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INFLUENCE OF P38 INHIBITOR ON OXIDATIVE STRESS DEVELOPMENT IN RAT BICEPS FEMORIS MUSCLE DURING EXPERIMENTAL METABOLIC SYNDROME MODELING

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Oxidative stress is a typical pathological process, which underlies development of many diseases. Metabolic syndrome disrupts physiological supply of metabolites to the cell leading to energy deficiency, due to low grade inflammation development, and oxidative stress, caused by mitochondria impairment. It has been already shown that redox sensitive transcriptional factors like NF-κB play a crucial role in pathogenesis of metabolic syndrome and its complications. At the same time, there are few mentions of role of mitogenic cycle controlling factors such as p38-MAPK cascade in pathogenesis of metabolic syndrome. The purpose of the study was to evaluate influence of SB203580 administration on production of superoxide anion radical, activities of antioxidant enzymes, intensity of lipid peroxidation and protein damage in rat biceps femoris muscle during metabolic syndrome modeling. Studied basic superoxide anion radical production, its production from microsomal electron transport chain, its production from mitochondrial electron transport chain, superoxide dismutase and catalase activity, concentration of malondialdehyde and oxidatively modified proteins. SB203580 administration to animals, on which we modeled metabolic syndrome, led to decrease in basic superoxide production in rat biceps femoris muscle by 25.7 % compared to metabolic syndrome group. Superoxide anion radical production from microsomal electron transport chain decreased by 16.0 % and from mitochondrial electron transport chain decreased by 14.6 % compared to indicators of metabolic syndrome group. SB203580 administration to animals, on which we modeled metabolic syndrome, increased superoxide dismutase and catalase activities by 93.1 % and by 98.4 %, respectively, compared to metabolic syndrome group. Concentration of malondialdehyde and oxidatively modified proteins in rat biceps femoris muscle decreased by 21.1 % and by 22.8 % compared to metabolic syndrome group. Administration of SB203580 attenuates oxidative stress development in rat biceps femoris muscle during metabolic syndrome by decreasing reactive oxygen species production, increasing antioxidant defense and lowering intensity of lipid and protein damage.

Key words: skeletal muscles, oxidative stress, antioxidants, metabolic syndrome, carbonyl stress, p38, MAPK.

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ВПЛИВ ІНГІБІТОРА P38 НА РОЗВИТОК ОКСИДАТИВНОГО СТРЕСУ В ДВОГОЛОВОМУ М'ЯЗІ СТЕГНА ЩУРІВ ПІД ЧАС МОДЕЛЮВАННЯ ЕКСПЕРИМЕНТАЛЬНОГО МЕТАБОЛІЧНОГО СИНДРОМУ

Оксидативний стрес – типовий патологічний процес, який лежить в основі розвитку багатьох захворювань. Метаболічний синдром порушує фізіологічне постачання метаболітів до клітини, що призводить до енергетичного дефіциту через розвиток низькоінтенсивного запалення та оксидативного стресу, спричиненого порушенням функції мітохондрій. Вже було показано, що редокс-чутливі транскрипційні фактори, такі як NF-κB, відіграють вирішальну роль у патогенезі метаболічного синдрому та його ускладнень. Водночас, мало згадується про роль факторів, що контролюють мітогенний цикл, таких як каскад p38-MAPK, у патогенезі метаболічного синдрому. Метою цього дослідження є оцінка впливу введення SB203580 на продукцію супероксидного аніон-радикала, активність антиоксидантних ферментів, інтенсивність перекисного окислення ліпідів та пошкодження білків у двоголовому м'язі стегна щурів під час моделювання метаболічного синдрому. Досліджували базову продукцію супероксидного аніон-радикала, його продукцію від мікросомального ланцюга електрон-транспорту, його продукцію від мітохондріального ланцюга електрон-транспорту, активність супероксиддисмутази та каталази, концентрацію малонового діальдегіду та окисно-модифікованих білків. Введення SB203580 тваринам, на яких ми моделювали метаболічний синдром, призвело до зниження продукції основного супероксиду в двоголовому м'язі стегна щурів на 25,7 % порівняно з групою метаболічного синдрому. Продукція супероксидного аніон-радикалу з мікросомального електроно транспортного ланцюга зменшилася на 16,0 %, а з мітохондріального електроно транспортного ланцюга – на 14,6 % порівняно з показниками групи метаболічного синдрому. Введення SB203580 тваринам, на яких ми моделювали метаболічний синдром, збільшило активність супероксиддисмутази та каталази на 93,1 % та 98,4 %, відповідно, порівняно з групою метаболічного синдрому. Концентрація малонового діальдегіду та окисно-модифікованих білків у двоголовому м'язі стегна щурів зменшилася на 21,1 % та на 22,8 % порівняно з групою метаболічного синдрому. Введення SB203580 послаблює розвиток оксидативного стресу в двоголовому м'язі стегна щурів під час метаболічного синдрому шляхом зменшення вироблення активних форм кисню, посилення антиоксидантного захисту та зниження інтенсивності пошкодження ліпідів та білків.

