

Correction of Disorders in Tissue Perfusion, Blood Coagulation and Fibrinolysis with Orbita Apparatus on Terahertz Waves of Cell Metabolites

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ABSTRACT

This article contains information on principle of operation, technical parameters and possible application of *Orbita* apparatus for hemodynamic, fibrinolytic and peripheral perfusion disorders treatment. A single exposure to terahertz waves emitted by *Orbita* apparatus, corresponding to frequencies of molecular absorption and emission spectra of atmospheric oxygen (129.0 GHz), completely cures coagulant and fibrinolytic disorders of animals with acute immobilization stress. A course of treatment with electromagnetic waves corresponding to frequencies of molecular absorption and emission spectra of nitrogen oxide (150.176 - 150.664) leads to normalization of disrupted peripheral tissue perfusion parameters of animal undergoing treatment and stimulates basal and induced output of nitrogen oxide. This leads to decrease in peripheral vascular resistance to microcirculation and increase in blood flow to microvasculature. Experimental data provided in this article serves as a proof of viability of *Orbita* apparatus for treatment of coagulant, fibrinolytic and tissue perfusion disorders.

Keywords: *Orbita* Apparatus; Terahertz Waves; Nitrogen Oxide; Atmospheric Oxygen; Stress; Peripheral Perfusion; Coagulation; Fibrinolytic Disorders

1. Introduction

Nowadays, development of new drug-free methods of hemodynamic disorder treatment is a subject of intense study. One of such methods is application of low-intensive millimeter and submillimeter radiation [1-4].

In recent years, a new branch of information therapy has emerged—terahertz therapy [5]. Terahertz frequency band makes for an interesting research subject because molecular absorption and emission spectra (MAES) of various cell metabolites (NO, CO, active forms of oxygen etc.) belong to this band [6].

Our experimental studies resulted in creation of *Orbita* apparatus whose principle of operation is based on application of terahertz electromagnetic waves, corresponding to frequencies of molecular absorption and emission spectra of nitrogen oxide (150.176 - 150.664 GHz) and atmospheric oxygen (129.0 GHz) [7], emitted by special terahertz wave generators created for the first time.

There is evidence of positive effect of the said frequencies energy deposition on rheological properties of blood and platelet functional activity [8,9], coagulant and fibrinolytic activity [10], blood gas and electrolyte con-

centration [11], lipid peroxidation and antioxidative activity [12,13], functional status of thyroid body [14], primary indices of metabolic status [15], concentration of adrenocorticotrophic hormone in blood [16], receptor system of formed blood elements [17], state of vascular endothelium [18] and microcirculation [19].

The purpose of this research work is to study the effect of emission produced by *Orbita* apparatus on peripheral tissue perfusion as well as coagulation and fibrinolytic activities of blood of white rats affected by acute and continuous immobilization stress.

2. Materials and Methods

A group of 120 male non-pedigree albino rats with average weight of 180 - 220 g was chosen as a test subject. All animals were exposed to similar conditions. An acute and continuous immobilization stress caused by a single or daily 3-hour long immobilization performed to test animals for 5 consecutive days was chosen a model system of coagulation and fibrinolytic activities and microcirculation disorders.

The studied animals were divided into 3 groups of 15

animal each: 1st group—control group, 2nd group—comparison group (animals with acute and continuous immobilization stress), 3rd group was comprised of animals exposed to a single terahertz radiation (generated by *Orbita* apparatus) equal to frequency of atmospheric oxygen (129.0 GHz) for 5, 15 and 30 minutes (respectively) during acute stress and daily terahertz radiation equal to frequencies of nitrogen oxide (150.176 - 150.664 GHz) after every 3-hour long immobilization for 5 consecutive days.

Orbita, a terahertz therapy apparatus, contains two radiation heads: one emitting 129 GHz frequency bands and the other 150 GHz ones. 129 GHz frequency band is one of spectral lines in second zone (120 - 132 GHz) of molecular absorption spectrum of nitrogen oxide (NO). [6].

The apparatus electrical power is supplied by (220 ± 22) V power networks with standard frequency of (50 ± 0.5) Hz.

Maximal electrical power consumption of this apparatus does not exceed 15 VA. The apparatus climate class conforms to boreal climate requirements as prescribed by category 4.2 of GOST (State Standard) No. 15150: working temperature range -10°C to 35°C , maximal relative humidity -80% at 25°C ; ambient pressure range -84 to 106.7 kPa (630 to 800 mmHg).

