Galician Medical Journal

Scientific and Practical Journal of Ivano-Frankivsk National Medical University

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Effectiveness of Correction of Metabolic Disturbances in Rats with Combined Iodine and Iron Deficiencies

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Abstract. The research deals with the study of lipid and protein peroxidation, antioxidant defense, metabolism of nitric oxide, blood lipid spectrum and protein metabolism in rats with combined deficiencies of iodine and iron and determines the effectiveness of correction of detected changes by microelements, antioxidants and nitric oxide donators. The research was carried out on rats weighting 120-150 g that were divided into five research groups: Group I included animals with iodine deficiency (the comparison group, n=30); Group II comprised animals with combined iodine and iron deficiencies (n=30); Group III included animals with correction of combined iodine and iron deficiencies using iodine-containing drugs (potassium iodide, n=30); Group IV comprised animals with correction of combined iodine and iron deficiencies using iodine-containing drugs and iron hydroxide (n=30); Group V consisted of animals with correction of combined iodine and iron deficiencies using iodine, iron, antioxidants, nitric oxide donators (n=30). In order to induce iodine deficiency all animals were kept on iodine-deficient diet for 45 days and received merkazolil with drinking water (7.5 mg/100 g body weight). Iron deficiency was induced by daily intraperitoneal injection of chelator deferoxamine at a dose of 20 mg/100 g body weight since 31st to 45th days of the experiment. The correction was carried out by addition of potassium iodide (50 mg daily for 30 days), iron hydroxide (2.5 mg daily for 20 days), alpha-tocopherol acetate (20 mg daily for 30 days), L-arginine hydrochloride (2.5 g daily for 20 days) to diet. The control group consisted of 30 animals, which received intraperitoneal injection of physiologic solution (0.2 ml/100 g body weight) since 31st to 45th days of the experiment. Iodine deficiency and combined microelement deficiency were found to be accompanied by impaired thyroid profile as indicated by a decrease in serum levels of free triiodothyronine (fT3) and thyroxine (fT4) on the background of increased TSH. In animals with iodine deficiency the activation of lipid peroxidation, the suppression of most studied antioxidant enzymes (excluding the catalase and glutathione peroxidase the levels of which increased) and the development of dyslipidemia were observed. Iron deficiency negatively affected prooxidant-antioxidant homeostasis in rats, potentiated changes in blood lipid spectrum that could significantly increase the risk for the development of disturbances associated with goiter. The correction of revealed changes was effective when using potassium iodide. The effectiveness and rationale of adding iron hydroxide, antioxidants (alpha-tocopherol acetate) and nitric oxide donators (L-arginine hydrochloride) to the therapeutic scheme for correcting metabolic disturbances and preventing comorbid pathology in hypothyroid dysfunction were determined

Keywords: hypofunction of the thyroid gland; iodine deficiency; prooxidant-antioxidant homeostasis; nitric oxide; lipid status

Problem statement and analysis of the recent research

Cellular oxidative stress is one of the pathogenetic factors of comorbid pathology. Excessive intensification of peroxidation processes contributes to disturbances of microcirculation, metabolism and the development of hypoxia, which also induces lipid peroxidation (LPO) [7, 10]. Oxidative modifications of proteins (OMP) can outpace the attack upon membrane lipids by free radicals [6]. Under such conditions, the antioxidant system (AOS) cannot withstand aggressive reactive oxygen species (ROS). The AOS also includes nitric oxide (NO) being the most regulated endogenous antioxidant [17, 18]. Nowadays significant progress has been made in the study of disruption of pro-antioxidant balance in thyroid homeostasis disorders, including the iodine deficiency [10]. At the same time, there are limited data on the features of metabolic processes on the background of combined deficiencies of essential micronutrients. The study of the effects of

combined iodine and iron deficiencies on the human body is of great interest because iron plays a key role in oxidoreduction and cellular respiration, is an active center of peroxidase being responsible for the transformation of iodine into the organic form and binding of the iodine-containing tyrosine residues to thyroglobulin, as well as the prevalence of iron deficiency anemia in endemic regions [2, 14]. One of such effects is a violation of metabolism, primarily lipid metabolism. The development of secondary dyslipidemia is known to belong to early markers of hypothyroidism [11]. However, it is assumed that violation of lipid metabolism occurs only in comorbidity.