Ключові слова: скелетні м'язи, оксидативний стрес, антиоксиданти, метаболічний синдром, карбонільний стрес, p38, MAPK.

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Oxidative stress is a typical pathological process, which underlies development of many diseases. Oxidative stress is initiated by increased production of reactive oxygen species (ROS), which include: superoxide anion radical (SA), hydroxyl radical, singlet oxygen, hydrogen peroxide etc. Any disruption of

metabolic supply to the cell energy-producing apparatus leads to increased production of ROS and may initiate oxidative stress in organs and tissues.

Metabolic syndrome (MetS) disrupts physiological supply of metabolites to the cell leading to energy deficiency, due to low grade inflammation development, and oxidative stress, caused by mitochondria impairment [2]. It has been already shown that redox sensitive transcriptional factors like NF-κB play a crucial role in pathogenesis of MetS and its complications [1]. Activation of transcriptional factors, which control antioxidant enzymes gene expression, like Nrf-2, usually displays protective effects on MetS-induced tissue damage [1]. At the same time, there are few mentions of role of mitogenic cycle controlling factors in pathogenesis of MetS. One of the key mitogenic cycle controlling factors is p38-mitogen activated protein kinase (MAPK) cascade. This cascade has a close interaction with transcription factor NF-κB and may be involved in pathogenesis of MetS [4].

One of the main pathogenetic links in MetS development is increase in insulin resistance. Increased insulin resistance may lead to fat accumulation in skeletal muscles with subsequent loss of muscle tissue, leading to sarcopenia [8]. Despite the fact that pathological mechanisms associated with MetS, such as hyperglycemia and inflammation, have been associated with changes in skeletal muscle fiber composition, metabolism, insulin sensitivity, mitochondrial function, and strength, it is still under scientific debate whether skeletal muscles are bystanders or influencers during metabolic syndrome development [10].

Therefore, role of p38-MAPK activation in skeletal muscles during metabolic syndrome in oxidative stress development is still studied insufficiently.

The purpose of the study was to evaluate the influence of SB203580 administration on production of superoxide anion radical, activities of antioxidant enzymes, intensity of lipid peroxidation and protein damage in rat biceps femoris muscle during metabolic syndrome modeling.

Materials and methods. In order to conduct our study, we randomly selected 24 mature male Wistar rats weighing 200-260 g from Poltava State Medical University vivarium. For two weeks prior the experiment, animals were kept on standard diet and 12/12 light/dark cycle. The animals were divided into 4 groups of 6 animals each:

I – Control group. Animals from this group received manipulations similar to those of the other groups, but instead of the active substances, they received a 0.9 % solution of sodium chloride.

II – Metabolic syndrome group (MetS group). MetS was reproduced by using a 20 % fructose solution as the only source of water for 60 days [5].

III – SB203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole, Sigma-Aldrich) administration group (SB203580 group). SB203580 was administered intraperitoneally in a dose 2 mg/kg once every 3 days for 60 days [13].

IV – Administration of SB203580 on the background of MetS modelling (MetS+ SB203580 group). Animals from this group received SB203580 intraperitoneally in a dose 2 mg/kg once every 3 days and had 20 % fructose solution as the only source of water for 60 days.

