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COMPARATIVE ASSESSMENT OF THE INTENSITY OF OXIDATIVE STRESS IN VARIOUS EXPERIMENTAL MODELS

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The possibilities of modeling oxidative stress in vivo include a fairly wide range of effects from the introduction of xenobiotics to the use of a temperature factor, irradiation, etc. On the basis of the Amur Medical Academy, pharmacologists summarized many years of experience in using various models of the formation of oxidative stress in a warm-blooded organism - the processes of peroxidation lipids were induced by exposure to low and high temperatures, ultraviolet irradiation, and a low-frequency alternating magnetic field. The intensity of lipid peroxidation processes in various models was assessed by the degree of accumulation of diene conjugates, lipid hydroperoxides, malondialdehyde and the level of ceruloplasmin, vitamin E, and catalase activity in the blood of laboratory animals on days 7, 14, and 21 of the experiments. The results of a comparative assessment of the intensity of oxidative stress in various experimental models showed that the most pronounced changes in the antioxidant status are caused by the cooling of animals and exposure to ultraviolet rays, and the latter model triggers a shift in the balance to the prooxidant side by the end of the first week of the experiment, which is confirmed by the accumulation of lipid peroxidation products by 48 -61% and a decrease in the activity of the components of the antioxidant system by 31-33% compared with the control. Thermal exposure to rats and the effect of a low-frequency alternating magnetic field causes less pronounced, but more stable changes in the dynamics from 7 to 21 days in the state of the prooxidant/antioxidant system, which, similarly to models using hypothermia and ultraviolet light, allows us to ascertain the formation of oxidative stress.

Keywords: experimental models, hypothermia, hyperthermia, ultraviolet irradiation, low-frequency variable magnetic field, oxidative stress, lipid peroxidation products, antioxidant system, rats.

Today, there is no doubt about the dominant role of oxidative stress in the pathogenesis of many diseases and pathological conditions, and therefore, since the end of the last century, pharmacologists have been actively engaged in the search for effective drugs that prevent and/or level the consequences of excessive intensity of lipid peroxidation processes [3, 5, 6, 11, 12]. Naturally, when testing pharmacocorrectors at the preclinical stage, the question arises of how to model oxidative stress [1, 4, 8]. Various models of stress are known with the use of xenobiotics, temperature effects, ionizing and ultraviolet radiation, etc. [2, 7, 9, 10]. Due to the variety of existing models of oxidative stress, it is quite logical that the researcher faces the question of choosing an adequate model. For several decades, the Department of Pharmacology of the Amur State Medical Academy of the Ministry of Health of Russia has successfully used models of oxidative stress induced by cold exposure (since the 80s of the last century), thermal exposure (since the 2000s), ultraviolet irradiation (since 2007) , alternating magnetic field of low frequency (since 2020). The accumulated experience of the effectiveness of these experimental models became the reason for presenting in this paper the results that reflect the comparative aspects of the induction of lipid peroxidation (LPO) processes in vivo in dynamics, in order to facilitate the problem of choosing a model for novice researchers.

The purpose of the study is a comparative assessment of the intensity of oxidative stress in various experimental models.

Material and methods. The experiments were carried out on outbred male rats weighing 200–250 g, obtained from the nursery of the Central Scientific Research Laboratory of the AGMA, Blagoveshchensk. The animals were kept in a vivarium under natural light under conditions of controlled temperature (22 ± 2) 0C and humidity (65 ± 10)% of the air with free access to water and standard food. The experiments were carried out in accordance with the National Standard of the Russian Federation GOST R 53434 - 2009 "Principles of Good Laboratory Practice", Order of the Ministry of Health and Social Development of the Russian Federation of August 23, 2010 No. 708n "On Approval of the Rules of Laboratory Practice". All conducted studies are approved by the Local Ethics Committee of the Amur State Medical Academy and comply with the regulatory requirements for conducting preclinical experimental studies.

Oxidative stress in laboratory animals was modeled by the following actions:

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;

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3. Ultraviolet irradiation - daily irradiation of rats (exposure time - 3 min) in an ultraviolet chamber [1] for 21 days;

