Substantiation of the choice of the model for the formation of oxidative stress in preclinical studies

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Abstract. At the preclinical stage, a comparative assessment of various models of the formation of oxidative stress by exposure to high and low temperatures, ultraviolet irradiation, and the influence of a low-frequency alternating magnetic field was carried out. Exposure to laboratory animals was carried out daily for 21 days. It has been established that the modeling of oxidative stress by exposure to ultraviolet rays on rats allows, by the end of the first week of the experiment, to induce an increase in the intensity of lipid peroxidation processes with the accumulation of lipid peroxidation products by 48-61% and a decrease in the activity of antioxidant components by 31-33% in comparison with the control, which exceeds similar parameters on day 7 in models of hypothermia, hyperthermia and magnetic induction. Cold exposure in rats is accompanied by a more pronounced shift in the antioxidant status towards the prooxidant side by the end of the third week, which is confirmed by an increase in the concentration of lipid peroxidation products by 45-67% and a decrease in the level of components of the antioxidant system by 28-37% relative to the control. In general, the prooxidant effect in vivo of all the studied effects was confirmed, which we recommend as experimental models for the formation of oxidative stress at the preclinical stage, substantiating in the work the possibility of using each effect depending on the purpose of the pharmacological study.

1 Introduction

Stress causes a variety of physiological and biochemical changes in the body, which are used to assess the stress status of animals. Blood profiles, serum hormones, enzymes, and physiological conditions such as body temperature, heart rate, and respiration rate of animals are the most commonly used biomarkers of stress in animal production [1-11]. With prolonged exposure to various stress factors, stress immunodeficiency occurs in

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animals. At an early stage of stress, catecholamines enter the blood intensively, promoting the activation of glycogenolysis and the rapid mobilization of energy resources with the simultaneous production of corticosteroids that suppress the activity of T-lymphocytes. Under chronic stress, impaired functional and metabolic parameters do not have time to normalize due to the active secretion of catecholamines and corticosteroids; against this background, the number of all T-lymphocyte subpopulations decreases and, accordingly, their functional activity decreases, resulting in secondary immunodeficiencies, accompanied by the development of chronic pathological processes [1 , 9].

Oxidative stress is an imbalance between the formation of reactive oxygen species and the ability of endogenous antioxidant systems to take up reactive oxygen species, where they suppress the antioxidant activity of components of antioxidant systems. Oxidative stress plays a decisive role in the pathogenesis of diseases associated with hypoxia during fetal development and postnatal life: an excess amount of reactive oxygen species leads to irreversible damage to cell membranes, DNA, and other cellular structures through the oxidation of lipids, proteins, and nucleic acids [12, 13]. Critical analysis and regular monitoring of the benefits of using some experimental strategies in terms of the formation of oxidative stress allow us to evaluate the possibilities of creating new pharmacological treatments [5].

To date, one of the directions for optimizing pharmacotherapy is the addition of treatment regimens with antioxidant drugs, since oxidative stress is a pathogenetic link in many diseases and pathological conditions [2, 4, 12, 14-18]. The search for effective pharmacocorrectors-antioxidants and/or the establishment of antioxidant properties in known drugs starts from the preclinical stage, where oxidative stress in laboratory animals is modeled by introducing xenobiotics, exposure to hypo- and hyperthermia, ionizing and ultraviolet radiation, etc. [14, 15, 8, 11]. The novice pharmacological researcher faces the question of choosing an adequate model of oxidative stress [16]. And since we have tested various models of the formation of oxidative stress in the experiment, an attempt to reveal the advantages of each on the basis of the results obtained in this work is, in our opinion, of some interest.

The purpose of this work is to evaluate the effectiveness of various models of oxidative stress formation at the preclinical stage. To achieve this goal, it is necessary to perform a certain number of tasks:

- simulate oxidative stress by cold and heat exposure, ultraviolet irradiation and the action of a low frequency alternating magnetic field on rats;
- to identify the conditions for the formation of oxidative stress during hypo- and hyperthermia, ultraviolet irradiation and under the action of a low-frequency alternating magnetic field.

