

COMPARATIVE EVALUATION OF THE EFFECT OF LOW-INTENSITY ELECTROMAGNETIC NOISE SIGNALS IN THE MICROWAVE RANGE ON THE INDUCTION OF METABOLIC AND VASCULAR ADAPTATION REACTIONS IN EXPERIMENTAL THERMAL BURNS

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Abstract. Currently, we continue to actively develop a high-tech rehabilitation method of microwave therapy using sub- and millimeter-wave radiation sources. However, the question of the feasibility of its use in the rehabilitation of patients with burn injuries is not sufficiently studied. In this connection, the works analyzing the effectiveness of the influence of different micro-wave ranges on the nature and degree of development of adaptive metabolic and vascular reactions in the early period of trauma, which affect the outcome of the burn, become particularly relevant. The study was carried out on male rats of the Wistar line under conditions of experimental contact thermal burn of the IIIA degree on an area of 20% of the dorsal surface of the body. A 7-day course of point non-contact exposure to broadband microwave emitters of various ranges with a radiation power of 10 μ W on the occipital protuberance area was started after the rats were released from anesthesia when the contact burn was applied. The vascular status, the state of oxidative stress and energy homeostasis were assessed by the intensity of oxidative processes, antioxidant protection, and the total microcirculation index with a wavelet analysis. The obtained data reliably revealed a more pronounced favorable effect on the level of adaptive reactions in the range of 150,179-150.664 GHz, which contains the frequencies of radiation and absorption of nitric oxide. This should be taken into account when choosing frequency-energy parameters and developing a potential method of rehabilitation in the acute period of burn injury.

Keywords: burn, electromagnetic radiation, microwaves, microcirculation, oxidative stress.

List of Abbreviations

AP – acupuncture points
EMR – electromagnetic radiation
LI – low-intensity
EHF – extremely high frequency radiation
THzR – terahertz range
CThB – contact thermal burn
SAS – sympathoadrenal system
GHz – Gigahertz
MSRA – molecular spectrum of radiation and absorption
NO – nitric oxide molecule
O₂ – oxygen molecule
FRO – free radical oxidation
LPO – lipid peroxidation
TAA – total antioxidant activity
tg2 α – an indicator that characterizes the rate of decline of FRO processes in plasma
S – the chemiluminescence light sum for 30 seconds.
MDA – malonic dialdehyde

SOD – superoxide dismutase
LDH – lactate dehydrogenase
GR – glutathione reductase
G1-6-fDH – glucose-6-phosphate dehydrogenase
BHL – biochemiluminescence
LDF – Laser Doppler flowmetry
MI – microcirculation indicator

Introduction

The problem of burn injuries was actualized all over the world against the background of increased man-made disasters and local military conflicts (Shabanov *et al.*, 2016; Sidelnikov *et al.*, 2019; VanLieshout *et al.*, 2018). Experience shows a close relationship between the quality of medical care in the acute period (from the first hours to the next 5–7 days from the moment of injury) and the outcomes of burn injury. This encourages the search for innovative technologies for early rehabilitation of burned pa-

tients (Alekseev *et al.*, 2020). Burns of the IIIA degree, in which the epidermis and partially the dermis die in the wound (Ubbink *et al.*, 2015; Agadzhanova, 2020), lead to local and systemic pathological changes, the development of severe dysmetabolic changes, microcirculation disorders and inhibition of the body's protective reactions. This requires the use of combination therapy (Bobrovnikov, Alekseev, 2017; Saeidinia *et al.*, 2017; Fayazov *et al.*, 2019). Modern local and general effects on the body by physical factors in burns should activate adaptive-compensatory reactions involving SAS, which plays a leading role in the process of adaptation of the body (Tapbergenov *et al.*, 2019). Despite the successes achieved, this problem has remained highly relevant and has gained interdisciplinary significance (Podoyntsyna *et al.*, 2015; Bailey *et al.*, 2018).

