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ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ



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The Efficiency of Modeling Oxidative Stress in Rats by Exposure to Noise Compared to Exposure to Hyperthermia or Magnetic Field

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Abstract

Introduction. *In vivo* model for experimental creation of the necessary pathological processes is an important element of scientific research planning. Numerous series of experiments have demonstrated the relevance of modeling oxidative stress in laboratory rats by exposure to hyperthermia, magnetic field and noise. The problem of finding the advantages of each particular prooxidant factor in modeling stress response underlies the current experiment, and its expediency is induced by the need to generate a robust response of the prooxidant/antioxidant system, with statistically significant changes of its parameters, at various periods of time. The study aims at conducting a comparative assessment of the effect of noise, hyperthermia, and magnetic field on the intensity of lipid peroxidation processes in rats.

Materials and Methods. The study was conducted at the Research Laboratory of Amur Medical Academy in 2023–2024. The experiment involved 120 white rats divided into four equal in number groups. The animals in the first group (intact) were not subjected to any impacts; the animals in the second group (experimental group 1) were subjected to hyperthermia; the animals in the third group (experimental group 2) were exposed to magnetic field; and the animals in the fourth group (experimental group 3) were exposed to noise. On 7th, 14th, and 21st days of the experiment the rats were decapitated (10 animals from each group) and their blood was sampled for analysis. Oxidative stress markers were determined using the standard techniques; the results were analysed using the Mann-Whitney and Kruskal-Wallis tests. The critical significance level was set to 0.05 for all assessment procedures.

Results. With regard to the influence on the accumulation degree of conjugated dienes, statistically significant advantages of noise model over the magnetic field model were recorded ($p=0.000005$ on 14th and 21st days) and over hyperthermia model ($p=0.002039$ on 14th day; $p=0.001837$ on 21st day). With regard to malondialdehyde, noise exposure surpassed hyperthermia by the end of the experiment ($p=0.000561$). With regard to ceruloplasmin, the advantages of the noise model over hyperthermia model were established ($p=0.0167980$ on 7th day; $p=0.004813$ on 21st day), as well as over the magnetic field model ($p=0.000005$ at all control points). In relation to vitamin E, the noise model advantages over the magnetic field ($p=0.000006$ on 21st day) were revealed.

Discussion and Conclusions. Significant advantages of the noise-exposure model over the hyperthermia- and magnetic-field-exposure ones in modeling the oxidative stress were established, along with the respective changes in prooxidant/antioxidant system components. By the end of the first, second, and third weeks of the experiment, the statistically significant deviation of oxidative stress markers in laboratory animals occurred under exposure to noise, unlike exposure to temperature and magnetic field. In future, studies on the acoustic load influence on the adaptive potential of warm-blooded organisms are planned to probe possible pharmacological medications to negative influence of noise.

Keywords: modeling, oxidative stress, exposure to noise, hyperthermia, magnetic field, lipid peroxidation, antioxidant status, rats






Declaration on Compliance with the Principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes: the authors declare that all research was conducted in compliance with the principles of Good Laboratory Practice.

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Оригинальное эмпирическое исследование

Эффективность моделирования окислительного стресса у крыс воздействием шума в сравнении с гипертермией и магнитным полем

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Аннотация

Введение. Экспериментальная модель создания необходимых патологических процессов в условиях *in vivo* является важным элементом при планировании научных исследований. Многочисленными сериями экспериментов была показана состоятельность моделирования окислительного стресса воздействием гипертермии, магнитного поля, шума на лабораторных крыс. Вопрос о преимуществах моделирования стресс-реакции конкретным прооксидантным фактором стал основанием для проведения настоящего эксперимента ввиду необходимости формирования надежной ответной реакции в различные временные интервалы со статистически значимым изменением параметров прооксидантной/антиоксидантной системы. Цель исследования — сравнительная оценка влияния шума, гипертермии и магнитного поля на интенсивность процессов липопероксидации у крыс.

