

PROCESSES OF PEROXIDATION OF LIPIDS AND PROTEINS IN ORGANS OF RABBITS CONSIDERING THE AGE-OLD ASPECT

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The content of products of lipid peroxidation and products of oxidizing modification of proteins were investigated in the brain, heart and the longest back muscle of New Zealand breed rabbits. During investigations it was set, that concentration of the hydroperoxide of lipids (HPL) during research period was the lowest in the longest back muscle 4,79-5,63 u/g of tissue. The increase of content of TBA-RC was marked in the heart of rabbits with 2,24 mmol/ g of tissue in one-day old animals to 4,85 mmol/g of tissue in 90-day old animals. The oxidizing modification of proteins (OMP) products are the perspective marker of free-radical oxidization level estimation, as this index have the protracted period of disintegration, as compared to products of lipoperoxidation. High content of dinitrophenylhydrazines of basic and neutral character was seen in the brain of one day old rabbits. However, with age, content of OMP products diminished in the organs of the investigated animals, that testified weakening of free-radical processes and activating of the system of antioxidant defense.

Keywords: peroxidation of lipids, oxidizing modification of proteins, rabbits, brain, heart, the longest back muscle.

Providing of population with valuable foodstuffs, especially animal origin, remains one of major tasks of agricultural production. Intensive technologies of maintenance of agricultural animals, in particular rabbits, are a strong stress factor that leads to destabilization of metabolic processes and as a result there is a decline of meat productivity. At these terms of conducting of rabbit breeding an important task is an increasing of productivity, a storing population of rabbits and an improvement quality of received products. The literature data of testify an important role of the system of antioxidant defense in the mechanisms of adaptation of rabbits to the conditions of existence and action of stress factors [5, 6, 14]. The violation of activity of the antioxidant system of defense (AOS) and the absence of it's control of the processes of peroxidation can become a factor of development of abnormal peroxidation and can cause different diseases [1, 7, 18]. Processes of peroxidation of lipids and proteins may be effectively corrected by preparations of external antioxidants taking into account the age-old features of functioning of AOS.

The objective of research was to learn progress of processes of peroxidation of lipids and proteins in the organism of New Zealand breed rabbits of different age.

Materials and methods

Investigations were conducted on the New Zealand breed rabbits in Limited Liability Company (LLC) "Gregut" Fastiv District, Kyiv Region. On the base of principle of analogues (age and weight) two groups of animals were formed - control and experience (100 heads in each). Animals were fed with the standard mixed fodder balanced on all indexes of feeding with free access to water. All period of the research lasted for 90 days. During conducting of experiment the principles of bioethics, legislative norms and requirements according to suggestion of "European convention about defense of vertebrates, that are used for experience and scientific aims" (Strasbourg, 1986) and "General ethic principles of experiments on the animals" accepted by First National congress on bioethics (Kyiv, 2001) were observed.

Materials for the research were heart, brain and the longest muscle of the back (musculus longissimus dorsi) that were taken away after slaughter animals 1 -, 15 -, 30 -, 45 -, 60 -, 75 - and 90-days old. The state of processes of peroxidation of lipids (POL) and proteins was determined by the generally accepted methodologies on maintenance the hydroperoxide of lipids (HPL), thiobarbituric acid reactive substances (TBA-RS), keto-dinitrophenylhydrazines (KDNPhH) and alpha-dinitro phenylhydrazines (ADNPhH) of neutral and basic character [2, 10, 13]. The obtained data were processed by the program Microsoft Excel with the use of t- criterion.

Results and discussions

There is a great amount of the works dealing with the study of the functional state of an antioxidant defense system of animals of different kinds, such as pigs, quails, rats [3, 7, 11, 15]. However progress of processes of lipid peroxidation in the organism of New Zealand breed crawls of the is not investigated enough. Research of mechanisms of lipids peroxidation and proteins oxidation in the organism of New Zealand breed rabbits gives an opportunity to work out the reasonable methods of correction the processes of peroxidation, improve functioning of the system of antioxidant defense.

Peroxidation of lipids (POL) plays an important role in adjusting of the oxidizing phosphorylating of cells and permeability of cellular membranes [4, 5]. Excessive formation of reactive oxygen species can be a reason of damage and cell death [3, 6, 7]. The obtained data testify that the processes of lipid peroxidation in the organism of rabbits of different experimental groups flow with different intensity and do not have the clearly expressed tissue specificity (Table 1).