Primary technical parameters of this apparatus are as follows:

- 1) Central frequency of generator with KRPГ. 431111.001-01 head: 150.0 ± 0.75 GHz;
- 2) Radiation power output: 100 μW ;
- 3) Available time range for therapeutic treatment duration: 1 - 99 minutes;
- 4) Increment of available time range for therapeutic treatment duration: 1 minute;
- 5) Maximal electrical power consumption: 15 VA;
- 6) Operating mode setup time: no more than 1 minute;
- 7) Continuous work time: no less than 8 hours;
- 8) Expected reliability parameters of apparatus operation (at confidence factor $P = 0.8$) shall be as follows:
 - Mean time to failure (T_0): no less than 400 hours;
 - Average useful life ($T_{\text{сн}}$): no less than 5 years;
 - Mean time to recovery ($T_{\text{в}}$): no more than 3 hours.

Terahertz therapy apparatus consists of MES30A-2PIJ regulated medical type power supply unit (+9 V) with HO5VV-F networks cable, control and display unit, membrane keyboard and extremely-high frequency (EHF) wave emitter. Control and display unit contains analog signal generator and general purpose control and display element.

Analog signal generator of terahertz therapy apparatus performs functions as follows:

- Generation of continuous voltage (+5 V) for general purpose control and display element power supply;

- Amplification and stabilization of electric current running through Gunn diode of emitters connected to apparatus;
- Connection/disconnection of EHF wave emitter to/from electric power supply network of generator during reception of control signal from microcontroller of general purpose control and display element;
- Generation of audible signals during reception of control signal from microcontroller of general purpose control and display element;
- ЯКУЛ.467851.016 general purpose control and display element of terahertz therapy apparatus performs functions as follows;
- Reception and processing of signals from membrane keyboard;
- Control over EHF wave emitters power supply and piezoelectric emitter of analog signal generator;
- Provision of information about set operating mode and duration treatment on liquid-crystalline display (LCD).

General purpose control and display element consists of PIC16F876A microcontroller, LCD and keyboard controller and MT-16S2D-2VLB symbol (alphanumeric) LCD produced by МЭИТ company. Electrical power supply of General purpose control and display element is provided by L7805CV voltage regulator. The regulator transforms +9 V voltage provided by MES30A-2PIJ power supply unit to +5 V continuous voltage.

LCD and keyboard controller is built on CE110 microchip. This microchip uses CMDS technology, has a low power consumption rate (≈ 2 mA) and requires only few external discrete components. For example, this microchip does not require connection to crystal oscillator as its clock frequency is generated by a simple RC-generator. The microchip is connected to electric power supply via CE110 basic connection layout.

PIC16F876A microcontroller produced by MicroChip company is an eight-bit RISC-based processor. This processor is produced by CMOS FLASH/EEPROM method which provides its low power consumption and high speed performance. Memory size of microcontroller is as follows: FLASH memory -8 KB, EEPROM memory -256 Byte (B), data memory -368 B. Microcontroller can operate in a wide voltage range (from 2 to 5.5 V). The microcontroller design allows application of crystal oscillator.

PIC16F876A microcontroller of terahertz therapy apparatus performs functions as follows:

- Reception and transfer of data and control signals to CE110 LCD and keyboard controller via I^2C interface as regulated by a software program recorded into microcontroller;
- Transmission of +5 V voltage pulse to analog signal generator in order to connect/disconnect emitter to/from

power supply as regulated by a software program recorded into microcontroller;

- Transmission of +5 V voltage pulse to analog signal generator in order to switch 3И-18 piezoelectric emitter on/off and generate audible signals as regulated by a software program recorded into microcontroller. All software is recorded in controller memory.

The radiation heads consist of brass hull with waveguide channel with 1.6×0.8 mm section implanted with Gunn diode with in-feed.

Radiation-producing waveguide flange has a thread that can be used to attach a PTFE safety cap transparent for terahertz waves (quenching -0.3 ± 0.1 dB). Radiation head hull is encased in protective shroud with an identification nameplate containing a six-digit code.

Head power supply is provided by regulated power supply source of control and display unit. Pulse operation of radiation head is provided by switching on impulse power supplied to Gunn diode. In the process of operation the head produces terahertz radiation.

Coagulation and fibrinolytic properties of blood of white affected by acute immobilization stress were studied with a series of tests performed on CGL 2110 "Solar" (Minsk, the Republic of Belarus) turbidimetric coagulation meter using reagents provided by NPO (research and production association) "RENAM" (Moscow, Russia).