Материалы и методы. Исследование проведено в научно-исследовательской лаборатории Амурской медакадемии в 2023–2024 гг. В эксперименте участвовало 120 белых крыс, которых разделили на четыре равные по численности группы. В первой группе (интактная) животных не подвергали каким-либо воздействиям; во второй группе (подопытная 1) животных подвергали гипертермии; в третьей группе (подопытная 2) — воздействию магнитного поля; в четвертой группе (подопытная 3) – воздействию шума. На 7, 14, 21-й дни эксперимента крыс декапитировали (по 10 голов из каждой группы) и производили забор крови для анализа. Определение маркеров окислительного стресса проводили по общепринятым методикам, результаты анализировали с применением критериев Манна-Уитни и Краскела-Уоллиса. Во всех процедурах оценки критический уровень значимости принимался равным 0,05.

Результаты исследования. Статистически значимые преимущества по влиянию на степень накопления диеновых конъюгатов зарегистрированы у модели шумового влияния над магнитным полем ($p=0,000005$, 14-й и 21-й дни) и гипертермией ($p=0,002039$, 14-й день; $p=0,001837$, 21-й день). Что касается малонового диальдегида, шумовое воздействие превзошло гипертермию к концу опыта ($p=0,000561$).

Обозначены преимущества шумовой модели в отношении церулоплазмина над гипертермией ($p=0,0167980$, 7-й день; $p=0,004813$, 21-й день) и магнитным полем ($p=0,000005$ во все контрольные точки); в отношении витамина E – над магнитным полем ($p=0,000006$, 21-й день).

Обсуждение и заключение. Установлены значимые преимущества модели шумового воздействия над гипертермией и магнитным полем в плане моделирования окислительного стресса и характерных изменений компонентов прооксидантной/антиоксидантной системы. В отличие от температурного воздействия и магнитной нагрузки, под влиянием шума у лабораторных животных формируются статистически значимые отклонения маркеров окислительного стресса к концу первой, второй и третьей недель экспериментального воздействия. В дальнейшем предполагается изучение влияния акустической нагрузки на адаптационный потенциал теплокровного организма с целью апробации потенциальных фармакокорректоров негативного воздействия шума.

Ключевые слова: моделирование, окислительный стресс, шумовое воздействие, гипертермия, магнитное поле, перекисное окисление липидов, антиоксидантный статус, крысы

Декларация о соблюдении принципов Европейской конвенции о защите позвоночных животных, используемых для экспериментов и других научных целей: авторы заявляют, что все проведенные исследования соответствовали принципам конвенции и правилам надлежащей лабораторной практики.

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Introduction. When conducting pharmacological and pathophysiological preclinical studies, a young specialist often faces the problem of choosing an experimental model for inducing the processes, which need to be studied [1–7]. It is possible to induce lipid peroxidation (LPO) processes in cell membranes with subsequent formation of the oxidative stress through exposure to hyperthermia, magnetic field and noise. For many years these models have been tested and tried at the Department of Pharmacology of Amur Medical Academy to work out the dose and duration of the exposure (daily, course, etc.) [8–10]. Due to the increase of acoustic load intensity on humans and animals from ever to year [11–16], the studies aimed at investigating the effects of noise on warm-blooded organisms are becoming particularly relevant. All of the above-mentioned factors can trigger the free-radical reactions, however, it's worth to compare and determine the efficiency of various models in projecting the specific oxidative stress markers and demonstrating the time frame of prooxidant/antioxidant system parameters respond to stress factors. In our opinion, reliability in revealing maximum possible changes in the “lipid peroxidation/antioxidant defence” system parameters and the highest percentage of probability of oxidative stress development indicate the advantages of the experimental model and its usability in preclinical research.

In the frame of the experiment with laboratory rats, the study aims to conduct a comparative analysis of the effects of noise, hyperthermia and magnetic field on the intensity of lipid peroxidation processes in biomembranes.

