included the sections of the wall of the ascending aorta, the aortic arch, and the descending aorta taken from 26 sexually mature white male rats weighing 100 - 160 g. For morphometric examination, a series of photos of the aortic wall was taken using a Meiji MT4300 LED microscope with an x40 objective, x10 ocular. The measurements were carried out using the Image J software. The development of micro - and macroangiopathies in experimental animals with 8-week streptozotocin-induced diabetes mellitus was histologically confirmed. Statistical analysis revealed a significant difference in all morphometric parameters of the components of the aortic wall and the vessels of its hemomicrocirculatory bed after 8 weeks of experimental diabetes as compared with the normal values, control values, and in values obtained at the 6-week period of the experiment. The study has demonstrated clear dependence between the severity of destructive changes in the aortic wall and sections of its hemomicrocirculatory bed and the duration of the experiment.

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INFLUENCE OF MELATONIN ON AGE-RELATED CHANGES OF CARBOHYDRATE METABOLISM AND ANTIOXIDANT CAPACITY IN THE BLOOD OF ALLOXAN DIABETIC RATS

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Melatonin and its metabolites have potent antioxidant/anti-inflammatory properties, and they have proven to be highly effective in a variety of disorders linked to inflammation and oxidative stress. The object of this experimental research was to ascertain the influence of aging on the level of basal glycemia and activities of glucose-6-phosphate dehydrogenase [EC1.1.1.49], pyruvate kinase [EC 2.7.1.40] and glutathione reductase [EC1.6.4.2] in erythrocytes of alloxan diabetic rats on the background of melatonin injections. Methods: We used 100 male Wistar rats, two age groups: the - 2-month (adult), and II - 4-month (old). Alloxan diabetes was evoked via injecting the rats with a 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg. Four days after diabetes induction, rats were divided into diabetic (untreated) and melatonin-diabetic group (10 mg/kg, daily and intraperitoneally for six weeks). Blood was taken from the tail vein evaluate the basal glycemia on 5-th and 47-th day after the injection of alloxan. Rats were sacrificed at the 47-th day of the experiment accordance with the ethical treatment of animals. Determinations of the enzymes activities were by standard methods. Statistical analysis was performed using Statistica 10 StatSoft Inc. Results. The level of basal glycemia on the fifth day of the experiment in animals of both groups increased on average by 115% from baseline values. We founded that on 47-th day this index was higher in group of old rats on 20% more than in adult rats. Pyruvate kinase activity in erythrocytes of adult and old animals with diabetes decreased by 34% and 51% respectively compared with the control. glucose-6-phosphate dehydrogenase activity in erythrocytes of adult and old animals with diabetes decreased by 25% and 44% respectively compared with the control on 47-th day. The changes may be the result of age-related disorders of glucose metabolism due to disturbances in free radical mechanisms. Glutathione reductase activity in erythrocytes of adult and old animals with diabetes decreased by 30% and 36% respectively compared with the control on 47-th day. A 42-days injection of melatonin to the alloxan diabetic rats of both groups contributed to a normalization of the level of basal glycemia, the activities of pyruvate kinase and glutathione reductase in the rat blood, as well as to a considerable increase of the activity of glucose-6-phosphate dehydrogenase, whose level exceeded by average 9% this particular index in the control group of animals. Under the influence of melatonin increase activity of glucose-6-phosphate dehydrogenase in the blood of rats may be due to the increasing number of substrate for glucose-6-phosphate dehydrogenase (stimulating the flow of glucose into cells and its phosphorylation) and direct action. Conclusion. In this case melatonin probably increases use of glucose for regeneration of $NADPH_2$ and aerobic oxidation of glucose that indicate an acceleration of antioxidative protection and energy production in blood of adult and old diabetic rats.

Key words: melatonin, blood, alloxan diabetes, aging, rats.

This study is conducted as a part of research project "Morphofunctional and biochemical substantiation of dysfunctions of neurosecretory structures of the brain and endocrine glands and hepatorenal system of rats in experimental pathology, in the age aspect and ways of its correction", State Registration No. 0119 U101345.

