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AGE DEPENDET FEATURES OF LIPID PEROXIDATION REGULATION IN BLOOD FERTILITY OF MALE RATS

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This work is devoted to the research of age-dependent features of lipid peroxidation regulation in blood and fertility of rats. Such methods as cytochemical and biochemical were used. Age–dependent changes of lipid hydro peroxide contain were investigated, state of enzymatic and non-enzymatic antioxidant system in blood plasma and erythrocytes of rats of 1-, 3-,12- and 24-months old have been studied then studied the fertility of male rats: motility sperm, sperm content, morphology. The content of trace elements (Zn, Mg, Cu) in blood and seminal plasma and spermatozoons was investigated.

Obtained results allow to make a conclusion, that the role of enzymatic glutton dependent antioxidant system of animals in LPO inhibition increases with aging. In young (1 month old) and old groups (24 months old) male rats were infertile, adult group (12 months) were fertile. It was shown, that fertility of animals depended on aging. In the case with young and old male rats the age may explain higher content of Cu and lower content Mg and Zn in blood, seminal plasma and spermatozoids than in group 12 months age.

The results of the research are the following: 1) The status of the antioxidant system depends on aging. 2) The content of helatable metals (zinc, cuprum, magnesium) in blood and seminal plasma and spermatozoons depends on aging. 3) The fertility depends on aging.

Key words: fertility, helatable metals, Cu, Zn, Mg, aging, antioxidant fermentation and non-fermentative system.

Єщенко Ю.В., Бовт В.Д., Романова М.Д. ВПЛИВ ВІКУ НА ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ У КРОВІ І ФЕРТИЛЬНІСТЬ САМЦІВ ЩУРІВ / Запорізький національний університет, 69600, Україна, Запоріжжя, вул. Жуковського, 66.

Дослідження присвячене впливу віку на регуляцію перекисно-окиснюваних процесів у крові, а також на фертильність у самців щурів. Використано цитохімічні і біохімічні методи. Досліджувались залежні від віку зміни в перекисному окисненні ліпідів та їх зв'язок з чоловічою фертильністю. Дослідження проводилися на безпородних самцях щурів віком 1, 3, 12 і 24-місяці. Визначали стан ферментативних та неферментативних систем крові, також було вивчено фертильність за такими показниками, як рухливість сперматозоїдів, їх кількість та морфологія. Крім того оцінювали вміст хелатоутворюючих металів (Zn, Mg, Cu) у плазмі крові та спермі і їх вміст у сперматозоїдах.

Встановлено, що найнижча активність антиоксидантної системи крові була у молодих та старих щурів, а найбільша – у дорослих, аналогічні результати були отримані для показників фертильності та стану металолігандного гомеостазу.

Результати дослідження: 1) стан антиоксидантних систем залежить від віку; 2) вміст хелатоутворюючих металів (Zn, Mg, Cu) в плазмі крові, спермі та в сперматозоїдах залежать від віку; 3) фертильність залежать від віку.

Ключові слова: фертильність, хелатоутворюючі метали, Cu, Zn, Mg, вік, антиоксидантні ферменти та не ферментативні системи.

Ещенко Ю.В., Бовт В.Д., Романова М.Д. ВЛИЯНИЕ ВОЗРАСТА НА ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ В КРОВИ И ФЕРТИЛЬНОСТИ САМЦОВ КРЫС / Запорожский национальный университет, 69600, Украина, Запорожье, ул. Жуковского, 66.

Исследование посвящено влиянию возраста на регуляцию перекисных окислительных процессов в крови, а также на фертильность самцов крыс. Были использованы: цитохимические и биохимические методы. Исследовались зависящие от возраста изменения в перекисном окислении липидов и их связь с мужской фертильностью. Исследования проводились на беспородных самцах крыс возрастом 1, 3, 12 и 24-месяца. Определяли состояние ферментативных и неферментативных систем крови, также изучена фертильность по таким показателям, как подвижность сперматозоидов, их количество и морфология. Кроме того, оценивали содержание хелатообразующих металлов (Zn, Mg, Cu) в плазме крови и спермы и их содержание в сперматозоидах.

