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Research Article

Effect of endothelial dysfunction on metabolic syndrome development

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ABSTRACT
Endothelial dysfunction in metabolic syndrome is a closely associated state and forms a vicious circle leading to metabolic and cardiovascular disorders. Inflammation activates the expression of pro-inflammatory mediators of inducible form of NO-synthesis, which leads to the synthesis of peroxynitrite in the body. In the dynamics of experimental metabolic syndrome expressed changes in endothelial dysfunction indicators are observed on the 60th day of experience.

Keywords: metabolic syndrome, endothelium, blood, cytokines, nitrogen oxide, homocysteine, Willebrand factor.

INTRODUCTION

According to WHO, between 40 and 60 million people in Europe suffer from metabolic syndrome (MS). A study was conducted in Sweden and Denmark, which introduced new approaches to the diagnosis of MS. The results demonstrated that even with undisturbed glucose tolerance, MS was detected in 10% of women and 15% of men (Chazova I.E., Mychka V.B., Metabolic syndromyarterial hypertension //Consiliummedicum. - – 2002. - №11. -pp.587–590.). When glucose tolerance develops, the percentage of glucose detection increases to 42% in women and 64% in men, and in the presence of diabetes mellitus to 78% and 84%, respectively (Hayashi T., Boyko E.J., Leonetti D.L.etal. Viscerala diposity and the risk of impaired glucose tolerance. A prospective study among Japanese Americans//DiabetesCare.-2003.-Vol.26.-pp.650-655.) One of the key epidemiological trends is the steady increase in the total population of persons with metabolic syndrome. In this regard, there is a natural increase in the prevalence of lesions of target organs - heart, blood vessels, kidneys A combination of a number of risk factors for the development and progression of cardiovascular disease, including obesity, carbohydrate tolerance, dyslipidemia, proinflammatory and prothrombotic conditions, play a role in the development of these lesions as hemodins in MS (Butrova S.A., 2001); Ginzburg, M.M.; Kryukov, N.N., 2002; Uzbekova, N.R. et al., 2013) increase the risk of serious cardiovascular complications by a factor of 2-4 compared to healthy people (Chasova, I.E.; Mychka, V.B., 2002; Zang, S.L., Chen, X., Hsieh, T.J. et al., 2002).

To date, the Republic of Uzbekistan has developed a pathophysiological model of metabolic syndrome development (S.A.Saidov, 2006) and a system of treatment of patients with metabolic syndrome (N.R.Uzbekova, 2017). Clinical and biochemical peculiarities of MS have been studied quite fully, but the mechanisms of disruption of interorgan and intersystem relations have not been clarified yet, and the role of endothelial dysfunction has not been established.

The analysis of the scientific and medical documentation shows that the available literature does not contain exhaustive data on the role of dysfunction of endothelium, interleukins, and nitroergic system disorders in metabolic syndrome.

The study aims to assess the molecular mechanisms of abnormal oxidation processes in the liver, kidneys and endothelium in the development of metabolic syndrome in the experiment.

MATERIAL AND RESEARCH METHODS

The experiments were performed on 24 male rabbits, each with a bodyweight of 2050-3400 g, divided into 2 groups (12 rabbits each) depending on the purpose of the study and treatment method: the 1st (control) - intact rabbits; the 2nd - animals with a simulated metabolic syndrome.

Metabolic syndrome in rabbits was caused by S.A.Saidov's method (2006). For this purpose, a

5% sugar solution was added to the animals' drinker, and crystalline cholesterol was added to the feed daily in a dose of 250 mg/kg of body weight. At the same time, insulin was administered to rabbits subcutaneously in a dose of 0.1 units/100 g, a day later. The duration of the experiment was 2 months.

The content of cytokines in blood serum was carried out by the method of immunoenzyme analysis in the CRL TMA analyzer of Stat-Fax (USA) using a set of test-systems produced by "Citokin" LLC (St. Petersburg), their content was expressed in pg/ml.