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is the major product of the pineal gland that functions as a regulator of sleep, circadian rhythm, and immune function. Melatonin and its metabolites have potent antioxidant/anti-inflammatory properties, which have been proven as highly effective in a variety of disorders associated with inflammation and oxidative stress [3]. The studies on animals and humans have documented that short-term use of melatonin is safe, even when overdosed. Similarly, randomized clinical trials indicate the long-term melatonin treatment causes only mild adverse effects comparable to placebo [2].

In recent years, a considerably increasing number of people have been diagnosed as having

diabetes mellitus (DM) [6]. The increasing incidence of type 1 diabetes coupled with advances in treatment of type I diabetes has resulted in an unprecedented number of older adults living with and managing type I diabetes I [11].

It is known that metabolism in human body changes throughout ontogenesis [5]. Aging is characterized by a progressive deterioration in physiological functions and metabolic processes. The age-related loss of cells in vital tissues and organs is regarded as a factor resulting in oxidative stress and inflammation [5]. Oxygen free radicals of mitochondrial origin seem to be involved in aging [15]. The rate of mitochondrial oxygen radical generation of post-mitotic tissues is negatively correlated with animal longevity.

DM is characterized by metabolic disturbances. The most obvious symptom of diabetes, hyperglycemia, is caused by inadequate uptake of glucose from the blood. DM is manifested by hyperglycemia due to an absolute or relative lack of insulin and/or insulin resistance [1, 4]. A clinical diagnosis of dementia is likely preceded by a period of cognitive decline during which one's ability to properly manage blood sugar level may be impacted; this is an especially important limitation in the population of older adult individuals with type 1 diabetes when self-care plays such an important role in disease management [11].

Glucose-6-phosphate dehydrogenase (insulin-dependent enzyme) is the first enzyme of pentose phosphate pathway. This enzyme accelerates the dehydrogenase reactions in oxidative stage of pentose phosphate pathway that results in $NADPH_2$ production. The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase using $NADPH_2$ as a source of reducing electrons in erythrocytes, liver and other body tissue [7]. Glutathione system is one of the main antioxidant. The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase using $NADPH_2$ as a source of reducing electrons in erythrocytes and other tissue of body [9]. Ontogenetic changes in the antioxidant system and carbohydrate metabolism including glycolysis in the blood of rats with DM receiving melatonin are less studied.

The purpose of this experimental study was to ascertain the effect of aging on the level of basal glycemia (BG) and activity of glucose-6-phosphate dehydrogenase (G6PD, [EC1.1.1.49]), pyruvate kinase (PK, [EC 2.7.1.40]), and glutathione reductase (GR, [EC1.6.4.2]) in erythrocytes of rats with alloxan-induced diabetes during the course of melatonin injections.

Methods

This study is consistent with the standards and policies of The Regulations on biological experimentation with animals (1977), the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes drawn up by the Council of Europe (Strasbourg, 1986), as well as with the directions of International Committee of Medical Journals Editors (ICMJE), and "Bioethical expertise of preclinical and other scientific researches conducted on animals" (Kyiv, 2006). The study included 140 male Wistar rats of two age groups: I group included 2-month (adult rats), and the II group included 4-month (old rats). Diabetes was modelled by injecting the rats with a 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg. Four days after diabetes induction, rats were divided into diabetic (untreated) group and diabetic group, which received intraperitoneal injections of melatonin in a dose of 10 mg/kg daily for six weeks. Blood was taken from the tail vein to evaluate the BG on 5-th and 47-th day after the injection of alloxan. Rats were euthanized on the 47-th day of the experiment in accordance with the ethical treatment of animals. The enzyme activities were assessed by applying standard methods [16].

Statistical analysis was performed using Statistica 10 StatSoft Inc. To determine an adequate method of statistical estimation of the average difference between the study groups, we used preliminary check distribution quantities in samples. To verify the normality distribution, the calculation of the Shapiro-Wilk test was applied. When the result ranges were not subject to normal distribution, statistical processing was performed using a non-parametric method, the Mann-Whitney test. Differences were considered to be statistically significant at $p \leq 0.05$.

Results and discussion

The BG level (fig. 1) on the fifth day of the experiment in the animals of both groups increased on average by 117% compared to baseline values. We founded that on 47-th day this index was higher in the group of old rats by 22% than in adult rats.

