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LIPID AND PROTEIN PEROXIDATION IN RATS WITH IODINE DEFICIENCY, INSULIN RESISTANCE AND IN TERMS OF THEIR COMBINATION

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S u m m a r y. The examination of lipid and protein peroxidation, antioxidant defense in rats with iodine deficiency (ID), insulin resistance (IR) and in terms of their combination was studied. It was noticed that ID causes activation of free radical oxidation of lipids. IR increasingly affects the peroxidation of proteins in examined tissues. In case of ID and IR combination, the activation of peroxidation against the background of significant inhibition of anti-radical protection was observed.

K e y w o r d s: iodine deficiency, insulin resistance, peroxidation, antiradical defense.

INTRODUCTION

Deficiency of thyroid hormones leads to the disruption of metabolic processes, dysfunction of organs and systems, and multi-organ pathology. The wide range of influence of thyroid hormones hinders the disclosure of pathogenic mechanisms of hypothyroid dysfunction. Thus, in case of iodine deficiency (ID) several pathological processes develop, e.g. glomerular filtration, tubular reabsorption and secretion are reduced in the kidneys, causing fluid retention, edema and weight gain, violations of trabecular structure, damage to the structure of hepatocytes in the liver [4]. The teeth are also affected by such changes manifested as the lesions of the periodontal tissues and oral mucous membrane [2, 8]. It is also interesting to study the relationship of hypothyroidism with other concomitant endocrine diseases and conditions, particularly with insulin resistance (IR).

It is known that in subclinical hypothyroidism, the process of insulin transport to target tissues, and its uptake by insulin-sensitive cells is disturbed. The development of secondary dyslipidemia and violation of pro oxidative-anti oxidative homeostasis are other important factors facilitating the progression of pathological conditions.

THE AIM OF THE STUDY

The purpose of the research is the study of lipid and protein peroxidation, antioxidant defense in rats with ID, IR and in terms of their combination.

MATERIALS AND METHODS

The study group consisted of 90 male rats weighing 150-180 g which were divided into three groups. The rats from group 1 (n=30) had ID induced; to achieve that, the animals were kept on iodine deficiency diet for 45 days [1]. The rats from group 2 (n=30) had IR induced by adding of 10% solution of fructose to the drinking water for 8 weeks [11]. The animals from group 3 had model IR due to iodine deficiency diet. Animal euthanasia was performed by decapitation under ketamine anesthesia (100 mg/kg of body weight). Processes of peroxidation in the blood serum, kidneys, liver, dental pulp and oral mucous membrane were assessed by diene conjugates

content (DC), active products that respond to thiobarbituric acid (TBA-AP) [9], and products of oxidative modification of proteins (OMP) [5]. Antioxidant protection was characterized by the activity of catalase (C), ceruloplasmin (CP), superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR) and saturation of transferrin with iron (STR) in the blood serum [3]. For comparison, the corresponding indexes were determined in 30 intact animals (control group) that were fed standard diet, kept at standard temperature and light regime of vivarium. All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals, and the European Council directive of 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee. Quantitative research results were analyzed using mathematical software package Statistic Soft 7.0 using the Student's t-test. Statistically significant difference was considered at $p<0.05$.

RESULTS

In the animals with ID, the activation of lipid peroxidation (LPO) was observed in most studied tissues (Table 1). Thus, in the rats from group 1, DC content increased in the kidneys up to 73.58% ($p<0.01$), and it doubled in the dental pulp ($p<0.02$), in the oral mucosa – eight times ($p<0.05$), and TBA-AP content increased in the liver 2.15 times ($p<0.05$), in the dental pulp 55.88% ($p<0.02$), and in the oral mucosa 41. 42% ($p<0.05$) compared with the reference values. Activation of protein peroxidation (PPO) was found only in the pulp of teeth, where the content of most fractions of OMP increased 2.00-2.75 times ($p<0.02$) compared to the reference range (Table 2). However, the reduction of PPO products by 77.34 - 82.50% ($p<0.02$) was noted in the blood serum, compared to the corresponding values in the control animals.

The activation of LPO in the studied organs and tissues was also observed in the rats with IR (Table 1). In particular, in the animals from group 2 DC content increased 4.07 times in the kidneys ($p<0.001$), in the liver to 36.84% ($p<0.05$) and in the oral mucosa 15.5 times ($p<0.001$) compared to the control animals. The increase in the end product of lipid peroxidation was more pronounced. Thus, the content of TBA-AP increased in the blood serum to 41.17% ($p<0.05$), in the dental pulp to 46.48% ($p<0.001$), and in the oral mucosa it increased 2.40 times ($p<0.001$) compared to reference values. Co-directed were the changes of PPO: the content of OMP fractions in the kidneys increased 3.00-4.07 times ($p<0.02$), in the dental pulp 3.25-7.25 times ($p<0.02$) and in the oral mucosa 4.00-6.15 times ($p<0.05$) compared to the control animals.

