DOI 10.26724/2079-8334-2022-1-79-179-183
UDC [616.12:599.323.4]:612.08

T.A. Voroshilova, V.I. Shepitko, Ye.V. Stetsuk, O.Ye. Akimov, G.S. Puzyryov
Poltava State Medical University, Poltava

CHANGES IN NITRIC OXIDE PRODUCTION AND DEVELOPMENT OF OXIDATIVE STRESS IN RATS HEART DURING PROLONGED TRIPTORELIN-INDUCED CENTRAL DEPRIVATION OF LUTEINIZING HORMONE SYNTHESIS

e-mail: stetsuk78@gmail.com

Disruption of synthesis of luteinizing hormone may lead to testosterone deficiency. And testosterone deficiency leads to increased risk of cardiovascular mortality and aggravates coronary artery disease. On the 180th day we observed the biggest shift towards M1 phenotype. This event coincided with the increased SAR production. Since M1 polarized tissue macrophages have the ability to produce reactive oxygen and nitrogen species we can speculate that highest lipid peroxidation observed in our study on the 180th day of the experiment is connected with changes in macrophage polarization towards predominance of M1 phenotype. Therefore, we can conclude, that decrease in oxidative damage to the heart on the 30th day of the experiment can be connected to lower concentration of luteinizing hormone. On later terms of the experiment activation of xanthine oxidase/uric acid signaling due to the lack of testosterone causes the development of oxidative stress. Decrease in activity of antioxidant enzymes during peak production of SAR (180th day) can be explained by exhaustion of these enzymatic systems.

Keywords: nitric oxide, oxidative stress, heart, rats, luteinizing hormone, triptorelin

Т.А. Ворошилова, В.І. Шепітько, Є.В. Стецук, О.Є. Акімов, Г.С. Пузирьов

ЗМІНИ ПРОДУКЦІЇ ОКСИДУ АЗОТУ ТА РОЗВИТКУ ОКСИДАЦІЙНОГО СТРЕСУ У СЕРЦІ ЩУРІВ ПРИ ДОВГОТРИВАЛІЙ ЦЕНТРАЛЬНІЙ ДЕПРИВАЦІЇ СИНТЕЗУ ЛЮТЕЇНІЗУЮЧОГО ГОРМОНУ ТРИПТОРЕЛІНОМ

Порушення синтезу лютеїнізуючого гормону може призвести до дефіциту тестостерону. А дефіцит тестостерону призводить до підвищення ризику серцево-судинної смертності та посилює ішемічну хворобу серця. На 180-й день ми спостерігали найбільший зсув у бік фенотипу М1. Ця подія збіглася зі збільшенням виробництва SAR. Оскільки поляризовані макрофаги М1 мають здатність продукувати активні форми кисню та азоту, можна припустити, що найвище перекисне окислення ліпідів, яке спостерігалося в нашому дослідженні на 180-й день експерименту, пов'язане зі зміною поляризації макрофагів у бік переважання фенотипу М1. Таким чином, можна зробити висновок, що зниження окисного ураження серця на 30-ту добу експерименту може бути пов'язане із зниженням концентрації лютеїнізуючого гормону. На більш пізніх термінах експерименту активація сигналізації ксантиноксидази/сечової кислоти через брак тестостерону викликає розвиток окисного стресу. Зниження активності антиоксидантних ферментів під час піку продукції SAR (180-а добу) можна пояснити виснаженням цих ферментних систем.

Ключові слова: оксид азоту, окислювальний стрес, серце, щури, лютеїнізуючий гормон, трипторелін.

The study is a fragment of the research project "Experimental morphological study of cryopreserved placenta transplants action diphereline, ethanol and 1 % methacrylic acid on the morphofunctional status in a number of internal organs", state registration No. 0119U102925.

