Research Letter

The usage of THz radiation of nitrogen oxide occurrence in blood antioxidant characteristics correction and lipid peroxidation process under stress conditions

Vyacheslav F. Kirchuk, Alexander A. Tsymbal
Saratov State Medical University n.a. V.I. Razumovsky, Saratov, Russia

Received 18 May 2013, Accepted 23 July 2013

© 2013, Kirchuk V.F., Tsymbal A.A.
© 2013, Russian Open Medical Journal

Abstract: There has been studied influence of terahertz waves of molecular and absorption spectrum occurrence of 150.176-150.664 GHz nitrogen oxide on intensity of lipid peroxidation processes and blood antioxidant characteristics in 75 white outbred male-rats of 180-220 g under the immobilization stress condition. The tests were performed with account of lipid peroxidation metabolites – lipid hydroperoxides, malondialdehyde. The states of blood antioxidant activity were peroxide erythrocyte resistance, superoxide dismutase and catalasa, general sulfhydryl groups and vitamin E. There has been displayed that under the influence of the THz-band of 150.176-150.664 GHz nitrogen oxide occurrence there was a total normalization of lipid peroxidation processes and of functional antioxidants activity (on the background of stress at white rats).

Keywords: lipid peroxidation, antioxidants, THz radiation, nitrogen oxide

Cite as Kirchuk VF, Tsymbal AA. The usage of THz radiation of nitrogen oxide occurrence in blood antioxidant characteristics correction and lipid peroxidation process under stress conditions. Russian Open Medical Journal 2013; 2: 0303.

Correspondence to Prof. Vyacheslav F. Kirchuk. Address: Department of normal physiology n.a. I.A. Chuevsky, Saratov State Medical University n.a. V.I. Razumovsky, 112, Bolshaya Kazachiya str., Saratov, 410012, Russia. E-mail: normal@yandex.ru

Introduction
The problem of an adequate treatment of acute post-stress diseases (ischemic heart disease, instable angina, acute myocardial infarction, etc.) is still topical as for clinical medicine and for fundamental studies as well. An importance of these diseases is connected with their prevalence and the role they play in death rate and population disability, in an economic damage [2]. It is proved that the stress-reaction activation is caused by stereotyped and biologically important change of intracellular compartment, organism. So the signal is pro- and antioxidant disequilibrium towards the activation of lipid peroxidation process in biological membranes and fluids [2]. Under the influence of extreme stimulus biological oxidation and lipid peroxidation processes consistently increase, and as well there is a depression of functional activity of enzymatic and nonenzymatic components of antioxidant system [2]. Increase of lipid peroxidation products level (comparing with permanent level) is considered as a universal mechanism of insult to cell in different pathological states, including cardiovascular system pathology [2, 4].

This means that the correction of lipid peroxidation intermediate products and normalization of blood antioxidant activity is pathogenetically grounded.

THz-band is electromagnetic waves of \(10^7...10^9\) GHz or with wavelength from 3 mm to 30 \(\mu\)m [3]. THz-band biophysical effects give perspectives to innovations in biomedicine technologies: “THz-band therapy” and “THz-band diagnostics” [3]. Terahertz waves of molecular and absorption spectrum occurrence (MASO) of 150.176-150.664 GHz nitrogen oxide [3, 7] occurrence provoke much interest, because nitrogen oxide is a regulator of physiological, pathophysiological and biochemical processes in cells and in organism as well [7, 17, 18]. Today there have been discussed positive results of influence of THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence on changed indices of metabolism, blood gases and electrolytes, fibrinolysis [11-15].

In connection with it the study aimed researching the influence of the THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence on antioxidant blood properties and lipid peroxidation process under the experimental stress.

Material and Methods
There 75 blood samples of white outbred male-rats of 180-220 g have been studied. Experimental animals were placed into the specially prepared department the access to which was controlled. The immobilization stress (the rats were fixed in prone position for 3 hours [6]) was as a model of intensification of lipid peroxidation and antioxidant system depression. The research has been performed in 5 groups of animals, 15 rats in each of the groups: group 1 – control group (intact animal); group 2 – comparison group (animals are under the immobilization stress); 3, 4, and 5 groups – experimental animals which have been under the single irradiation during 5, 15, 30 minutes (also on the background of immobilization). To eliminate the influence of seasonal and circadian dependence on lipid peroxidation and blood antioxidant activity processes, the experiments had been...
organized in autumn at the first part of the day. All of the animals were in the same conditions during the experiment.