Significant changes in biological substrates of peroxide decomposition in the animals with IR combined with ID were observed (Table 1). Thus, DC content in the kidneys, dental pulp and oral mucosa significantly exceeded reference values: 9.1 times ($p<0.001$), 2.43 times ($p<0.001$) and 22 times respectively. Similar changes were noted regarding the end products of lipid peroxidation whose content increased 2.03-3.11 times in all investigated tissues ($p<0.05$) compared to the reference values. It is worth noting that enhanced free radical oxidation of proteins occurred under these experimental conditions. Thus, the content of most OMP fractions in all studied tissues increased 5.75-8.44 times ($p<0.05$) compared to the animals from the control group (Table 2).

Comparative analysis of indicators of lipid peroxidation in animals from groups 2 and 3 found a significant increase in their content: in the kidneys 41.76% ($p_{1-3}<0.05$), in the liver 32.26% ($p_{1-3}<0.05$), in the dental teeth 17.65% ($p_{1-3}<0.05$), in the oral mucosa 63.64% ($p_{1-3}<0.01$). At the same time, the activation of PPO was most pronounced only in the dental pulp and oral mucosa, where the content of most OMP fractions increased 2.70-6.71 times ($p_{1-3}<0.01$). The comparison of the indexes of lipid peroxidation in the groups 2 and 3 found lipid oxidation in the kidneys indicated by a significant increase in TBA-AP - 2.37 times ($p_{2-3}<0.001$) in the animals with a combined pathology. These changes occurred against the backdrop of enhanced protein peroxidation. Thus, the E_{430} fraction content increased in the kidneys, liver, dental pulp and oral mucosa 2.86 times ($p_{2-3}<0.05$), 2.06 times ($p_{2-3}<0.05$), 43.48% ($p_{2-3}<0.05$), and 2.11 times ($p_{2-3}<0.02$), respectively, compared with the animals with IR (Table 2).

Activated peroxide processes were accompanied by the redistribution of activity of the enzymes of antioxidant defense (AOD). Specifically, in the rats from group 1 we noted a decrease in activity of GR and GP – by 42.10% ($p<0.05$) and 76.50% ($p<0.05$) respectively compared to the reference values, indicating the

exhaustion of antioxidant reserves for ID conditions (Table 3). By contrast, in the animals with IR, multi-directed changes of components of anti-radical protection were observed. Thus, C activity was lower by 66.7 % ($p<0.05$), whereas the GP activity increased by 63.2% ($p<0.05$) compared with the values in the control group of rats. The animals from group 3 were found to have increased activity of GP –2.37 times ($p<0.05$) amid of falling STR – by 42.34% ($p<0.05$) compared to the reference values.

A comparative analysis of indicators revealed increased activity of GP and GR - 3.9 times ($p_{1-2}<0.01$) and 6.3 times ($p_{1-2}<0.01$) in the animals with IR compared to the animals from group 1. In the animals from group 3, the activity of GP and GR was 5.63 times higher ($p_{1-3}<0.05$) and 4.50 times ($p_{1-3}<0.05$) respectively compared to the reference reduction in STR 47.62% ($p_{1-3}<0.02$) in comparison to the animals with iodine deprivation. At the same time, in the rats with IR combined with ID, the activity of SOD and CP was reduced 19.89% ($p_{2-3}<0.05$) and 46.96% ($p_{2-3}<0.001$) respectively, compared to isolated conditions of IR (Table 3).

