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EFFECTIVENESS OF CORRECTION OF METABOLIC DISORDERS WITHIN MYOCARDIUM TISSUE OF RATS WITH HYPOTHYROIDISM ON THE BACKGROUND OF COMBINED IODINE AND COPPER DEFICIT

Abstract. *In experiments on rats with hypothyroidism on the background of combined iodine and copper deficit (HTG_{I+Cu}) the changes of oxygen dependent processes and activity of NO-synthases in myocardium were studied and effectiveness of correction of found changes by microelements, antioxidants and donators of nitric oxide (NO) was ascertained. It was found that HTG_{I+Cu} lead to excessive peroxidation and enhancement of NO-synthases activity (mainly by inducible – iNOS) in myocardial tissue to analogical indexes in animals with isolated iodine deficit. The introduction of Potassium iodide to experimental animals stabilizes the passing of metabolic processes. The carrying out complex correction by microelements, antioxidants and donators of NO is accompanied by more significant positive therapeutic dynamics.*

Key words: *hypothyroidism, iodine deficit, copper deficit, peroxide processes, NO-synthases, correction of metabolic disoders.*

Introduction. One of the leading nexus of disorders of heart operant behavior is deemed to be changes of metabolic processes in it, first of all – activation of peroxide destruction of proteins and lipids on the background of hypoactivity of antioxidant defense system of organism [8]. Disorder of pro-antioxidant homeostasis is possible upon conditions of hypothyroidism (HTG). It is common that combined deficit of microelements, in particular of iodine and copper, is capable to potentiate the influence of actual factors on an organism. Wherein deceleration of copper content in myocardium may be negatively reflected on energy metabolism of heart and acted as risk factor of progression of cardiological pathology [1]. Nitric oxide (NO) may decelerate the reaction behavior of peroxidation. Such a peculiar anti-oxidative effect of NO may be biologically important way of detoxication of potentially dangerous reactive oxygen intermediates [19]. Taking into consideration the role of NO in neurohumoral regulation of physiological functions of cardiovascular system, figuring out of changes of indices of NO-synthases system upon conditions of microelementosis is of some interest.

Objective: to assess the history of oxygen dependent reactions and activity of NO-synthases in myocardium of rats with HTG on the background of combined iodine and copper deficit and to figure out effectiveness of correction of detected changes caused by microelements, antioxidants and donators of NO.

Materials and methods. Researches were carried out on more than 180 white non-pedigree male rats of about 150-180 g. weight each. Animals were allocated on five experimental groups: rats with HTG on the background of iodine deficit (HTG I, 1st group, 30 animals), rats with HTG on the background of combined iodine and copper deficit (HTGI+Cu, 2nd group, 30 animals), upon conditions of HTGI+Cu correction by: potassium iodide (3nd group, 30 animals), potassium iodide and copper sulfate (4th group, 30 animals), potassium iodide, copper sulfate, α-tocopherol acetate and L-arginine hydrochloride (5th group, 30 animals). Reference group consisted of 30 intact rats. Animals from experimental groups were being on iodine deficiency diet [15] during 45 days. Copper deficit was modeled by addition of chelate of D-penicillamine (cuprenil, „Polfa" Kutno Pharmaceutical Company, Poland, 100mg/100g of body weight) to the drinking

water from 25 to 45 day of research [14]. HTGI+Cu correction was being carried out by potassium iodide (iodide-100, Nycomed Merck KGaA, Denmark, 50 mcg of iodide preorally daily during 30 days) [2], copper sulfate (0,09 mg/100g of body weight preorally daily during 30 days) [5]. α-tocopherol acetate was utilized as anti-oxidant (Kyiv Vitamin Factory, Ukraine, 20mg/kg of body weight preorally daily during 30 days) [12]. Donator of NO L-arginine hydrochloride (tivortin-aspartate, "Yuria-Pharm", Ukraine, 2.5g/day) was provided for the animals preorally daily during last 21 days of research [10]. Keeping, feeding and euthanasia of rats were being complied with regulations of current international requirements regarding humane treatment of animals.