The irradiation was the THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence with the help of “KBH-NO” generator. 3 cm² skin area above the metasternum had been irradiated. Irradiator had been located 1.5 cm above the body of an animal. The generator radiating power was 0.7 mW, the power density that arrives at the 3 cm² skin area was 0.2 mW/cm² [9].

Blood sampling for the experiment was collected by transmyocardial acupuncture into the plastic test tubes. 3.8% sodium citrate fluid was used as a blood anticoagulant in proportion 9:1. The condition of lipid peroxidation processes were evaluated according to the concentration of lipid peroxidation intermediate products – lipid hydroperoxides, malondialdehyde. To estimate the degree of autoxidation and cytolysis syndrome progressing there had been used index of molecules of an average mass in blood. Integrative indices of the blood antioxidant system condition under the immobilization stress were peroxide erythrocyte resistance (PER), as well as cell fraction activity in high-molecular composition of enzymatic component – superoxide dismutase (SOD) and catalase, and low-molecular composition of nonenzymatic component – general sulfhydril groups (SH-groups) and vitamin E.

Experiments on animals had been performed according to: Geneva Convention “International Guiding Principles for Biomedical Research Involving Animals” (Geneva, 1990), the order of Ministry of health of the USSR № 755 dated 12.08.1977 “About measures of further perfecting of organizational works using experimental animals” (as of 20 Oct 2006), World Medical Association’s Declaration of Helsinki (editing October, 2000).

Statistical analysis of the results had been done with the help of Statistica 6.0 soft. To reveal accuracy of change in indices of lipid peroxidation and blood antioxidant activity there had been tested a hypothesis for the type of distribution (Shapiro-Wilk statistics) and equality of dispertion (Levene's test). In case of normal distributions and the fulfillment of a equality of dispertion condition, Students’ t-test was used. In the case of the distributions different from normal – Mann-Whitney U-test.

**Results**

It is displayed that in rats under the immobilization stress the process of lipid peroxidation (LPO) had been extremely activated. This is followed by an increase (statistically valid comparing with the control group) of toxical lipid peroxidation intermediate products – lipid hydroperoxides, malondialdehyde (Table). Overconcentration of the LPO products at immobilization stress was followed by cytolysis syndrome progressing. This was clear from the overconcentration of the molecules of an average mass in blood (Table). At rats under the immobilization stress there had been fixed extreme depression of enzymatic and nonenzymatic components of antioxidant system. This came out in statistically valid decrease of SOD and catalase potency in erythrocytes, decrease of the amount of general SH-groups and vitamin E in blood serum. There had been also revealed progressive reduction of erythrocytes peroxide resistance (Table).

**Table. Level of lipid peroxidation intermediate products and antioxidant potency indices under immobilization stress on the background of electromagnetic irradiation of THz-band of 150.176-150.664 GHz nitrogen oxide occurrence**