Установлено, что низкая активность антиоксидантной системы крови была у молодых и старых крыс, а наивысшая – у взрослых, аналогичные результаты были получены для показателей фертильности и состояния металлолигандного гомеостаза.

Выводы из результатов: 1) состояние антиоксидантных систем зависит от возраста; 2) содержание хелатообразующих металлов (Zn, Mg, Cu) в плазме крови, сперме и в сперматозоидах зависят от возраста; 3) фертильность зависит от возраста.

Ключевые слова: фертильность, хелатообразующие металлы, Cu, Zn, Mg, возраст, антиоксидантные ферменты, неферментативные системы.

INTRODUCTION

On the biological trace elements Zn, mg and Cu are important in reproduction [1-3]. Helatable metals (Zn, Mg, Cu) has been intensively studied, there deficiency leads to gonadal dysfunction [1-3], decreases testicular weight and causes shrinkage of seminiferous tubules [4-7].

Helatable metals are necessary for reproduction because the gonads are the most rapidly growing tissues in body and vital enzymes involved in nuclear acid and protein synthesis are metal-enzymes [1-4].

The three cations (cu, Zn, Mg) stimulate or inhibit progressive motility depending on the concentration of each. At high concentration, these elements individually or jointly, impair fertility among the patients with normal sperm density [4-7].

Recent studies hypothesized that insufficient intake of Zn can impair and make the sperm cell highly susceptible to oxidative damage [4-6]. High levels of free radicals may overwhelm the antioxidant strategies, which is associated with low quality of sperm [4-6].

Nowadays, one of the most universally recognized representations of the mechanisms of aging in the Harman's hypothesis is about the key role of free radicals in infringement of structure and functions of bio membranes and macromolecules with aging [7, 8]. While the tendency to a simplified treatment of free radical of aging, as an activation of free radical processes biostructures damage with age prevails in literature [7, 8].

The purpose of the presented work was the investigation of the level of LPO products and also the state of enzymatic and non-enzymatic antioxidant system in plasma and erythrocytes, content of trace elements (Zn, Mg, Cu) in blood and seminal plasma and their relation to fertility of male rats of 1-, 3-,12- and 24- months old.

MATERIALS AND METHODS

We used 40 male rats of 1-, 3-, 12- and 24- month age, per 10 animals in each group. The 1 month age rats were a control group. After decapitation the animal's blood was collected in heparinized test-tubes, plasma and erythrocytes were obtained frozen and kept in liquid nitrogen in polyethylene ampoules 0,5 ml in each.

Before measuring the LPO products and activities of enzymatic and non- enzymatic antioxidant system, ampoules were heated in water bath at 37C. It had shown earlier, that fast freezing-thawing out didn't considerably influence the value of the measured parameters [9].

The resistance of erythrocytes to a spontaneous hemolysis was defined spectrophotometrically at 543 nm according to Jagera method [10]. In experiments we used fresh heparinized blood. The degree of spontaneous hemolysis of erythrocytes was expressed in % to template hemolysis of erythrocytes. Antioxidant activity (AOA) Of blood plasma was determined from the ability of plasma to impale brake the accumulation of thiobarbituric acid – active LPO products in suspensions of yolk lipoproteins [11]. A spectrum of absorption of TBA - active product was recorded on a two beam spectrophotometer «Specord UV VIS», then was absorbed at 532-580 nm [9].

The efficiency of quenching of OA-radicals by plasma of blood was defined from the activity of plasma to break the destruction of deoxyribose's by AO-radical [9]. Antiradical activity was calculated in the same way as AOA.

The definition of lipid hydro peroxide in blood plasma was carried out according to Asakava [9]. A spectrum of adsorption of the painted product was recorded on a two beam spectrophotometer «Specord UV VIS» then we measured the absorption at 535-520 nm. The content of hydro peroxides was expressed in equivalent amounts of malondialdehyde (MDA).