Table 1

The content of lipid peroxidation products in the organs of rabbits (M±m; n=5)

Age, days	Brain		Heart		The longest back muscle	
	HPL, U/g of tissue	TBA-RS, mmol/g of tissue	HPL, U/g of tissue	TBA-RS, mmol/g of tissue	HPL, U/g of tissue	TBA-RS, mmol/g of tissue
1	7.52±0.06	62.11±1.04	7.64±0.04	2.24±0.15	5.01±0.05	19.59±0.35
15	8.68±0.09***	55.58±0.85**	7.98±0.03	2.66±0.17	5.38±0.06**	23.24±0.77**
30	9.23±0.05***	54.01±1.27	7.31±0.07	2.84±0.23	5.63±0.04**	23.09±0.59
45	8.58±0.07***	56.06±1.96	7.49±0.03	3.26±0.45	5.81±0.07*	23.81±0.44
60	8.28±0.09*	56.45±1.44	7.31±0.04	4.79±0.49*	4.83±0.08***	23.15±0.43
75	9.08±0.11**	56.27±1.37	7.68±0.05	4.82±0.25	4.79±0.09	23.87±0.41
90	9.23±0.13	55.04±0.67	7.82±0.06	4.85±0.26	5.09±0.08*	23.36±0.68

Note: here and further in the tables * - p≤0,05; ** - p≤0,01; *** - p≤0,001 – compared with preceding age period.

Intensity of free-radical processes is largely conditioned by the features of cellular metabolism [14, 16]. The determination of the content of HPL in organs has an important value for the estimation of POL activation. A concentration of HPL during all investigated period was the lowest in the longest back muscle and hesitated within the limits of 4,79-5,63 U/g of tissue. At the same time in the longest muscle of back of 15-days old rabbits it was seen a reliable increase of content of TBA-RS on 18,6 % in compare with one day-old rabbits, but on the next stages this index did not change substantially (Table 1).

The content of HPL in the brain of rabbits was higher at 90-days old animals. This index exceeded at 1,2 times the index of one-day old rabbits. It testifies about intensity of processes of lipid peroxidation in a postnatal period, in fact a molecules in brain is the first organ that yields to the processes of free-radical oxidation. It should be marked that content of TBA-RS in the brain of rabbits with age diminished and in 90-days old was at 12 % lower then the indexes of one-day old animals (Table 1). These changes can explained by increasing of functioning of antioxidant defense system and increase of the level of animals adaptation to production conditions.

In heart tissue the reliable difference between the content of HPL in rabbits of different age was not found. It was set act that the content of TBA-RS in the heart tissues at the end of investigation increased at 2,2 times, compared with the start of the experiment. Unconcerned changes of the content of TBA-reactive substances with hydroperoxides of lipids take place because these products appear from HPL that can yield to repeated oxidation and rendering by glutathione dependent enzymes that confirms by obtained data about activation of glutathione link of the system of antioxidant defense of organism [5, 7, 9].

Proteins are involved in all vitally important processes. Therefore a study of dynamics of their content in the tissues of animals is one of an important indexes of the physiology state of the organism. Proteins are needed for growth and development of animals, synthesis of enzymes and hormones. The early indicator of damage of cells at the terms of free-radical oxidation is oxidizing modification of proteins (OMP). It is considered that OMP plays a key role in molecular mechanisms of oxidative stress and is a starting factor for oxidation destruction of other molecules, in particular, lipids and nucleic acids [6, 14].

Destruction of proteins is more reliable marker of oxidation damages of tissues than lipids peroxidation. Products of OMP are more stable when compare with the peroxides of lipids that quickly metabolize under the action of peroxidase and low-molecular antioxidants [15, 18].

In the most of dinitrophenylhydrazines (DNPhH) belongs to KDNPhH and ADNPhH of neutral character. It was set that the content of KDNPhH of neutral and basic character was the highest in the tissues of rabbits brain, however there was a tendency of declining of these indexes with age (Table 2). But the amount of these products was a significantly lower in the heart and in the longest back muscle.

Table 2

The content of products of oxidizing modification of proteins in the rabbits' organs
(OE/g of tissue, $M \pm m$; $n=5$)

Age, days	Neutral character products		Basic character products	
	KDNPhH, $\lambda = 356$	ADNPhH, $\lambda = 370$	KDNPhH, $\lambda = 430$	ADNPhH, $\lambda = 530$
Brain				
1	60.27±0.47	45.77±0.93	38.71±0.59	11.17±0.39
15	57.72±0.52**	44.59±0.49	37.73±0.35	9.89±0.36*
30	58.61±0.39	43.81±0.74	36.26±0.61*	9.21±0.42
45	57.62±0.43	43.51±0.63	34.79±0.35*	8.13±0.39
60	57.82±0.44	42.24±0.42	33.12±0.25**	7.06±0.33*
75	56.45±0.33*	41.94±0.33	32.54±0.39	6.76±0.39
90	56.25±0.32	41.16±0.35	31.95±0.52	6.17±0.38
Heart				
1	55.47±0.59	46.84±0.57	26.56±0.42	10.19±0.48
15	52.53±0.29**	41.85±0.33***	23.72±0.51**	8.23±0.36**
30	47.14±0.29***	40.38±0.59*	20.68±0.42**	6.96±0.33*
45	44.69±0.57**	38.91±0.59	18.62±0.51*	5.88±0.47
60	41.16±0.35***	34.98±0.63**	15.19±0.59**	4.21±0.39*
75	37.83±0.28***	33.12±0.67	13.52±0.43*	3.92±0.35
90	33.91±0.52***	29.79±0.52**	11.17±0.36**	2.94±0.36
The longest back muscle				
1	42.53±0.36	34.99±0.39	34.31±0.64	9.98±0.53
15	40.47±0.29**	32.34±0.47**	31.75±0.42**	8.92±0.42
30	37.83±0.36***	29.79±0.67*	29.11±0.45**	7.25±0.41*
45	35.57±0.51	25.97±0.35***	24.41±0.57***	5.09±0.39**
60	34.59±0.45	24.59±0.36*	20.48±0.42***	4.61±0.45
75	32.24±0.51**	22.54±0.35**	18.82±0.45**	3.82±0.27
90	30.77±0.57	20.48±0.55*	17.15±0.49*	3.14±0.37