<table>
<thead>
<tr>
<th>Indices</th>
<th>Intact animals (n = 15)</th>
<th>Immobilization stress (n = 15)</th>
<th>Irradiation on the background of stress during:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 minutes (n = 15)</td>
<td>15 minutes (n = 15)</td>
<td>30 minutes (n = 15)</td>
</tr>
<tr>
<td>Lipid peroxidation, unit of optimal density (UOD)/ml</td>
<td>3.49 ± 1.01</td>
<td>7.45 ± 2.22  P &lt; 0.01</td>
<td>7.22 ± 2.20  P &lt; 0.05, P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.01 ± 2.0  P &lt; 0.05, P &lt; 0.05</td>
<td>3.96 ± 1.22  P &gt; 0.05, P &lt; 0.01, P &lt; 0.05</td>
</tr>
<tr>
<td>Malondialdehyde, mcmol/ml</td>
<td>3.64 ± 1.11</td>
<td>7.65 ± 2.55  P &lt; 0.01</td>
<td>7.30 ± 2.21  P &lt; 0.05, P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.82 ± 1.85  P &lt; 0.05, P &gt; 0.05</td>
<td>4.01 ± 1.77  P &gt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>Molecules of an average mass, unit of exp.</td>
<td>0.25 ± 0.2</td>
<td>0.47 ± 0.4  P &lt; 0.05</td>
<td>0.46 ± 0.4  P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>exp. (blood serum)</td>
<td></td>
<td>0.44 ± 0.4  P &lt; 0.05, P &gt; 0.05</td>
<td>0.29 ± 0.21  P &gt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>SH-sur groups, mM/l</td>
<td>2.01 ± 1.21</td>
<td>0.84 ± 0.85  P &lt; 0.05</td>
<td>0.81 ± 0.77  P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>(blood serum)</td>
<td></td>
<td>1.11 ± 1.20  P &lt; 0.05, P &gt; 0.05</td>
<td>1.80 ± 1.22  P &lt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>Catalase,Uu/l (erythrocytes)</td>
<td>3.44 ± 1.15</td>
<td>8.02 ± 2.22  P &lt; 0.01</td>
<td>7.88 ± 2.20  P &lt; 0.05, P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.22 ± 1.84  P &lt; 0.05, P &gt; 0.05</td>
<td>4.0 ± 1.20  P &gt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>SOD, c.u./ml</td>
<td>373.81±12.0</td>
<td>246.23 ± 10.1  P &lt; 0.01</td>
<td>245.01 ± 11.0  P &lt; 0.05, P &gt; 0.01</td>
</tr>
<tr>
<td>(erythrocytes)</td>
<td></td>
<td>285.5 ± 12.2  P &lt; 0.05, P &lt; 0.01</td>
<td>341.22 ± 12.2  P &gt; 0.05, P &lt; 0.05, P &lt; 0.01</td>
</tr>
<tr>
<td>PER, c.u. (erythrocytes)</td>
<td>1.57 ± 0.85</td>
<td>3.24 ± 1.22  P &lt; 0.05</td>
<td>3.16 ± 1.65  P &lt; 0.05, P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.09 ± 1.24  P &lt; 0.05, P &gt; 0.05</td>
<td>2.00 ± 1.25  P &gt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
<tr>
<td>Vitamin E, UOD/ml</td>
<td>20.11 ± 4.44</td>
<td>11.71 ± 3.20  P &lt; 0.01</td>
<td>11.53 ± 3.21  P &lt; 0.05, P &gt; 0.05</td>
</tr>
<tr>
<td>(blood serum)</td>
<td></td>
<td>14.22 ± 3.22  P &lt; 0.05, P &lt; 0.05</td>
<td>18.86 ± 4.77  P &gt; 0.05, P &lt; 0.05, P &lt; 0.05</td>
</tr>
</tbody>
</table>

Data presented as mean and standard deviation (SD).

P<comparing with the group of intact animals; P<comparing with the group of animals under the immobilization stress; P<comparing with the group of animals after 5-minute irradiation on the background of stress; P<comparing with the group of animals after 15-minute irradiation on the background of stress.
As a result in immobilization stress there is a defect of all components (enzymatic and nonenzymatic) in antioxidant cell barrier, and lipid peroxidation processes are activated.

The 5-minute irradiation of THz-band electromagnetic waves of 150.176-150.664 GHz nitrogen oxide occurrence on animals under the immobilization stress does not cause much change of blood antioxidant system or in lipid peroxidation. This is evident from the absence of statistically valid differences in basic parameters of blood antioxidant activity and lipid peroxidation in this group comparing with the groups of animals under the immobilization stress. At the same time, there had been fixed statistically significant differences in experimental indices comparing with the group of control (Table).

During the 15-minute irradiation of THz-band electromagnetic waves of nitrogen oxide occurrence on the background of the immobilization stress, there had been revealed partial (but more evident than during the 5-minute irradiation) normalization of lipid peroxidation process and antioxidant activity. This is clear from the reduction of concentration of toxical intermediate products of lipid peroxidation, and partial recovery of blood antioxidant properties (Table).

The 30-minute irradiation of the same occurrence causes total normalization of lipid peroxidation processes, which is evident from the decrease of concentration of toxical intermediate products of lipid peroxidation as it is at intact animals (Table). Functional activity of enzymatic and nonenzymatic components if antioxidant cell reaction had also been recovered and statistically valid does not differ from the indices of intact animals (Table).

The given irradiation mode promotes total normalization of lipid peroxidation processes and recovery of antioxidant system activity of enzymatic and nonenzymatic components.

Therefore on the base of the given facts we may conclude that there a positive influence of the THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence on blood antioxidant properties and lipid peroxidation processes at animals under the immobilization stress. The 30-minute mode of irradiation had been the most effective for the lipid peroxidation recovery. The 5-minute irradiation produced very low positive effect, and the 15-minute irradiation it is partially positive.

Discussion

The problem of stress, adaptation and prevention of stress damage is the most important in modern biology and medicine [17]. One of the main pathogenetic mechanisms of acute and chronic stress-reactions is the defect of a structure and functions of biological cell membranes and tissues, disorganization of consolidated visceral system of an organism [16].

