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ANTIOXIDANT ACTION OF MELATONIN IN THE BRAIN OF ALLOXAN DIABETIC RATS

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

Abstract. *Possible protective effect of melatonin as an antioxidant against alloxan-induced diabetic brain injury in rats was shown in this study.The introduction of melatonin to alloxan diabetic rats led to a decrease in them of the level of basal glycemia BG, as well as - a stabilization of the indices of the body's disturbed antioxidant defense, namely activities of glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G-6-PhD), content of malonic dialdehyde (MDA) and glutathione (G-SH) in rats brain.*

Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a neuroendocrine hormone, which is synthesized primarily by the pineal gland [8]. The synthesis and secretion of melatonin are regulated by light intensity [17]. It was found that melatonin functions to regulate the sleep cycle in the early study [2]. Further investigation revealed that melatonin also has antioxidant and anti-inflammatory functions [18]. It has also been shown to regulate lipid and glucose metabolism [1, 5]. Importantly, recent research suggests that melatonin plays an important role in various cardiovascular diseases, including myocardial ischemia-reperfusion injury [20, 21], atherosclerosis [10,6], hypertension [3,11], heart failure [4,16], and drug-induced myocardial injury [13]. In the past year, several studies have focused on the mechanism of the protection of melatonin on cardiovascular diseases [7].

Mitochondrial alterations related to diabetic encephalopathy include increased mitochondrial fission, excessive reactive oxygen species (ROS) levels, augmented levels of both lipid peroxidation and nitrite, and decreased levels of total antioxidant. In addition, it has been suggested that diabetes-induced oxidative stress increases the levels of proinflammatory cytokines, which enhances neuronal degeneration. Therefore, mitochondrial oxidative damage contributes, at least in part, to the development of diabetic encephalopathy [14].

Further studies are required about these issues for the development of therapeutic strategies to ameliorate the impact of diabetic encephalopathy and other complications of diabetes. In this regard, nutraceuticals with antioxidant properties have been used as alternative treatments to slow and/or prevent the inherent complications of diabetes. A candidate belonging to this group of nutraceuticals is melatonin. Moreover, improvement in glycemic control, plasma lipid profile, and atherogenic index has been observed in diabetic patients consuming melatonin.

Uncontrolled diabetes mellitus (DM) results in neuronal damage caused by increased intracellular glucose. The addition of natural products as complementary therapy can reduce neuronal complications [12].

The main objectives of this study was to determine whether melatonin supplementation protect rat brains in alloxan-induced diabetic rats via estimating lipid peroxidation and anti-oxidant status of brain.

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 brain of alloxan diabetic rats.

Methods

Research performed in compliance with the Rules of the 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 researches 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 (10 mg/kg, daily and orally for one week). 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 evaluate the BG level with the use of OneTouchUltra (LifeScan, USA). Rats were

sacrificed at the twelfth day from the beginning of the experiment accordance with the ethical treatment of animals. The brain tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. The homogenate, 5% 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/min was used for measurement of activities of enzymes. Brain oxidant status was assessed by measuring of MDA, GSH levels, GR, GPx, G-6-PhD activities. Determinations of the enzymes activities were by standard methods [19]. 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 held preliminary check distribution quantities in samples. According to the criteria Shapiro-Wilk, which is used to assess the normality of distribution in the sample volume n≤50, all samples not received data on deviation of the distribution of samples from normal (p>0,05). Given these data, the use of Mann-Whitney test was considered sufficient for valid conclusions. Differences were considered to be statistically significant at $p \leq 0,05$.

Results

Insertion of melatonin (Fig.) for 7 days helped to reduce 1,8 times compared with the baseline, basal glucose level in the group of animals with overt diabetes, indicating its hypoglycemic action.

The brain is particularly prone to oxidative damage owing to its high rate of oxygen consumption. The metalloproteins superoxide dismutase, catalase, and GPx provide the first line of antioxidant defense against reactive oxygen species through enzyme-catalyzed dismutation of O_2- to H_2O_2, which is further reduced to oxygen and water [15].

Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, thereby deplete the activity of the antioxidative defense system and thereby promote the generation of free radicals [9].

