Effects of Terahertz Irradiation at Nitric Oxide Frequencies on Intensity of Lipoperoxidation and Antioxidant Properties of the Blood under Stress Conditions

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 8, pp. 166-169, August, 2009
Original article submitted December 3, 2008

The effects of terahertz irradiation at the nitric oxide frequencies (150.176-150.664 GHz) on the intensity of lipoperoxidation and antioxidant properties of the blood were examined on albino rats subjected to immobilization stress. This terahertz irradiation completely normalized LPO processes and functional activity of antioxidants in stressed rats.

Key Words: *lipoperoxidation; antioxidants; terahertz irradiation; nitric oxide*

The processes of biological oxidation and LPO are up-regulated under stress conditions, which creates prerequisites for accumulation of toxic lipoperoxidation intermediates and decrease in functional activity of antioxidants [10]. Specifically, elevation of concentration of LPO products above the steady-state level is considered as a universal mechanism of cell damage in pathological states, *e.g.* under conditions of cardiovascular pathology [2,10]. Therefore, correction of the amount of intermediate products of lipoperoxidation and normalization of activity of the blood antioxidant system seem to be reasonable from the pathogenetic viewpoint.

Electromagnetic waves within the frequency range of 10^2-10^4 GHz corresponding to wavelengths of 3 mm to 30 μ are referred to as terahertz radiation [3]. The biophysical effects of these waves open new vista for the development of novel biomedical technologies such as terahertz therapy, diagnostics, and prevention [3]. In the experiments, the most promising are the electromagnetic waves corresponding to molecular spectra of NO radiation and absorption (150.176-150.664 GHz) [3,7], because NO belongs to universal regulators of physiological, pathophysiological, and biochemical processes in cells and in the organism [9,11].

Our aim was to study the effects of terahertz irradiation at the frequencies of 150.176-150.664 GHz on the intensity of lipoperoxidation and antioxidant properties of the blood in rats subjected to experimental stress.

MATERIALS AND METHODS

We examined blood serum specimens from randombred male albino rats (n=75) weighing 180-220 g maintained under vivarium condition in Saratov State Medical University. Immobilization stress, *i.e.* fixation of the rats in the supine position for 3 h was used as a physiological model activating lipoperoxidation [5]. The rats were randomized into five equal groups consisting of 15 rats. Group 1 rats were intact control; group 2 rats were immobilized and not irradiated (control); groups 3 and 4 rats were subjected to a single irradiation session for 15 and 30 min, respectively. Irradiation of a skin site (3 cm^2) located over the xiphoid process of the sternum was performed with a KVCh-NO apparatus in a frequency range of 150.176-

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0007-4888/09/1482-0200

150.664 GHz. The radiator was placed at the distance of 1.5 cm above the skin. The radiation power was 0.7 mW with the power flow of 0.2 mW/cm^2 [6].

Group 5 rats was formed to confirm our assumption that LPO processes are modulated by terahertz irradiation at the nitric oxide frequencies 150.176-150.664 GHz. These rats were irradiated for 30 min with electromagnetic waves of extremely high frequencies (EHF) at 53.54 GHz produced by an Yav'-1 generator and subjected to immobilization stress. The skin (3 cm^2) above the xiphoid process of the sternum was irradiated. The radiator was placed at the distance of 1.5 cm above the skin. The power flow was 0.7 mW/cm^2 [6]. During the experiments, all rats were kept under the same conditions.

The blood was drawn into plastic tubes via cardiocentesis. The blood samples were stabilized with 3.8% sodium citrate in 9:1 ratio. Lipoperoxidation processes were assessed by blood content of MDA and lipid hydroperoxides, intermediate products of lipoperoxidation. The degree of autointoxication and the development of cytolysis syndrome were evaluated by the content of medium-molecular-weight molecules in the blood. The integral indices of activity of blood antioxidant and antiradical defense systems were peroxide resistance of erythrocytes, vitamin E content, total content of sulfhydryl groups (SH-groups), and activities of SOD and catalase [10].

Experiments were carried out in strict adherence to World Medical Association Declaration of Helsinki (2000).

The data were statistically processed using Statistica 6.0 software. The hypotheses on the distribution types were analyzed using Shapiro—Wilk test. Since the most data were not normally distributed we used Mann—Whitney *U* test. The differences were significant at $p<0.05$.

RESULTS

In rats subjected to immobilization stress, LPO processes were dramatically activated, which was seen from significant accumulation (in comparison with the control) of MDA and lipid hydroperoxides, intermediate products of lipoperoxidation (Table 1). Excessive accumulation of LPO products during immobilization stress was accompanied by the development of cytolysis syndrome manifested in excessive accumulation of medium-molecular-weight molecules in the blood (Table 1).

