Original article

Restoration of blood rheological properties with the help of THz-band electromagnetic waves of nitrogen oxide molecular spectrum occurrence

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Abstract: A continuing relevance of the study is in researching of new non-drug methods of stress traumas correction. Stress is the main reason of different diseases and, first of all, of a cardiovascular system failure. The study *aims* the researching of the effect of electromagnetic waves of 150.176–150.664 GHz nitrogen oxide molecular and absorption spectrum occurrence. This is the most important natural regulator of endocellular interactions; it also influences the rheological blood indices in experimental stress-reaction of white male-rats. The blood rheological properties research was organized with the help of the rotation viscosimeter with a free-floating cylinder "АКР-2" [trasliteration from Russian: "a ka er 2"]. *The result* of the experiments displayed that: 1) the exposure of electromagnetic waves of 150.176–150.664 GHz nitrogen oxide molecular and absorption spectrum occurrence is able to regenerate (depending on a time mode) acute stress-dependent blood viscosity defects and the functional erythrocytes activity – their deformability and aggregation; 2) a 5-minute exposure of electromagnetic waves of the same frequencies band does not have influence with the stress-damaged blood viscosity and functional erythrocytes activity; 15-minute and 30-minute modes of exposure of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide are effective and totally regenerate stress-dependent blood viscosity defects and functional erythrocytes activity – their deformability and aggregation.

Keywords: hemorheology, electromagnetic exposure, THz-band, nitrogen oxide, erythrocytes, viscosity.

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Introduction

Stress situations (physical and psychological overstrains, emotional overloads) are the most important causative factors leading to a cardiovascular system pathology. It is commonly known that all of these factors are stress-factors; they cause general adaptation syndrome or the stress-reaction. This is accompanying by the amount of the complex of syndromes – hypertensive disease, hypercoagulability, hyperlipidaemia, depression and agitated state, cardiovascular system failure, immunodeficiency [1]. Nitrogen oxide is the prime natural regulator of endocellular interactions [2-5]. It is a neuromediator, potent hemostasis factor, antiaggregant, endogenic vasodepressor [3, 6-8], and it fulfils many important functions in an organism. It is indicated that nitrogen oxide has a stress-limiting effect [9].

Nowadays much attention is given to the point of the interaction of biological objects with THz-band, and 150.176–150.664 GHz nitrogen oxide molecular and absorption spectrum occurrence [10-12].

THz-therapy can be a new perspective non-drug method in treatment of many diseases [13]. The THz-band (100GHz – 10THz) is interesting by having molecular and absorption spectrum occurrence of the major cell metabolites (NO, O2, CO2, CO, OH- etc.) [13].

In vitro experiments in irradiation of THz-band electromagnetic waves of nitrogen oxide molecular and absorption spectrum occurrence (150.176–150.664 GHz) on platelet rich plasma and a whole blood indicated the influence of the given band on restoration of blood rheological properties in patients with stable and unstable angina when the thrombocyte and erythrocyte aggregation processes are defected [14].

The results of the experiments with irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence and 129.0 GHz atmospheric oxygen displays positive energetic influence of the given frequencies on the functional thrombocyte activity [11, 15], clotting and fibrinolytic blood properties [16], arterial blood gas and electrolytes [17], lipid peroxidation process and antioxidant blood properties [18, 19], functional thyroid condition [20], the major metabolic status indices [21], corticosterone level [22], blood corpuscle receptor system [23], endothelium condition [24], microcirculation [12].

This research **aimed** to study the effect of electromagnetic waves of 150.176–150.664 GHz nitrogen oxide molecular and absorption spectrum occurrence on the rheological blood indices in experimental stress-reaction of white rats.

