ORIGINAL ARTICLE

PATHOGENETIC ASPECTS OF METABOLIC SYNDROME IN EXPERIMENTAL ANIMALS

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ABSTRACT

The aim: Was to study the state of the nitric oxide system, LPO and antioxidant system in the body of experimental animals in simulated metabolic syndrome. The aim of the study was to study the state of the nitric oxide system, lipid peroxidation and antioxidant system in the body of experimental animals in simulated MS.

Materials and methods: The study was performed on 20 white male Wistar rats. Male control rats (n = 10) were fed a normal control diet. Male rats of the main group (n = 10) were fed a diet high in fat (over 60 % energy from fats) for 16 weeks, thus modeling the development of MS. The indicators of the prooxidant and antioxidant system, as well as the nitric oxide system were determined by photospectrographic method.

Results: In animals with simulated MS, intensification of lipoperoxidation (statistically significantly higher level of TBA-active products 1.84 times), depletion of antioxidant protection (statistically significantly lower level of superoxide dismutase 2 times), activation of nitric oxide system (statistically significantly higher NO-synthase level 2.15 times) were found compared with intact animals.

Conclusions: In animals with simulated MS, activation of lipid peroxidation processes, depletion of antioxidant protection and increased levels of nitrooxidative stress were found.

KEY WORDS: metabolic syndrome, peroxidation, free radicals, antioxidant protection, nitrooxidative stress

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INTRODUCTION

The prevalence of metabolic syndrome (MS), which includes a cluster of cardiovascular risk factors associated with obesity and insulin resistance, has recently increased dramatically and has become epidemic in many developed countries. This pathology is characterized by metabolic disorders such as hypertriglycerinemia, decreased levels of high-density lipoprotein, increased levels of low-density lipoprotein, insulin resistance, abnormal glucose tolerance and hypertension, which in combination with genetic predisposition to risk of type 2 diabetes, atherosclerosis and kidney, liver and heart disease [1].

MS is one of the most studied pathologies in the world due to the fact that such metabolic disorders are associated with common diseases of modern man - atherosclerosis, hypertension, type 2 diabetes, obesity. According to modern ideas, MS is characterized by a set of disorders of systemic regulation of lipid, carbohydrate, protein and other types of metabolism under the influence of external and internal factors. Separation of MS is of significant clinical importance, because on the one hand this condition is reversible, and on the other hand, it is necessary to address the tactics of such a patient due to the fact that among people with MS risk of coronary heart disease or stroke 3 times higher, with a significant increase in mortality from cardiovascular disease [2]. The effectiveness of the use of different criteria for the detection of MS is unequal, which requires the necessary discussion and comparative analysis of existing diagnostic criteria and requires further in-depth study of biochemical parameters in MS.

One of the aspects associated with the development of MS and concomitant pathology is the excess of reactive oxygen species, which initiate peroxidation processes and cause damage to various cellular components. The accumulation of products of lipid and carbohydrate metabolism triggers detoxification reactions, including free radical processes [3]. Free radical processes are aimed at maintaining homeostasis, but at high intensity they can lead to the development of oxidative stress. Oxidative stress is one of the triggers that help to activate the body's cellular adaptation. The ratio of the activity of antioxidant systems and the amount of peroxidation products may vary depending on the state of the organism, the effects of various environmental factors. A normal stress response

may be accompanied by a short-term increase in reactive oxygen species. This is due to the reaction of adaptation of the organism in extreme conditions, in which reactive oxygen species play the role of secondary messengers, participating in the transmission of signal transduction, in the expression of a number of genes. The result is a timely mobilization of antioxidant protection, which reduces the level of reactive compounds, thereby preventing the manifestations of their toxic effects [4]. The toxic effect of reactive oxygen species is manifested in conditions of oxidative stress, which is accompanied by a sharp intensification of free radical processes and a decrease in the activity of antioxidant protection. Intensification of free radical processes and the development of oxidative stress is one of the pathogenetic links of many diseases, including cardiovascular, inflammatory, and aging. There are many works devoted to the study of free radical oxidation in various pathological conditions. However, data on the development of oxidative stress in MS is clearly insufficient, and this applies primarily to studies of the activity of antioxidant enzymes [5].

