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MELATONIN IMPROVES MITOCHONDRIAL FUNCTION AND DECREASES OXIDATIVE STRESS IN GUMS OF DIABETIC RATS

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It is known that melatonin not only carries out control of circadian and seasonal biorhythms in the human body, but also helps to maintain oxidative antioxidant homeostasis and normoglucemia in the body. The purpose of the study was to determine the effect of melatonin on the content of reduced glutathione and the activities of glutathione peroxidase, catalase and superoxide dismutase, as well as the content of TBC reactive compounds and protein carbonyl content in the cytosol, and the activities of succinate dehydrogenase and H^+-ATP-ase in the mitochondria of the gums of rats with alloxan diabetes mellitus. Research methods. Animals were divided into 5 groups: 1) control; 2) rats with apparent diabetes – basal glycemia levels ≥ 8.0 mmol / l; 3) rats with apparent diabetes who, from day 5 after administration of aloxane, received daily melatonin 10 mg / kg of weight during 7 days daily at 8:00 per os; 4) rat with impaired glucose tolerance - basal glycemia level ≤ 6.9 mmol / l; 3) rats with impaired glucose tolerance, which were similarly administered within 7 days of melatonin. Results and discussion. In gingival tissues of rats with apparent diabetes, reduction in the content of reduced glutathione by 30% and increased activity of glutathione peroxidase by 32% were observed, respectively, when compared with control rats. The activity of catalase and superoxide dismutase decreased by 18% and 46% in the group of diabetic rats than in the control group. In the group of animals with impaired glucose tolerance, the activity of catalase was 25% higher than control. The content of TBC reactive compounds increased in groups of diabetic rats and with impaired glucose tolerance by 65% and 36% respectively, while the level of oxidized proteins in animals with diabetes increased by 52% compared to control. In the mitochondrial fraction of gum cells, decrease in the activity of succinate dehydrogenase and H + -ATPase in animals with diabetes was found to be 68% and 41%, respectively, as compared to control. Weekly daily administration to rats with apparent diabetes melatonin at a rate of 10 mg / kg contributed to the normalization of the glutathione system and basal glycemia we studied. Conclusion: These results demonstrate that melatonin supplementation prevents gingival mitochondrial dysfunction induced by diabetes in association with decreased oxidative stress.

Key words: antioxidative system, mitochondria, aloxane diabetes, gums, melatonin, rats.

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Introduction

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 [6]. The metalloproteins SOD, CAT, 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 [11].

Melatonin, a hormone secreted by the pineal gland, has remarkable antioxidant properties [13].

Diabetic gingivitis [8] is a diabetic complication related to the metabolic alterations featuring diabetes. Diabetes is characterized by increased lipid peroxidation, altered glutathione redox status, exacerbated levels of ROS, and mitochondrial dysfunction.

Succinate dehydrogenase (SDH) or succinate-coenzyme Q reductase (SQR) or respiratory Complex II is an enzyme complex, found in many bacterial cells and in the inner mitochondrial membrane of eukaryotes. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain [9]. ATPases in mitochondria are the prime producers of ATP, using the proton gradient generated by oxidative phosphorylation [5].

Taking this into consideration, the aim of this work was to evaluate the effects of melatonin intake in gingival mitochondrial function and cytosolic oxidative status in alloxan-induced diabetic rats.

The aim was to determine the influence of melatonin on basal levels of glucose (BG) in the blood, levels of protein carbonyl content and thiobarbituric acid reactive compounds (TBCRC), reduced glutathione (GSH), activities of glutathione peroxidase [EC 1.11.1.9] (GPx), superoxiddysmutase [EC 1.15.1.1] (SOD) and catalase [EC 1.11.1.6] (CAT) in cytosolic fraction of gums, activities of succinate dehydrogenase [EC 1.3.5.1] (SDH) and H^+-ATP-ase [EC 3.6.1.3] in gingival mitochondria of alloxan diabetic rats.