The objective of the research was to evaluate the course of the oxygen-dependent reactions, antiradical protection, changes in nitric oxide synthesis and blood lipid spectrum in rats with combined iodine and iron deficiencies, as well as to determine the effectiveness of correction of revealed changes in micronutrients, antioxidants and nitric oxide (NO) donators.

Material and methods

The research was carried out on rats weighting 120-150 g that were divided into five research groups: Group I (n=30) included animals with iodine deficiency (the comparison group); Group II (n=30) comprised animals with combined iodine and iron deficiencies; Group III (n=30) included animals with correction of combined iodine and iron deficiencies using iodine-containing drugs; Group IV (n=30) comprised animals with correction of combined iodine and iron deficiencies using iodine- and iron-containing drugs (n=30); Group V (n=30) consisted of animals with correction of combined iodine and iron deficiencies using iodine- and iron-containing drugs, antioxidants (alpha-tocopherol acetate), NO donators (L-arginine hydrochloride). In order to induce iodine deficiency all animals were kept on iodine-deficient diet for 45 days and received merkazolil with drinking water (7.5 mg/100 g body weight) [15, 19]. Iron deficiency was induced by daily intraperitoneal injection of chelator deferoxamine at a dose of 20 mg/100 g body weight since 21st to 45th days of the experiment [14]. The correction was carried out by addition of potassium iodide (50 mg daily for 30 days) [3], iron hydroxide (2.5 mg daily for 14 days) [14], α-tocopherol acetate (20 mg daily for 30 days) [30], L-arginine hydrochloride (2.5 g daily for 20 days) [12] to diet. The control group consisted of 30 animals, which were kept on standard ration, under ordinary room temperature and standard lighting conditions of vivarium. They received intraperitoneal injection of physiologic solution (0.2 ml/100 g body weight) since 31st to 45th days of the experiment. Euthanasia was performed using decapitation under ketamine anesthesia (100mg/kg body weight). Keeping, feeding and euthanasia were performed according to the international guidelines for the Humane Treatment and Management of Animals (1986, 2007).

The thyroid status of animals was assessed by serum levels of free triiodothyronine - fT_3, thyroxine - fT_4, thyroid stimulating hormone (TSH). The status of LPO was assessed by the accumulation of diene conjugates (DC) and TBA-reactive substances (TBARS) in the blood serum [4, 9]. The level of serum protein peroxidation (PP) was determined by the number of OMP using spectrophotometry at wavelengths of 356, 370, 430, 530 nm [7]. Serum nitrite ion levels were determined to assess the NO system [5]. The status of the AOC was evaluated by the activity of catalase, ceruloplasmin (CP), glutathione peroxidase (GP), glutathione reductase (GR), superoxide dismutase (SOD), transferrin saturation (TSAT) [1, 8, 16]. Blood lipid spectrum was studied by the levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels and atherogenic coefficient (AC). Serum levels of total protein were also determined.

Statistical analysis was performed using modern computer programs (Statistic Soft 7.0). The distribution of the studied parameters was checked for each sample using the Shapiro-Wilk test. There was determined whether the distribution of data samples corresponded to the standard normal (Gaussian) distribution. In case of two normal distributions equality of general dispersions was checked using Levene's test. Then, the samples were compared using Student's t-test. The difference was considered statistically significant at $p<0.05$.