Content of products of protein peroxidation (PP) and lipid peroxidation (LP) was being determined within homogenate of myocardium. The level of oxidative protein modifications (OPM) was being detected resting on the quantity of their products via spectophotometery when longitude of wave (356, 370, 430, 530) nm [6]. The state of LP was being assessed resting on accumulation of diethenoid conjugates (DC) of polyunsaturated fatty acids [3] and active products that respond to thiobarbituric acid – TBA-AP [7]. For characterization of NO synthesis system within myocardium homogenate NO-synthases activity was being determined: neuronal (nNOS), inducible (iNOS), constitutive (cNOS) [11].

Statistical processing of results was being carried out resting on statistical software package Statistica 7,0.

Results and discussion. Research outcomes showed (see table), that progression of HTG_{I+Cu} caused activation of processes of peroxidation of proteins and lipids. Thus within myocardium of 2nd experimental group of animals the increase of OPM was detected (E_{430} - 2,6 times, $p<0,001$), content of DC – by 48,2% ($p<0,02$) to data of intact animals. Amplifying of PP and LP processes may act as risk factor of ischemic heart injury. It ought to be emphasized that the level of E_{430} OPM in animals with combined microelementosis was 2,2 times ($p<0,001$) higher than in animals with HTG_I. Because of OPM products are more stable than metabolites of lipid peroxidation, the level of protein destruction may act as marker of oxidative damages of myocardium when HTG. Upon such conditions NOS activity increased by 25,5% ($p_{1-2}<0,001$) to the analogical indice of animals from the 1st experimental group, generally by means of iNOS, which activity increased by 51,8% ($p_{1-2}<0,05$) to the analogical indices in animals with HTG_I. It is noteworthy that increase of expression and activity of exactly iNOS appears due to myocardial infraction, hypertension, angor pectoris, cardiomyopathy, cardiac distress and other pathological processes [4].

Conducting of correction of microelement imbalance caused inhibition of peroxidation processes within the tissue of myocardium (see table). Thus introducing of potassium iodide into animals caused to decrease of peroxide destruction of proteins predominantly, that characterized decrease of content of OPM products of $E_{356, 370, 430}$ fractions by 92,0-94,9% ($p_{2-3}<0,001$), and also of TBA-AP – by 68,6% ($p_{2-3}<0,001$) to the analogical indices in animals before correction. Increase of cNOS activity by 91,5% ($p_{2-3}<0,001$) to data of animals from the 2nd experimental group attracts attention. Wherein protecting abilities of NO (vasodilation, inhibition of aggregation processes, opening of K^+(ATF)-channels, control of coronary circulation and heart beats) [9] are connected with cNOS.

In animals from the 4th experimental group was being observed predominant decrease of PP content (level of $E_{356-430}$ fractions decreased by 65,1-79,5 %, $p_{2-4}<0,001$) and LP (DC content decreased by 56,0 %, $p_{2-4}<0,001$) to the analogical indices in animals from the 2nd experimental group (see table). In animals was detected the decrease of activity of iNOS by 45,7 % ($p_{2-4}<0,01$) on the background of increase of NOS activity by 37,1 % ($p_{2-4}<0,01$) and cNOS activity – by 2,8 times ($p_{2-4}<0,01$) to the analogical indices in animals with microelementosis. Dynamics of such the changes shows the weakening of absolute risk of accompanying heart pathology development upon conditions of correction of thyroid homeostasis by microelements.

Carrying out of complex correction caused more evident changes of processes of free-radical oxidation of proteins and lipids within the myocardium tissue. Thus, level of OPM E_{356}, E_{370} and E_{430} fractions in myocardium decreased by 59,0-82,6 % ($p_{2-5}<0,01$), content of DC and TBA-AP – by 85,0% and 73,4% ($p_{2-5}<0,001$) respectively to the analogical data in animals

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Table

Indices of peroxidation of proteins and lipids, NO-synthases activity in myocardium of rats with hypothyroidism on the background of iodine deficit, combined iodine and copper deficit and upon conditions of correction by potassium iodide, copper sulfate, α-tocopherol acetate and L-arginine hydrochloride.

