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**Efficacy of Microelements, Antioxidants and Nitric Oxide Donators Use for the Correction of NO-Synthase System in the Myocardium of Rats with Hypothyroid Dysfunction on the Background of Combined Iodine and Selenium Deficiency**

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***Abstract.*** The performance of NO-synthase system in the myocardium of rats with iodine deprivation, on the background of combined iodine and selenium deficiency and under conditions of iodine correction (potassium iodide); iodine and selenium (potassium iodide, selenium active); iodine, selenium, antioxidants, donators of NO (potassium iodide, selenium active, α-tocopherol acetate, L-arginine hydrochloride) was analyzed in the article. The development of hypothyroid dysfunction was detected to be accompanied by inhibition of NO-synthase system in the myocardium, mainly through inducible NOS. Potassium iodide and selenium active had positive influence on the metabolism of nitric oxide in the myocardium. This could be the result of restoration of thyroid gland functional capacity. The introduction of α-tocopherol acetate and L-arginine hydrochloride was accompanied by increase in constitutive NOS activity. Comparative analysis of experimental research on the capabilities of hypothyroid dysfunction correction on the background of microelements imbalance using potassium iodide, simultaneous administration of selenium and potassium iodide, involving α-tocopherol acetate and L-arginine hydrochloride to the scheme of correction confirms the importance of individual approach to the prevention and correction of thyroid disease manifestations.

***Keywords: iodine deficiency; selenium deficiency; NO-synthase system; cardiovascular system.***

**Problem statement and analysis of the recent research**

According to the high prevalence of cardiovascular diseases in endemic regions, the research of NO-synthase system indexes and the possibility of including the donators of nitric oxide (NO) for prevention of the diseases associated with goiter are scientifically interesting [3, 4]. The development of secondary dyslipidemia is the risk factor of cardiac pathology under the conditions of hypothyroid dysfunction (HD) [5, 8, 10]. However, data about the changes in NO system under conditions of HD are limited [6]. At the same time NO belongs to the reactive oxygen species and it is powerful, the most regulated endogenous antioxidant that reduces the production of free radicals, which stimulate oxidative stress mainly by NF-kβ factor and dymetylarhinin inhibition [9, 14, 15]. Significant changes in the system of NO metabolism can complement pathogenesis link of the cardiovascular system disorders on the background of HD.

**The objective of the research** was to study the changes of NO-synthase system indexes in the myocardium of rats with HD under the conditions of iodine deprivation, combined iodine and selenium deficiency and determine efficacy of microelements, antioxidants and donators of NO.

**Materials and methods of the research**

The research was conducted on 150 nonlinear sexually matured male rats weighting 150-180 g. The animals were divided into such research groups: rats with HD on the background of iodine deficiency ($HD_I$, 1st research group – comparison group); rats with HD on the background of combined iodine and selenium deficiency ($HD_{I+Se}$, 2nd research group); animals with HD on the background of combined iodine and selenium deficiency, which received iodine (3rd research group); iodine and selenium drugs (4th research group); iodine, selenium, antioxidants, donators of NO drugs (5th research group). The control group consisted of 30 intact animals kept on standard nutrition, ordinary temperature and light regimen of vivarium. HD (1-5th experimental groups) was modeled by adding mercazolilum to animals feed

("Health", Ukraine) in a dose of 7.5 mg/100g of body weight during 15 days [13]. Animals of all research groups were kept on iodine deficient diet during the experiment [16]. Selenium deficiency (2-5$^{th}$ research groups) was modeled by the addition of balanced selenium deficient ration of natural ingredients to the basal diet during 45 days [1]. HD correction (3-5$^{th}$ research groups) was made by adding potassium iodide to feed (iodide-100, Nycomed Merck KGaA, Germany) at the rate of 50 mg per day during 30 days [12]. Selenium replacement therapy was performed by adding selenium active ("Elit-farm" Dnipropetrovsk, Ukraine) to drinking water at the rate of 5 mkg/day during 20 days [2]. Animals of the 5$^{th}$ research group received antioxidant α-tocopherol acetate (20 mg/kg) during 30 days of the experiment and donator NO L-arginine hydrochloride (tivortin-aspartate, "Yuriya Farm", Kyiv, Ukraine) during the last 20 days [11].

Euthanasia was made by decapitation under the ketamine anesthesia (100 mg/kg of body weight). Keeping, feeding and euthanasia were performed due to international requirements about humane relationship to animals and conventional national norms of bioethics (Strasburg, 1986; Kiev, 2001). The system of NO metabolism in the myocardium was estimated by the activity of NO-synthases: general (NOS), constitutive (cNOS) and inducible (iNOS).

Statistical processing was conducted using the modern package of computer programs (Statistic Soft 7.0). Shapiro-Wilka test was used for each of the selection to check if the distribution of examined index was normal. According to this test the distribution of these selections was determined to correspond to Gaussian distribution. In the case of two normal distributions the equality of general dispersions was checked using Levene test, after that the selections were compared with the help of t- Student's t-test. The difference was considered statistically significant in case of $p<0.05$.

