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DYNAMICS OF CHANGES IN THE INDICES OF LIPID PEROXIDATION AND ANTIOXIDANT SYSTEM IN RATS WITH ALLOXAN DIABETES, MODELED ON THE BACKGROUND OF THE PRELIMINARY TOXICITY OF HEAVY METAL SALTS

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ABSTRACT

***Aim**: to study the processes of lipid peroxidation and the antioxidant defense system in rats with alloxan-induced diabetes against the background of isolated and combined exposure to lead and chromium salts.*

***Materials and methods**: an experimental study was conducted on 90 outbred male rats weighing from 230 to 270 g, divided depending on the intoxication caused into 8 subgroups: intact; subgroup "lead"; subgroup "chrome", subgroup "lead+chrome"; subgroup – alloxan diabetes in intact rats – "control"; subgroup – alloxan diabetes in rats exposed to lead – "lead + alloxan"; subgroup – alloxan diabetes in rats exposed to chromium - "chromium + alloxan"; subgroup - alloxan diabetes in rats that were simultaneously exposed to lead and chromium - "lead + chromium + alloxan". The rats were slaughtered under ether anesthesia after 30 days of metal priming, as well as on days 3 and 14 after the administration of alloxan. After decapitation, the liver was removed from the animals, washed with cold saline and frozen, then a 10% homogenate was prepared, where the content of diene conjugates, malonic dialdehyde, catalase and superoxide dismutase activity. The concentration of lead and chromium in the blood of rats was determined at the end of a 30-day priming using inductively coupled plasma atomic emission spectrometry.*

***Results:** Experimental alloxan diabetes, modeled in terms of the combined action of compounds of lead and chromium, in contrast to alloxan diabetes on isolated background of their action was characterized by a decrease in SOD enzyme activity compared to the level before the introduction of diabetogen. The combined effect of these metals reduces the resistance β-cells of the pancreas to the diabetogenic action of alloxan to a greater extent than their isolated effects. Isolated exposure to lead acetate resulted in increased diene levels conjugates by 13%, the isolated effect of potassium bichromate - by 18%, while the combined effect of lead and chromium - by 50% compared to intact rats. Malon content dialdehyde in the "lead" group and in the "chromium" group increased by 13%, in the "lead + chromium" group – by 68% in relation to intact animals. Increased lipid peroxidation processes in liver cells led to the activation of superoxide dismutase, the activity of which increased by 8% in the lead group, by 21% in the chromium group, and by 45% in the lead + chromium group compared to intact rats. While isolated exposure to lead and chromium had virtually no effect on the enzymatic activity of catalase, the combined influence of metals led to an increase in catalase activity by 13% compared to intact animals.*

***Conclusions**: isolated exposure to both lead and chromium activates the processes of lipid peroxidation in the liver of animals to approximately the same extent, does not affect the enzymatic activity of catalase, but the introduction of potassium dichromate activates superoxide dismutase by 12% more than exposure to lead acetate. The combination of these metals activates the processes of lipid peroxidation and the activity of superoxide dismutase to a greater extent than the isolated action of metals, and unlike the isolated action of lead and chromium, the combination of metals increases the activity of catalase.*

***Key words:** experiment, alloxan diabetes, oxidative stress, lipid peroxidation, antioxidant system, intoxication, lead, chromium*

Due to exposure to harmful agents of relatively low intensity, the occurrence or absence of diseases is largely determined by the state of the body's adaptive systems, however, their long-term, combined or combined influence leads to disadaptation and disregulation disorders, leading to the depletion of protective mechanisms, which in turn contributes to the development and/ or worsening of the disease [1,2]. Any adaptation or pathological process occurs against the background of the formation of reactive oxygen species and the intensification of free radical oxidation of biosubstrates [3]. At the same time, despite outstanding research in the field of studying diabetes mellitus and related metabolic disorders, the presence of numerous screening programs for the timely detection of the disease, many unresolved questions remain for the diabetes service and science in general [4]. Thus, not enough attention is paid to the study of the effects of heavy metal compounds on the development of diabetes mellitus, although

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there is evidence in the literature that diabetes mellitus develops more often in people employed in industries associated with lead, chromium, nickel and other heavy metals [5]. In this case, it is important to establish the nature of the influence of heavy metals not only on the development, but also on the aggravation of diabetes.

