Metabolic Effects of Electromagnetic EHF Radiation and Ozone Therapy in the Engraftment of the Skin Flap

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Abstract: - The aim of this study was to evaluate the effect of ROS on the intensity of oxidative and energy metabolism in the blood of rats with an operational model of ischemia of the dorsal skin flap in vivo. Our studies have allowed us to establish that the modeling of an extensive skin defect is accompanied by pronounced shifts in oxidative metabolism in the blood plasma of animals. At the same time, in the absence of pathogenetic treatment, signs of oxidative stress are formed, including the intensification of free radical processes and the inhibition of general antioxidant activity in combination with the accumulation of an increased amount of lipoperoxidation products (in particular, malondialdehyde). Conducting experimental therapy with the introduction of ozone or treatment with electromagnetic radiation of the EHF band allows partially compensating for these metabolic disorders, however, the most optimal option is a combination of these factors within a single scheme. It should be noted that the results obtained are of great applied importance for the creation of innovative technologies for the complex medical rehabilitation of orthopedic-traumatological patients as a tool for influencing reparative processes in the lesion to restore and maintain tissue structures.

Key-Words: - reactive oxygen species, wound, skin flap, ozone therapy, EMR therapy, metabolism.

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1 Introduction

Despite significant achievements in the field of tissue engineering, cellular technologies, and regenerative medicine, the problem of adequate restoration of full-fledged skin in case of injury remains relevant. Therefore, it is expedient to search for and evaluate the effectiveness of new methods for stimulating reparative skin regeneration, [1], [2]. Taking into account the fact that one of the most significant mechanisms of post-traumatic tissue damage is oxidative stress, [2], [3], [4], [5], [6], which develops as a result of excessive activation of free radical processes, impaired functioning of antioxidant defense systems, for the treatment of ischemia that occurs during the formation of a dorsal skin flap, it is advisable to use a regulator of the state of pro- and antioxidant systems, which can be reactive oxygen species (ROS), including ozone, [7], [8], [9].

The sanogenetic significance of ozone in medicine is associated with its high redox potential, which accompanies antibacterial, immunomodulatory, anti-inflammatory, antiviral, cytostatic, analgesic, and antihypoxic effects, and also promotes oxidative detoxification of the body, [9], [10]. The biological effects of ozone depend on its concentration and the presence of target enzymes in the cell. At elevated concentrations, ozone reduces the body's resistance to bacterial infections, quickly oxidizes many amino acids, and inactivates antioxidant enzymes, thereby disrupting the course of many biochemical processes. In small doses, ozone is used to stimulate repair and regeneration, ensure rapid wound healing, and activate the immune response, [2], [11], and in large doses, to fight pathogens, [12].

However, in the literature, there is still no convincing data on the complete safety of the use of ozone, which encourages further research work. Until now, the final idea of the pathways and physicochemical aspects of the action of ROS has not been formed, [6]. In this regard, it is relevant to

identify all kinds of "points of application" of ROS in living biological systems, including the influence of these factors on the body at the molecular, cellular, and systemic levels, [13], [14]. Therefore, it is important to study the mechanisms of action of ROS on the functional state of the pro- and antioxidant systems of the body, and the activity of oxidoreductases under conditions of experimental oxidative stress in vivo.

An additional causative agent that causes the formation of ROS is physical effects. One of these organizations represents electromagnetic attacks that induce free radical processes in biological objects. Therefore, this agent also has pro-regenerative properties, but no such data are currently available. In addition, the effects of a sanogenic combined source of ROS (including medical ozone) and the reflection of the consequences are not fully applied.
This study aimed to evaluate the effect of ROS on the intensity of oxidative and energy metabolism in the blood of rats with an operational model of ischemia of the dorsal skin flap in vivo.

