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Changes of lipid oxidation products content in ventricles of the rat heart as a result of electrolyte-steroid cardiomyopathy and correction of this condition

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Abstract

Cardiovascular disease is one of the leading causes of death in the world. One of such pathologies is metabolic cardiomyopathy. This pathology includes dysfunction of amino acids, lipids, mitochondria, and disease of products accumulation. Conditions that can cause such changes include congenital and acquired hormone levels. A good example is the long-term use of dexamethasone for the treatment of patients with allergies or rheumatology diseases. Electrolyte disturbances are often a concomitant consequence of disease or lifestyle. They also contribute to heart damage under these conditions. Cell damage often begins with damage to cell lipid membranes and is accompanied by accumulation of lipid peroxidation products. The purpose of our study was to investigate diene, triene conjugates and thiobarbituric acid reactive substances levels in the animal ventriculs heart tissue of both sex in conditions of long-term action of dexamethasone and high concentration (4%) solution of

sodium chloride in drink water. L-carnitine, as an antioxidant and having the ability to influence to energetic metabolism, apoptosis and transcription of DNA may be a promising means for correction disorders. The experiment was performed with the use of 96 mature white nonlinear rats. Dexamethasone with long-term use promotes the accumulation of diene, triene conjugates and thiobarbituric acid reactive substances (malondialdehyde is main) in the ventricular myocardium of rats of both sexes, more in animals with high content (4%) of NaCl in drinking water, and in males, compared with females. L-carnitine has the ability to significantly offset the negative effects of dexamethasone on the content of lipoperoxidation products in the ventricular myocardium of rats of both sexes, including against the background of increased salt content in drinking water.

Key words: lipid peroxidation; dexamethasone; sodium chloride load; L-carnitine.

Introduction

Cardiovascular disease is one of the leading causes of death and hospitalization in many countries. This is due to standard risk factors such as high blood pressure, psycho-emotional overload, hypokinesia or obesity. An important issue of experimental research is the identification of non-standard risk factors of the cardiovascular system in order to strengthen preventive measures [1]. Metabolic cardiomyopathies include dysfunction of amino acids, lipids and mitochondria, as well as disease of accumulation [8]. Metabolic cardiomyopathy develops in various pathological conditions and is associated with systemic metabolic disorders. They can be acquired in adulthood or congenital [2, 3, 4]. Disorders of lipid metabolism include systemic carnitine deficiency and abnormalities of carnitine palmitoyltransferase, with long-chain acyl-CoA dehydrogenase and multiple deficiency of acyl-CoA dehydrogenase. Disorders of mitochondrial metabolism also affect the complex I, II, III, IV and V [3, 9].

Prolonged adverse conditions on the background of the underlying disease can lead to a cascade of adverse effects. Currently, attention is focused on oxidative stress, as one of the main determinants of endothelial dysfunction in cardiovascular disease. The main reason for all the negative effects of glucocorticoids is that dexamethasone induces excessive production of reactive oxygen species, causing dysregulation of physiological processes [1, 6, 7].

In addition, changes in energy metabolism can also increase oxidative stress in various metabolic disorders. The catabolism of substances such as glucose and fatty acids promotes the accumulation of reduced coenzymes (NADPH and FAD), which transfer their electrons to

the respiratory chain [5], allowing the production of ATP by oxidative phosphorylation. The relative contribution of each of these substances as energy sources depends on their presence in the cell.

Since the production of reactive oxygen species in mitochondria occurs during the transport of electrons to molecular oxygen in the respiratory chain, the influx of electrons due to excessive oxidation of fatty acids contributes to their additional formation in the myocardium [6].

During this process, polyunsaturated fatty acids, which have the structure of phospholipid, glycolipid, glyceride or sterol, present in the cell membrane are converted into various harmful products such as peroxides, alcohols, aldehydes and hydroxy fatty acids. With increased peroxidation of membrane lipids also increases the viscosity of the lipid bilayer by reducing the degree of saturation of fatty acids. Excess lipoperoxides inactivate sulfhydryl groups of proteins - enzymes, inhibits oxidative phosphorylation, which leads to impaired membrane permeability [5, 10]. One of the main end products of lipid peroxidation is malonic dialdehyde. Non-oxygen radicals are generally known as reactive oxygen species.

