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STUDY OF THE PATHOGENETIC MECHANISMS OF THE PLEIOTROPIC ACTION OF ANGIOTENSIN II TYPE 1 RECEPTOR BLOCKERS IN METABOLIC SYNDROME

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Abstract

The article is aimed to study the dynamics of parameters of carbohydrate and lipid metabolism regulation, markers of associated systemic inflammation in experimental model of the metabolic syndrome in rats, and to estimate the effectiveness of its pathogenetic treatment using the angiotensin II type 1 receptor antagonist azilsartan. Materials and methods. The research was performed on 30 white Wistar rats of the specific pathogen-free category weighing 280 - 290 g, age 2.5 months (10 weeks). To induce the metabolic syndrome 24 weeks feeding with 60% fructose content was used. For experimental treatment angiotensin II type 1 receptor antagonist azilsartan ("Edarbi", Takeda, Japan) was used at a dosage of 1 mg/kg per day.

Experimental modeling of metabolic syndrome was accompanied with the development of visceral obesity, alteration of carbohydrate and lipid metabolism such as hyperglycemia, increase of cholesterol and triglyceride levels. Also the changes of the molecular expression of metabolic markers were observed: statistically significant decrease of expression glucose transporter type 4 and peroxisome proliferator-activated receptors. The development of metabolic syndrome was accompanied with associated inflammatory and acute phase systemic reactions. It was confirmed by the increased expression of toll-like receptor 4 and significant increase of C-reactive protein in blood plasma as compared to control levels. It has been shown, that the treatment of experimental metabolic syndrome by the angiotensin II type 1 receptor antagonist azilsartan prevents development of visceral obesity, disorders of regulation of carbohydrate and lipid metabolism and associated inflammatory reaction. We have suggested that this effect is associated with the ability of angiotensin II type 1 receptor antagonists, especially new-generation drugs (azilsartan), to induce peroxisome proliferator-activated receptors activation. Development of metabolic syndrome is accompanied by dysregulation of carbohydrate and lipid metabolism and development of an associated inflammatory response and acute phase reaction. The use of the new generation of an angiotensin II type 1 receptor antagonists (asilsartan) on the background of experimental metabolic syndrome effectively reduces the severity of metabolic and acute phase reactions.

Keywords:*. metabolic syndrome, associated inflammation, angiotensin II type 1 receptor antagonist, azilsartan*

Introduction

The studies devoted to pathogenetic mechanisms of development of metabolic syndrome (MS), as well as the search for new effective methods for its treatment, are among the most urgent tasks of modern medical science. The urgency of this problem is caused byrampant spread of MS all over the world. This problem is especially actual in industrialized countries, where a significant part of the population is exposed to a number of typical risk factors: hypodynamia, eating high-calorie and easily digestible food, chronic stress, etc. For example, according to report of the US National Health and Nutrition Health and Examination Survey for 1999 - 2006, the prevalence of metabolic syndrome in the United States

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was 34% [*Lin S.X., Pi-Sunyer E.X., 2007*]. According to WHO in Europe the spread of MS in men was 27.0%, in women - 19.7% [*Hu G. et al., 2004*]. At the same time, the number of people suffering from MS continues to grow, and according to WHO forecasts it will reach 300 million people by 2025. Another reason for the riveted attention of researchers to the problem of MS is the high risk of developing severe chronically progressive diseases with an unfavorable general prognosis: atherosclerosis, coronary heart disease, hypertension, type 2 diabetes mellitus (DM 2), cancer [*Boden-Albala B et al., 2012; O'Neill S, O'Driscoll L, 2015; Micucci C et al., 2016*].