Glutathione peroxide activity of plasma and erythrocytes was determined by spectrophotometric at 350 nm in a medium containing 50 mM K, Na – phosphate buffer, pH 7,4, 1 mM EDTA, 0,15 mM NAD PH, 1mM GSH, 0,6 mM MA_2O_2, 3 mM NaN_3 and 1,0 U/ml glutathione reductase as it was described [12]. The activity was expressed in nmol NADPH /ml of plasma or for 1 mg of erythrocytes protein in 1 mg of erythrocytes in 1 minute, using the factor of molar extinction that is 6,22 x 103 $M^{-1}cm^{-1}$.

Glutathione reductase activity of erythrocytes was determined by spectrophotometric at 340 nm in a medium, containing 50 mM K-phosphate the buffer, pH 7,4, 1 mM EDTA, 0,16 mM NADPH, 1mM GSSG, as it was described [9]. The activity was expressed in nmol NADPH an 1 mg in 1 minute.

Glucose-6-phosphate dehydrogenase activity of erythrocytes was measured by spectrophotometric from the speed of NADP reduction [10].

Semen parameters analysis on microscopic examination sperm morphology was evaluated according to the criteria by Kruger [10].

Measurement of Cu, Mg, Zn in semen and blood plasma: semen samples were centrifuged at 600 g for 10-fold by deionized water. Levels of Cu, Mg, Zn were measured by atomic absorption spectrophotometer [2, 6].

Measurement of Cu, Mg, Zn in spermatozoons: seminal smears were fixed for 5 min formalin vapors before dithizone staining 0,2% water ammonia solution of this reagent for Zn was used [2, 6]. Reagent of Mg was lumomagneson [2, 6], of Cu lumocuppheron [10]. Metals content in spermatozoids was evaluated with semi quantitative methods.

The first one was carried out using the following criteria 1- weak, 2- moderate, 3-pronounced reaction intensity. The score for 100 cells was counted, using colored sales [14]. The number of spermatozoids was expressed as its number per 1 mml of the semen. The results are presented as X ±SD: testing for significance is performed using Students – test.

RESULTS AND DISCUSSION

The data submitted in the table 1 changes in content of LPO products and the state of enzymatic and non-enzymatic antioxidant system in plasma and erythrocytes of blood of rats of different ages.

The data submitted in the table testify, that blood considerably decreased with an average age of animals and during further aging a little bit increased, having remained authentically lower at the level of young 1-month rats. The activity of the basic enzyme, utilizing hydro peroxides of lipids of plasma, H-dependent glutathione peroxidase – on the contrary, sharply increased by average age. So highly expressed the increasing of glutathione peroxidase activity by the 12-month age in rats was also found in erythrocytes of blood.

Glutathione-s-transferase activity in blood plasma of 12-month old animals was also authentically over activity among the young 1-month old rats. During further aging by 24-month, the activity of the studied glutathione-dependent antioxidant enzymes a little bit reduces, remaining considerably higher among the young (glutathione peroxidase erythrocytes) or at level of 1- month old rats (glutathione peroxidase and glutathione-s-transferase of plasma).

Table 1 – The changes in content of LPO products and the state of enzymatic and non-enzymatic antioxidant system in plasma and erythrocytes of blood of male rats of different age (1,3, 12, 24 month, n = 10 in all group.)

Measurable parameters	Age, month			
	1	3	12	24
Hydro peroxide of lipids (nmol MDA/ml plasma)	5,41 ±0,22	4,12±0,17*	3,61±0,14**	3,72±0,19*
Glutathione peroxidase activity (nmol NADPH/ml plasma per minute)	1932±153	2081±199	2814±222*	1922±321
Glutathione transferase activity (nmol XDNB/ml plasma per minute)	90,6±3,7	101,3±3,5	123,8±11,1*	95,9±7,9
Glutathione peroxidase activity (nmol NADPA/mg protein erythrocytes per minute)	2,88±0,22	2,24±0,27*	1,82±0,08*	2,48±0,23
Glutathione reductase activity (nmol NADPA/mg protein erythrocytes per minute)	2,89±0,24	2,24±0,18*	1,85±0,83*	2,46±0,21
Glucosed-6-phosphate dehydrogenase activity (nmol NADPH/mg protein erythrocytes per minute)	82,9±6,8	72,2±5,5	57,9±5,1*	63,9±4,9*
Degree spontaneous hemolysis erythrocytes, in % to complete	26,1±0,9	16,9±1,1*	18,6±1,1*	21,1±0,5*
Antioxidant activity of plasma, in %	53,9±7,8	53,8±4,5	24,9±4,9*	19,9±5,1*
Antiradical activity of plasma, in %	64,2±2,3	62,8±2,9	58,5±3,1	54,1±3,1*

Note: * P<0,05 compare with 1 month age rats group.

