UDC 577.17:612.826.33:616.379-008.64]-092.9

CHANGES OF THE ANTIOXIDANT DEFENCE IN KIDNEYS OF ALLOXAN DIABETIC RATS UNDER MELATONIN ACTION

O.Yu. Kushnir, I.M. Yaremii

Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi

Key words: *antioxidative system, melatonin, alloxan diabetes, kidney, rats.*

Clinical and experimental pathology. Vol.17, №3 (65), P.2.- P.83-87.

DOI:10.24061/1727-4338.XVII.3.65.2018.162

E-mail: kushnir@bsmu.edu.ua

Objective. *The aim was to determine the influence of melatonin on basal levels of glucose (BG), malonic dialdehyde (MDA), reduced glutathione (GSH) levels, glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G-6-PhD) activities in the kidney of alloxan diabetic rats.*

Materials and methods. *Diabetes was induced in male Wistar rats by single i.p. injection of alloxan (170 mg/kg). Four days after diabetes induction, rats were divided into diabetic (untreated) and melatonin-diabetic group (5 mg/kg, daily and orally for two weeks). Among diabetic rats were rats with preserved normoglycemia (impaired glucose tolerance - IGT) and rats with diabetes mellitus (DM) BG ≥ 8 mmol/l. Blood was taken from the tail vein to evaluate the BG level. Rats were sacrificed on the 19th day from the beginning of the experiment in 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*. In DM group of rats activity of GR, GPx, G-6-PhD, the level of GSH decreased 45%, 18%, 48%, 49% respectively in compareison with the control. The level of MDA was found to be higher 90% in DM group and on 55% in IGT group respectively than in control. In the group of rats with IGT activity of GR, GPx, G-6-PhD, the level of GSH increased 42%, 18%, 82%, 55% respectively as compared with control. Melatonin injections were efficient for normalization of these indexes under study.*

Conclusion. *The introduction of melatonin to alloxan diabetic rats for two weeks daily lead to a decrease of basal glycemia (BG) level in them, as well as stabilization of disorder indices of antioxidant defense system, namely, GR, GPx, G-6-PhD activity, content of MDA and G-SH in rat kidneys.*

Ключові слова: *антиоксидантна система, мелатонін, алоксановий діабет, нирки, щури.*

Клінічна та експериментальна патологія Т.17, №3 (65), Ч.2.- С.83-87.

ЗМІНИ АНТИОКСИДАНТНОГО СТАТУСУ В НИРКАХ ЩУРІВ З АЛОКСАНОВИМ ДІАБЕТОМ ПІД ДІЄЮ МЕЛАТОНІНУ

О.Ю. Кушнір, І.М. Яремій

Мета роботи *- полягала в тому, щоб визначити вплив мелатоніну на базальний рівень глюкози (БГ), вміст малонового диальдегіду (МДА), рівень глутатіону (GSH), глутатіонредуктази (ГР), глутатіонпероксидази (ГП), глюкозо-6-фосфат-дегідрогенази (Г-6-фДГ) в нирках щурів з алоксановим діабетом.*

Матеріал і методи. *Діабет був індукований у самців пацюків роду Вістар за одиничним інтраперетонеальним введення алоксану (170 мг / кг). Через чотири дні після індукції цукрового діабету (ЦД) щурів розподіляли на діабетичну (нелікована) та мелатонін-діабетичну групу (5 мг / кг, щодня та перорально протягом двох тижнів). Серед діабетичних тварин були щури зі збереженою нормоглікемією (порушення толерантності до глюкози - ПТГ) та щури із ЦД (БГ ≥8 ммоль / л). Кров забирали з хвостової вени, щоб оцінити рівень БГ. Декапітація щурів була на 19-й день з початку експерименту відповідно до етичного поводження з тваринами. Визначення активності ферментів були стандартними методами. Статистичний аналіз проводився за допомогою Statistica 10 StatSoft Inc.*