According to modern concept, MS is a multifactorial pathology with a hereditary predisposition. It is characterized by a complex and multi-component pathogenesis. Hereditary factors are most often associated with the polymorphism of a group of genes responsible for the regulation of carbohydrate and lipid metabolism, immunological reactivity, endocrine regulation [*Hu G.et al.,2004; Sandholt CH. et al., 2015*]. Currently, researchers pay special attention to the genes responsible for the synthesis of peroxisome proliferator-activated receptors (PPAR-γ) proteins, which play a key role in insulin signal transduction [*Attila P et al., 2016; Agrawal N et al., 2018*]. The environmental factors include hypodynamia, an imbalance in the diet with a predominance of light carbohydrates. A key mechanism for the implementation of a genetic predisposition is the development of insulin resistance, which acts as the main marker of MS. In most cases, the main mechanism of insulin resistance is blockade of post-receptor mediators. At the same time, a violation of post-receptor transduction causes compensatory hyperinsulinemia. As the syndrome progresses, β-cells are depleted, and insulin resistance is complicated by insufficient insulin production [*Gobato AO et al., 2014*]. A significant role in the pathogenesis of MS is attributed to toll-like receptors (TLRs), which participate in the first link in immune defense reactions. TLRs recognize nonspecific bacterial antigens in infectious diseases, lipopolysaccharides [*Krikun G et et al., 2012*] of intestinal bacteria in autoendotoxemia, endogenous ligands (free fatty acids, triglycerides) and initiate reactions of innate and acquired immunity. Saturated fatty acids can act as ligands of TLR 4. It has been proven that experimental obesity of mice modeled using a diet rich with fat causes increase the expression of TLR 2 and TLR 4 in fat cells and hepatocytes [*Roncon-Albuquerque R, et al, 2008*]. It has been established, that mutations in the gene encoding TLR 4 significantly increase the risk of atherosclerosis, coronary heart disease increases, but decreases the concentration of circulating pro-inflammatory cytokines, fibrinogen, adhesins, C-reactive protein (CRP) and other inflammatory markers. As a result, activation of TLR 4 leads to subclinical systemic inflammation [*Zhongxia Ren Z et al, 2018*].

Currently, the use of PPAR activators is considered as one of the promising areas of pathogenetic therapy of the MS. Fibrates, thiazolidones, angiotensin II type 1 receptor antagonists(ARB1), which are widely used in clinical practice to treat arterial hypertension and type 2 diabetes, have this effect [*Ernsberger P., Koletsky R. J., 2007*].

Thus, the *purpose* of the research is to study the dynamics of parameters of regulation of carbohydrate and lipid metabolism, markers of associated systemic inflammation in experimental model of the metabolic syndrome in rats, and to estimate the effectiveness of its pathogenetic treatment using the angiotensin II type 1 receptor antagonist azilsartan.

Material and methods

The experimental study was performed on 30 white Wistar rats of the SPF category weighing 280 - 290*g* (FSIBC RAS nursery "Pushchino") age 2.5 months (10 weeks). The experiment was approved by the ethics committee of V.I. Vernadsky Crimean Federal University (protocol No. 1 dated January 17, 2018). To induce the metabolic syndrome the fructose model was used. It is based on feeding with 60% fructose content on the basis of the standard solid feed - FFR (Research Diet, USA) [*Ishimoto T et al, 2012; Park J et al., 2015; Chou C et al., 2017*]. The duration of feeding was 24 weeks. As a drug for experimental correction, the angiotensin II type 1 receptor antagonist azilsartan ("Edarbi", Takeda, Japan) was used at a dosage of 1 *mg/kg* per day. The drug was administered orally using a probe, starting from the 14th week of feeding.

For the study, 3 groups of animals were formed:
1) Metabolic syndrome without correction (n = 10)
2) Metabolic syndrome on the background of the use of Azilsartan ("Edarbi", Takeda) (n = 10)

3) The control group of intact animals (n = 10)

During the experiment, the following research methods were used:

- somatometric - weighing rats, weighing adipose tissue;
- biochemical - routine methods for determining the level of glucose in the blood, lipidogram;
- enzyme-linked immunosorbent assay (ELISA) - expression of TLR4, glucose transporter type 4 (GLUT 4), PPAR-g, C-reactive protein. CUSABIO BIOTECH Co, Ltd sets were used for enzyme-linked immunosorbent assay.

All measurements and studies were carried out using measuring instruments that passed metrological calibration and auxiliary equipment that was certified at the Center for Collective Use of Scientific Equipment "Molecular Biology" of the Medical Academy named after S.I. Georgievsky (structural unit) V.I. Vernadsky Crimean Federal University. Statistical analysis was carried out in the environment of the program Statistica 10.0 using parametric (Student's T-test) and non-parametric criteria (Wilcoxon W-test).

