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METHOD FOR CORRECTING ENDOTHELIAL DYSFUNCTION

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Abstract

The vascular wall endothelium is a metabolically active tissue whose dysfunction is a mandatory pathogenetic link in almost all cardiovascular diseases: coronary heart disease, hypertension, chronic heart failure, atherosclerosis, etc.

There is no doubt that the altered functional activity of vascular wall endothelial cells requires the active monitoring and correction. Given the high degree of comorbidity and pharmacological (drug) load of patients with cardiovascular diseases, and sometimes polypragmasia, it is advisable to use non-drug (complementary) methods of correcting endothelial functions in this group of patients.

The studied influence of terahertz electromagnetic waves at the frequencies of 150.176-150.664 GHz on the altered functional activity of the vascular wall endothelium was in the experiment. For its evaluation, the level of biologically active substances of endothelial origin that primarily affect vascular tone was studied: endothelial NO-synthase, endothelin-1, asymmetric dimethylarginine, the total concentration of nitrite/nitrate ions, which is a generally recognized method for evaluating the activity of nitric oxide production.

Irradiation of experimental animals was made with electromagnetic waves at the frequencies of nitric oxide 150.176-150.664 GHz on a skin area of 3 cm above the xiphoid process of the sternum. The emitter of electromagnetic waves was located at a distance of 1.5 cm above the surface of the animal's body. We used a "NO" emitter with a radiation power of 0.7 mW, the power density falling on a skin area of 3 cm in size was equal to 0.2 mW/cm^2. For irradiation of animals, a portable medical device of terahertz therapy "Orbit" was used the radiation dose was determined by the power density falling on the skin and the specified irradiation time. The duration of a single exposure was 15 and 30 minutes.

It was indicated that 15-minute exposure to terahertz waves leads to partial normalization of the profile of endothelium-dependent biologically active substances, and at 30 minute exposure, there is a complete statistically significant recovery of the concentration of biologically active substances produced by the endothelium.

Basing on the data achieved, it is reasonable to recommend the complementary use of terahertz electromagnetic waves at nitrogen oxide frequencies to correct altered endothelial functions.

Keywords: *endothelium, endothelial dysfunction, nitric oxide, endothelial NO-synthase, asymmetric dimethylarginine, endothelin-1, terahertz waves, terahertz technology, "Orbita" apparatus.*

Introduction

Nowadays, the issues of preserving and maintaining the health of citizens are foremost among the strategic tasks of the state's development.

The main cause of premature mortality in the world is cardiovascular diseases. According to the WHO report "World health statistics", the annual mortality rate from cardiovascular diseases among the world's population as a whole is 17.7 million, of which 7.4 million deaths are due to coronary heart disease and its exacerbations (acute coronary syndrome, acute myocardial infarction, unstable angina, etc.) [*Mensah G et al., 2017*].

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One of the leading chains in the pathogenesis of these diseases is the pathology of the vascular wall endothelium, its structure and functions [*Patti G, 2005; Sitia S et al., 2010; Hubert A et al., 2020*].

Having diseases of the cardiovascular system, the vascular endothelium appears as a primary target organ, since the endothelial lining of vessels participates in the regulation of vascular tone, the synthesis of inflammatory factors, fibrosis and their inhibition, performs barrier functions, and has a regulatory effect on the hemocoagulation system and platelet function [*Drexler H, Hornig B, 1999; Kirichuk V et al., 2008; Hubert A et al., 2020; Lespagnol E et al., 2020; Maida C et al., 2020*].

Patients with cardiovascular disease in most cases, these are comorbid patients. The accumulation of various nosologies is naturally accompanied by an active pharmacological (medicinal) load, which may or may not be justified. Polypragmasia ("drugs killers") simultaneous administration of a large number of drugs, including unreasonably. Polypragmasia in persons 60-69 years old occurs in 28.6%, and at the age of ≥80 years in 51.8% [*Walckiers D et al., 2015; Carmona-Torres J et al., 2018*].

