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ROLE OF NF-KB IN CONNECTIVE TISSUE DEGRADATION IN RAT HEART DURING EXPERIMENTAL METABOLIC SYNDROME

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In recent years more attention has been paid to regulatory functions of connective tissue's extracellular matrix. The aim of this study is to evaluate influence of NF-κB activation inhibitor on concentration of different fractions of glycosaminoglycans, L-hydroxyproline and sialic acids in rat heart during metabolic syndrome modelling. Research was conducted on 24 male Wistar rats weighing 190–260 g divided into 4 groups: control group; ammonium pyrrolidinedithicarbamate (PDTC) administration group; metabolic syndrome group (MetS group); combined administration of PDTC and MetS modelling group. Administration of PDTC during MetS modelling decreased total glycosaminoglycans, heparin-heparan and keratan-dermatan fraction concentration by 22.53 %, by 21.66 % and by 59.64 %, respectively, but increased chondroitin fraction by 62.1 % compared to MetS group. Concentration of L-hydroxyproline and sialic acids decreased by 26.95 % and by 17.37 %, respectively. Activation of nuclear transcription factor NF-κB in rat heart during metabolic syndrome modelling leads to increased intensity of extracellular matrix degradation.

Key words: NF-κB, connective tissue, heart, glycosaminoglycans, L-hydroxyproline, metabolic syndrome.

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РОЛЬ NF-KB У ДЕГРАДАЦІЇ СПОЛУЧНОЇ ТКАНИНИ В СЕРЦІ ЩУРІВ ПІД ЧАС ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ

В останні роки все більше уваги приділяється регуляторним функціям позаклітинного матриксу сполучної тканини. Метою цього дослідження була оцінка впливу інгібітора активації NF-κB на концентрацію різних фракцій глікозаміногліканів, L-гідроксипроліну та сіалових кислот у серці щурів під час моделювання метаболічного синдрому. Дослідження проводили на 24 щурах-самцях лінії «Вістар» масою 190–260 г, розділених на 4 групи: контрольна; група введення амонію піролідиндитікарбамату (ПДТК); група метаболічного синдрому (група МетС); група комбінованого введення ПДТК і моделювання МетС. Застосування ПДТК під час моделювання МетС зменшувало загальний вміст глікозаміногліканів, концентрацію гепарин-гепаранової та кератан-дерматанової фракцій на 22,53 %, на 21,66 % та 59,64 % відповідно, але збільшувало концентрацію хондроїтинової фракції глікозаміногліканів на 62,1 % порівняно з групою МетС. Концентрація L-гідроксипроліну та сіалових кислот зменшилася на 26,95 % та 17,37 % відповідно. Активація ядерного фактора транскрипції NF-κB у серці щурів під час моделювання метаболічного синдрому призводить до підвищення інтенсивності деградації позаклітинного матриксу.

Ключові слова: NF-κB, сполучна тканина, серце, глікозаміноглікани, L-гідроксипролін, метаболічний синдром.

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Connective tissue plays an important role in heart physiology: starting from being a part of the structure of the heart valves and finishing by being one of the regulators of heart regeneration and hypertrophy. In recent years more attention is paid to regulatory functions of connective tissue's extracellular matrix (ECM). It was shown in the study of Johnson B.B. et al., that perlecan, a heparan sulfate proteoglycan, is vital for cardiomyocytes differentiation and maturation [5]. Haploid insufficiency of genes responsible for perlecan synthesis leads to reduced heart tissue thickness and force generation, while cardiomyocytes grown on a perlecan-rich environment in presence of abundance of amino acids are enlarged and display features typical of hypertrophic growth [5]. Cardiac ECM is critically involved in cardiac homeostasis, which can be proven by the fact, that accumulation of chondroitin sulfate glycosaminoglycans exacerbates heart failure by augmenting inflammation and fibrosis during chronic heart failure due to pressure overload [4]. However, at acute stage of pressure overload chondroitin sulfate glycosaminoglycans play a cardioprotective role [4]. Therefore, changes in heart glycosaminoglycans fractions may play a key role in pathogenesis of many heart diseases.