RESULTS

As a result of the research it was found that experimental modeling of the metabolic syndrome is accompanied by visceral obesity in rats, which is one of the main clinical criteria of MS. The mass of visceral fat of rats in the experimental group with the MS model without treatment increased more than twice ($p<0.001$) compared to control group. At the same time the use of azilsartan on the background of the development of MS prevented weight gain. In this group, the rat visceral fat mass was not statistically different from the control values.

It should be noted that the modeling of MS in rats for a long time was not accompanied by the development of hyperglycemia. Apparently this is due to long-term compensation by means of hyperinsulinemia. Since the 18th week only, the glucose level began to rise in comparison to the control, which in our opinion is evidence of the development of insulin resistance. By the 24th week, the average value of blood glucose in rats of the control group by the 24th week of the study was 5.8 mmol/l, while in the group with the MS model, this indicator was 6.8*mmol/l*, which is 17.2% ($p<0.05$) higher than control values. At the same time the average glucose level in MS group on the background of azilsartan use decreased even in comparison to the control and amounted to 5.0 mmol/l, which is 36% ($p<0.01$) lower than in the same group without treatment (Fig. 1).

Among the major markers of metabolic sindrome, an important role belongs to lipid metabolism indicators. The results of the studies showed that experimental modeling of MS is accompanied by characteristic changes in these parameters. Thus, the level of total cholesterol increased by 6.5% ($p<0.01$), and the level of triglycerides increased by 33% ($p<0.001$). The use of azilsartan on the background of MS modeling led to a decrease in the level of total cholesterol by 9.3% ($p<0.05$), and triglycerides - by 30.1% ($p<0.001$) compared with the similar indicators of rats with metabolic syndrome. At the same time indicators reached control values (Fig. 2).

The development of MS was not accompanied by statistically significant changes in the expression of the main transmembrane glucose transporter

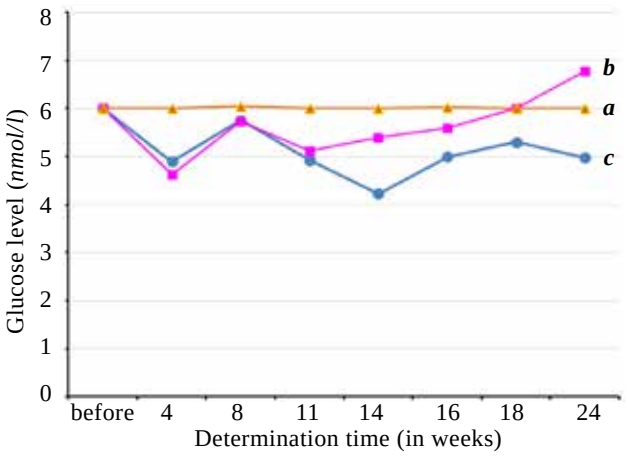

FIGURE 1. *Glucose level in metabolic syndrome*
NOTE: ***a****- control group;* ***b*** *- metabolic syndrom group and* ***c*** *- metabolic syndrom with azilsartan*

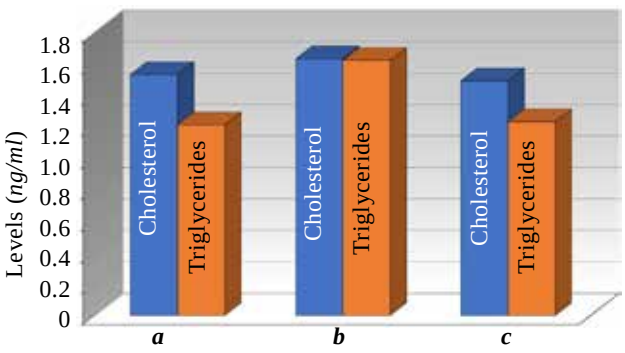

FIGURE 2. *Levels of cholesterol and triglycerides in metabolic syndrome*
NOTE: *Meaning of notation* ***a***, ***b*** *and* ***c*** *- as in Figure 1.*

GLUT 4 as compared to control values. However, the treatment of experimental MS by the administration of azilsartan led to an increase in the expression of GLUT 4 by more than 13 times ($p<0.001$) compared to a similar model without treatment.