Currently, microwave therapy is actively developing using sub- and millimeter-wave radiation sources, which corresponds to the THzR and EHF frequency ranges. It has been shown that the LI EMR EHF and THzR system has a homeostatic effect on metabolic and microcirculatory disorders, especially at frequencies containing NO and O₂ MSRA, since these molecules are involved in most biochemical reactions (Ivanov *et al.*, 2016; Polyakova *et al.*, 2016; Deryugina *et al.*, 2017; Lukin *et al.*, 2018). The optimal way to deliver of EMR to the body is the impact on the AP. This is explained by their biophysical features due to the increased concentration of extraneuronal transmitters (Spasova *et al.*, 2007; Savin *et al.*, 2017). An important role of AP in maintaining electromagnetic homeostasis has been experimentally proved (Limansky *et al.*, 2013). In this context, the correlation relationships between the disorders of the body's functions under external influences were investigated and a scheme of the bioresonance interaction of radiation with variations in their characteristics was constructed (Subbotina, Yashin, 2018). However, the use of microwave puncture therapy in the early rehabilitation of patients with burns has not been practically studied. In this regard, the assessment of the effectiveness of irradiation of AP by microwaves with different fre-

quency characteristics is particularly relevant. The criteria for this assessment can be the dynamics of microcirculation, the level of FRO and LPO. In this paper, the analysis of the character and degree of influence of various broadband bands of sub- and millimeter-wave EMR ranges on the induction of metabolic and vascular adaptation reactions in the acute period of experimental thermal trauma in vivo is carried out. The differences were determined by comparing the data obtained with the indicators of intact animals and rats with CThB without subsequent irradiation.

Materials and Methods

The experiments were carried out on 25 male Wistar rats weighing 310–360g. in accordance with the requirements of bioethics and the rules of laboratory practice (GPL), the Geneva Convention for the Protection of Animals «International Guiding Principles for Biomedical Research Involving Animals» (Geneva, 1990), Order of the Ministry of Health of the Russian Federation № 267 of 19.06.2003 «On approval of the rules of laboratory practice». The study was approved by the Local Ethics Committee of the Privolzhsky Research Medical University of the Ministry of Health of the Russian Federation, in accordance with the provisions of the Helsinki Declaration of 1975, revised in 2008.

The animals were kept in the same conditions, free drinking regime and standard nutrition were observed until the end of the experiment. We used a model of CThB of IIIA degree, which was applied under intramuscular anesthesia from a combination of «Zoletil-100» («VirbacSanteAnimale», France) at a dose of 10 ml/kg and «Xylavet» («Interchemie», Netherlands) at a dose of 10 mg/kg on the depilated surface of the back of the animal on an area of 20% of the body surface by a single three-second contact of the isolated skin area with a thermal heating element (Peretyagin *et al.*, 2018). The rats were randomly divided into five groups of equal numbers ($n = 5$): 2 control groups and 3 experimental groups. Contact rats (control 1) and animals with CThB without subsequent irradiation (control 2) served as controls.

The rats of the experimental groups immediately after coming out of anesthesia began to receive a course of daily non-contact (0.5 cm above the surface of the skin) exposure to LI EMR over the center of the occipital protuberance in the projection of TAGV.14. Radiation power – 10 mcW, power density – 0.05 mW/cm², exposure – 10 minutes. The exposure was carried out from the first to the seventh day after the application of CThB. For the duration of the procedure, the animals were placed in a special pencil case.

Radiation sources: certified device «Amphit-0.2/10-01» (frequency range 53.57 ... 78.33 GHz), developed by limited liability company Fiztech (Lobachevsky university, Russia); experimental model of the EMR noise signal based on the device «Amphit-0.2/10-01» (frequency range 130.0...170.0 GHz); experimental noise signal generator (frequency range 150,176...50.664 GHz), developed by IFM RAS (Russia). The noise mode of EMR allows the body to adjust itself to the resonance to the desired frequency, the used radiation parameters exceed 3 times the threshold sensitivity of the cells, which minimizes possible side effects.

Animals of the 3rd group (experiment 3) were irradiated with EMR EHF 53, 57...78.33 GHz, which does not contain the frequencies of messenger molecules. Animals of the 4th group (experiment 4) received the exposure to EMR THzR 130.0...170.0 GHz (contains MSRA O₂ and NO). Animals of the 5th group (experiment 5) received the EMR THzR 150.176...150.664 GHz (contains MSRA only NO). The animals were removed from the experiment under anesthesia after decapitation after the seventh radiation session.