Luteinizing hormone controls the fertility of male organisms by regulating the production of testosterone. Disruption of synthesis of luteinizing hormone may lead to testosterone deficiency. Such disruption may be the result of treatment of prostate cancer by triptorelin-based drugs, which can be considered as the "golden standard" for such disease [12]. Therefore, prolonged treatment by triptorelin may cause testosterone deficiency.

Testosterone plays several important roles in the functioning of the cardiovascular system. And testosterone deficiency leads to an increased risk of cardiovascular mortality and aggravates coronary artery disease. Testosterone replacement therapy can be used as an effective remedy to correct these changes in the heart, albeit it works partially and not in all clinical cases [13]. The cause of such partial effectiveness may lie in the absence of data about molecular mechanisms of changes, which take place heart during testosterone deficiency.

Testosterone is involved in the regulation of the activity of proteins, responsible for the handling of Ca^{2+}-ions in the heart. Some authors provide evidence, that testosterone deficiency improves the state of the heart after myocardial infarction [6]. Testosterone deficiency also prevents the development of myocardial hypertrophy at later stages after myocardial infarction [3]. At the same time, testosterone deficiency may cause the development of oxidative stress and propagate endothelial dysfunction by intruding into nitric oxide production [8]. Testosterone can influence the polarization of tissue macrophages. In physiological concentrations of testosterone, tissue macrophages tend to retain their anti-inflammatory polarization (M2 phenotype) [5]. In the situation where there is a deficiency in testosterone production of macrophages may transit to their pro-inflammatory polarization (M1 phenotype).

Therefore, the effects of prolonged deficiency of testosterone caused by central deprivation of luteinizing hormone synthesis may lead to controversial changes in heart oxidative metabolism and they demand study in detail.

The purpose of the study was to establish the changes in inducible and constitutive NO-synthase isoforms activities, arginase activity, production of superoxide anion radical, superoxide dismutase and catalase activities and concentration of malondialdehyde in rat heart during prolonged central deprivation of luteinizing hormone synthesis by triptorelin injection.

Materials and methods. The experiments were carried out on 60 sexually mature male white rats of the Wistar line weighing 140–160 g. Rats were divided into 2 groups: the control group (10) and the experimental group (25). Animals from the experimental group were injected subcutaneously with diphereline (Triptorelin embonate) at a dose of 0.3 mg of the active substance per kg of body weight for 365 days, while the control group received an injection of saline [2]. Animals were kept in standard vivarium conditions of the Poltava State Medical University. Experimental animals were sacrificed in strict compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes"; (Strasbourg, 1986), as well as with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001). Animals from the experimental group were removed from the experiment on the 30^{th} day (n=5), on the 90^{th} day (n=5), on the 180^{th} day, on the 270^{th} day and on the 365^{th} day of modelling of central deprivation of luteinizing hormone synthesis.

All biochemical studies were carried out in 10 % homogenate of the heart using Ulab 101 spectrophotometer.

The activity of inducible NO-synthase (iNOS) and constitutive isoforms of NO-synthase (cNOS) were determined by an increase of nitrite (NO_2^-) concentration after incubation in Trys-buffer solution (pH=7.4) containing 320 mM L-arfinine, 8 mM NADPH and specific iNOS inhibitor (aminoguanidine) [9]. The activity of arginase (ARG) was evaluated by an increase of L-ornithine concentration in the phosphate buffer solution (pH=7.0) containing 0.2 ml of 10 % tissue homogenate and 0.3 ml 24 mM solution of L-arginine [14]. Basic production of superoxide anion radical (SAR) was determined by the growth of diformazan concentration, formed in the reaction of SAR with nitro blue tetrazolium [16]. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, while catalase activity was determined by the amount of hydrogen peroxide, which remained after its catalase-dependent reduction [9]. The concentration of free malondialdehyde (MDA) was determined by reaction with 1-methyl-2-phenylindole [7].