Результати*. У групі щурів з ЦД активності ГР, ГП, Г-6-фДГ та рівень GSH знижувалися на 45%, 18%, 48% та 49%, відповідно, порівняно з контролем. Встановлено, що рівень МДА вищий на 90% у групі з ЦД і на 55% у групі тварин з ПТГ, ніж у контролі. У групі щурів з ПТГ активність ГР, ГП, Г-6-фДГ та рівень GSH збільшені на 42%, 18%, 82% та 55% відповідно, порівняно з контролем. Ін'єкції мелатоніну були ефективними для нормалізації цих досліджуваних показників.*

Висновок. *Ін'єкції мелатоніну діабетичним щурам щоденно впродовж двох тижнів призвели до зниження в останніх рівня базальної глікемії, як і до нормалізації показників порушення антиоксидантної системи захисту, а саме активності ГР, ГП, Г-6-фДГ, вмісту МДА та GSH в нирках щурів.*

ИЗМЕНЕНИЯ АНТИОКСИДАНТНОГО СТАТУСА В ПОЧКАХ КРЫС С АЛЛОКСАНОВЫМ ДИАБЕТОМ ПОД ДЕЙСТВИЕМ МЕЛАТОНИНА

А.Ю. Кушнир, И.Н. Яремий

Ключевые слова: *антиоксидантная система, мелатонин, аллоксановый диабет, почки, крысы.*

Клиническая и экспериментальная патология Т.17, №3 (65),Ч.2.-С.83-87.

Цель работы *- состояла в том, чтобы определить влияние мелатонина на базальный уровень глюкозы (БГ), содержание малонового диальдегида (МДА), уровень глутатиона (GSH), глутатионредуктазы (ГР), глутатионпероксидазы (ГП), глюкозо-6-фосфатдегидрогеназы (Г-6 ФДГ) в почках крыс с алоксанового диабетом.*

Материал и методы. *Диабет был индуцированный у самцов крыс рода Вистар по единичному интраперетонеальному введению аллоксана (170 мг / кг). Через четыре дня после индукции сахарного диабета (СД) крысы делили на диабетическую (нелеченная) и мелатонин-диабетическую группу (5 мг / кг, ежедневно и перорально в течение двух недель). Среди диабетических животных были крысы с сохраненной нормогликемией (нарушение толерантности к глюкозе - НТГ) и крысы с СД (БГ ≥8ммоль/л). Кровь забирали из хвостовой вены, чтобы оценить уровень БГ. Декапитация крыс была на 19-й день от начала эксперимента в соответствии с этическими нормами обращения с животными. Определение активности ферментов были стандартными методами. Статистический анализ проводился с помощью Statistica 10 StatSoft Inc.*

Результаты. *В группе крыс с СД активности ГР, ГП, Г-6-ФДГ и уровень GSH снижались на 45%, 18%, 48% и 49% соответственно по сравнению с контролем. Установлено, что уровень МДА выше на 90% в группе с СД и на 55% в группе животных с ПТГ, чем в контроле. В группе крыс с ПТГ активность ГР, ГП, Г-6-ФДГ и уровень GSH увеличены на 42%, 18%, 82% и 55% соответственно по сравнению с контролем. Инъекции мелатонина были эффективными для нормализации этих исследуемых показателей.*

Вывод. *Инъекции мелатонина алоксандиабетичным крысам привели к снижению в последних уровня базальной гликемии, так же как и нормализации показателей антиоксидантной системы защиты, а именно активности ГР, ГП, Г-6-фДГ, содержания МДА и восстановленного глутатиона в почках крыс.*

Introduction

Completed in accordance with the planned research work "Stressed morphofunctional and biochemical changes in the structures of chronoperiodical and hepatorenal systems in mammals" 0114 U002472 - Fundamental.

Diabetes is a disease which disturbs carbohydrate metabolism and leads to the hyperglycemia.

