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DYNAMICS OF PEROXIDATION PROCESSES IN MALE RABBITS UNDER EXPERIMENTAL LPS-INDUCED OXIDATIVE STRESS

The article presents the results of an experimental study on the creation of a model of male infertility due to lipopolysaccharide-induced oxidative stress. The objectives of the research were to establish the dynamics of peroxidation processes in the blood serum of male rabbits during acute and chronic intake of LPS solution as OS inducers. A significant increase in the content of DC by 1.85 times (P<0.001), TBA-RC by 80.8% (P<0.001) and stable metabolites of the NO cycle by 88.0% (P<0.001) with a single administration of LPS solution was noted. An increase in the intensity of lipoperoxidation processes during chronic intake was observed as was evidenced by an increase in the content of DC by 52.1% (P<0.001), TBA-RC by 42.5% (P<0.001) and NO_x by 42.3% (P<0.001).

Keywords: *lipopolysaccharide, prooxidant-antioxidant balance, diene conjugates, thiobarbiturate-active products, Nitric oxide cycle.*

Introduction. The cases of reduced male reproductive capacity are explained by an oxidative imbalance in the reproductive system and the influence of toxic radicals on the survival and fertilizing ability of sperm depending on the season and the influence of endogenous factors [1–3].

The influence of bacterial endotoxins – lipopolysaccharides (LPS) on the body of males and their reproductive function is of interest to researchers in the field of reproductive endocrinology as it is among common factors which initiate an increase in the synthesis of reactive oxygen species. Concentrated feeds used in feeding males can be contaminated with various bacterial agents, and as a result of their decay these feeds are contaminated with LPS. Ingested and/or accumulated in the body of animals they cause oxidative stress (OS) [4, 5].

The mechanism of the negative effect of LPS on reproductive capacity was shown in the studies in vivo (rat model) and in vitro (boar sperm). The occurrence of LPS-induced mitochondrial dysfunction mediated by the activation of oxidative phosphorylation was noted [6, 7]. Therefore, the determination of LPS effect as OS

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inducers on the quality indicators of male ejaculates, the peculiarities of their hormonal and metabolic indicators is a relevant problem.

The goal of the work. Experimental studies were made to determine the dynamics of changes in OS markers in male rabbits during single and chronic administration of LPS solution as an experimental model of LPS-induced OS.

Materials and methods. Three groups of sexually mature rabbits were formed. They were kept on a standard diet and had free access to water. Animals of experimental group 1 (n=5) were given a single intraperitoneal injection of LPS solution from *E. coli* O111:B4 (Sigma) of 4 mg/kg of body weight (0.18 LD_{50}) according to the method by Halawa et al., 2018 [8]. The males of experimental group 2 (n=5) were chronically exposed to LPS at a dose equivalent to 1:10 LD_{50} for 14 days according to the method by Brecchia et al., 2010 [9]. A control group (n=5) was injected with 0.9% sodium chloride solution.

OS markers were spectrophotometrically determined in blood serum samples: the number of diene conjugates (DC) in the heptane-isopropanol extract at a wavelength of λ=233 nm based on the value of the molar extinction coefficient for conjugated dienes of polyunsaturated higher fatty acids, thiobarbiturate-active products (TBA-RC) – according to the method based on the binding of malondialdehyde with thiobarbituric acid with the formation of a stable trimethine complex at a wavelength of λ=532 nm and the content of stable metabolites of the Nitrogen oxide (NO_x) cycle – according to the method based on the reaction in which Cadmium in the presence of zinc reduces nitrate to nitrite, for which thawed deproteinized blood serum samples were incubated after adding Griess reagent and examined on a spectrophotometer at a wavelength of λ=546 nm [10].

Statistical processing of experimental results to determine biometric indicators (average values and their errors, comparison of average values according to the Student's criterion) was carried out using the Microsoft Excel program.

Results of research and discussion. The introduction of LPS in the form of a solution caused the expected effects in the body of males and caused a significant increase in the intensification of peroxidation processes, as evidenced by the dynamics of the content of OS markers in the blood serum of rabbits.

The influence of LPS on the dynamics of peroxidation processes during acute intake is shown in fig. 1.