For the biochemical analysis of the enzymatic activity, blood stabilized with sodium citrate (1:9) was used. The activity of FRO processes in plasma and red blood cells was studied by the method of induced biochemiluminescence on the device BHL-06 (N. Novgorod), coupled with an IBM computer. The BHL-06 device is designed for recording light fluxes that occur in biological samples as a result of enzymatic and chemical processes. The following parameters of the chemiluminogram were evaluated: tg2a – an indicator reflecting TAA;

S is an indicator that reflects the potential ability of a biological object to perform LPO. The intensity of the LPO was determined by the level of MDA, activity of the SOD (Sirota, 2012). The activity of catalase and LDH in the direct (LDHdir) and reverse (LDHrev) reactions, GR and Gl-6-fDH was determined by spectrophotometric method (Sibgatullina *et al.*, 2011).

The dynamics of microblood flow in the border area of burn injury was monitored before the injury and on the 7th day after CThB under combined anesthesia according to the LDF method (Kozlov *et al.*, 2012). The integral MI, which characterizes the degree of perfusion of the tissue volume per unit of time, was evaluated using a laser blood flow analyzer LAKK-M («Lazma», Russia) with the use of a wavelet analysis, which allows us to evaluate the components of microvessel tonus based on the values of the amplitudes of microcirculation fluctuations (Krupatkin, Sidorov, 2013).

Statistical data processing was carried out using the program Statistica 6.0 (StatSoft, Inc.), which was used to calculate the arithmetic mean of the indicators and the error of the average. The significance of the differences between the indicators was determined using the Student's t-test. Differences at $p < 0.05$ were considered statistically significant.

Results

The study of biochemical parameters of oxidative and energy metabolism in animals of the 2nd control group against the background of CThB in the absence of radiation revealed the development of oxidative stress. This was confirmed by statistically significant differences in the studied parameters compared to intact rats ($p < 0.05$). This was manifested in the activation of FRO in the blood (an increase in the concentration of MDA in red blood cells by 20%, in plasma – by 11%, the S index – by 42% in plasma and by 7% in red blood cells) and a decrease in the specific activity of antioxidant enzymes (SOD, catalase, GR, Gl-6-fDH) in red blood cells and TAA in blood plasma. A decrease in the activity of LDHdir and LDHrev by 39% and 30%, respectively, against the back-

ground of CThB (compared with intact animals) led to lactic acidosis and, as a result, the development of hypoxia.

The general dynamics of the indicators of the antioxidant and energy systems under the influence of course irradiation of rats with different frequency ranges in the conditions of experimental burn injury is presented in Table 1.

From the presented data, it follows that a 7-day course of daily exposure to broadband LI EMR on AP in the skin projection of the center for vegetative regulation of animals with experimental thermal trauma at frequencies of 130.0-170.0 GHz and 150.179-150.664 GHz led to a decrease in the intensity of LPO in blood plasma. This was most clearly manifested when EMR was irradiated at a frequency of 150.179-150.664 GHz (by 31%), which corresponds to the MSRA of nitric oxide. A similar trend was typical for the dynamics of MDA. Thus, the S index remained significantly higher than the control values of intact rats by 14.84% ($p = 0.001$) and the concentration of MDA in erythrocytes – by 70.25% respectively under the influence of EMR THzR in the frequency range of 130.0-170.0 GHz, where NO and O₂ MSRA are present. These indicators returned to the state of physiological norm in animals exposed to EMR THzR with a range of 150.179-150.664 GHz.

When exposed to radiation at frequencies of 150.179-150.664 GHz, corresponding to the MSRA NO (experiment 5), there was a significant ($p < 0.05$) increase in the specific activity of SOD, catalase, GR and Gl-6-fDH of red blood cells, as well as an increase in the level of TAA in blood plasma relative to the control group (2) by 95%, 63%, 30%, 32%, 49%, accordingly. Under the influence of THzR EMR with frequency spectra of 130.0-170.0 GHz and 150.179-150.664 GHz, the activity of SOD and TAA in 4 and 5 experimental groups was significantly reduced compared to intact rats (control 1). The activity of GR and GL-6-fDH in the group of animals exposed to THzR EMR only at the NO frequency was normalized. The red blood cells of these animals had increased peroxide resistance, which increased their re-

sistance in the fight against active oxygen forms formed against the background of thermal trauma.