All manipulations with laboratory animals were approved by the Bioethics Commission of the Poltava State Medical University (Record № 206 from 24.06.2022). Animals were removed from experiment under thiopental anesthesia by blood sampling from the right heart ventricle.

The object of the study was a 10 % homogenate of the biceps femoris muscle of rats. Tissue homogenate was prepared by grinding 1 g of biceps femoris muscle with 9 ml of Tris-HCl buffer solution (pH=7.4). Received mixture of tissue and buffer was centrifugated for 30 minutes at 3000 g. Upper layer was taken for biochemical studies. We studied following biochemical parameters: basic SA production, its production from microsomal electron transport chain (ETC), SA production from mitochondrial ETC, superoxide dismutase (SOD) and catalase activity, concentration of malondialdehyde (MDA) and oxidatively modified proteins (OMP) [7].

Statistical analysis of obtained results was performed in Microsoft Office Excel using the extension Real Statistics 2019. In order to evaluate statistical significance of differences between groups we used non-parametric Kruscal-Wallis test (non-parametric ANOVA) followed by paired comparisons by Mann-Whitney U-test with Bonferroni correction. The difference was counted as statistically significant if $p<0.05$.

Results of the study and their discussion. MetS modeling led to development of oxidative stress in rat biceps femoris muscle (Table 1). Key features of MetS-induced oxidative stress in rat biceps femoris muscle were: increased production of SA from mitochondrial and microsomal ETCs, decreased SOD and catalase activities, and elevated intensity of lipid peroxidation and protein damage.

Table 1

Parameters of the pro- and antioxidant balance in rat biceps femoris muscle under the condition of modeling the metabolic syndrome and SB203580 administration (M±SE)

Parameters	Groups			
	Control, n=6	MetS, n=6	SB203580, n=6	SB203580 +MetS, n=6
Basic SA production, nmol/s per g	0.63±0.01	1.09±0.01 *	0.68±0.01 */#	0.81±0.02 */#/^
SA production from microsomal ETC, nmol/s per g	9.04±0.21	15.07±0.06*	9.77±0.25 */#	12.66±0.45 */#/^
SA production from mitochondrial ETC, nmol/s per g	10.71±0.14	16.85±0.10*	11.27±0.17 */#	14.39±0.09 */#/^
SOD activity, c.u.	5.97±0.25	4.20±0.17 *	7.62±0.64 */#	8.11±0.39 */#
Catalase activity, μkatal/g	0.728±0.02	0.376±0.004 *	0.899±0.01 */#	0.746±0.005 #/^
Free MDA concentration, μmol/g	5.27±0.14	22.22±0.39 *	9.21±0.36 */#	17.54±0.17 */#/^
OMP content, c.u.	0.097±0.002	0.228±0.005 *	0.122±0.001 */#	0.176±0.002 */#/^

Note: * – data is statistically significantly different from control group ($p<0.05$). # – data is statistically significantly different from MetS group ($p<0.05$). ^ – data is statistically significantly different from SB203580 group ($p<0.05$).

Blockade of p38-MAPK cascade activation by SB203580 administration to healthy animals led to increase in basic SA production in rat biceps femoris muscle by 7.9 % compared to control group. SA production from microsomal ETC increased by 8.1 % and from mitochondrial ETC increased by 5.2 % compared to indicators of control group. SB203580 administration to healthy animals increased SOD and catalase activities by 27.6 % and 23.5 %, respectively, compared to control. Concentration of MDA and OMP in rat biceps femoris muscle increased by 74.8 % and by 25.8 % compared to control.

SB203580 administration to healthy animals led to decrease in basic SA production in rat biceps femoris muscle by 37.6 % compared to MetS group. SA production from microsomal ETC decreased by 35.2 % and from mitochondrial ETC increased by 33.1 % compared to MetS group. SB203580 administration to healthy animals increased SOD and catalase activities by 81.4 % and 139.1 %, respectively, compared to MetS group. Concentration of MDA and OMP in rat biceps femoris muscle decreased by 58.6 % and by 46.5 % compared to MetS group.

