Effects of Terahertz Radiation at Atmospheric Oxygen Frequency of 129 GHz on Blood Nitrite Concentrations under Conditions of Different Types of Stress against the Background of Administration of Nonselective Inhibitor of Constitutive NO-Synthases

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We studied the effect of terahertz radiation at atmospheric oxygen frequency 129 GHz on blood nitrite concentration in different types of experimental stress against the background of administration of nonselective inhibitor of constitutive NO-synthases. Normalizing effects of radiation on blood nitrite dynamics in animals with acute stress was shown after 15-min exposure and in animals with chronic stress after 30-min exposure. No positive effect of terahertz radiation was observed on altered blood nitrite concentration in male rats after preliminary administration of nonselective constitutive NO-synthase isoform inhibitor L-NAME.

Key Words: *nitrites; stress; constitutive NO-synthases; terahertz radiation; active cell metabolites*

Endothelial dysfunction is an initial stage in the pathogenesis of cardiovascular diseases [5]. Endothelial dysfunction is characterized by reduced production of vasodilating and antithrombotic agents and associated with predominant synthesis of vasoconstrictive and procoagulant factors, which results in increased vascular tone and provokes thrombosis [5].

NO is one of the most important factors synthesized by the endothelium. It is a unique second messenger in terms of its nature and mechanism of action in majority of body cells [9]. Endogenous NO is constantly synthesized in organs, tissues, and cells from amino acid L-arginine in reactions catalyzed by NO-synthases [10]. NO undergoes oxidation into nitrites and then into nitrates.

Reduced production of NO by the endothelium was demonstrated at early stages of cardiovascular disease; this manifests by reduced blood concentration of its stable metabolites, nitrites and nitrates [5].

Available methods for drug correction of the NO system are often insufficiently effective; they require thorough laboratory and clinical control during usage and associated with a wide spectrum of contradictions and side effects; high costs of this therapy is also worthy of note. Moreover, clinical use of organic nitrates as NO donors is associated with the development of resistance and cross-resistance, which can aggravate endothelial dysfunction and even more reduce NO production by the endothelium [5].

Thus, improvement of methods for NO synthesis regulation and the search for alternative therapeutic and experimental approaches are important problems.

The objective of the study was to investigate the effects of terahertz waves at frequency of molecular emission and absorption spectrum of atmospheric oxygen (129 GHz) on post-stress changes in nitrite blood concentration and against the background of

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administration of non-selective inhibitor of constitutive NO-synthases.

MATERIALS AND METHODS

Blood samples from outbred white male rats (n=110) weighing 180-220 g were examined. Immobilization stress was used for modeling disturbances in NO system. Single 3-h fixation in the supine position was used as the model of acute stress. Daily 3-h immobilization in the supine position for 5 days was used as the model of chronic stress. The animals were divided into 11 groups (10 animals in each). Group 1 (control) comprised intact rats; groups 2 and 3 (reference groups) consisted of rats subjected to acute and chronic immobilization; groups 4, 5, and 6 (experimental) included animals exposed to single irradiation in the beginning of acute immobilization for 5, 15, and 30 min, respectively; groups 7, 8, and 9 (experimental) included rats subjected to daily irradiation for 5 days for 5, 15, and 30 min, respectively, in the beginning of each immobilization; groups 10 and 11 included animals exposed to acute stress, single irradiation (in the beginning of immobilization) with terahertz waves for 15 and 30 min respectively against the background of administration of nonselective inhibitor of constitutive NO-synthases L-NAME.

To eliminate season and circadian effects on the dynamics of blood nitrite concentrations, experiments were carried out in autumn in first half of the day. All animal were kept under the same conditions during the experiment.

Against the background of acute or chronic stress, the animals were irradiated for 5, 15, or 30 min with electromagnetic waves at a frequency of atmospheric oxygen molecular spectrum of emission and absorption 129 GHz using KVCh-O_2-Orbita apparatus (skin area 3 cm^2 above the xiphoid process of the sternum). The radiator was positioned at a distance of 1.5 cm above the body surface. Power density for 3 cm^2 skin area was 100 μW/cm^2 [4,7].