Indices	Reference group (n=30)	1st experimental group (animals with iodine monodeficit, n=30)	2nd experimental group (animals with combined iodine and copper deficit, n=30)	3rd experimental group (correction of microelementosis by potassium iodide, n=30)	4th experimental group (correction of microelementosis by potassium iodide and copper sulfate, n=30)	5th experimental group (correction of microelementosis by potassium iodide and copper sulfate, α-tocopherol acetate, L-arginine hydrochloride n=30)
OPM, E_{356}, c.u.	1,66±0,13	1,83±0,02	1,95±0,08	0,15 ± 0,02$^{\#\#}$ $p_{2-3} < 0,001$	0,68 ± 0,27** $p_{2-4} < 0,01$	0,80 ± 0,31* $p_{2-5} < 0,01$
OPM, E_{370}, c.u.	1,74±0,13	1,89±0,02	2,01±0,11	0,16 ± 0,01$^{\#\#}$ $p_{2-3} < 0,001$	0,57 ± 0,32$^{\#}$ $p_{2-4} < 0,01$	0,67 ± 0,42$^{\#}$ $p_{2-5} < 0,02$
OPM,E_{430}, c.u.	0,74±0,12	0,88±0,04	1,95±0,11## $p_{1-2}<0,001$	0,10 ± 0,01$^{\#\#}$ $p_{2-3} < 0,001$	0,40 ± 0,25 $p_{2-4} < 0,001$	0,34 ± 0,09$^{\#}$ $p_{2-5} < 0,001$ $p_{3-5} < 0,05$
OPM, E_{530}, c.u.	0,06±0,08	-	-	0,03 ± 0,008	-	-
DC, c.u./ml	1,35±0,17	1,70±0,06	2,00±0,13**	4,75 ± 2,67	0,88 ± 0,11$^{\#}$ $p_{2-4} < 0,001$	0,30 ± 0,09 $p_{2-5} < 0,001$ $p_{4-5} < 0,01$
TBA-AP, nM/ml	3,70±0,26	3,79±0,13	3,98±0,19	1,25 ± 0,3 $^{\#\#}$ $p_{2-3} < 0,001$	2,1 ± 1,16	1,78 ± 0,07* $p_{2-5} < 0,05$
NOS (nM/min×mg)	16,57±1,11	13,44±0,48*	16,86±0,36 $p_{1-2}<0,001$	18,78 ± 2,94	5,73 ± 0,82** $p_{2-4} < 0,001$ $p_{3-4} < 0,01$	15,29 ± 0,93$^{\#}$ $p_{4-5} <0,01$
iNOS (nM/min×mg)	11,96±1,65	7,05±1,21*	10,70±0,63 $p_{1-2}<0,05$	14,74±0,56$^{\#}$	15,87 ± 2,07**	12,75 ± 1,74$^{\#}$
cNOS (nM/min×mg)	4,55±0,61	5,43±0,78	4,11±0,66	11,7±0,83$^{\#\#}$ $p_{2-3} < 0,001$	17,24 ± 2,94** $p_{2-4} < 0,01$	5,64 ± 1,07 $p_{3-5} < 0,01$ $p_{4-5} < 0,001$

from the 2nd experimental group. Upon such conditions cNOS activity in rats from the 5th experimental group was less by 51,8 % (p_{3-5}<0,01) to the analogical data in rats with HTG_{I+Cu} that were receiving potassium iodide.

Conclusions. Progression of HTGI+Cu is accompanied by activation of PP, LP processes within myocardium on the background of increase of NOS activity, generally by means of iNOS. Conducting of correction of detected changes by the potassium iodide caused to inhibition of free-radical processes on the background of particular increase of activity of protector cNOS. Engaging to the scheme of microelement and anti-oxidative complexes contributed enhancing of therapy efficiency and more evident stabilization of pro-oxidative and anti-oxidative balance of myocardium.