The content of KDNPhH of neutral character in heart of rabbits at the end of investigation diminished at 39 %; in the longest muscle of back at 28 %, compared with the beginning of investigation. The research of ADNPhH of basic and neutral character showed the less content of these products in heart and the longest muscle of back. So, on the 90-th day of investigation in heart of rabbits the content of ADNPhH of neutral character diminished at 36,4 %, in the longest muscle of back at 41,5 %, compared with one day-old rabbits.

The fluctuations of the content of different products of OMP have the features that, probably, it is related to the terms of their formation. So, dityrozin, that is a doubled molecule of tyrosine appears mainly at the direct action of reactive oxygen on species protein molecules. In the same time, Carbonyl derivatives of proteins may be formed through the direct oxidation of amino acid or due to reactions with products of lipid peroxidation and glycol oxidation.

Thus, conducted complex investigations of various indexes of free-radical oxidation of lipids and proteins in the organs of New Zealand breed rabbits allowed more valuably describe peroxidation processes. In rabbits heart negative cross-correlation connection ($r=-0,9$) was educed between content of TBA-RS and DNPhH of neutral and basic character. It was also marked moderate negative connection ($r=-0,6$) between content of HPL and OMP products of neutral and basic character in the brain.

However, the content of TBA-RS and OMP in the brain have moderate degree of linear correlation ($r=+0,55$). The content of POL products in brain of rabbits has a positive cross-correlation connection ($r=+0,9$). Oxidizing modification of proteins is an early sign of damage of tissues at free-radical damage mediated pathologies that is why the indexes of OMP can be used as one of the indexes of the state of oxidative stress at pathological states. At development of most pathological processes exactly of proteins, but not lipids and nucleic acids are effective traps for the active forms of oxygen. Proteins may be degraded in cells from a few hours to several days, and the products of lipid peroxidation are subject to detoxification within minutes

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ПЕРОКСИДНЕ ОКИСНЕННЯ ЛІПІДІВ І БІЛКІВ В ОРГАНАХ КРОЛІВ: ДОСЛІДЖЕНО У ВІКОВОМУ АСПЕКТІ

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Досліджено вміст продуктів пероксидного окиснення ліпідів і продуктів окисної модифікації білків у мозку, серці та найдовшому м'язі спини кролів новозеландської породи. У ході досліджень було встановлено, що концентрація гідропероксидів ліпідів протягом усього дослідного періоду була найнижчою у

найдовшому м'язі спини 4,79–5,63 ОЕ/г тканини. Відмічено підвищення вмісту продуктів, що реагують з тіобарбітуровою кислотою у серці кролів з 2,24 ммоль/г тканини в однодобовому віці до 4,85 ммоль/г тканини у віці 90 діб. Продукти окисної модифікації білків є перспективним маркером оцінки вільнорадикального окиснення, оскільки характеризуються тривалішим періодом розпаду, порівняно з продуктами ліпопероксидації. Найвищий вміст динітрофенілгідрозонів основного та нейтрального характеру спостерігали у мозку однодобових кроленят. Проте з віком вміст продуктів окисної модифікації білків у органах досліджуваних тварин зменшувався, що свідчить про пригнічення вільнорадикальних процесів і активацію системи антиоксидантного захисту.

Ключові слова: пероксидне окиснення ліпідів, окисна модифікація білків, кролі, мозок, серце, найдовший м'яз спини.

ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ И БЕЛКОВ В ОРГАНАХ КРОЛИКОВ: ИССЛЕДОВАНО В ВОЗРАСТНОМ АСПЕКТЕ

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Продукты ОМБ являются перспективным маркером оценки свободнорадикального окисления, поскольку имеют длительный период распада, по сравнению с продуктами липопероксидации. Высокое содержание динитрофенилгидразонов основного и нейтрального характера наблюдали в мозге односуточных крольчат. Однако с возрастом содержание продуктов ОМБ в органах исследуемых животных уменьшалось, что свидетельствует об ослаблении свободнорадикальных процессов и активации системы антиоксидантной защиты.

Ключевые слова: перекисное окисление липидов, окислительная модификация белков, кролики, мозг, сердце, длиннейшая мышца спины.