2 Materials and Methods

In the experiment, 25 male rats of the Wistar line weighing 250-300 g were used, obtained from the Stolbovaya branch of the Federal Center of Biomedical Research (Moscow). All animals were kept in standard vivarium conditions in cages with free access to food and water on the diet. Working conditions with rats corresponded to the rules of the European Convention ET/S 129, 1986, and Directives 86/609 ESC, [15]. The animals were divided into 5 groups: group 1 – intact (healthy rats, n=5), group 2 – control – operated animals without any effects (n=5), and Group 3 - experimental– operated animals with ROS treatment in the postoperative period (n=5). Animals of the fourth (experimental) group (n=5) were irradiated daily (once a day for seven days) with a modified device "Amphit-0,2/10-01", [16], [17], with a noise frequency range (53-78 GHz). During the session, the animals received a dose of EMI of 0.12 MJ (in accordance with the 10-minute exposure exposure). The area of the base of the ischemic flap was exposed to contact radiation. Rats of the fifth group (n=5) received combined exposure to EMR radiation and ozone therapy procedures.

In the intact group, no manipulations were performed during the study. In rats of the experimental and control groups on the depilated back under

intramuscular anesthesia (Zoletil 60 mg/kg + Xyl 6 mg/kg) was used to cut out a 3×10 cm skin flap on a feeding leg with an axial type of blood circulation, including the skin and its skin muscle with a base on a horizontal line connecting the corners of the shoulder blades, [17]. Then the flap was placed in place without tension and sewn with nodular seams with atraumatic suture material 4.0 (Fig. 1). This led to the occurrence of acute circulatory disorders, which made it possible to use this model to study both the positive and negative effects of ROS on the "survival" of the flap. The maintenance of animals after surgery was solitary.

In the postoperative period, the animals of the experimental group received daily treatment with ROS for 14 days. Animals of the third group were treated daily with Levoxime gel (in the morning) and ozone cream (in the evening) with an ozonide content of at least 1500 mg O2/kg (Medozons LLC, Nizhny Novgorod), 1 ml of 0.9% NaCl solution with a saturating concentration of ozone in the oxygen-ozone mixture from the Medozons ozonator was injected intraperitoneally.-Systems – 3000 mcg / l and a dose of O3 – 0.6 mcg per animal. The concentration of O3 in saline solution was determined using an ozone analyzer in liquid media IKOZH-5 (certificate of conformity RU.C. 31.001.A No. 29545-05, Kirov). As part of the gel, Levoxime is the main ingredient (by weight) Xymedone (8%) is a pyrimidine drug. In addition, the gel contains levomycetin succinate (2%), as well as fillers. Xymedone (1-(β-hydroxyethyl)-4,6-dimethyl-1,2-dihydro-2-oxopyrimidine), has proven itself well in the treatment of purulent and burn wounds of soft tissues due to its high antioxidant, reparative, anti-inflammatory, antibacterial activity with minimal toxicity and hypoallergenic, [18].

Rats were taken out of the experiment on the 14th day after surgery by decapitation with the preliminary cutting of the carotid artery under combined anesthesia (Zoletil (60 mg/kg) + Xyl (6 mg/kg)). The blood was stabilized with sodium citrate (1:9). Plasma and erythrocytes washed twice in saline solution by centrifugation for 10 min at 1600 g on a CM-6 centrifuge were used for research. In plasma and suspended erythrocytes in saline solution (1:4), the activity of free radical oxidation (SRO) processes was studied using the method of induced biochemiluminescence, [2], [3], [7], on the biohemiluminometer BHL-06 (N.Novgorod). The following parameters were evaluated: tg 2α – an indicator that characterizes the rate of decline of SRO processes in plasma and indicates total antioxidant activity (AOA); S – chemiluminescence light sum for 30 seconds. – reflects the potential ability of a biological object to lipid peroxidation (LPO); EPR – erythrocytes peroxide resistance, characterizes the degree of

severity of LPO in erythrocytes. To assess the intensity of LPO, the level of the secondary product of free radical oxidation – malonic dialdehyde (MDA) in plasma and erythrocyte hemolysate was determined (1:10), [19].

Among the enzymes representing the first link of the antioxidant defense system, the activity of superoxide dismutase (SOD), which converts the superoxide radical into the electroneutral form of H2O2, and catalase, which recycles peroxide, were analyzed. The activity of SOD was determined in the hemolysate of washed erythrocytes (1:10) by inhibiting the formation of the product of autoxidation of adrenaline, [20]. To assess catalase activity in erythrocyte hemolysate (1:100), a spectrophotometric method was used based on determining the rate of decomposition of H_2O_2 by catalase of the test sample with the formation of water and oxygen, [21].