Another, no less important aspect of research in the pathogenesis of MS is the study of the role of mediators of intercellular interaction, which include nitric oxide and its metabolites. Nitric oxide is a universal regulator of various biochemical processes. Previously, the role of nitric oxide was associated only with inflammation, but it is involved in many physiological and pathophysiological reactions of the body, including pure apoptosis reactions. Nitric oxide and its metabolites are essential in the development of complications of MS. It is known that MS is accompanied by endothelial dysfunction, which is characterized by increased production of nitric oxide. Therefore, the increased attention of researchers is focused on this problem. Insufficient or excessive production of nitric oxide characterizes the presence of endothelial dysfunction, which is associated with a violation of the antioxidant system under the action of free radical oxidation [6]. This phenomenon is a major risk factor for the occurrence and complication of various diseases, including MS.

It is known that nitric oxide can cause both protective and damaging effects. It plays a significant role in the processes that regulate the production of free radicals. Its molecule itself as one of the reactive forms of oxygen is involved in the initiation of oxidative stress, which has independent antioxidant properties. However, the pathogenetic mechanisms that explain the role of nitrooxidative stress, lipid peroxidation (LPO) processes, the state of the antioxidant system in the development of MS and its complications have not been fully studied [8]. There are no clear criteria that would allow to have an idea of the course of this pathology, to allow to predict the course of the disease and to prevent undesirable consequences. Equally important is the search for effective, low-cost and prognostically successful methods of treating MS.

Thus, the role of nitric oxide activity, LPO processes, antioxidant enzymes in the pathogenesis of systemic disorders in MS remains unclear.

THE AIM

The aim of the study was to study the state of the nitric oxide system, LPO and antioxidant system in the body of experimental animals in simulated MS.

MATERIALS AND METHODS

The study was performed on 20 white male Wistar rats weighing 200–250 g (age 9–10 weeks), which were kept in standard vivarium conditions (air temperature: (22 ± 2) ° C, humidity - 30-60 %, light / dark cycle: 12/12 hours). Male control rats (n = 10) were fed a normal control diet. Male rats of the main group (n = 10) were fed a diet high in fat (over 60 % energy from fat) for 16 weeks [9], thus modeling the development of MS.

At the end of the experiment, the animals were decontaminated by decapitation under thiopental anesthesia. The experiment complied with the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and the European Union Directive 2010/10/63 EU on animal experiments. The Commission on Bioethics of Ternopil National Medical University named after I. Gorbachevsky (Prototol No. 12 of November 4, 2020) did not find any violations of moral and ethical norms during this study.

Determination of the content of TBA-active products (TBA-AP) was performed using the photospectrographic method. The principle of the method is the ability of secondary products of lipid peroxidation, namely malonic dialdehyde, when interacting with thiobarbituric acid (TBA) at high temperatures in an acidic environment to form a colored complex, the intensity of which is directly proportional to the content of TBA-active products (TBA-AP). Studies have been performed in and serum [10]. The content of TBK-AP was expressed in μmol / l serum.

The level of *ceruloplasmin* (CP) was also determined by photospectrographic method [11]. Principle of the method: oxidation of p-phenylenediamine in the presence of ceruloplasmin leads to the formation of colored products. The amount of ceruloplasmin is proportional to the intensity of the color. The study was subjected to blood serum without traces of hemolysis. The result was expressed in mg / l.

The principle of the method [12] for determining the activity of *catalase* (CT) is based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate. The study was subjected to blood serum. Catalase activity was determined by photospectrometric method and expressed in mcat / l.

The principle of the method for determining the content of *reduced glutathione* (GSH) is that the interaction of 5,5'-dithiobis2-nitrobenzoic acid (Elman's reagent) with free SN groups of reduced glutathione forms a thionitrophenyl anion, the amount of which is directly proportional to the group content of S [13]. The concentration of reduced glutathione in serum was expressed in mmol / l.

The *total antioxidant activity* of blood serum (TAA) was determined by photospectrographic method. The principle

Table I. Indicators of prooxidant-antioxidant system in the serum of experimental rats (M±m)

Indicator	Groups of animals	
	Intact rats (n=10)	Rats with MS (n=10)
	Blood plasma	
CP, mg / l	302,1±14,9	634,5±25,2*
CT, mcat / l	0,88±0,04	2,03±0,78*
ГSH, mmol / l	3,90±0,29	2,03±0,18*
TAA, %	59,43±4,09	32,65±2,36*
TBA-AP, µmol / l	8,50±0,49	15,76±1,09*
	Liver	
SOD, units / g	0,77±0,04	0,36±0,03*

Note: * - statistically significant significance of the difference between indicators compared with the control group.

of the method is the ability of TAA to inhibit the formation of peroxidation products in the homogenate of the rat brain [14]. TAA was expressed in%.