Material and Methods

In order to support business objectives there had been studied whole blood samples of 75 white male-rats of 180-220 g. Immobilization stress was used as a model of blood rheological properties failure at patients with stable and unstable angina. There were 5 sets of experiments: 15 untreated rats -the control group; 15 animals in the immobilization stress condition, and a group of 15 animals under the 5-, 15- and 30-minute irradiation (on the background of the immobilization stress respectively) of the generator "КВЧ-NO-Орбита" {Traisliteration from Russian: "ka ve tche en o orbita"} (*Orbita* apparatus) worked on 150.176–150.664 GHz. The *Orbita* apparatus consists of: the Gunn diode generator, match membrane closed by round metal diaphragm (inner diameter 4 mm), body and shielded power cable. There is a power adapter as well with a supply transformer, rectifier, voltage regulator for the Gunn diode and electromagnetic oscillation frequency modulator.

The major specifications:

1. Operating frequency – 150.0±0.3 GHz (wave length (WL) 1,991-1,997 mm);
2. Frequency modulation 100 Hz; frequency deviation – 1 GHz;
3. Radiant power – 0.7 mW;
4. Parasitic oscillation suppression in 129 – 143 GHz band – 52 dB maximum.

The machine operates on mains power supplies of 220±10% V voltage alternating current, and it is optimized for the operation at a temperature of +10 – +35 ^{0}C and atmosphere relative humidity up to 80% (at a temperature of +25 ^{0}C). Continuous service time is up to 2 hours.

The structure of the molecular THz-band electromagnetic waves of nitrogen oxide occurrence is formed with the help of this generator according to the methods of the generator complex created by the researchers of the Saratov Central Scientific Institute of Measuring Equipment (Saratov, Russia). These methods are realized in quasi-optical extremely high frequency generator complex of determinate noise modeling for biophysical studies.

3 cm² skin area above the metasternum had been irradiated. Irradiator had been located 1.5 cm above the body of an animal. The radiant power was 0.7 mW, and the radiant power concentration on the 3 cm² skin area – 0.2 mV/cm^2 . The irradiation dosage depended on the power concentration on the skin area and the irradiation time mode. The length of a single irradiation was 5, 15 and 30 minutes.

Due to the fact that blood is not a non-Newtonian fluid, there was used a rotational viscometry to study blood flow phenomena.

The research was organized with the help of the rotation viscosimeter with a free-floating cylinder "АКР-2" {trasliteration from Russian: "a ka er 2"} [25]. This equipment conforms to a standard of the viscosity instrumentations of biological fluids:

- allows to study blood viscosity and its components (blood plasma, blood serum) under the specific rate-of-shear from 300 sec^{-1} to 5 sec^{-1};
- keeps constant temperature in sample cells;
- it is possible to organize experiments in a relatively short time;
- many fluid samples are not required.

Blood sampling was realized by right heart acupuncture. Heparin (40 U/ml) was as a blood anticoagulant (Gedeon Richter, Hungary) as it does not provoke polyplasmia which can be caused by using sodium citrate in proportion 1:9.

The rheological study was realized after 15-20 minutes since blood samples had been taken. The measurement was begun at a 300 sec^{-1} rate-of-shear to exclude sludging of blood [25].

The 0.85 ml samples of the materials were poured into a plastic measuring cell, then they had been thermostating for 5 minutes in special analyzer cells. After that dry metal cylinder was put into the cell with blood at an angle of 45^0 . The ability of the cylinder to float free (with no air bubbles between the cylinder and the cell wallside) in a sample was the major criterion of the correct filling of a measuring camera. Total time of the whole blood sample studying was no more than 10-15 minutes. The measurement was organized under the constant temperature condition in the measuring cell (37^{0}C), which is good for the most exact result [25, 26].

The researching of blood rheological properties was realized in the following rate band – 300, 200, 150, 100, 50, 20, 10, 5 sec^{-1} . It was done for the most correct estimate of the blood flow properties condition, and it was performed according to Yaroslavl agreement (Yaroslavl, Russia, 2003).

The given band was chosen because blood viscosity at low-rate-of-shear (up to 10-20 sec^{-1}) is typical to microvasculature, to lesser and medium arteries in 20-100 sec^{-1} interval, and high-rate-of-shear (more than100-150 sec^{-1}) mold the blood-stream in large-bore arterial vessels [28, 29]. Erythrocytes aggregability and deformability, their form and size influence much on blood viscosity, especially in vessels with low-rate-of-shear (microcirculation). These processes are the base of the non-Newtonian blood behavior that is to determine blood viscosity dependence on the rate-of-shear [28, 29].