Materials and Methods. The experiment was conducted in 2023 – 2024 in the research laboratory of Amur Medical Academy (Blagoveshchensk) in compliance with the requirements for preclinical studies and approved by the local Ethics Committee (extract from protocol No. 9 of December 7, 2022). For the experiment, 120 white mongrel male rats aged 2 – 3 months weighing 200 – 250 g were used. The rats were divided into four groups of equal size: in the first group (intact group) the animals were not exposed to any effects; in the second group (experimental group 1) the animals were exposed to hyperthermia ($+40\pm 2$ °C 45 min/day daily for 7, 14, 21 days); in the third group (experimental group 2) animals were exposed to a magnetic field (0.4 millitesla 180 min/day daily for 7, 14, 21 days); in the fourth group (experimental group 3) animals were exposed to noise (95–105 dB 60 min/day daily for 7, 14, 21 days). No lethal cases were recorded during the experimental exposures.

Rats were decapitated on the 7th, 14th, and 21st day of the experiment (10 animals from each group). The animals' blood was collected into chilled tubes with heparin and centrifuged at 3000 rpm for 15 min. The resulting blood serum was stored at -20°C until the time of the study. The intensity of lipid peroxidation processes was assessed by examining the content of: conjugated dienes (by the method of I.D. Stalnaya), malondialdehyde (by colouring

reaction of the thiobarbituric acid test), and the main components of the antioxidant system (AOS) – ceruloplasmin (by the method of V.G. Kolb), vitamin E (by the method of R.Zh. Kiselevich). In the work, the following equipment was used: spectrophotometer KFK-2mp (Zagorsk Optical-Mechanical Plant, Russia), UNICO spectrophotometer (United Products & Instruments, USA), and photoelectrocolorimeter Solar PV 1251 C (SOLAR JSC, Belarus).

Statistical processing of the results was performed in the Microsoft Excel 2016 (Microsoft) and the Statistica.10.0 software packages (Statsoft Inc., USA). Quantitative indicators were analysed for compliance with normal distribution using the Shapiro-Wilk test (number of animals in groups $n < 50$). Based on the quantitative analysis and graphical representation of frequency histograms, it was found that the predominant part of the quantitative data did not correspond to the normal type of distribution, therefore the results were described by calculating the median (Me), lower and upper quartiles (Q1; Q3). Intergroup comparison by the quantitative indicator was performed using the Mann-Whitney U-test; the statistical significance of intragroup changes of parameters in dynamics was determined using the Wilcoxon test; to compare values in more than two samples, taking into account the abnormal distribution of quantitative data, a non-parametric alternative to univariate (intergroup) analysis of variance (ANOVA) was used — the Kruskal-Wallis test. The critical significance level was set to 0.05 in all assessment procedures.

Results. It was established that exposure to noise, magnetic field and hyperthermia triggers unidirectional non-specific processes in the organism associated with an increase in the intensity of free radical (peroxide) oxidation of biomembrane lipids and formation of oxidative stress, manifested by the accumulation of LPO products in the blood plasma of experimental rats. Thus, upon the experimental exposures, the concentration of conjugated dienes (Table 1) has statistically significantly increased relative to that in intact animals: by the end of the first week of the experiment by 23% (hyperthermia, $p < 0.05$), by 39% (noise, $p < 0.05$) with the tendency towards an increase of the parameter, by 13% (magnetic field); by the end of the second week, the level of the primary peroxidation product has significantly increased by 27% (hyperthermia), 16% (magnetic field), 49% (noise, $p < 0.05$); by the end of the third week — by 31%, 17%, and 58%, respectively ($p < 0.05$). On the whole, the experimental exposures can be arranged in the descending order by their efficiency in inducing the diene conjugation in lipids as follows: noise > hyperthermia > magnetic field.

The secondary product of lipid peroxidation — malondialdehyde (MDA), responded to the exposure to the studied factors by a statistically significant increase compared to the intact group at all control time points (Table 2): on the 7th day of the experiment, MDA increased by 37% (hyperthermia), by 45% (magnetic field),

by 53% (noise) ($p < 0.05$); on the 14th day — by 40%, 49% and 47% respectively ($p < 0.05$); on the 21st — by 29%, 44% and 61% ($p < 0.05$). This allows us to arrange the experimental exposures by the model efficiency as follows: noise > magnetic field > hyperthermia.