To examine nitrite concentrations, the blood was taken into plastic tubes using cardiac puncture immediately after completion of acute or chronic (5 day long) immobilization.

Serum nitrite concentration was determined using Griss reagent according to the method described by V. B. Karpyuk *et al.* [3].

To elucidate the mechanism of beneficial effects of terahertz radiation at a frequency of atmospheric oxygen 129 GHz on altered blood nitrite concentrations after the stress, we used competitive constitutive NO-synthase inhibitor L-NAME (N_G-Nitro-L-arginine methyl ester, Sigma). The inhibitor was administered intramuscularly in a dose of 25 mg/kg 7 h before acute immobilization.

The experiments were performed in accordance to the principles of the Declaration of Helsinki (October 2000).

The data were processed statistically using Statistica 6.0 software. The differences were significant at p<0.05.

RESULTS

Statistically significant reduction of serum nitrite concentration was observed in rats subjected to acute or chronic immobilization stress (Tables 1, 2). The most pronounced reduction of serum nitrite concentration took place under conditions of chronic stress (Table 2). Thus, acute and chronic stress was associated with disturbances in NO production, which undoubtedly required correction (Tables 1 and 2).

Terahertz irradiation at a frequency of atmospheric oxygen 129 GHz for 5 min of the animals under

TABLE 1. Dynamics of Blood Nitrite Concentrations (μg/ml) after Acute Stress against the Background of Exposure to Terahertz Radiation at Atmospheric Oxygen Frequency 129 GHz in Rats

Intact Animals	Stress	Irradiation against the background of stress		
		5 min	15 min	30 min
0.76 (0.66; 0.80)	0.21 (0.20; 0.24) P_1<0.01	0.35 (0.25; 0.45) P_1<0.05; P_2<0.05	0.62 (0.50; 0.72) P_1>0.05; P_2<0.01 P_3<0.05	0.82 (0.61; 0.97) P_1>0.05; P_2<0.01 P_3<0.05; P_4<0.05

Note. Here and in Tables 2, 3: for each case, medians and the lower (25%) and upper (75%) quartiles are presented (within brackets) for the corresponding number of measurements. P_1: in comparison with intact animals; P_2: in comparison with animals exposed to immobilization stress; P_3: in comparison with animals exposed to 5 min radiation against the background of the stress; P_4: in comparison with animals exposed to 15 min radiation against the background of the stress.

conditions of acute stress only partially restored serum nitrite concentration (Table 1). Daily irradiation for 5 days against the background of chronic immobilization did not normalized altered serum nitrite concentration in male rats (Table 2).

Irradiation of acutely stressed animals with terahertz electromagnetic waves at a frequency of atmospheric oxygen 129 GHz for 15 min resulted in complete statistically significant normalization of blood nitrite concentration (Table 1). Daily 15-min exposure of stressed animals to terahertz radiation for 5 days only partially restored blood nitrite concentrations in male rats (Table 2).

Irradiation of animals against the background of acute immobilization stress with electromagnetic waves of the specified frequency for 30 min completely normalized altered NO production (Table 1). Daily 30-min irradiation of chronically immobilized animals for 5 days also completely normalized NO production, which manifested in a lack of statistically significant differences in serum nitrite concentrations between intact and irradiated animals (Table 2).

However, variability range of serum nitrite concentrations in animals exposed to terahertz irradiation for 30 min at a frequency of atmospheric oxygen 129 GHz against the background of acute stress was wider than variability of the parameter in control animals (Table 1). In some cases, nitrite concentration surpassed the level of intact control (Table 1).

Irradiation of stressed rats with terahertz waves at a frequency of atmospheric oxygen 129 GHz for 15 and 30 min against the background of preliminary administration of nonselective constitutive NO-synthase inhibitor did not normalize altered serum nitrite concentration: nitrite concentrations in animals subjected to irradiation against the background of preliminary L-NAME administration significantly differed from the control (Table 3).