Melatonin administration reduced the BG level twice as much in adult rats and 2.2 times in old rats compared with the indexes DM animals of relevant groups, which did not receive melatonin correction. Thus, we can suggest melatonin administration is effective in the normalization of BG level in both DM groups as the BG levels did not differ from control. Earlier reports [8, 9] concluded that the hypoglycemic action of melatonin could be partly due to amelioration in beta-cells of pancreatic islets.

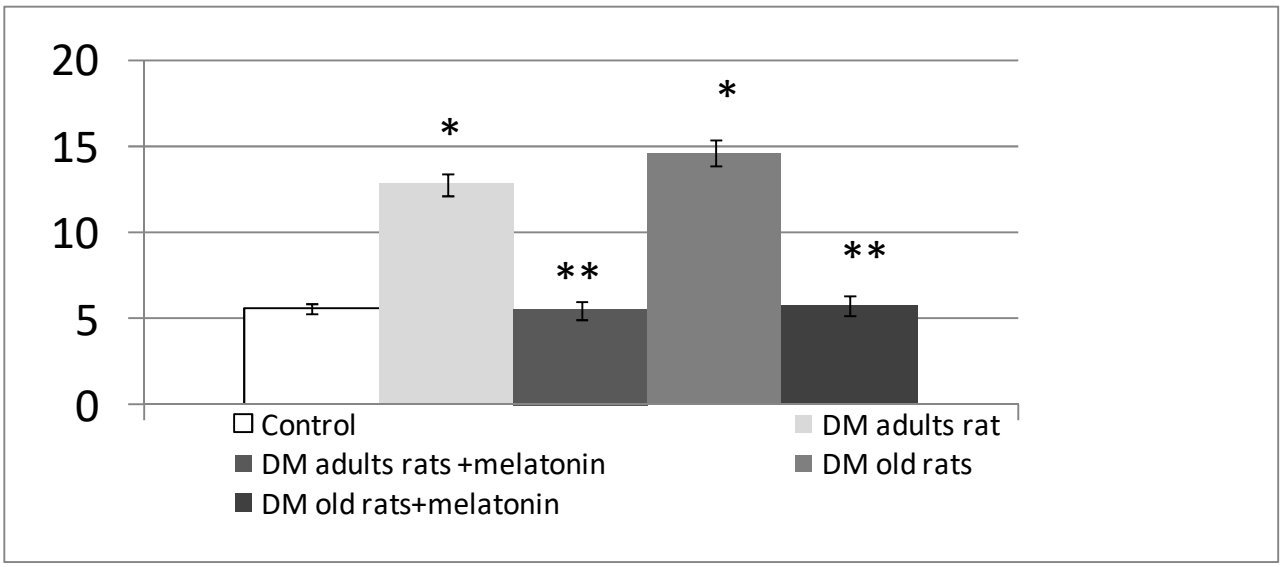

*Fig.1. The level of basal glucose (mmol/l) in blood of rats, (n=6, x±Sx): 1. *, ** - changes are reliable (p≤0.05). 2. * - control; ** - rats with DM.*

PK is an enzyme that catalyses the final step of glycolysis, i.e. it catalyses the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), yielding one molecule of pyruvate and one molecule of ATP. PK activity (fig. 2) in erythrocytes of adult and old animals with diabetes decreased by 34% and 51% respectively compared with the control. The tendency to decrease the PK activity can be explained by the fact that PK is regulated by insulin, which production is lowered in alloxan-induced diabetes mellitus.