Thus, all the tested experimental exposures are efficient with regard to increasing the level of oxidative stress markers. In this context, magnetic field more significantly fosters the accumulation of the secondary product of LPO, hyperthermia — the primary one, whereas, exposure to noise proved to have stable, more pronounced efficiency in modeling stress, which was

confirmed by the results of the Kruskal-Wallis rank analysis of variance (Table 3): at all control time points, statistically significant changes in conjugated dienes and malondialdehyde were obtained in rats exposed to noise load, compared to intact animals. At the same time, statistically significant advantages of the noise exposure model over the magnetic field model ($p = 0.000005$, 14th and 21st day) and over hyperthermia model ($p = 0.002039$, 14th day; $p = 0.001837$, 21st day) with regard to the level of conjugated dienes were recorded. With regard to MDA, noise exposure exceeded hyperthermia by the end of the experiment ($p=0.000561$).

Table 1

The effect of noise, magnetic field, and high temperatures on the concentration of conjugated dienes in the blood plasma of experimental and intact rats (nmol/ml, Me [Q₁;Q₃])

Groups of animal	Days of experiment		
	7 th	14 th	21 st
Intact, n = 30	36.0 [35.5; 36.2]	35.6 [35.0; 35.9]	36.0 [35.8; 36.2]
High temperatures, n = 30	44,2 * [44.0; 44.6]	45,1 * [44.0; 45.5]	47,1 * [46.9; 47.5]
Magnetic field, n = 30	40.6 * [40.2; 41.0]	41.4 * [40.9; 42.0]	42.1 * [41.8; 42.7]
Noise, n = 30	49.9 * [49.5; 50.3]	53.2 * [53.0; 53.5]	57.0 */** [56.6; 57.8]

Note. Here and in Tables 2, 4, 5:

* $p < 0.05$ compared to intact animals on the same day of the experiment (according to the Mann-Whitney test);

** $p < 0.05$ compared to animals on the 7th day of the experiment (according to the Wilcoxon test)

Table 2

The effect of noise, magnetic field, and high temperatures on the concentration of malondialdehyde in the blood plasma of experimental and intact rats (nmol/ml, Me [Q₁;Q₃])

Groups of animals	Days of experiment		
	7 th	14 th	21 st
Intact, n = 30	3.8 [3.7; 4.0]	4.3 [4.2; 4.5]	4.1 [3.9; 4.4]
High temperatures, n = 30	5.2 * [5.0; 5.5]	6.0 */** [5.9; 6.1]	5.3 * [5.0; 5.5]
Magnetic field, n = 30	5.5 * [5.3; 5.8]	6.4 */** [6.2; 6.6]	5.9 * [5.7; 6.0]
Noise, n = 30	5.8 * [5.7; 6.0]	6.3 */** [6.0; 6.5]	6.6 */** [6.4; 7.0]

Table 3

Results of the Kruskal-Wallis rank analysis of variance and two-sided p-values for multiple comparisons of the concentration of lipid peroxidation products in blood plasma of rats exposed to hyperthermia, magnetic field, and noise