This given situation requires the search for complementary (non-drug) ways to target the main links in the pathogenesis of endothelial dysfunction. Electromagnetic waves at the frequencies of active cellular metabolites can be perspective from the very opinion.

THz waves (T-rays) are named the electromagnetic waves with a frequency diapason 10^2-10^4 *GHz* (or with the lengths from 3 *mm* till 30 micro millimeters) [*Smye S et al., 2001; Rothman L et al., 2013; Svistunov A et al., 2017*]. Biophysical and biomedical effects of electromagnetic waves of THz diapason is a new field of research.

The features of *THz* rays are:

1. maximum energy quantum hν is twice higher than in classical EHF diapason;
2. belong to the "informative", nonthermal affects, as integral heating of radiating objects in the experiment doesn't exceed 0.1°C;
3. simultaneously as radio waves, they penetrate through many nontransparent solid materials, as light, *THz* waves can be focused , using this while constructing medical tools, but a special interest of scientists to THz waves is connected with that point that indeed there are frequency molecular spectra of radiation and absorption of the most important cellular metabolites (NO, O_2, CO_2, CO, OH). In the connection with this, prerequisites are created setting the opportunities of regulating directly their metabolism process in biological environment using the electromagnetic waves of *THz* diapason [*Smye S et al., 2001; Rothman L et al., 2013; Svistunov A et al., 2017*].

The greatest interest in experimental science is caused by electromagnetic waves of molecular spectrum of radiation and absorption of molecular oxygen and NO, since molecular oxygen and NO performs the function of one of the universal regulators of physiological, pathophysiological and biochemical processes in the cell and in the body as a whole [*Bian K, Murad F, 2003; Bian K, Murad F, 2007; Kapuganti J et al., 2020*].

On the basis of numerous studies Institute of Radio technique and electronics RAS named after V.A. Kotelnikov (Moscow, RF) and "Central Scientific Institute of Measuring Equipment" CIME (Saratov, RF) an innovative device for terahertz therapy "Orbit" was created, it forms electromagnetic waves of terahertz range at frequencies of active cellular metabolites (NO, molecular oxygen, etc.) [*Kirichuk V et al., 2006; Kirichuk F, Tsymbal A, 2011; Tsymbal A, Kirichuk V, 2011*].

Consequently, the purpose of this research is to study the peculiarities of the influence of terahertz electromagnetic waves at the frequencies of active cellular metabolites 150,176 – 150.664 *GHz* on the functional state of the vascular wall endothelium in stressed male rats in a chronic experiment.

To overcome it is possible, due to the uniting the knowledge and will of all doctors in the world

Materials and methods

The blood samples of 60 white outbred rat-males weighing 180-220 *g* were studied, they were obtained from the vivarium of the University. The experimental animals were placed into a specially equipped room, the access to which was limited.

As a model, imitating the endothelial dysfunction there was used the chronic immobilization stress – fixation of white male rats in the back position for 3 hours, every day, for 7 days.

The study was conducted in 4 groups of animals of 15 individuals in each: 1st group – control (intact animals); 2nd group – comparison, animals in a state of chronic immobilization stress; 3rd and 4th groups – experimental, in which animals were exposed to fractional mode for 15 and 30 minutes, respectively, against the background of each immobilization for 7 days.

Irradiation of experimental animals under prolonged immobilization stress was made with electromagnetic waves at the frequencies of the molecular spectrum of nitric oxide 150.176-150.664 *GHz* on a skin area of 3 *cm* above the xiphoid process of the sternum. The emitter of electromagnetic waves was located at a distance of 1.5 *cm* above the surface of the animal's body. The "NO" emitter was used, and the radiation power was 0.7 *mW*, and the power density falling on a 3 *cm* area of skin was 0.2 mW/cm^2. The radiation dose was determined by the power density falling on the skin and the specified irradiation time. The duration of daily exposure for 5 days was 15 and 30 minutes. In the control and comparison groups, the same manipulations accompanying irradiation were performed as in the animals of the experimental groups.

