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RESEARCH OF FREE RADICAL AND TISSUE ENERGY SUPPLY PROCESSES ON RATS SUBJECTED TO CONCURRENT TETRACHLORMETHANE AND ADRENALINE IMPACT AFTER MEXIDOL ADMINISTRATION

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Summary

Experiments on rats subjected to simultaneous impact of tetrachlormethane and increased adrenaline doses, revealed activated protein oxidative modification and lipoperoxidation. This caused suppression of tissue energy supply that is confirmed by decreased cytochrome oxidase and succinate dehydrogenase. The detected disorders were found to induce cardiocyte cytolysis, indicated by increased blood serum kreatine phosphokinase and lactate dehydrogenase activity alongside with its decrease in rats' myocardium after the impact. Mexidol, used as an antioxidant, revealed positive influence on free radical oxidation processes as well as on the normalization of myocardial bioenergetics.

Key words: tetrachlormethane, adrenaline, protein oxidative modification, lipoperoxidation, energy supply, Mexidol.

Introduction. According to the World Health Organization and European Cardiology Society, acute cardiac pathology is ranking first in the world-wide cause of death rating [15]. The yearly death rate from cardiovascular diseases is over 7

million people that is 12.8 per cent of the total mortality. Every sixth man and every seventh woman in Europe die of myocardial infarction [13]. In view of such a deplorable statistics, continuous efforts for the improvement of therapeutic efficacy as well as numerous scientific research in the domain seem to be fully justified. Mortality from acute coronary insufficiency is related to age, concomitant pathology as well as to timeliness and quality of medical aid [14].

This experimental investigation deals with the peculiarities of acute myocardium lesion at the background of toxic hepatitis. Besides, the efficacy of Mexidol, as a drug of choice for the treatment of this comorbid pathology, was estimated. We have undertaken the study of energy exchange dynamics and the state of cellular membranes in the animals, subjected to the remedial administration of this antioxidant and membrane-protective drug that inhibits lipoperoxidation, increases superoxide dismutase activity, lipid-protein ratio alongside with decreasing membrane viscosity.

Mexidol was shown to modulate the activity of membrane-bound enxymes (free phosphodiesterase, adenylate cyclase and acetylcholinesterase) and receptor complex (benzodiazepine, GABA and acetylcholine) that contributes to the preservation of biomembranes structure and functionality along with enhancing aerobic glycolysis compensatory activation and reducing the degree of oxidation suppression within the Krebs cycle under hypoxia, with increased ATP and phosphocreatine content, as well as activating mitochondria energy synthesis and cell membrane stabilization [3].

The objective of the research is to study the dynamics of bioenergy values and organ-specific enzymes activity as well as their correlation ratio with free radical oxidation values under combined acute myocardium lesion by increased adrenaline doses and toxic tetrachlormethane liver injury, both untreated and treated.

Materials and methods. Experiments were carried out on 36 non-linear albino male rats (b.w.170-200g), which had been kept on the standard vivarium ration.

Toxic liver damage was induced by intraperitoneal introduction of 50% tetrachlormethane oil solution, 1.0 ml/kg [10]. Acute adrenaline myocardium lesion

was induced by single intramuscular injection of 0.18% adrenaline hydrotartrate solution (“Darnytsya”, Ukraine), 0.5 mg/kg [11].

Mexidol, a drug with antioxidant and antihypoxant effect (Medical centre “Ellara”, Pokrov, Russia), was used as a remedy. The dosage was calculated basing on the application instruction and using Yu.R. Rybolovlev’s species sensibility ratios and his method of converting human dosage into the rat one [8]. The drug was introduced intraperitoneally, 50 mg/kg, three times, concurrently adrenaline introduction.

The rats were euthanized with thiopental sodium in 3, 24 and 48 hours after starting adrenaline introduction at the background of 7 days’ acute toxic hepatitis. The animals were grouped as 1) intact; 2) afflicted; and 3) subjected to Mexidol.

Myocardium, liver, blood and blood serum of afflicted rats were studied concerning the content of TBA-active products (TBA-AP) [9]. The intensity of oxidative protein modification was determined according to the method of R. Levine (Dubinina’s modification) by recording the optical density of aliphatic aldehyde- and ketodinitrophenylhydrazines of basic (OPM_{370}) and neutral type (OPM_{430}) [1]. The activity of succinate dehydrogenase (SDH) mitochondrial enzymes [7] and cytochrome oxidase (CO) [7], as the values of energy supply oxidation, was investigated. Kreatine phosphokinase (KPK) [4] and lactate dehydrogenase (LDH) [5] activity in the samples was determined using kinetic method.