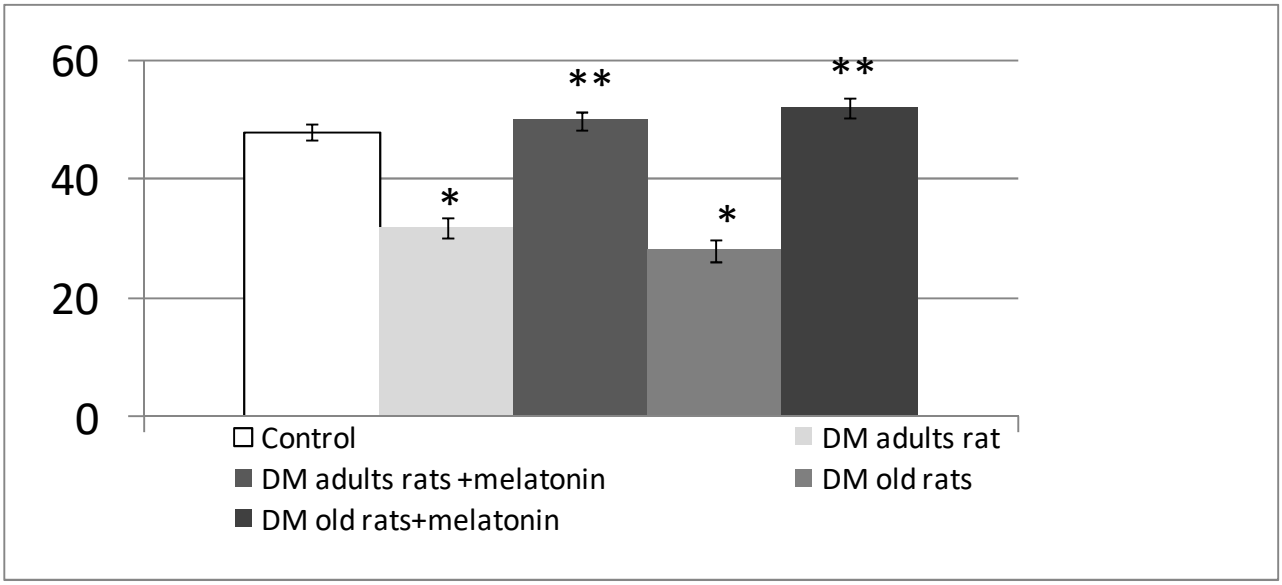

*Fig.2. The level of pyruvate kinase (mkmol/min·g (Hb)) in blood of rats, (n=6, x±Sx): 1. *, ** - changes are reliable (p≤0.05). 2. * - control; ** - rats with DM.*

The injection of melatonin in a dose of 10 mg/kg was conducive to a normalization of the PK activity of carbohydrate metabolism in the group of old and adult animals with diabetes compared with control.

The study has demonstrated that melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor substrate-1 / phosphoinositide 3-kinase (IRS-1/PI-3-kinase) pathway that implies, at the molecular level, its role in glucose homeostasis and possibly in diabetes [7].

G6PD activity (fig. 3) in erythrocytes of adult and old animals with diabetes decreased by 25% and 44% respectively compared with the control on 47-th day. This is associated with lowered production of $NADPH_2$ [10].