4. Low frequency alternating magnetic field (LF LF) - daily exposure of rats to LF MF (exposure duration - 3 hours), created by a system of Helmholtz rings (diameter 1 meter), powered by an alternating current source with a frequency of 50 Hz, with a magnetic field induction of 0,4 mT for 21 days. The exposure time for each experimental exposure was tested by multiple studies at the preliminary stage in order to select the optimal exposure duration that induces a shift in the equilibrium in the LPO/AOS system to the prooxidant side with the formation of oxidative stress. All exposures to animals were carried out under adequate conditions of humidity and ventilation. The controls in each experimental model were intact animals under standard vivarium conditions. Animals were slaughtered by decapitation on the 7th, 14th, and 21st days of the experiments, 10–12 rats from the control and experimental groups. After decapitation of the animals, the blood was collected into cooled tubes with heparin, centrifuged at 3000 rpm for 15 min, the obtained blood serum was stored at –20 0C until the moment of the study. The intensity of lipid peroxidation processes was assessed by examining the content of diene conjugates (DC), lipid hydroperoxides (HL) according to the methods developed by I.D. V.G. Flask [3], 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. These techniques are reflected in our previously published works [5, 7, 9, 10]. The following instruments were used in the work: a KFK-2mp spectrophotometer, a UNICO spectrophotometer, a Solar PV 1251 C photoelectric colorimeter. Statistical processing of the results was carried out using the Student's test (t) using the Statistica v.6.0 program, differences were considered significant at $p < 0.05$. When presenting the data, the results obtained in the control groups (intact rats) are conditionally taken as one (100%), the graphical representation of the dynamics of the LPO/AOS components in the experimental animals (subjected to various influences) reflects the percentage deviation from the control.

Results and discussion. Cold exposure to rats induces an increase in the activity of lipid peroxidation processes with the accumulation of LPO products and a decrease in the activity of AOS components in the blood of cooled animals: the content of DC increases by

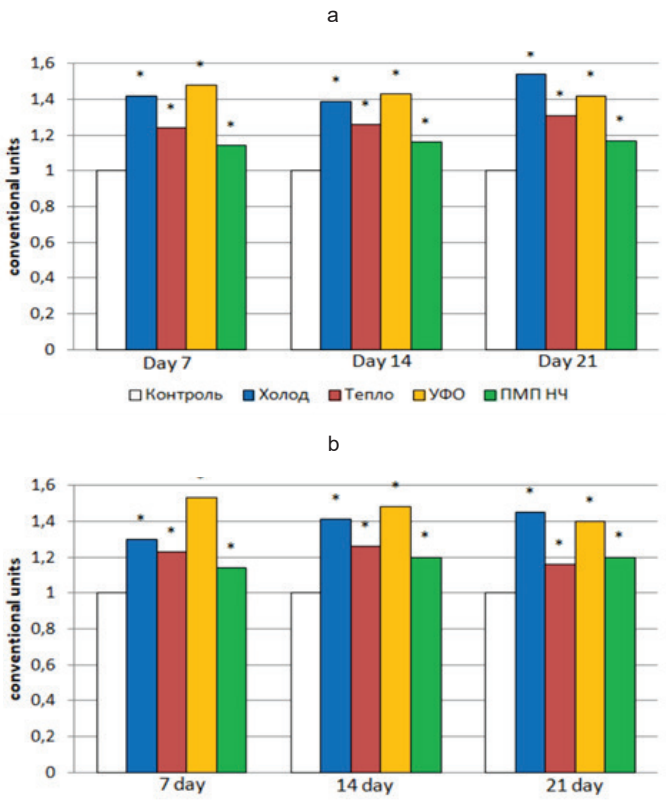

Fig. 1. Dynamics of diene conjugates (a) and lipid hydroperoxides (b) in intact (control) and exposed laboratory animals. In Fig. 1-3: * - significance of differences in relation to control at $p < 0.05$