A similar trend was observed for PPAR-γ receptors. The experimental modeling of MS was accompanied with the decrease of PPAR-γ expressionby 49% ($p<0.01$) compared to control level. Use of azilsartan on the background of development of MS caused increase PPAR-g expression more than 3 times ($p<0.001$), significantly exceeding the level of the control group (Fig. 3).

The development of the metabolic syndrome was accompanied by the development of an associated inflammatory response. This is statement can be based on a 4-times increase of the TLR 4 expression ($p<0.001$). The use of azilsaratan as a pathogenetic treatment drug led to a decrease in the level of TLR 4 expression by more than 5 times ($p<0.001$) compared with the same group without treatment. At the same time, the indicator approached the control level (Fig. 4).

In our study C-reactive protein was used as the marker indicating development of the systemic acute phase reaction in MS. The results of our studies showed that in experimental model of MS this parameter increased more than 3 times ($p<0.001$) compared with the control level, while in case of development of MS on the background of experimental treatment by the angiotensin II type 1 receptor antagonists CRP increased only 66, 8% ($p<0.01$) higher than control level. Thus, the level of CRP was 2 times lower ($p<0.001$) compared to the group with the MS model without treatment (Fig. 5).

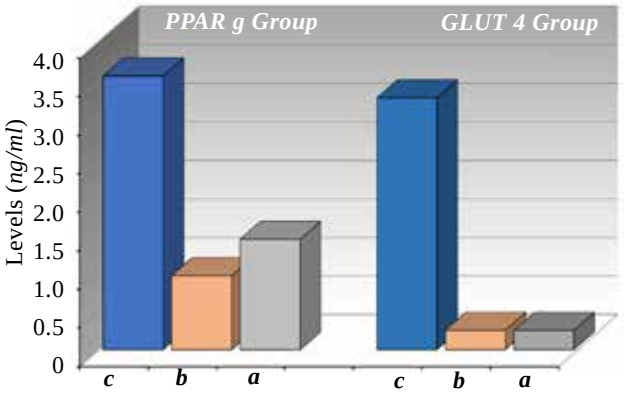

Figure 3. *Molecular expression in metabolic syndrome*
Note: *Meaning of notation* ***a***, ***b*** *and* ***c*** *- as in Figure 1.*

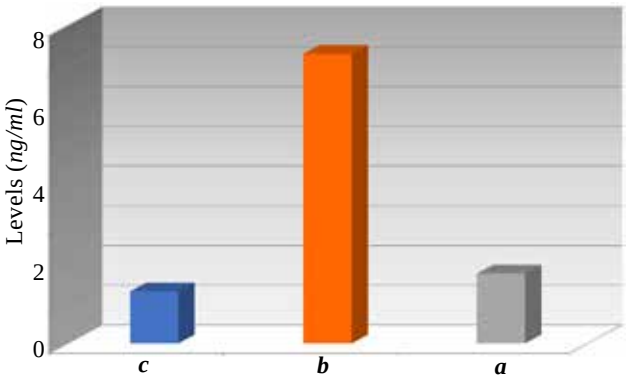

Figure 4. *TLR 4 expression in metabolic syndrome*
Note: *Meaning of notation* ***a***, ***b*** *and* ***c*** *- as in Figure 1.*

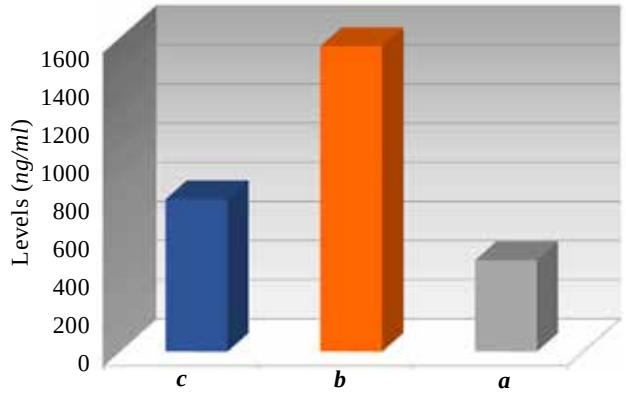

Figure 5. *CRP concentration in metabolic syndrome*
Note: *Meaning of notation* ***a***, ***b*** *and* ***c*** *- as in Figure 1.*