The results of the study displayed that effenter component of a stress-dependent cell disorganization is an activation of a lipid peroxidation and antioxidant system depression (Table). This fact is consistent with the literature data about the LPO as a general metabolic component of stress reaction [2, 4]. It is a reaction on an overimpression and, as well can cause concentration of lipid peroxidation intermediate products and decrease of a functional activity of antioxidant systems [2, 4].

Revealed fact of an overconcentration of lipid peroxidation intermediate products in blood under the immobilization stress condition is caused by the defect of functional activity of enzymatic and nonenzymatic components. This is the result of the reduction of the following indices: SOD and catalase potency, vitamin E level in blood, general SH-groups in blood serum and erythrocytes peroxide resistance comparing with the control group (Table). It should be noticed that vitamin E deficiency which had been revealed during the experimental stress, displays destabilization of mitochondrial, lysosomal, cytoplasmic membranes, because, as it is known, lipid-soluble antioxidants (vitamin E also) settle in biological membranes mainly, and protect them from free-radical destruction [4]. At the same time significant depression of SOD potency (Table) causes an overconcentration of superoxide anion-radical. Although this radical has less reactivity to lipids, protein components of biomolecules and nucleic acids, it causes quite evident disorganization in these structures and their dysfunction. This is confirmed by the results of our research according to which malondialdehyde and lipid peroxidation are overconcentrated under the immobilization stress condition (Table). The last ones, as we know, are toxical intermediate products of lipid peroxidation induced with the process of interaction of active form of oxygen and polysaturated fatty acids of biomembranes – linoleic acid and arachidonic acid [2].

30-minutes irradiation of the THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence totally normalize antioxidant system potency – its enzymatic and nonenzymatic components and lipid peroxidation processes.

Concerning positive influence of the THz-band of 150.176-150.664 GHz nitrogen oxide occurrence on defected LPO processes and antioxidant status of animals under the stress conditions, it is necessary to notice the following. It is known that nitrogen oxide is as a stress-limit factor, it limits exhaust of pituitary hormones, catecholamines in synaptic connections and from adrenal body [10, 16, 18]. Nitrogen oxide also prevents an increase of intracellular factor IV concentration, enlarges antioxidant enzymes activity and encoding genes, activates Hsp70 immune protein synthesis, modifies and stabilizes phospholipid bilayer of biomembranes, energetic and flexible cell supply, activity of transport and receptor systems of membranes, cell irritation, many of intracellular metabolic processes [12, 15, 16, 18]. There was demonstrated that THz-band of nitrogen oxide occurrence that had been performed alongside with a stress agent action, prevents stress-dependent changes progressing in an antioxidant system of an organism and decreases LPO activity. This may be connected with an increase of reactivity of free endogenic nitrogen oxide or an increase of its concentration because of the influence of the THz-band of MASO of 150.176-150.664 GHz nitrogen oxide occurrence directly on NOS and their catalysis [1, 6-9]. It should be noticed that the THz-band irradiation of nitrogen oxide occurrence had evident stress-limiting effect [5, 10], because of the overconcentration of corticosterone and catecholamines as well [10].

Conclusion

The presented data show possibility of correcting of lipid peroxidation processes and dysfunction of antioxidant potency with the help of the THz-band of 150.176-150.664 GHz nitrogen oxide occurrence.

Conflict of interest

This study has been fit in to the Russian academy of medical science “Scientific research of Povolzhsky region of 2008-2010”, “Experimental research of the influence of radioimpulse radiation on functional white rats...
(bioobjects) condition under different types of stress". Confirmed by the Presidium of the RAMS (23 April 2008, protocol no.72).

Reference

1. Andronov EV. Terragerz irritation at the nitric oxide frequency 240 GHz and platelet activity in white rats in the state of oxidative stress on background of injection of endothelium no-syntase inhibitor. Journal of New Medical Technologies 2008; (3): 14-16. [Article in Russian]
12. Kirichuk VF, Tsyymbal AA, Krenitsky AP. Experiment of basic indices of metabolic status recovery with the help of THz-band of 150,176-150,664 GHz nitrogen oxide occurrence. Biomeditsynskaya radioelectronika (Biomedical Radioelectronics) 2011; (1): 30-35. [Article in Russian]
15. Tsyymbal AA. Changes in blood protein assay, glutathione-S-transferase activity, glucose and ceruleoplasmin concentration during the influence of THz-band of 150,176-150,664 GHz nitrogen oxide occurrence under acute stress. Biomeditsynskaya radioelectronika (Biomedical Radioelectronics) 2011; (8): 30-35. [Article in Russian]