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 NADPH₂ 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 1 diabetes has resulted in an unprecedented number of older adults living with and managing type 1 diabetes [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 NADPH₂ production. The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase using NADPH₂ as a source of reducing electrons in erythrocytes, liver and other body tissue [7]. Glutathione system is one of the main antioxidants. The cell regenerates reduced glutathione in a reaction catalyzed by glutathione reductase using NADPH₂ 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 Statistics 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 ≤ 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.
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.

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 NADPH2 [10].
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.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.**

**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\textsubscript{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...
degredation. The intensity of such protein glyca-
tion 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 stimula-
tion 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 me-
tabolism, phospholipid metabolism, and redox
homeostasis. Beyond its direct free radical scav-
enging and indirect antioxidant effects, melatonin
has a variety of physiological and metabolic ad-
vantages that may enhance its ability to limit ox-
diative stress [10].

Conclusion. In this case melatonin probably
increases use of glucose for regeneration of
NADPH₂ and aerobic oxidation of glucose that in-
dicate an acceleration of antioxidative protection
and energy production in blood of adult and old
diabetic rats.

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нормалізації рівня базальної глікемії, активності піруваткінази та глутатіонредуктази в крові, а також значителному збільшенню активності глікозо-6-фосфатдегідрогенази, рівень активності якої перевищив у середньому на 9% цей конкретний показник у контрольній групі діабетичних щурів.

Мелатонін та його метаболіти володіють потенційними антиоксидантними та противітовими 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 встановлено вплив старіння на рівень базальної глікемії та активність глікозо-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 модулювання 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 щурів 5% розчину алоксану моногідрату внутрішньочеревно з розрахунку 170 мг/кг. У кожній з діабетичних груп було вибірено групу тварин без корекції та групу тварин, яким вводили мелатонін інтратонітально 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 мелатоніну. Декапітацію тварин проводили на 47-й день експерименту відповідно до етичного поводження з тваринами. Визначення активності ферментів проводилося 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 зросла 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 знижилась на 34% і 51% відповідно порівняно з контролем. Активність глікозо-6-фосфатдегідрогенази в еритроцитах дорослих та старих тварин з діабетом знижилась на 25% та 44% відповідно порівняно з контролем на 47-й день. Зміни можуть бути наслідком вікових порушень метаболізму глікози через порушення механізмів вільних радикалів.

Активність глутатіонредуктази в еритроцитах дорослих та старих тварин з діабетом зменшилася на 30% та 36% відповідно порівняно з контролем на 47-й день. Введення мелатоніну впродовж 42 днів щоденно діабетичним щурам обох груп сприяло нормалізації рівня базальної глікемії, активності піруваткінази та глутатіонредуктази в крові щурів, а також значному збільшенню активності глікозо-6-фосфатдегідрогенази, рівень активності якої перевищив у середньому на 9% цей конкретний показник у контрольній групі тварин. Під впливом мелатоніну підвищення активності глікозо-6-фосфатдегідрогенази в крові щурів може бути обумовлене збільшеним кількістю субстрата для глікозо-6-фосфатдегідрогенази (стимулювання надходження глікози в клітини та фосфориливання) і безпосередньою дією. Висновок. У цьому дослідженні мелатонін, імовірно, збільшує використання глікози для регенерації НАДФН2 та аеробного окислення глікози, що відкриває можливість для проаналізування антиоксидантного захисту та вироблення енергії в крові дорослих та старих діабетичних щурів.

Реферат
ВПЛИВ МЕЛАТОНІНУ НА ВІКОВУ ЗАЛЕЖНІСТЬ ЗМІН БАЗАЛЬНОГО ОБМІНУ ТА АНТИОКСИДАНТНОГО ЗАХИСТУ В КРОВІ ЩУРІВ З АЛОКСАНОВИМ ДІАБЕТОМ
Яремій І.М., Кушнір А.Ю.
Ключові слова: мелатонін, кров, алоксановий діабет, старіння, щури.