Discussion

The trends of the parameters which were studied in the MS model demonstrated the complete viability of the model based on feeding solid food with 60% fructose content. Most of the indicators used as markers of MS showed an expected response to the application of this experimental model. In addition, studies have shown that the use of azilsartan since the 14th week of MS induction not only corrects metabolic and functional disorders, but often prevents their development. Thus, a two-fold statistically significant weight gain of visceral adipose tissue of rats in the model of MS was actually prevented by the administration of azilsartan. We have suggested that this effect is associated with the ability of angiotensin II type 1 receptor antagonists, especially new-generation drugs (azilsartan), to induce PPARγ activation. PPARγ is known to play an important role in the regulation of the differentiation and functioning of adipocytes and of lipids inside them. PPARγ along with SREBP-1c (sterol regulatory element binding protein) and hepatic receptor X (LXRα), regulates

the expression of genes that control the level of fatty acids in adipocytes [*Ahmadian M. et al., 2013; Lu Han et al., 2017*]. If the assumption about the ability of ARB1 to activate PPARγ is true, then it is logical to assume that the lack of an increase in blood glucose levels in the simulation of MS against the background of azilsartan is due to the same effect. This may be due to the fact that PPARγ enhances the expression of the main transmembrane glucose transporter GLUT 4. This was confirmed by the results of our study, which indicate a parallel multiple increase in both PPARγ and GLUT 4 in experimental modeling of MS on the background of azilsartan use.

To date, the fact of the participation of the systemic inflammatory component in the pathogenesis of MS has been confirmed by numerous studies. A significant role in this process belongs to TLR. Of particular interest is the participation of TLR 4 in the regulation of the secretion of adipokines - hormonally active substances secreted by adipocytes and performing an important function in the regulation of physiological and pathological processes both locally, in adipose tissue, and at the system level. It has been experimentally proven that TLR 4 adipocytes can be activated by the action of free fatty acids followed by the launch of NF-κB signaling pathways, which caused the secretion of monocytic chemotactic protein (MCP-1), as well as adiponectin. According to the literature, adiponectin exhibits antagonistic relationships with TLR 4, and feedback is observed between them. It is known that adiponectin inhibits the production of pro-inflammatory cytokines, such as TNF-α, the synthesis of which is activated by lipopolysaccharides [*Ahmadian M. et al., 2013*]. Thus, it is logical to assume that TLR 4 acts as a link between metabolic disorders and the development of a systemic inflammatory response. The results of our study showed that the modeling of the metabolic syndrome is accompanied by a multiple increase in the molecular expression of TLR 4. At the same time, the use of the ARB1 azilsartan preparation significantly prevented the growth of the studied marker. Similar trend was characteristic for another marker of systemic inflammation – CRP, which is considered to be the classical criterion for the intensity of acute phase reactions.

A characteristic feature of the experimental metabolic syndrome is the fact that the applied model is the result of exposure to solely environmental factors. A hereditary factor does not take part in its formation, in particular, the polymorphism of the genes responsible for the expression of factors regulating both metabolic processes and the associated inflammatory component. This gives an indirect basis to suggest the priority of the role of modifiable environmental factors in the development of the metabolic syndrome. This suggestion has unconditional reservation on the species specificity of both the genes responsible for the regulation of carbohydrate and lipid metabolism and the nature of the usual diet. It is also noteworthy that the preparation ARB1 azilsartan, not having the stated direct metabolic or anti-inflammatory effects, has a expressive corrective effect on the development of MS.

CONCLUSION

1. The obtained results indicate that experimental modeling of the metabolic syndrome is accompanied by dysregulation of carbohydrate and lipid metabolism, which are characterized by the development of hyperglycemia, hypercholesterolemia, a decrease in the molecular expression of the main transmembrane transporter GLUT 4 and PPAR-g receptors.

2. Modeling of MS is accompanied by the development of an associated inflammatory response and acute phase reaction, as evidenced by an increase in TLR4 levels and CRP levels in blood plasma.

3. The use of the new generation of an angiotensin II type 1 receptor antagonists (asilsartan) on the background of experimental modeling of MS effectively reduces the severity of metabolic and acute phase reactions.

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