Perspectives of further investigations. Determination of microelement balance within organism with the aim of research of aetiopathogenesis of comorbide pathology on the background of HTG; finding out of opportunities of engaging of microelements, anti-oxidants and donators of NO to the scheme of treatment of metabolic abnormalities of myocardium with the aim of complex treatment and early detection of cardiological pathology upon conditions of thyroid dysfunction.

References:

1. Аникеева Т.В. Изменения реологических свойств крови при ишемической болезни сердца / Т. В. Аникеева // Международный медицинский журнал. – 2010. – № 2. – С. 35-37.

2. Воронич–Семченко Н. М. Біохімічні показники сироватки крові щурів з гіпотиреозом в умовах корекції препаратом йодид–100 / Н. М. Воронич–Семченко // Фізіологічний журнал. – 2007. – № 6. – С. 73-77.

3. Гаврилов В. Б. Вимірювання дієнових кон'югатів в плазмі крові по ІФ–поглинанню гептанових і ізопропанольних екстрактів / В. Б. Гаврилов, А. Р. Гаврилова, Й. Ф. Хмара // Лабораторное дело. – 1988. – № 2. – С. 60-63.

4. Гарматина О. Ю. Влияние индукции iNOS на экспрессию iNOS белка в разных отделах сердца при ишемии–реперфузии миокарда / О. Ю. Гарматина, А. А. Мойбенко, А. Г. Портниченко // Клиническая и экспериментальная патология. – 2004. – Т. 3, № 1.– С. 20-24.

5. Демко Е. Б. Влияние нарушеного соотношения некоторых микроэлементов (йода, меди, кобальта, магния) на щитовидную железу на фоне оптимального и несбалансированого питания: автореф. дис. на соиск. науч. степ. д–ра мед. наук: спец. 14.00.07 / Е. Б. Демко. – Смоленськ, 1972. – 33 с.

6. Дубініна О. Ю. Окислювальний стрес і окислювальна модифікація білків / О.Ю. Дубіна // Медична хімія. – 2001. – Т. 3, № 2. – С. 5-12.

7. Коробейникова Е. Н. Модифікація визначення продуктів перекисного окислення ліпідів у реакції з тіобарбітуровою кислотою // Лабораторное дело. – 1989. – № 7. – С. 8-10.

8. Крюк Ю.Я. Особенности проявления оксидативного стресса при гипотиреозе разной степени тяжести в эксперименте / Ю.Я. Крюк, Л.В. Махнева, С.Е. Золотухин, Д.С. Битюков // Патологія. – 2011. – Т.8, №2. – С. 62-65.

9. Кульматицький А. В. Оксид азоту та перекисне окислення ліпідів у гострому періоді повторного ішемічного інсульту / А. В. Кульматицький, В. М. Шевага, М. С. Білобрин // Клінічна та експериментальна патологія. – 2011. – Т. X, № 4. – С. 49-56.

10. Романюк А. М. Сперматогенна функція в умовах впливу солей важких металів і корекції препаратом «Тівортін» / А. М. Романюк, С. В .Сауляк, Р. А. Москаленко // Лікарська справа. – 2012. – № 1–2. – С. 123-128.

11. Сумбаев В.В. Влияние ДДТ на активность синтазы оксида азота в печени, легких и головном мозге крыс / В. В. Сумбаев, И. М. Ясинская // Современные проблемы токсикологии. – 2000. – № 3. – С. 3-7.

12. Тучак О. І. Стан системи перекисного окислення ліпідів за умов корекції гіпотиреозу α–токоферолом / О. І Тучак // Актуальні проблеми сучасної медицини. Вісник Української медичної стоматологічної академії. – 2009. – № 4(28). – С. 143-146.

13. D–penicillamine – induced copper deficiency in suckling mice: neurogical abnormalities and brain mitochondrial enzyme activities / Masahiko Yamamoto [et al.] // Developmental Brain Research. – 1990. – Vol. 55. – P. 51-55.

14. Martinez-Galan J.R. Early effect of iodine deficiency on radial glial cells of the hippocampus of the rat fetus / J.R. Martinez–Galan, P.Pedraza, M. Santacana // J. Clin.Invest. – 1997. – Vol. 99. – P. 2701-2709.