

THE CHANGES OF PROOXIDANT-ANTIOXIDANT AND ENERGY HOMEOSTASIS IN RATES WITH IMPAIRED GLUCOSE TOLERANCE

Serhii Guranych

Ivano-Frankivsk National Medical University, Ivano-Frankivsk, Ukraine

Abstract. Topicality. Violation of glucose utilization leads to disruption of all types of metabolic processes. Particularly dangerous in the prognostic aspect is the combination of carbohydrate metabolism disorders and other endocrine pathologies, in particular hypothyroid dysfunction.

The objective of the study was to examine prooxidant-antioxidant changes of blood serum, teeth pulp and oral mucosa in rats with impaired glucose tolerance on the background of iodine deficiency.

Materials and Methods. The studies were carried on 60 female rats, which were divided into two research groups - rats with impaired glucose tolerance and insulin resistance on the background of iodine deficiency. Lipid peroxidation processes were evaluated due to the content of diene conjugates and active products that react with thiobarbituric acid in blood serum, teeth pulp and oral mucosa. Antioxidant defence of blood serum was characterized by catalase, ceruloplasmin, superoxide dismutase, glutathione peroxidase, glutathione reductase activity and iron transferrin saturation. Energy metabolism was examined by the activity of succinate dehydrogenase, malate dehydrogenase and lactate dehydrogenase in blood serum.

Results. Impaired glucose tolerance was found to lead to the activation of lipoperoxidation, mainly due to the accumulation of final products of lipid peroxidation in all studied tissues against the background of redistribution of the activity of antiradical enzymes. Under these experimental conditions, different changes in the activity of energy synthesis enzymes were observed. The development of combined endocrine pathology has led to more pronounced changes in the prooxidant-antioxidant system and significant suppression of serum dehydrogenase activity. **Conclusions.** The development of insulin resistance on the background of iodine deficiency causes the activation of oxygen-dependent processes in periodontal tissues against the background of reduced antiradical defence and disruption of energy synthesis system.

Keywords: *impaired glucose tolerance, iodine deficiency, prooxidant-antioxidant system, energy homeostasis.*

Problem statement and analysis of the latest researches

The course of many diseases is known to be closely related to the activation of oxidative stress and impaired antioxidant defence of the organism [2]. In particular, hyperglycemia provokes the formation of excessive amounts of free radicals, which are the cause of peroxide fragmentation of biosubstrates. Accumulation of oxygen intermediates, acidosis, release of intralysosomal enzymes have a toxic effect on various cellular organelles, for example, mitochondria [7]. On the other hand, in the process of energy supply of the organism at the cellular and subcellular levels, active oxygen metabolites play a significant role in the course of redox reactions [1]. Therefore, it is necessary to take into account the relationship of peroxidation reactions with energy intracellular processes, because in the case of a decrease in the intensity of oxidative phosphorylation, excessive consumption of oxygen in other metabolic reactions, in particular, lipid peroxidation (LP) is possible [10]. In turn, antioxidant system of organism provides the elimination of oxygen metabolites excess by involving them in energy metabolism and promotes the activity of synthetic processes. Therefore, glycation of antiradical enzymes leads to the reduction of protective system effectiveness, which makes tissues more vulnerable to oxidative and carbonyl stress [4].

The objective of the research was to study the prooxidant-antioxidant changes in blood serum, teeth pulp and oral mucosa in rats with impaired glucose tolerance on the background of iodine deficiency (ID).

Materials and methods

The studies were performed on 60 female rats weighing 150-180 g, which were divided into two research groups, 30 animals in each. The 1st group included rats with insulin

resistance (IR); 2nd - rats with IR on the background of ID. The state of IR was modelled by adding a 10% solution of fructose to the drinking water of animals for eight weeks [8]. ID was reproduced by keeping animals on a diet with limited iodine intake for two months [9]. The control group (n=30) included intact animals, which were kept on a standard diet, normal temperature and light regime of the vivarium.

Carbohydrate metabolism was assessed by blood glucose in fasting conditions, serum immunoreactive insulin (IRI) and glycosylated hemoglobin, followed by calculation of HOMA-IR index (Homeostasis Model Assessment Insulin Resistance). Thyroid status was determined by the content of free triiodothyronine (fT₃), thyroxine (fT₄), thyroid-stimulating hormone of adenohypophysis (TSH), and the index fT₃/fT₄ was calculated. The concentration of iodine was examined to determine the state of iodine supply of rats in single portions of urine, which were collected by the method of exchange cells. The processes of LP in blood serum, teeth pulp and oral mucosa were evaluated by the content of diene conjugates (DC) and active products that react to thiobarbituric acid (TBA-RP). Antioxidant defence of blood serum was characterized by the activity of catalase (K), ceruloplasmin (CP), superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR) and iron transferrin saturation (STr). Energy metabolism was examined by the activity of succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) in blood serum.