The blood viscosity increases much at the rate-of-shear reduction below 10 sec^{-1}, which is connected with reversible red cells adhesion. This spurious blood viscosity decreases with increase of rate-of-shear caused by the aggregate destruction. There are no any erythrocyte aggregates at the rate-of-shear more than 100 sec^{-1}, discoid erythrocytes become elliptical with membrane rotation around the entocyte. Since this moment blood behaves as a non-Newtonian fluid – the blood viscosity becomes constant, symptomless (aorta, capillaries) [28, 29].

Erythrocytes aggregation (rouleaux formation) is one of the major indices of a blood viscosity, so its influence on toughness curve changes is rather important. The erythrocytes aggregation evidence was estimated by an index of erythrocytes aggregation (IEA) [25]. IEA was calculated as a quotient of the value of viscosity measured at 20 sec^{-1} by the value of viscosity measured at 100 sec^{-1}.

Erythrocytes deformability is one of the basic phenomena; it allows erythrocytes go through the vessels the diameter of which is commensurate to the size of erythrocytes themselves.

The decrease of the blood viscosity at the rate-of-shear more than 100 sec^{-1} is connected with erythrocytes deformability. Erythrocytes deformability index (EDI) was measured as a proportion of the value of blood viscosity measured at 100 sec^{-1} and the value of viscosity measured at 200 sec^{-1} [25].

Experiments on animals had been performed according to Geneva Convention "International Guiding Principles for Biomedical Research Involving Animals" (Geneva, 1990).

Statistical analysis of the results had been done with the help of nonparametric analysis with Mann-Whitney T-test ("Statistica 6.0" soft, USA). During the statistical analysis of the results there were the following probability characteristics of stochastic variables [median, lower (25%) and upper quartiles (75%)] which had validity not less than 95% (P-value < 0,05).

Table 1. Whole blood viscosity (in mPa•c) at male-rats under acute stress-reaction and different time modes of irradiation of THz-band electromagnetic waves of molecular and absorption spectrum of 150,176-150,664 GHz nitrogen oxide occurrence

Rate-of-shear	*Control (n=15)*	*Acute stress (n=15)*	*Acute stress together with the irradiation during…*		
			5 min (n=15)	*15 min (n=15)*	*30 min (n=15)*
300 sec^{-1}	2.4 (2.3, 2.5)	3.2 (3.0, 3.5) P_1<0.001	3.2 (3.0, 3.5) P_1<0.001; P_2=0.709	2.5 (2.3, 2.7) P_1=0.852; P_2<0.001	2.4 (2.3, 2.5) P_1=0.934; P_2<0.001
200 sec^{-1}	2.4 (2.3, 2.5)	3.2 (3.0, 3.5) P_1<0.001	3.2 (3.0, 3.5) P_1<0.001; P_2=0.708	2.5 (2.3, 2.7) P_1=0.851; P_2<0.001	2.4 (2.3, 2.5) P_1=0.851; P_2<0.0001
150 sec^{-1}	2.4 (2.3, 2.5)	3.3 (3.0, 3.6) P_1<0.001	3.2 (3.0, 3.6) P_1<0.001; P_2=0.589	2.5 (2.3, 2.7) P_1=0.177; P_2<0.001	2.4 (2.3, 2.6) P_1=0.604; P_2<0.001
100 sec^{-1}	2.5 (2.4, 2.6)	3.6 (3.4, 3.9) P_1<0.001	3.5 (3.3, 3.8) P_1<0.001; P_2=0.561	2.7 (2.5, 2.9) P_1=0.056; P_2<0.001	2.5 (2.4, 2.6) P_1=0.724; P_2<0.001
50 sec^{-1}	2.8 (2.7, 3.0)	4.2 (4.1, 4.5) P_1<0.001	4.2 (3.7, 4.6) P_1<0.001; P_2=0.771	3.0 (2.8, 3.3) P_1=0.064; P_2<0.001	2.9 (2.7, 3.0) P_1=0.493; P_2<0.001
20 sec^{-1}	3.4 (3.1, 3.6)	5.2 (5.1, 5.7) P_1<0.001	5.0 (4.7, 5.3) P_1<0.001; P_2=0.164	3.6 (3.3, 4.0) P_1=0.105; P_2<0.001	3.4 (3.2, 3.6) P_1=0.966; P_2<0.001
10 sec^{-1}	3.7 (3.4, 4.0)	6.0 (5.5, 6.2) P_1<0.001	5.8 (5.7, 6.2) P_1<0.001; P_2=0.966	4.0 (3.6, 4.4) P_1=0.16; P_2<0.001	3.8 (3.7, 3.9) P_1=0.506; P_2<0.001
5 sec^{-1}	4.2 (4.0, 4.4)	6.5 (6.2, 6.8) P_1<0.001	6.4 (6.2, 6.8) P_1<0.001; P_2=0.933	4.3 (4.0, 4.8) P_1=0.455; P_2<0.001	4.2 (4.1, 4.3) P_1=0.803; P_2<0.001