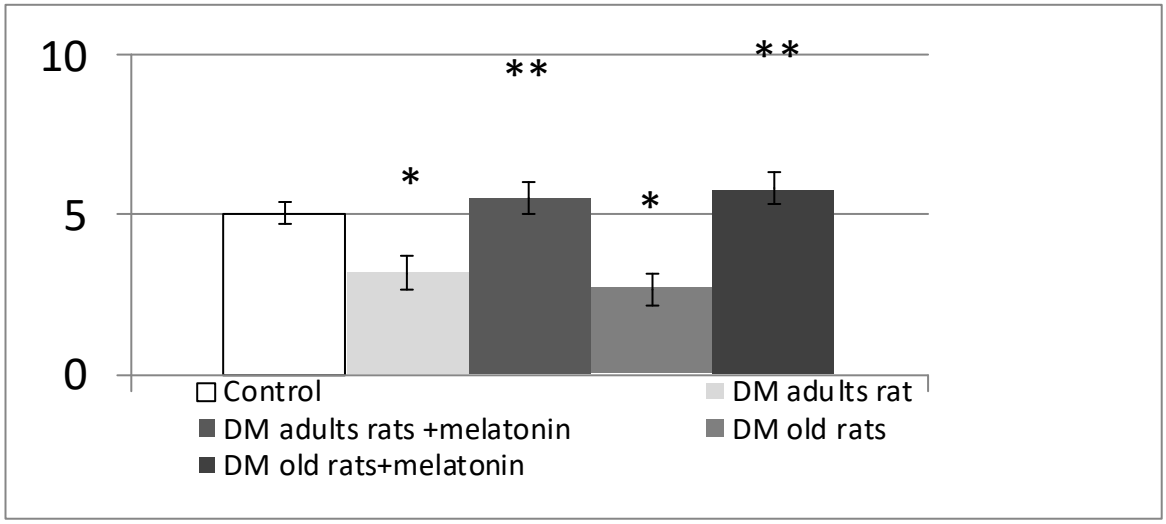

*Fig.3. The level of Glucose-6-phosphate dehydrogenase (nmol/min·g (Hb)) in blood of rats, (n=6, x±Sx): 1. *, ** - changes are reliable (p≤0.05). 2. * - control; ** - rats with DM.*

GR activity (fig. 4) in erythrocytes of adult and old animals with diabetes decreased by 30% and 36% respectively compared with the control on 47-th day. The changes may result from age-related disorders of glucose metabolism due to disturbances in free radical mechanisms. Moreover, hyperglycemia leads to increased free radical mechanism and oxidative modification of protein (insulin and insulin-dependent enzyme) in old rats [14].

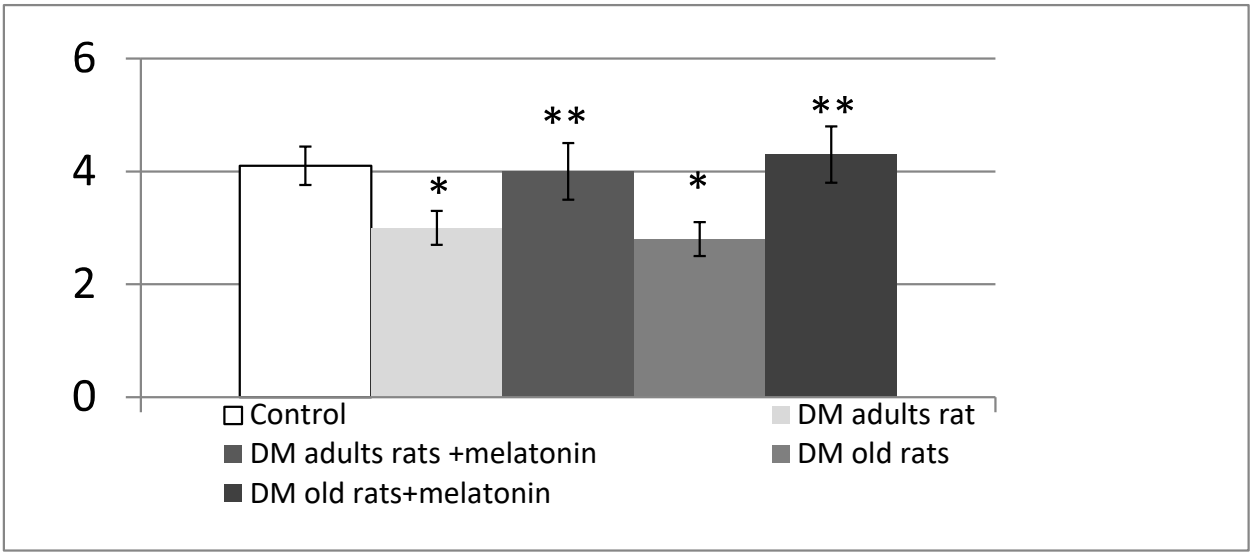

*Fig.4. The level of Glutathione reductase (mkmol/min·g (Hb)) in blood of rats, (n=6, x±Sx): 1. *, ** - changes are reliable (p≤0.05). 2. * - control; ** - rats with DM.*

These results demonstrate the degenerative role of hyperglycemia in cellular reducing equivalent homeostasis and antioxidant defence, and provide further evidence that pharmacological correction by applying antioxidants may quite effective in the prevention of the pro-oxidant manifestations of diabetes and protection of redox status of the cells. ROS react with some amino acids, producing molecules from modified, denatured and non-functioning proteins that in further may be responsible for oxidative stress.

Decreased activity of GR leads to decline in the reduced glutathione level. These changes may result from age-related disorders of free radical metabolism and age-related deficiency of $NADPH_2$ [11, 12].