Primary characteristics of hemostatic system coagulation include activated partial thromboplastin time (APPT), prothrombin time, international normalized ratio (INR), fibrinogen concentration, thrombin time and factor XIII activity in blood plasma. Anticoagulant blood potency was studied by determination of activity of natural anticoagulants such as antithrombin-III and protein C contained in blood plasma. Fibrinolytic blood potential was studied with Hageman-dependent and streptokinase-induced euglobulin fibrinolysis by calculating plasminogen reserve index.

Disruption of dermal microvasculature was studied by laser Doppler flowmetry (LDF) on the 6th day since the start of tests with 2nd version of "JIAKK-02" blood flow laser analyzer (produced by NPP (research and production enterprise) "Lazma", Russia) and LDF 2.20.0.507 WL software program. In order to immobilize tested animal, it was injected (intramuscularly) with a combination of zoletyl (produced by Virbac Sante Animale, France; doze: 0.05 ml/kg) and xylazine (produced by "Interchemie", France; doze: 1 ml/kg). Sensor of laser blood flow analyzer is attached to the back surface of right foot with an atraumatic plaster. Standard record time was 7 minutes.

Statistical analysis of obtained data was performed with the help of Statistica 6.0 program. Distribution type hypothesis were checked (Shapiro-Wilk test). A majority of obtained data does not comply with Gaussian distribu-

tion law, thus, Mann-Whitney U-test was used to compare data.

3. Results and Discussion

Exposure of animals affected with immobilization stress to terahertz radiation equal to frequency of atmospheric oxygen (129.0 GHz) for 5 minutes did not cause significant changes of disrupted coagulant and fibrinolytic parameters as evidenced by a lack of statistically significant discrepancies between characteristic parameters of coagulation cascade and fibrinolysis in comparison to non-involved animals. However, there is a statistically significant discrepancy between values of primary characteristics of hemostatic system coagulation and fibrinolytic activity of blood in comparison to non-involved animals (**Tables 1 and 2**).

It should be noted that exposure of animals affected with acute immobilization stress to electromagnetic waves from the aforementioned range for 15 minutes lead to partial normalization of characteristic parameters of coagulation cascade and fibrinolysis, *i.e.* APPT and thrombin time were recovered completely and had no statistically significant discrepancies with the same parameters measured for non-involved animals. However, all other hemostatic parameters had statistically significant discrepancies of those of non-involved animals (**Tables 1 and 2**).

According to test results, perfusion parameter (M) of male rats, affected by continuous stress and exposed to a series of treatment with *Orbita* apparatus, recovered completely despite having statistically significant discrepancies from the same parameter measured for animals in control group (**Table 3**). A statistically significant increase of root mean square deviation of perfusion parameter and variation coefficient is observed for animals of the aforementioned group compared to animals affected with continuous stress and not exposed to terahertz wave radiation (**Table 3**). It should also be noted that the value of root mean square deviation of perfusion parameter and variation coefficient did not reach the control group value which is an evidence of incomplete recovery of micro blood flow modulation and mechanisms of its regulation.

According to results of amplitude-frequency analysis of LDF-charts, a statistically significant increase of amplitudes of vasomotor and endothelial oscillations was observed for male rats, affected by continuous stress and subjected to a series of exposure to electromagnetic waves equal to frequencies of molecular absorption and emission spectra of nitrogen oxide, compared to animals affected with continuous immobilization stress and not exposed to terahertz wave radiation (**Table 4**). This means that vasodilating activity of endothelium increased (by activation of basal nitrogen oxide production) and

Table 1. Primary characteristics of hemostatic system coagulation measured after exposure of white rats, affected with experimentally induced stress, to terahertz electromagnetic waves equal to frequencies of molecular absorption and emission spectra of atmospheric oxygen (129.0 GHz) for various time.

