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EFFECT OF LONG-TERM CENTRAL BLOCKING OF HORMONE RELEASE WITH TRIPTORELIN ON OXIDATIVE STRESS MARKER ACTIVITY IN THE LIVER OF RATS

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Over the past two decades, oxidative stress has been a significant concern among researchers in the field of biology worldwide. Stress can be defined as a process of altered biochemical homeostasis resulting from psychological, physiological, or environmental factors. Insufficient amounts of testosterone can worsen liver damage caused by obesity. Oxidative damage is linked to the cause of many diseases, such as cardiovascular disease, neuronal degeneration, and cancer, and also influences the aging process. The experiment was conducted on 60 sexually mature male white rats. The animals were divided into 3 groups. Group 1 – control rats injected with saline (10 animals). Group 2 – rats injected subcutaneously with diphereline (triptorelin) at a dose of 0.3 mg of active ingredient per kg of body weight with drug activity for 12 months (25 animals). Group 3 – rats that were administered a triptorelin solution at the rate of 0.3 mg of active ingredient per kg of body weight to simulate central deprivation of luteinizing hormone synthesis with the addition of quercetin to the diet using a gastric tube based on the body weight of the animals three times a week (25 animals). Triptorelin induces oxidative damage to hepatocytes by increasing the production of reactive oxygen species and decreasing the activity of antioxidant enzymes. Oxidative damage to liver cells begins at the molecular and cellular levels as early as the 1st month of observation. Additional administration of quercetin reduces manifestations of oxidative stress by increasing the activity of antioxidant enzymes and reducing the production of reactive oxygen species.

Key words: liver, hepatocyte, oxidative stress, testosterone, luteinizing hormone, quercetin, triptorelin.

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ВПЛИВ ДОВГОТРИВАЛОГО ЦЕНТРАЛЬНОГО БЛОКУВАННЯ РЕЛІЗІНГГОРМОНУ ТРИПТОРЕЛІНОМ НА МАРКЕРНУ АКТИВНІСТЬ ОКИСНОГО СТРЕСУ В ПЕЧІНЦІ ЩУРІВ

Протягом останніх двох десятиліть окислювальний стрес був однією з найгостріших проблем серед біологічних дослідників у всьому світі. Стрес можна визначити як процес зміненого біохімічного гомеостазу, спричиненого психологічними, фізіологічними або екологічними причинами. Недостатньо кількість тестостерону може посилити пошкодження печінки, викликане ожирінням. Окислювальні пошкодження пов'язані з причиною багатьох захворювань, таких як серцево-судинні захворювання, дегенерація нейронів і онкологія, а також впливають на процес старіння. Експеримент проведено на 60 статевозрілих білих щурах самцях. Тварини були розділені на 3 групи. 1 група – контрольна, щури яким вводився фізіологічний розчин (10 тварин). 2 група – щури, яким підшкірно вводили диферелін (трипторелін) у дозі 0,3 мг діючої речовини на кг маси тіла з активності препарату протягом 12 –ти місяців (25 тварин). 3 група – щури, яким вводили розчин триптореліну із розрахунку 0,3 мг діючої речовини на кг маси тіла для моделювання центральної депривації синтезу лютеїнізуючого гормону з додаванням кверцетину до харчування за допомогою гастрального зонду з перерахунку на масу тіла тварин тричі на тиждень (25 тварин). Трипторелін призводить до окисного пошкодження гепатоцитів через збільшення виробництва активних форм кисню та зниження активності антиоксидантних ферментів. Окислювальне пошкодження клітин печінки починається на молекулярному та клітинному рівнях вже на 1-й місяць спостереження. Додаткове введення кверцетину зменшує прояви окисного стресу за рахунок підвищенням активності антиоксидантних ферментів і зниженням продукції активних форм кисню.

Ключові слова: печінка, гепатоцит, оксидативний стрес, тестостерон, лютеїнізуючий гормон, кверцетин, трипторелін.