The state of the NO-ergic system in the homogenate of the liver and kidneys was estimated by the content of stable metabolites of NO (NO_2^- и NO_3^-); nitrogen oxide synthase activity and peroxynitrite level (ONO_2^{--}).

The level of NO was determined by the sum of nitrite and nitrate metabolites (NO_2^- и NO_3^-) - the method of P.P.Golikov and others. (2000).

The level of peroxynitrite (ONO_2-) was determined by oxidation with hydroxylamine (NH_2O^-) of the formed peroxynitrite in the reaction $ONOO^- + NH_2O^- \xrightarrow{Cu^{2+}} NO_2^- + NO + H_2O$ (Belkina L.M., Smirnova E.A., Terekhina O.L., Kruglov S. V., Boychuk, E.S. The role of nitrogen oxide in the pathogenesis of alloxane diabetes (in Russian) // Bulletin on Biol. 2012. T. 154, № 11. pp. 555–558.).

Statistical processing of results. Digital material was statistically processed on a personal computer using a package of applications for statistical analysis.

RESULTS OF THE STUDY AND THEIR DISCUSSION

In recent years, real prerequisites have been created to decipher the nature of immunological disorders in various pathologies. It should be noted that in the presence of inflammation, a cascade of immunological reactions is launched, which triggers the activation of proinflammatory mediators. Immunological markers of inflammation include immune activation indices - cytokines and immune complexes. According to the literature, type I lymphocytes mainly synthesize IL-2, INFγ, TNFα and IL13, while type II Th-lymphocytes are mostly IL-4, IL-5, IL-6, IL-12. Cytokines primarily regulate the development of local protective reactions in tissues with the participation of different types of blood cells, endothelium of connective tissue and epithelium. Inflammation develops in response to damage and penetration of pathogens into tissues with the participation of proinflammatory cytokines, which include IL-1, IL-6 and TNF-α, chemokines and some other cytokines. These cytokines are synthesized in the inflammation area, mainly by macrophage cells activated by pathogen cell wall components and in response to tissue damage.

An increase in the level of proinflammatory cytokines in plasma is considered to be a factor that increases the severity of the disease. It has been established that TNF-α is capable of suppressing the contractility of arteriomyocytes and lowering blood pressure [Smirnov A.V., Esayan A.M. Chronic kidney disease: on the road to unity of ideas/// Nephrology. –2002. -№4. - pp.11–17.]. This cytokine is produced mainly by macrophages. One cannot ignore the ability of TNF-α to express Fas-antigen on target cells, thus preparing them for apoptosis.

Cytokines secreted by activated T cells can control macrophage activity, Scavenger receptor expression and metalloproteinase secretion. Moreover, secreted by T cells and macrophages, they modulate smooth muscle cell proliferation, nitrogen oxide formation, and apoptosis.

It should be noted that most cytokines are immunoactive compounds secreted by immunocompetent cells locally in the inflammation zone. The main immunoregulatory effect of cytokines is paracrine, but their systemic effect is known to be endocrine effect [Guidance on endocrine gynecology /Edited by.E.M.Vikhlyaeva. -2000. – p. 586].

Violation of the regulation of the inflammatory process leads to a significant change in its flow. Today, most researchers give priority to cytokines in the regulation of the inflammatory process, which in relation to inflammation are divided into 2 groups: pro- and anti-inflammatory. Cytokines are molecules that change the metabolism and functional activity of cells and are themselves produced by cells; therefore, the change in their level is determined by the state of cytokine producing cells.

The high levels of proinflammatory cytokines - IL-1β, IL-6, IL-8 and TNF-α - have been found to cause a systemic inflammatory reaction, which is accompanied not only by an increase in the production of a number of cytokines but also by a subtle balance between individual cytokines, ligands and their receptors and receptor antagonists. There is evidence of a serious imbalance, depending on the activity of inflammatory and fibrous processes, between IL-1β and its receptor antagonist (IL-6).

TNF-α is the primary mediator of inflammation, which is involved in the pathogenesis of most infectious and immunopathological diseases. TNF-α appears to play a significant role in coordinating the inflammatory response and the cytokine cascade. It increases the phagocytic

activity of micro- and macrophages in low concentrations and stimulates the production of interleukins.