Metabolic syndrome (MetS) is a complex condition involving disturbances in synthesis and utilization of many energetic substrates, often resulting in increased insulin resistance. MetS is often complicated by heart failure due to development of oxidative damage to the heart tissues [11]. Oxidative stress development regardless of organ or tissue is controlled/mediated by redox-sensitive transcriptional

factors like NF-κB, Nrf-2, STAT-3, SIRT-1 etc. [13]. It was proven, that transcriptional factor NF-κB plays an important role in cardiac remodeling and has influence on the structural composition of heart ECM [1]. However, the exact role of NF-κB activation during metabolic syndrome development in remodeling of heart ECM remains elusive.

The purpose of the study was to evaluate influence of NF-κB activation inhibitor on concentration of different fractions of glycosaminoglycans, L-hydroxyproline and sialic acids in in rat heart during experimental metabolic syndrome modelling.

Materials and methods. Our study was performed on 24 male Wistar rats weighing 190–260 g. Animals were procured from Vivarium of Poltava State Medical University. For experimental purposes animals were divided into 4 groups containing 6 animals each. First group was a control group. Second group was ammonium pyrrolidinedithicarbamate (PDTC), a selective inhibitor of transcriptional factor NF-κB activation, administration group. Third group was experimental metabolic syndrome group (MetS group). Forth group was group of combined administration of PDTC and MetS modelling. Metabolic syndrome was induced by method of Mamikutty N. et al., which involved changing drinking water to 20 % fructose solution for 60 days [6]. PDTC was administered intraperitoneally at a dose 76 mg/kg thrice a week for 60 days [8].

The object of the study was 10 % tissue homogenate of rat heart. Tissue homogenate was prepared with 0.2 M Tris-buffer solution (pH=7.4) with ration 1 g of tissue to 9 ml of buffer solution.

In 10 % heart tissue homogenate following parameters were studied: total concentration of glycosaminoglycans (GAG), concentration of heparin-heparan fraction of GAG, concentration of keratan-dermatan fraction of GAG, concentration of chondroitin fraction of GAG, concentration of free L-hydroxyproline, concentration of sialic acids [9].

In order to evaluate metabolic syndrome development in rat blood we studied: concentration of glucose, triglycerides (TG), total cholesterol (TC), concentration of low and very low density lipoproteins (LDL-C) and high density lipoproteins (HDL-C). For evaluation of all blood parameters we used respective assays produced by Filisit Diagnostika (Ukraine). We calculated insulin resistance indexes (TyG and TG/HDL-C) according to recommendations of Zhang Y. et al. [15].

Statistical analysis was performed in Microsoft Office Excel program with extension Real Statistics 2018. To evaluate statistical significance of differences between groups Kruskal-Wallis ANOVA method followed by pair-wise Mann-Whitney were used. To avoid multiple comparisons error Bonferroni correction was used. Differences were counted as statistically significant if $P<0.05$.

Results of the study and their discussion. Administration of PDTC to rats led to decrease in glucose and LDL-C concentration in blood by 8.0 % and 15.6 % respectively compared to control group (Table 1). Modelling of MetS led to development typical metabolic changes in blood of rats: hyperglycemia, hyperlipidemia, dyslipidemia and development of insulin resistance, as evidenced by increase of TyG and TG/HDL-C indexes compared to control group of animals. Abovementioned changes proved the development of MetS and effectiveness of selected experimental model.

Table 1

Metabolic changes in rat blood and insulin resistance indexes under conditions of metabolic syndrome and blockade of NF-κB activation by PDTC (M±m)

Parameters	Groups			
	Control, n=6	PDTC administration, n=6	MetS, n=6	PDTC administration on the background of MetS, n=6
Glucose, mg/dL	70.02±1.10	64.47±0.70 *	148.30±1.99 *	99.45±2.53 */#/^
TG, mg/dL	79.30±5.06	90.42±5.09	243.09±4.96 *	168.98±11.13 */#/^
TC, mg/dL	45.64±0.55	46.58±1.51	68.32±0.94 *	57.24±0.68 */#/^
LDL-C, mg/dL	6.48±0.19	5.47±0.21 *	10.66±0.58 *	7.85±0.36 */#/^
HDL-C, mg/dL	21.38±0.60	22.61±0.35	14.83±0.38 *	20.52±0.58 #/^
TyG	7.92±0.07	7.97±0.05	9.80±0.02 *	9.02±0.08 */#/^
TG/HDL-C	3.75±0.33	3.99±0.20	16.48±0.75 *	8.31±0.69 */#/^