The activity of non-enzymatic antioxidant system of blood presented in this work is characterized by the amount AOA of plasma, the ability, to quench OH-radical and by the degree of erythrocytes resistance to a spontaneous hemolysis, in contrast to activity of enzymatic antioxidant system considerably decreased by an average age in 2,2 times and by 24-months in 2,7 times in comparison with 1-month animals. The resistance of erythrocytes to a spontaneous hemolysis and antiradical activity of plasma decreased in 1,2 times by 24-month age.

Obtained results allow to make a conclusion, that the role of enzymatic glutton – dependent antioxidant system of blood of animals in LPO inhibition increases with aging. Here it is important to note that glucose-6-phosphate dehydrogenase and glutathione reductase activities in erythrocytes decreased during the aging of animals. According to this reason it is possible to expect, that activation of POL may be more expressed in old rats than in young rats under certain long extreme conditions, which cause oxidation of GSH and NADPH. As a whole, received results about age changes of certain parameters, which characterize the condition of LPO and antioxidant system of blood, testify that during relatively late periods of enzymatic system. In this concentration more complete supply with reducing equivalents (NADPH and GSH) of fermentative antioxidant system of the young and old male rats' organisms may be an effective

way to inhibit the occurrence and development of age related pathologies of free radical etiology. Average values of sperm parameters in group of male rat can be observed in table 2.

Table 2 – Age and semen parameters in 4 studied groups.

Variable	Age, month			
	1	3	12	24
Sperm content ($x10^6$)	2,78±0,23	14,25±0,31**	17,81±0,45***	3,21±0,46**
Motility, %	2,31±0,12	54,21±0,42***	63,91±7,81***	3,42±0,34*
Normal morphology, %	3,25±0,31	17,18±1,12***	25,14±2,25***	4,47±1,81*

Note: *P <0.05, **P <0,01, ***P <0,001 compare with 1 month age rats group.

In this group young (1 month old) and old (24 months old) male rat was infertile and other rats were fertile. The fertility of animal depends on aging.

Then content of helatable metals (Zn, Mg, Cu) in blood and seminal plasma in male rat is shown in table 3.

Table 3 – The content of metals (Zn, Mg, Cu) in blood and seminal plasma in 4 studied groups.

Variable	Age, month			
	1	3	12	24
Zn (in blood plasma, µmol/l)	7,73±0,81	10,15±0,79***	12,24±2,56***	6,54±1,35*
Zn (in seminal plasma, µmol/l)	103,02±3,01	110,25±8,42*	136,51±2,32**	94,28±3,25*
Mg (in blood plasma, µmol/l)	0,77±0,08	1,04±0,16***	1,24±0,09***	0,41±0.06**
Mg (in seminal plasma, µmol/l)	10,21±2,31	12,42±1,62	15,37±2,74**	8,31±1,35
Cu (in blood plasma, µmol/l)	22,19±3,91	17,07±1,12***	15,71±2,42***	18,82±1,79*
Cu (in seminal plasma, µmol/l)	271,31± 15,21	191,82±3,74**	175,05±8,94***	239,39±14,31

Note: *P<0.05, **P<0,01, ***P<0,001 compare with 1 month age rats group.

The situation with the control group (1-month old) and old group (24-months old) may explain higher content of Cu and lower content of Zn and Mg in blood and seminal plasma. The situation with the group (12-months old) may explain higher content of Zn, Mg and lower content of Cu in blood and seminal plasma . The content helatable metals in blood and seminal plasma depends on aging. Table 4 shows the content of helatable metals in spermatozoons.

Table 4 – The content of metals (Zn, Mg, cu) in spermatozooons of male rats.