In stressed rats, inhibition of both enzymatic and non-enzymatic components of the antioxidant system was observed. This manifested in significant changes in SOD and catalase activities in erythrocytes and a decrease in concentration of total SH-groups and vitamin E in blood serum. We also observed progressive impairment of peroxidation resistance of the erythrocytes: the content of hemolyzed erythrocytes increased (Table 1).

Thus, our study showed that activation of free radical oxidation is the efferent element of stress-dependent disorganization of cells. This observation completely agrees with the data showing that LPO activation is a general metabolic stage of stress reaction, which develops immediately in response to extraordinary stimulation and in turn, can initiate accumulation of intermediate products of lipoperoxidation and moderation of functional activity of the antioxidant system [2,10].

Irradiation of immobilized rats with terahertz waves within the range of 150.176-150.664 GHz for 15 minutes led to partial normalization of LPO processes and antioxidant activity: concentration of intermediate LPO products decreased and antioxidant properties of the blood partially recovered (Table 1).

Similar irradiation applied for 30 min normalized LPO processes: the concentration of intermediate LPO products returned to a level characteristic of intact rats (Table 1). Functional activity of enzymatic and non-enzymatic stages of the antioxidant protection of the cells was also restored and did not significantly differ from the level observed in intact rats (Table 1).

Therefore, the chosen mode of irradiation completely normalized the course of LPO processes and restored activity of the antioxidant system.

Irradiation of immobilized rats with EHF electromagnetic waves at the frequency of 53.54 GHz for 30 min led to only partial normalization of lipoperoxidation processes and antioxidant activity: the content of toxic intermediate LPO products somewhat decreased and antioxidant properties of the blood partially recovered (Table 1).

Thus, 30-min electromagnetic irradiation in the terahertz range at the NO frequencies of 150.176-150.664 GHz is the most efficient irradiation mode to restore disturbed LPO processes as well as enzymatic and non-enzymatic mechanisms of the antioxidant cell protection.

NO plays an important role of stress-limiting factor. It limits the release of pituitary stress hormones and catecholamines from the synaptic structures and adrenal glands [9]. In addition, NO prevents elevation of intracellular calcium, increases activity of antioxidant enzymes and expression of the corresponding genes. Moreover, it activated synthesis of the protective proteins Hsp70, stabilizes and modifies phospholipid bilayer of biomembranes, energy and plastic supply to cells, activity of membrane transport and receptor systems, cell excitability, numerous intracellular metabolic processes, and cell-cell interactions [9,11].

TABLE 1. Effect of Electromagnetic Irradiation of Terahertz Frequencies in NO-Related Range of 150.176-150.664 GHz on the Content of Intermediate Products of Lipoperoxidation and Blood Antioxidant Activity in Stressed Rats

Index	Intact rats (n=15)	Stress (n=15)	Terahertz electromagnetic irradiation		Irradiation with EHF waves for 30 min (n=15)
			15 min (n=15)	30 min (n=15)	
Lipid hydroperoxides, optical density unit/ml	3.49 (2.01; 4.0)	7.45 (5.60; 8.02) $p_1<0.01$	5.01 (4.80; 5.45) $p_1<0.05$; $p_2<0.05$	3.96 (2.22; 4.55) $p_1>0.05$; $p_2<0.01$; $p_3<0.05$	6.00 (5.55; 7.49) $p_1<0.05$; $p_2<0.05$; $p_3<0.05$; $p_4<0.01$
MDA, μmol/ml	3.64 (2.80; 4.11)	7.65 (5.22; 8.65) $p_1<0.01$	6.82 (5.88; 7.77) $p_1<0.05$; $p_2>0.05$	4.01 (2.33; 4.55) $p_1>0.05$; $p_2<0.01$; $p_3<0.05$	6.55 (4.33; 7.29) $p_1<0.05$; $p_2<0.05$; $p_3>0.05$; $p_4<0.01$
Medium-molecular-weight molecules, ex. units	0.25 (0.22; 0.30)	0.47 (0.30; 0.51) $p_1<0.05$	0.44 (0.38; 0.50) $p_1<0.05$; $p_2>0.05$	0.29 (0.2; 0.3) $p_1>0.05$; $p_2<0.01$; $p_3<0.05$	0.40 (0.25; 0.49) $p_1<0.05$; $p_2>0.05$; $p_3>0.05$; $p_4<0.01$
SH-groups, mmol/liter	2.01 (1.62; 2.74)	0.84 (0.84; 1.15) $p_1<0.05$	1.11 (0.71; 1.22) $p_1<0.05$; $p_2<0.05$	1.80 (1.64; 2.12) $p_1>0.05$; $p_2<0.05$; $p_3<0.05$	1.73 (1.65; 2.14) $p_1>0.05$; $p_2<0.05$; $p_3<0.05$; $p_4>0.01$
Catalase, μU/liter (erythrocytes)	3.44 (2.80; 3.77)	8.02 (7.01; 8.87) $p_1<0.01$	6.22 (5.44; 6.84) $p_1<0.05$; $p_2<0.05$	4.00 (3.55; 4.22) $p_1>0.05$; $p_2<0.01$; $p_3<0.05$	6.66 (5.22; 8.01) $p_1<0.05$; $p_2<0.05$; $p_3>0.05$; $p_4<0.01$
SOD, arb. unit (erythrocytes)	373.81 (320.1; 398.1)	246.23 (220.1; 264.2) $p_1<0.01$	285.5 (270.1; 301.1) $p_1<0.05$; $p_2<0.01$	341.22 (321.5; 382.6) $p_1>0.05$; $p_2<0.01$; $p_3<0.01$	267.33 (218.3; 295.3) $p_1<0.05$; $p_2<0.05$; $p_3<0.05$; $p_4<0.01$
PRE, arb. unit	1.57 (1.27; 1.87)	3.24 (3.01; 4.44) $p_1<0.05$	3.09 (2.85; 3.64) $p_1<0.05$; $p_2>0.05$	2.00 (1.44; 2.41) $p_1>0.05$; $p_2<0.05$; $p_3<0.05$	3.00 (2.27; 4.00) $p_1<0.05$; $p_2>0.05$; $p_3>0.05$; $p_4<0.05$
Vitamin E, opt. dens. unit/ml	20.11 (16.1; 22.4)	11.71 (8.24; 14.66) $p_1<0.01$	14.22 (12.2; 16.8) $p_1<0.05$; $p_2<0.05$	18.86 (16.3; 20.2) $p_1>0.05$; $p_2<0.01$; $p_3<0.05$	12.20 (10.23; 14.45) $p_1<0.01$; $p_2>0.05$; $p_3<0.05$; $p_4<0.01$