Statistical processing of the research results was performed using the mathematical software package StatisticSoft 7.0. Student's t test was used to assess the significance of differences between groups. The difference at p<0.05 was considered as statistically significant.

Table 1. Indexes of carbohydrate metabolism in intact animals, rats with insulin resistance under conditions of adequate iodine supply and iodine deficiency (M±m)

Scheme of the experiment, groups of animals	Glucose, mmol/l	Immunoreactive insulin, mkU/l	Glycosylated Hb, μ mol of fructose/g Hb	HOMA-IR index
Intact animals (control group, n=30)	4.42±0.14	13.71±0.43	3.76±0.41	2.69±0.12
Insulin resistance (1 st group, n=30)	10.72±0.55****	17.86±0.62****	6.92±1.12*	8.50±0.52****
Insulin resistance on the background of iodine deficiency (2 nd group, n=30)	11.80±0.58****	19.49±0.44****	7.88±1.03***	10.22±0.56****

Note: Here and in the following tables * - $p<0.05$; *** - $p<0.01$; **** - $p<0.001$ - a reliable difference between the indexstoanalogical values in intact animals

Results

Impaired glucose tolerance in rats of the 1st research group was indicated by changes of carbohydrate metabolism (Table 1). Thus, in animals with IR an increase of blood glucose by 2.4 times ($p<0.001$), blood serum IRI - by 30.3 % ($p<0.001$) and glycosylated hemoglobin in the blood - by 84.0 % ($p<0.05$) in comparison with analogical indexes in rats of the control group was found. The development of IR was confirmed by elevation of HOMA-IR index by 3.2 times ($p<0.001$) in animals of the 1st research group relative to the corresponding indicators of intact rats.

The significant violations of thyroid homeostasis under the conditions of IR development compared with the control were not found (Table 2).

Impaired glucose tolerance led to the activation of LP processes, mainly due to the accumulation of end products of lipoperoxidation (Table 3). In particular, in rats of the 1st research group an increase in the content of TBA-RP in all studied tissues was noticed. Thus, under such experimental conditions, the content of the final products of LP increased by 70.0 % ($p<0.05$) in blood serum, by 86.8 % ($p<0.001$) in teeth pulp and by 2.4 times ($p<0.05$) in oral mucosa relative to control values. At the same time, the opposite changes in the content of DC, namely a pronounced increase in oral mucosa against the background of a decrease in the teeth pulp relative to the data in intact animals was found.

Activation of peroxide processes under IR conditions was accompanied by a redistribution of the antioxidant enzymes activity (Table 4). In particular, the activity of K in animals of the 1st research group decreased by 66.7 % ($p<0.05$) with a simultaneous increase in GP activity by 63.2 % ($p<0.05$) compared to the similar indexes in the control group of rats.

The development of IR led to the changes of energy metabolism. Thus, in rats of the 1st research group a significant

decrease of LDH and SDH activity by 97.6 % ($p<0.02$) and by 66.3 % ($p<0.05$), respectively, compared to the similar indexes of the control group of rats was found (Table 4). However, the value of MDH in rats with IR underwent opposite changes, as indicated by an increase in enzyme activity by 62.8% ($p<0.02$) relative to baseline.

Combined endocrinopathy was characterized by significant disorders of carbohydrate metabolism, that was indicated by changes in biochemical parameters. Thus, in rats of the 2nd research group an increase in blood glucose by 2.7 times ($p<0.001$), serum IRI - by 42.2 % ($p<0.001$) and glycosylated hemoglobin in blood - by 2.1 times ($p<0.001$) in comparison to the analogical indexes in rats of control group was found (see table 1). Under such experimental conditions, HOMA-IR index increased almost by four times ($p<0.001$) relative to the values in intact animals. It should be noted that the development of IR on the background of ID led to a significant suppression of the functional activity of the thyroid gland, which was manifested by a decrease in fT_3 and fT_4 content by 46.2 % ($p<0.001$) and 42.5% ($p<0.001$), respectively, with a simultaneous increase of TSH concentration by 53.9 % ($p<0.01$) compared to baseline data (see table 2).