42-day melatonin course received by rats with alloxan-induced diabetes mellitus in both groups contributed to a normalization of the BG level, of PK and GR activity in the blood, as well as considerable increased the G6PD activity, whose level exceeded by average 9% this particular index in the control group of animals. The ability of melatonin increases activity of G6PD in the blood of rats may be due to the increasing number of substrate for G6PD (stimulating the glucose flow into cells and its phosphorylation) and direct action.

Diabetic hyperglycemia due to free radical production causes protein glycation and oxidative

degeneration. The intensity of such protein glycation is estimated by using some biomarkers such as glycated haemoglobin. Reduction of enzyme activities can be due to glycosylation. Melatonin can inhibit glycation by reducing the generation of reactive carbonyl or dicarbonyl groups either from fructosamine or glucose, probably due to stimulation of glucose transport to skeletal muscle cells and preventing of ROS formation in conditions of hyperglycemia.

That is known data [15] that in liver, the best metabolites to predict age were in glucose metabolism, phospholipid metabolism, and redox homeostasis. Beyond its direct free radical scavenging and indirect antioxidant effects, melatonin has a variety of physiological and metabolic advantages that may enhance its ability to limit oxidative stress [10].

Conclusion. In this case melatonin probably increases use of glucose for regeneration of $NADPH_2$ and aerobic oxidation of glucose that indicate an acceleration of antioxidative protection and energy production in blood of adult and old diabetic rats.

References

1. Aly HF, Mantawy MM. Comparative effects of zinc, selenium and vitamin E or their combination on carbohydrate metabolizing enzymes and oxidative stress in streptozotocin induced-diabetic rats. Eur Rev Med Pharmacol Sci. 2012;16(1):66-78.
2. Andersen LP, Gogenur I, Rosenberg J, Reiter RJ. The safety of Melatonin in Humans. Clin. Drug. Investig. 2016;36(3):169-75.
3. Banaei S, Ahmadiasl N, Alihemmati A. Comparison of the Protective Effects of Erythropoietin and Melatonin on Renal Ischemia-Reperfusion Injury. Trauma Mon. 2016; 21(3):e23005.
4. Buonfiglio D, Parthimos R, Dantas R. Melatonin Absence Leads to Long-Term Leptin Resistance and Overweight in Rats. Front Endocrinol (Lausanne). 2018; 9: 122.
5. Favero G, Rodella LF, Nardo L, Giugno L, Cocchi MA, Borsani E, Reiter RJ, Rezzani R. A comparison of melatonin and α-lipoic acid in the induction of antioxidant defences in L6 rat skeletal muscle cells. Age (Dordr). 2015;37(4):9824.
6. Gesmundo I, Villanova T, Banfi D, et al. Role of Melatonin, Galanin, RFamide Neuropeptides QRFP26 and QRFP43 in the Neuroendocrine Control of Pancreatic β-Cell Function. Front Endocrinol (Lausanne). 2017; 8: 143.
7. Ivankov YI, Oleshchuk OM, Datsko TV, Fedonyuk LY. Features of indicators of prooxidant-antioxidant homeostasis, carbohydrate metabolism and liver morphological changes with the introduction of melatonin in type 2 experimental diabetes. Bulletin of morphology. 2016; 22(2):253-6.
8. Kushnir A, Davydenko I. Influence of melatonin on condition of the Langergans isles of the pancreas in alloksan diabetic rats. World of Medicine and Biology. 2009;5(4):31-35.
9. Kushnir OYu, Yaremii IM, Shvetsv VI, Shvets NV. Influence of melatonin on carbohydrate metabolism in the kidney of alloxan diabetic rats. Fiziol. journ., 2017;63(4):64 – 71.
10. Manchester LC, Coto-Montes A, Boga JA, et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res. 2015;59(4):403-19.
11. Marcheva B, Ramsey KM, Buhr ED, et al. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature. 2010;466(7306):627-31.
12. Mary E. Lacy, Paola Gilsanz, Andrew J. Karter Long-term Glycemic Control and Dementia Risk in Type 1 Diabetes. Diabetes Care. 2018 Nov; 41(11): 2339-2345.
13. Oberlin D and Buettner C. How does leptin restore euglycemia in insulin-deficient diabetes? J Clin Invest. 2017;127(2):450–453.
14. Ortiz-Avila O, Esquivel-Martínez M, Olmos-Orizaba BE, et al. Avocado Oil Improves Mitochondrial Function and Decreases Oxidative Stress in Brain of Diabetic Rats. J Diabetes Res. 2015;2015:485759. doi: 10.1155/2015/485759.
15. Riekelt H. Houtkooper, Carmen Argmann, Sander M. Houten The metabolic footprint of aging in mice. Sci Rep. 2011; 1: 134.
16. Szpetnar M, Luchowska-Kocot D, Boguszewska-Czubara A, Kurzepa J. The Influence of Manganese and Glutamine Intake on Antioxidants and Neurotransmitter Amino Acids Levels in Rats' Brain. Neurochem Res. 2016; 41(8):2129-2139.