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Prostate cancer is the leading cause of cancer death among men in Northern and Western Europe, ranking first and second in their respective regions. It is the most common cancer among men in Europe, with serious consequences for the healthcare system. Every year, around 450,000 European men are diagnosed with prostate cancer [2]. Delayed diagnosis may lead to higher rates of disease metastasis, which is a morbid condition that coincides with high mortality rates and long-term negative impacts on quality of life [5, 8]. Organized re-screening has been shown to lead to early detection, which can reduce suffering and death from prostate cancer. Modern tools and strategies can optimize the process of detecting cancer when it poses a threat to the patient [7]. Therefore, the treatment and diagnosis of prostate cancer is a pressing problem in modern medical science.

One approach to treating prostate cancer is either chemical or surgical castration with drugs aimed at blocking the releasing hormone, which in turn blocks testosterone synthesis. It is considered a key risk factor for prostate cancer progression [15]. However, recent scientific discoveries have provided grounds for revising this paradigm [11].

The reason for this change in views is damage to organs and tissues in testosterone deficiency against the background of the simultaneous influence of external and internal pathogenic factors. Testosterone deficiency makes brain tissue vulnerable to oxidative damage caused by the stress response [13]. Therefore, oxidative stress can be considered an imbalance between the body's prooxidants and antioxidants. Over the past two decades, oxidative stress has been one of the most pressing concerns among biological researchers worldwide. Stress can be defined as a process of altered biochemical homeostasis caused by psychological, physiological, or environmental causes.

Insufficient testosterone can exacerbate liver damage caused by obesity [8]. In both situations (stress response and obesity), increased reactive oxygen species formation and decreased antioxidant defense play a leading role in pathogenesis. The cause of increased tissue damage during testosterone deficiency may also be reduced activity of antioxidant enzymes such as superoxide dismutase isoforms [2]. Oxidative damage is linked to the cause of many diseases, such as cardiovascular disease, neuronal degeneration, and cancer, and also influences the aging process. At the same time, it has been shown in the scientific literature that increased testosterone concentration leads to prostatic hyperplasia through the activation of redox transcription factors and oxidative damage [6, 10].

The purpose of the study was to identify biochemical markers of damage to rat liver cells at the tissue and cellular levels, specifically to investigate the processes of reactive oxygen species production and the intensity of lipid peroxidation during prolonged triptorelin-induced central blockade of releasing hormone.

Materials and methods. The experiment was conducted on 60 sexually mature white male rats weighing 140–160 g. Liver tissues were used as the material for the study. The animals were divided into 3 groups. Group 1 – control rats injected with saline (10 animals). Group 2 – rats injected subcutaneously with diphereline (triptorelin) [4] at a dose of 0.3 mg of active ingredient per kg of body weight with drug activity for 365 days (25 animals). Group 3 – rats that were administered a triptorelin solution at the rate of 0.3 mg of active ingredient per kg of body weight to simulate central deprivation of luteinizing hormone synthesis with the addition of quercetin to the diet using a gastric tube based on the body weight of the animals three times a week (25 animals). Animals were sacrificed after 1, 3, 6, 9, and 12 months by overdose of ether anesthesia. The animals were kept under standard conditions in the vivarium of Poltava State Medical University.

All research and euthanasia of experimental animals were conducted by 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).

The process of euthanasia of animals was carried out by overdose of ether anesthesia. Small liver fragments were fixed according to standard techniques and embedded in paraffin blocks. Sections 4 μm thick were made from these blocks and stained with hematoxylin and eosin [1]. A comprehensive study of histological preparations was performed on a BIOREX-3#5605 light microscope. Quantitative counting of microcirculatory vessels was performed in the fields of view by visual assessment using a light microscope with a digital microfilter and software adapted for these studies. Photography was performed using a DCM 900 digital micrograph attachment using special software for these studies.