DISCUSSION

The analysis of indicators of free radical oxidation found activation of lipid peroxidation in the studied tissues under conditions of ID. The intensity of free radical processes, AOD status primarily depends on the nature of the course of metabolic processes in different tissues that undergo changes in conditions of hypothyroid dysfunction. Under conditions of IR, the processes of protein peroxidation prevailed. Increase in OMP is the result of an imbalance between the processes that regulate protein synthesis and oxidation, and reduction of proteases activity that break down selectively oxidized forms of proteins. One of the causes of OMP changes may be uncontrolled intensification of LPO. It is known that LPO products, including TBA-AP, reacting with lysine residues of proteins, cause their degradation with the formation of various cytotoxic compounds. OMP may include a direct fragmentation of proteins or cause denaturation, and their partial or complete loss of functions. In turn, especially metal-enzymes (SOD, C, GP) are first to be subjected to oxidative destruction. Any system which forms hydrogen peroxide and restores trivalent iron to bivalent, or bivalent to monovalent copper, can cause selective modification of proteins. Partitioned and denaturated proteins are the substrates for intracellular proteases [6, 7]. It is believed that the negative effect of OMP in cells is due to the fact that oxidized proteins are a source of free radicals that deplete stocks of cellular antioxidants. Thus PPO is not the only trigger of pathological processes but also their earliest marker. Dynamics of changes of the products of PPO reflects the degree of oxidative lesions in the cells and reserve-adaptive capabilities of the organism. It is necessary to emphasize that combined endocrine pathology is accompanied by a substantial violation of all studied links of pro-oxidative-antioxidant homeostasis towards the activation of peroxidation. Such activation of oxygen-dependent reactions under these conditions can be associated with oxidative potential of corresponding tissues, and with the degree of involvement of certain organs can lead to the formation of adaptive processes, depletion anti-radical defense under the conditions for potentiation of negative mutual influence of ID and IR [9, 10]. It should be noted that under isolated ID, the activation of peroxidation of both proteins and lipids was revealed in the dental pulp and oral mucosa, which indicates a significant risk of dental disease development in endemic goiter regions.

CONCLUSIONS

ID causes activation of LPO in the blood serum, kidneys, liver and organs of dental pulp. IR increasingly affects the peroxidation of proteins. In the combined ID and IR, the activation of peroxidation against the background of significant inhibition of anti-radical protection is observed. The obtained results can provide a theoretical basis for clinical observations that facilitate timely diagnosis, and treatment. Prevention of complications of combined endocrine pathologies can help improve the quality of life of patients.

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T a b l e 1. The content of diene conjugates and products responding to thiobarbituric acid in the blood serum, kidneys, liver, dental pulp and oral mucosa in rats with iodine deficiency (ID), insulin resistance (IR) and their combination (M±m)

Group of animals	Diene conjugates, cu·ml				
	Blood serum	Kidneys	Liver	Pulp of teeth	Oral mucosa
Intact animals (n=30)	0.45±0.14	0.14±0.07	0.57±0.13	0.07±0.005	0.04±0.01
1st research group (ID, n=30)	0.31±0.10	0.53±0.09**	0.62±0.13	0.14±0.001$^{\#\#}$	0.32±0.12*
2nd research group (IR, n=30)	0.36±0.15	0.57±0.02$^{\#\#}$	0.78±0.10*	0.03±0.002$^{\#\#}$ p_{1-2}<0.001	0.62±0.02$^{\#\#}$ p_{1-2}<0.05
3rd research group (IR combined with ID, n=30)	0.64±0.24	0.91±0.10$^{\#\#}$ p_{1-3}<0.05 p_{2-3}<0.02	0.82±0.02 p_{1-3}<0.05	0.17±0.01$^{\#\#}$ p_{1-3}<0.05 p_{2-3}<0.001	0.88±0.03$^{\#\#}$ p_{1-3}<0.01 p_{2-3}<0.001
	Products responding to thiobarbituric acid, nmol·ml				
Intact animals (n=30)	3.13±0.81	4.50±0.67	3.50±0.15	0.38±0.03	0.99±0.14
1st research group (ID, n=30)	4.86±0.89	5.23± 0.84	7.53±0.72*	0.68±0.05$^{\#\#}$	1.69±0.24*
2nd research group (IP, n=30)	5.32±0.18*	5.92±1.01	7.56±0.80	0.71±0.02$^{\#\#}$	2.38±0.18$^{\#\#}$ p_{1-2}<0.05
3rd research group (IR combined with ID, n=30)	14.01±0.23$^{\#\#}$ p_{1-3}<0.001 p_{2-3}<0.001	0.64±0.01$^{\#\#}$ p_{2-3}<0.02	8.03±0.96	2.97±0.82*	6.34±1.02*

*p<0,05; **p<0,01; $^{\#\#}$p <0,001 to analogical indexes in intact animals; p with Arabic numerals - reliable difference between the indexes of correspondent research groups (here and in the next tabl.).