Diabetes mellitus can damage the eyes, kidneys, nerves and heart. Microvascular and macrovascular disorders are the leading causes of morbidity and mortality in diabetic patients [11]. Hyperglycemia can increase the parameters of lipid peroxidation and oxidative stress in which free radicals play the main role in the pathogenesis of these complications. Therefore, antioxidants which defeat oxidative stress should be able to prevent and repair free radicals induced damages. Although free radicals result in kidney damage, atherosclerosis, diabetes, heart disease, nephrotoxicity and hepatotoxicity, clinical trials do not definitely confirm a substantial impact on diabetic damage.

Alloxan diabetes was reported to induce oxidative stress and generates reactive oxygen species (ROS) [8]. In the presence of intracellular thiols, especially glutathione, alloxan generates ROS in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a stage of final iron-catalysed reaction, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity and the ensuing state of insulin-dependent 'alloxan diabetes'.

Melatonin (N-acetyl-5-methoxytryptamine) is the major product of the pineal gland, which functions as a regulator of sleep, circadian rhythm, and immune function. 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 [2].

The aim of the study was to determine the influence of melatonin on basal levels of glucose (BG), malonic dialdehyde (MDA), reduced glutathione (GSH) levels, glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G-6-PhD) activities in the kidney of alloxan diabetic rats.

Materials and methods

Research was conducted in compliance with the Rules of work using experimental animals (1977) and the Council of Europe Convention on the Protection of Vertebrate Animals used in experiments and other scientific purposes (Strasbourg, 1986), according to directions of International Committee of Medical Journals Editors (ICMJE), as well as "Bioethical expertise of preclinical and other scientific research conducted on animals" (Kyiv, 2006). Diabetes was induced in male Wistar rats by single i.p. injection of alloxan (170 mg/kg). Four days after diabetes induction rats were divided into diabetic (untreated) and melatonin-diabetic group (5 mg/kg daily and orally for two weeks) [1]. There were rats with preserved normoglycemia (impaired glucose tolerance - IGT) and rats with diabetes

mellitus (DM) BG≥ 8 mmol/l among diabetic rats. Blood was taken from the tail vein to evaluate the BG level using OneTouchUltra (LifeScan, USA). Rats were sacrificed on the 19th day from the beginning of the experiment in accordance with the ethical treatment of animals. The kidney tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. The 5% homogenate in ice-cold 0,25 mM tris-HCl-buffer (pH 7,4) was made using a homogenizer. The supernatant of the homogenate, prepared by ultracentrifugation for 10 min at 3000g was used for measurement of activities of the enzymes. Oxidant status was assessed by measuring of MDA, GSH levels, GR, GPx, G-6-PhD activities. Determinations of the enzymes activities were fulfilled by standard methods [3, 4, 12, 13].

Statistical analysis was performed by means of Statistica 10 StatSoft Inc. To determine an adequate method of statistical estimation of the average difference between groups under study preliminary check distribution quantities was carried out in samples. According to the criteria Shapiro-Wilk, used to assess the normality of distribution in the sample volume n≤50, data concerning distribution deviation from normal (p>0,05) were not obtained in all samples. Taking into consideration these data, the use of Mann-Whitney test was considered as sufficient for valid conclusions. Differences were considered to be statistically significant at $p \le 0,05$.

Results and discussion

Melatonin injections caused a sharp decrease 57% at the elevated serum glucose level in DM group of rats as compared with BG level before treatment.

It has been detected, that melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor substrate-1 / phosphoinositide 3-kinase (IRS-1/PI-3-kinase) pathway, which implies, at the molecular level, its role in glucose homeostasis and possibly in diabetes [9].

It is concluded [7] that the hypoglycemic action of melatonin could be partly due to amelioration of the beta-cells of pancreatic islets.

Alteration in the function and structure of antioxidant protein enzymes may be also due to non-enzymatic glycation thus, detoxification of free radicals is realized by means of oxidative stress strengthening in diabetes [5].