Реферат

ВЛИЯНИЕ МЕЛАТОНИНА НА ВОЗРАСТНУЮ ЗАВИСИМОСТЬ ИЗМЕНЕНИЙ УГЛЕВОДНОГО ОБМЕНА И АНТИОКСИДАНТНОЙ ЗАЩИТЫ В КРОВИ КРЫС С АЛОКСАНОВЫМ ДИАБЕТОМ

Яремий И.М., Кушнир А.Ю.

Ключевые слова: мелатонин, кровь, алоксановый диабет, старение, крысы.

Мелатонин и его метаболиты владеют потенциальными антиоксидантными и противовоспалительными свойствами, а также доказана их высокая эффективность при использовании в лечении заболеваний, сопровождающихся нарушением антиоксидантной системы защиты и воспалением. Объектом этого экспериментального исследования было установить влияние старения на уровень базальной гликемии и активность глюкозо-6-фосфатдегидрогеназы [КФ1.1.1.49], пируваткиназы [КФ2.7.1.40] и глутатионредуктазы [КФ1.6.4.2] в эритроцитах крыс с аллоксановым диабетом на фоне введения мелатонина. Материалы и методы. Мы использовали 100 крыс самцов Wistar, две возрастные группы: 2-месячный (взрослый) и II - 4-месячный (старый). Алоксановый диабет вызвали путем инъекции крысам 5% раствора аллоксана моногидрата внутрибрюшинно из расчета 170 мг/кг. В каждой из диабетических групп выделили группу животных без коррекции, а также группу крыс, которым вводили мелатонин интраперитонеально из расчета 10 мг/кг массы тела животного каждый день. Кровь отбирали из хвостовой вены для оценки базальной гликемии на 5-й и 47-й день после введения аллоксана. Декапитацию животных проводили на 47-й день эксперимента в соответствии с принципами этического обращения с животными. Определение активности ферментов проводилось стандартными методами. Статистический анализ проводили с помощью Statistica 10 StatSoft Inc. Результаты. Уровень базальной гликемии на пятый день эксперимента у животных обеих групп вырос в среднем на 115% от значений контроля. Мы установили, что на 47-й день этот показатель был выше в группе старых крыс на 20% больше, чем у взрослых крыс. Активность пируваткиназы в эритроцитах взрослых и старых животных с диабетом снизилась на 34% и 51% соответственно по сравнению с контролем. Активность глюкозо-6-фосфатдегидрогеназы в эритроцитах взрослых и старых животных с диабетом снизилась на 25% и 44% соответственно по сравнению с контролем на 47-й день. Изменения могут быть следствием возрастных нарушений метаболизма глюкозы из-за нарушения механизмов свободных радикалов. Активность глутатионредуктазы в эритроцитах взрослых и старых животных с диабетом уменьшилась на 30% и 36% соответственно по сравнению с контролем на 47-й день. Введение мелатонина в течение 42 дней ежедневно диабетическим крысам обеих групп способствовала

нормализации уровня базальной гликемии, активности пируваткиназы и глутатионредуктазы в крови крыс, а также значительному увеличению активности глюкозо-6-фосфатдегидрогеназы, уровень активности которого превысил в среднем на 9% этот конкретный показатель в контрольной группе животных. Под влиянием мелатонина повышение активности глюкозо-6-фосфатдегидрогеназы в крови крыс может быть обусловлено увеличением количества субстрата для глюкозо-6-фосфатдегидрогеназы (стимулирование поступления глюкозы в клетки и фосфорилирования) и непосредственным действием. Вывод. В этом исследовании мелатонин, вероятно, увеличивает использование глюкозы для регенерации $НАДФН_2$ и аэробного окисления глюкозы, свидетельствует об ускорении антиоксидантной защиты и выработки энергии в крови взрослых и старых диабетических крыс.