The research results were processed according to the Statistica 6.0 program, with the help of which the arithmetic mean of the indicators and the error of the average were calculated. 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.

3 Results and Discussion

It was found that in rats with skin flap modeling with basic local treatment, there is a moderate but statistically significant increase in the intensity of free radical processes ($p<0.05$; Fig. 1). This creates prerequisites for the development of secondary oxidative damage to cells and tissues that occurs in response to the presence of an extensive defect of the skin.

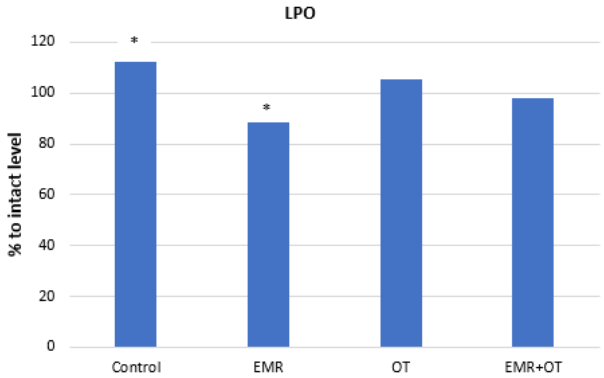

Fig. 1: The intensity of lipoperoxidation in rat blood plasma under various influences (EMR electromagnetic radiation of the EHF band, OT - ozone therapy, EMR+OT – a combination of EHF therapy and ozone therapy; "*" - the statistical significance of differences relative to intact rats $p<0.05$)

It was revealed that different variants of the applied experimental therapy have an unequal effect on the intensity of lipid peroxidation. Thus, the use of EHF radiation significantly reduces the parameter under consideration (by 11.3% relative to the level characteristic of intact animals; $p<0.05$). At the same time, ozone therapy and combined exposure retain the value of the indicator at the physiological level, but the latter option is statistically significantly lower than rats who received only basic treatment (by 14.4%; $p<0.05$).

The second component of the analysis was the total antioxidant activity of animal blood plasma (Fig. 2). It was found that in the control group of rats, this parameter was reduced by 9.8% relative to healthy animals ($p<0.05$). Together with a significant increase in intensive lipoperoxidation in operated rats, this indicates the presence of oxidative stress in them, which needs pathogenetic correction. The possibilities of three experimental therapy options were studied in the framework of the study.

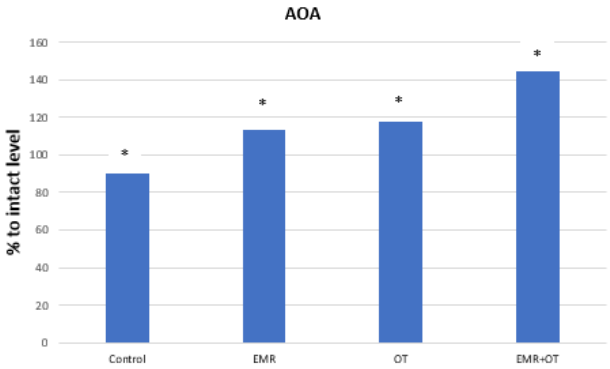

Fig. 2: Total antioxidant activity of rat blood plasma under various influences (EMR electromagnetic radiation of the EHF band, OT - ozone therapy, EMR+OT – a combination of EHF therapy and ozone therapy; "*" – statistical significance of differences relative to intact rats $p<0.05$)

It was shown that all the tested factors contributed to an increase in the antioxidant activity of the biological fluid, and the isolated use of ozone therapy and electromagnetic radiation provided an increase in the level of indicators approximately equally compared with intact animals (+13.3% and + 17.7% for these factors, respectively; $p<0.05$ for both cases). Interestingly, their combination made it possible to achieve a cooperative effect, which was manifested in an increase in the antioxidant potential of blood plasma by 44.2% relative to healthy rats ($p<0.05$). Taking into account the fact that no increase in the intensity of lipoperoxidation

was recorded in this mode, it can be argued that it makes it possible to realize the most complete antioxidant effect.