Parameter	Noninvolved animal (n = 15)	Animal under stress (n = 15)	Exposure to radiation under stress for the time provided below		
			5 minutes (n = 15)	15 minutes (n = 15)	30 minutes (n = 15)
APPT, s	24.8 (21.3; 26.3)	19.0 (17.9; 20.0) P ₁ < 0.05	19.9 (18.1; 21.1) P ₁ < 0.05; P ₂ > 0.05	23.4 (21.2; 25.5) P ₁ > 0.05; P ₂ < 0.01 P ₃ < 0.05	25.1 (22.8; 27.6) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ > 0.05
Prothrombin time, s	19.5 (17.3; 21.2)	15.0 (14.0; 18.0) P ₁ < 0.01	16.1 (15.0; 17.8) P ₁ < 0.05; P ₂ > 0.05	17.2 (16.2; 20.1) P ₁ < 0.05; P ₂ < 0.05 P ₃ > 0.05	18.8 (17.5; 21.5) P ₁ > 0.05; P ₂ < 0.01 P ₃ < 0.05; P ₄ < 0.05
International normalized ratio (c.u.)	1.22 (0.88; 1.44)	0.86 (0.77; 1.11) P ₁ < 0.05	0.99 (0.80; 1.20) P ₁ < 0.05; P ₂ > 0.05	1.01 (0.91; 1.44) P ₁ < 0.05; P ₂ < 0.01 P ₃ > 0.05	1.11 (1.00; 1.35) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ < 0.01
Thrombin time, s	22.0 (19.6; 23.5)	15.0 (14.2; 18.3) P ₁ < 0.01	16.4 (15.0; 18.7) P ₁ < 0.05; P ₂ > 0.05	20.8 (19.0; 23.3) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05	21.2 (20.0; 24.4) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ > 0.05
Fibrinogen concentration, g/l	2.0 (1.68; 2.22)	3.7 (1.71; 3.75) P ₁ < 0.05	3.2 (2.0; 3.5) P ₁ < 0.05; P ₂ > 0.05	2.9 (2.1; 3.3) P ₁ < 0.05; P ₂ < 0.05 P ₃ > 0.05	2.4 (1.75; 2.5) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ > 0.05
Factor XIII activity in blood plasma, %	50.0 (45.0; 70.0)	75.0 (60.0; 95.0) P ₁ < 0.01	70.0 (57.0; 80.0) P ₁ < 0.01; P ₂ > 0.05	57.0 (44.0; 60.1) P ₁ < 0.05; P ₂ < 0.05 P ₃ < 0.05	54.0 (45.2; 65.0) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.01; P ₄ < 0.05

Notes: APPT—activated partial thromboplastin time; An average value (median), lower and upper quartiles (25%, 75%) from a corresponding number of measurements of every parameter are provided in the table; Discrepancies with $P < 0.05$ were considered statistically significant; P₁—comparison with uninvolved animal group; P₂—comparison with a group of animal with induced immobilization stress; P₃—comparison with a group of animal exposed to radiation under stress for 5 minutes; P₄—comparison with a group of animal exposed to radiation under stress for 15 minutes.

Table 2. Changes of characteristic parameters of anticoagulant and fibrinolytic activities of blood of white rats, affected with experimentally induced stress, after exposure to terahertz electromagnetic waves equal to frequencies of molecular absorption and emission spectra of atmospheric oxygen (129.0 GHz) for various time.

Parameter	Noninvolved animal (n = 15)	Animal under stress (n = 15)	Exposure to radiation under stress for the time provided below		
			5 minutes (n = 15)	5 minutes (n = 15)	5 minutes (n = 15)
Antithrombin III, s	30.0 (25.0; 36.0)	24.8 (21.0; 30.1) P ₁ < 0.05	23.0 (20.0; 27.0) P ₁ < 0.05; P ₂ > 0.05	25.0 (23.0; 30.0) P ₁ < 0.05; P ₂ > 0.05 P ₃ < 0.05	32.0 (27.0; 35.0) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ < 0.05
Protein C activity, c.u.	0.87 (0.60; 1.0)	0.54 (0.30; 0.61) P ₁ < 0.05	0.61 (0.55; 0.77) P ₁ < 0.05; P ₂ > 0.05	0.66 (0.60; 0.77) P ₁ < 0.05; P ₂ < 0.01 P ₃ > 0.05	0.80 (0.77; 1.1) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ < 0.05
HPa—kininogenase-dependent fibrinolysis, min	14.0 (12.0; 16.1)	24.0 (19.0; 27.0) P ₁ < 0.01	22.4 (20.0; 25.0) P ₁ < 0.05; P ₂ > 0.05	18.1 (15.0; 20.0) P ₁ < 0.05; P ₂ < 0.01 P ₃ < 0.05	15.7 (13.0; 19.0) P ₁ > 0.05; P ₂ < 0.05 P ₃ < 0.05; P ₄ < 0.05
Streptokinase-induced euglobulin fibrinolysis, s	130.0 (110.0; 160.0)	180.0 (160.0; 200.0) P ₁ < 0.01	171.0 (155.0; 190.0) P ₁ < 0.05; P ₂ > 0.05	150.0 (130.0; 170.0) P ₁ < 0.05; P ₂ < 0.01 P ₃ < 0.01	138.5 (125.0; 150.0) P ₁ > 0.05; P ₂ < 0.01 P ₃ < 0.01; P ₄ < 0.05
Plasminogen reserve index, %	70.1 (61.5; 86.6)	50.5 (42.1; 60.0) P ₁ < 0.01	46.3 (30.3; 50.5) P ₁ < 0.01; P ₂ > 0.05	62.3 (50.1; 70.6) P ₁ < 0.05; P ₂ < 0.05 P ₃ < 0.05	66.1 (55.5; 80.5) P ₁ > 0.05; P ₂ < 0.01 P ₃ < 0.01; P ₄ < 0.05

Notes: see Table 1.