THE PURPOSE OF OUR STUDY

was to study the processes of lipid peroxidation (LPO) and the antioxidant defense system (AOD) in rats with alloxan diabetes against the background of isolated and combined exposure to lead and chromium salts.

MATERIALS AND METHODS

The experimental study was carried out on 90 outbred male rats weighing from 230 to 270 g. The maintenance, care of the animals and their removal from the experiment were carried out in accordance with the order of the Ministry of Health of the Republic of Kazakhstan dated July 25, 2007 No. 442 "On approval of the rules for conducting preclinical research, medical and biological experiments and clinical trials in the Republic of Kazakhstan". Depending on the intoxication caused, the experimental animals were divided into 8 subgroups: 1 subgroup - intact animals, which were injected with an equal volume of 0.9% NaCl solution for 30 days; subgroup 2 – rats that received a 1% solution of lead acetate at a dose of 15 mg/kg body weight daily for 30 days ("lead" subgroup); subgroup 3 – rats that received a 0.5% solution of potassium dichromate at a dose of 5 mg/kg body weight, also daily orally for the same period – "chromium"; subgroup 4 – rats that received combined metal priming by daily oral administration of potassium bichromate + lead acetate for 30 days – "lead + chromium"; subgroup 5 – alloxan diabetes in intact rats – "control"; subgroup 6 – alloxan diabetes in rats exposed to lead – "lead + alloxan"; subgroup 7 – alloxan diabetes in rats exposed to chromium - "chromium + alloxan"; Subgroup 8 – alloxan diabetes in rats that were simultaneously exposed to lead and chromium – "lead + chromium + alloxan".

After 30 days, diabetes mellitus was modeled in animals of the first four subgroups with a 5% solution of alloxan (Reakhim) at a dose of 15 mg per 100 g of body weight by a single subcutaneous injection after a 48-hour fast [6,7]. The rats were slaughtered under ether anesthesia after 30 days of metal priming, as well as on days 3 and 14 after the administration of alloxan. After decapitation, the liver was removed from the animals, washed with cold saline and frozen, then a 10% homogenate was prepared, where the content of diene conjugates (DC), malonic dialdehyde (MDA) [8], catalase and superoxide dismutase (SOD) activity [9,15]. The concentration of lead and chromium in the blood of rats was determined at the end of a 30-day priming using inductively coupled plasma atomic emission spectrometry.

Static processing of the material was carried out using the statistical software package Statistica 6 ("StatSoft" USA). The reliability of intergroup differences was assessed using Student's t-test.

RESULTS AND DISCUSSION

Studies have shown that in animals on the 30th day of isolated and combined exposure to metals in the liver, there was an increase in the content of lipid peroxidation products - DC and MDA, which indicated the activation of lipid peroxidation processes. The obtained data are presented in Table 1.

Table 1
Indicators of LPO and AOD in the liver after 30 days of priming with metals (M±m)

Indicators	Subgroups of Animals			
	Intact	Lead	Chromium	Lead+Chrome
DC (μmol /ml)	0.8±0.05	0.9±0.03*	0.94±0.02*	1.2±0.02*
MDA (μmol /ml)	0.8±0.04	0.9±0.03*	0.9±0.01*	1.34±0.02*
SOD (U/ml/min)	8.36±0.1	9.0±0.2*	10.1±0.2*	12.1±0.6*
Catalase (cat/l)	678±1.2	682±1.9	680±6.4	766.9±9.1*

Note: * - differences are significant compared to intact rats ($p \leq 0.05$)

Thus, from Table 1 it is clear that isolated exposure to lead acetate (the "lead" subgroup) led to an increase in the level of DC by 13%, isolated exposure to potassium bichromate (the "chromium" subgroup) - by 18%, while the combined influence of lead and chromium (subgroup "lead + chromium") led to an increase in DC content by 50% compared to intact rats. The MDA content in the "lead" subgroup and in the "chrome" subgroup increased by 13%, in the "lead + chromium" subgroup – by 68% in relation to intact animals. Activation of LPO processes in liver cells, in turn, led to activation of SOD, the activity of which increased by 8% in the "lead" subgroup, by 21% in the "chrome" subgroup, and by 45% in the "lead + chromium" subgroup. compared with intact rats. At the same time, isolated exposure to lead and chromium had virtually no effect on the enzymatic activity of catalase; the combined influence of metals led to an increase in catalase activity by 13% compared to intact animals (Fig. 1).