Statistical processing of the study results was carried out using the Microsoft Office Excel software and the Real Statistics 2019 extension to it. The nonparametric Mann-Whitney test was used to determine the statistical significance of differences between the groups. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. Influence of prolonged triptorelin administration on nitric oxide cycle. Prolonged central deprivation of luteinizing hormone synthesis by triptorelin injection caused a sharp decline in iNOS activity (decreased by 58.21 % compared to the control group) on the 30^{th} day of the experiment (table 1).

On the 90^{th} day, the iNOS activity remained decreased by 40.30 % compared to the control group but was elevated by 42.86 % compared to the 30^{th} day of the experiment. On the 180^{th} day of the experiment iNOS activity increased by 72.39 % compared to the control group and by 188.75 % compared to the 90^{th} day of the experiment. On the 270^{th} day of the experiment iNOS activity decreased by 26.84 % compared to the 180^{th} day, but remained by 26.12 % higher than in the control group. On the 365^{th} day the iNOS activity was higher than in the control group by 21.64% and statistically did not differ from the 270^{th} day.

There were no changes in the activity of cNOS compared to the control group on 30^{th}, 180^{th} and 270^{th} day of the experiment. However, we observed an increase in cNOS activity on the 90^{th} and 365^{th} days of the experiment compared to the control by 6.17 % and 3.30 % respectively. It is worth mentioning that cNOS activity decreased on the 180^{th} day by 7.43 % compared to the 90^{th} day.

The ARG activity increased on the 30^{th} day by 7.05 %. On the 90^{th} day, ARG activity decreased by 22.41 % compared to the control group and by 27.52 % compared to the 30^{th} day. The ARG activity was decreased on the 180^{th} by 37.34 % compared to the control group and by 19.25 % compared to the 90^{th} day. On the 270^{th} day of the experiment, the ARG activity increased by 25.83 % compared to the 180^{th} day but was still by 21.16 % lower than in the control group. On the 365^{th} day of the experiment ARG activity was by 15.77 % lower than in the control group, but increased by 6.84 % compared to the 270^{th} day.

Table 1

Activity of nitric oxide cycle enzymes and biochemical parameters of oxidative stress in rat heart during prolonged central deprivation luteinizing hormone synthesis (M±m).

Biochemical parameters	Control group	Experimental group				
		30th day	90th day	180th day	270th day	365th day
iNOS activity, μmol/min. per g of protein	1.34±0.03	0.56±0.06 *	0.80±0.04 */**	2.31±0.07 */**	1.69±0.09 */**	1.63±0.07 *
cNOS activity, μmol/min. per g of protein	0.0583±0.0005	0.0598±0.0005	0.0619±0.0005 */**	0.0573±0.0008 **	0.0583±0.0002	0.0602±0.0003 */**
ARG activity, μmol/min. per g of protein	2.41±0.03	2.58±0.03 *	1.87±0.02 */**	1.51±0.03 */**	1.90±0.03 */**	2.03±0.03 */**
SAR production, nmol/s per g of tissue	1.37±0.01	1.25±0.02 *	1.62±0.02 */**	1.95±0.01 */**	1.82±0.01 */**	1.67±0.01 */**
SOD activity, c.u.	1.93±0.14	2.81±0.18*	1.96±0.04**	1.32±0.09*/**	1.71±0.13	1.56±0.14
Catalase activity, μkat/g of tissue	0.292±0.003	0.333±0.001*	0.277±0.002 */**	0.210±0.001 */**	0.241±0.002 */**	0.261±0.001 */**
MDA concentration, μmol/g of tissue	10.33±0.04	8.85±0.08 *	12.74±0.08*/**	16.90±0.12 */**	14.40±0.06 */**	13.49±0.06 */**
NO_2^- concentration, nmol/L	7.84±0.11	4.99±0.28 *	9.48±0.11 */**	8.88±0.18 */**	7.78±0.16 **	7.11±0.16 */**

Note: * – indicates that data is statistically significantly different from the control group; ** – indicates that data is statistically significantly different from the previous term of the experiment

The nitrite concentration in rat hearts decreased by 36.35 % on the 30th day of the experiment compared to the control group. On the 90th day, it increased compared to the control group and to the 30th day of an experiment by 20.92 % and 47.36 % respectively. On the 180th day, nitrite concentration increased by 13.27 % compared to the control and decreased by 6.33 % compared to the 90th day. On the 270th day of the experiment, there were no statistically significant changes in nitrite concentration compared to the control group, but nitrite concentration was decreased compared to the 180th day by 12.39 %. On the 365th day of the experiment, nitrite concentration decreased by 9.31 % compared to the control group and by 8.61 % compared to the 270th day.