A parallel study of the dynamics of MI showed a decrease of tissue perfusion by 21% compared to intact animals (Fig.1) during the CThB without subsequent irradiation (control 2). This is natural for the pathogenesis of thermal trauma.

The intensity of blood flow in the animals from the 3 experimental group compared with the rats of the 2nd control group (CThB without treatment) significantly ($p < 0.05$) increased by 29% and exceeded the normal parameters of the animals of the 1st control group by only 11%.

The greatest increase in the level of tissue perfusion was observed in rats exposed to THzR EMR in the ranges of 130.0-170.0 GHz and 150.179–150.664 GHz. The microcirculation indicator was comparable in 4 and 5 experimental groups. A significant increase of 39% compared to control 1 and 73% compared to control 2 was recorded in 4 and 5 experimental groups. This fact confirms the vasodilatation effect of nitric oxide, which is included in both frequency ranges.

The greatest increase in fluctuations in the ranges of endothelial (by 23%) and cardiac (by 15%) frequencies in relation to the control group (2) ($p < 0.05$) was observed in the 3 experimental group under the influence of EMR EHF 53.57-78.33 GHz.

The factors of active regulation underwent noticeable changes compared to the control (2) values (a decrease in the endothelial component by 32% and an increase in the myogenic component by 22%) and the factors of passive regulation increased according to the respiratory and cardiac components by 21% and 20%, respectively ($p < 0.05$) in group 4 under the influence of EMR THzR 130.0–170.0 GHz. An even more pronounced dynamics compared to the control 2 was registered in group 5, where the irradiation was carried out by THzR EMR with a frequency of nitric oxide (150.179-150.664 GHz). This led to a decrease in endothelial tone by 26% and respiratory tone – by 41% ($p < 0.05$).

Table 1

Dynamics of indicators of antioxidant and energy systems in groups

Groups	Control 1 (intact)	Control 2 (CThB)	Experiment 3 53.57-78.33 GHz	Experiment 4 130-170 GHz	Experiment 5 150,179-150.664 GHz
Biochemical parameters					
GR, nmol NADPH / min×mg of protein	89,99±7,10	62,03±5,24*	68,88±2,67*	75,94±6,02**	80,53±4,39**
GI-6-fDH, nmol NADPH/min×mg of protein	42,03±2,11	30,23±1,44*	35,90±2,00**	37,42±1,00**	39,87±0,68**
LDHdir, nmol NADH/min×mg of protein	39,78±3,12	24,27±1,09*	29,55±0,89**	30,90±1,04**	32,56±2,00**
LDHrev, nmol NADH/min×mg of protein	164,54±13,34	114,85±10,06*	128,97±7,53*	139,64±6,10**	147,65±7,48**
LPOplasma, RVU	10,58±0,52	14,99±0,75*	12,50±0,68**	12,15±0,59**	11,43±0,51**
TAA, RVU	0,91±0,03	0,51±0,02*	0,79±0,03**	0,59±0,02**	0,75±0,02**
LPOerythrocytes, RVU	9,79±0,411	10,47±0,44*	7,82±0,33**	9,35±0,47**	6,42±0,32**
SOD, RVU / mg of protein	917,67±21,11	332,52±7,65*	613,35±14,32**	702,52±15,66**	648,69±14,98**
Catalase, RVU / mg of protein	30,24±1,01	17,56±0,98*	21,99±1,53**	25,38±1,11**	28,55±0,86**
MDAplasma, micmol/l	1,07±0,01	1,19±0,09*	0,99±0,02**	1,025±0,06**	1,02±0,07**
MDAerythrocytes, micmol/l	5,95±0,07	10,97±1,23*	8,79±0,28**	10,12±1,23*	7,96±1,07**

Note:

* – differences are statistically significant compared to intact rats ($p < 0.05$);** – differences are statistically significant compared to CThB controls ($p < 0.05$).