Blockade of p38-MAPK cascade activation by SB203580 administration to animals, on which we modeled MetS, led to increase in basic SA production in rat biceps femoris muscle by 28.6 % compared to control group. SA production from microsomal ETC increased by 40.0 % and from mitochondrial ETC increased by 34.4 % compared to indicators of control group. SB203580 administration to animals, on which we modeled MetS, increased SOD activity by 35.8 %, while catalase activity remained unchanged compared to control. Concentration of MDA and OMP in rat biceps femoris muscle increased by 232.8 % and by 81.4 % compared to control.

SB203580 administration to animals, on which we modeled MetS, led to decrease in basic SA production in rat biceps femoris muscle by 25.7 % compared to MetS group. SA production from microsomal ETC decreased by 16.0 % and from mitochondrial ETC decreased by 14.6 % compared to indicators of MetS group. SB203580 administration to animals, on which we modeled MetS, increased SOD and catalase activities by 93.1 % and by 98.4 %, respectively, compared to MetS group. Concentration of MDA and OMP in rat biceps femoris muscle decreased by 21.1 % and by 22.8 % compared to MetS group.

SB203580 administration to animals, on which we modeled MetS, compared to SB203580 administration to healthy animals increased basic SA production in rat biceps femoris muscle by 19.1 %. SA production from microsomal ETC increased by 29.6 % and from mitochondrial ETC increased by 27.7 % compared to indicators of SB203580 group. SB203580 administration to animals, on which we modeled MetS, decreased catalase activity by 17.0 %, while SOD activity remained unchanged compared to SB203580 group. Concentration of MDA and OMP in rat biceps femoris muscle decreased by 90.4 % and by 44.3 % compared to SB203580 group.

An increase in ROS production in MetS group observed in our study correlates with studies of other scientists and is most likely connected to the increase in insulin resistance and activation of redox sensitive transcriptional factors in rat muscle tissue [1, 6]. MetS development causes a complex of changes in cell metabolism [6]:

1. Through increased glucose accumulation excessive amount of sorbitol is formed.
2. Excess of sorbitol leads to depletion of $NADPH_2$ and increased ROS production.
3. Increased ROS production induces accumulation of advanced glycation end product (AGE).

4. AGEs activate protein kinase C, which in turn trough MAPK can activate transcriptional factor NF-κB.

5. Activation of transcriptional factor NF-κB exacerbates ROS production.

Substance SB203580 is a potent and highly selective inhibitor of p38-MAPK cascade [9]. Considering the crosstalk between p38-MAPK and Nrf-2 we can assume that increase in SOD and catalase activity observed in SB203580 group and SB203580+MetS group are not connected to the increase of expression of antioxidant genes, but are caused by decrease in workload on these enzymes due to decrease in ROS production. The main reason for such assumption is that p38-MAPK inhibitor SB203580 can lower the activation of Nrf-2 [12].

OMP represent the intensity of carbonyl stress developing in skeletal muscles during MetS modeling caused by excessive glucose accumulation. The decrease in OMP content caused by SB203580 administration on the background of MetS modeling is connected with ability of p38-MAPK inhibitor to decrease intensity of reactive carbonyl compounds formation [9].

Activation of p38-MAPK/NF-κB during MetS modeling may cause a shift in tissue macrophage polarization towards predominance of M1 (pro-inflammatory) phenotype. Blockade of p38-MAPK/NF-κB cascade by SB203580 administration restores physiological predominance of M2 (anti-inflammatory) polarization of macrophages in skeletal muscles, which also may be one of mechanisms of ROS production decrease [11].

A decrease in intensity of lipid peroxidation in skeletal muscle observed in group of SB203580 administration to rats with modelled MetS is consistent with the results of Chen W et al., who showed that inhibition of p38-MAPK partially removed anisomycin-induced ferroptosis [3].

An increase in MDA and OMP content in group of SB203580 administration to heathy animals may be a sign of light toxicity of the drug.

Interaction of p38-MAPK with transcriptional factor NF-κB in skeletal muscles under physiological and pathological conditions requires further investigation.

Conclusion

Administration of SB203580 attenuates oxidative stress development in rat biceps femoris muscle during metabolic syndrome by decreasing reactive oxygen species production, increasing antioxidant defense and lowering intensity of lipid and protein damage.

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