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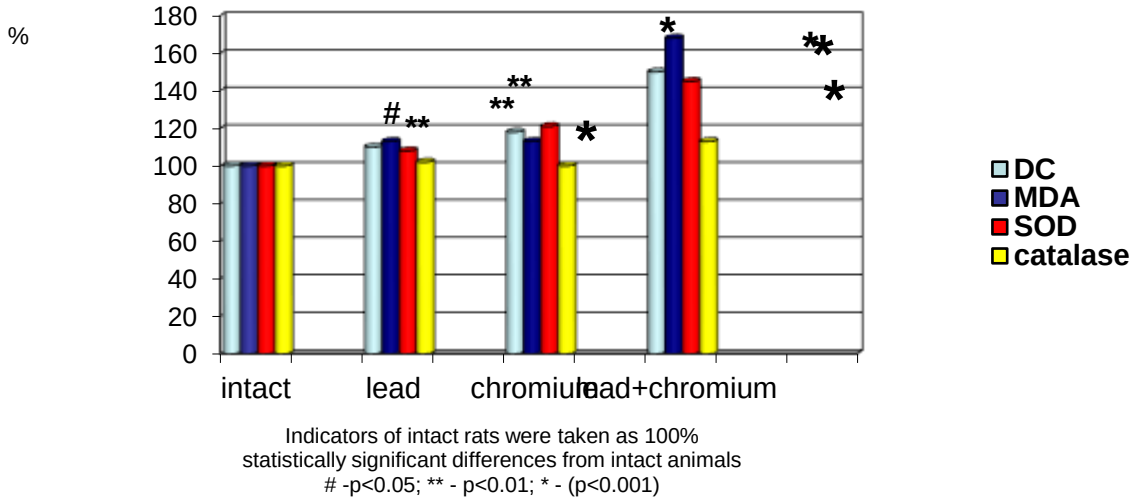

Figure 1. Indicators of the LPO-AOP system in the liver of rats exposed to isolated and combined exposure to lead and chromium for 30 days

Thus, studies have shown that isolated exposure to both lead and chromium activates lipid peroxidation processes in the liver of animals to approximately the same extent and does not affect the enzymatic activity of catalase, but the introduction of potassium dichromate activated SOD by 12% more than exposure to acetate lead Meanwhile, the combination of these metals activated lipid peroxidation processes and SOD activity to a greater extent than the isolated effect of metals, and in contrast to the isolated effect of lead and chromium, the combination of metals increases the activity of catalase.

A comparative analysis of the "lead + chromium" subgroup with animals under conditions of isolated exposure to metals revealed that the shift in the equilibrium in the LPO-AOP system in the case of simultaneous exposure to metals was more significant $K_{LPO} / _{AOD} = 2$ than in the "lead" subgroups $K_{POL} / _{AOZ} = 1.1$ and "chrome" K_{POL} / AOZ = 1.3.Summarizing the above, it can be noted that simultaneous exposure to metals leads to more pronounced activation of free radical oxidation (FRO) processes, as a result of which an excess of free radicals induces LPO, which in turn can lead to greater damage to pancreatic β- cells.

Alloxan diabetes in laboratory animals is a model of insulin-dependent diabetes, in the pathogenesis of which the progressive death of pancreatic β-cells plays a key role [10,11]. In our experiment, the development of diabetes with the administration of alloxan was accompanied by further activation of lipid peroxidation processes and a decrease in AOP, probably due to selective damage to pancreatic β-cells by alloxan, which, as a result of conversion to dialuric acid, generates free radicals and promotes the production of reactive oxygen species that cause rupture DNA and death of insulinocytes [12,13,16].

Table 2
Dynamics of LPO and AOP parameters in the liver after alloxan administration (M± m)

Indicators	Subgroups Of Animals							
	Intact+ Alloxan		Lead+Alloxan		Chromium+Alloxan		Lead+ Chrome+ Alloxan	
	3 days	14 days	3 days	14 days	3 days	14 days	3 days	14 days
DC (μmol/l)	1.11+0.04 **	1.04±0.02	1.1+0.05 **	1.01±0.01	1.12+0.04 **	1.06±0.03	1.22±0.02	1.16±0.03
MDA (μmol/l)	1.05±0.02 **	1.2±0.01▼	1.01±0.02 **	1.2±0.01▼	1.1±0.02 **	1.3±0.02▼	1.4±0.01 **	1.5±0.02 ▼
SOD (U/ml/min)	12.5±0.2 **	10.14±0.2 ▼	13.4±0.4 **	10.42±0.5 ▼	13.38±0.4 **	10.2±0.4▼	12.02±0.6	10.7±0.3 ▼
Catalase (cat/l)	756.2±3.5 **	692.7±5.7 ▼	720.2±5.4 **	698±3.7▼	722.2±5 **	697±4.6▼	778±5	771.2±11.1

