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# Features of the system of antioxidant protection and lipid peroxidation in microangiopathies against type 2 diabetes mellitus

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## Abstract

The article presents the results of an experimental study of the AOP and LPO system in animals simulated complications of the microcirculatory tract (diabetic retinopathy, diabetic nephropathy) on the background of type 2 diabetes. It was found that in the group of animals with type 2 diabetes increased the level of DC, TBCproducts and MDA; catalase and FG levels decreased. In animals with simulated DN, AOP disorders were more pronounced compared to animals with DR. This trend can be explained by the fact that in addition to hyperglycemia in these animals, the excessive formation of free radicals contributes to metabolic shifts in ischemic areas of tissues and organs. Disturbance of balance in AOP and LPO in type 2 diabetes triggers a pathogenetic cascade of development of complications of the microcirculatory tract and is accompanied by a tendency to further increase the generation of reactive oxygen species and activation of POL in microangiopathies.

**Key words: antioxidant system; diabetes mellitus; diabetic retinopathy; diabetic**

**nephropathy; lipid peroxidation.**

**Introduction.** Incidence of diabetes mellitus (DM) and its prevalence is progressive is increasing in all countries of the world, despite significant achievements in diabetology and huge funds invested by the governments of various countries in scientific research, dedicated to the problem of diabetes, its prevention and complications. According to forecasts experts of the International Diabetes Federation, the number of patients with type 2 diabetes by 2025 year will reach about 380 million people [8]. The main cause of mortality with type 2 diabetes there are macro- and microvascular complications, which are believed to be the cause hyperglycemia, which is the same factor risk of developing atherosclerosis and the heart vascular mortality, as well as the level of total and arterial cholesterol pressure [9].

One of the most important links in pathogenesis of type 2 diabetes and its complications is considered oxidative stress and its cause the result is the intensification of peroxide lipid oxidation (POL). It has been proven that type 2 diabetes is a free radical on pathology. Relevance and not necessity of forecasting violations in system of antioxidant protection of both spoken also by the fact that the cascade is free radical reactions are launched even before clinical manifestation of type 2 diabetes and the first years of the disease [4]. Even in patients with newly diagnosed diabetes of the 2nd type, the activation of oxidative stress is already present. It is manifested by an increase in oxidative modification of biological molecules and intensification of POL.

The degree of oxidative stress closely related to the weakening of the enzymatic antioxidant system (AOS), the duration of the disease and the degree of decompensation of carbohydrate metabolism [8].

Oxidative stress is at the root the development of many late complications of diabetes 2 types, micro- and macroangiopathies and neuropathies. At the same time, the significance of hyperglycemia in the initiation and potentiation of the generation of reactive oxygen species proven both on experimental models and in clinical studies [12].

Oxidative stress, as an imbalance between intensity of formation of active forms oxygen, hydrogen peroxide and free radicals, on the one hand, and the activity of AOS – on the other hand, it plays an important role in the pathogenesis of complications in type 2 diabetes.

However, the impact of disease duration and late complications on the severity of oxidative stress in type 2 diabetes remains an open question and also whether oxidative stress depends on degree of compensation for exchange violations [13, 14].

**The aim of work** – to evaluate parameters of free-radical oxidation in chronic complications (diabetic retinopathy (DR) and diabetic nephropathy (DN) of type 2 diabetes.

**Materials and methods.** Experimental studies performed on 24 white non-linear male rats weighing 240-280 g, which were divided into 4 experimental groups (6 animals in each): 1st group – intact control – animals that were kept on the standard food ration of the vivarium; 2nd group – animals that were reproduced with streptozotocin diabetes [5]; 3rd group – animals, which after administration of streptozotocin and nicotinamide reproduced the DR model [3]; 4th – animals that after administration of streptozotocin and nicotinamide reproduced the DN model [1].

The streptozotocin model was used to reproduce type 2 diabetes: to rats once intravenously administered streptozotocin at a dose of 65 mg/kg [5]. Streptozotocin solution was prepared in 0.1 M citrate buffer pH 4.5. In order to reduce the diabetogenic effect streptozotocin 15 minutes before it introduction, nicotinamide was administered intraperitoneally at a dose of 230 mg/kg, for due to which animals develop moderate and stable basal hyperglycemia. After 1 week, a glucose tolerance test was performed to determine fasting and after 30, 60, 90 blood glucose levels and 120 min after intragastric introduction of 40% glucose solution by dose 3 g/kg and glycemic levels from 9.0 to 14 mmol/l.

DR was reproduced by introduction erythropoietin solution subcutaneously three times per week, 6 Units per 100 g of body weight within 6 months [3]. In animals that were simulated DN, the diet was based on high fat diet for 35-40 weeks animals showed signs of DN – proteinuria, decreased glomerular velocity filtering [1].