Note: TG – triglycerides, TC – total cholesterol, LDL-C – concentration of low and very low density lipoproteins, HDL-C – high density lipoproteins, PDTC – Ammonium pyrrolidinedithiocarbamate, MetS – metabolic syndrome.

* – the data are statistically significantly different from the control group ($P<0.05$).

– the data are statistically significantly different from the experimental metabolic syndrome group ($P<0.05$).

^ – the data are statistically significantly different from the group of PDTC administration ($P<0.05$).

Administration of PDTC on the background of MetS modelling led to decrease of blood sugar level, removed hyperlipidemia, lowered the insulin resistance and improved the balance between low-density lipoproteins and high-density lipoproteins. Thus, administration of PDTC may be considered as an effective means of correction of glucose and lipid changes in blood induced by metabolic syndrome.

Administration of PDTC to rats did not change the levels of free L-hydroxyproline and sialic acids compared to control group of animals (Tab. 2). At the same time administration of PDTC increased total GAG level by 8.6 % and led to increase of heparin-heparan and keratan-dermatan fractions by 36.57 % and 20.14 %, respectively, while concentration of chondroitin fraction decreased by 7.7 % compared to control group.

Table 2

Parameters extracellular matrix degradation in the heart of rats under conditions of metabolic syndrome and blockade of NF-κB activation by PDTC (M±m)

Parameters	Groups			
	Control, n=6	PDTC administration, n=6	MetS, n=6	PDTC administration on the background of MetS, n=6
GAG concentration:				
Total, μmol/l	1.62±0.01	1.76±0.01*	2.53±0.01*	1.96±0.03 */#/^
Heparin-heparan fraction, μmol/l	0.361±0.005	0.493±0.004*	0.577±0.009*	0.452±0.004*/#/^
Keratan-dermatan fraction, μmol/l	0.442±0.006	0.531±0.003*	1.405±0.010*	0.567±0.004 */#/^
Chondroitin fraction, μmol/l	0.834±0.012	0.770±0.004*	0.543±0.005*	0.880±0.009 */#/^
Concentration of free L-hydroxyproline, μmol/g	0.473±0.008	0.448±0.021	0.757±0.046*	0.553±0.022*/#/^
Concentration of sialic acids, mg/g	5.81±0.03	5.71±0.16	7.60±0.33*	6.28±0.03 */#/^

Note: PDTC – Ammonium pyrrolidinedithiocarbamate, MetS – metabolic syndrome.
* – the data are statistically significantly different from the control group (P<0.05).
# – the data are statistically significantly different from the experimental metabolic syndrome group (P<0.05).
^ – the data are statistically significantly different from the group of PDTC administration (P<0.05).

Modelling of MetS led to increase of concentrations of free L-hydroxyproline, sialic acids and total GAG in the heart of rats compared to control group. The highest increase in concentration of GAG was observed in keratan-dermatan fraction, while content of chondroitin fraction in the heart of rats decreased.

Administration of PDTC on the background of MetS modelling led to increase of total GAG concentration in the rat heart by 20.99 % compared to control group, and by 13.36 % compared to PDTC administration group, while compared to MetS group total GAG concentration decreased by 22.53 %. Concentration of heparin-heparan fraction of GAG under these conditions increased by 25.21 % compared to control group, decreased by 8.3 % compared to PDTC administration group and by 21.66 % compared to MetS group. Concentration of keratan-dermatan fraction of GAG under these conditions increased by 28.28 % compared to control group and by 6.8 % compared to PDTC administration group, but decreased by 59.64 % compared to MetS group. Concentration of chondroitin fraction of GAG under these conditions increased by 5.5 % compared to control group, by 14.29% compared to PDTC administration group and by 62.1 % compared to MetS group.