2 Materials and methods

The studies were carried out on the basis of the Central Research Laboratory (CRL8) of the Amur State Medical Academy (Blagoveshchensk) on 150 outbred male rats weighing 200–250 g in accordance with the National Standard of the Russian Federation GOST R 53434–2009 “Principles of Good Laboratory Practice”, Order of the Ministry health and social development of the Russian Federation dated August 23, 2010 No. 708n "On approval of the rules of laboratory practice". All conducted studies comply with the regulatory requirements for preclinical experimental studies.

The animals were kept in a vivarium under natural light under conditions of controlled temperature (22 ± 2) °C and humidity (65 ± 10)% of the air with free access to water and standard food.

Oxidative stress in rats was modeled by the following interventions:

1. Cold exposure - daily cooling of rats (exposure duration - 3 hours) in the conditions of the "Fentron" climate chamber (Germany) at a temperature regime of -150C for 21 days;

2. Thermal exposure - daily overheating of rats (exposure duration - 45 min) under the conditions of an air laboratory thermostat TVL-K (St. Petersburg) at a temperature regime of +40±1-2°C for 21 days;

3. Ultraviolet irradiation - daily irradiation of rats (exposure time - 3 min) in an ultraviolet chamber [14] for 21 days;

4. Variable magnetic field of low frequency (VFM LF) - daily exposure of rats to VFM LF (duration of exposure - 3 hours), created by a system of Helmholtz rings (1 meter in diameter), powered from a 50 Hz AC source, with magnetic field induction of 0.4 mTl, for 21 days.

Exposure time for each experimental exposure was tested by multiple studies at the preliminary stage in order to select the optimal duration of exposure, inducing the formation of oxidative stress. All exposures to the animals were performed under adequate humidity and ventilation conditions. The control were intact animals (n=30) under standard vivarium conditions.

The animals were slaughtered by decapitation on days 7, 14, and 21 of the experiment, 10 rats each from the control and experimental groups. After animals were decapitated, blood was collected in cooled tubes with heparin, centrifuged at 3000 rpm for 15 min, and the obtained blood serum was stored at -20°C until investigation.

The intensity of lipid peroxidation (LPO) processes was assessed by examining the content of diene conjugates (DC), lipid hydroperoxides (HL) according to the methods developed by I.D. Stalnoj, malondialdehyde (MDA) by color reaction with thiobarbituric acid, the main components of the antioxidant system (AOS) - ceruloplasmin according to the method of V.G.Kolba , vitamin E according to the method of R.Zh. Kiselevich, catalase according to the method modified by E.A. Borodin in the blood of rats [14]. These techniques are reflected in our previously published works [15, 3, 10, 18].

The following devices were used in the work: a KFK-2mp spectrophotometer, a UNICO spectrophotometer, and a Solar PV 1251 C photoelectrocolorimeter.

Statistical processing of the results was carried out using the Styudenta (t) test using the Statistica v.10.0 program; differences were considered significant at $p < 0.05.3$.

3 Results

The results of the study showed that the impact of all the studied factors has a prooxidant effect on a warm-blooded organism: lipid peroxidation products accumulate relative to the control (intact rats), in particular, diene conjugates by 42% (day 7), 39% (day 14), 54% (21 day of the experiment) under conditions of hypothermia, by 24%, 26%, 31% respectively - under conditions of hyperthermia, by 48%, 43%, 42% - under ultraviolet irradiation, by 14%, 16%, 17% - under conditions of alternating low frequency magnetic field; lipid hydroperoxides - by 30%, 41%, 45% by the end of the first, second and third weeks of the experiment against the background of cold exposure, by 23%, 26%, 16%, respectively - against the background of thermal exposure, by 53%, 48%, 40 % - against the background of ultraviolet irradiation, by 14%, 14%, 20% - against the background of an alternating magnetic field of low frequency; malondialdehyde - by 53% (7 days), 74% (14 days), 67% (21 days of the experiment) under hypothermia, by 39%, 39%, 32%, respectively - under hyperthermia, by 61%, 48% , 40% - under conditions of ultraviolet irradiation, by 46%, 45%, 46% - under conditions of an alternating magnetic field of low frequency (Table 1).