Materials and 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 research conducted on animals” (Kyiv, 2006). Diabetes was induced in male Wistar rats by single i.p. injection of alloxan (170 mg/kg) [7]. Four days after diabetes induction, rats were divided into diabetic (untreated) and mela-

tonin-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 ≥ 8,0 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 gingival 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. Gingival cytosolic oxidant status was assessed by measuring of GSH level, SOD, CAT, and GPx activities. Determinations of the enzymes activities were by standard methods [12].

In the process of oxidative modification of proteins in the radicals of the aliphatic amino acid residues, aldehyde and ketone groups are formed. They interact with 2,4-dinitrophenylhydrazine (2,4-DNPH) to form 2,4-dinitrophenylhydrazones with a specific absorption spectrum. Aldehyde- and keto-derivatives which are neutral in nature are determined at a wavelength of 370 nm [2]. The method of TBCRC determination is based on a spectrophotometric determination of the trimetinic colored complex formed from the TBCRC interaction with thiobarbituric acid [1].

Mitochondria were isolated by differential centrifugation in the isolation buffer [14]. Energy function of mitochondria was estimated by determination of succinate dehydrogenase activity [3] and H^+-ATP-ase [4].

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 $n \leq 50$, 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 and Discussion

Insertion of melatonin for 7 days helped to reduce 1.9 times compared with the baseline, basal glucose level in the group of animals with overt diabetes, indicating its hypoglycemic action.

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 [6].

To access the protein oxidation mediated by glycation process, the levels of protein carbonyl content (tabl. 1) were measured. The level of protein carbonyl groups was significantly increased in DM by 52% compared with control, whereas melatonin treatment significantly suppressed an increase in protein carbonyl content. When comparing with index of diabetic rats, the percentage reduction of carbonyl content by melatonin was found to be 19%.

The biochemical function of GPx is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. Glutathione neutralizes ROS, both directly and through GPx. We have found the level of GSH decreases by 30% and activity of GPx increases by 32% in DM group of animals compared with control. Reduced content of GSH in gums under DM, presumably due to both inhibition of its synthesis and enhanced use by GPx to neutralize hydrogen peroxide and other hydroperoxides, formed due to increased free radical oxidation of lipids and biopolymers in gum tissue. The activities of CAT and SOD were found to be lesser on 18% and 46% in DM group of rats than in control. In group of animals with IGT activity of CAT was on 25% higher than control possible due to absence of hyperglycemia. Melatonin injections was helpful for normalization this index under study.

TBCRC are formed as a result of lipid peroxidation that can be used to measure lipid peroxides after reacting it with thiobarbituric acid. The level of TBCRC was found to be higher on 65% in DM group and on 36% in IGT group respectively than in control. So, the lipid peroxidation was increased in diabetic gums. Melatonin partly prevented diabetes-induced increase in TBCRC in gingival tissues.

According to the results obtained, it may be postulated that melatonin inhibits glycation by reducing the generation of reactive carbonyl or dicarbonyl groups either from fructosamine or glucose, probably due to stimulation of glucose transport to cells [1] and preventing of ROS formation in conditions of hyperglycemia.

Reduced levels of the mitochondrial enzyme SDH (tabl. 2), the main element of complex II of electron transport chain, were observed in gingival mitochondria of DM rats which were on 68% less than control. Energy metabolism defects have been identified according decrease of the H^+-ATP-ase activity on 41% compared with control. Melatonin injections was helpful for normalization this index under study.

Melatonin, as it is known [10], stimulates the utilization of glucose by tissues, it promotes an increase in the tissues of ATP concentration, the restoration of disturbed under diabetes mellitus oxidant-antioxidant homeostasis. A week daily administration to rats with DM melatonin at a rate of 10 mg / kg contributed to a decrease in basal glycemia and normalization of all of our indices.