Concentration of free L-hydroxyproline in the rat heart under conditions of PDTC administration on the background of MetS modelling increased by 16.91 % compared to control group and by 23.44 % compared to PDTC administration group, but decreased by 26.95 % compared to MetS group. Concentration of sialic acids in the rat heart under conditions of PDTC administration on the background of MetS modelling increased by 8.1 % compared to control group and by 10.0 % compared to PDTC administration group, but decreased by 17.37 % compared to MetS group.

Increase in collagenolytic activity observed in MetS group, as evidenced by increase of L-hydroxyproline concentration in heart tissue, is coherent with study of Olgar Y. et al. [10]. Olgar Y. et al. showed in their research that high carbohydrate fed rats have increased activities of matrix metalloproteinases (MMP) 2 and 9 [10]. Decrease in L-hydroxyproline observed in group of combined influence of PDTC and MetS can be explained by ability of PDTC to inhibit activation of transcriptional factor NF-κB, which directly controls the expressions of genes of MMP-2 and MMP-9 [14]. The absence of statistically significant decrease in L-hydroxyproline concentration in group of PDTC administration is likely connected to the fact, that physiological remodeling of connective tissue ECM is performed by other types of MMP, which is not controlled by transcriptional factor NF-κB.

Increase in concentration of sialic acids in heart tissues during experimental modelling of metabolic syndrome can be the result of desialization process caused by elevated activity of different isoforms of neuraminidase. It was proven, that activation of neuraminidase-1 contributes greatly to development of heart failure, and, taking into account the results of our study, we can assume, that activation of neuraminidase-1 is one of the mechanisms of heart failure development during metabolic syndrome [12]. Transcriptional factor NF-κB does not have direct control over neuraminidase-1 expression. On contrary, neuraminidase-1 over expression may cause activation of transcriptional factor NF-κB. Being a redox-sensitive, transcriptional factor NF-κB is connected with vicious circle of reactive oxygen species production (ROS), which in turn may form a mutually stimulating circle with neuraminidase-1 expression [2]. Therefore, a decrease in sialic acids content observed in our study under conditions of combined

influence of PDTC and MetS is most likely connected to the ability of PDTC to inhibit transcriptional factor NF-κB activation, thus lowering ROS production and decreasing neuraminidase-1 expression by breaking ROS- neuraminidase-1 vicious circle.

An increase in concentration of heparin-heparan fraction of GAG in rat heart during metabolic syndrome can be viewed as adoptive response since GAGs of this fraction have beneficial effects on myocardium [5]. According to the results of our research chondroitin-mediated mechanism of heart failure development during our model of metabolic syndrome is highly unlikely, since concentration of this fraction of GAG is decreased during metabolic syndrome modelling.

The limitation of our study is that we did not evaluated separate concentration of keratan sulfate and dermatan sulfate fractions of GAG in rat heart. The reason for increase of keratan-dermatan fraction of GAG in MetS group may be connected to several factors: either increase in Lumican (keratan sulfate derivate) concentration or increase in Endocan (dermatan sulfate derivate) concentration or their combination. Both Endocan and Lumican are markers of developing heart failure [3, 7]. Therefore, increase in concentration of keratan-dermatan fraction of GAG in MetS group can be considered a negative prognostic sign, while its decrease in group of combined influence of PDTC and MetS proves effectiveness of PDTC in treatment of MetS-induced influence on GAG content in rat heart.

Conclusion

Activation of nuclear transcription factor NF-κB in rat heart during metabolic syndrome modelling leads to increased intensity of extracellular matrix degradation as evidenced by increase in total glycosaminoglycans and sialic acids, as well as, by heightened collagenolysis.

Pharmacological blockade of NF-κB activation by ammonium pyrrolidinedithicarbamate is an effective mean of countering of changes in lipid and glucose metabolism in blood and useful tool for prevention of extracellular matrix degradation in rat heart under conditions of metabolic syndrome development.

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