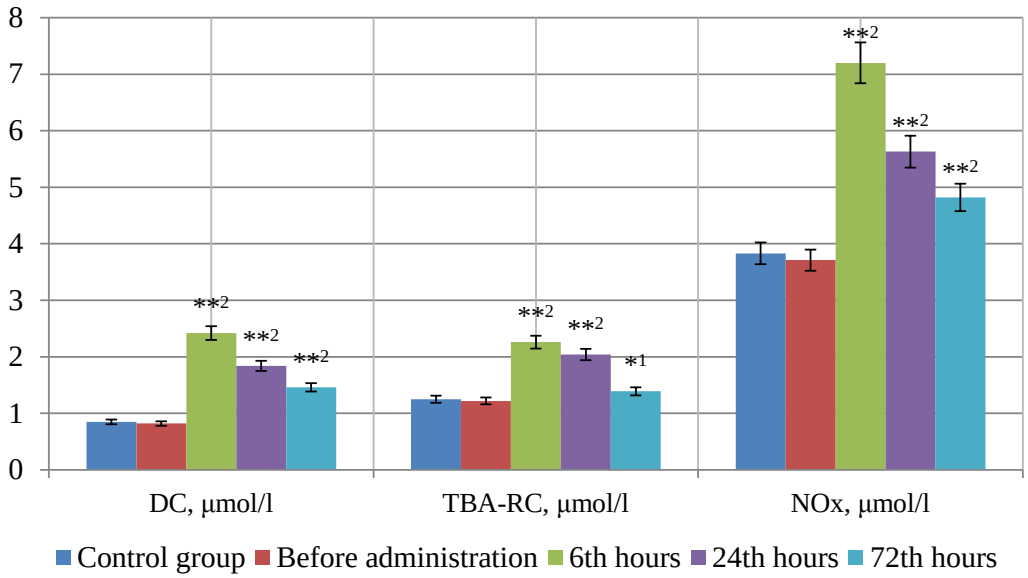

Fig. 1. Dynamics of male rabbits serum blood OS markers content after acute intake of LPS solution.

Notes: * P < 0.05; ** P < 0.001 – statistically significant changes in relation to the control group; [1] P < 0,05; [2] P < 0,001 – statistically significant changes in relation to the group of animals before administration.

6 hours after the introduction of LPS a significant increase in the content of markers OS – DC by 1.85 times (P<0.001), TBA-RC by 80.8% (P<0.001) and stable metabolites of the NO cycle by 88.0% (P<0.001) were noted <0.001).

Then a gradual decrease of oxidative load was noted – after 24 hours the amount of DC decreased by 24.0% (P<0.001) compared to the data after 6 hours and was 1.17 times higher than the control indicator (P<0.001), TBA-RC by 9.7% (P<0.05) and by 63.2% (P<0.001), respectively. The concentration of stable metabolites of the NO cycle underwent similar changes. It was by 21.8% (P<0.001) lower than after 6 hours, but by 47.0% (P<0.001) higher than the control group.

After 72 hours the content of OS markers remained high, although a tendency to decrease peroxidation processes was noted compared to the indicators of the group 24 hours after LPS administration. Thus, the content of DC was higher than the control indicators by 71.8% (P<0.001), NO_x by 25.9% (P<0.001), and TBA-RC by 11.2% (P<0.05), but compared to the data of the group after 24 hours they were lower by 20.7% (P<0.001), 14.4% (P<0.001) and 31.9% (P<0.001), respectively.

Similar dynamics of changes were obtained by other researchers. In particular, with a single administration of 5 mg/kg of body weight to male Wistar rats significant damage to gonadal tissues and an imbalance in the hormonal background were observed compared to intact animals [11], while in the experiment on mice their negative effect was noted on the morphology of gonads and quality characteristics of

sperm [8]. Therefore, the obtained results allow to use LPS solution as OS inducers to create an experimental model of male infertility.

A single injection of LPS determined by oxidative imbalance contributes to the disruption of spermatogenesis processes, in particular, by delaying the maturation of meiosis germ cells during the leptotene/zygotene phase (at stages IX-XIII) [12]. It was proven in the prolonged experiment on mice that LPS had a negative effect not only on the qualitative characteristics of sperm, but also on the processes of spermatogenesis, which tended to recover over time [13].

To establish the separate effects of LPS exposure on the processes of lipid peroxidation the content of OS markers during chronic intake of LPS solution was determined. The results of the experimental study are shown in Table 1.

Table 1

Dynamics of the content of oxidative stress markers in blood serum of rabbits after chronic intake of LPS solution

Indicator	Animal groups:				
	control (n=5)	experiment (n=5)			
		before administration	15th day	30th day	45th day
Diene conjugates, μmol/l	1,28±0,04	1,30±0,03	1,94±0,06***[3]	1,73±0,07***[3]	1,46±0,04
Thiobarbiturate-active products, μmol/l	0,88±0,03	0,91±0,03	1,25±0,03***[3]	1,15±0,03***[3]	0,98±0,04*[1]
Stable metabolites of the NO cycle, μmol/l	3,93±0,12	3,92±0,10	5,59±0,13***[3]	4,77±0,19**[2]	4,33±0,09*[1]

Notes: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – statistically significant changes in relation to the control group; [1]$P < 0,05$; [2]$P < 0,01$; [3]$P < 0,001$ – statistically significant changes in relation to the group of animals before administration.