Diabetes mellitus produces disturbances in the lipid profile of body making the cells more susceptible to lipid peroxidation (Patricia, 2009). Experimental studies demonstrate that polyunsaturated fatty acids in cell membrane are extremely prone to be attacked by free radicals due to the presence of multiple bonds. Lipid hyperperoxides (LHP) through intermediate radical reactions produce fatty acids that generate highly reactive and toxic lipid radicals that form new LHP (Matough et al., 2012). A critical biomarker of oxidative stress is Lipid peroxidation which is the most explored area of research when it comes to ROS (Hatice et al., 2004). MDA are formed as a result of lipid peroxidation that can be used to measure lipid peroxides after its reaction with thiobarbituric acid.

MDA levels (tab.1) were found to be higher 90% in DM group and 55% in IGT group respectively than in control. So, the lipid peroxidation was increased in diabetic kidney. Melatonin partly prevented diabetes-induced increase in MDA levels in the kidney.

Diabetes induces alterations in activity of enzymes GPx and GR (tab.1). These enzymes are found in cell that metabolizes peroxide to water and converts glutathione disulfide back into glutathione (Maritim et al., 2003). Any alteration in their levels will make the cells prone to oxidative stress and, hence, cell injury [6].

Table

Changes of the antioxidant defence in kidney of diabetic rats, (n=6, x±S)

Indexes / Groups	MDA, mkmol /g	G-SH, mkmol/g	GPx, nmol/min×mg	G-6-PhD, nmol/min× mg	GR, nmol/min× mg
1.Control group	24,8±0,68	4,1±0,03	146,8±7,33	4,3±0,09	5,1±0,11
2. DM	47,2±0,78^{a}	2,1±0,04^{a}	120,3±8,15^{a}	2,2±0,07^{a}	2,8±0,12^{a}
3. DM + melatonin	25,5±0,58^{b}	4,2±0,03^{b}	148,2±8,23^{b}	4,6±0,08^{b}	5,2±0,12^{b}
4. IGT	38,6±0,52^{a,b}	6,3±0,04^{a,b}	173,2±9,5^{ab}	7,8±0,1^{a,b}	7,3±0,14^{a,b}
5. IGT + melatonin	22,8±0,56^{b,c}	4,3±0,02^{b,c}	150,0±7,9^{b,c}	4,4±0,09^{b,c}	5,2±0,12^{b,c}

1. a, b, c - changes are reliable (p≤0,05).
2. a - concerning intact rats;
   b - concerning rats with diabetes mellitus;
   c – concerning rats with IGT.

On the other hand, GR, GPx, G-6-PhD activity also depends on hyperglycemia presence. In DM group of rats GR, GPx, G-6-PhD activity decreased 45%, 18%, 48% according to the control rats. We have found the level of GSH to be lower 49% in DM group of animals compared with control. These results are consistent with the degenerative role of hyperglycemia on cellular reducing

equivalent homeostasis and antioxidant defense, and provide further evidence that pharmacological intervention of antioxidants may have significant implications in the prevention of the prooxidant feature of diabetes and protects redox status of the cells.

In the group of rats with preserved normoglycemia (IGT) GR, GPx, G-6-PhD activity increased 42%, 18%, 82% respectively in comparison with control rats. Increase of G6PhD activity under conditions of diabetes with IGT is probably a compensatory reaction aimed to reduce of ROS. It was found that the level of GSH increased 55% in comparison with the control.

NADPH2 reducing equivalents (produced in this reaction) are used for regeneration of glutathione from its oxidized form due to action of NADPH2-dependent glutathione reductase. Glutathione neutralizes ROS, both directly and through GPx.

Melatonin injections were helpful for normalization of these indexes under study.

It means that melatonin probably increases use of glucose for regeneration of NADPH2 and aerobic oxidation of glucose is indicative of an acceleration of antioxidative protection and energy production in the kidney of diabetic rats.

The actions of melatonin on radical metabolizing/producing enzymes may be mediated by the Keap1-Nrf2-ARE pathway. Beyond its direct free radical usage and indirect antioxidant effects, melatonin has a variety of physiological and metabolic advantages that may enhance its ability to limit oxidative stress [10].

So, melatonin not only neutralizes reactive oxygen species, but also acts through the stimulation of several antioxidative enzymatic systems in kidney of alloxan diabetic rats.