All biochemical studies were carried out in 10 % homogenate of testis tissue using Ulab 101 spectrophotometer. 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 [12]. Superoxide dismutase (SOD) activity was determined by inhibition of adrenaline autooxidation, while catalase activity was determined by the amount of hydrogen peroxide, remained after its catalase-dependent reduction [12]. The free malondialdehyde (MDA) concentration was determined by reaction with 1-methyl-2-phenylindole [12]. 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. When we studied markers of oxidative stress over 12 months, we found that the production of superoxide anion radical fell sharply in the first month of observation compared to the control group of animals and amounted to 1.04±0.015 nmol/s on g in tissue at $p<0.01$, which is 41 % less than in the control group. A sharp increase characterized the 3rd month of observation, the indicator being only 23 % higher than the control indicators, and as much as 108 % higher than the indicator of the 1st month. The 6th month of observation of the superoxide anion radical index was characterized by an increase in the index by 52 % compared to the control group and by 24 % from the index of the previous stage of the experiment. 9th month – the indicator was 2.21±0.008 nmol/s on g in tissue at $p<0.05$, which is 26 % higher than the control indicators but 18 % lower than the previous period of the experiment. The 12th month showed a tendency for the indicator to return to the control figures, but the difference between them was small, amounting to only 4 % and 17 % lower than the previous values (Table 1).

Table 1

Changes in markers of oxidative stress in the liver during central triptorelin blockade with the addition of quercetin (M±m)

	Production of SAR, nmol/s on g of tissue	Activity of SOD, c.u.	Activity of catalase, μkat/g of tissue	Concentration of free MDA, μmol/g of tissue
Control group	1.76±0.050	10.12±1.540	0.379±0.001	14.04±0.201
1 m	1.04±0.015**	3.12±0.192**	0.19±0.002**	14.39±0.205
3 m	2.16±0.019*	5.23±0.065	0.21±0.031**	15.81±0.139*
6 m	2.68±0.031**	4.62±0.249**	0.21±0.003**	19.54±0.098**
9 m	2.21±0.008**	5.94±0.358*	0.28±0.001*	18.27±0.046**
12 m	1.83±0.011*	7.86±0.463*	0.30±0.001*	17.02±0.059*

Note: * – indicates that data is statistically significantly different as compared to the control group ($p<0.05$), ** – indicates that data is statistically significantly different compared to control group ($p<0.01$).

SOD activity in liver tissue showed a sharp decline in the index in the 1st month of observation and was 3.12±0.192 c.u., at $p<0.01$, which is 69 % of the control values. In the 3rd month, the activity of the indicator gradually begins to recover and is 52 % of the control values, which is a 68 % increase from the previous values (1st month). In the 6th month, there was a slight decrease in the indicator from the control and previous values by 54 % and 12 %, respectively. The activity in the 9th month was 5.94±0.358 c.u., at $p<0.05$, 41 %, and 29 %, respectively, compared to the control and 6th month of observation values. The 12th month of observation shows a tendency to restore the indicator to the control values, but its amount is 22 % less.

The lowest catalase activity was observed in the 1st month of the experiment, which was 50 % lower than the control group – 0.19±0.002 μkat/g in tissue at $p<0.01$. Starting from the 3rd month, the indicator recovers to control values, but gradually by 45 % (3rd), 45 % (6th), 25 % (9th), and 21 % (12th), which corresponds to an increase in catalase activity over time.

The MDA concentration increased by 2 % (1st), 13 % (3rd), and 39 % (6th) from the control value and gradually decreased by 6 % (9th) and 7 % (12th) during the observation months compared to the previous periods.

Under physiological conditions, the Luteinizing hormone stimulates interstitial endocrinocytes of the testicles to produce testosterone. Therefore, under conditions of blockade of luteinizing hormone production, a sharp decrease in testosterone levels in the body will occur, which is the predominant goal when using triptorelin [13]. Thus, we noted a clear trend towards increased lipid peroxidation processes in the liver of rats with prolonged blocking of releasing hormone to luteinizing hormone production, which in turn leads to a decrease in testosterone production. Any change in homeostasis, and our case, a decrease in testosterone, leads to an increase in the production of free radicals, significantly exceeding local tissues' detoxification capacity. These excess free radicals then interact with other molecules within cells and cause oxidative damage to proteins, membranes, and genes. In the process, even more free radicals are often formed, causing a chain of destruction. Oxidative damage is linked to the cause of many diseases, such as cardiovascular disease, neuronal degeneration, and cancer, and also influences the aging process.