The level (tab.) of MDA was found to be higher

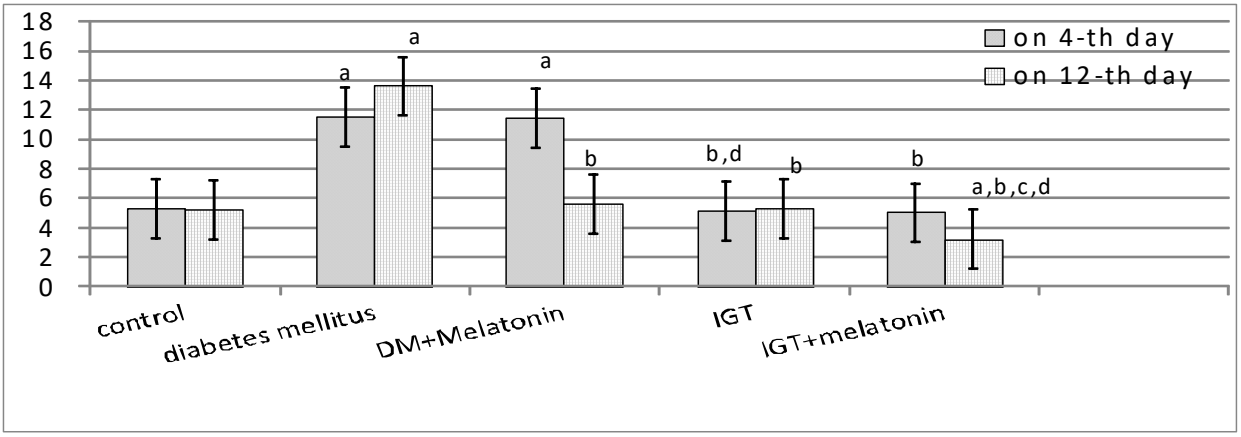

Fig.The level of basal glycemia (mmol/l) in blood of rats, (n=6, x±Sx): 1. a, b, c - changes are reliable (p≤0,05). 2. a - concerning intact rats; b - concerning rats with diabetes mellitus; c - concerning rats with IGT; d - concerning indices on 4-th day

Таблиця

Поліморфні варіанти гена APO-1/Fas (rs2234767) як чинники ризику патології щитоподібної залози з урахуванням показників апоптозу та неспецифічного

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	18,0±0,48	2,2±0,02	155,8±10,4	3,2±0,08	3,5±0,12
2. DM	32,1±0,58^{b}	1,3±0,04^{a}	124,2±9,4^{a}	1,5±0,12^{a}	2,6±0,18^{a}
3. DM + melatonin	20,2±0,67^{b}	2,3±0,03^{b}	160,2±9,34^{b}	3,6±0,10^{b}	3,7±0,14^{b}
4. IGT	26,4±0,63^{a,b}	3,4±0,04^{a,b}	182,0±11,1^{ab}	7,0±0,12^{a,b}	5,2±0,20^{a,b}
5. IGT + melatonin	20,7±0,55^{b,c}	2,3±0,02^{b,c}	152,0±8,4^{b,c}	4,0±0,06^{b,c}	3,8±0,15^{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; d - concerning indices on 4-th day

on 75% in DM group and on 47% in IGT group respectively than in control. So, the lipid peroxidation (MDA) was increased in diabetic brain. Melatonin partly prevented diabetes-induced increase in MDA in brain.

On the other hand, GSH level, GR, GPx, G-6-PhD activities also depend on the presents of hyperglycemia. In DM group of rats the level of GSH and activities of GR, GPx, G-6-PhD were decreased on 42%, 27%, 20%, 54% respectively compare with control rats. 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) level of GSH and activities of GR, GPx, G-6-PhD were increased on 55% and 49%, 17%, 48% respectively compare with control rats. Increase of G6PhD activity in condition of diabetes with preserved normoglycemia (IGT) is probably a compensatory reaction aimed to reduce of ROS.

NADPH2 reducing equivalents (that are 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 helped to normalize parameters of antioxidant body defense.

Conclusions

Melatonin was found to be excellent for strengthening the antioxidative defense system, reducing the generation of ROS and damaging oxidative substances, and maintaining membrane fluidity in the brain of diabetes-induced rats. Supplementation of melatonin modulated diabetic brain injury and can be potentially used for preventing diabetic neurodegenerative sequelae.