During chronic administration of LPS a significant increase in the intensity of lipoperoxidation processes was observed, which was evidenced by an increase in the content of OS markers on the 15th day of the experiment – DC by 52.1% ($P<0.001$), TBA-RC by 42.5% ($P<0.001$) and NO_x by 42.3% ($P<0.001$).

On the 30th day of the experiment a decrease in OS manifestations in males was noted. Thus, the content of DC was higher than the control indicators by 35.4% ($P<0.001$), TBA-RC by 30.9% ($P<0.001$), and stable metabolites of the NO cycle by 21.4% ($P<0.01$). It should be noted that these indicators were lower than those of the group on the 15th day of the study, namely: NO_x by 14.7% ($P<0.01$), TBA-RC by 8.0% ($P<0.05$), and the number of DC tended to decrease.

At the same time, at the end of the study the oxidative load was less significant – the amount of TBA-RC was by 14.8% (P<0.05) less than the data of the group on the 30th day and had a tendency to increase compared to the control group, while the content of DC decreased by 15.6% (P<0.05), but was higher than the control group by 14.2% (P<0.05). The content of stable metabolites of the NO cycle remained by 10.2% (P<0.05) more than the control indicators, and had a tendency to decrease compared to the previous group.

The obtained results in terms of the increase in the intensity of peroxidation processes in the blood serum of rabbits are consistent with the results of studies of the content of malondialdehyde and the antioxidant status of rat gonads after the use of LPS [14]. Dynamics of changes in the oxidative load parameters of male rabbits throughout the experiment are shown in fig. 2.

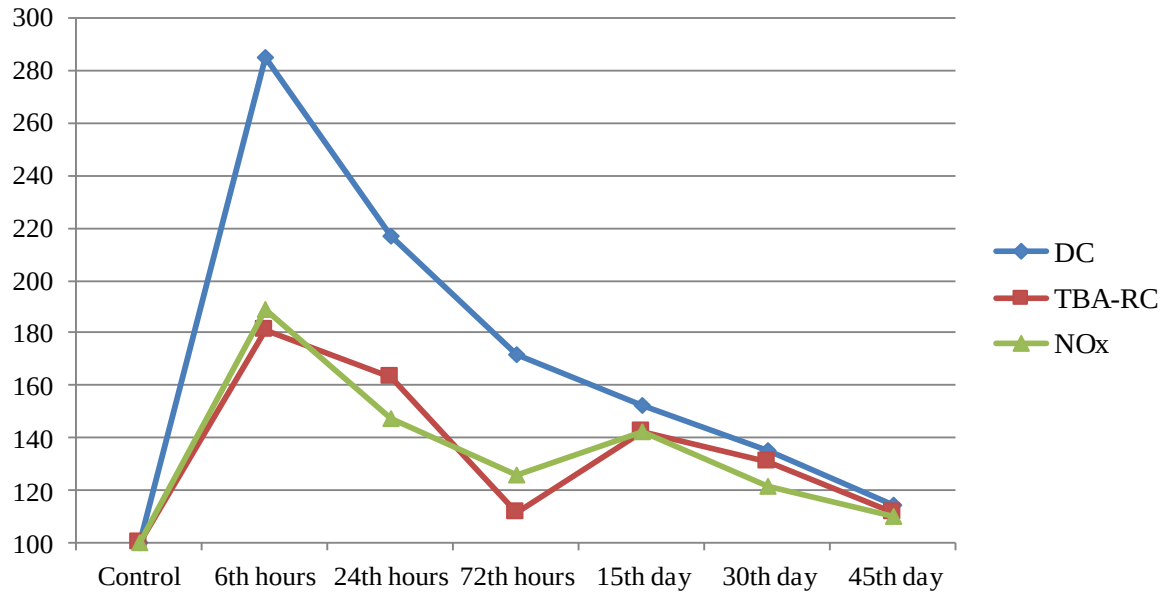

Figure 2. Oxidative load parameters of male rabbits organisms under the influence of LPS solution.

Note: Indicators of OS markers on control group are taken as 100%.

In a chronic experiment on rabbits, we established the presence of a pronounced oxidative imbalance in the blood serum, which can contribute to the negative dynamics of sperm quality and structural characteristics of spermatozoa after LPS administration, as shown in the study of Collodel G. et al. [15]. In addition, chronic administration of lipopolysaccharides can cause changes in the dynamics of growth factors, which will contribute to morphological changes in the tissues of the gonads and is a leading factor in male reproductive capacity, as shown in a study on white mice [16]. In general, the results of our research showed the possibility of creating an improved experimental model of male infertility under LPS-induced OS.