Intensity of cytochemical reaction, sing	Age, month			
	1	3	12	24
Zn (dithizone)	0,86±0,07	1,27±0,14**	1,38±0,09**	0,95±0,07**
Mg (lumomagnesone)	0,32±0,05	0,94±0,61***	1,07±0,08***	0,74±0,05**
Cu(lumocuppherone)	1,04±0,04	0,85±0,07**	0,49±0,05***	0,76±0,08*

Note: *P<0.05, **P<0,01, ***P<0,001 compare with 1 month age rats group.

The situation with the control group (1-month old) and old group (24-monthes old) may explain higher content of Cu and lower content of Zn and Mg in spermatozoons. The situation with the group (12-month) may explain higher content of Zn, Mg and lower content of Cu in spermatozoons. The content helatable metals in spermatozoons depends on aging.

In future we will study the method of correction of alteration in metal ligand homeostasis, antioxidant system and male fertility which depends on aging.

CONCLUSION

1. The status of the antioxidant system depends on aging. The antioxidant activity was lower in groups (1 and 24-months old) and higher in 12 months old rats.
2. The content of the helatable metals (Mg, Cu, Zn) in blood and seminal plasma and spermatosoons depends on aging, in case with young and old rats male may explain higercontent of Cu and lower content Zn and Mg in blood and seminal plasma in spermatozoons.
3. The fertility depends on aging. The fertility was lower in young and old groups (1 and 24-months old) and higher in adult 12 months old.

LITERATURE

1. Zalupus R.K Cellular and molecular biology of metal / R. K. Zalupus, J. Koropatnich. – New York : CRC Press, – 2010. – 442 p.
2. Valle B.L. Zink biochemistry, physiology, toxicology and clinical pathology / B.L. Valle. – Biofactors. – 1988. – Vol. 1. – P. 31–36.
3. Tudor R. Zinc in health and chronic disease / R. Tudor, P. Zalewski, R. Rathaike // J. Nutr. Health Aging. – 2005. – Vol. 1. – P. 31–36.
4. Єщенко Ю.В. Стрес і метаболізм металів / Ю.В. Єщенко – Запоріжжя: ЗНУ, 2010. – 268 с.
5. Yunagimachi R. Mechanism of fertilization in animals / R. Yunagimachi, G. Mastroianni, J.D. Biggers // Fertilization and embryonic development *in vitro*. – New York : Plenum press. – 1981. – P. 81–187.
6. Novel association between sperm reactive oxygen species production, sperm morphological defeets and the sperm deformity index / [Aziz N., Saleh R.A. Sharma R.K., et all.] // Fertil. Steril. – 2004. – 349 p.
7. Sikka S.L. Role of oxidative stress and antioxidants in male infertility/ S.L. Sikka, M. Rajasekaran, W.J. Hellstrom // J. Androl. – 1995. – Vol. 1. – P. 464–468.
8. Harman D. Free radicals in aging / D. Harman // Mol. Cell Biochem. – 1988. – Vol. 84 (2). – P. 155–161.
9. A spectrometric method for the determination of hydroperoxides in liposomes / [Cimato A., Facorro G., Aguirre F. at all.] // Spectrochim Acta A Mol Biomol Spectrosc., 1998. – Vol. – 54 A (12). – P. 2001–2008.
10. Thomas T. Chemical laboratory diagnosis / T. Thomas. – Frankfurt: Verlagsgeseeschaft, 1998 – 172 p.

REFERENCES

1. Zalupus R.K Cellular and molecular biology of metal / R. K. Zalupus, J. Koropatnich. – New York : CRC Press, – 2010. – 442 p.
2. Valle B.L. Zink biochemistry, physiology, toxicology and clinical pathology / B.L. Valle. – Biofactors. – 1988. – Vol. 1. – P. 31–36.
3. Tudor R. Zinc in health and chronic disease / R. Tudor, P. Zalewski, R. Rathaike // J. Nutr. Health Aging. – 2005. – Vol. 1. – P. 31–36.
4. Eshhenko Ju.V. Stres i metabolizm metaliv / Ju.V. Eshhenko– Zaporizhzhja: ZNU, 2010. – 268 s.