Superoxide dismutase (SOD) activity was also determined by photospectrographic method [15]. Liver tissue homogenate was taken for the study. The activity of the enzyme was determined by its ability to inhibit the recovery of nitrotetrazolium blue. The percentage of inhibition was expressed in units / g.

The total content of *nitrates and nitrites* was determined by the Griss method after reduction of nitrates to nitrites with cadmium [16]. Calculations were performed according to the calibration schedule, using sodium nitrite as a standard. The content of nitrates and nitrites was expressed in mmol / l of blood serum.

The total activity of *NO-synthase* (NOS) in blood serum was determined colorimetrically by the amount of nitrates and nitrites formed in the incubation medium [17]. The amount of nitrates and nitrites formed was determined as described below.

Statistical processing of the obtained research results was processed using the software Excel («Microsoft», USA) and Statisticav.10.1. (Statsoft, USA), by the method of variation statistics using the Mann-Whitney U-test and the Student's test. Changes at $p < 0.05$ were considered statistically significant.

RESULTS

We have established a tendency to intensify lipoperoxidation processes and reduce the protective resources of antioxidant protection. Statistically significantly higher indicators of the content of TBA-AP in the serum of animals with simulated MS were established. As the results of our studies showed, the level of TBA-AP increased statistically significantly 1.84 times in animals with simulated MS (Table 1). There were also statistically significantly lower activity rates of SOD, TAA, and the level of SH-groups in the studied animals with simulated MS compared with intact animals. However, the content of CP and CT activity in our experiment increased. Thus, we found an increase in the content of CP in the blood plasma of animals with simulated MS in 2.1 times compared with the control group of animals. CT activity increased statistically significantly in the study group of animals by 2.3 times compared with the control. At the same time, in our experiment, the activity of SOD (2 times), TAA (1.8 times) and the content of GSH (1.9 times) decreased statistically significantly. This is apparently due to the depletion of the pool of antioxidant enzymes and the negative course of MS in experimental animals. The obtained data show that experimental MS contributes to oxidative and nitrooxidative stress, depletion of the antioxidant defense system.

When evaluating nitric oxide parameters, we recorded the development of endothelial dysfunction in rats with

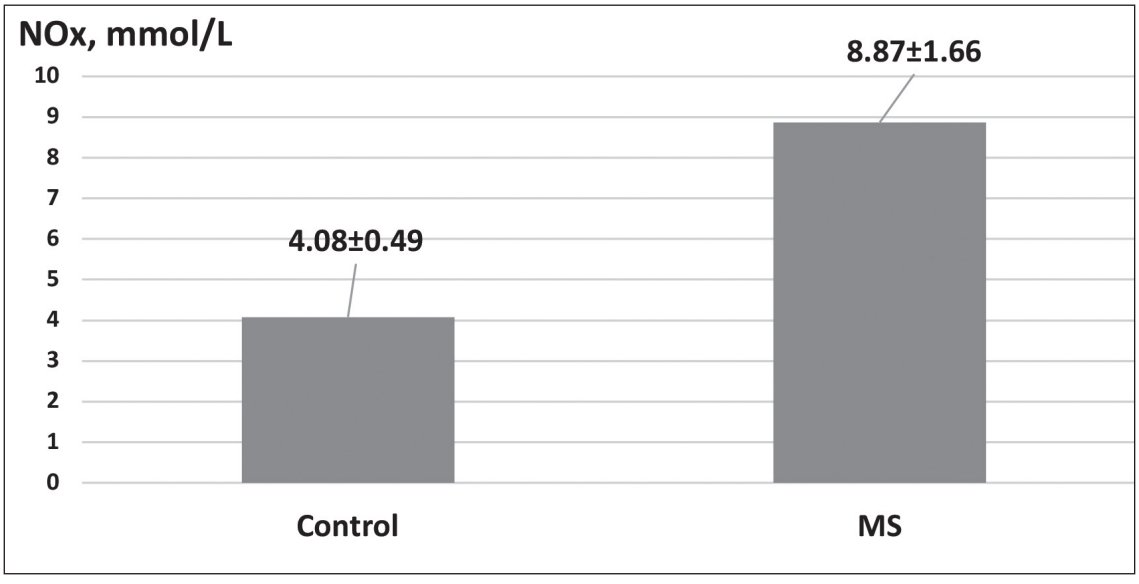

Fig. 1. Concentration of nitrates and nitrites (NOx) in the serum of experimental rats