For irradiation of animals, a portable medical device of terahertz therapy "Orbita" was used, developed in "JSC Central research Institute of measuring equipment" (Saratov) (registration certificate No FSR 2009/05497, license No 99-03-002043 dated June 7, 2010) [*Kirichuk V et al., 2006*].

It is shown that the most physiological and effective is the fractional mode of action of terahertz waves on biological objects of different levels of organization. This mode of exposure was first used in this study [*Kirichuk V, Tsymbal A, 2011; Tsymbal A, Kirichuk V, 2011; Svistunov A et al., 2017*]. Fractional exposure mode "3/5" is an alternation of irradiation of a biological object – 3 minutes and 5 minutes – a break (no irradiation). The effective irradiation period was 15 and 30 minutes, the total exposure period was 40 and 80 minutes, respectively [*Kirichuk V, Tsymbal A, 2011; Tsymbal A, Kirichuk V, 2011; Svistunov A et al., 2017*].

The blood sampling from rats in all the experiments was carried out in an isolated quiet room, to which the animal was brought in an individual cage.

The blood was taken from experimental animals in plastic tubes by heart puncture. If necessary, a 3.8% solution of sodium citrate in a ratio of 9:1 was used as a blood stabilizer.

The level of endothelial nitric oxide synthase (eNOS) in blood plasma was quantitatively determined by solid-phase enzyme immunoassay using a set of reagents from Uscn Life Science Inc. (China, Wuhan).

Despite the fact that NO-synthase reaction in the endothelium is the main but not the only source of NO and nitrite ions, for the past several years, determination of total concentration of nitrite/nitrate ion is a well-recognized method for assessing activity eNOS and nitric oxide production in human body. The concentration of nitrites in the blood serum of experimental animals was defined using the L. Green method with modifications and use of the Griss-Ilosvay reagent [*Miranda K et al., 2001*].

The concentration of asymmetric dimethylarginine (ADMA) in blood serum was determined by solid-phase enzyme immunoassay using the test system "ADMA ELISA Kit Immundiagnostik AG" (Germany).

Quantitative determination of the level of endothelin-1 (1-38) in blood serum was made by solid-phase enzyme immunoassay using reagents from Biomedica gruppe (Austria).

The results were processed using the Statistica for Windows program (version 13.3). Hypotheses about the type of distributions (the Shapiro-Wilk test) are tested. It was found that the studied indicators are not described by the law of normal distribution, so further studies of dependencies were performed using

nonparametric statistics using The U-criterion of the Mann-Whitney test. During statistical processing of the obtained data, the main probabilistic characteristics of random variables were calculated: the average value and the lower (25%) and upper (75%) quartiles, which had a confidence of at least 95% (p-value < 0.05) and the Fisher Z-test, which is a derivative of the Mann-Whitney U-test.

Results

As a result of the performed biochemical studies, indicated that in male rats under chronic stress, the functional activity of the vascular endothelium changes, which is expressed in a change in the concentration of biologically active substances of endothelial origin (table).

Thereby, there is not only a statistically significant decrease in the content and activity of eNOS in the blood of experimental animals, but also an increase in the concentration of its endogenous competitive inhibitor – ADMA. Asymmetric dimethylarginine prevents the conversion of arginine to citrulline under the action of NO-synthase (eNOS), i.e. it disrupts the synthesis of nitric oxide. We found a correlation between the concentration of ADMA in blood plasma and the level of total no production. Simultaneously with these changes in the blood of the animals, the concentration of big ET-1 (1-38) increased statistically significantly (table).

Accordingly, chronic immobilization stress leads to changes in the functional activity of the vascular endothelium, which is naturally accompanied by the changes in the content and activity of its bioactive substances in the blood.