Thus, administration of nonselective constitutive NO-synthase inhibitor L-NAME to stressed animals completely blocked the normalizing effect of terahertz irradiation at a frequency of atmospheric oxygen 129 GHz on altered serum nitrite concentrations (Table 3).

It is known that the effect of terahertz electromagnetic irradiation in cells and biological fluids can be mediated by reactive oxygen species, which continuously appear as a result of enzymatic reaction due to changes in hydration of protein molecules and increased activity of NADP-H-oxidase, cyclooxygenase, xanthine oxidase; as a result, their concentration is maintained at a stable level [8]. Reactive oxygen species in turn can stimulate soluble guanilate cyclase via calcium ions, which leads to accumulation of cy-

TABLE 2. Dynamics of Blood Nitrite Concentrations (μg/ml) after Chronic Stress against the Background of Exposure to Terahertz Radiation at Atmospheric Oxygen Frequency 129 GHz in Rats

Intact Animals	Stress	Irradiation against the background of stress		
		5 min	15 min	30 min
0.76 (0.66; 0.80)	0.19 (0.18; 0.22) $P_1<0.01$	0.22 (0.19; 0.24) $P_1<0.01$; $P_2>0.05$	0.50 (0.42; 0.62) $P_1<0.05$; $P_2<0.05$ $P_3<0.05$	0.69 (0.58; 0.84) $P_1> 0.05$; $P_2<0.01$ $P_3<0.01$; $P_4<0.05$

TABLE 3. Dynamics of Blood Nitrite Concentrations (μg/ml) in Stressed Animals against the Background of L-NAME Administration and Irradiation with Terahertz Waves at Frequency of Atmospheric Oxygen

Intact Animals	Stress	L-NAME administration and irradiation against the background of acute stress	
		15 min	30 min
0.76 (0.66; 0.80)	0.21 (0.20; 0.24) $P_1<0.01$	0.11 (0.11; 0.15) $P_1<0.01$; $P_2<0.05$	0.12 (0.1; 0.16) $P_1<0.01$; $P_2<0.05$ $P_4>0.05$

clic guanosine monophosphate in vascular endothelial cells, activation of NO-synthase, and enhanced NO production [2,6]. It is also indirectly supported by the lack of positive effects of terahertz radiation on altered blood nitrite concentration against the background of L-NAME administration [1].

Thus, 15-min radiation regimen is most effective for normalization of altered nitrite blood concentration after acute immobilization, whereas 30-min regimen is most effective after chronic immobilization. Administration of L-NAME to irradiated animals against the background of acute stress prevented the normalizing effects of terahertz radiation on blood nitrite concentration, which attests to involvement of constitutive NO-synthases into the mechanisms of positive effects of this radiation.

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REFERENCES

1. E. V. Andronov, *Vestn. Nov. Med. Tekhnol.*, **15**, No. 3, 14-16 (2008).
2. M. S. Volin, K. A. Davidson, P. M. Kaminski, *et al.*, *Biokhimia*, No. 63, 958-965 (1998).
3. V. B. Karpyuk, Yu. S. Chernyak, and M. G. Shubich, *Klin. Lab. Diagnost.*, No. 5, 16-19 (2000).
4. V. F. Kirishuk. *Apparatus for Exposure to Extremely High Frequency Electromagnetic Waves*. Useful model patent # 66961 dated 10 October 2007.
5. V. F. Kirichuk and P. V. Glybochko, *Endothelium Dysfunction*, Saratov (2008).
6. V. F. Kirichuk, C. V. Sukhova, O. N. Antipova, *et al.*, *Boimed. Radiotekhnol.*, No. 12, 20-25 (2008).
7. A. P. Krenitskiy, A. V. Mayborodin, O. V. Betskiy, *et al.*, *Biomed. Tekhol. Radiotekhnol.*, No. 8, 35-47 (2001).
8. M. M. Potselueva, A. V. Pustovidko, Yu. V. Evtodienko, *Dokl. AN*, No. 3, 415-418 (1998).
9. E. Anggärd, *Lancet*, **343**, 1199-1206 (1994).
10. L. G. Ignarro, F. Murad, *Adv. Pharmacol.*, **34**, 510-516 (1995).