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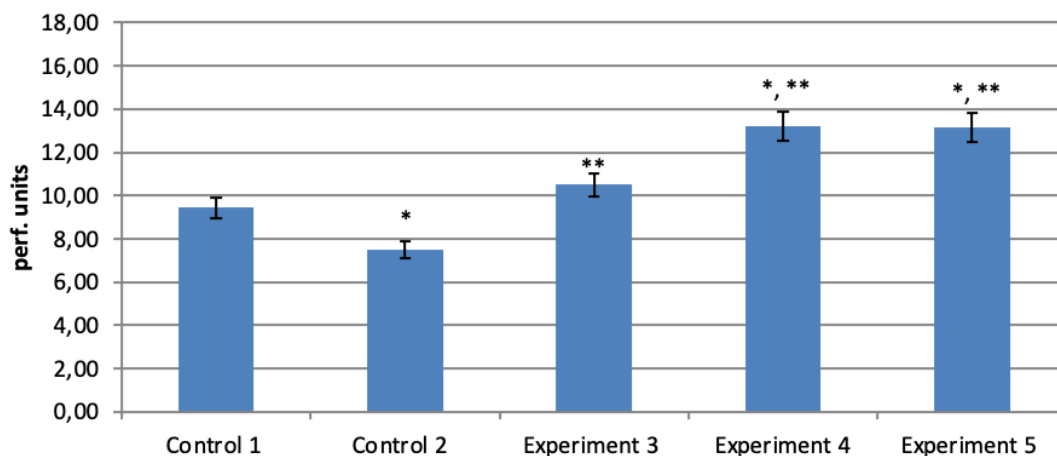


Fig. 1. The level of the microcirculation indicator (MI) in the experimental treatment of thermal trauma. Note: * – differences are statistically significant compared to intact rats ($p < 0.05$); ** – differences are statistically significant compared to CThB controls ($p < 0.05$)

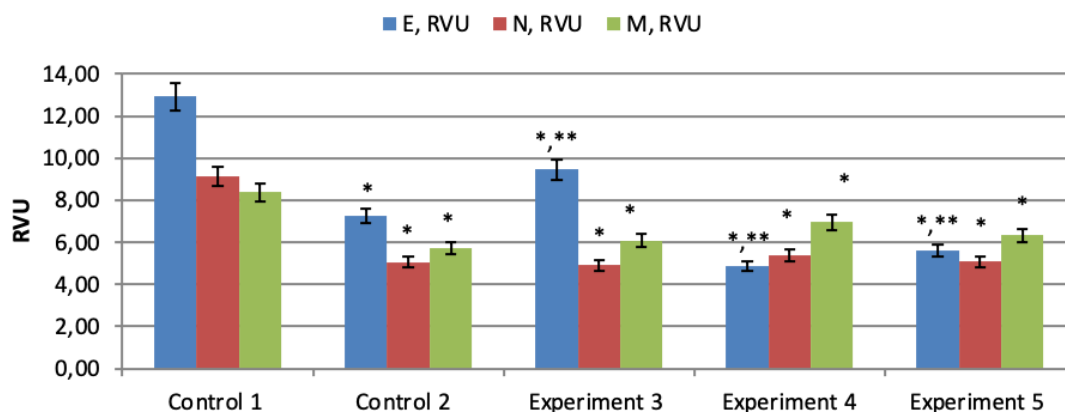


Fig. 2. Dynamics of factors of active regulation of microcirculation under the influence of different EMR ranges of EHF and THzR on animals in the CThB state. Note: E – endothelial factor, M-myogenic factor, N-neurogenic factor, * – differences are statistically significant compared to intact rats ($p < 0.05$); ** – differences are statistically significant compared to CThB controls ($p < 0.05$)