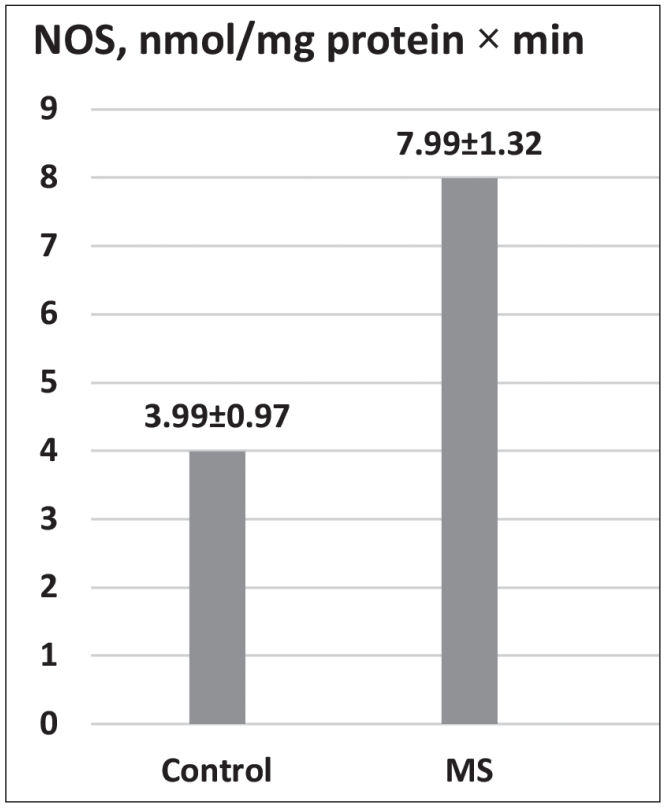

Fig. 2. Indicators of NO-synthase activity in the liver of experimental rats

simulated MS. There was a statistically significant increase in the concentration of nitrates and nitrites (NOx) in the serum of rats with experimental MS in 2 times compared with the control (Fig. 1).

We also found an increase in NO-synthase activity in the liver homogenate of animals by 2.15 times (Fig. 2). It is obvious that the increase in total NO-synthase activity registered by us is a consequence of activation of the inducible form of this enzyme, and the obtained fact of increase of nitrates and nitrites in blood serum of animals with the simulated metabolic syndrome is a consequence of excessive formation of nitric oxide and NOS. quickly converted to NOx.

Thus, the dynamics of the indicators established by us testifies to the development of nitrooxidative stress and imbalance in the prooxidant-antioxidant system in rats with simulated MS.

DISCUSSION

The obtained data show that experimental MS contributes to oxidative and nitrooxidative stress, depletion of the antioxidant defense system. We obtained data increasing the concentration of nitrates and nitrites (NOx) in the serum of rats by 2 times and increasing the activity of NO synthase in the liver homogenate of animals by 2.15 times with experimental MS compared with the control. It is obvious that the registered increase in the total activity of NO synthase is a consequence of activation of the inducible form of this enzyme, and our increase in nitrates and nitrites in the serum of animals with simulated MS is a consequence of excessive formation of nitric oxide and-NOS, which is unstable molecule and rapidly converted to NOx. At the same time, the products of partial oxygen reduction - superoxide-anion-radical - accumulate in the inflammatory focus [11-19]. In MS, high levels of NO in the body lead to interaction with the superoxide anion radical and the release of peroxynitrite anion, which in turn damages the vascular endothelium, which promotes the oxidation of lipids in cell membranes. In the presence of peroxynitrite or its breakdown products, thiol radicals of glutathione are formed, as a result of which the latter of the antioxidant is converted into a prooxidant, thereby initiating the processes of lipid peroxidation. In animals with experimental MS there is an increase in TBA-AP in 1.84 times.