<u>Days of experiment</u>	<u>Groups</u>	<u>Rank (mean)</u>	<u>Intact</u>	<u>Hyperthermia</u>	<u>Magnetic field</u>	<u>Noise</u>
			p (two-sided)			
Conjugated dienes						
7 th	<u>Intact</u>	5.5000		0.156675	1.000000	0.000645
	<u>Hyperthermia</u>	25.500	0.156675		1.000000	1.000000
	<u>Magnetic field</u>	15.500	1.000000	1.000000		0.074218
	<u>Noise</u>	37.450	0.000645	1.000000	0.074218	
14 th	<u>Intact</u>	5.5000		0.156675	1.000000	0.000000
	<u>Hyperthermia</u>	25.500	0.156675		1.000000	0.002039
	<u>Magnetic field</u>	15.500	1.000000	1.000000		0.000005
	<u>Noise</u>	55.300	0.000000	0.002039	0.000005	
21 st	<u>Intact</u>	5.5000		0.156675	1.000000	0.000000
	<u>Hyperthermia</u>	25.500	0.156675		1.000000	0.001837
	<u>Magnetic field</u>	15.500	1.000000	1.000000		0.000005
	<u>Noise</u>	55.500	0.000000	0.001837	0.000005	
Malondialdehyde						
7 th	<u>Intact</u>	5.5000		1.000000	0.054836	0.000531
	<u>Hyperthermia</u>	18.350	1.000000		1.000000	0.191444
	<u>Magnetic field</u>	28.200	0.054836	1.000000		1.000000
	<u>Noise</u>	37.800	0.000531	0.191444	1.000000	
14 th	<u>Intact</u>	5.5000		0.598197	0.004150	0.016423
	<u>Hyperthermia</u>	21.550	0.598197		1.000000	1.000000
	<u>Magnetic field</u>	33.900	0.004150	1.000000		1.000000
	<u>Noise</u>	31.000	0.016423	1.000000	1.000000	
21 st	<u>Intact</u>	5.5000		1.000000	0.009442	0.000000
	<u>Hyperthermia</u>	18.100	1.000000		1.000000	0.000561
	<u>Magnetic field</u>	32.200	0.009442	1.000000		0.307174
	<u>Noise</u>	50.300	0.000000	0.000561	0.307174	

The antioxidant system decreased its activity in response to the influence of prooxidant factors, specifically, the level of ceruloplasmin in the blood plasma of experimental rats (Table 4) significantly decreased by 31% (hyperthermia), 16% (magnetic field), 39% (noise) by the

end of the first week of experimental exposure ($p < 0.05$); by 33%, 15% and 42% respectively — by the end of the second week ($p < 0.05$); by 27%, 16% and 50% by the end of the third week ($p < 0.05$).

Against this background, the concentration of vitamin E (Table 5) became statistically significantly lower upon exposure to magnetic field only towards the end of the experiment; to noise — on the 14th day (17%, $p < 0.05$) and 21st day (31%, $p < 0.05$); to hyperthermia — on the 7th day (28%, $p < 0.05$), 14th day (30%, $p < 0.05$) and 21st day (29%, $p < 0.05$).

Thus, the respond of ceruloplasmin was more pronounced, when oxidative stress was modeled by acoustic load (noise > hyperthermia > magnetic field), while the

respond of vitamin E was more pronounced in temperature exposure model (hyperthermia > noise > magnetic field). This was reflected in the overall results of the rank analysis of variance (Table 6), which confirmed the advantages of the noise model over hyperthermia model ($p=0.0167980$, 7th day; $p=0.004813$, 21st day) and over magnetic field model ($p=0.000005$ at all control time points) with regard to ceruloplasmin; whereas, with regard to vitamin E — advantages over the magnetic field model ($p=0.000006$, 21st day).

Table 4

The effect of noise, magnetic field, and high temperatures on the concentration of ceruloplasmin in the blood plasma of experimental and intact rats ($\mu\text{g/ml}$, Me [Q1;Q3])

Groups of animals	Days of experiment		
	7 th	14 th	21 st
Intact, n = 30	25.9 [25.8; 26.2]	26.0 [25.8; 26.5]	26.0 [25.6; 26.4]
High temperatures, n = 30	18.0 * [17.8; 18.2]	17.3 * [16.9; 18.0]	19.0 * [18.8; 19.1]
Magnetic field, n = 30	21.8 * [21.5; 22.1]	22.1 * [21.5; 22.4]	21.9 * [21.4; 22.0]
Noise n = 30	15.7 * [15.5; 16.0]	15.0 * [14.6; 15.3]	13.0 * [12.9; 13.4]

Table 5

The effect of noise, magnetic field and high temperatures on the concentration of vitamin E in the blood plasma of experimental and intact rats ($\mu\text{g/ml}$, Me [Q1;Q3])