Note. PRE is peroxide resistance of erythrocytes. The median value and the lower and upper quartiles (25%, 75%) calculated for the corresponding number of experiments are shown. p_1: compared to intact rats, p_2: compared to immobilized (stressed) rats, p_3: compared to stressed rats subjected to 15-min terahertz irradiation, p_4: compared to 30-min terahertz irradiation.

Irradiation with terahertz waves in the NO-related frequency range during the action of a stressor agent prevents the development of the stress-dependent changes in the antioxidant system of the organism and moderates LPO activity. These effects can be related to elevation of reactivity of the free endogenous NO or its accumulation due to the action of terahertz electromagnetic irradiation at the frequencies of NO molecular spectrum directly on various NO-synthases resulting in their catalization [1,6,7,8]. It should be also mentioned that terahertz irradiation is characterized by a pronounced stress-limiting effect [4].

The data presented attest to possibility of correcting the disturbed LPO processes and insufficient functional activity of the antioxidants by terahertz irradiation within NO-related frequency range of 150.176-150.664 GHz.

REFERENCES

1. E. V. Andronov, *Vest. Nov. Med. Tekhnol*., **15**, No. 3, 14-16 (2008).
2. V. A. Baraboi, *Usp. Sovr. Biol*., **111**, No. 6, 923-931 (1991).
3. O. V. Betskii and A. P. Krenitskii, *Biomed. Tekhnol. Radioelectr*., No. 12, 3-6 (2003).
4. V. F. Kirichuk, O. N. Antipova, A. N. Ivanov, *et al*., *Ibid*., No. 11, 12-20 (2004).
5. V. F. Kirichuk, O. N. Antipova, A. P. Krenitskii, *et al*., A *Method of Prevention and Correction of Stress-Induced Damages to Living Organism*. *Patent of RF*, No. 2284837, October 10, 2006.
6. V. F. Kirichuk, O. N. Antipova, A. A. Tsymbal, *et al*., *Med. Tekh*., No. 1, 29-33 (2006).
7. V. F. Kirichuk, A. N. Ivanov, O. N. Antipova, *et al*., *Byull. Eksp. Biol. Med.,* **145**, No. 1, 81-84 (2008).
8. V. F. Kirichuk, A. P. Krenitskii, and A. V. Maiborodin, *Biomed. Tekhnol. Radioelectr*., No. 10, 95-108 (2002).
9. I. Yu. Malyshev and E. B. Manukhina, *Biokhimiya*, No. 63, 992-1000 (1998).
10. N. P. Chesnokova, E. V. Ponukalina, M. N. Bizenkova, *et al*., *Activation of Free-Radical Oxidation As Efferent Stage of Typical Pathological Processes* [in Russian], Saratov (2006).
11. E. Anggard, *Lancet*, **343**, 1199-1206 (1994).