Table 1. The concentration of lipid peroxidation products in laboratory animals of the control and experimental groups, M±m.

Indicators	Terms of experience	Control (n=30)	Hypothermia (n=30)	Hyperthermia (n=30)	UVI (n=30)	VMF LF (n=30)
DC, nmol/ml	day 7	34.4±3.1	48.8±2.2*	42.7±1.8	50.9±2.9*	39.2±2.2
	day 14	35.4±2.0	49.2±2.5*	44.6±1.4*	50.6±2.8*	41.1±1.0*
	day 21	31.2±1.6	48.0±1.8*	40.9±1.2*	44.3±2.3*	36.5±0.9*
LH, nmol/ml	day 7	26.0±1.8	33.8±1.1*	32.0±1.2*	39.8±2.5*	29.6±1.3
	day 14	25.0±2.0	35.3±1.2*	31.5±1.1*	37.0±2.2*	28.5±1.5
	day 21	28.6±1.5	41.5±1.1*	33.2±1.0*	40.0±2.0*	34.3±1.2*
MDA, nmol/ml	day 7	3.8±0.1	5.8±0.2*	5.3±0.3*	6.1±0.4*	5.5±0.4*
	day 14	3.8±0.2	6.6±0.4*	5.3±0.2*	5..6±0.3*	5.5±0.3*
	day 21	4.4±0.3	7.3±0.4*	5.8±0.2*	6.2±0.4*	6.4±0.3*

Note. Here and in Table 2: * - significance of differences in relation to control at $p < 0.05$.

The accumulation of lipid peroxidation products is accompanied by a decrease in the activity of the components of the antioxidant system in the blood of experimental animals in comparison with the control: under cold exposure, the concentration of ceruloplasmin decreases by 31%, 34%, 34% by the end of the first, second and third weeks of the experiment, respectively, vitamin E - by 23%, 19%, 28%, catalase - by 16%, 20%, 37%; with thermal exposure - by 30%, 33%, 26%, respectively (ceruloplasmin), by 27%, 30%, 28% (vitamin E), by 19%, 29%, 24% (catalase); with ultraviolet irradiation - by 33%, 36%, 29%, respectively (ceruloplasmin), by 31%, 31%, 27% (vitamin E), by 31%, 32%, 30% (catalase); when exposed to an alternating low-frequency magnetic field - by 17%, 15%, 16%, respectively (ceruloplasmin), vitamin E - by 10% by the end of the experiment (only a downward trend was recorded on days 7 and 14), catalase - by 20% , 15%, 14%, respectively (Table 2).

Table 2. Concentration of components of the antioxidant system in laboratory animals of the control and experimental groups, M±m.

Indicators	Terms of experience	Control (n=30)	Hypothermia (n=30)	Hyperthermia (n=30)	UVI (n=30)	VMF LF (n=30)
Cerulo-plasmin, mcg/ml	day 7	30.0±1,9	20.7±1.7*	21.0±2.0*	20.1±2.5*	24.9±1.0*
	day 14	28.8±1.4	19.0±1.5*	19.3±1.8*	18.4±2.9*	24.5±0.8*
	day 21	26.8±1.4	17.7±1.6*	19.8±1.9*	19.0±2.0*	22.5±0.7*
Vitamin E, mcg/ml	day 7	48.7±2.6	37.5±1.9*	35.6±2.5*	33.6±2.8*	44.0±2.1
	day 14	47.5±2.2	38.5±1.6*	33.3±2.8*	32.8±2.9*	42.9±2.5
	day 21	45,8±1.9	33.0±1.8*	33.0±2.2*	33.4±2.8*	41.2±0.6*
Catalase, µmol H_2O_2 $g^{-1}c^{-1}$	day 7	93.0±2.7	78.1±3.1*	75.3±3.4*	64.2±3.5*	74.4±3.3*
	day 14	95.2±3.2	76.2±3.9*	67.6±3.7*	64.7±3.8*	80.9±3.5*
	day 21	97.0±3.5	61.1±4.2*	73.7±3.3*	67.9±3.0*	83.4±3.1*