Oxidative stress was assessed by the following indicators: 1) the level of TB active products in the liver homogenate by reaction with thiobarbituric acid by the method of Uchiyma M. & Michara M. in modifications of I. A. Volchegorsky (1989) [7]; 2) content of diene conjugates (DC) according to the method of Steel I.D. in modification Skornyakova V.I. (1988) [7]; 3) concentration of malondialdehyde (MDA) according to by the method of I. D. Stalna et al. (1987) [11]; 4) catalase content according to the method of Gavrilyuk M. A. (1988) [7].

The state of the antioxidant protection system was characterized by its content of reduced glutathione (RG) in the homogenate of the liver, which was determined spectrophotometrically by reaction with Elman's reagent [7].

When working with animals, the International Medical Code was observed of ethics (Venice, 1983), "European Convention for the Protection of Vertebrate Animals Used for

Experimental and Other Scientific Purposes" (Strasbourg, 1986), "General Ethical Principles of Animal Experimentation", adopted by the First National congress on bioethics (Kyiv, 2001), Directive 2010/63/EU of European Parliament and Council on the protection of animals used for scientific purposes, the Law of Ukraine "On the Protection of Animals from of cruel treatment" No. 440-IX from 01/14/2020 [10]

Statistical processing of the obtained results was carried out with the help of the "Statistica 10.0" program. The probability of differences between the indicators of the control and experimental groups was determined by Student's and Fisher's tests. The level of reliability was accepted at $p<0.05$.

**Results of study and their discussion.** It is known that chronic hyperglycemia leads to the development of oxidative stress due to increased generation of reactive oxygen species in mitochondria, non-enzymatic glycosylation of proteins and autoxidation of glucose, and the increased level of free fatty acids, which arises as a result of insulin resistance - due to mitochondrial dysfunction, b-oxidation in peroxisomes and lipoperoxidation [4]

In animals of the CP group reliable was observed increasing the content of DC on 47.4% ($p<0.05$) in the liver homogenate compared with by intact control animals, which indicated oxidative damage of lipids hepatocyte membranes, as the above compounds are intermediates and final products of POL, respectively (Fig. 1, Fig. 2).

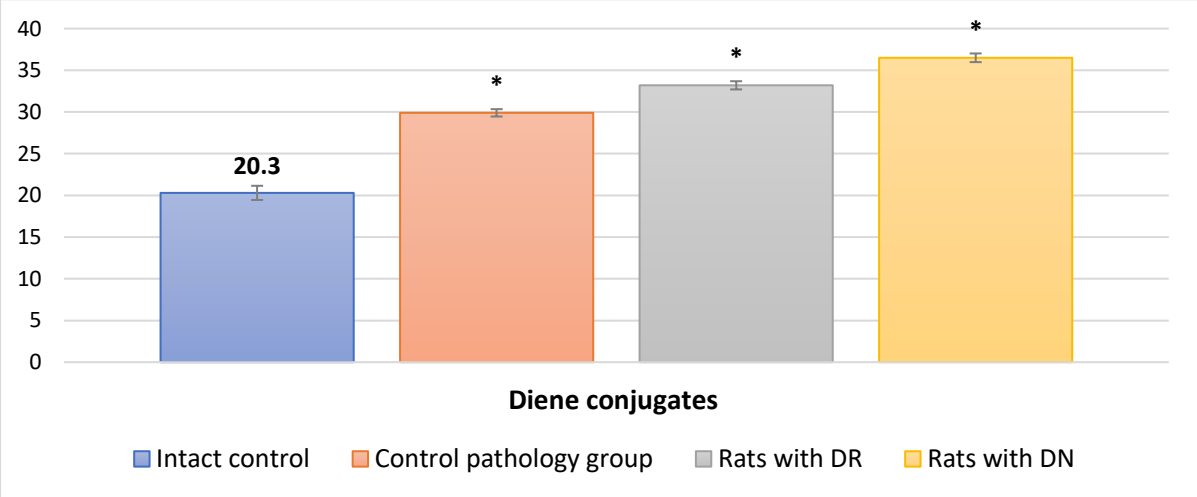


Fig. 1. DC content in rats with background microvascular complications type 2 diabetes

*Note:* * - $p<0.05$ relative to the indicators of the intact group of animals.