Data presented as median (Me), lower and upper quartiles (25%, 75%).

P_1 – differences accuracy comparing with the control group; P_2 – differences accuracy comparing with the acute stress group.

Table 2. Indices of the erythrocytes functional activity at male-rats under the acute stress-reaction and different time modes of irradiation of THz-band electromagnetic waves of molecular and absorption spectrum of 150,176-150,664 GHz nitrogen oxide occurrence

Indices	*Control (n=15)*	*Acute stress (n=15)*	*Acute stress together with the irradiation during…*		
			5 min (n=15)	*15 min (n=15)*	*30 min (n=15)*
Erythrocytes deformability index (RVU)	1.05 (1.04, 1.08)	1.09 (1.07, 1.13) P_1=0.004	1.10 (1.07, 1.12) P_1=0.004; P_2=0.693	1.06 (1.04, 1.08) P_1=0.851; P_2=0.006	1.06 (1.04, 1.09) P_1=0.299; P_2=0.018
Erythrocytes aggregation index (RVU)	1.33 (1.3, 1.36)	1.45 (1.43, 1.49) P_1<0.001	1.42 (1.37, 1.46) P_1=0.001; P_2=0.171	1.34 (1.31, 1.38) P_1=0.599; P_2<0.001	1.32 (1.31, 1.34) P_1=0.633; P_2<0.001

Data presented as median (Me), lower and upper quartiles (25%, 75%).

P_1 – differences accuracy comparing with the control group; P_2 – differences accuracy comparing with the acute stress group.

Results

In the group of rats with immobilization stress the results of the experiment displays statistically-valid increase of the whole blood viscosity at low and at high-rate-of-shear as well (comparing with the control group (*Table* 1)). The IEA and EDI are also statistically-valid increased (P<0.05) comparing with the control group (*Table* 2). This displays an increase of the erythrocytes aggregability and their flexibility.

During the 5-minute irradiation there was found out that the irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence on stressed animals does not affect much the whole blood viscosity and functional erythrocytes activity as well (their aggregation and deformability). This is evident from the fact that there are no statistically-valid differences in blood viscosity at any experimental rates-of-shear and functional erythrocytes activity indices in the group with the 5-minute irradiation (on the background of an acute stress), and the fact of the statistically-valid differences of a high degree of validity comparing with the control group (*Tables* 1 and 2).

During the 15-minute irradiation there was found out that the irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence on animals under the immobilization stress displayed total restoration of blood viscosity up to the level of the animals from the control group on all of the rates-of-shear typical to lesser, medium and large-bore arterial vessels [28, 29]. This is evident from the fact that there are no statistically-valid differences in the indices in the control group, and the fact of the statistically-valid differences of a high degree comparing with the analogical groups with the acute stress (*Table* 1).