Under conditions of these humoral disorders, metabolic processes were accompanied by more pronounced changes. In particular, the intensity of free radical oxidation of lipids increased, mainly due to the final products of lipoperoxidation. Thus, the content of TBA-RP in blood serum, teeth pulp and oral mucosa increased by 4.5 times ($p<0.001$), by 7.8 times ($p<0.05$) and by 6.4 times ($p<0.05$), respectively, compared with the data of the control group of animals (see table 3). It should be noted that an increase in the content of intermediate products of LP was observed only in the pulp of the teeth, where the level of DC was 2.4 times ($p<0.0001$) higher relative to the baseline. During the comparative analysis of

Table 2. Indicators of thyroid status in intact animals, rats with insulin resistance under conditions of adequate iodine supply and iodine deficiency (M±m)

Scheme of the experiment, groups of animals	Free triiodothyronine (fT_3), pmol/l	Free thyroxine (fT_4), pmol/l	Thyroid stimulating hormone (TSH), mIU/l	fT_3/fT_4	Iodine in urine, mcg/l
Intact animals (control group, n=30)	6.60±0.41	29.96±1.26	0.13±0.02	0.22±0.01	99.02±0.92
Insulin resistance (1 st group, n=30)	5.33±0.55*	26.51±2.81	0.17±0.02	0.23±0.01	71.35±1.67****
Insulin resistance on the background of iodine deficiency (2 nd group, n=30)	3.55±0.63***	17.22±1.64**** $p_{1-2}<0.05$	0.20±0.01***	0.29±0.02**	8.63±0.65 **** $p_{1-2}<0.001$

Note: Here and in the following tables ** - $p<0.02$ - a reliable difference between the indexstoanalogical values in intact animals

Table 3. Changes of the diene conjugates (DC) and active products that react to thiobarbituric acid (TBA-RP) content in blood serum, teeth pulp and oral mucosa in intact animals, rats with insulin resistance under conditions of adequate iodine supply and iodine deficiency (M±m)

Scheme of the experiment, groups of animals	DC, cu·ml			TBA-RP, nmol/ml		
	Blood serum	Teeth pulp	Oral mucosa	Blood serum	Teeth pulp	Oral mucosa
Intact animals (control group, n=30)	0.45±0.14	0.07±0.005	0.04±0.01	3.13±0.81	0.38±0.03	0.99±0.14
Insulin resistance (1 st group, n=30)	0.36±0.15	0.03±0.002 ****	0.62±0.02 ^{##}	5.32±0.18*	0.71±0.02 ****	2.38±0.18 ^{##}
Insulin resistance on the background of iodine deficiency (2 nd group, n=30)	0.64±0.24	0.17±0.01**** p _{1,2} <0.001	0.08±0.03 p _{1,2} <0.001	14.01±0.23 **** p _{1,2} <0.001	2.97±0.82*	6.34±1.02*

lipoperoxidation intensity under the conditions of isolated and combined endocrinopathy, the content of DC in the teeth pulp (5.7 times, p_{1,2}<0.001) and TBA-RP in the blood serum (2.6 times, p_{1,2}<0.001) reliably increased.

Activation of free radical reactions under the conditions of IR on the background of ID was accompanied by the depletion of antiradical reserves (see table 4). Thus, in animals of the 2nd research group STr decreased by 46.3 % (p<0.05) relative to control values. The changes of the activity of antioxidant enzymes during the comparative analysis of parameters in rats with combined and isolated endocrinopathies were unidirectional. In particular, the activity of SOD and CP of rats with IR on the background of ID decreased by 19.9% (p_{1,2}<0.05) and 47.0 % (p_{1,2}<0.001), respectively, relative to analogical values of animals of the 1st research group.

The changes in the system of energy metabolism under conditions of combined endocrinopathy were manifested by suppression of the activity of serum dehydrogenases (see table 5). The depression of SDH (by 90.4 %, p<0.01), MDH (by 9.9%, p<0.02) and LDH (97.1 %, p<0.02) activity relative to the indexes of control group of rats was noted. The comparative analysis of the activity of energy synthesis enzymes in animals with impaired glucose tolerance and in rats with changes of hydrocarbon metabolism on the background of ID showed a significant deceleration of the macroergs formation. In particular, the activity of LDH and MDH in animals of the 2nd research group decreased by 71.5 % (p_{1,2}<0.05) and by 44.7% (p_{1,2}<0.05), respectively, compared to the values in rats with isolated IR.