Results and discussion

Iodine deficiency and combined microelement deficiency were found to be accompanied by impaired thyroid homeostasis as indicated by a decrease in serum levels of free triiodothyronine (fT3) and thyroxine (fT4) by 50.07-74.52% ($p<0.001$) and 85.14-92.70% ($p<0.001$), respectively on the background of increased TSH (by 23.53%, $p<0.05$).

In rats of Group I the activation of lipid peroxidation (an increase in TBARS by 2.10 times, $p<0.001$), decreased OMP (by 36.13-30.29%), the disturbances of blood lipid spectrum (elevated TG levels - by 55.70%, elevated levels of TC - by 70.0%, elevated LDL cholesterol levels - by 88.89%, $p<0.001$) were observed. The activity of GR, SOD, CP, TSAT decreased by 14.17-68.42% ($p<0.001$), however, the activities of catalase (by 42.94%, $p<0.001$) and GP (by 50.0%, $p<0.001$) increased compared to those in intact rats (Table 1).

Table 1

Parameters of lipid and protein peroxidation, metabolism of nitric oxide, antioxidant system, blood lipid spectrum, protein metabolism in rats with hypofunction of the thyroid gland secondary to combined deficiencies of iodine and iron and under conditions of correction with potassium iodide, iron hydroxide, α-tocopherol acetate and L-arginine hydrochloride

Parameters	Intact animals (n=30)	Group I (animals with iodine deficiency, n=30)	Group II (animals with combined iodine and iron deficiencies, n=30)	Group III (correction of combined iodine and iron deficiencies with potassium iodide, n=30)	Group IV (correction of combined iodine and iron deficiencies with potassium iodide and iron hydroxide, n=30)	Group V (correction of combined iodine and iron deficiencies with potassium iodide, iron hydroxide, α-tocopherol acetate and L-arginine hydrochloride, n=30)
DC, IU/ml	0.49±0.02	0.19±0.01$^{\#}$	0.59±0.05 p_{1-2}<0.001	0.68±0.03$^{\#}$	0.50±0.02 p_{3-4}<0.001	0.56±0.03 p_{3-5}<0.05
TBARS, nmol/ml	3.35±0.19	7.05±0.24$^{\#}$	8.12±0.31$^{\#}$ p_{1-2}<0.05	3.39±0.11 p_{2-3}<0.001	3.27±0.08 p_{2-4}<0.001	3.73±0.18 p_{2-5}<0.001 p_{4-5}<0.05
OMP, E_{356}, IU	3.10±0.14	1.98±0.08$^{\#}$	3.31±0.14 p_{1-2}<0.001	0.69±0.06$^{\#}$ p_{2-3}<0.001	2.23±0.08$^{\#}$ p_{2-4}<0.001 p_{3-4}<0.001	2.10±0.07$^{\#}$ p_{2-5}<0.001 p_{3-5}<0.001
OMP, E_{370}, IU	3.06±0.13	2.13±0.09$^{\#}$	3.03±0.12 p_{1-2}<0.001	0.74±0.03$^{\#}$ p_{2-3}<0.001	2.14±0.06$^{\#}$ p_{2-4}<0.001 p_{3-4}<0.001	1.91±0.07$^{\#}$ p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.05
OMP,E_{430}, IU	0.80±0.02	0.89±0.05	1.01±0.06**	0.25±0.02$^{\#}$ p_{2-3}<0.001	0.73±0.01** p_{2-4}<0.001 p_{3-4}<0.001	0.69±0.04* p_{2-5}<0.001 p_{3-5}<0.001
OMP, E_{530}, IU	0.14±0.01	0.16±0.01	0.13±0.01 p_{1-2}<0.05	0.04±0.004 p_{2-3}<0.001	0.04±0.001$^{\#}$ p_{2-4}<0.001	0.04±0.002$^{\#}$ p_{2-5}<0.001
Nitrite ion, mcmol/l	38.76±1.27	40.84±0.57	21.13±0.49$^{\#}$ p_{1-2}<0.001	46.30±1.28$^{\#}$ p_{2-3}<0.001	36.64±1.37 p_{2-4}<0.001 p_{3-4}<0.001	39.69±0.80 p_{2-5}<0.001 p_{3-5}<0.001
C, mg H_2O_2/ml	10.55±0.40	15.08±0.39$^{\#}$	13.59±0.37$^{\#}$ p_{1-2}<0.05	3.75±0.34$^{\#}$ p_{2-3}<0.001	3.81±0.22$^{\#}$ p_{2-4}<0.001	2.06±0.17$^{\#}$ p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.001
GP, mcmol/1gHb per min	0.20±0.01	0.30±0.01$^{\#}$	0.38±0.01$^{\#}$ p_{1-2}<0.05	0.35±0.02**	0.29±0.01$^{\#}$ p_{2-4}<0.001 p_{3-4}<0.05	0.24±0.03* p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.01
GR, nmol/min/mgB	0.19±0.01	0.06±0.004$^{\#}$	0.56±0.03$^{\#}$ p_{1-2}<0.001	0.38±0.04$^{\#}$ p_{2-3}<0.01	0.41±0.03$^{\#}$ p_{2-4}<0.01	0.47±0.03$^{\#}$ p_{2-5}<0.05 p_{3-5}<0.05
SOD,%	35.50±1.23	30.47±0.33$^{\#}$	10.10±1.34$^{\#}$ p_{1-2}<0.05	40.20±0.97** p_{2-3}<0.001	42.40±0.57$^{\#}$ p_{2-4}<0.001	44.20±1.63$^{\#}$ p_{2-5}<0.001 p_{3-5}<0.05
CP, IU	57.00±4.14	46.38±3.00*	48.34±2.18	46.34±3.47	52.19±1.66	86.43±2.65$^{\#}$