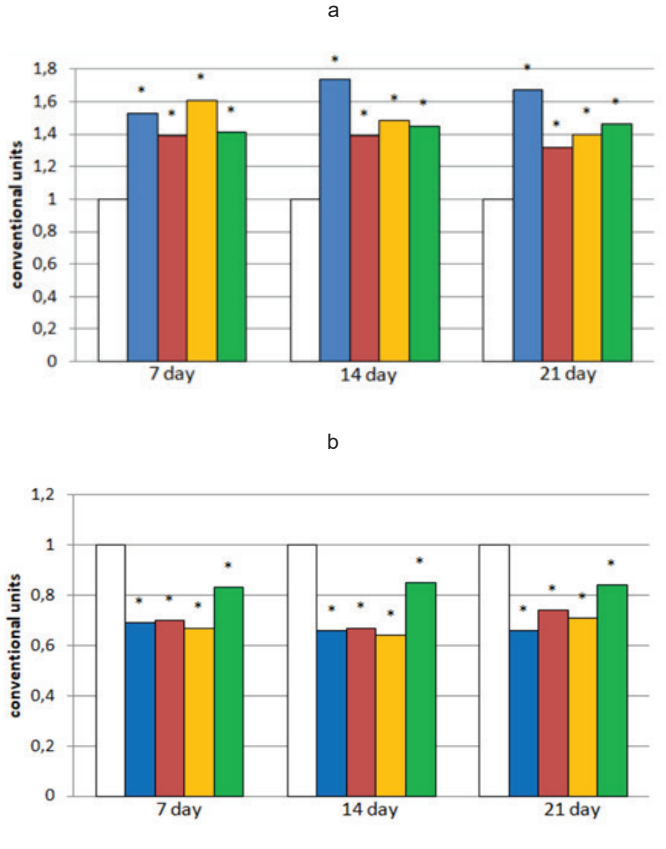

Fig. 2. Dynamics of malondialdehyde (a) and ceruloplasmin (b) in intact (control) and exposed laboratory animals

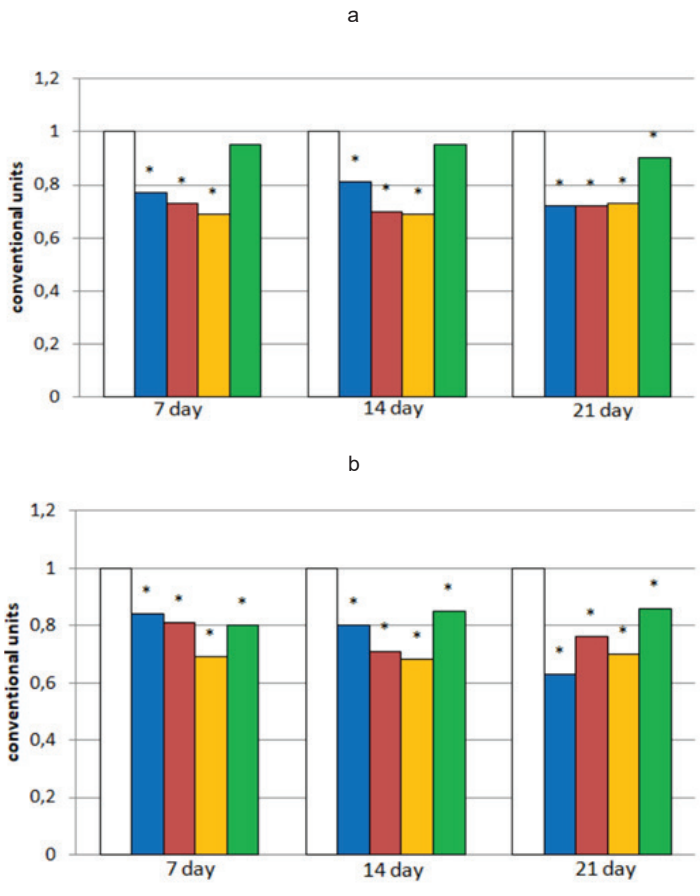

Fig. 3. Dynamics of vitamin E (a) and catalase activity (b) in intact (control) and exposed to various effects of laboratory animals

