n=28); group 5, experimental controls subjected to 3-hour immobilization stress and receiving L-NAME; group 6, acutely immobilized experimental rats exposed to THF-irradiation at NO EAMS frequencies of 150.176-150.664 GHz (n=32); and group 7, acutely immobilized experimental rats exposed to THF-irradiation at NO EAMS frequencies of 150.176-150.664 GHz and receiving L-NAME (n=15).

Blood was drawn via puncture of the right subdivisions of the heart. Plasma concentration of nitrites was determined with Griess reagent [5].

The data were statistically processed with Statistica 6.0 software. The hypotheses on the distribution normality was verified using Shapiro–Wilk test. Since most of the data did not conform to normal distribution, the data were compared using nonparametric Mann–Whitney U test.

RESULTS

In experimental rats subjected to 30-min THF-irradiation, a trend to elevation of the plasma concentration of nitrites was noted, although comparison with the control group revealed no significant difference in this parameter, which probably results from NO deposition [10]. Injection of L-NAME to intact (non-irradiated) rats blocked NO-synthase component of the NO circle, which was

TABLE 1. Effect of Acute Immobilization Stress and THF-Irradiation at NO EAMS Frequencies of 150.176-150.664 GHz on Plasma Concentration of Nitrites in Rats

Concentration, μg/ml	Intact rats			Acute immobilization stress		Acute immobilization stress with THF-irradiation for 30 min	
	control (n=15)	THF-irradiation for 30 min (n=15)	irradiation of L-NAME-treated rats(n=15)	L-NAME-free (n=28)	L-NAME-treated (n=15)	L-NAME-free (n=32)	L-NAME-treated (n=15)
Nitrites	0.503 (0.499; 0.595)	0.625 (0.375; 0.667) Z_1=0.82; p_1=0.414120	0.125 (0.125; 0.333) Z_1=3.27; p_1=0.001065	0.328 (0.228; 0.380) Z_1=3.98; p_1=0.000068	0.2 (0.125; 0.333) Z_2=3.56; p_2=0.000373; Z_4=0.15; p_4=0.884574	0.473 (0.333; 0.629) Z_1=0.70; p_1=0.486474; Z_2=2.65; p_2=0.007995	0.250 (0.125; 0.250) Z_3=3.99; p_3=0.000072; Z_4=0.11; p_4=0.905604

Note. Z_1, p_1: compared to the control group; Z_2, p_2: compared to acutely immobilized rats; Z_3, p_3: compared to acutely immobilized rats exposed to 30-min THF-irradiation; Z_4, p_4: compared to intact L-NAME-treated rats.

seen from a pronounced decrease in plasma nitrite concentration compared to the control group (Table 1).

Immobilization for 3 h significantly decreased the level of plasma nitrites (Table 1) indicating deficiency of NO in the blood plasma of stressed rats. This observation agrees with published data on the effect of stress on NO production: long-term and severe stress resulted in deficiency of NO in the organism, which attests to collapse of the stress-control systems and disadaptation of the organism to the stressor [8-10].

Irradiation of male albino rats subjected to immobilization stress with THF-waves at NO EAMS frequencies significantly increased the level of plasma nitrites in comparison with group 4 rats subjected to immobilization stress without electromagnetic radiation (Table 1). Persistently low level of plasma nitrites in acutely immobilized rats and its stability under THF-irradiation of immobilized and L-NAME treated rats (Table 1) indicates involvement of NO-synthase component of NO cycle in the realization of THF-irradiation effect.

Up-regulation of activity of NO-system by electromagnetic irradiation at terahertz frequencies resulting in elevation of plasma nitrites makes it possible to develop novel and efficient non-medicamentous methods to correct supression of endogenous NO synthesis, which can be used to treat some cardiovascular diseases characterized by deficiency of NO production such as ischemic heart disease and arterial hypertension.

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