Table 1: Change in the content (pg/ml) of cytokines in blood serum in the dynamics of metabolic syndrome

Group	TNF-α	IL-1	IL-6
Intact	9,21±0,580	23,66±0,881	24,62±1,763
20th day	23,56±0,602***	44,17±3,032***	42,27±1,142***
40th day	27,52±0,291*** ^ ^ ^	48,03±1,510*** ^	46,03±1,771***
60th day	24,65±0,89***°	56,2±3,194*** ^ ^ ^°	59,9±2,843*** ^ ^ ^°°°

Note: * - Differences in the intact group data are significant (*** - P<0.001), ^ - Differences in the intact group data are significant (^ - P<0.05, ^ ^ ^ - P<0.001), ° - Differences in the group data are significant (° - P<0.05, ° - P<0.001)

The results of the study of cytokines content in blood serum in the dynamics of metabolic syndrome development are presented in Table 1. As can be seen from the data presented in the table, the content of TNF-α in serum on the 20th, 40th and 60th day of the study significantly increased in 2.6, 3.0 and 2.7 times, respectively, compared to the intact group. The most pronounced increase in its content is on the 40th day of the study.

The study of IL-1 and IL-6 showed a dynamic increase in their content at all times. IL-1 on the 20th, 40th and 60th day of the study increases in 1.9, 2.0 and 2.4 times compared to the intact group. IL-6 also increases by 1.7, 1.9 and 2.4 times on the 20th, 40th and 60th day of the study, respectively, compared to the intact group.

Therefore, in the blood serum of rabbits with metabolic syndrome, there is a sharp expression of proinflammatory cytokines, especially TNF-α on the 40th day, IL-1 and IL-6 on the 60th day of the experiment.

The effects of TNF-α are probably partly related to its ability to activate the synthesis of NO-synthase, thereby increasing the nitrogen oxide content of the tissues. The expression of the iNOS gene by macrophages, the release of large amounts of nitrogen oxides for a long time and its interaction with superoxide anion, leads to the formation of peroxynitrite, which has an exclusively cytotoxic effect. On the other hand, nitrogen oxide acts as a regulator of the processes of neoplasms, accumulation, maturation and fibrosis of collagen, increases the secretion of cytokines, mediating the growth of fibroblasts and blood vessels, and influences the different links of the repair process. Taking into account the above mentioned, it has caused interest in studying some parameters of NO-ergic system in rabbits with experimental metabolic syndrome.

It is known that inflammation activates the expression of proinflammatory mediators and inducible form of NO-synthase, the combination of which leads to cellular alteration. Detached by activated macrophages and neutrophils in large amounts of nitrogen oxide interacting with superoxide anion, forms peroxynitrite and nitrogen dioxide, hydroxyl anion, etc. have an extremely strong cytotoxic effect.

Nitrogen oxide acts as a regulator of the processes of neoplasms, accumulation, maturation and fibrosis of collagen. It also enhances the secretion of cytokines, which mediates the growth of fibroblasts and vessels, thus affecting the various links in the reparation process. Taking into account the abovementioned, it was interesting to study some parameters of NO-ergic system in the dynamics of metabolic syndrome development.

The results of the study of NO-ergic system blood parameters in the dynamics of metabolic syndrome development are presented in Table 2.

Table 2: Indicators of NO-ergic system of blood in the dynamics of development metabolic syndrome

Group	NO, micromol/L	eNOS, μmol/min/l	iNOS, μmol/min/l	ONO_2^- μmol/l
Intact	7,06±0381	10,36±0,254	0,114±0,013	0,115±0,007
20th day	7,73±0,43	8,36±0,11***	0,86±0,02***	0,29±0,016***
40th day	8,5±0,41*	8,2±0,12***	0,91±0,016***	0,32±0,014***
60th day	4,2±0,31*** ^ ^ ^°	7,2±0,55*** ^	0,99±0,034*** ^ ^ ^°	0,42±0,034*** ^ ^°

Note: * - Differences in the intact group data are significant (*** - P<0.001), ^ - Differences in the intact group data are significant (^ - P<0.05, ^ ^ ^ - P<0.001), ° - Differences in the group data are significant (° - P<0.05, ° - P<0.001)

We found a reliable increase in nitrogen oxide content on the 40th day of the study by 20.4% compared to the intact group. On the 60th day of the study there is a decrease in nitrogen oxide content by 40.8% compared to the intact group.