The development of oxidative stress in liver tissues may be associated with a decrease in testosterone concentration under the influence of triptorelin. Testosterone has the property of reducing the number of macrophages polarized by the pro-inflammatory phenotype (M1) and increasing the number of macrophages polarized by the anti-inflammatory phenotype (M2) [10, 11]. Thus, decreased testosterone concentration may lead to increased production of reactive oxygen species by liver macrophages. Testosterone can also reduce the production of reactive oxygen species by mitochondria [4]. Systemic testosterone deficiency in the body may be an etiological factor in the increased production of reactive oxygen species (ROS) in the liver [10]. It is worth noting that testosterone deficiency leads to increased ROS formation in the testes of rats and in remote organs, such as the heart [11]. The administration of testosterone in case of its deficiency helps reduce the production of ROS and enhances the antioxidant protection of tissues [3].

Since the structural structure of the liver did not change in the first stages of the experiment (1st and 3rd months of observation), it can be stated that changes in the liver during this period occur mainly at the cellular and subcellular levels. This statement is supported by the increased level of lipid peroxidation and increased production of SAR against the background of a decrease in the volume and area of the hepatocyte nucleus. The highest intensity of lipid peroxidation and SAR production was observed in the 6th month of the experiment, coinciding with the first signs of structural changes at the tissue level. A decrease in the activity of constitutive isoforms of NO-synthases may lead to their uncoupling and cause the increased production of SAR observed in our study. As it is known that testosterone can positively modulate the activity of endothelial and neuronal nitric oxide synthases, testosterone deficiency can reduce their activity [14]. Testosterone deficiency also affects the functional state of mitochondria, leading to increased production of SAR from the mitochondrial electron transport chain (mtETC) [3]. Exogenous testosterone administration to the body leads to improved mitochondrial function and reduced SAR

formation from mtETL [9]. The main mechanisms of the positive effect of testosterone on mitochondria are the activation of the Nrf-2/ARE and AMPK transcriptional cascades [3]. Testosterone-induced activation of the Nrf-2/ARE cascade contributes to enhanced mitochondrial antioxidant defense and reduces mitochondrial membrane damage caused by excessive production of SAR from mtETC [3]. As already noted above, under conditions of prolonged blockade of luteinizing hormone synthesis with triptorelin, fatty liver disease develops, which may be a consequence of a decrease in AMPK activity, which activates lipolysis and enhances the utilization of energy metabolites to increase ATP formation [9]. Thus, the increase in the production of superoxide anion radical in the liver of rats may be due to the lack of inhibitory effect of testosterone on liver mitochondria, which is accompanied by depletion of antioxidant enzymes and the development of oxidative stress, which can be compensated by the administration of bioflavonoids, namely quercetin, which is a potent antioxidant.

Further studies are needed to assess the role of the inducible NF-kB isoform of NO-synthase and to identify the sources of SAR production in developing morphological and metabolic changes in the liver during prolonged central deprivation of testosterone synthesis.

Conclusion

Blockade of luteinizing hormone synthesis with triptorelin from the 1st to the 12th month leads to oxidative damage to rat liver tissues at all studied periods due to increased production of reactive oxygen species and decreased antioxidant protection. Oxidative damage to liver cells begins at the molecular and cellular levels as early as the 1st month of observation. SOD activity in liver tissue showed a sharp decline in the indicator in the 1st month of observation, with a gradual recovery in the 3rd month. The indicator activity gradually recovers to control values by the 12th month of observations. The lowest catalase activity was observed in the 1st month of the experiment, which was 50 % lower than the control group. Starting from the 3rd month, the indicator returns to control values, but gradually, corresponding to an increase in catalase activity over time. MDA concentration increased from the 1st to the 6th month of observation, with a gradual recovery to the control value by the 12th month. Additional administration of quercetin reduces manifestations of oxidative stress by increasing the activity of antioxidant enzymes and reducing the production of reactive oxygen species.