References. 1.Agil A, Navarro-Alarcon M, Ruiz R, et al. Beneficial effects of melatonin on obesity and lipid profile in young Zucker diabetic fatty rats. J Pineal Res 2011; 50:207-212. 2.Arendt J, Bojkowski C, Folkard S, et al. Some effects of melatonin and the control of its secretion in humans. Ciba Found Symp. 1985; 117:266-283. 3.Benova T, Viczenczova C, Radosinska J, et al. Melatonin attenuates hypertension-related proarrhythmic myocardial maladaptation of connexin-43 and propensity of the heart to lethal arrhythmias. Can J Physiol Pharmacol. 2013; 91:633-639. 4.Dominguez-Rodriguez A, Abreu-Gonzalez P, Reiter RJ. The potential usefulness of serum melatonin level to predict heart failure in patients with hypertensive cardiomyopathy. Int J Cardiol. 2014; 174:415-417. 5.Espino J, Pariente JA, Rodriguez AB. Role of melatonin on diabetes-related metabolic disorders. World J Diabetes. 2011; 2:82-91. 6.Favero G, Rodella LF, Reiter RJ, et al. Melatonin and its atheroprotective effects: a review. Mol Cell Endocrinol. 2014; 382:926-937. 7.Hang Sun, Aaron M. Gusdon, Shen Qua. Effects of melatonin on cardiovascular diseases: progress in the past year. Curr Opin Lipidol. 2016; 27(4): 408-413. doi: 10.1097/MOL.0000000000000314 8.Hardeland R, Pandi-Perumal SR, Cardinali DP. Melatonin. Int J Biochem Cell Biol. 2006; 38:313-316. 9.Hong JH, Kim MJ, Park MR, Kwag OG, Lee IS, Byun BH, Lee SC, Lee KB, Rhee SJ. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. Clin Chim Acta. 2004; 340(1-2):107-115. 10.Hu ZP, Fang XL, Fang N, et al. Melatonin ameliorates vascular endothelial dysfunction, inflammation, and atherosclerosis by suppressing the TLR4/NF-kappaB system in high-fat-fed rabbits. J Pineal Res. 2013; 55:388-398. 11.Hung MW, Kravtsov GM, Lau CF, et al. Melatonin ameliorates endothelial dysfunction, vascular inflammation, and systemic hypertension in rats with chronic intermittent hypoxia. J Pineal Res. 2013; 55:247-256. 12.Khedr N. Fish Oil and Wheat Germ Oil Supplementation Modulates Brain Injury in Streptozotocin-Induced Diabetic Rats. J Diabetes. 2016. doi: 10.1111/1753-0407.12518. 13.Liu X, Chen Z, Chua C. Melatonin as an effective protector against doxorubicin-induced cardiotoxicity. Am J Physiol Heart Circ Physiol. 2002; 283:H254-H263. 14.Ortiz-Avila O, Esquivel-Martinez M, Olmos-Orizaba BE, Saavedra-Molina A, Rodriguez-Orozco AR, Cortes-Rojo C. 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.Qian Guo, Young-Nam Kim, Bog-Hieu Lee. Protective effects of blueberry drink on cognitive impairment induced by chronic mild stress in adult rats. Nutr Res Pract. 2017; 11(1): 25-32. doi: 10.4162/nrp.2017.11.1.25/ 16.Sehirli AO, Koyun D, Tetik S, et al. Melatonin protects against ischemic heart failure in rats. J Pineal Res. 2013; 55:138-148. 17.Skene DJ, Arendt J. Human circadian rhythms: physiological and therapeutic relevance of light and melatonin. Ann Clin Biochem. 2006; 43:344-353. 18.Sun H, Huang FF, Qu S. Melatonin: a potential intervention for hepatic steatosis. Lipids Health Dis. 2015; 14:75. 19.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. doi: 10.1007/s11064-016-1928-7. 20.Tan DX, Manchester LC, Reiter RJ, et al. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998; 25:184-191. 21.Yang Y, Sun Y, Yi W, et al. A review of melatonin as a suitable antioxidant against myocardial ischemia-reperfusion injury and clinical heart diseases. J Pineal Res. 2014; 57:357-366.

АНТИОКСИДАНТНОЕ ДЕЙСТВИЕ МЕЛАТОНИНА В МОЗГЕ КРЫС С АЛЛОКСАНОВЫМ ДИАБЕТОМ

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Резюме. В статье показан возможный защитный эффект мелатонина как антиоксидантного средства против аллоксаном-провоцируемых нарушений в мозге крыс. Инъекции мелатонина аллоксандиабетичним крысам привели к снижению в последних уровня базальной гликемии, так же как и нормализации показателей нарушения антиоксидантной системы защиты, а именно: активности глутатионредуктазы, глутатионпероксидазы, глюкозо-6-фосфатдегидрогеназы, содержания малонового альдегида и восстановленного глутатиона в мозге крыс.

Ключевые слова: антиоксидантная система, мелатонин, аллоксановый диабет, мозг, крысы.

АНТИОКСИДАНТНА ДІЯ МЕЛАТОНІНУ В МОЗКУ ЩУРІВ З АЛОКСАНОВИМ ДІАБЕТОМ

О.Ю. Кушнір, І.М. Яремій

Резюме. У статті показаний можливий захисний ефект мелатоніну як антиоксидантного засобу проти алоксан-провокованих порушень у мозку щурів. Ін'єкції мелатоніну аллоксандіабетичним щурам призвели до зниження в останніх рівня базальної глікемії, а також до нормалізації показників порушення антиоксидантної системи захисту, а саме: актив-

ності глутатіон редуктази, глутатіон пероксидази, глюкозо-6-фосфатдегідрогенази, вмісту малонового альдегіду та відновленого глутатіону в мозку щурів.

Ключові слова: антиоксидантна система, мелатонін, алоксановий діабет, мозок, щури.

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