All experiments were carried out with strict adherence to the regulations and provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and General ethical Principles of Experiments on Animals (Kyiv, 2001) [2, 12]. Variation statistics method with the use of Student criterion was involved in the statistical treatment of the results [6].

Results and discussion. Oxidative protein modification (OPM) is known to be an early indicator of the cell damage in free radical oxidation. OPM is suggested to play a key role in the molecular mechanisms of oxidative stress and to act as a trigger for the oxidative destruction of other molecules, e.g. lipids and nucleic acids. Investigation of the OPM revealed constantly growing content of neutral aldehyde-

and keto derivatives (OPM_{370}) in the blood serum of Group 2 (afflicted rats). On the 48th experimental hour it was 190% higher as compared to the intact group. Meanwhile, investigation of the OPM in the in the blood serum of Group 3 (subjected to Mexidol) revealed 12.6%, 9.8% and 12.5% decrease within 3, 24 and 48 hours, respectively, as compared to Group 2. Analogous tendency occured in the tissues under study, in particular, the content of neutral aldehyde-and keto derivatives (OPM_{370}) was found to increase in afflicted rats throughout the investigation term, being at most 227% higher as compared to the intact rats within 24 and 48 hours.

In Group 3, the value was found to decrease by 6%, 9% and 13% within 3, 24 and 48 hours, respectively, compared to untreated animals. In the liver of afflicted rats, the content of neutral aldehyde-and keto derivatives (OPM_{370}) was found to be 108%, 103% and 92% higher within corresponding terms as compared to the intact group. However, after treatment the value was shown to decrease by 145, 9% and 8% within 3, 24 and 48 hours, respectively.

The content of basic aldehyde-and keto derivatives (OPM_{430}) was found to undergo similar, even more marked changes. In the blood serum, the value increased by 600%, 630% and 500% within corresponding terms as compared to the intact group, whereas in Group 3 it was found to decrease by 30%, 22% and 13% within 3, 24 and 48 hours, respectively, as compared with Group 2. In the liver of afflicted rats, the content of basic aldehyde- and keto derivatives (OPM_{430}) was growing gradually throughout the investigation term, being 302% higher compared to the intact group. In Group 3, the value was found to decrease by 9%, 13% and 27% within corresponding terms as compared to Group 2. In the myocardium of afflicted rats, the value was shown to increase by 269%, 237% and 126% within 3, 24 and 48 hours, respectively, as compared to intact animals, whereas in Group 3 it was found to decrease by 9%,19% and 22% within corresponding terms compared to untreated rats.

Concurrent tetrachlormethane and adrenaline impact activates free radical processes, resulting in the systemic accumulation of lipoperoxidation after-products. This is confirmed at the determination of TBA-active products. The blood serum value for Group 2 was found to increase 2.3, 4.3 and 3.4 times within 3, 24 and 48

hours, respectively, as compared with Group 1. In Group 3, the TBA-AP content was 1.1, 1.6 and 1.3 times lower within 3, 24 and 48 hours, respectively, as compared with the Group 2. The similar dynamics of the value is observed in studied tissues. The liver concentration of TBA-active products was found to increase 1.3, 1.6 and 1.4 times in Group 2 as compared with the Group 1 within corresponding terms. In Group 3, the value was found to decrease 1.2, 1.3 and 1.1 times within experimental term as compared with Group 2. In the myocardium, the value for Group 2 increases by 1.3 within 3 hours and by 1.2 within 24 and 48 hours as compared with Group 1. In the animals, subjected to remedial Mexidol, the value was found to decrease by 1.1 within 3 and 24 hours and by 1.2 within 48 hours in comparison with untreared rats.

Activated free radical oxidation processes lead to changes in the cellular energy supply. Cytochrome oxidase (CO), the end enzyme of respiratory chain providing transportation of electrons from the cytochrome to the oxygen, is essential for the energy metabolism. In the conditions of concurrent damage, the CO activity was suppressed as compared with the intact group. In the blood serum, the enzyme activity decreased by 6%, 12% and 13% within 3, 24 and 48 hours. In Group 3, the value was found to grow and exceeded that in Group 2 by 5%, 10% and 11% respectively, within corresponding experimental terms (Fig. 1).

The similar regularity was traced with the CO activity in the liver, in which it was found to be 25%, 29% and 31% lower in 3, 24 and 48 hours after adrenaline introduction, respectively, as compared to Group 1. In Group 3, the value was found to increase by 8%, 5% and 9% within corresponding terms in 24 and 48 hours. In the myocardium, the enzyme activity was also suppressed, decreasing progressively by 33%, 38% and 40% within 3, 24 and 48 hours, respectively, as compared to the control group. In Group 3, the value was found to increase by 12 %, 5% and 20 % within 3, 24 and 48 hours, as compared to Group 2.