Antioxidants counteract the harmful effects of oxidative stress. L-carnitine is required for the transport of long-chain fatty acids across the mitochondrial membrane for betta-oxidation. Therefore, in this study we tried to study the protective effects of L-carnitine against the effects of oxidative stress. L -carnitine is a tool that protects antioxidant enzymes from oxidative damage, preventing the processes of lipid peroxidation of lipids.

Purpose of the research:

The study of lipid peroxidations process in both sex animals under the influence of dexamethasone and high concentration of sodium chloride in drinking water and the correction of the disorders by L-carnitine.

Materials and methods

The experimental studies were performed with the use of 96 mature white nonlinear rats aged 5-6 months, weighing 0.17-0.23 kg. The glucocorticoids dexamethasone was introduced in a dose of 350 mg / kg for 15 days. Half of the animals taken 4% sodium chloride solution in drinking water. The correction of the pathological changes was performed using L-carnitine ("Ahvantar", Ersel Pharma, per os) in a dose of 200 mg / kg for 19 days. The significance of differences between the results (minimum significance of $p < 0.05$) was assessed using criteria by Newman-Keuls (software BioStat, AnalystSoft Inc., version 6). The content of diene, triene conjugates (DC, TC) and thiobarbituric acid reactive substances (TBARS) was determined in ventricles homogenates by spectrophotometry method.

Results and discussion

In the study of the content of DC in the ventricular myocardium of intact rats, as well as in the use of L-carnitine in animals of both sexes, no significant difference was found. Prolonged administration of dexamethasone to both females and males rats caused a significant increase in the content of the studied metabolite (2.1 and 2.4 times, respectively) ($p < 0.001$; table 1). Comparing the data of animals of both sexes, it was found that the level of DC by 9.9% ($p = 0.02$) was higher in males.When using L-carnitine for protective purposes in animals treated with dexamethasone, the content of DC decreased 2.1 times in females and 2 times in males ($p < 0.001$) If we compare these groups with intact animals, the use of L-carnitine for protective purposes was quite effective, because the concentrations of DC in these groups did not differ significantly (including between animals of different sexes).

Table 1. Diene conjugates value in the experimental animals

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	Diene conjugates level in ventricules of heart, 10^{-3} CU/kg, M ± m							
Intake of usual quantity NaCl	0,263 ± 0,010	0,255 ± 0,008	0,252 ± 0,009	0,248 ± 0,008	0,551 ± 0,033*	0,606 ± 0,020*§	0,269 ± 0,009^	0,299 ± 0,011^
Intake of 4% solution NaCl	0,298 ± 0,011	0,289 ± 0,014	0,281 ± 0,012	0,284 ± 0,012	0,750 ± 0,041*#	0,854 ± 0,028*#§	0,318 ± 0,013^	0,420 ± 0,016*^#§

Note: * $p<0,05$ − significance of differences in relation to control groups of animals;
^ $p<0,05$ – significance of differences in relation to dexamethasone groups of animals;
# $p<0,05$ – significance of differences in relation to animals with normal level of sodium chloride; § $p<0,05$ – significance of differences between the male and female rats.

When comparing intact groups of rats with control animals that received drinking water with high (4%) NaCl content, no significant difference between the content of the studied indicator was found in both females and males. However, when comparing the content of this metabolite of lipoperoxidation in animals of different sexes that received salt, no differences were found. It should be noted that L-carnitine did not change the concentration of diene conjugates in the myocardium of the ventricles of the heart in both females and males with high salt content in the diet, as well as compared with animals receiving water without high salt and L- carnitine (with a naturally significant difference between animals of both sexes also not found).

With long-term use of dexamethasone in animals with high salt content in the diet revealed a significant increase in the content of the studied indicator (2.5 and 3 times, respectively; $p < 0.001$) in both females and males, which was higher (1.4 times; $p < 0.001$) for rats of both sexes, compared with animals that did not receive additional salt (males are 13.8% higher than females; $p < 0.05$).