42%, 39%, 54% by the end of the first, second and third weeks of the experiment, respectively, relative to intact rats (Fig. 1), GL - by 30%, 41%, 45%, respectively (Fig. 2), MDA - by 53%, 74%, 67% (Fig. 3); against this background, the concentration of ceruloplasmin decreases by 31%, 34%, 34%, respectively (Fig. 4), vitamin E - by 23%, 19%, 28% (Fig. 5), catalase - by 16%, 20%, 37 % (Fig. 6). When using the thermal model of the experiment, the growth of lipid peroxidation products relative to the control (intact animals) was 24% (day 7), 26% (day 14), 31% (day 21) in relation to DC (Fig. 1), 23%, 26%, 16%, respectively, in relation to GL (Fig. 2), 39%, 39%, 32% in relation to MDA (Fig. 3), which was accompanied by a decrease in the level of ceruloplasmin by 30%, 33%, 26%, respectively (Fig. 4), vitamin E - by 27%, 30%, 28% (Fig. 5), catalase - by 19%, 29%, 24% (Fig. 6). Ultraviolet irradiation (UVR) of laboratory animals leads to the accumulation of DC by 48%, 43%, 42% by the end of the first, second and third weeks of the experiment (Fig. 1), GL - by 53%, 48%, 40%, respectively (Fig. 2), MDA - by 61%, 48%, 40% (Fig. 3); under these conditions, the content of ceruloplasmin decreases by 33%, 36%, 29%, respectively (Fig. 4), vitamin E - by 31%, 31%, 27% (Fig. 5), catalase - by 31%, 32%, 30 % (Fig. 6) in comparison with similar parameters in animals of the control group. Exposure to PMF NPs in rats is accompanied by an increase in the content of DC by 14%, 16%, 17% by the end of the first, second and third weeks of the experiment, respectively, relative to intact rats (Fig. 1), GL - by 14%, 14%, 20%, respectively (Fig. 2), MDA - by 46%, 45%, 46% (Fig. 3); the decrease in the content of ceruloplasmin was 17%, 15%, 16% on days 7, 14, 21 of the experiment (Fig. 4), vitamin E - by 10% by the end of the experiment (only a downward trend was recorded on days 7 and 14) (Fig. 5), catalase - by 20%, 15%, 14% (Fig. 6). As a result of a comparative assessment of various models of the formation of oxidative stress in laboratory animals, we can state an earlier response (already by the end of the first week of the experiment) of the LPO/AOS system with a shift to the prooxidant side when exposed to ultraviolet rays on a warm-blooded organism, which, in our opinion, is due to - firstly, 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 UV conditions; secondly, with the genus of laboratory animals (rats), for which exposure to ultraviolet radiation is the most pronounced stress factor in comparison with other effects studied by us. In turn, cold exposure leads to a stable accumulation of lipid peroxidation products against the background of a decrease in AOS activity by the end of the third week of the experiment, which exceeds the previous model in parameter values and can be used in experiments of sufficient duration, for example, when studying the antioxidant activity of phytopreparations, a lasting effect from the use of which develops, as a rule, after 3-4 weeks. It is important to note the absence of significant fluctuations from the 7th to the 21st day of all determined indicators when using the thermal model, the range of which was from 0 to 10%, and the PMF LF, where the changes were in the range from 0 to 6%, which indicates stable and unidirectional processes occurring in vivo, however, the concentration of LPO products/AOS components during hyperthermia was 1.5-2 times higher than similar parameters under magnetic induction conditions.

Thus, 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; stable changes in the LPO/AOS system in models of hyperthermia and magnetic induction are more adequate when testing different doses of new antioxidants or registered drugs tested for the presence of antioxidant activity.

Conclusions.

1. Modeling oxidative stress by exposure to ultraviolet rays on laboratory animals 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 AOS components by 31-33% in comparison with the control, which exceeds similar parameters on day 7 in models of hypo-, hyperthermia and magnetic induction.

2. Cold exposure in rats is accompanied by a more pronounced shift in the LPO/AOS system 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 AOS components

by 28-37% relative to intact animals.

3. Induction of LPO processes under in vivo conditions by hyperthermia and PMF LF leads to a more stable state of the LPO/AOS system in dynamics from 7 to 21 days of the experiment, according to which, when assessing the values of the main parameters, it is possible to ascertain the formed oxidative stress.

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PREVALENCE OF FUNCTIONAL GASTROINTESTINAL DISEASES IN SCHOOLCHILDREN OF KRASNOYARSK BY ROME IV CRITERIA

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The prevalence of functional gastrointestinal diseases (FGDs) in adolescents of 11-18 years old in school (500 individuals) and in a specialized gastroenterology unit (141 individuals), identified by questioning the Russian-language version of the QPGS - RIV questionnaire (Questionnaire on Pediatric Gastrointestinal Symptoms, Rome IV Version) was analyzed. As a result, the prevalence of FGDs in school was as follows: functional dyspepsia (FD) - 5.3%, irritable bowel syndrome (IBS) - 0.6%, abdominal migraine (AM) - 1.0%, functional abdominal pain syndrome (FAPS) - 0.2%, functional constipation (FC) - 5.3%. In the study profile of recurrent abdominal pain (RAP) among children in hospital, FD was 73.6%, IBS - 22.6%, FAPS - 3.8%, 17% of children had both FD and IBS. Compared to the previous version, according to the new criteria, instead of IBS, the FD diagnosis prevailed (due to a decrease of the criterion for the prevalence of pain syndrome, as well as the inclusion of postprandial distress syndrome (PDS) for diagnosis), and the IBS incidence rate decreased threefold (due to the new limiting criteria).

Keywords: adolescents, recurrent abdominal pain, functional gastrointestinal diseases, prevalence, ROME IV.

Introduction: The new Rome criteria of FGDs for revision IV (ROMEIV) were introduced in May 2016 (Table 1).

Significant changes mainly affected functional dyspepsia (FD) and irritable bowel syndrome (IBS). Currently, FD is divided into two independent forms: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) both in adults and in children. The criteria for diagnosing IBS have been corrected, i.e. in the previous version, along with abdominal pain, two more conditions should have occurred: amelioration after bowel move-