Table 1
Changes of the antioxidant defence in gums of diabetic rats, (n=6, x±S $\bar{x}$)

Indexes / Groups	G-SH, μmol/g	GPx, μmol/min×mg	CAT, mmol/min×mg	SOD, OD/min×mg	TBCRC, μmol/g of tissue	Protein carbonyl (370нм), mmol/g protein
1.Control group	93.0±3.5	437±31	103.2±3.54	0.25±0.012	21.5±1.09	2.04±0.08
2. DM	65.3±4.2[a] p=0.0032	574±28[a] p=0.014	84.5±5.27[a] p=0.015	0.14±0.009[a] p=0.00093	35.4±2.28[a] p=0.0024	3.11±0.19[a] p=0.003
3. DM + melatonin	94.4±3.8[b] p=0.0031	448±38[b] p=0.024	93.3±4.19	0.18±0.011[b] p= 0.019	27.8±1.23[b] p=0.015	2.52±0.18[a. b] p=0.035 p=0.048
4. IGT	97.8±5.0[b] p=0.0034	461±34[b] p=0.028	129.0±8.97[a.b] p= 0.024 p=0.0057	0.29±0.018[b] p= 0.00088	29.1±2.05[a.] p=0.014	2.53±0.21
5. IGT + melatonin	102.0±4.9[b] p=0.0022	455±28[b] p=0.013	105.0±5.22[b.c] p=0.02 p=0.043	0.26±0.015[b] p=0.0012	20.2±1.72[b.c] p=0.0027 p=0.013	2.17±0.09[b] p=0.0049

Note: 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

Table 2
Changes of mitochondrial energy function in gums of diabetic rats, (n=6, x±S $\bar{x}$)

Indexes / Groups	SDH, nmol/min×mg	H^+-ATP-ase, μmol (iP)/min×mg
1.Control group	10.87±2.860	0.37±0.023
2. DM	3.52±1.435[a] p=0.047	0.21±0.019[a] p=0.0027
3. DM + melatonin	9.03±1.81[b] p=0.041	0.29±0.011[a. b] p= 0.01 p= 0.0031
4. IGT	10.75±3.0598	0.38±0.015
5. IGT + melatonin	10.2±2.352	0.41±0.03

Note: 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

Conclusion

Melatonin improves gingival mitochondrial function in diabetic rats preventing impairment of mitochondrial respiration. Melatonin also decreased ROS levels and lipid peroxidation and improved the GSH level as well. These results demonstrate that melatonin supplementation prevents gingival mitochondrial dysfunction induced by diabetes in association with decreased oxidative stress.

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Реферат

МЕЛАТОНІН ПОКРАЩУЄ МІТОХОНДРІАЛЬНУ ФУНКЦІЮ ТА ПОПЕРЕДЖАЄ ОКИСНЮВАЛЬНИЙ СТРЕС В ЯСНАХ ДІАБЕТИЧНИХ ЩУРІВ

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Ключові слова: антиоксидантна система, мітохондрії, алоксановий діабет, ясна, мелатонін, щурі.

Відомо, що мелатонін не лише здійснює в організмі людини контроль циркадіанних і сезонних біоритмів, але й сприяє підтриманню в організмі оксидантно-антиоксидантного гомеостазу та нормоглікемії. Мета дослідження: з'ясувати вплив мелатоніну на вміст глутатіону відновленого й активності глутатіонпероксидази, каталази та супероксиддисмутази, а також вмісту ТБК-реактивних сполук і окисно модифікованих білків в цитозолі, та активність сукцинатдегідрогенази та H^+-АТФ-ази – в мітохондріях ясен щурів із алоксановим цукровим діабетом. Методи дослідження. Тварин було розділено на 5 груп: 1) контроль; 2) щури з явним діабетом – рівень базальної глікемії ≥ 8,0 ммоль/л; 3) щури з явним діабетом, яким починаючи з 5-ої доби після введення алоксану впродовж 7-ми днів щоденно о 8:00 per os вводили мелатонін з розрахунку 10 мг/кг маси; 4) щури з порушеною толерантністю до глюкози – рівень базальної глікемії ≤ 6,9 ммоль/л; 3) щури з порушеною толерантністю до глюкози, яким аналогічно впродовж 7-ми днів вводили мелатонін. Результати й обговорення. У тканинах ясен щурів із явним діабетом відзначалося зниження вмісту відновленого глутатіону на 30% та зростання активності глутатіонпероксидази на 32% відповідно при порівнянні з контрольними щурами. Активність каталази та супероксиддисмутази знизилися на 18% і 46% відповідно в групі щурів із діабетом ніж у контролі. В групі тварин з порушеною толерантністю до глюкози активність каталази виявилася на 25% вищою, ніж контроль. Вміст ТБК-реактивних сполук зріс в групах щурів із діабетом та порушеною толерантністю до глюкози на 65% та 36% відповідно, тоді як рівень окисно модифікованих білків у тварин з діабетом підвищився на 52% при порівнянні з контролем. У мітохондріальній фракції клітин ясенних тканин встановлено зниження активності сукцинатдегідрогенази та H^+-АТФ-ази в тварин з діабетом на 68% та 41% відповідно порівняно з контролем. Тижневе щоденне введення щурам із явним діабетом мелатоніну з розрахунку 10 мг/кг сприяло нормалізуванню досліджуваних нами показників глутатіонової системи та базальної глікемії. Висновок: Ці результати вказують на те, що уведення мелатоніну запобігає виникненню мітохондріальних порушень, викликаних діабетом, та зниженню окисного стресу.