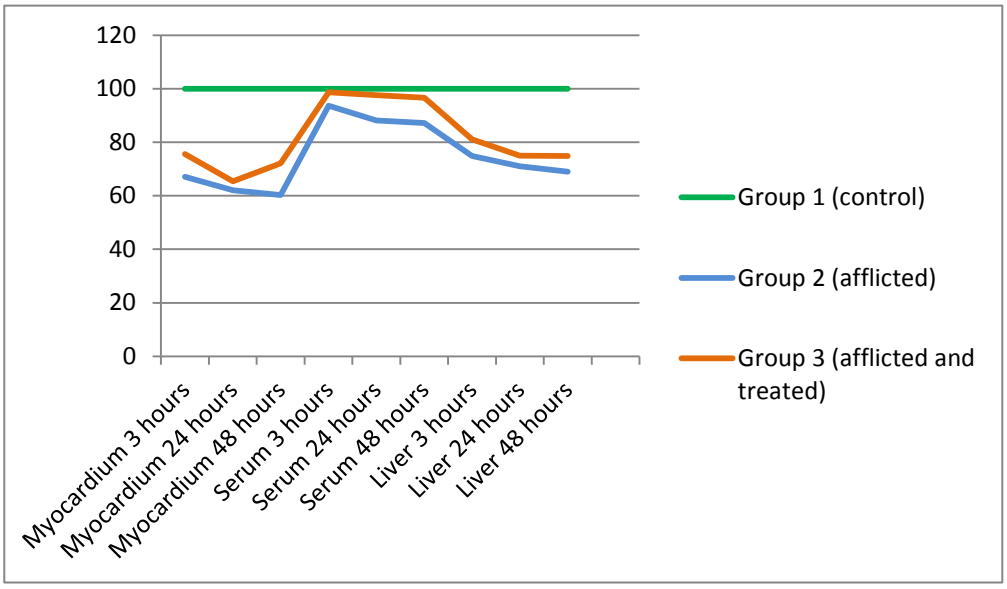

Fig. 1. Dynamics of cytochrome oxidase activity in the blood serum and tissues of rats, both untreated and subjected to Mexidol, concurrently affected by toxic doses of adrenaline and tetrachlormethane (percentage).

For more detailed study of the tissue respiration process, succinate dehydrogenase (SDH), as a mitochondrial enzyme, was investigated. Within 3 hours after adrenaline administration, the enzyme activity in the blood serum of Group 3 was found to decrease by 26%, whereas within 24 and 48 hours – by 30% and 22%, respectively (Fig. 2). In Group 3, the value was found to grow by 10%, 20% and 22% within corresponding terms. In the liver, this regularity was maintained, the value being 12% lower on the 3d investigation hour and kept decreasing by 38% and 33% within 24 and 48 hours, respectively, as compared with the control group. In Group 3, the value grew by 2%, 18% and 12% times within 3, 24 and 48 hours, respectively. In the myocardium, the SDH was also found to decrease, though less markedly, in the experimental Group 2: within 3 hours after adrenaline administration – by 4%, the maximum 17% decrease being noted within 24 hours, with the value further remaining 17% lower against the control within 48 hours. In Group 3, the value was found to grow by 2%, 7% and 5% within corresponding experimental terms.

Concurrently, determination of kreatine phosphokinase and lactate dehydrogenase activity in the blood serum and tissues of experimental groups was conducted in order to compare free radical and cytolytic activity.