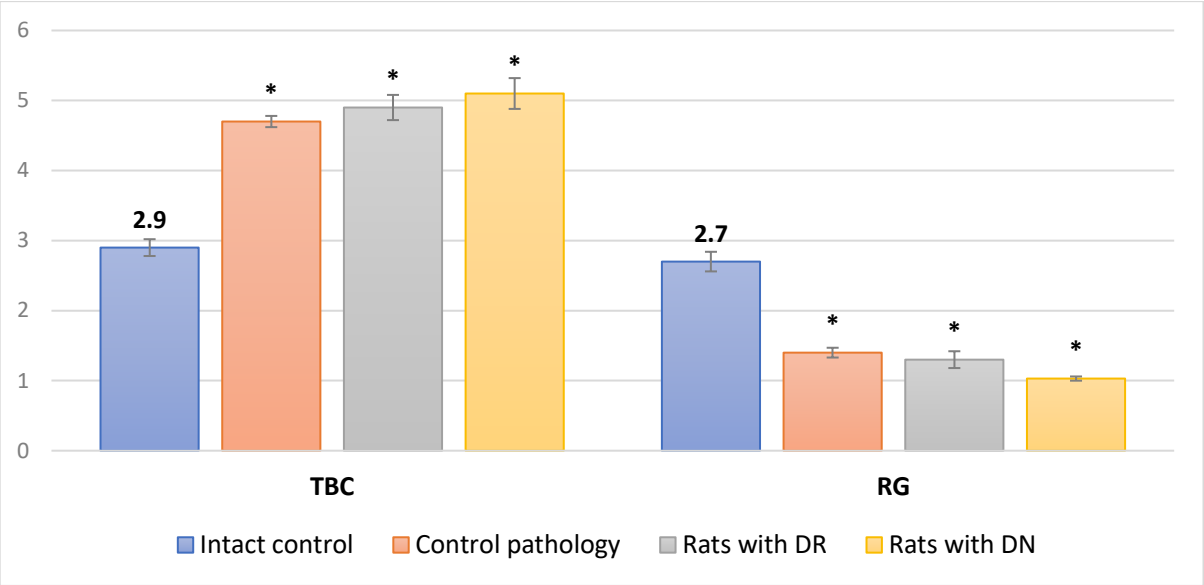


Fig. 2. The content of TBK-AP and RG in rats with microvascular complications against the background of type 2 diabetes

*Note:* * - p<0.05 relative to the indicators of the intact group of animals.

Also was noted the increase of DC in animals that were simulated DR and DN. Their content was in 1.7 times higher (p<0.05) and in 1.8 times (p<0.05) compared to the intact group of rats.

In the group of animals with control pathology, an increase in the content of TBC-reactants was established in 1.6 times (p<0.05) compared to intact control animals. Animals with DR have this indicator increased in 1.7 times (p<0.05), and animals with DN – 1.8 times (p<0.05) compared to intact animals.

At the same time, the development of type 2 diabetes correlated with depletion of antioxidant defenses, as evidenced on 2.0 times decrease in RG in animals with DM type 2 (p<0.05); in 2.1 times in animals with type 2 diabetes and DR (p<0.05); in 2.6 times in animals with type 2 diabetes and DN (p<0.05) relative to a group of intact animals.

The functional basis of antioxidant protection is formed by glutathione system, which includes the glutathione and enzymes that catalyze the reactions of its reverse transformation. RG – the central component of AOS, the main sulfur-containing antioxidant that protects sulfhydryl groups of globin, erythrocyte membrane, divalent iron from the action of oxidants [2].

When studying the indicators of MDA, it was established that its level also changed. In the group of animals with type 2 diabetes, the content MDA was in 3.6 times higher (p<0.05); in animals with DR – in 4.1 times (p<0.05); in animals with DN – in 4.4 times

(p<0.05) relatively a similar indicator in a group of animals intact control (Table 1).

*Table 1*

Study of some indicators of LPO in microangiopathic complications against the background of type 2 diabetes (X±Sx, n=6)

| Indicator | Intact control | Control pathology | Rats with DR | Rats with DN |
|---|---|---|---|---|
| MDA, μmol/l | 4,0±0,2 | 14,2±0,5* | 16,4±1,0* | 17,6±1,1* |
| Catalase, mmol/l | 0,30±0,003 | 0,22±0,015* | 0,19±0,017* | 0,16±0,012* |

*Note:* * - p<0.05 relative to the indicators of the intact group of animals.

Catalase level in rats with type 2 diabetes decreased in 1.4 times (p<0.05); in animals with DR – in 1.6 times (p<0.05); in animals with DN – in 1.9 times (p<0.05) compared to group of intact animals.

So, excess free generation radicals leads to the development of endothelial dysfunction, modification lipoproteins, hyperviscosity and hypercoagulation. In this case, it can be argued that violation of the balance in AOS and POL at diabetes mellitus type 2 triggers a pathogenetic cascade of microcirculatory complications and is accompanied by a tendency towards an even greater increase in the generation of reactive oxygen species and activationPOL.

It is worth noting the tendency to more pronounced oxidative stress in rats with complications of type 2 diabetes. Apart from this, in animals with simulated DN, AOS violation was more pronounced compared to animals with DR. This trend can be explained by the fact that, in addition to hyperglycemia in these animals is excessive the formation of free radicals contributes to metabolic shifts in ischemic zones tissues and organs.

**Conclusions**

1. When studying the indicators of AOS and POL was found that in the group of animals with type 2 diabetes, the level of DC increased, TBK products and MDA; the executioner's level catalasa and RG decreased.

2. In animals with simulated DN, disturbances of AOS was more pronounced compared to animals with DR. This tendency can be explained by the fact that in addition to hyperglycemia in these animals, excessive formation of free radicals contributes to metabolic damage in ischemic areas of tissues and organs.

3. Disturbance of the balance in AOS and POL in type 2 diabetes triggers a

pathogenetic cascade of microcirculatory complications and is accompanied by a tendency to even greater increase in the generation of reactive oxygen species and activation of POL in microangiopathies.

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