Реферат

МЕЛАТОНИН УЛУЧШАЕТ МИТОХОНДРИАЛЬНУЮ ФУНКЦИЮ И ПРЕДУПРЕЖДАЕТ ОКИСЛИТЕЛЬНЫЙ СТРЕСС В ДЕСНАХ ДИАБЕТИЧЕСКИХ КРЫС

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Ключевые слова: антиоксидантная система, митохондрии, алоксановый диабет, десна, мелатонин, крысы.

Известно, что мелатонин не только осуществляет в организме человека контроль циркадианных и сезонных биоритмов, но и способствует поддержанию в организме оксидантно-антиоксидантного гомеостаза и нормогликемии. Цель исследования: выяснить влияние мелатонина на содержание глутатиона восстановленного и активности глутатионпероксидазы, каталазы и супероксиддисмутазы, а также содержания ТБК-реактивных соединений и окислительно модифицированных белков в цитозоле, и активность сукцинатдегидрогеназы и H^+ -АТФазы - в митохондриях десен крыс с алоксановым сахарным диабетом. Методы исследования. Животных было разделено на 5 групп: 1) контроль; 2) крысы с явным диабетом - уровень базальной гликемии ≥ 8,0 ммоль / л; 3) крысы с явным диабетом, которым начиная с пятого дня после введения аллоксана в течение 7-ми дней ежедневно в 8:00 per os вводили мелатонин из расчета 10 мг / кг массы; 4) крысы с нарушенной толерантностью к глюкозе - уровень базальной гликемии ≤ 6,9 ммоль / л; 3) крысы с нарушенной толерантностью к глюкозе, которым аналогично в течение 7-ми дней вводили мелатонин. Результаты и обсуждение. В тканях десны крыс с явным диабетом отмечалось снижение содержания восстановленного глутатиона на 30% и рост активности глутатионпероксидазы на 32% соответственно при сравнении с контрольными крысами. Активность каталазы и супероксиддисмутазы снизились на 18% и 46% соответственно в группе крыс с диабетом. В группе животных с нарушенной толерантностью к глюкозе активность каталазы оказалась на 25% выше, чем контроль. Содержание ТБК-реактивных соединений выросло в группах крыс с диабетом и нарушенной толерантностью к глюкозе на 65% и 36% соответственно, тогда как уровень окислительно модифицированных белков у животных с диабетом повысился на 52% при сравнении с контролем. В митохондриальной фракции клеток десневых тканей установлено снижение активности сукцинатдегидрогеназы и H^+АТФ-азы в животных с диабетом на 68% и 41% соответственно по сравнению с контролем. Недельное ежедневное введение крысам с явным диабетом мелатонина из расчета 10 мг / кг способствовало нормализации исследуемых нами показателей глутатионовой системы и базальной гликемии. Вывод: Эти результаты указывают на то, что введение мелатонина предотвращает митохондриальне нарушения, вызванные диабетом, и снижает окислительный стресс.