						p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.01
TSAT, IU	0.43±0.02	0.25±0.01#	0.21±0.04#	1.32±0.08# p_{2-3}<0.001	0.42±0.02 p_{2-4}<0.001 p_{3-4}<0.05	0.42±0.02 p_{2-5}<0.001 p_{3-5}<0.001
TG, mmol/l	0.79±0.05	1.23±0.05#	0.23±0.02# p_{1-2}<0.001	0.32±0.03# p_{2-3}<0.05	0.56±0.02# p_{2-4}<0.001 p_{3-4}<0.001	0.72±0.04 p_{2-5}<0.001 p_{3-5}<0.001 p_{4-5}<0.01
TC, mmol/l	1.41±0.04	2.27±0.07#	2.57±0.11# p_{1-2}<0.05	2.02±0.08# p_{2-3}<0.001	1.41±0.04 p_{2-4}<0.001 p_{3-4}<0.001	1.32±0.07 p_{2-5}<0.001 p_{3-5}<0.001
LDL cholesterol, mmol/l	0.18±0.01	0.34±0.02#	0.33±0.01#	0.26±0.01# p_{2-3}<0.001	0.22±0.01* p_{2-4}<0.001 p_{3-4}<0.05	0.28±0.02# p_{2-5}<0.05 p_{4-5}<0.05
HDL cholesterol, mmol/l	1.03±0.04	1.08±0.03	0.62±0.02# p_{1-2}<0.001	0.98±0.03 p_{2-3}<0.001	1.01±0.03 p_{2-4}<0.001	0.84±0.04** p_{2-5}<0.001 p_{3-5}<0.05 p_{4-5}<0.01
AC, IU	0.40±0.03	1.19±0.02#	1.66±0.04# p_{1-2}<0.001	0.99±0.08# p_{2-3}<0.001	0.59±0.07* p_{2-4}<0.001 p_{3-4}<0.01	1.04±0.13# p_{2-5}<0.001 p_{4-5}<0,05
Total protein,g/l	45.5 ▯1.13	74.95 ▯0.54#	72.80 ▯1.20#	48.65 ▯1.82 p_{2-3}<0.001	74.15 ▯0.50# p_{3-4}<0.001	73.00 ▯1.15# p_{3-5}<0.001

Notes:
* – p<0.05, ** – p<0.01, # – p<0.001 compared to those in animals of the control group;
p with Arabic numeral – statistically significant difference in the parameters of different groups.