A 15-minute exposure to terahertz waves at the frequencies of nitric oxide 150,176-150.664 *GHz* in male rats under chronic stress leads to partial recovery of the studied parameters. Thus, irradiation causes a statistically significant decrease in the level of asymmetric dimethylarginine (ADMA), which is an endogenous competitive inhibitor of eNOS. Against the background of normalization of L-arginine/NO-metabolism, the functional activity and level of endothelial NO-synthase in the blood of ir-

Table

Biologically active substances secreted by the vascular endothelium in male rats under chronic immobilization stress and exposed to terahertz radiation for 15 and 30 minutes

Indices	Intact animals (n=15)	Chronicle stress (n=15)	Terahertz wave irradiation at nitrogen oxide frequencies 150.176-150.664 *GHz*	
			15 minutes (n=15)	30 minites (n=15)
Blood content of eNOS, (*pg/ml*)	**451.17** (423.7; 489.9)	**287.24** (227.8; 335.2) Z_1=4.11; P_1=0.002	**369.77** (318.2; 410.2) Z_1=0.52; P_1=0.604 Z_2=3.87; P_2=0.001	**389.19** (341.1; 414.9) Z_1=0.35; P_1=0.723 Z_2=3.36; P_2=0.002
Concentration of stable metabolites of NO-nitrites. (*μg/ml*)	**0.77** (0.66; 0.80)	**0.22** (0.18; 0.27) Z_1=2.84; P_1=0.004	**0.68** (0.63; 0.82) Z_1=0.46; P_1=0.632 Z_2=2.05; P_2=0.041	**0.81** (0.61; 0.97) Z_1=0.47; P_1=0.633 Z_2=4.62; P_2=0.001
Contents of big ET-1 (1-38). (*fmol/ml*)	**10.39** (8.65; 11.61)	**16.01** (12.87; 17.98) Z_1=3.24; P_1=0.002	**13.29** (11.01; 16.74) Z_1=0.72; P_1=0.004 Z_2=2.31; P_2=0.611	**12.11** (11.04; 13.44) Z_1=0.60; P_1=0.547 Z_2=2.10; P_2=0.035
The level of ADMA. (*μmol/l*)	**0.41** (0.21; 0.59)	**1.55** (0.93; 1.52) Z_1=3.97; P_1=0.001	**0.74** (0.57; 0.82) Z_1=0.96; P_1=0.336 Z_2=2.29; P_2=0.021	**0.69** (0.49; 0.88) Z_1=0.44; P_1=0.677 Z_2=2.88; P_2=0.003

Note: eNOS – *endothelial nitric oxide synthase,* ***big ET-1 (1-38)*** *– big endothelin-1 (1-38),* ***ADMA –*** *asymmetric dimethylarginine. In each case, the average value (median), lower and upper quartiles (25%, 75%) from the corresponding number of measurements are given. Z_1, P_1 – compared to the control group; Z_2, P_2 – compared to a group of animals under stress*

radiated animals increased, this was expressed in the restoration of production of NO by endothelial cells, and, consequently, its stable metabolites-nitrites in the blood serum of male rats. With the above changes, the concentration of cyclic polypeptide – big ET-1 decreased (1-38), but it did not reach the level of statistically significant values (table).

Therefore, electromagnetic radiation of the terahertz range at the frequencies of nitric oxide 150.176-150.664 *GHz* at the exposure of waves for 15 minutes can only partially normalize the altered endocrine activity of the vascular wall endothelium in experimental animals under stress.

Irradiation of male white rats under chronic immobilization stress with terahertz waves at 150.176-150.664 *GHz* nitric oxide frequencies for 30 minutes resulted in a statistically significant increase in the content and activity of eNOS, compared with a group of animals under stress that were not exposed to terahertz radiation. Thus, the level of eNOS increased to 379 *PG/ml*, and the level of stable nitric oxide metabolites-nitrites – to 0.82 μg/*ml*. Simultaneously with the normalization of the concentration and activity in the blood of eNOS, there was a statistically significant 2-fold decrease in the ADMA level to 0.62 *mmol*/*l*. Against the background of irradiation with these waves, the level of big ET-1 (1-38) was completely normalized within 30 minutes and its concentration in animals was restored to 12.13 *fmol*/*l* (table).

Thereby, irradiation with electromagnetic waves of terahertz range at frequency NO 30 minutes of white male rats on the background of chronic immobilization completely restores the functional activity of vascular endothelium, resulting in normalization of endothelium-dependent biologically active substances.