4 Discussion

Thus, the results of the experiment confirm the formation of oxidative stress under conditions of exposure to low and high temperatures, ultraviolet irradiation and a low-

frequency alternating magnetic field, and an earlier response (already by the end of the first week of the experiment) of the lipid peroxidation / antioxidant system with a shift to the pro-oxidant side it is registered under the influence of ultraviolet rays on a warm-blooded organism, which, in our opinion, is associated, firstly, with the genus of laboratory animals (rats), for which exposure to ultraviolet radiation is the most pronounced stress factor in comparison with other effects that we study; secondly, with the mechanism of action of ultraviolet rays and the formation of free radicals from valence-saturated lipid molecules in biological systems at the initial stage of chain nucleation under ultraviolet irradiation, which becomes a trigger mechanism in inducing lipid peroxidation processes and changing the "ionic conjuncture" in cells and tissues [10]. This leads to an increase in the permeability of cell membranes, the accumulation of calcium cations in the cytoplasm, followed by the activation of hydrolytic enzymes (phospholipase A2) and damage to intracellular structures, aggravated by increased activity of lysosomal enzymes due to peroxide damage to lysosome membranes.

Further accumulation of lipid peroxidation products during ultraviolet irradiation inactivates the most important membrane enzymes (glucose-6-phosphate dehydrogenase, cytochrome P-450, monoamine oxidase, mitochondrial redox-chain electron transporters, etc.), which potentiates further increase in membrane ion permeability and leads the cell to destruction. In turn, cold exposure leads to stable accumulation of lipoperoxidation products against the background of reduced activity of the antioxidant system by the end of the third week of the experiment, which exceeds the values of the parameters of the previous model. During adaptation of the warm-blooded organism to cold, disproportion in the hormonal and energetic status of anabolic processes against the background of deficiency of bioenergetic resources and tissue hypoxia, based on the mismatch between tissue oxygen demand and its delivery, leads to damage of lysosome membranes with release of autolytic enzymes into intercellular space, which, similar to the previous model of experimental exposure, contributes to damage of cell membranes.

Taking into account the pronounced adaptive potential of laboratory animals with respect to hypothermia, the cold model of oxidative stress can be used in experiments of sufficient duration, for example, when studying the antioxidant activity of medicinal plant preparations, the lasting effect of which develops, as a rule, after 3-4 weeks [12]. It is important to note the absence of significant fluctuations from the 7th to the 21st day of all determined parameters when using a thermal model and a low-frequency alternating magnetic field, which indicates stable and unidirectional processes occurring in vivo, however, the concentration of lipid peroxidation products / antioxidant system of the antioxidant system during hyperthermia was 1.5-2 times higher than similar parameters under magnetic induction conditions.

This fact allows us to state more pronounced negative changes in the body of laboratory animals when exposed to high temperatures, less pronounced - when exposed to a low frequency alternating magnetic field. These experimental models of the formation of oxidative stress can be recommended for use in pharmacological studies at the preclinical stage when studying the pharmacokinetic and pharmacodynamic characteristics of drugs with an antioxidant effect.

5 Conclusion

The prooxidant effect on laboratory animals of hypo- and hyperthermia, ultraviolet rays and a low-frequency alternating magnetic field has been confirmed. Depending on the purpose of modeling oxidative stress in a warm-blooded organism, we recommend ultraviolet irradiation of laboratory animals if it is necessary to create an experimental model in a shorter time, cold exposure - if it is necessary to maintain a shift in lipid peroxidation /

antioxidant system to the pro-oxidant side for three or more weeks; stable changes in the lipid peroxidation / antioxidant system in models of hyperthermia and magnetic induction are more adequate when testing different doses of new antioxidants or when studying registered drugs tested for the presence of antioxidant activity.

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