As noted earlier, iNOS activation promotes cellular alteration and triggers a cascade mechanism of apoptosis induction. According to V.P.Skulachev (2001), individual cell organelles, namely, mitochondria, carrying out breathing and oxidative phosphorylation, undergo apoptosis. Taking into account that the mechanism of action of the toxic effect of nitrogen oxide is based on its binding to hem-containing enzymes and their inhibition, it can be assumed that a high level of oxide will contribute to cell death. However, it is not the nitrogen oxide itself that has a toxic effect, but its metabolites with active oxygen forms, in particular, peroxynitrite.

The results of the study showed a multidirectional change in the activity of eNOS and iNOS in blood serum in metabolic syndrome. The activity of eNOS decreases significantly in all terms of the study in comparison with intact animals. If the activity of this enzyme on the 20th and 40th days of the experiment decreases by 19.3% and 20.8%, then on the 60th day of the study the decrease in its activity is more pronounced in comparison with the previous study terms and amounts to 30.5% in comparison with intact animals.

The activity of iNOS on the 20th and 40th days of the experiment in comparison with intact animals increases significantly 7.5 and 8.0 times. But on the 60th day of the study the activity of this enzyme increases in comparison with intact animals - 8.7 times, $P < 0.05$, and with the previous terms of research in 15.1 and 8.8% respectively.

The increase of ONO_2-comparison with intact animals in the dynamics of metabolic syndrome development was established. On the 20th, 40th and 60th day of the study, the content of peroxynitrite 2.5; 2.8 and 3.6 times respectively increased in comparison with intact animals. From the presented data, it is clear that the most pronounced increase in ONO_2- falls on the 60th day of the study.

Therefore, an important factor of NO-system functioning disorders is the imbalance in the activity of enzymes eNOS and iNOS and, as a consequence, a high level of blood ONOO-.

As noted above, inflammation activates the expression of proinflammatory mediators and inducible form of NO-synthase, the combination of which leads to cellular alteration. Detected by activated macrophages and neutrophils in large amounts of nitrogen oxide, interacting with superoxide anion, forms peroxynitrite and nitrogen dioxide, hydroxyl anion and others, which have an extremely strong cytotoxic effect. At the same time, nitrogen oxide acts as a regulator of the processes of neoplasms, accumulation, maturation and fibrosis of collagen, increases the secretion of cytokines, mediating the growth of fibroblasts and blood vessels, thereby affecting the various links in the process of repair.

Thus, summarizing the results obtained, it can be said that the metabolic syndrome observed activation of proinflammatory cytokines and induced synthesis of peroxynitrite in the body.

The nature of the relationship between endothelial function and surrounding tissues is poorly understood. To date, there is a notion of endothelial dysfunction, which is understood as an imbalance between mediators, providing in normal course of all endothelium-dependent processes. The pathogenetic role of endothelial dysfunction has been proved in a number of the most widespread diseases and pathological conditions, but it has been studied little in chronic liver diseases (Kolomoets N.M., 2001, Petrischev N.N., 2003).

Recently, one of the markers of endothelial dysfunction has been considered vWF, which acts as rheological glue, a kind of bridge, connecting the receptors of the thrombocytic membrane with subendothelial structures of the damaged vessel wall. The Willebrand factor is a complex multidimensional adhesive glycoprotein synthesized by endothelial cells and megacariocytes. The formation of vWF in vessels differs significantly from region to region: in the lungs, heart, skeletal muscles, high levels of t-RNA vWF, and low levels in the kidneys and liver. The most essential function of this factor is that it is a carrier-stabilizer for procoagulant protein FVIII: C, which circulates in serum in the form of non-covalent binding complex and is a protein of adhesion in hemostasis processes. Some studies have found a natural increase in the plasma vWF level at significant vascular lesions (Petrischev N.N., 2003).