Discussion

Oxidative stress that occurs under IR conditions is the result of autooxidation of glucose, non-enzymatic glycosylation and weakening of antioxidant defence [5]. Conducted researches indicated the activation of LP in animals with IR. Thyroid hormones are known to regulate all metabolic processes in the organism, including carbohydrate metabolism [4, 6]. The analysis of the obtained indexes revealed the more significant violations of glucose utilization in animals with combined endocrinopathy, which confirmed the induction of IR development on the background of hypothyroid dysfunction. Glycation of antioxidant enzymes reduced the effectiveness of defence systems and made cells more vulnerable to free radicals action [3], that was confirmed by activation of peroxide processes in the teeth pulp and oral mucosa of experimental animals. Therefore, the changes of prooxidant-antioxidant homeostasis can disrupt the functional and structural properties of periodontal tissues under conditions of hyperglycemia and ID.

It should be noted that between the changes of carbohydrate metabolism and energy supply of cells close metabolic relationships were revealed. Disorders of energy metabolism under conditions of IR occurred as a result of significant reduction in the production of macroergic compounds due to disruption of respiratory chain and intracellular glucose deficiency. From the other side, hypoxia slowed down the course of glycogenesis reactions and reduced the activity of intracellular glucose transporters [10]. Taking into account that SDH reflected the energy potential of the cell and had a

Table 4. Changes of the activity of serum antioxidant enzymes in intact animals, rats with insulin resistance under conditions of adequate iodine supply and iodine deficiency (M±m)

Scheme of the experiment, groups of animals	Catalase mg H ₂ O ₂ · ml	Superoxide dismutase, IU·mg hemoglobine	Glutathione peroxidase μmol·mg protein	Glutathione reductase, nmol·min·mg protein	Ceruloplasmin, cu	Saturation of transferrin with iron, cu
Intact animals (control group, n=30)	10.85±1.78	35.50±6.99	0.19±0.03	0.17±0.05	56.49±21.43	0.41±0.08
Insulin resistance (1 st group, n=30)	3.61±2.60*	28.00±1.00	0.31±0.04*	0.25±0.05	37.71±1.60	0.37±0.01
Insulin resistance on the background of iodine deficiency (2 nd group, n=30)	6.74±2.53	22.43±1.51 p _{1,2} <0.05	0.45±0.14	0.18±0.05	20.00±2.63 p _{1,2} <0.001	0.22±0.01*

Table 5. Activity of succinate dehydrogenase (SDH), malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) in the serum of intact animals, rats with insulin resistance under conditions of adequate iodine supply and iodine deficiency (M±m)

Scheme of the experiment, groups of animals	SDH, nmol/mg·min	MDH, μmol/(min·mg)	LDH, Mkkat/l
Intact animals (control group, n=30)	39.71±10.60	1.21±0.17	46.19±14.93
Insulin resistance (1 st group, n=30)	13.38±4.12*	1.97±0.20**	1.12±0.23**
Insulin resistance on the background of iodine deficiency (2 nd group, n=30)	3.82±0.15# p ₁₋₂ <0.05	1.09±0.30# p ₁₋₂ <0.05	1.33±0.33**

powerful antioxidant effect, the determination of its activity was not only a biomarker of energy synthesis, but also characterized the oxidative metabolism of cells, including periodontal tissues. The severe suppression of SDH activity in animals with IR on the background of ID may indicate disorders of energy and prooxidant-antioxidant homeostasis under conditions of endocrinopathies, which aggravates the course of dental and maxillofacial system diseases.

Conclusions

The development of IR on the background of ID causes the activation of oxygen-dependent processes in periodontal tissues against the background of suppression of antiradical defence and disruption of energy synthesis. Accumulation of lipoperoxides in teeth pulp and oral mucosa deepens the development of inflammatory and destructive changes, especially in combination with reactogenic influence of glucose excess. Decreased efficiency of energy-synthetic processes under conditions of endocrinopathies can cause hypoxic damage of the dental-alveolar complex components and more pronounced metabolic disorders in them. Therefore, in order to timely diagnose, comprehensive treatment and targeted prevention of dental complications in case of IR and ID development, it is necessary to take into account all links of metabolic disorders that occur in the context of endocrine diseases.

Perspectives for further research

Complex study of all parts of the metabolic imbalance and cascade of biochemical reactions that occur under conditions of IR and ID will expand existing knowledge about the pathophysiological mechanisms of periodontal tissue damage under conditions of endocrinopathies.

Ethics Policy

All experiments were carried out according to the legislation of Ukraine (Law of Ukraine № 3447-IV «On protection of animals from cruel treatment», 2006), the rules of European Convention for the protection of vertebrate animals used in experimental research and for other scientific purposes (Strasbourg, 1986) and approved by the Ethics Commission of Ivano-Frankivsk National Medical University (protocol №94/17 from 16.02.2017).

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Conflict of Interests

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