Analyzing changes in nitric oxide cycle during prolonged triptorelin-induced luteinizing hormone synthesis deprivation we should note the following:

1. The lowest activity of ARG and the highest activity of iNOS was observed the on 180th day of the experiment.

2. The highest nitrite concentration was observed on the 90th day of the experiment.

3. The cNOS activity increased on the 90th day against the background of decreased iNOS activity.

Oxidative stress development in rat heart during prolonged central deprivation of luteinizing hormone synthesis by triptorelin.

On the 30th day of the experiment, SAR production decreased by 8.76 %. On the 90th day, SAR production increased by 18.25 % compared to the control group and by 29.60 % compared to the 30th day. Production of SAR on the 180th day in rat heart increased by 42.34 % compared to the control and by 20.37 % compared to the 90th day. On the 270th day of the experiment, SAR production increased by 32.85 % compared to the control and decreased by 6.67 % compared to the 180th day. On the 365th day of the experiment, SAR production increased by 21.90 % compared to the control and decreased by 8.24 % compared to the 270th day.

The SOD activity statistically significantly increased on the 30th day of the experiment by 45.60 % and decreased by 31.61 % on the 180th day. It is worth mentioning that SOD activity decreased by 30.25 % on the 90th day compared to the 30th day. SOD activity also decreased by 32.65 % on the 180th day compared to the 90th day.

Catalase activity increased by 14.04 % on the 30th day of the experiment compared to the control group. On the 90th day of the experiment, catalase activity decreased by 5.14 % compared to the control and by 16.82 % compared to the 30th day. On the 180th day of the experiment, catalase activity decreased

by 28.08 % compared to the control and by 24.19 % compared to the 90th day. On the 270th day of the experiment, catalase activity decreased by 17.47 % compared to the control and increased by 14.76 % compared to the 180th day. On the 365th day of the experiment, catalase activity decreased by 10.62 % compared to the control and increased by 8.30 % compared to the 270th day.

The MDA concentration decreased by 14.33 % on the 30th day of the experiment compared to the control group. On the 90th day of the experiment, MDA concentration increased by 23.33 % compared to the control and by 43.95 % compared to the 30th day. On the 180th day of the experiment, MDA concentration increased by 63.60 % compared to the control and by 32.65 % compared to the 90th day. On the 270th day of the experiment, MDA concentration increased by 39.40 % compared to the control and decreased by 14.79 % compared to the 180th day. On the 365th day of the experiment, MDA concentration increased by 30.59 % compared to the control and decreased by 6.32 % compared to the 270th day of the experiment.

Analyzing the changes in oxidative-antioxidative balance, it is worth noting the following:

1. On the 180th day of the experiment, we observed the highest intensity of lipid peroxidation and SAR production together with the lowest activity of antioxidant enzymes.

2. On the 30th day of the experiment, we established that oxidative damage to the heart was the lowest and activity of antioxidant enzymes was the highest.

3. There was a tendency to decrease in oxidative damage to the heart starting from the 180th day of the experiment.

The activity of iNOS may be used as a marker of polarization of macrophages according to M1 phenotype, while ARG activity is a clear marker of M2 phenotype. This is due to the fact that these enzymes are expressed by macrophages polarized by specific phenotype and they cannot be expressed together [4]. We can state that at the end of the experiment polarization of macrophages of the heart shifts towards prevalence of M1 phenotype. On the 180th day, we observed the biggest shift towards the M1 phenotype. This event coincided with the increased SAR production. Since M1 polarized tissue macrophages have the ability to produce reactive oxygen and nitrogen species we can speculate that the highest lipid peroxidation observed in our study on the 180th day of the experiment is connected with changes in macrophage polarization towards the predominance of M1 phenotype.