**Results of the research and their discussion**

The inhibition of NO-synthases activity in myocardium of animals with $HD_I$ was detected (Table 1), particularly NOS activity decreased by 24.7% ($p<0.01$), iNOS – by 33.4% ($p<0.01$) and changes of cNOS were not reliable to control. The selenium deficiency negatively affected the studied parameters as evidenced by reduction of NOS (by 54.4%, $p<0.001$), iNOS (by 40.9%, $p<0.001$) and cNOS (by 43.2%, $p<0.01$) in comparison to similar parameters in rats with iodine mono deficiency. We can assume that the combined deficiency of microelements can be especially dangerous with age, because age inhibition of NOS activity leads to reduction of NO synthesis, which is one of the physiological mechanisms of aging [7].

*Table 1*

Changes of NO-synthase system in the myocardium of rats with hypothyroid dysfunction under conditions of potassium iodide, selenium,
α-tocopherol acetate and L-arginine hydrochloride correction (M±m)

| Groups of animals | NOS, nmol/min · mg | iNOS, nmol/min · mg | cNOS, nmol/min · mg |
|---|---|---|---|
| Intact animals (n=30 ) | 12.81±0.66 | 9.26±0.55 | 3.47±0.29 |
| 1$^{st}$ research group ($HD_I$, n=30) | 9.64±0.49** | 6.17±0.55** | 3.54±0.38 |
| 2$^{nd}$ research group ($HD_{I+Se}$, n=30) | 4.40±0.24* $p_{1-2}<0.001$ | 2.41±0.19* $p_{1-2}<0.001$ | 2.01±0.19** $p_{1-2}<0.01$ |
| 3$^{rd}$ research group (correction $HD_{I+Se}$ by potassium iodide, n=30) | 12.05±0.52 $p_{2-3}<0.001$ | 9.29±0.33* $p_{2-3}<0.001$ | 2.64±0.28$^{\#}$ |
| 4$^{th}$ research group (correction $HD_{I+Se}$ by potassium iodine and selenium, n=30) | 13.82±0.59 $p_{2-4}<0.001$ $p_{3-4}<0.05$ | 10.46±0.27 $p_{2-4}<0.001$ $p_{3-4}<0.05$ | 3.30±0.21 $p_{2-4}<0.01$ |
| 5$^{th}$ research group (correction $HD_{I+Se}$ by potassium iodine, selenium, α-tocopherol acetate, L-arginine hydrochloride, n = 30) | 16.73±0.30* $p_{2-5}<0.001$ $p_{3-5}<0.001$ $p_{4-5}<0.01$ | 11.43±0.23** $p_{2-5}<0.001$ $p_{3-5}<0.001$ $p_{4-5}<0.05$ | 5.28±0.16* $p_{2-5}<0.001$ $p_{3-5}<0.001$ $p_{4-5}<0.001$ |

In animals of 3$^{rd}$ research group (correction of microelementosis by potassium iodide) NOS index in myocardium increased by 2.7 times ($p_{2-3}<0.001$) and iNOS – by 3.9 times ($p_{2-3}<0.001$) compared to the data before correction. In rats of the 4$^{th}$ research group the increase in NOS by 3.1 times ($p_{2-4}<0.001$), iNOS – by 4.3 times ($p_{2-4}<0.001$) and cNOS - by 64.2% ($p_{2-4}<0.01$) was detected in comparison to analogic data in animals of 2$^{nd}$ research group. Herewith, NOS exceeded the analogic data in rats that received potassium iodide mono therapy by 14.7% ($p_{3-4}$<0.05) and iNOS – by 12.6% ($p_{3-4}$<0.05).

The involvement of NO donators to the correction scheme was determined due to the research results according to the relation between the level of selenium in blood serum and the risk of progression of coronary heart disease, coronary arteries disease and mortality as a result of cardiovascular diseases [6]. In rats of 5th research group NOS activity increased by 3.8 times ($p_{2-5}$<0.001), iNOS – by 4.7 times ($p_{2-5}$<0.001), cNOS – by 2.6 times ($p_{2-5}$<0.001) in comparison to the data in animals with $HD_{I + Se}$ before the correction and to indexes in animals that received only potassium iodide: NOS increased by 38.8% ($p_{3-5}$<0.001), iNOS – by 23.0% ($p_{3-5}$<0.001), cNOS – twice ($p_{3-5}$<0.001). At the same time, NOS activity in rats of 5th research group increased by 21.1% ($p_{4-5}$<0.001), iNOS and cNOS in heart homogenate tended to increase and exceeded data in animals of 4th research group by 9.3% ($p_{4-5}$<0.05) and by 60.0% ($p_{4-5}$<0.001) respectively.

Comparative analysis of experimental research results of HD correction opportunities on the background of combined deficiency of selenium and iodine by potassium iodide, simultaneous administration of selenium and potassium iodide, involving α-tocopherol acetate and L-arginine hydrochloride to the schemes of correction confirms the importance of individual approach to the prevention and correction of thyroid pathology manifestations.

**Conclusions**

1. The changes in NO system under the conditions of hypothyroidism may increase the cardiovascular risk, especially with age.
2. Selenium deficiency potentiates the NO-synthase system violation in the myocardium of animals with HD on the background of iodine deficiency. Selenium has strong cardio protective effect.
3. The correction of thyroid homeostasis should be made individually taking into account the microelements panel (iodine and selenium supply level) and HD manifestations.
4. The obtained results can complement HD pathogenesis and be the experimental reasoning for improving the prevention of comorbid pathology in conditions of hypothyroidism.

**Prospects of further research** involve examination of the correlation between indicators of thyroid status, NO-synthase and antioxidant systems, lipid spectrum of blood, structural changes in myocardium and vessels under the conditions of microelements imbalance.

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