In animals with combined deficiencies of essential micronutrients the course of the oxygen-dependent processes was activated, as indicated by increased serum DC (by 20.41%, p<0.05), TBARS (by 2.42 times, p<0.001) OMP (by 26.25%, p<0.01) compared to the control group (Table 1). It is necessary to note that iron deficiency increases the processes of lipid and protein peroxidation. In particular, in animals of Group II an increase in DC (by 3.11 times, p_{1-2}<0.001), TBARS (by 15.18%, p_{1-2}<0.05), OMP (by 13.48-67.17%, p_{1-2}<0.05) was observed compared to the comparison group (animals with iodine deficiency).

Under such conditions, the activity of antioxidant enzymes was multidirectional, and the nature and severity of the identified changes worsened in microelementosis (Table 1). In particular, in animals of Group II there was found an increase in the activity of catalase (by 28.82%, p<0.001), GP (by 26.67%, p<0.001), GR (by 9.33 times, p<0.001) on the background of inhibition of SOD (by 71.55%, p<0.001) and TSAT (by 53.49%, p<0.001) compared to the control group. The activation of individual enzymes of the AOS could be a result of increased peroxidation. Combined microelement deficiency was accompanied by exhaustion of antiradical reserve as indicated by decreased activity of catalase (by 9.88% p_{1-2}<0.05), SOD (by 67.85%, p_{1-2}<0.05) and TSAT (by 84.00%, p_{1-2}<0.05) compared to animals of Group I. The decrease in serum levels of nitrate ions (by 46.49%, p<0.001 and 48.26%, p1-2<0.001 compared to the control group and comparison group) may reduce antioxidant capacity of blood serum. These biochemical changes may indicate an inadequate response of antiradical mechanisms to oxidative stress in animals.

Microelement disbalance was accompanied by the development of dyslipidemia, as indicated by elevated serum levels of TC (by 1.82 times, p<0.001), LDL (by 83.33%, p<0.001) and AC (by 4.15 times, p<0.001) on the background of decreased levels of HDL cholesterol (by 39.81%, p<0.001) and TG (by 80.89%, p<0.001) compared to the control group (Table 1). When conducting a comparative analysis of indicators of Group I and Group II elevated serum levels of TC (by 13.22%, p_{1-2}<0.05) and AC (by 39.50%, p_{1-2}<0.001) together with decreased levels of HDL cholesterol (by 42.59%, p_{1-2}<0.001) and TG (by 81.30%, p<0.001) were detected.

The total serum protein concentration in animals of Group II increased by 58.43% ($p<0.001$) compared to similar data in intact animals. There was no statistically significant difference in this parameter between animals of Group I and Group II.

Injection of potassium iodide resulted in stabilization of processes of prooxidant-antioxidant homeostasis. In particular, in rats of Group III the concentration of TBARS in the blood serum decreased by 58.25% ($p_{2-3}<0.001$), the number of OMP reduced by 69.13-79.15% ($p_{2-3}<0.001$) compared to the data before the correction. Serum nitrite ion levels increased by almost four times ($p_{2-3}<0.001$) and TSAT increased by 6.29 times ($p_{2-3}<0.001$). The increase in TSAT may be due to the redistribution of iron concentration between different tissues. Reduced serum levels of TC (by 21.40%, $p_{2-3}<0.001$), LDL cholesterol (by 21.21%, $p_{1-2}<0.001$) HDL cholesterol (by 58.06%, $p_{1-2}<0.001$), resulting in reduced AC (by 40.36%, $p_{1-2}<0.001$) indicated the effectiveness of the correction. The concentration of TBARS, OMP, LDL cholesterol and TP was similar to those in intact animals. Such changes may be due to the improvement of functional ability of the thyroid gland.