Conclusion

The administration of melatonin to alloxan diabetic rats for two weeks daily lead to a decrease of basal glycemia (BG) level in them, as well as stabilization of the indices disturbance of antioxidant defense system, namely, GR, GPx, G-6-PhD activity, content of MDA and G-SH in rat kidneys.

Prospects for further research

Research will continue in the chosen scientific direction.

Список літератури

1.Banaei S, Ahmadiasl N, Alihemmati A. Comparison of the Protective Effects of Erythropoietin and Melatonin on Renal Ischemia-Reperfusion Injury. Trauma Mon [Internet]. 2016[cited 2018 Jul 22];21(3):e23005. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5124127/doi:10.5812/traumamon.23005

2.Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27(2):189-200. doi: 10.1385/ENDO:27:2:189

3.Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J Med Life. 2016;9(3):259-62.

4.Djindjic B, Kostic T, Radovanovic Z, Djindjic N, Lazovic M, Zivic M, et al. The contributions of fasting and postprandial blood glucose increments to oxidative stress and inflammation in dyslipidemic type 2 diabetic patients with stable ischemic heart disease. Int J Cardiol. 2017;227:611-6. doi: 10.1016/j.ijcard.2016.10.089

5.Gerush I, Boichuk T, Yaremii I, Kushnir O, Gerush O. Effects of melatonin on the glutathione system in the blood of alloxan diabetic rats. In: 22nd IUBMB & 37th FEBS Congress; 2012 Sep 4-9; Seville, Spain. FEBS Journal. 2012;279(1):88.

6.Gumieniczek A, Wilk M. Nitrosative stress and glutathione redox system in four different tissues of alloxan-induced hyperglycemic animals. Toxicol Mech Methods. 2009;19(4):302-7. doi: 10.1080/15376510902839762

7.Кушнір ОЮ, Давиденко ІС. Вплив мелатоніну на стан острівців Лангерганса підшлункової залози алоксандіабетичних щурів. Світ медицини та біології. 2009;(4):31-5.

8.Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26. doi: 10.1007/s00125-007-0886-7

9.Lin GJ, Huang SH, Chen YW, Hueng DY, Chien MW, Chia WT, et al. Melatonin prolongs islet graft survival in diabetic NOD mice. J Pineal Res. 2009;47(3):284-92. doi: 10.1111/j.1600-079X.2009.00712.x

10.Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res. 2015;59(4):403-19. doi: 10.1111/jpi.12267

11.Rahimi-Madiseh M, Malekpour-Tehrani A, Bahmani M, Rafieian-Kopaei M. The research and development on the antioxidants in prevention of diabetic complications. Asian Pac J Trop Med. 2016;9(9):825-31. doi: 10.1016/j.apjtm.2016.07.001

12.Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of Berberis aristata root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol. 2009;123(1):22-6. doi: 10.1016/j.jep.2009.02.038

13.Власова СН, Шабунина ЕИ, Переслегин ИА. Активность глутатион-зависимых ферментов эритроцитов при хронических заболеваниях печени у детей. Лабораторное дело. 1990;(8):19-21.

References

1.Banaei S, Ahmadiasl N, Alihemmati A. Comparison of the Protective Effects of Erythropoietin and Melatonin on Renal Ischemia-Reperfusion Injury. Trauma Mon [Internet]. 2016[cited 2018 Jul 22];21(3):e23005. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5124127/ doi: 10.5812/traumamon.23005

2.Carrillo-Vico A, Guerrero JM, Lardone PJ, Reiter RJ. A review of the multiple actions of melatonin on the immune system. Endocrine. 2005;27(2):189-200. doi: 10.1385/ENDO:27:2:189

3.Ceban E, Banov P, Galescu A, Botnari V. Oxidative stress and antioxidant status in patients with complicated urolithiasis. J Med Life. 2016;9(3):259-62.