With the combined use of glucocorticoids with L-carnitine, the level of diene conjugates decreased 2.4 times in females and 2 times in males rats (in males it was 32.2% higher compared to females). It should be noted that compared with control animals, this figure did not differ in females and was 45.5% ($p < 0.05$) higher in males, and compared with animals receiving dexamethasone and L-carnitine with normal content salts in drinking water, was not significant in females and 40.4% higher in males ($p < 0.05$). Thus, L-carnitine on the background of elevated levels (4%) of NaCl in drinking water prevented the accumulation of this toxic metabolite of lipid peroxidation in the ventricles of the ventricles of females, which also confirms the lack of significant difference in DC concentrations in the heart of rats of this sex. As for the effectiveness of L-carnitine in males exposed to dexamethasone for a long time and high salt content in drinking water, the protective effect could not be achieved- the content of diene conjugates in the ventricular myocardium of the latter was higher than the level of intact animals by 64.7% ($p < 0.05$).

When examining the content of TC in the ventricular myocardium of rats, a significant difference between the sexes, as well as the use of L-carnitine in animals of both sexes was not detected. Prolonged use of dexamethasone caused an increase in the content of TC (2.1 times and 2.4 times, respectively; $p < 0.001$, table 2) in the ventricular myocardium of both females and males rats. Comparing the data of animals of both sexes in the study group, it was found that the level of TC was 10.7% higher in males ($p < 0.05$).

When using L-carnitine for protective purposes in animals treated with dexamethasone, the TC content decreased 1.9 times in females and 2 times in males ($p < 0.001$). When comparing the results of this group with intact animals that received L-carnitine for protective purposes, it was found that the concentration of TC in these groups did not differ significantly, including between animals of different sexes.

When comparing the data of intact groups of rats with control animals that received drinking water with high (4%) NaCl content in both females and males, no significant difference between the content of the studied indicator was found. Differences in the content of this lipoperoxidation metabolite in animals of different sexes receiving salt, when compared with each other, were also not significant. It should be noted that the introduction

of rats only L-carnitine did not change the concentration of TC in the ventricular myocardium in both females and males with high salt in the diet, as well as in comparison with animals receiving water without high salt and L-carnitine (no significant difference between animals of both sexes was also found).

Table 2. Triene conjugates value in the experimental animals

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	Triene conjugates level in ventricules of heart, 10^{-3} CU/kg, M ± m							
Intake of usual quantity NaCl	0,269 ± 0,008	0,262 ± 0,008	0,259 ± 0,008	0,256 ± 0,008	0,562 ± 0,023*	0,622 ± 0,025*§	0,291 ± 0,014^	0,309 ± 0,013^
Intake of 4% solution NaCl	0,299 ± 0,013	0,288 ± 0,015	0,285 ± 0,013	0,760 ± 0,027*#	0,882 ± 0,028*#§	0,333 ± 0,010^#	0,429 ± 0,015*^#§	0,299 ± 0,013

Note: * p<0,05 − significance of differences in relation to control groups of animals;
^ p<0,05 – significance of differences in relation to dexamethasone groups of animals;
# p<0,05 – significance of differences in relation to animals with normal level of sodium chloride; § p<0,05 – significance of differences between the male and female rats.

With long-term use of dexamethasone in animals with high salt content (4%) in the diet showed a significant increase in the content of the studied indicator in both females and males (2.5 and 3 times, respectively; p <0.001), which was higher for both sexes (1.4 times; p <0.01), compared with rats that did not receive additional salt (males were 16% higher than females; p <0.05). With the combined use of glucocorticoids with L-carnitine, the level of triene conjugates decreased 2.3 times in females and 2.1 times in males (p <0.001). In males it was 29.0% higher than in females (p <0.05). It should be noted that in comparison with control animals that received only NaCl, this figure did not differ in females and was 43.7% higher in males (p <0,01), and compared with intact - by 23.8 % (p <0.05) and 64, 0% higher, respectively, in females and males rats (p <0.01). However, the use of L-carnitine on the background of elevated levels of NaCl in drinking water led to an increase in the content of triene conjugates in the myocardium of ventriculs in females of this group by 23.8% (p <0.05), and in males - by 64 0% (p <0.01), compared with intact animals.