T a b l e 2. The oxidative modification of proteins (AU·g protein) in the blood serum, kidneys, liver, dental pulp and oral mucosa in rats with iodine deficiency (ID), insulin resistance (IR) and their combination (M±m)

	E_{356}, nm	E_{370}, nm	E_{430}, nm	$E_{530,}$ nm
	Blood serum			
Intact animals (n=30)	3.09±0.82	3.05±0.79	1.21±0.47	0.14±0.05
1st research group (ID, n=30)	0.54±0.25##	0.63±0.21#	0.27±0.09#	0.05±0.03
2nd research group (IR, n=30)	1.21±0.75	1.18±0.55	0.47±0.18	0.06±0.01
3rd research group (IR combined with ID, n=30)	0.91±0.35**	0.97±0.12*	0.44±0.05*	0.08±0.02
	Kidneys			
Intact animals (n=30)	0.26±0.09	0.15±0.03	0.40±0.17	0.04±0.01
1st research group (ID, n=30)	0.32±0.04	0.40±0.13	0.18±0.05	0.03±0.01
2nd research group (IR, n=30)	0.78±0.02## p_{1-2}<0.001	0.61±0.13#	0.28±0.05	0.03±0.01
3rd research group (IR combined with ID, n=30)	1.26±0.49	1.15±0.43*	0.80±0.17 p_{1-3}<0.01 p_{2-3}<0.05	0.04±0.01
	Liver			
Intact animals (n=30)	0.38±0.10	0.41±0.16	0.31±0.08	0.04±0.01
1st research group (ID, n=30)	0.27±0.09	0.36±0.08	0.30 ±0.09	0.09 ±0.01#
2nd research group (IR, n=30)	0.54±0.01 p_{1-2}<0.05	0.79±0.04 p_{1-2}<0.05	0.34±0.05	0.08±0.01
3rd research group (IR combined with ID, n=30)	0.58±0.10	0.86±0.03*	0.70±0.01 p_{2-3}<0.05	0.10±0.02
	Pulp of teeth			
Intact animals (n=30)	0.10±0.01	0.08±0.04	0.08±0.01	-
1st research group (ID, n=30)	0.20±0.004##	0.22±0.003#	0.17±0.002##	0.15±0.01
2nd research group (IR, n=30)	0.59±0.16* p_{1-2}<0.05	0.58±0.15# p_{1-2}<0.05	0.26±0.06*	0.04±0.01 p_{1-2}<0.001
3rd research group (IR combined with ID, n=30)	0.72±0.04## p_{1-3}<0.001	0.22±0.04*	0.46±0.03## p_{1-3}<0.001 p_{2-3}<0.05	0.01±0.002* p_{1-3}<0.001 p_{2-3}<0.05
	Oral mucosa			
Intact animals (n=30)	0.13±0.01	0.14±0.01	0.09±0.01	0.007±0.003
1st research group (ID, n=30)	0.14±0.04	0.16±0.04	0.12±0.05	0.01±0.002
2nd research group (IR, n=30)	0.80±0.24* p_{1-2}<0.05	0.82±0.21# p_{1-2}<0.01	0.36±0.08* p_{1-2}<0.05	0.03±0.01*
3rd research group (IR combined with ID, n=30)	0.94±0.01## p_{1-3}<0.001	0.96±0.01## p_{1-3}<0.001	0.76±0.03## p_{1-3}<0.001 p_{2-3}<0.02	0.05±0.002 p_{1-3}<0.001

#p <0,02 to analogical indexes in intact animals;
p with Arabic numerals - reliable difference between the indexes of correspondent research groups.

T a b l e 3. Changes in the activity of antioxidant enzymes in the blood serum of rats with iodine deficiency (ID), insulin resistance (IR) and their combination (M±m)

Group of animals	Catalase mg H_2O_2 ·ml	Superoxide dismutase, cu·mg hemoglobine	Glutathione peroxidase µmol·mg protein	Glutathione reductase, nmol·min·mg protein	Ceruloplasmin, cu	Saturation of transferrin with iron, cu
Intact animals (n=30)	10.85±1.78	35.50±6.99	0.19±0.03	0.17±0.05	56.49±11.43	0.41±0.08
1st research group (ID, n=30)	9.27±3.34	32.00±1.41	0.08±0.03*	0.04±0.02*	42.96±14.79	0.42±0.06
2nd research group (IP, n=30)	3.61±2.60*	28.00±1.00 $p_{1-2}<0.05$	0.31±0.04* $p_{1-2}<0.01$	0.25±0.05 $p_{1-2}<0.01$	37.71±1.60	0.37±0.01
3rd research group (IR combined with ID, n=30)	6.74±2.53	22.43±1.51 $p_{2-3}<0.05$	0.45±0.14 $p_{1-3}<0.05$	0.18±0.05 $p_{1-3}<0.05$	20.00±2.63 $p_{2-3}<0.001$	0.22±0.01* $p_{1-3}<0.02$