Our data are consistent with the results of a number of studies, which found that in MS conditions there is increased activity of lipoperoxidation, and the oxidative stress that develops acts as an important pathogenetic mechanism of dysregulatory changes in metabolism. Changes in the prooxidant-antioxidant system under experimental MS can also contribute to the progression of metabolic disorders that are accompanied by the accumulation of lipids in cells [20]. Accordingly, free radical oxidation of lipids leads to increased levels of free fatty acids, triglycerides, cholesterol. It is known that the products formed in the intermediate stages of the peroxide cascade, in particular ketodienes and conjugated trienes, have a higher thermodynamic stability, as a result of which they are the initiators of numerous damaging effects at the level of biomembranes [21].

We can predict that the excessive accumulation of toxic products of lipoperoxidation obtained by us may further exacerbate existing damage, preceded by the appearance of more significant shifts in metabolism in MS. Overall antioxidant status is a limiting factor in the increased intensity of LPO. Activation of LPO processes leads to an imbalance in the antioxidant defense system, which deepens the development of complications in MS.

The most common in the literature data is the assessment of the intensity of free radical processes on the concentration of products of intermediate and final peroxidase catalysis: malonic dialdehyde, diene conjugates, ketodienes and trienes. The antioxidant system is studied by the activity of enzymes - SOD, CT, and indicators of non-enzymatic system of antioxidant protection - the content of GHS (SH-groups) [22].

According to the results of our own research, we have obtained data that are consistent with data from domestic and foreign studies. In particular, there is a tendency to intensify LPO processes and to reduce the protective resources of antioxidant protection. There were statistically significantly higher indicators of the content of TBA-AP, as well as statistically significantly lower indicators of activity of SOD, TAA, and the level of SH-groups in the studied animals with simulated MS compared to intact animals. However, the content of CP and CT activity in our experiment increased. Thus, we found an increase in the content of CP in the plasma of animals with

simulated MS in 2.1 times compared with the control group of animals, which may be due to the fact that the specific SOD activity of ceruloplasmin in rats is associated with an additional site for copper ion binding in this enzymes [23-24]. Since CP is to some extent able to inhibit the respiratory explosion of neutrophils due to SOD activity, it can be assumed that this mechanism of protection against oxidative stress associated with inflammation in animals with MS is more pronounced.

Also in our study, we found an increase in catalase activity in animals with MS in 2, 3 times compared with the control group of animals. The increase in the activity of the studied enzyme is associated with the inclusion of compensatory mechanisms of the antioxidant defense system. However, in our experiment, the activity of SOD (2 times), TAA (1.8 times) and the content of GHS (1.9 times) was statistically significantly reduced, which is obviously associated with the depletion of the pool of antioxidant enzymes and negative course of MS in experimental animals.

Under the conditions of our experiment, the level of free radical oxidation and inhibition of antioxidant complexes increased, which leads to the accumulation of free radicals in the serum of animals with simulated MS. In general, the presence of inflammation, hypoxia and oxidative stress enhances the synthesis of cytokines that express inducible NO-synthase, and the latter generates high levels of NO production. Changes in the processes of LPO, the state of antioxadants, the intensity of nitric oxide formation cause the development of MS [25-26]. Therefore, the question of studying the intensity of the course of pathological free radical oxidation, which causes a violation of the integrity of the vascular endothelium in MS, remains relevant. Studies confirm the fact that the activity of free radical reactions contributes to severe vascular endothelial cell dysfunction. In turn, it plays a leading role in the violation of vascular tone and the development of atherosclerotic lesions of the arteries. In this regard, the endothelium, its functions and correction of their disorders require new searches for their correction and prevention of complications of MS [27-28]. Thus, the results indicate the important role of nitrooxidative stress, activation of lipoperoxidation processes, disturbances in the system of pro- and antioxidant protection in the formation and progression of MS and the need to seek its surgical correction.

CONCLUSIONS

In animals with simulated metabolic syndrome, activation of lipid peroxidation processes (at a statistically significantly higher level of TBA-AP), depletion of antioxidant protection (at a statistically significantly lower level of SOD) and an increase in nitrooxidative stress (at a statistically significantly higher level of nitric oxide) were found. These disorders are due to the deepening of metabolic imbalance in the development of metabolic syndrome.

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