5. Yunagimachi R. Mechanism of fertilization in animals / R. Yunagimachi, G. Mastroianni, J.D. Biggers // Fertilization and embryonic development in vitro. – New York : Plenum press. – 1981. – P. 81–187.
6. Novel association between sperm reactive oxygen species production, sperm morphological defeets and the sperm deformity index / [Aziz N., Saleh R.A. Sharma R.K., et all.] // Fertil. Steril. – 2004. – 349 p.
7. Sikka S.L. Role of oxidative stress and antioxidants in male infertility/ S.L. Sikka, M. Rajasekaran, W.J. Hellstrom // J. Androl. – 1995. – Vol. 1. – P. 464–468.
8. Harman D. Free radicals in aging / D. Harman // Mol. Cell Biochem. – 1988. – Vol. 84(2). – P. 155–161.
9. A spectrometric method for the determination of hydroperoxides in liposomes / [Cimato A., Facorro G., Aguirre F. at all.] // Spectrochim Acta A Mol Biomol Spectrosc., 1998. – Vol. – 54 A (12). – P. 2001–2008.
10. Thomas T. Chemical laboratory diagnosis / T. Thomas. – Frankfurt: Verlagsgeseeschaft, 1998 – 172 p.

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ЗМІНА РІВНЯ СТІЙКОСТІ УВАГИ СТУДЕНТІВ І ТА ІІІ КУРСІВ У ПРОЦЕСІ НАВЧАННЯ

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У статті розглянуто проблеми стійкості уваги в студентів І і ІІІ курсів біологічного факультету. Мета дослідження – провести порівняльний аналіз змін рівня стійкості уваги студентів І і ІІІ курсів із різним типом темпераменту. Порівняльний аналіз отриманих даних показав, що студенти ІІІ курсу загалом краще виконували завдання та мали кращі результати порівняно зі студентами-першокурсниками протягом усього навчального року, що можна пояснити кращою адаптацією до навчання у ВНЗ.

Ключові слова: увага, типи темпераменту, холерик, сангвінік, флегматик, стійкість уваги.

Кучковский О. Н., Сичова С. О. ИЗМЕНЕНИЕ УРОВНЯ УСТОЙЧИВОСТИ ВНИМАНИЯ СТУДЕНТОВ І И ІІІ КУРСА В ПРОЦЕССЕ ОБУЧЕНИЯ / Запорожский национальный университет; 69600, Украина, Запорожье, ул. Жуковского, 66

В статье рассмотрены проблемы устойчивости внимания у студентов І и ІІІ курсов биологического факультета. Целью нашего исследования является сравнительный анализ изменений уровня внимания студентов І и ІІІ курсов с разным типом темперамента. Сравнительный анализ полученных данных устойчивости внимания показал, что студенты ІІІ курса в целом лучше выполняли задания и имели лучшие результаты по сравнению со студентами-первокурсниками в течение всего учебного года, что можно объяснить лучшей адаптацией к обучению в ВУЗе.

Ключевые слова: внимание, типы темперамента, холерик, сангвиник, флегматик, стойкость внимания.

Kuchkovsky O.N., Sichova S.O. CHANGE OF THE LEVEL OF STABILITY OF ATTENTION IN THE PROCESS OF STUDENTS TRAINING I AND III OF THE COURSE / Zaporozhye National University; 69600, Ukraine, Zaporozhye, Zhukovsky str., 66

The problem of studying of attention is now becoming increasingly important. This is primarily due to the high dynamics of life, which becomes increasingly important health problem students, facilitating their living and learning. Today a growing number of children who have lost interest in the study decreased their intellectual level, decreased concentration, all this necessitates the development of practical psychological and pedagogical methods to increase the potential of students. Attention is inherently one of the phenomena of approximately & D personality. And the psychological impact is directed to the content of the image, a phenomenon thought. Attention plays an important role in the regulation of intellectual activity. It focuses, directs the human mind at any given time on any real or ideal objects – objects, events, events, images, thoughts, etc. Attention occupies a special place among mental phenomena, speaking an integral part of learning, feeling and will.

Attention review and as a function of internal control over compliance mental action programs for their implementation. Development of such monitoring improves the performance of any activity and its