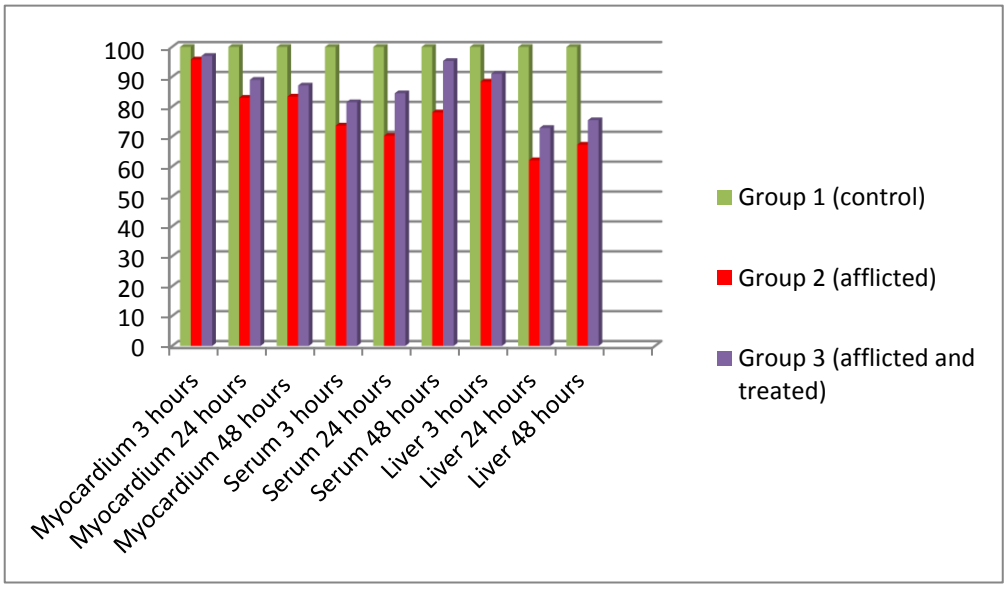

Fig. 2. Dynamics of succinate dehydrogenase activity in the blood serum and tissues of rats concurrently affected by toxic doses of adrenaline and tetrachlormethane, and subsequently untreated and subjected to Mexidol therapy (percentage).

Kreatine phosphokinase (KPK) was found to increase in the blood serum of Group 2 animals 2.8, 3.1 and 3 times within 3, 24 and 48 hours, respectively, as compared with the control Group 1, whereas in Group 3 the value with positive dynamics was found to decrease by 4%, 9% and 31% within corresponding terms as compared to the similar value in the previous group. In the myocardium, the inverse dynamics of the enzyme activity was observed. In comparison with Group 1, KPK myocardium activity in Group 2 decreased 2.1, 2.5 and 2.6 times within 3, 24 and 48 hours following cardiotoxin introduction. In treated animals, the enzyme activity was growing progressively and was higher than that in the previous group by 19%, 20% and 22%, corresponding to experimental terms.

A resembling tendency was observed in the dynamics of lactate dehydrogenase (LDH) activity. In the blood serum of Group 2 animals, the particular value was found to be 2.7, 3.7 and 4.3 times the value in Group 1 within 3, 24 and 48 hours after cardiotoxin introduction (Table 1).

Positive dynamics occurred in group 3, and LDH activity decreased by 12% within 48 hours. As to the liver, in Group 2 this value was 1.7 time lower within 3 and 24 hours and 1.8 time – within 48 hours as compared to the controls. In Group 3,

the enzyme activity was found to grow within all experimental terms, eventually surpassing the group 2 value by 21%.

Table 1. Dynamics of lactate dehydrogenase activity in the blood serum (M units/l) and tissues (M units/100g) of rats concurrently affected by toxic doses of adrenaline and tetrachlormethane, and subsequently untreated or subjected to Mexidol therapy.

Study Object	Term	Group 1	Group 2	Group 3
Blood Serum	3 hours	156,7	420,7	403,0
	24 hours		581,8	525,4
	48 hours		672,5	593,9
Liver	3 hours	124,1	74,6	78,8
	24 hours		73,8	78,2
	48 hours		68,8	83,1
Myocardium	3 hours	123,5	71,7	73,0
	24 hours		65,1	75,5
	48 hours		69,0	87,6

Group1- intact rats; Group 2 - concurrently affected by toxic doses of adrenaline and tetrachlormethane; Group 3 - concurrently affected by toxic doses of adrenaline and tetrachlormethane, subsequently subjected to mexidol therapy

In the myocardium, the LDH activity decreased and was found to be 1.7, 1.9 and 1.8 times lower the value of Group 1 within corresponding experimental terms, and, likewise in the liver, was found to increase in Group 3 rats, surpassing Group 2 value by 27 % within 48 hours.

Such a dynamics of enzymes activity confirms aggravation of membrane-destructive processes, followed by the adrenaline-furthered passage of the intracellular enzyme proteins into the blood, thus determining the positive effect of the chosen remedial method.

Conclusions. Concurrent tetrachlormethane and adrenaline damage to rats has been found to activate systemic oxidative processes, indicated by increased content of both TBA-active and oxidative protein modification products in all organs. Because of this, tissue energy supply is disturbed and depression of cytochrome oxidase and

succinate dehydrogenase, the tissue respiration enzymes, is observed. Cardiocytes cytolysis has been proven to occur in this pathology (increased blood serum kreatine phosphokinase and lactate dehydrogenase activity alongside with its decrease in the myocardium). Used as a remedy for the disturbances detected, Mexidol, an antioxidant, has been shown to effect the studied values positively, thus enabling to recommend the drug administration in the complex therapy of toxic lesions to the liver and the myocardium.

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