Testosterone has a complex and controversial influence on iNOS. There is evidence that testosterone can inhibit iNOS activity and ameliorate inflammation caused by neuroglia [1]. On the other hand, testosterone promotes the expression of iNOS genes in the prostate and stimulates inflammation [10]. The rapid decrease in iNOS activity on the 30th day of the experiment probably resulted in a reduced amount of nitric oxide in the heart. This event, on the background of reduced testosterone level, can activate xanthine oxidase/uric acid signalling [11]. Activation of this signalling pathway enables the formation of nitric oxide from nitrate-nitrite reduction. This can explain the low quantity of nitrites in the heart, observed on the 30th day and their peak concentration on the 90th day. As evidence of insufficient nitric oxide production by oxidative pathway (NO-synthase-dependent) in the heart, we can count the increase in cNOS activity, since the only possible mechanism of increase of endothelial and neuronal NO-synthase activity is elevated transcription and translation of their respective genes. Our findings do not contradict the information about the ability of testosterone to promote endothelial NO-synthase activity since we blocked luteinizing hormone rather testosterone. In addition, decreased luteinizing hormone can elevate cNOS activity [9]. Therefore, we can conclude, that decrease in oxidative damage to the heart on the 30th day of the experiment can be connected to a lower concentration of the luteinizing hormone. In later terms of the experiment activation of xanthine oxidase/uric acid signalling due to a decrease of testosterone causes the development of oxidative stress. Decrease in activity of antioxidant enzymes during peak production of SAR (180th day) can be explained by exhaustion of these enzymatic systems. Their further increase in activity is caused by activation of redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) in response to increased reactive oxygen species production and testosterone deficiency [15].

Conclusion

Central deprivation of luteinizing hormone synthesis for 365 days leads to the development of oxidative stress in rat hearts. The peak of oxidative damage to the heart under these conditions happens on the 180th day of the central deprivation of luteinizing hormone synthesis.

Production of nitric oxide during central deprivation of luteinizing hormone synthesis undergoes complex changes from an initial decrease of NO-synthase-dependent nitric oxide production on the 30th and 90th day to overproduction of nitric oxide from NO-synthases on the 365th day of the experiment.

References

1. Abdel-Aziz AM, Gamal El-Tahawy NF, Salah Abdel Haleem MA, Mohammed MM, Ali AI, Ibrahim YF. Amelioration of testosterone-induced benign prostatic hyperplasia using febuxostat in rats: The role of VEGF/TGFβ and iNOS/COX-2. Eur J Pharmacol. 2020 Dec 15; 889:173631. doi: 10.1016/j.ejphar.2020.173631.

2. Akhigbe RE, Hamed MA, Odetayo AF, Akhigbe TM, Ajayi AF, Ajibogun FAH. Omega-3 fatty acid rescues ischaemia/perfusion-induced testicular and sperm damage via modulation of lactate transport and xanthine oxidase/uric acid signaling. Biomed Pharmacother. 2021 Oct; 142:111975. doi: 10.1016/j.biopha.2021.111975.

3. Akseh S, Karimi MA, Safaie N, Valizadeh A, Rahmanpour D, Pezeshkian M, Nouri M, Faridvand Y, Jodati A. The serum levels of testosterone in coronary artery disease patients; relation to NO, eNOS, endothelin-1, and disease severity. Horm Mol Biol Clin Investig. 2021 Sep 9. doi: 10.1515/hmbci-2021-0026.