Note: ** - differences are significant compared to the corresponding initial indicators;
▼ – compared with the corresponding indicators on the 3rd day (P ≤0.05)

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Table 2 shows that on the 3rd day after the administration of alloxan in the subgroup of rats "intact + alloxan" - control, there was an increase in the content of DC by 39% and MDA by 31%, SOD activity increased by 50%, catalase by 12%. compared to the corresponding source data. In the "lead + alloxan" subgroup, on the 3rd day after the administration of alloxan, the content of DC increased by 22%, MDA by 12%, SOD activity by 50%, catalase by 6% compared with the corresponding initial indicators. In the "chromium + alloxan" subgroup during these periods, the content of DC increased by 19%, MDA – by 22%, the enzymatic activity of SOD increased by 32%, catalase – by 6% compared to the initial data.

The introduction of alloxan in the "lead + chromium + alloxan" subgroup on the 3rd day had virtually no effect on the level of DC, as well as on the activity of SOD, the activity of catalase tended to decrease, while the level of MDA increased by 4% relative to the initial data.

When assessing the state of balance in the LPO-AOP system on the 3rd day after the administration of alloxan in the "lead + chromium + alloxan" subgroup, the balance coefficient was equal to 2.1, while in the control subgroup it was 1.1; in the "lead + alloxan" subgroup - 1 and in the "chromium + alloxan" subgroup - 1.2.

The correlation analysis carried out revealed a close positive relationship between the indicators of LPO and AOD processes $r = +1$, indicating the presence of a balance in the LPO-AOD system. In addition, the decrease in the value of the balance coefficient of the LPO-AOP system compared with that on the 30th day of priming indicates that we administered alloxan to rats of this group against the background of preliminary adaptation to the toxic effects of lead, since the enzymatic link of the cell AOD, represented SOD and catalase, to the introduction of the second pathogenic factor, were able to adequately respond and to some extent suppress the development of SRO. In the "chromium + alloxan" subgroup, a close negative correlation was observed $r = -0.88$ ($p < 0.01$). All of the above indicates the development of a more pronounced imbalance in the LPO-AOP system and, possibly, a breakdown in adaptive capabilities in animals of this subgroup, in contrast to the control and "lead + alloxan" subgroup.

Correlation analysis in the subgroup "lead + chromium + alloxan" revealed an inverse functional relationship between indicators of lipid peroxidation processes and antioxidant protection factors, the rank correlation coefficient was equal to $r = -1$. An increase in the balance coefficient and the presence of a negative correlation indicated the inability of the enzymatic component of antioxidants to influence FRO processes in this subgroup of animals.

Thus, on the 3rd day of experimental alloxan diabetes (EAD), against the background of the combined effect of lead and chromium, compared with EAD, in intact and under conditions of isolated influence of metals, a pronounced imbalance was found in the LPO-AOP system towards the accumulation of pro-oxidants.

On the 14th day of EAD in the control subgroup, compared to day 3, the level of DC did not change noticeably, the MDA content increased by 14%, the enzymatic activity of SOD decreased by 19%, and catalase by 8%. At the same time, in the "lead + alloxan" subgroup, the content of DC tended to decrease, MDA increased by 19%, the activity of SOD and catalase decreased by 22% and 3%, respectively, compared to the data on the 3rd day. In the "chromium + alloxan" subgroup, the content of DC remained at the same level, on the contrary, the level of MDA increased by 18%, the activity of SOD and catalase significantly decreased: SOD - by 24%, catalase - by 4% compared with the corresponding indicators on the 3rd day. The DC level in the "lead + chromium + alloxan" subgroup was the highest compared to all experimental subgroups. Thus, in the control subgroup the DC content was 90%, in the "lead + alloxan" subgroup - 87%, in the "chromium + alloxan" subgroup - 91% compared to the same indicator in the "lead + chromium + alloxan" subgroup. A similar pattern was observed on the part of MDA: the content of this indicator in the control and "lead + alloxan" subgroups was 80%, in the "chromium + alloxan" subgroup - 87% compared to the MDA level in the "lead + chromium + alloxan" subgroup.