Conclusions and prospects for further research. The obtained results showed the effect of LPS as inducers of OS in the body of rabbits. It will allow to study their influence on reproductive capacity and to develop effective ways of correcting LPS-induced OS:

1. With a single injection of LPS solution a significant increase in the content of DC by 1.85 times (P<0,001), TBA-RC by 80.8% (P<0,001) and stable metabolites of the NO cycle by 88.0% (P<0,001) was noted.

2. 72 hours after the injection a tendency to decrease peroxidation processes was noted. Thus, the content of DC was higher than the control indicators by 71.8% (P<0.001), NO_x by 25.9% (P<0.001), and TBA-RC by 11.2% (P<0.05), but compared to the data of the group after 24 hours they were smaller by 20.7% (P<0.001), 14.4% (P<0.001) and 31.9% (P <0.001), respectively.

3. During the chronic administration of LPS a significant increase in the intensity of lipoperoxidation processes was observed, which was evidenced by an increase in the content of OS markers on the 15^{th} day of the experiment - DC by 52.1% (P<0.001), TBA-RC by 42.5% (P< 0.001) and NO_x by 42.3% (P<0.001).

4. Moreover, at the end of the study the oxidative load was less significant - the amount of TBA-RC was by 14.8% (P<0.05) less than the data of the group on the 30^{th} day and had a tendency to increase compared to the control group, while the DC content decreased by 15.6% (P<0.05), but was higher than the control group by 14.2% (P<0.05). The content of stable metabolites of the NO cycle remained by 10.2% (P<0.05) more than the control indicators, and had a tendency to decrease compared to the previous group.

The perspective of further research is to establish indicators of sperm quality and hormonal background in male rabbits during experimental LPS-induced OS, as a model of male infertility and development of means of its correction.

ДИНАМІКА ПРОЦЕСІВ ПЕРОКСИДАЦІЇ У САМЦІВ КРОЛІВ ЗА ЕКСПЕРИМЕНТАЛЬНОГО ЛПС-ІНДУКОВАНОГО ОКСИДАТИВНОГО СТРЕСУ / Кошевой В.І., Науменко С.В.

Вступ. *Провідним патогенетичним механізмом неплідності самців є стан оксидативного стресу. Серед поширених факторів, що ініціюють підвищення синтезу активних форм кисню, зацікавленість дослідників галузі репродуктивної ендокринології викликає вплив бактеріальних ендотоксинів – ліпополісахаридів на організм самців і їх репродуктивну функцію.*

Метою роботи *було передбачено встановлення динаміки змін маркерів пероксидації у самців кролів за одноразового та хронічного введення розчину ліпополісахаридів як експериментальної моделі ліпополісахарид-індукованого оксидативного стресу.*

Матеріали і методи досліджень. *Були сформовані три групи статевозрілих кролів: дослідна 1 (n=5), тваринам якої одноразово інтраперитонеально вводили 4 мг/кг маси тіла*

(0,18 LD_{50}) розчину LPS from E. coli O111:B4 (Sigma), дослідна 2 (n=5), самці якої зазнавали хронічного впливу ЛПС, у дозі еквівалентній 1:10 LD_{50} упродовж 14 діб та контрольна група (n=5), яким вводили 0,9 % розчин натрію хлориду. У пробах сироватки крові спектрофотометрично визначали маркери ОС: кількість дієнових кон'югатів, тіобарбітурат-активних продуктів і вміст стабільних метаболітів циклу Нітроген оксиду.

Результати досліджень та їх обговорення. *Введення ЛПС у формі розчину спричиняло очікувані ефекти в організмі самців і викликало значне збільшення інтенсифікації процесів пероксидації, про що свідчить динаміка вмісту маркерів ОС у сироватці крові кролів. Через 6 годин після введення ЛПС відмічали значне збільшення вмісту маркерів ОС. Через 72 години вміст маркерів ОС залишався високим, хоча відмічали тенденцію до зниження пероксидаційних процесів порівняно з показниками групи через 24 години після введення ЛПС. Подібні зміни відмічали й за хронічного введення розчину ЛПС.*

Висновки та перспективи подальших досліджень. *Отримані результати показали вплив ЛПС як індукторів ОС у організмі кролів: за одноразового введення розчину ЛПС відмічали значне збільшення вмісту ДК в 1,85 разів, ТБК-АП на 80,8 % та стабільних метаболітів циклу NO на 88,0 %, тоді як за хронічного введення відбувалося збільшення вмісту маркерів ОС: ДК – на 52,1 %, ТБК-АП – на 42,5 % і NO_x – на 42,3 %. Перспективою подальших досліджень передбачено встановлення показників якості сперми і гормонального фону у самців кролів за експериментального ЛПС-індукованого ОС, як моделі неплідності самців й розробка засобів її корекції.*

Ключові слова: *ліпополісахарид, прооксидантно-антиоксидантний баланс, дієнові кон'югати, тіобарбітурат-активні продукти, цикл Нітрогену оксиду.*

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