We also assessed shifts in the concentration of the secondary product of lipoperoxidation – malondialdehyde – in the blood plasma of rats of the formed groups (Fig. 3). It was found that only in operated animals receiving exclusively basic treatment, there was an increase in this indicator compared to the level characteristic of healthy rats (+10.1%; $p<0.05$). In the remaining groups, a decrease in the concentration of the metabolite in question relative to intact values was observed, most pronounced with combined exposure (-43.1%; $p<0.05$), which fully corresponds to the dynamics of the intensity of free radical processes and the antioxidant potential of the biological fluid. Therefore, such an option, including simultaneous courses of EHF therapy and ozone therapy, provides the maximum antioxidant systemic effect.

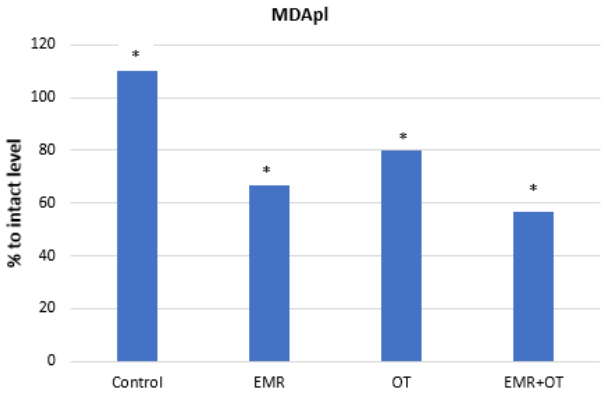

Fig. 3. Concentration of malondialdehyde in rat blood plasma under various influences (EMR electromagnetic radiation of the EHF band, OT - ozone therapy, EMR+OT – a combination of EHF therapy and ozone therapy; "*" – the statistical significance of differences relative to intact rats $p<0.05$)

This indicates that there is no psychogenic influence on the result of evaluating the effectiveness of the considered technology of individualized metabolic correction based on the parameters of crystallogenic activity of blood serum, which characterize both mineral and organic components of the latter.

In general, the results obtained indicate that all the studied options for exposure (isolated application of electromagnetic fields, ozone therapy, and their combinations) have a modulating effect on free radical processes in the blood and tissues of animals. Under the conditions of modeling an engrafting skin flap, oxidative stress develops in the blood of animals, which is characterized by the intensification of lipid peroxidation and inhibition of the antioxidant potential. According to the results obtained in this study, wound treatment with both electromagnetic radiation and the use of ozonized creams contributes to partial relief of laboratory signs of oxidative stress. At the same time, if for ozone therapy this fact was known earlier, which was shown, in particular, in our previous publications, [2], then for electromagnetic radiation in the literature there is only evidence of a pro-regenerative effect in relation to bone and cartilage tissue, [22], [23], [24], [25]. In this regard, the data obtained indicate the feasibility of the combined use of the studied physicochemical agents.

4 Conclusion

Our studies have allowed us to establish that the modeling of an extensive skin defect is accompanied by pronounced shifts in oxidative metabolism in the blood plasma of animals. At the same time, in the absence of pathogenetic treatment, signs of oxidative stress are formed, including the intensification of free radical processes and the inhibition of general antioxidant activity in combination with the accumulation of an increased amount of lipoperoxidation products (in particular, malondialdehyde).

Conducting experimental therapy with the introduction of ozone or treatment with electromagnetic radiation of the EHF band allows partially compensating for these metabolic disorders, however, the most optimal option is a combination of these factors within a single scheme. It should be noted that the results obtained are of great applied importance for the creation of innovative technologies for the complex medical rehabilitation of orthopedic-traumatological patients as a tool for influencing reparative processes in the lesion to restore and maintain tissue structures.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

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-Irina E. Sazonova: Investigation, Formal analysis and Writing – original draft and Writing – review & editing.
-Anna G. Soloveva: Investigation, Formal analysis and Writing – original draft and Writing – review & editing.
-Alexandra S. Fedotova, Lida K. Kovaleva: Investigation, Formal analysis and Writing – original draft & editing.

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Conflict of Interest

The authors have no conflicts of interest to declare.