Despite the fact that in the subgroup "lead + chromium + alloxan" the maximum intensification of lipid peroxidation processes was observed, the enzymatic activity of SOD and catalase, on the contrary, decreased. Thus, SOD activity was 89% ($p \leq 0.05$), catalase tended to decrease compared to the 3rd day. When comparing these indicators with other experimental subgroups, it was found that the activity of SOD in all the studied subgroups was not statistically significantly different, while the activity of catalase in the control subgroups (intact + alloxan) was 89.8%, "lead + alloxan" – 90.2%, "chrome + alloxan" - 90.3% compared to the same indicator in the "lead + chromium + alloxan" subgroup.

Thus, on the 14th day of EAD, against the background of the combined action of lead and chromium, maximum activation of lipid peroxidation processes and an increase in catalase activity were observed compared with EAD in intact animals, as well as against the background of isolated exposure to metals.

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Analysis of the state of balance of the LPO-AOP system in the "lead + chromium + alloxan" subgroup on the 14th day of EAD showed a further shift in the balance compared to the 3rd day: coefficient $K_{LPO}/_{AOD} = 3.2$, i.e. 52% more than on the third day, and 220% higher compared to intact animals. In the comparison subgroups, an imbalance of these processes was also observed and the coefficient of balance of the LPO-AOP system was 3.5 in the control subgroup, 2.7 in the "lead + alloxan" subgroup and 3.8 in the "chromium + alloxan" subgroup (Fig. 2).

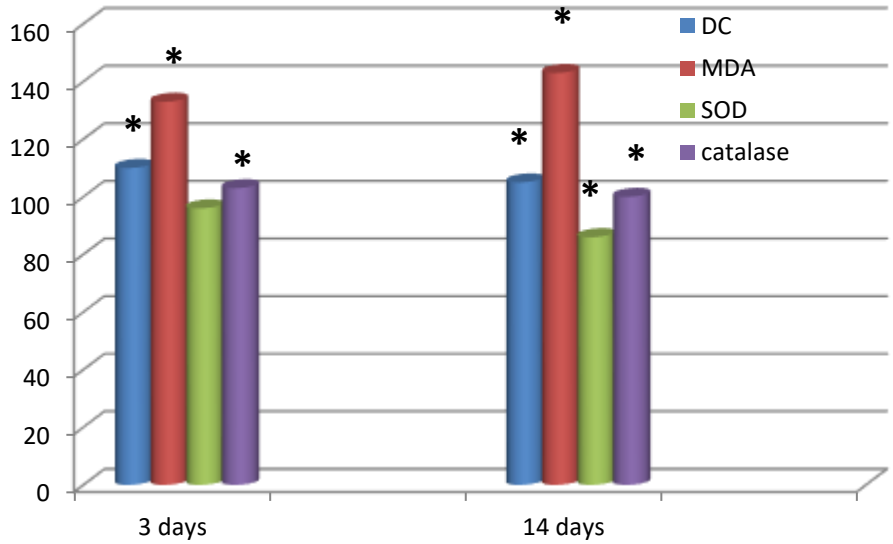

Figure 2. Indicators of the LPO-AOP system in rats with EAD against the background of lead-chromium intoxication

CONCLUSION

The administration of alloxan against the background of the combined action of lead and chromium, in contrast to other subgroups of animals, led to a decrease in SOD activity below the initial level on the 14th day of diabetes. Meanwhile, in the control and "lead + alloxan" subgroups, although there was a decrease in SOD activity at the specified time, the indicators remained higher compared to the initial data. At the same time, in the "chromium + alloxan" subgroup, the SOD content on the 14th day of EAD did not differ significantly from the corresponding initial value.

Oxidative stress in diabetes mellitus is a vicious circle due to an increase in the sources of free radicals, potentiation of toxic effects and changes in the activity of AOD components, which leads to tissue damage [14,17].

Summarizing the above, we can conclude that experimental alloxan diabetes modeled under conditions of the combined action of lead and chromium compounds, in contrast to alloxan diabetes against the background of their isolated action, was characterized by a decrease in the enzymatic activity of SOD compared to the level before the introduction of the diabetogen. Considering the role of SOD as a key factor in antioxidant protection against the damaging effects of alloxan, it can be assumed that the combined effect of lead and chromium reduces resistance β- cells of the pancreas to the diabetogenic effect of alloxan to a greater extent than their isolated effect.

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