Реферат

ВПЛИВ МЕЛАТОНІНУ НА ВІКОВУ ЗАЛЕЖНІСТЬ ЗМІН ВУГЛЕВОДНОГО ОБМІНУ ТА АНТИОКСИДАНТНОГО ЗАХИСТУ В КРОВІ ЩУРІВ З АЛОКСАНОВИМ ДІАБЕТОМ

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Ключові слова: мелатонін, кров, алоксановий діабет, старіння, щури.

Мелатонін та його метаболіти володіють потенційними антиоксидантними та протизапальними властивостями, а також доведено їхню високу ефективність при використанні у лікуванні захворювань, що супроводжуються порушенням антиоксидантної системи захисту та запаленням. Об’єктом цього експериментального дослідження було встановити вплив старіння на рівень базальної глікемії та активність глюкозо-6-фосфатдегідрогенази [КФ1.1.1.49], піруваткінази [КФ2.7.1.40] та глутатіонредуктази [КФ1.6.4.2] в еритроцитах щурів з алоксановим діабетом на фоні уведення мелатоніну. Матеріали та методи. Ми використовували 100 щурів самців Wistar, дві вікові групи: 2-місячний (дорослий) та II - 4-місячний (старий). Алоксановий діабет викликали шляхом ін'єкції щурам 5% розчину алоксану моногідрату внутрішньочеревно з розрахунку 170 мг/кг. У кожній з діабетичних груп було виділено групу тварин без корекції та групу тварин, яким вводили мелатонін інтраперитонеально з розрахунку 10 мг на кг маси тварини. Кров відбирали з хвостової вени для оцінки базальної глікемії на 5-й і 47-й день після введення алоксану. Декапітацію тварин проводили на 47-й день експерименту відповідно до етичного поводження з тваринами. Визначення активності ферментів проводилось стандартними методами. Статистичний аналіз проводили за допомогою Statistica 10 StatSoft Inc. Результати. Рівень базальної глікемії на п’ятий день експерименту у тварин обох груп в середньому зростав на 115% від значень контролю. Ми встановили, що на 47-й день цей показник був вищим у групі старих щурів на 20% більше, ніж у дорослих щурів. Активність піруваткінази в еритроцитах дорослих і старих тварин з діабетом знизилася на 34% і 51% відповідно порівняно з контролем. Активність глюкозо-6-фосфатдегідрогенази в еритроцитах дорослих та старих тварин із діабетом знизилася на 25% та 44% відповідно порівняно з контролем на 47-й день. Зміни можуть бути наслідком вікових порушень метаболізму глюкози через порушення механізмів вільних радикалів. Активність глутатіонредуктази в еритроцитах дорослих та старих тварин із діабетом зменшилась на 30% та 36% відповідно порівняно з контролем на 47-й день. Введення мелатоніну впродовж 42 днів щоденно діабетичним щурам обох груп сприяла нормалізації рівня базальної глікемії, активності піруваткінази та глутатіонредуктази у крові щурів, а також значному збільшенню активності глюкозо-6-фосфатдегідрогенази, рівень активності якого перевищив у середньому на 9% цей конкретний показник у контрольній групі тварин. Під впливом мелатоніну підвищення активності глюкозо-6-фосфатдегідрогенази в крові щурів може бути обумовлене збільшенням кількості субстрату для глюкозо-6-фосфатдегідрогенази (стимулювання надходження глюкози в клітини та фосфорилювання) і безпосередньою дією. Висновок. У цьому дослідженні мелатонін, ймовірно, збільшує використання глюкози для регенерації $НАДФН_2$ та аеробного окислення глюкози, що свідчить про прискорення антиоксидантного захисту та вироблення енергії в крові дорослих та старих діабетичних щурів.