When evaluating the effectiveness of the correction of detected changes with potassium iodide and iron hydroxide there was detected the increase in adaptive-compensatory capabilities of resistance to oxidative stress in rats. Thus, in animals of Group IV the concentration of TBARS in the blood serum decreased by 59.73% ($p_{2-4}<0.001$), the number of OMP reduced by 37.72-67.00% ($p_{2-4}<0.05$) on the background of increase in serum nitrite ion levels by 73.40% ($p_{2-4}<0.001$), activity of SOD by 4.20 times ($p_{2-4}<0.001$), TSAT by twice ($p_{2-4}<0.001$) compared to those in animals with combined microelement deficiency before the correction. The activity of catalase, GP and GR in animals of Group IV decreased by 23.68-71.94%, $p_{2-4}<0.001$) compared to animals of Group II. The parameters of blood lipid spectrum changed as follows: the levels of TG and HDL cholesterol increased by 2.43 times (p2-4<0.001) and 62.90% (p2-3<0.001), respectively, the levels of TC and LDL cholesterol reduced by 45.14% (p2-4<0.001) and 33.34% (p2-4<0,001), respectively. The decrease in AC by 64.46% (p2-4<0.001) may indicate the probability of decrease in absolute risk of developing cardiac pathology in adequate correction of thyroid dysfunction considering adequate supply with essential microelements. The addition of iron sulfate to the correction scheme resulted in the decrease in serum content of DC by 26.47% ($p_{3-4}<0.001$), serum nitrite ion levels reduced by 20.86% ($p_{3-4}<0.001$), the levels of GP and TC reduced by 17.14% ($p_{3-4}<0.05$) and 30.12% ($p_{3-4}<0.001$), respectively. There was a reduction in the levels of LDL cholesterol by 15.38% ($p_{3-4}<0.05$), AC reduced by 40.40% ($p_{3-4}<0.01$) and the level of TP increased by 52.42% ($p_{3-4}<0.001$).

The addition of α-tocopherol acetate and L-arginine hydrochloride to the correction scheme was accompanied by stabilization of the processes of oxygen-dependent metabolism, in particular, the concentration of DC, TBARS and nitrite ion in the blood serum in animals of Group V did not differ from those in the control group and the level of OMP in rats of this group was even lower than that in intact animals. Under such conditions, the activity of GP and TSAT did not differ from that in the control group, and the activity of GR, COD and the CP even exceeded that in the control group by 2.47 times ($p<0.001$), by 24.51% ($p<0.001$) and 51.63% ($p<0.001$), respectively. And only catalase activity was significantly lower than in intact animals. On the background of complex therapy serum levels of TG and TC also reached the control level, but the level of LDL cholesterol was higher than that in intact animals by 55.56% ($p<0.001$) and the level of HDL cholesterol exceeded control values by 18.45% ($p<0.001$).

Conclusions

Iodine deficiency was found to be accompanied by imbalance in the system of LP/PP/AOS/NO being more pronounced in animals in combined deficiencies of iodine and iron. Potassium iodide and iron sulfate are effective in correcting metabolic disorders. The addition of α-tocopherol acetate and L-arginine hydrochloride to the correction scheme improves the effectiveness of therapy, activation of antioxidant defense, reduces the progression of oxidative stress and normalizes lipid and protein metabolism.

Prospects for further research

We are going to study the relationship between iron levels and functional ability of the thyroid gland; to perform clinical observation in order to determine iron balance in patients with hypothyroidism as well as to clarify the possibilities of using iron supplements, antioxidants and NO donators simultaneously with potassium iodide in medical practice to improve the effectiveness of treatment of hypofunction of the thyroid gland and prevent the development of comorbidities.

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