The experiment with the functional erythrocytes activity (their aggregation and deformability) also displays total restoration of their functions under the experimental 15-minute irradiation of the given occurrence. This is evident from the fact that there are no statistically-valid differences between aggregation and deformability indices in the experimental group of the animals, and from the fact of the statistically-valid differences comparing with the group with the acute stress (*Table* 2).

During the 30-minute irradiation there was found out that the irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence on the stressed animals affects blood viscosity: there was total restoration of blood viscosity at low, medium and high-rate-of-shear. This is evident from the fact that there are no statistically-valid differences in the blood viscosity indices at any experimental rate-of-shear in the group with the 30-minute irradiation and in the control group. There are no statistically-valid differences of a high degree comparing with the acute stress group (*Table* 1).

The 30-minute irradiation of the experimental occurrence facilitates the restoration of erythrocytes functional properties, which influences positively the whole haemorrheology. This is evident from the fact that there are no statistically-valid differences between the indices of the control group and the 30-minute irradiation group, and the fact of the statistically-valid differences between the experimental group of male-rats and the acute stress group (*Table* 2).

So, as follows from the analysis, the conclusions are:

1) the irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence (depending on the time mode) is able to restore stress-dependent failures of blood viscosity and functional erythrocytes activity (deformability and aggregation);

2) the 5-minute irradiation of the given occurrence (on the background of the acute stress) does not affect much the restoration of the stressed blood viscosity and functional erythrocytes activity;

3) the 15- and 30-minute irradiation of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence are effective and totally recover stress-dependent blood viscosity and functional erythrocytes activity (deformability and aggregation).

Discussion

Blood rheological properties, as it is known, are characterized by the flow conditions when there is the blood flow determined by the geometry of the vessel and differential arteriovenous pressure, flow properties of blood corpuscles (erythrocytes mostly): their deformability and aggregation, interaction with high molecular weight proteins and all osmotic components, blood flow property [26, 28-30]. It is well known that blood plasma proteins influence the erythrocytes deformability and aggregation. So, fibrinogen, its degradation products concentrate on the erythrocyte membrane, and as a result cause the aggregation and an increase of the erythrocytes deformability. It has been argued that the erythrocytes deformability increase in plasma with a high fibrinogen (and its degradation products) level is the protecting mechanism of an organism, which prevents an acute blood flow failure. The findings about an increase of erythrocyte aggregation and their flexibility growth under the immobilization stress do not contradict with the findings (experiments with different pathological states) of other authors [31-33]. This also may be connected with fibrinogen increase in blood and its degradation products caused by fibrinolytic blood potential [34, 35].

It is found out that there is a decrease of erythrocyte aggregation [36] under the nitrogen oxide donators in vitro and in vivo as well. Hemorheology indices restoration, including erythrocyte aggregation process, at animals under the immobilization stress of THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence, can be connected with the rise of endogenic nitrogen oxide concentration and its activity increase. The general idea is that the THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence is a physiological regulator of NO-system based on its interaction with NO-synthase [37]. Besides, one of the factors preventing erythrocyte aggregation process is a coulomb repulsion [28, 29]. Antiaggregant effect of the THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence can be connected with its ability to stabilize erythrocyte membrane [38, 39].

An important rheological index is erythrocyte deformability and its changes are one of the factors that determine an oxygen delivery to tissues [40, 41]. The restoration of the erythrocyte deformability under the immobilization stress at THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence can be also explained by nitrogen oxide concentration and its activity increase, as we can see it from the information about the role of nitrogen oxide as a factor of erythrocyte membrane deformability decrease [42]. Besides, under the nitrogen oxide influence the vessels geometry changes are possible because of their dilation [4, 7, 43]. So this can be one more factor which may affect the restoration of rheological blood properties under stress at electromagnetic waves of nitrogen oxide occurrence.

Conclusion

Therefore, THz-band electromagnetic waves of 150.176–150.664 GHz nitrogen oxide occurrence can be used for restoration of the microcirculation failure, also connected with changes in rheological blood properties at the experimental animals. Afterwards this method can be effective for patients.

Conflict of interest: none declared.

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