Groups of animals	Days of experiment		
	7 th	14 th	21 st
Intact, n = 30	45.9 [45.5; 46.2]	45.8 [45.3; 46.2]	45.8 [45.4; 46.3]
High temperatures, n = 30	33.2 * [32.8; 33.9]	32.2 * [31.8; 32.6]	32.9 * [32.8; 33.8]
Magnetic field, n = 30	42.1 [42.0; 42.6]	43.4 [43.1; 43.8]	41.1 * [40.8; 41.3]
Noise n = 30	41.2 * [40.8; 41.5]	38.0 * [37.5; 38.2]	31.6 */** [31.2; 32.0]

Table 6

Results of the Kruskal-Wallis rank analysis of variance and two-sided p-values for multiple comparisons of the concentration of antioxidant system components in blood plasma of rats exposed to hyperthermia, magnetic field and noise

Days of experiment	Groups	Rank (mean)	Intact	Hyperthermia	Magnetic field	Noise
			p (two-sided)			
Ceruloplasmin						
7 th	Intact	55.500		0.025058	1.000000	0.000000
	Hyperthermia	30.950	0.025058		0.937068	0.016798
	Magnetic field	45.500	1.000000	0.937068		0.000005
	Noise	5.5000	0.000000	0.016798	0.000005	
14 th	Intact	55.500		0.007813	1.000000	0.000000
	Hyperthermia	28.400	0.007813		0.428483	0.050511
	Magnetic field	45.500	1.000000	0.428483		0.000005
	Noise	5.5000	0.000000	0.050511	0.000005	
21 st	Intact	55.500		0.075708	1.000000	0.000000
	Hyperthermia	33.600	0.075708		1.000000	0.004813
	Magnetic field	45.500	1.000000	1.000000		0.000005
	Noise	5.5000	0.000000	0.004813	0.000005	
Vitamin E						
7 th	Intact	55.500		0.000005	1.000000	0.162544
	Hyperthermia	15.500	0.000005		0.001935	0.150995
	Magnetic field	45.400	1.000000	0.001935		1.000000
	Noise	35.600	0.162544	0.150995	1.000000	
14 th	Intact	55.500		0.000000	1.000000	0.103517
	Hyperthermia	11.800	0.000000		0.000240	0.057122
	Magnetic field	45.500	1.000000	0.000240		1.000000
	Noise	34.400	0.103517	0.057122	1.000000	
21 st	Intact	55.500		0.000379	1.000000	0.000000
	Hyperthermia	22.600	0.000379		0.050511	0.503283
	Magnetic field	45.500	1.000000	0.050511		0.000006
	Noise	6.0000	0.000000	0.503283	0.000006	

Discussion and Conclusion. Taking into account a combination of facts, including changes in the parameters of the prooxidant/antioxidant system upon the exposure to various stress factors, significant advantages of oxidative stress modeling by noise were established, which surpassed the hyperthermia and magnetic load models by the capacity to induce the lipid peroxidation processes. Importantly, at all control time points, the acoustic load model triggered a cascade of reactions in a warm-blooded organism leading to the increase of the peroxidation processes

intensity. This phenomenon can be explained by defining the targets for noise exposure. In the sporadic publications the effect of sound waves in targeting biological membranes, and in particular cell membrane proteins, has been described [17]. Exposure to noise alters the conformational and functional properties of integral and surface proteins, which first of all affects the cellular permeability and cation-anion imbalance, with all the attendant consequences [18]. In this context, the development of membrane enzymopathy should be taken into account, as it couples the

increase of the intensity of free-radical lipid oxidation (lipid peroxidation) processes, which are the key components of the protein-lipid system of biomembranes. Like a stone falling from a top of a mountain, the increased intensity of these reactions triggers a cascade of processes associated with the excessive load on the endogenous antioxidants. This is precisely the reason for endogenous antioxidant system depletion, and the present study has confirmed a decrease in the activity of its main components.

Thus, modeling oxidative stress in the organisms of laboratory animals by exposure to noise has proven its efficiency, based on significant changes of antioxidant parameters by the end of the first, second and third weeks of experimental exposure. The present research implies further study of the acoustic stress effect on the adaptive potential of the warm-blooded organisms aimed at testing the potential pharmacocorrectors for treatment of negative noise effect

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