4. Babcock MC, DuBose LE, Witten TL, Stauffer BL, Hildreth KL, Schwartz RS, Kohrt WM, Moreau KL. Oxidative Stress and Inflammation Are Associated With Age-Related Endothelial Dysfunction in Men With Low Testosterone. J Clin Endocrinol Metab. 2022 Jan 18; 107(2):e500–e514. doi: 10.1210/clinem/dgab715.

5. Barbonetti A, D'Andrea S, Francavilla S. Testosterone replacement therapy. Andrology. 2020 Nov; 8(6):1551-1566. doi: 10.1111/andr.12774.

6. Botté MC, Lerrant Y, Lozach A, Bérault A, Counis R, Kottler ML. LH down-regulates gonadotropin-releasing hormone (GnRH) receptor, but not GnRH, mRNA levels in the rat testis. J Endocrinol. 1999; 162(3): 409–415. doi:10.1677/joe.0.1620409.

7. Fernandes Corrêa RA, Ribeiro Júnior RF, Mendes SBO, Dos Santos PM, da Silva MVA, Silva DF, Biral IP, de Batista PR, Vassallo DV, Bittencourt AS, Stefanon I, Fernandes AA. Testosterone deficiency reduces the effects of late cardiac remodeling after acute myocardial infarction in rats. PLoS One. 2019 Mar 21; 14(3):e0213351. doi: 10.1371/journal.pone.0213351.

8. Kirby M, Hackett G, Ramachandran S. Testosterone and the Heart. Eur Cardiol. 2019 Jul 11;14(2):103–110. doi: 10.15420/ecr.2019.13.1.

9. Meng X, Li X, Xu X, Li P, Chen Y, Fu X, Xu X. Elevated luteinizing hormone contributes to atherosclerosis formation by inhibiting nitric oxide synthesis via PI3K/Akt pathway. Vascul Pharmacol. 2019 Oct;121:106582. doi: 10.1016/j.vph.2019.106582.

10. Ren X, Fu X, Zhang X, Chen S, Huang S, Yao L, Liu G. Testosterone regulates 3T3-L1 pre-adipocyte differentiation and epididymal fat accumulation in mice through modulating macrophage polarization. Biochem Pharmacol. 2017 Sep 15; 140:73–88. doi: 10.1016/j.bcp.2017.05.022.

11. Ribeiro Júnior RF, Ronconi KS, Jesus ICG, Almeida PWM, Forechi L, Vassallo DV Testosterone deficiency prevents left ventricular contractility dysfunction after myocardial infarction. Mol Cell Endocrinol. 2018 Jan 15; 460:14–23. doi: 10.1016/j.mce.2017.06.011.

12. Stetsuk YeV, Kostenko VO, Shepitko VI, Goltsev AN. Influence of the 30-days central deprivation of testosterone synthesis on the morphological and functional features of rat testicular interstitial endocrinocytes and sustentocytes. World of Medicine and Biology. 2019; 4(70): 22833. doi: 10.26724/2079-8334-2019-4-70-228-233.

13. Yang S, Yuan HQ, Hao YM, Ren Z, Qu SL, Liu LS, Wei DH, Tang ZH, Zhang JF, Jiang ZS. Macrophage polarization in atherosclerosis. Clin Chim Acta. 2020 Feb; 501:142–146. doi: 10.1016/j.cca.2019.10.034.

14. Yelinska AM, Akimov OYe, Kostenko VO. Role of AP-1 transcriptional factor in development of oxidative and nitrosative stress in periodontal tissues during systemic inflammatory response. Ukr. Biochem. J. 2019; 91(1): 80–85. doi: 10.15407/ubj91.01.080.

15. Wang X, Huang L, Jiang S, Cheng K, Wang D, Luo Q, Wu X, Zhu L. Testosterone attenuates pulmonary epithelial inflammation in male rats of COPD model through preventing NRF1-derived NF-κB signaling. J Mol Cell Biol. 2021 May 7; 13(2):128–140. doi: 10.1093/jmcb/mjaa079.

Стаття надійшла 24.02.2021 р.