Discussion

Endothelial dysfunction is understood as an imbalance between endothelium-dependent substances that normally ensure the optimal course of all endothelium-mediated processes [*Kirichuk V et al., 2008*].

Endothelial dysfunction is the initial link of vascular damage. Experimental and clinical studies have indicated that abnormal endothelial function is associated with high cholesterol, diabetes, hypertension, and coronary heart disease [*Drexler H, Hornig B, 1999; Kirichuk V et al., 2008; Maida C et al., 2020; Hubert A et al., 2020; Lespagnol E et al., 2020*].

Endothelial dysfunction is a rather multifaceted process and it has been experimentally shown that its main manifestations are:

1. The changes in the level and bioavailability of NO, it is believed that this circumstance plays a key role in the onset of endothelial dysfunction under the influence of known risk factors for coronary heart disease.
2. Suppression of expression/inactivation of endothelial NO-synthase (the enzyme responsible for the synthesis of NO from L-arginine).
3. The increase in the concentration of the endogenous competitive inhibitor of NO synthase, ADMA. Asymmetric dimethylarginine prevents the conversion of arginine to citrulline under the action of NO-synthase, i.e. it disrupts the synthesis of nitric oxide. Finally, the combination of these factors eventually leads to a violation of L-arginine/NO-metabolism with a natural change in the functional activity of endothelial NO-synthase and the predominance of vasoconstrictor vascular reactions.
4. Increasing the production of large endothelial endothelin-1 and other vasoconstrictor substances by endothelial cells, which increases and supports the violation of L-arginine / NO-metabolism [*Bian K, Murad F, 2003; Bian K, Murad F, 2007; Ramadoss J et al., 2013; Matthaeus C et al., 2019; Kapuganti JG et al., 2020*].

Thus, the need to correct these changes is obvious. We have convincingly shown the possibility of using terahertz electromagnetic waves at no frequencies of 15 and 30 minutes to normalize endothelial functions in chronic stress in the experiment.

Speaking about the mechanisms of the identified effects, it is essential to highlight the following. The possibility of interaction of terahertz waves with NO-synthases is not excluded. We have shown that irradiation of male rats under chronic stress with terahertz waves at the no frequency for 30 minutes resulted in a statistically significant increase in the content and activity of

eNOS in the blood, and a decrease in the level of ADMA, which is an endogenous competitive inhibitor of eNOS. Against the background of normalization of L-arginine/NO-metabolism, the functional activity and level of endothelial NO-synthase in the blood of irradiated animals increased: this was expressed in the restoration of production of NO by endothelial cells, and, consequently, its stable metabolites-nitrites in the blood serum of male rats [*Gorren A, Mayer B, 1998*].

The result of this interaction may be an acceleration of intramolecular electron transfer (from FAD to FMN and from FMN to the oxidase domain), which leads to an increase in the rate of catalysis [*Gorren A, Mayer B, 1998*]. In addition, the interaction of THCH radiation with heme NO-synthase and/or guanylate cyclase – the main target of endogenous nitric oxide, leading to its transition to a high-spin state, which is accompanied by an increase in the affinity of NO-synthase to L-arginine and an increase in the activity of the enzyme [*Gorren A, Mayer B, 1998; Svistunov A et al.,2017*].

Consequently, when irradiated with THCH waves at frequencies NO 150,176-150.664 GHz, as well as the other frequencies, it is possible to increase the reactivity and/or concentration of endogenous nitric oxide.

Conclusion

Mechanisms of the influence of electromagnetic waves of the terahertz range at the frequencies of active cellular metabolites on biological systems are manifested, including at the tissue level.

The experimental data indicating the restoration of the functional state of the vascular wall endothelium and its endocrine activity when exposed to experimental animals with terahertz waves at the frequencies of active cellular metabolites, give grounds to recommend testing this method for correcting endothelial dysfunction in patients in clinical practice.

The conducted research creates prerequisites for further development, improvement and clinical testing of terahertz medical equipment for therapeutic sessions with electromagnetic waves of the terahertz range.

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