4.Djindjic B, Kostic T, Radovanovic Z, Djindjic N, Lazovic M, Zivic M, et al. The contributions of fasting and postprandial blood glucose increments to oxidative stress and inflammation in dyslipidemic type 2 diabetic patients with stable ischemic heart disease. Int J Cardiol. 2017;227:611-6. doi: 10.1016/j.ijcard.2016.10.089

5.Gerush I, Boichuk T, Yaremii I, Kushnir O, Gerush O. Effects of melatonin on the glutathione system in the blood of alloxan diabetic rats. In: 22nd IUBMB & 37th FEBS Congress; 2012 Sep 4-9; Seville, Spain. FEBS Journal. 2012;279(1):88.

6.Gumieniczek A, Wilk M. Nitrosative stress and glutathione redox system in four different tissues of alloxan-induced hyperglycemic animals. Toxicol Mech Methods. 2009;19(4):302-7. doi: 10.1080/15376510902839762

7.Kushnir AYu, Davydenko IS. Vplyv melatoninu na stan ostrivtsiv Lanherhansa pidshlunkovoi zalozy aloksandiabetychnykh schuriv [Influence of melatonin on condition of the Langergans isles of the pancreas in alloxandiabetic rats]. World of Medicine and Biology. 2009;(4):31-5. (in Ukrainian).

8.Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26. doi: 10.1007/s00125-007-0886-7

9.Lin GJ, Huang SH, Chen YW, Hueng DY, Chien MW, Chia WT, et al. Melatonin prolongs islet graft survival in diabetic NOD

mice. J Pineal Res. 2009;47(3):284-92. doi: 10.1111/j.1600-079X.2009.00712.x

10.Manchester LC, Coto-Montes A, Boga JA, Andersen LP, Zhou Z, Galano A, et al. Melatonin: an ancient molecule that makes oxygen metabolically tolerable. J Pineal Res. 2015;59(4):403-19. doi: 10.1111/jpi.12267

11.Rahimi-Madiseh M, Malekpour-Tehrani A, Bahmani M, Rafieian-Kopaei M. The research and development on the antioxidants in prevention of diabetic complications. Asian Pac J Trop Med. 2016;9(9):825-31. doi: 10.1016/j.apjtm.2016.07.001

12.Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of Berberis aristata root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol. 2009; 123(1):22-6. doi: 10.1016/j.jep.2009.02.038

13.Vlasova SN, Shabunina EI, Pereslegina IA. Aktivnost' glutation-zavisimykh fermentov eritrotsitov pri khronicheskikh diseases zabolevaniyakh pecheni u detey [The activity of the glutathione-dependent enzymes of erythrocytes in chronic liver diseases in children]. Laboratornoe delo. 1990;(8):19-21. (in Russian).

Відомості про авторів:
Яремій І. М. - доцент, к. біол. н. доцент кафедри біоорганічної і біологічної хімії та клінічної біохімії, Вищий державний навчальний заклад України "Буковинський державний медичний університет", м. Чернівці
Кушнір О. Ю. - к. мед.н., асистент кафедри біоорганічної і біологічної хімії та клінічної біохімії Вищий державний навчальний заклад України "Буковинський державний медичний університет", м. Чернівці

Сведения об авторах:
Яремий И. Н. - доцент, к. биол. н., доцент кафедры биоорганической и биологической химии и клинической биохимии Высшего государственного учебного заведения Украины "Буковинский государственный медицинский университет", г.Черновцы
Кушнир А. Ю. - к. мед.н., ассистент кафедры биоорганической и биологической химии и клинической биохимии Высшего государственного учебного заведения Украины "Буковинский государственный медицинский университет", г.Черновцы

Information about the autors:
Yaremii I. M. - docent, Candidate of Biological Sciences, docent Department of Bioorganic and Biological Chemistry and Clinical Biochemistry Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi
Kushnir O. Yu. - candidate of Medical Sciences, assistant Department of Bioorganic and Biological Chemistry and Clinical Biochemistry Higher State Educational Establishment of Ukraine "Bukovinian State Medical University", Chernivtsi

Стаття надійшла до редакції 12.05.2018
Рецензент – проф. Ю.Є. Роговий