References

1. Bahriy MM, Dibrova VA, Popadynets OH, Hryshchuk MI. Metodyky morfolohichnykh doslidzhen. Bahriy MM, Dibrova VA. redaktory. Vinnytsya: Nova knyha; 2016. 328. [in Ukrainian].
2. Bang WJ, Kim H, Oh CY, Jo JK, Cho JS, Shim M. Clinical significance of prostate volume and testosterone reduction on lower urinary tract symptoms in patients with prostate cancer undergoing androgen deprivation therapy. Sci Rep. 2022 Nov 2;12(1):18535. doi: 10.1038/s41598-022-21963-1.
3. Barjesteh F, Heidari-Kalvani N, Alipourfard I, Najafi M, Bahreini E. Testosterone, β-estradiol, and hepatocellular carcinoma: stimulation or inhibition? A comparative effect analysis on cell cycle, apoptosis, and Wnt signaling of HepG2 cells. Naunyn Schmiedebergs Arch Pharmacol. 2024 Aug;397(8):6121-6133. doi: 10.1007/s00210-024-03019-5.
4. 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.
5. Desai K, McManus JM, Sharifi N. Hormonal Therapy for Prostate Cancer. Endocr Rev. 2021 May 25;42(3):354-373. doi: 10.1210/endrev/bnab002.
6. Gur S, Alzweri L, Yilmaz-Oral D, Kaya-Sezginer E, Abdel-Mageed AB, Dick B, et al. Testosterone positively regulates functional responses and nitric oxide expression in the isolated human corpus cavernosum. Andrology. 2020 Nov; 8(6):1824-1833. doi: 10.1111/andr.12866.
7. Hao S, Östensson E, Eklund M, Grönberg H, Nordström T, Heintz E, et al. The economic burden of prostate cancer – a Swedish prevalence-based register study. BMC Health Serv Res. 2020 May 20;20(1):448. doi: 10.1186/s12913-020-05265-8.
8. Kimura T, Egawa S. Epidemiology of prostate cancer in Asian countries. Int J Urol. 2018 Jun; 25(6):524-531. doi: 10.1111/iju.13593.
9. Son SW, Lee JS, Kim HG, Kim DW, Ahn YC, Son CG. Testosterone depletion increases the susceptibility of brain tissue to oxidative damage in a restraint stress mouse model. J Neurochem. 2016 Jan;136(1):106-17. doi: 10.1111/jnc.13371.
10. Stetsuk YeV, Akimov OYe, Shepitko KV, Goltsev AN. Structural organization of stromal and parenchymal components of rat testes during central deprivation of testosterone synthesis on the 180 day of the experiment. World of medicine and biology. 2020; 72(2): 203-207. doi: 10.26724/2079-8334-2020-2-72-203-207.
11. Yassin A, AlRumaihi K, Alzubaidi R, Alkadhi S, Al Ansari A. Testosterone, testosterone therapy and prostate cancer. Aging Male. 2019 Dec; 22(4):219-227. doi: 10.1080/13685538.2018.1524456.
12. 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.
13. Yang J, Lin S, Zhang Y, Wu G, Yang Q, Lv Q, et al. Taurine Improves Sexual Function in Streptozotocin-Induced Diabetic Rats. Adv Exp Med Biol. 2017; 975 Pt 1:307-318. doi: 10.1007/978-94-024-1079-2_27.
14. Wang D, Li Y, Zhai QQ, Zhu YF, Liu BY, Xu Y. Quercetin ameliorates testosterone secretion disorder by inhibiting endoplasmic reticulum stress through the miR-1306-5p/HSD17B7 axis in diabetic rats. Bosn J Basic Med Sci. 2022 Apr 1;22(2):191-204. doi: 10.17305/bjbms.2021.6299.
15. Zhang J, Gallaher J, Cunningham JJ, Choi JW, Ionescu F, Chatwal MS, et al. A Phase 1b Adaptive Androgen Deprivation Therapy Trial in Metastatic Castration Sensitive Prostate Cancer. Cancers (Basel). 2022 Oct 25;14(21):5225. doi: 10.3390/cancers14215225.

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