The results of the study of endothelial dysfunction are shown in Table 3. As can be seen from the data in the table, the content of the Willebrand factor on the 20th, 40th and 60th day of the study is significantly increased by 50.8; 64.5 and 75.2% ($P<0.05$) compared to the intact group. It follows from the data presented that the content of this factor is most pronounced on the 60th day of the study, as its content is 16.2 and 6.5% higher than on the 20th and 40th days, respectively.

Table 3: Changes in endothelial dysfunction in the blood in the dynamics of metabolic syndrome

Group	FV, mcg/ml	ГЦ, μmol/l	ЭН-1, pg/ml
Intact	9,16±0,443	8,56±0,793	6,57±0,350
20th day	13,81±0,185***	23,63±0,49***	11,93±0,293***
40th day	15,07±0,251***^^^	25,9±0,54***^^	12,5±0,182***
60th day	16,05±0,963***^^^	29,25±1,05***^^^°°	13,9±0,934***^

Note: * - Differences in the intact group data are significant (*** - P<0.001), ^ - Differences in the intact group data are significant (^ - P<0.05, ^^ - P<0.01, ^^^ - P<0.001), ° - Differences in the intact group data are significant (° - P<0.01)

The results of the studies showed a marked increase in the content of HC in the blood in the dynamics of metabolic syndrome. The increase of HC on the 20th, 40th and 60th day of the experiment in 2,8; 3 and 3,4 times in comparison with intact animals was established. As can be seen from these data, the most pronounced increase in HC was established on the 60th day of the experiment. During this period of research, the content of HC was increased by 9.6% (P<0.05) compared to the 40th day of the experiment.

Endothelin-1 (Et-1) is a powerful vasoconstrictor peptide, which also regulates vascular angiogenesis. Et-1 is formed under the influence of many factors (adrenaline, thrombin, angiotensin, vasopressin) and is secreted inside the vascular wall, where specific high-affinity receptors are located [Petrischev N.N., 2003]. Several studies have shown an increase in the concentration of Et-1 in cirrhosis of the liver. According to some authors, Et-1 plays a specific role in modulation of liver vascular resistance, although its significance in the pathogenesis of chronic liver diseases is not quite clear (Petrischev N.N., 2003; Helmy A., Newby D.E., Jalan R., 2003).

In connection with the above, we have studied the content of endotheline-1 in blood serum dynamics of metabolic syndrome development. From the data of table 3.3, it is visible that the content of endotheline-1 increases all terms of research, especially more expressed, its increase is established on the 60th day of the experiment. During this period of research its content in comparison with intact animals was increased by 2.1 times (P<0.05).

Thus, the results of the conducted researches show the development of endothelial dysfunction in the dynamics of metabolic syndrome development. The most pronounced changes in these parameters are established on the 60th day of the experiment.

The conducted researches have shown the important role of endothelial dysfunction in the development of the metabolic syndrome. Endothelial dysfunction in MS is a closely associated condition and forms a vicious circle leading to metabolic and cardiovascular disorders.

The following conclusions can be drawn from the results obtained:

1. In the blood serum of rabbits with metabolic syndrome, there is a sharp expression of proinflammatory cytokines, especially FNO-α on the 40th day, IL-1 and IL-6 on the 60th day of the experiment. Inflammation activates the expression of proinflammatory mediators of the inducible form of NO-synthase, which results in the synthesis of peroxynitrite in the body.
2. In the dynamics of the experimental metabolic syndrome development, the expressed changes of endothelial dysfunction parameters are observed on the 60th day of the experiment.
3. The metabolic syndrome impairs the functioning of the NO-ergic system in the liver and kidneys of experimental animals, which was expressed in an increase in the content of nitrogen oxide and peroxynitrite, a multidirectional change in the activity of endothelial and inducible NO-synthase.

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