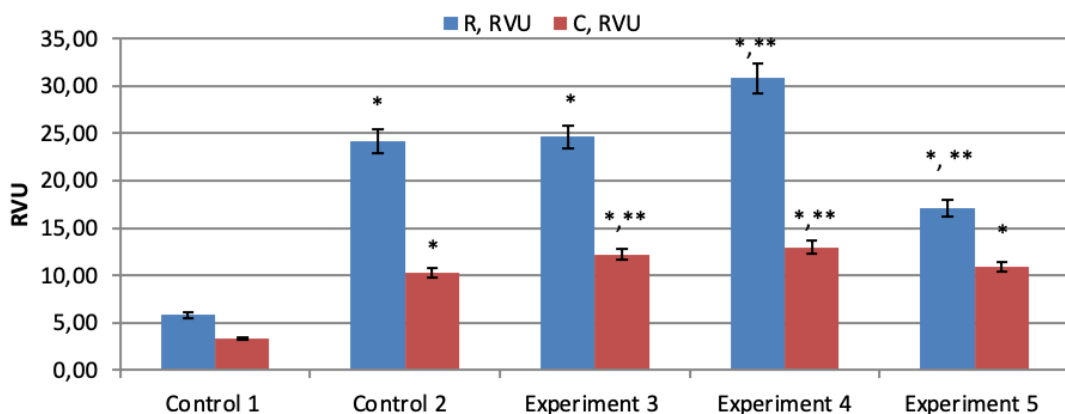


Fig. 3. Dynamics of passive factors of regulation under various factors of influence on a burn wound. Note: R – respiratory factor, C – cardiac component, * – differences are statistically significant compared to intact rats ($p < 0.05$); ** – differences are statistically significant compared to CThB controls ($p < 0.05$)

Discussion

Thus, the results of the comparative study of the influence of various frequency broadband ranges of microwaves allow us to expand the understanding of the degree and mechanism of their influence on the induction of protective metabolic and vascular reactions of the body in the conditions of experimental thermal trauma in vivo. Daily exposure to EMR for 7 days on burn animals led to the activation of energy metabolism in red blood cells, most pronounced in the frequency range of EMR THzR of nitric oxide, contributing to the utilization of lactic acid. This is probably due to the increased activity of LDH due to the interaction of NO with the sulfate groups of the active center of this enzyme.

LPO indicators in animals exposed to LI broadband EMR EHF in the range of 53.57–78.33 GHz during the CThB demonstrated a statistically significant antioxidant effect, manifested in a significant decrease of LPO in plasma and red blood cells (according to bioluminescence and MDA concentration) and an increase of TAA and the activity of antioxidant enzymes. In comparison with intact rats, the indicators of the prooxidant system were increased, and the antioxidant system were reduced. This confirms the results of previous studies (Polyakova A.G. *et al.*, 2016).

Important results of the study were the absence of a negative effect of all the EMR ranges on the processes of lipid peroxidation and antioxidant protection in the experimental groups. Exposure of THzR EMR with the frequency ranges of nitric oxide and oxygen MSRA compared to EHF EMR had a more pronounced antioxidant effect associated with an increase of TAA and the enzymatic activity of SOD, catalase, GR and Gl-6-fDH. Exposure to EMR with MSRA NO had a pronounced stimulating effect on the energy metabolism of red blood cells, manifested in an increase in LDH activity, had

a normalizing effect on the enzymatic activity of FRO in the blood, the activity of GR and Gl-6-fDH.

The irradiation by THzR EMR with a frequency of nitric oxide (150.179–150.664 GHz) led to a decrease in endothelial tone and respiratory tone, which confirms the decisive role of NO in the development of adaptive vascular reactions and coincides with the opinion of other researchers (Ivanov *et al.* 2016; Martusevich *et al.*, 2017). It was noted that the system regulation by 150.179–150.664 GHz was the most balanced against the background of improved venular link performance, which is confirmed by a decrease in the amplitude of respiratory oscillations.

Thus, the chains of interaction of various indicators of LPO, factors and components of microcirculation systems in the process of adaptation of the body to acute thermal stress are demonstrated. The role of nitric oxide as an endogenous vasodilator and neurotransmitter is shown, which statistically significantly positively modifies the state of oxidative blood metabolism and the main estimated indicators of vascular reactions, which indirectly confirms the readaptive possibilities of micro waves in the early post-burn period. However, we noted a pronounced normalizing effect when working at NO frequencies, which cannot be unambiguously interpreted as a purely positive one, taking into account the possible overstrain of the hypothalamic-pituitary-adrenal system during the further development of burn disease. The possibility of inversion of the physiological reaction with subsequent reduction of adaptive energy (Ushakova, 2009; Dmitriev *et al.*, 2010) requires the continuation of the initiated research and a differentiated approach to the development of a potential method for correcting vascular and metabolic reactions in the complex treatment of wounds and burns.

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