Table 3. Changes of perfusion parameter of animals affected by continuous stress and subjected to a course of treatment with terahertz electromagnetic waves equal to frequencies of molecular absorption and emission spectra of nitrogen oxide (150.176 - 150.664 GHz).

Parameter	Group	Control group	Animals affected with continuous immobilization stress	Animals affected with continuous immobilization stress and subjected to a course of terahertz waves treatment
Mean value of perfusion, pU		11.28 (9.91; 13.34)	7.12 (6.39; 8.63) $Z_1 = 3.93$ $P_1 = 0.000082$	10.86 (9.48; 12.47) $Z_1 = 0.55$ $P_1 = 0.579100$ $Z_2 = 3.22$ $P_2 = 0.001260$
Root mean square deviation of perfusion, pU		1.02 (0.75; 1.26)	0.29 (0.20; 0.33) $Z_1 = 4.49$ $P_1 = 0.000007$	0.54 (0.46; 0.67) $Z_1 = 3.18$ $P_1 = 0.001425$ $Z_2 = 3.91$ $P_2 = 0.000093$
Variation coefficient, %		8.6 (7.17; 10.87)	3.49 (2.80; 5.12) $Z_1 = 4.31$ $P_1 = 0.000017$	4.94 (4.64; 6.05) $Z_1 = 2.93$ $P_1 = 0.003283$ $Z_2 = 2.14$ $P_2 = 0.032388$

Notes: An average value (median—Me), lower and upper quartiles (25%, 75%) from a corresponding number of measurements of every parameter are provided in the table. Z_1, P_1 —comparison with control group; Z_2, P_2 —comparison with a group of animals with continuous stress.

Table 4. Amplitude-frequency characteristics of blood flow oscillations in microvasculature of male rats affected by continuous immobilization stress and subjected to a course of treatment with terahertz electromagnetic waves equal to frequencies of molecular absorption and emission spectra of nitrogen oxide (150.176 - 150.664 GHz).

Parameter	Group	Control group	Animals affected with continuous immobilization stress	Animals affected with continuous immobilization stress and subjected to a course of terahertz waves treatment
Maximal amplitude of endothelial oscillations, pU		2.08 (1.65; 2.81)	0.44 (0.30; 0.59) $Z_1 = 4.49$ $P_1 = 0.000007$	1.20 (0.78; 1.89) $Z_1 = 2.32$ $P_1 = 0.019820$ $Z_2 = 3.85$ $P_2 = 0.000121$
Maximal amplitude of vasomotor oscillations, pU		1.33 (1.16; 1.87)	0.32 (0.24; 0.38) $Z_1 = 4.49$ $P_1 = 0.000007$	0.94 (0.48; 1.44) $Z_1 = 2.10$ $P_1 = 0.035044$ $Z_2 = 3.72$ $P_2 = 0.000198$
Maximal amplitude of respiratory oscillations, pU		0.34 (0.25; 0.46)	0.11 (0.09; 0.20) $Z_1 = 3.38$ $P_1 = 0.000710$	0.21 (0.15; 0.32) $Z_1 = 1.88$ $P_1 = 0.059298$ $Z_2 = 2.23$ $P_2 = 0.025574$
Maximal amplitude of pulse oscillations, pU.		0.14 (0.11; 0.29)	0.06 (0.04; 0.07) $Z_1 = 4.23$ $P_1 = 0.000023$	0.08 (0.06; 0.11) $Z_1 = 2.57$ $P_1 = 0.009899$ $Z_2 = 2.29$ $P_2 = 0.021754$

Notes: see **Table 3**.

peripheral resistance decreased. Amplitudes of respiratory and pulse (cardiac) oscillation also increased (**Table 4**) which evidences an increment of blood flowing to microvasculature.

Thus, exposure to radiation emitted by *Orbita* apparatus is an effective method of non-drug treatment of blood perfusion disorders and normalization of coagulant and fibrinolytic activities of blood.

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