In the study of the content of TBARS in the ventricular myocardium of rats, no significant difference between the sexes, as well as the use of L-carnitine in intact animals of

both sexes was not detected. Prolonged administration of dexamethasone caused a significant increase in the content of the studied indicator (2.5 times and 2.8 times, respectively; p <0.001, Table 3) in both females and males rats. Comparing the data of animals of both sexes in the study group, it was found that the level of TBARS was 12.6% higher in males rats(p <0.05).

Table 3. TBARS value in the experimental animals

Rats	Groups of animals:							
	Control		L-carnitine		Dexamethasone		Dexamethasone + L-carnitine	
Sex:	female	male	female	male	female	male	female	male
The form of experiment	TBARS level in ventricules of heart, μmol/kg, M ± m							
Intake of usual quantity NaCl	0,499 ± 0,023	0,505 ± 0,021	0,445 ± 0,051	0,397 ± 0,037	1,255 ± 0,069*	1,413 ± 0,048*§	0,547 ± 0,033^	0,551 ± 0,020^
Intake of 4% NaCl	0,747 ± 0,033#	0,797 ± 0,027#	0,502 ± 0,024*	0,414 ± 0,028*	2,396 ± 0,110*#	2,643 ± 0,121*#§	0,900 ± 0,034*^#	1,012 ± 0,030*^#

Note: * p<0,05 − significance of differences in relation to control groups of animals;
^ p<0,05 – significance of differences in relation to dexamethasone groups of animals;
# p<0,05 – significance of differences in relation to animals with normal level of sodium chloride; § p<0,05 – significance of differences between the male and female rats.

When L-carnitine was used for protective purposes in animals treated with dexamethasone, the content of TBARS decreased by 2.3 times in females and 2.6 times in males rats (p <0.001). When comparing the obtained data of the study group with intact animals, it was found that the use of L-carnitine for protective purposes was effective. When comparing the content of TBARS in intact groups of rats with control animals treated with drinking water with high (4%) NaCl content, this figure was increased 1.5 times in females and 1.6 times in males (p <0.01). In animals of different sexes receiving saline, when compared with each other, the results were not reliable. It should be noted that L-carnitine reduced the concentration of TBARS in the ventricular myocardium of animals with high salt content in the diet of both females (by 32.8%; p <0.05) and males (by 48.1% ; p <0.02). When comparing the obtained data of the study group of rats with animals that consumed water without high salt content and L-carnitine, the changes were not significant. At the same time, no significant difference was found between animals of both sexes (table 3).

With long-term use of dexamethasone in animals with high salt content (4%) in the diet of both females and males rats, a significant increase in the content of TBARS (3.2 times and 3.3 times, respectively, p <0,001), which was 1.9 times higher (p <0,01) for rats of both sexes, compared with animals that did not receive additional salt (males 10.3% higher than females; p <0, 05). As a result of the use of L-carnitine as a protector in combination with a glucocorticoid, the levels of TBARS decreased 2.7 times in females and 2.6 times in males (p <0.001), but the data were higher by 20.5% and 27 , 0% in females and males rats, respectively, compared with the control (p <0,05). It should be noted that in relation to animals that did not receive salt, with this combination of drugs, the levels of TBARS were also significantly higher (64.4% in females and 83, 6% in males; p <0.02), and compared with intact animals - 1.8 times and 2 times (p <0.001), respectively, in females and males (table 3).

Conclusions

Dexamethasone with long-term use promotes the accumulation of diene, triene conjugates and TBARS in the ventricular myocardium of rats of both sexes, more in animals with high content (4%) of NaCl in drinking water, and in males, compared with females. L-carnitine has a pronounced protective effect on the heart of animals of both sexes on the level of diene, triene conjugates and TBARS in the ventricular myocardium of rats treated with dexamethasone for a long time, so their level remains at the same level as in intact animals. Against the background of increased salt content in drinking water, a similar pattern is observed, in particular, L-carnitine significantly reduces the content of lipoperoxidation products in the ventricular myocardium in females and males rats (2.7 times and 2.6 times, respectively (p<0.001). L-carnitine also reduced the content of TBARS by 2.4 times, diene conjugates - by 2.0 times (p <0.001) and 2.3 and triene conjugates - by 2.1 times (p <0.001), which indicates leveling the negative effects of dexamethasone. The same level of TBARS as in intact animals is observed in females relative to the content of diene conjugates.

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